Heart rate and blood pressure variability: association with white matter lesions and cognitive function following stroke

MD Thesis

Dr Andrew McLaren

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Supervisor: Professor Rose Anne Kenny

Co-supervisor: Professor Clive Ballard

Contents

Declaration

I wish to certify that no part of the material contained herein has been submitted by me for a degree in the University of Newcastle upon Tyne or any other institution.

I wish to state my independent role in submission of this Thesis. The hypotheses and content of the Thesis have followed my own design. Screening candidates enrolled within the Cognitive Function After Stroke study, subsequent interview and recruitment for cardiovascular investigations and Magnetic Resonance Imaging was performed by myself. Cardiovascular investigations were performed by myself. I obtained all clinical data from patient interview and clinical notes and entered clinical and cardiovascular data to the database. I performed all analyses on cardiovascular data.

The study arose from hypotheses generated by Professors Rose Anne Kenny and Clive Ballard who provided guidance and direction to the issues addressed within the Thesis and acted as supervisors. Dr Louise Allan contributed to study design. Sandra Davies contributed to clinical assessment of participants. Jean Scott provided administrative support and arranged clinical assessment. Elise Rowan designed and administered the central COGFAST database for collating clinical data and organised data input to the database. Emma Burton and colleagues designed MRI analysis systems and provided all MRI data. Sally Stephens, Sandra Campbell and Michael Bradbury performed all neuropsychometric investigations and provided all the CAMCOG and CDR data. Simon Kerr provided all cardiovascular data on control cases in Chapter 7. Nick Steen provided advice on statistical analysis.

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Glossary

30:15 ratio	The ratio of the longest to shortest RR intervals that occur around the 30^{th} and 15^{th} heart beats after rising from the supine to standing position
ACEi	Angiotensin converting enzyme inhibitor
AD	Alzheimer's disease
AF	Atrial fibrillation
BMI	Body mass index
BP	Blood pressure
BPM	Beats per minute
BRS	Baroreflex sensitivity: the quotient of induced change in pulse interval over the causing change in blood pressure
CAMCOG	The cognitive subsection of the Cambridge Mental Disorders of the Elderly Examination: an objective cognitive test, forming a mini-neuropsychological battery
Carotid sinus massage	Digital pressure over the carotid artery at the location of the carotid sinus
CDR	Cognitive Drug Research Assessment System: computerised tests of cognitive function measuring concentration and reaction time
CI	Confidence interval
COGFAST	Cognitive Function After Stroke study
Cold pressor	Immersion of the hand in cool water bath to provoke cardiovascular autonomic reflex, normally leading to a rise in blood pressure
COPD	Chronic obstructive pulmonary disease
CRT	Choice reaction time: CDR measure of reaction time to visual stimulus
CT	Computerised tomography
CV	Co-efficient of variation
DBP	Diastolic blood pressure

ΔDBP	Change in diastolic blood pressure during an autonomic test
ECG	Electrocardiogram
E/I ratio	Ratio of longest to shortest RR interval during respiration: longest interval occurs during expiration and shortest during inspiration
Ewing protocol	A series of cardiovascular autonomic reflex tests involving three measures of RR interval change and two measures of blood pressure change
FEV_1	Forced expiratory volume in one second
Finapres	Non-invasive blood pressure measurement device, able to record beat-to-beat blood pressure from the finger pulse (FINger Arterial PRESsure)
FVC	Forced vital capacity
High frequency (HF)	Frequencies between 0.15 and 0.40 Hertz from a power spectral analysis of heart rate or blood pressure variability: the HF power spectral density is the area under the curve for that band of frequencies
HR	Heart rate
ΔHR	Change in heart rate during an autonomic reflex test
HRV	Heart rate variability: measure of variation in heart rate or RR interval during a defined period
E-I difference	Difference between peak inspiratory heart rate and nadir expiratory heart rate during cycle of respiration
IMT	Intima media wall thickness
Isometric exercise	Skeletal isometric muscle exercise that provokes cardiovascular autonomic reflexes, normally a rise in blood pressure
LVH	Left ventricular hypertrophy
Low frequency (LF)	Frequencies between 0.04 and 0.15 Hertz from a power spectral analysis of heart rate (or blood pressure) variability: the LF power spectral density is the area under the curve for that band of frequencies
LVMI	Left ventricular mass index

Metronomic respiration	Timed and deep respiration at a set frequency for a defined period to provoke cardiovascular autonomic reflexes
MVC	Maximal voluntary contraction: measure of effort during isometric muscle exercise, usually expressed as a percentage
mmHg	Millimetres mercury: blood pressure measurement unit
MBP	Mean blood pressure
MI	Myocardial infarction
MMSE	Mini-mental state examination: a point item test of cognitive function
MRI	Magnetic resonance imaging
ms	millisecond
MSNA	Muscle sympathetic nerve activity: direct measure of sympathetic nerve traffic to skeletal muscle using transcutaneous microneurographic recording of superficial peripheral nerves
OCSP	Oxfordshire Community Stroke Project
Power band	Frequency bands of interest from power spectral analysis
Power spectral analysis (PSA)	Computerised measurement of RR interval or BP variation in the frequency domain: illustrates how variance distributes as a function of frequency
PP	Pulse pressure: difference between systolic and diastolic blood pressure
RR	The time interval between succesive R waves on the electrocardiogram
RSA	Respiratory sinus arrhythmia: the normal variation in heart rate with respiration
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
Spectral density	Area under the curve measurement following power spectral analysis (the 'power'): frequency plotted along *x* axis and amplitude

	along *y* axis
SRT	Simple reaction time: CDR measure of reaction time to visual stimulus
SSRI	Selective serotonin re-uptake inhibitor
SSS	Scandinavian Neurological Stroke Scale
Tilting	Elevating a subject from the supine to the upright position whilst secured to a powered tilting table, therefore the upright position is attained without normal skeletal muscle contraction
TIA	Transient ischaemic attack
VaD	Vascular dementia
Valsalva manoeuvre	Prolonged forced expiration with an open glottis, at a set pressure and expiration period, to increase intrathoracic pressure
Valsalva ratio (VR)	Ratio of longest to shortest RR intervals during Valsalva manoeuvre
WML	White matter lesion

1 Abstract

1.1 Background

Dementia presents a significant health care burden. Older post-stroke patients suffer high rates of dementia. Subcortical ischaemia may be an important mechanism of cognitive decline, particularly in older patients with cerebrovascular disease. It is hypothesised that abnormal heart rate and blood pressure variability will increase white matter lesion volume through hypoperfusion. This may lead to a subcortical pattern of cognitive decline characterised for example by deficits in attention and concentration.

1.2 Method

Stroke patients aged ≥ 75 years and free of dementia had a series of cardiovascular autonomic, brain imaging and neuropsychometric investigations performed more than three months following incident stroke. Autonomic reflex tests comprised blood pressure drop and RR interval change during standing and carotid sinus massage, blood pressure change during isometric exercise and cold cutaneous stress, blood pressure overshoot and heart rate change during Valsalva manoeuvre and heart rate variation during metronomic respiration. Heart rate variability and baroreflex sensitivity were measured over five minutes using power spectral analysis. Mean blood pressure, long term blood pressure variability and diurnal variation in blood pressure were measured by 24 hour ambulatory monitoring. Cortical white matter lesion volume was estimated from magnetic resonance imaging sequences of the brain at baseline and after an interval of two years. Annual neuropsychometric assessment included CAMCOG score and measures of reaction time and concentration using a series of visual and numerical tasks presented on computer (Cognitive Drug Research Assessment System).

1.3 Results

Stroke patients suffered deficits in both parasympathetic, sympathetic reflex tests and baroreflex sensitivity compared to an age matched group of stroke free control cases. There were no associations between individual cardiovascular autonomic tests and white matter lesion volume from the cross-sectional or longitudinal analyses. However a composite autonomic reflex score indicated a trend towards an association with white matter lesion volume at baseline. Systolic blood pressure variability, mean diastolic blood pressure and diabetes were significant independent predictor variables for baseline white matter lesion volume in stroke patients in sinus rhythm. Inclusion of all stroke cases (sinus rhythm and

atrial fibrillation) in regression analysis found an additional positive relationship for cardiac failure, and inverse relationship for forced vital capacity, with baseline white matter lesion volume as the dependent variable. Multiple regression of all risk factors with rate of white matter lesion volume increase over two years as the dependent variable yielded cardiac failure and ischaemic heart disease as positive and thiazide use as negative independent predictors. Daytime mean diastolic pressure was inversely associated with CAMCOG executive subscore at baseline. Increasing nocturnal dip in blood pressure was inversely associated with total CAMCOG score. There was a positive association between blood pressure rise during isometric exercise and vigilance tests, and an inverse association between Valsalva ratio and working memory. Decreasing high frequency baroreflex sensitivity was associated with impaired reaction time. None of the cardiovascular indices predicted deterioration in neuropsychometric function over one or two years.

1.4 Conclusion

Autonomic function is impaired in older stroke patients in the long term after stroke. These deficits are weakly associated with cross-sectional measures of sub-cortical performance but do not predict subsequent decline in cognitive function. Twenty-four hour blood pressure variability is associated with white matter disease and excessive nocturnal dipping is associated with impaired cognitive function. Again blood pressure variability does not help predict subsequent change in white matter lesion burden or cognitive function.

This study provides limited support for the hypoperfusion theory of post-stroke cognitive impairment. However it does not indicate a role for heart rate and blood pressure variability in the mechanism of increasing white matter disease or decline in cognition in the two years following stroke.

2 Epidemiology and pathology of vascular dementia

2.1 Classification

Vascular dementia is one of the three most common forms of dementia. The term describes a state of intellectual impairment due to organic brain failure resulting from vascular disease. There are several diagnostic criteria in current use. The format for most of these definitions is evidence of dementia, cerebrovascular disease and a temporal link between these two key features. However there are subtle differences in how these features are specified within each definition. Five of the most widely used diagnostic criteria are now discussed.

The American Psychiatric Association published the 'Diagnostic and Statistical Manual of Mental Disorders, Third Edition (DSM-III)' in 1980 (American Psychiatric Association, 1980). This has been updated on two occasions, DSM-III-R in 1987 (American Psychiatric Association, 1987) and the DSM-IV in 1994 (American Psychiatric Association, 1994). DSM-IV is widely used in routine clinical practice in the UK. It requires the presence of impairment in memory plus one other cognitive domain for dementia. Cerebrovascular disease can be either focal neurological symptoms or signs or laboratory evidence of ischaemic or haemorrhagic brain injury. Causal relationship is by clinical judgment. Inter-rater reliability is moderate (κ=0.59) (Chui, 2000). The DSM-III diagnosis for vascular dementia (VaD) was proven accurate by neuropathological criteria in 85% of 25 necropsy cases (Erkinjuntti, 1988).

The World Health Organization published *The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Clinical Guidelines* in 1993 (World Health Organization, 1993). This requires patchy distribution of cognitive deficits, focal neurological signs and clinical or laboratory evidence of significant cerebrovascular disease (CVD). There should be reasonable evidence of an aetiological link between CVD and dementia. Specificity was comparatively high when judged with other diagnostic criteria but this study used a 'standard clinical diagnosis', not neuropathological validation (Pohjasvaara, 1997).

The Hachinski Ischaemic Score (HIS) is a checklist of vascular risk factors and clinical features theoretically associated with VaD with a weighted score for each item (with various subsequent modifications) (Hachinski, 1975). Although widely known, it has a number of limitations. It does not specify the requirement for dementia (Homer, 1988; Rosen, 1980). Accuracy is less than 70% (Swanwick, 1996), specificity is 0.88, sensitivity is 0.43 (Gold, 1997) and inter-rater reliability is moderate (κ =0.59) (Chui, 2000; O'Neill, 1995). It has been superseded by more advanced criteria for research purposes.

The State of California Alzheimer Disease Diagnostic and Treatment Centers criteria for ischaemic vascular dementia (IVD) was formulated in 1992 (Chui, 1992). Note that only ischaemic damage is classified, dementia due to haemorrhagic brain injury is excluded. The cognitive deficit has to occur in more than one domain and be of any type. For *probable* IVD, there must be at least one infarct outside the cerebellum on neuroimaging plus evidence of at least 2 strokes from history, clinical signs or neuroimaging or a single stroke with a definite temporal link to dementia onset. *Possible* IVD amounts to evidence of a single stroke without a clear link to dementia onset or clinical and radiographic signs of extensive sub-cortical white matter disease. In the neuropathological validation study, sensitivity was 0.63 and specificity 0.64 (Gold, 1997). Inter-rater reliability is fair (κ=0.44) for probable and poor (κ=0.15) for possible IVD (Chui, 2000).

The National Institute of Neurological Disorders and Stroke – Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria for vascular dementia was published in 1993 (Roman, 1993). They are perhaps the most stringent diagnostic criteria. Memory plus at least 2 other cognitive domains must be impaired. *Probable* vascular dementia requires the following criteria: (a) cerebrovascular disease must be evident by virtue of clinical signs and a relevant neuroimaging abnormality and (b) the temporal relationship required is dementia onset within 3 months of stroke or an acute deterioration in cognitive function. *Possible* vascular dementia is diagnosed if neuroimaging evidence or the clear temporal association is absent. In neuropathological validation studies, sensitivity and specificity were 0.58 and 0.80 respectively in a group of geriatric inpatients with retrospective clinical diagnoses (Gold, 1997) but 0.43 and 0.95 in a community based post-mortem series (Holmes, 1999). Inter-rater reliability is moderate to substantial (κ= 0.42 – 0.72) (Chui, 1992; Lopez, 1994).

Thus the current diagnostic criteria for vascular dementia have a number of weaknesses. The underlying problem is that all these criteria were designed by consensus and not based on the gold standard of neuropathological data. However the issue is further complicated by the absence of recognised pathological criteria for vascular dementia. Thus one of the difficulties in this field is the the process of validation any of the clinical criteria. The pitfall of inconsistency between diagnostic criteria is underlined by studies documenting their poor concordance. The rate of diagnosis of vascular dementia has varied as much as fourfold between criteria (Chui, 2000; Pohjasvaara, 1997; Verhey, 1996; Wetterling, 1996) and even a tenfold difference between DSM III and ICD-10 in a community study (Erkinjuntti, 1997). DSM-IV tends to give the highest rate of diagnosis, NINDS-AIREN the lowest and ICD-10 plus ADDTC an intermediate rate. DSM-IV is valuable for community epidemiology by virtue of good sensitivity and absence of requirement for neuroimaging. ICD-10 also benefits

from not requiring scanning but sensitivity is poorer. NINDS-AIREN strength lies in excellent specificity and is widely used in research fields.

These consensus-led criteria have conceptual weaknesses (Bowler, 1999). The diagnosis of dementia is historically based on Alzheimer's disease but vascular dementia usually results in a different pattern of cognitive decline. Similarly the criteria rely on established dementia, but vascular dementia is a preventable illness, therefore early case identification at the stage of mild cognitive impairment will prove more useful for research and treatment. Some criteria rely on a history of stroke which may be lacking in cognitively impaired subjects with numerous vascular risk factors or neuropathologically proven vascular dementia (Emery, 1996). Another key difficulty is the emerging evidence of neuropathological overlap between vascular dementia and Alzheimer Dementia (Kalaria and Ballard, 1999; Rockwood, 2000). This situation has led to the pathological term 'mixed dementia' but this condition needs better recognition in diagnostic criteria.

Criteria should also take account of the subtypes of vascular dementia which may have fundamentally different pathophysiology and clinical presentation. One review identified 8 subtypes of vascular brain injury resulting in dementia (Loeb and Meyer, 1996). The most common presentations are listed first.

- Cortical infarcts due to large vessel disease
- Widespread subcortical disease due to small vessel disease (lacunar infarcts)
- Binswanger's disease (probably synonymous with small vessel disease)
- Mixed large and small vessel disease
- Mixed vascular disease and Alzheimer's disease.
- Single small strategic infarcts
- Haemorrhagic stroke disease
- Subcortical disease due to rare inheritable arteriopathies e.g. CADASIL

For a discussion of small vessel disease, see section 2.3.3. One of the most difficult areas of diagnosis is separating AD from subcortical vascular dementia since they share similar risk factors and an insidious onset of dementia (Ransmayr, 1998). In some instances subcortical disease lacks chronic focal neurological signs, increasing the difficulty of identifying vascular dementia. Further developments in diagnostic criteria need to define:

- Vascular aetiology, in subtypes
- Cognitive changes
- Neuropathological findings
- The temporal connection between evolution of vascular disease and cognitive decline
- Exclusion criteria for similar presentations of different forms of dementia.

2.1.1 Conclusion

There are a number of diagnostic criteria for vascular dementia developed by consensus. DSM-IV has good sensitivity whereas NINDS-AIREN has excellent specificity. Concordance is poor. Clinical diagnosis of mixed dementia is a problematic area. Neuropathological validation is required to improve diagnostic criteria.

2.2 Epidemiology

2.2.1 Incidence studies

The literature contains work on the incidence and prevalence of vascular dementia in both community and post-stroke cohorts. The characteristics of diagnostic criteria employed in each study should be borne in mind when comparing diagnostic rates. Vascular dementia has a poor prognosis, three year mortality being as high as 67% in over 85 year olds (Skoog, 1993).

Table 2-1 Incidence of Vascular dementia in community studies

Study author and location	Age range	Sample number (number screened)	Incidence all cause dementia/1000 (criteria)	Incidence VaD/1000 (criteria)	Incidence AD/1000 (criteria)
Cambridge (Brayne, 1995)	≥ 75	1173	133 (CAMDEX)	12 (95% CI 7-19) (CAMDEX)	27 (95% CI 16-44) (CAMDEX)
Japan (Yoshitake, 1995)	≥ 65	828	M 19.3 F 20.9 (DSM-IIIR)	M 12.2 F 9.0 (NINDS – AIREN)	M 5.1 F 10.9 (NINCDS – ADRDA)
Scotland (McGonigal, 1993)	40 - 64	6092 (hospital casenotes)		M 0.25 F 0.13 (HIS)	Probable: broad = 0.23 : 0.41
Liverpool (Copeland, 1999)	≥ 65	5222 (6035)	19 (AGECAT)	2.6 (ICD-10)	4.9 (ICD-10)
London (Boothby, 1994)	> 65	502 (656)	20 (DSM-III)	4 (HIS)	8 (NINCDS-ADRDA)
Canada (Hebert, 2000)	> 65	5747 (8623)		3.79* (ICD-10)	
Sweden (Hagnell, 1992)		2612		(lifetime risk M 34.5% F 19.4%)	
Sweden (Fratiglioni, 1997)	>74	987 (1473)	148 (DSM-III-R)	75-79 yrs M 12, F 20 ≥90 years M 15, F 87	

M, male: F, female: CAMDEX, Cambridge Examination for Mental Disorders of the Elderly: DSM, Diagnostic and Statistical Manual of Mental Disorders: NINDS-AIREN, National Institute of Neurological Disorders and

Stroke – Association Internationale pour la Recherche et l'Enseignement en Neurosciences: NINCDS-ADRDA. National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimers Disease and Related Disorders Association: HIS, Hachinski Ischaemic Score: AGECAT, see Copeland JRM 1999: ICD. International Classification of Diseases: * Incidence adjusted for mortality rate

Therefore a short screening interval is necessary to optimise case recognition. VaD is under-reported on death certificates (Thomas, 1997) and is under-represented in referrals to secondary care facilities (Nolan, 1998), presenting further pitfalls in epidemiological studies.

The incidence rates for VaD can vary markedly between studies, as shown in Table 2.1. In a meta-analysis of incidence rate studies in 1998 dementia rates rose exponentially with age (significant for all-cause and Alzheimer's disease, non-significant trend for VaD) (Jorm and Jolley, 1998). Some studies are hampered by small sample size (Boothby, 1994) or use of insensitive diagnostic tools resulting in high rates of undifferentiated dementia (Hebert, 2000). Note that in the McGonigal study (McGonigal, 1993) incidence rates were taken from referrals to secondary psychiatry services and extrapolated to the general population, therefore this was not a true community based survey. A review estimated an incidence rate of between 6 to 12 per 1000 for vascular dementia beyond the age of 70 (Hebert and Brayne, 1995). A review of epidemiological surveys in 1998 stated incidence rates of 20 to 40 per 100,000 in the 60 to 69 year old age range, and 200 to 700 per 100,000 in the over 80 years old age range (Leys, 1998).

2.2.2 Community prevalence studies

A comparative study of prevalence studies in Europe in 1991 (Rocca, 1991) identified 5 out of a possible 23 reports meeting their inclusion criteria. Prevalence rates markedly varied between studies. Prevalence increased with age and was generally higher in males. Subsequent prevalence studies are summarised in Table 2.2.

Table 2-2 Prevalence of Vascular Dementia in community studies

Study reference and location	Age range, sample size (screened)	Prevalence all cause dementia /1000 (criteria)	Prevalence vascular dementia /1000 (criteria)	Prevalence Alzheimer Disease /1000 (criteria)	Comments
Japan (Ueda, 1992)	≥ 65 887	67 (DSM-III)	31 (pathology)	15 (pathology)	50/59 dementia cases had autopsy
Sweden (Skoog, 1993)	>85 494	298 (DSM-III-R)	140 (clinical)	130 (NINCDS-ADRDA)	3 yr mortality 66.7% VaD 42.2% AD
Italy (Prencipe, 1996)	≥ 64 968 (1147)	80 (DSM-III)	22 (NINDS-AIREN)	52 (NINCDS-ADRDA)	AD 64% and VaD 27% of all dementias
Rotterdam (Ott, 1995)	55-106 7528	63 (DSM-III-R) 4 (55-59 yr) 432 (>95 yr)	10 (DSM-III-R)	45 (NINCDS-ADRDA)	AD 72% and VaD 16% of all dementias
India (Shaji, 1996)	> 60 2067	33.9 (DSM-III-R)	19 (ICD-10)	13 (ICD-10)	AD 41% and VaD 58% of all dementias
Honolulu (White, 1996)	71-93 3734 (4678)	93 (DSM-III-R) 21 (71-74yr) 330 (85-93yr)	18 (ADDTC) 42*	21 (NINCDS-ADRDA) 54*	14/1000 mixed (with AD or VaD) dementia
Hong Kong (Chiu, 1998)	>70 1034 (1503)	61 (DSM-IV)	18 (CAMDEX)	39 (DSM-IV)	
Egypt (Farrag, 1998)	>60	45	10	22	6/1000 mixed dementia
Korea (Woo, 1998)	>65 1674 (2171)	95 (NINCDS-ADRDA)	25 (HIS) M 31, F 21	45 (NINCDS-ADRDA) M 32, F 53	
Utah (Lyketsos, 2000)	≥65 5092 (5677)	65 (DSM-IV)	12 (NINDS-AIREN)	42 (NINCDS-ADRDA)	
Beijing (Wang, 2000)	≥65 3728	35 (DSM-III-R)	14 (ICD-10)	19 (ICD-10)	

M, male: F, female: DSM, Diagnostic and Statistical Manual of Mental Disorders: NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimers Disease and Related Disorders Association: NINDS-AIREN, National Institute of Neurological Disorders and Stroke – Association Internationale pour la Recherche et l'Enseignement en Neurosciences: ICD, International Classification of Diseases: ADDTC, Alzheimers's disease Diagnostic and Treatment Centers criteria: CAMDEX, Cambridge Examination for Mental Disorders of the Elderly: HIS, Hachinski Ischaemic Score: *primary or contributing cause; M=male, F=female

Some general comments can be made from the studies shown in Table 2.2. Vascular dementia rates clearly increase with age and vary between continents. In Western populations,

Alzheimer's disease is usually more prevalent than vascular dementia by a factor of 2 to 3, whereas in Asian populations prevalence rates are more even for these two causes of dementia. Prevalence rate for vascular dementia increase with age, for example rising from 10/1000 in over 60 years old in Egypt (Farrag, 1998) to 298/1000 for those aged over 85 years in Sweden (Skoog, 1993).

Prevalence rates in two reviews of vascular dementia were estimated to be 12-42/1000 for ages over 65 (Hebert and Brayne, 1995), or were quoted to vary from a minimum of 22/1000 (female) for the age range 70-79 to a maximum of 163/1000 (male) aged over 80 years (Leys, 1998). There's a consistent pattern for Alzheimer's disease to be more prevalent in Caucasian cohorts. VaD is often the more prevalent subtype in Asian and black cohorts.

2.2.3 Epidemiology of post-stroke dementia

Before describing the studies on post-stroke dementia, there are two points relevant to prevalence and incidence studies. Firstly the prevalence of pre-stroke dementia has been recorded at 10-16 % (Barba, 2000; Henon, 1997) which can account for approximately 1/3 of the post-stroke prevalence. Secondly the high attrition rate following stroke will influence the recorded dementia rates in survivors since dementia rates are likely to be much higher in the group who die early (Desmond, 1998a). Therefore timing of sampling is important.

A study of 463 consecutive stroke admissions in 1985 recorded 237 survivors at 6 months, 189 of whom were assessed using the Mini Mental State Examination (Ebrahim, 1985; Folstein, 1975). Twenty-two had an abnormal MMSE (of less than 21/30) thus 11.1% of testable survivors were considered demented. This is probably an underestimate in view of the low cut-off value on the MMSE; secondly it's likely the prevalence would be higher in those who were not assessable. A selective study of 108 lacunar infarct survivors from a series of 750 stroke inpatients excluded subjects with pre-existing dementia and diagnosed incident dementia using DSM-III-R criteria; 25 (23.1%) new cases of dementia were diagnosed over an average follow-up of 4 years, but the study does not quote annual incidence figures (Loeb, 1992). Cognitive function was recorded in 122 survivors at 6 months post-stroke from an initial inpatient cohort of 149 subjects (House, 1990). Twenty-six subjects (21%) had an MMSE less than 24/30. The same study also noted that 8/95 subjects deteriorated from a normal MMSE > 24 at 1 month to less than 24 at the 12 month post stroke period, giving a crude incidence rate of dementia of 8.4%.

A retrospective community study of dementia incidence in stroke survivors living in the community in Minnesota identified 971 individuals who suffered a first infarct with no evidence of previous dementia (Kokmen, 1996). Dementia incidence 3 months post-stroke was 7%, which was 8.8 times the predicted community rate. Interestingly they noted 40% of

these cases were Alzheimer's disease which was also much greater than the predicted community dementia incidence rate. The increased incidence of dementia in this cohort persisted but attenuated to twice the community rate over a 25 year follow-up period. The study does not quote which if any diagnostic criteria were employed.

Tatemichi published a series of papers on post-stroke dementia. From 927 consecutive ischaemic stroke admissions, 116 of 726 (16%) testable patients were demented at 10 days post-stroke (using clinical judgment only) with a subsequent 1 year incidence of 5.4% and 10.4% in 60 and 90 year olds respectively (Tatemichi, 1990). Another cohort were assessed at 3 months post-stroke using DSM-III-R, 26.3% (66/251) were demented compared with the community control rate of 3.2%, an odds ratio of 9.4 (95% CI 4.2-21.1) (Tatemichi, 1992). This study did not exclude subjects with pre-stroke cognitive impairment but found that 56% of the incident cases were probable vascular dementia and a further 36% were mixed Alzheimer's disease and VaD on clinical grounds. Finally, a prospective study of 184 post-stroke subjects over 60 years age had a dementia incidence of 8.4 per 100 person years compared with 1.3 in community controls, a relative risk of 5.5 (95% CI 2.5-11.1) (Tatemichi, 1994b).

2.2.4 Conclusions

Vascular dementia accounts for 10-30% of all dementia cases. The community incidence rate is at least 2.5/1000 per year in persons at risk, has a male preponderance and rises steeply with age. The prevalence is approximately 2% in those aged over 65 years and perhaps as much as 15% over 85 years age. The incidence is increased up to nine-fold in the first year following ischaemic stroke.

2.3 Pathology

Neuropathological studies on VaD highlight a number of key issues. Unfortunately the area tends to suffer from a confusing array of terminology. For example the numerous terms ascribed to small vessel disease, subcortical ischaemia, Binswangers disease, subacute arteriosclerotic encephalopathy and subacute ischaemic leukoencephalopathy are essentially describing the same process of bilateral ischaemic damage to the white matter (Roman, 1987). As previously discussed there are several subtypes of VaD and studies often benefit from subtype recognition in view of basic differences in aetiology. In particular the sudden onset of dementia following a cortical stroke due to large vessel disease or cardio-embolisation has different characteristics to the insidious onset of dementia from recurrent lacunar infarcts

from small vessel disease. Literature reports have so far tended to examine selective hospital based cohorts (Brayne, 1993) but more community based data is now emerging.

2.3.1 Neuropathological features

An important early study raised a number of issues in the definitive pathological changes of VaD (Tomlinson, 1970). This report described typical neuropathological changes in vascular dementia and indicated that larger volumes of infarcted tissue and presence of bilateral lesions played an important role in disease expression. An investigation of the validity of DSM-III VaD criteria found subjects had suffered bilateral infarcts in 96% cases particularly in the basal ganglia and temporal lobes. The volume of infarcted tissue (but not the number of infarcts) was proportional to the severity of dementia (Erkinjuntti, 1988). A study examining the neuropathology of subjects with only vascular lesions compared findings between demented and non-demented individuals, and concluded that infarct volume and number, lacunar state and white matter lesions were increased in dementia (del Ser, 1990). Macro-infarction involving cortical tissue can lead to dementia as shown in case series (del Ser, 1990; Hulette, 1997). Such large vessel disease often co-exists with small vessel disease (Brun, 1994) and many reports emphasise the role of the latter in vascular dementia. Vascular dementia subjects diagnosed by NINDS-AIREN criteria are characterised by diffuse white matter changes of arteriosclerosis, spongiosis etat crible and myelin loss (Erkinjuntti, 1996). A study comparing non-demented controls, cognitively normal subjects with cerebrovascular disease and dementia cases with only cerebrovascular disease (no/minimal AD changes) at post-mortem found that microvascular disease was the chief substrate of VaD, consisting of subcortical white matter damage, microinfarction and cribriform change: macro-infarction was rare in dementia (Esiri, 1997). Similar abnormalities of small infarcts, diffuse white matter rarefaction and reduced myelin were found in a group of 40 vascular dementia cases (diagnostic criteria not stated) (Englund, 1998).

2.3.2 Histochemistry and functional neuropathology

The reduction in cerebral acetylcholine activity is established in Alzheimer's disease but also occurs, to a lesser extent, in VaD (Perry, 1977). This finding has been replicated in studies which have also shown reduced serotinergic activity. Furthermore, in both these neurotransmitter disturbances occur at sites distant to infarcted tissue (Gottfries, 1994; Wallin, 1989). Recent functional neuroimaging studies indicate frontal lobe dysfunction in VaD. A study comparing normal controls, non-demented stroke subjects and sub-cortical stroke subjects with cognitive impairment indicated global cortical glucose metabolism is impaired in all groups with sub-cortical disease and deteriorated according to the degree of cognitive impairment. In addition sub-cortical infarction was associated with frontal regional

hypometabolism (Kwan, 1999). Cortical metabolic dysfunction correlates with the severity of subcortical small vessel disease in VaD (Sultzer, 1995). Memory failure in cognitively impaired subcortical stroke subjects correlates with reduction in frontal lobe glucose metabolism, whereas in Alzheimer's disease subjects memory failure correlates with hippocampal and temporal lobe hypometabolism (Reed, 2000). Frontal lobe hypometabolism and hypoperfusion is potentially useful as a diagnostic aide when differentiating between Alzheimer's disease and VaD (Nagata, 2000).

2.3.3 Pathophysiology of small vessel disease

Key research questions in VaD include the role of white matter disease, the influence of other non-vascular pathology and the concept of vascular dementia in the absence of stroke. Central to these issues is small vessel disease of subcortical white matter, which is clearly associated with vascular dementia (Esiri, 1997; Pantoni, 1996; Wallin and Blennow, 1991). The aetiology of small vessel disease is related to the lacunar hypothesis, reviewed by Bamford (Bamford and Warlow, 1988). Essentially pathological luminal narrowing of the microvasculature in the subcortex, particularly in the deep perforating vessels, renders the white matter vulnerable to ischaemic damage. Reduced or absent flow may lead to a well defined array of clinical syndromes referred to as lacunar stroke (Fisher, 1982). Lacunar damage is even more frequently asymptomatic (Tuszynski, 1989). Neuropathological proof of the lacunar hypothesis has been elusive owing to the low mortality rates immediately following lacunar stroke. There is evidence the hypothesis is valid from CT and MRI studies of acute lacunar stroke (Boiten and Lodder, 1991; Lindgren, 2000). The most common sites for lacunar stroke from necropsy studies are frontal lobe white matter, putamen, pons, parietal lobe white matter, thalamus and caudate nucleus in descending order of frequency (Dozono, 1991).

The pathophysiology of small vessel ischaemia is a crucial issue. Abnormal arteriole wall structure is certainly implicated. This was described by Fisher who examined the arterial lesions underlying 50 lacunes in 4 subjects; note that 36 lacunes were from just 1 subject (Fisher, 1969). In 90% of lacunes, it was associated with an arterial occlusion, usually due to "segmental arterial disorganisation"; this consisted of patchy layers of connective tissue obliterating the lumen, often exacerbated by haemorrhage within the abnormal vessel wall. A different occlusive lesion, fatty atheroma, was found in larger 300-500 micron arteries and was associated with larger volume infarcts with a more pronounced neurological deficit. Occlusion of a cerebral vessel leads to cellular hypoxia which triggers a chain of events from reduced ATP production, free radical production and finally irreversible glial injury; the severity of occlusion will influence the spatial and temporal dynamics of cellular injury

(Pulsinelli, 1992). In other words, a partial occlusion will theoretically produce cellular damage more slowly and in a smaller area than a complete and sudden occlusion.

The anatomy of the arterial tree supplying the white matter is important. Long tortuous arterioles branch out from the main cerebral arteries to supply white matter. Pial arteries on the cerebral surface also form perforating arteries; short and medium length vessels supply the cortex whilst longer 'medullary' penetrators continue to supply the deep white matter (Rowbotham and Little, 1965). The tissue between the 2 groups of perforators lies in a borderzone and is vulnerable to hypoperfusion (De Reuck, 1971). The perforators appear susceptible to hypertensive damage which results in lipohyalinosis, also known as hyaline arteriosclerosis or fibrohyalinosis. The arteriolar wall thickens due to deposition of hyaline, a material that shows a reddish homogenous appearance when stained with haemotoxylin and eosin and is predominantly composed of collagen (Zhang, 1994). The hyalinisation process tends to replace smooth muscle and elastic tissue elements within the arteriolar intima and media. More extreme hypertensive damage can lead to fibrinoid necrosis where the damaged vessel wall undergoes necrosis and is infiltrated by plasma constituents, leading to further thickening and luminal narrowing. Fibrinoid necrosis tends to occur in the larger arterioles (Brun, 1992) but is rarely cited in the neuropathology studies of vascular dementia which may be a reflection of difficulties in studying affected tissue immediately following lacunar infarction (or possibly due to inconsistent use of nomenclature).

Another key factor in small vessel disease may be cerebral autoregulation, the automatic process of maintaining constant blood flow to cerebral tissue in spite of changes in perfusion pressure. Abnormal autoregulation in the face of hypoperfusion may further impair blood flow and oxygen delivery to critically ischaemic white matter. This aspect is discussed further in a later section.

Therefore there are a number of variables determining the extent of ischaemic damage; (a) the severity of luminal narrowing in arterioles of varying calibre (b) presence of autoregulation and (c) susceptibility of different cell types to hypoxia (neurons > oligodendria > astrocytes). It follows that ischaemic damage is probably a spectrum from isolated damage involving a few neurons to frank infarction (Ostrow and Miller, 1993). The more severe the ischaemia, the more likely it will result in a large area of cellular necrosis, then gliosis and subsequent cavity formation and these larger lesions are usually termed lacunes. The ischaemic damage may occur within the territory of one deep perforator or between two perforators in the deep white matter, depending on the local perfusion and tissue demands of these at-risk sites (De Reuck, 1971; Marinkovic, 1985).

The morphological hallmarks of small vessel disease are arteriosclerotic perforating arteries, myelin loss and pallor, axonal loss, tissue rarefaction or spongiosis and small deep infarcts or lacunes (Akiguchi, 1997; Erkinjuntti, 1996; Ostrow and Miller, 1993). There are many neuroimaging studies on the associated CT or MRI changes of small vessel disease. These radiographic changes are a useful tool in investigating VaD. There is evidence of reduced blood flow in deep white matter correlating with the severity of small vessel disease as demonstrated on neuroimaging investigations (Kawamura, 1991a; Kobari, 1990). The clinical and pathological correlates of small vessel disease indices on CT and MRI are discussed in detail later.

Binswanger's disease (BD) is a term that receives prominence in the literature with the implication of being a separate pathologic entity. Clinical findings include dementia, frontal signs, pseudobulbar palsy, gait disturbance (marche a petit pas) and urinary incontinence (Roman, 1987). Binswangers' original description actually consisted of neuropathological findings in 8 subjects with vascular dementia who developed focal neurological signs: he found extensive white matter atrophy *without* focal infarcts, which he felt made this a distinct process from multi-infarct dementia (Binswanger, 1894). However his survey did not include microscopic studies in contrast to modern pathological descriptions which have demonstrated micro-infarction in Binswanger's disease (Pantoni and Garcia, 1995; van Gijn, 1998). It has been suggested that in Binswanger's disease, the majority of small vessel disease occurs in the very small arterioles (<150 micrometres) whereas lacunar infarcts tend to be seen in ischaemia of larger vessels (0.4-1.0 mm), thus Binswanger's disease and the lacunar state represent the extremes of a pathophysiological continuum (van Swieten, 1991a). Many authors have confirmed the impression that Binswanger's disease, along with the multitude of other associated terms, share the same distinctive and definitive underlying process of small vessel disease (Leys, 1999; van Gijn, 1998).

There is distinct small vessel pathology, cerebral amyloid angiopathy, which is characterised by amyloid deposition in the adventitia and media of small arteries and arterioles. These changes are classically seen in the cortical vessels and are associated with Alzheimer's disease, which contrasts with lipohyalinosis affecting the subcortex and its association with VaD (Kalaria, 1997b; Vinters, 1988). However, with the emerging evidence of overlap between Alzheimer's disease and VaD, this similarity of arteriolar luminal narrowing may be a link between the two processes, i.e. in the causation of cerebral damage.

2.3.4 The overlap between Vascular Dementia and Alzheimer's disease

Vascular dementia and Alzheimer's disease are frequently difficult to separate, in both clinical and pathological terms. A case series in 1988 observed the high prevalence of small vessel disease in neuropathogically verified Alzheimer's disease (Englund, 1988) and an epidemiological survey noted the increased incidence of Alzheimer's disease in a post-stroke cohort (Kokmen, 1996). The Nun Study was a clinicopathological survey of elderly nuns in North America which found that 61 from 102 cases met pathological criteria for Alzheimer's disease. For any given level of cognitive deficit, the burden of Alzheimer's disease neuropathology was significantly lower in cases with co-existent vascular pathology, inferring a lower threshold of Alzheimer's disease pathology for dementia in the presence of cerebrovascular disease (Snowdon, 1997). This finding has been replicated in a similar studies demonstrating poorer cognitive function in Alzheimer's disease cases with co-existent ischaemic changes when compared with pure Alzheimer's disease cases with comparative levels of Alzheimer's disease pathology (Esiri, 1999; Heyman, 1998; Nagy, 1997). Prospective clinicopathological studies have documented the high rate of mixed pathology (approximately one third) in elderly cases of dementia (Holmes, 1999; Xuereb, 2000). Vascular pathology was also found in 31% of clinically diagnosed dementia with Lewy bodies cases at autopsy (McKeith, 2000). Alzheimer's disease pathology occurred in 55% of subjects with clinically diagnosed VaD (Victoroff, 1995). These results suggest that pure dementia pathology is not common and dementia is most likely to ensue in individuals who suffer a mixture of neuropathology (Holmes, 1999). This theory is further supported by results from a study indicating an inverse relationship between the severity of Alzheimer's disease and cerebrovascular pathology in a group of predominantly Alzheimer's disease type pathology dementia cases (Goulding, 1999). Possible mechanisms for the association between Alzheimer's disease and vascular disease have been reviewed by Kalaria, which discussed potential common pathophysiology involving the blood-brain barrier, renin-angiotensin system and apoptosis, the role of apolipoprotein E and oxidative stress (Kalaria and Ballard, 1999). Alzheimer's disease -associated cerebral amyloid angiopathy could stimulate the same lesions as arteriosclerotic vascular disease. Evidence for the latter theory includes experiments on rat aorta which demonstrate that beta-amyloid (a pathological hallmark of Alzheimer's disease) interacts with endothelium to form superoxide radicals which (a) directly damage endothelial lining and (b) alters vascular tone by scavenging relaxing factor (Thomas, 1996).

A large community-based, multicentre neuropathological study has recently reported on pathological correlates of late-onset dementia in the first 209 subjects (CFAS, 2001).

Alzheimer's disease and VaD pathology were the major pathological correlates of cognitive impairment but there were no clear thresholds which predicted dementia status. One of the striking findings was the high frequency of neuropathology in individuals without clinical dementia. Small vessel disease was the most frequent cerebrovascular pathological feature but taken alone was not significantly related to dementia status. Only multiple vascular pathology was significantly more common in individuals with dementia than those without dementia and even then the difference was small (46% vs. 33%). Intermediate burdens of Alzheimer's disease pathology did not cause dementia in the face of additional VaD pathology, contrasting with the studies discussed above. The study highlights that other factors must determine if moderate burdens of Alzheimer and cerebrovascular pathology lead to cognitive failure.

2.3.5 Conclusion

Large vessel disease can cause sudden onset of dementia in the early aftermath of cortical stroke, typified by large infarct volumes and atherosclerotic disease of large arteries. Small vessel disease may lead to a more insidious dementia onset distant from the stroke event. Small vessel disease is characterised by arteriosclerosis, small lacunar infarcts, myelin pallor and spongiosis. Alzheimer's disease and vascular dementia frequently co-exist but the reasons for this overlap are not yet understood.

2.4 Risk factors

Risk factor analysis suffers from the problems of vascular dementia nosology. Variation in definitions of vascular dementia and risk factors under investigation can lead to design bias. Sample size may lead to types I and II errors; some studies discussed below have employed multiple significance testing, consequently increasing the prospect of chance differences of type I error. Vascular dementia is associated with a high mortality rate (Skoog, 1993), thus longer intervals in sampling during longitudinal studies run the risk of sampling bias. Despite these difficulties there are clear patterns in risk factors. The methodology of individual risk factor studies is beyond the scope of this review. Several large prospective cohorts form the basis for a number of risk factor reports, notably the Kungsholmen, Hisayama, Honolulu Heart Project, Canadian Study on Health and Ageing, Adult Health Study and Atherosclerotic Risk in the Community groups (Aguero-Torres, 1999; Lindsay, 1997; Ross, 1999; Ueda, 1992).

2.4.1.1 Blood pressure

Reports on hypertension provide an interesting insight on the pathogenesis of vascular dementia. Longitudinal studies in community cohorts conclude hypertension is an independent risk factor for VaD (Fujishima and Tsuchihashi, 1999; Hebert and Brayne, 1995;

Knopman, 2001; Launer, 2000; Meyer, 2000; Skoog, 1996; Yamada, 1999; Yoshitake, 1995). Due to their proven benefit in reducing stroke risk (Amery, 1985; Dahlof, 1991; MRC Working Party, 1992; Staessen, 1997b) it is likely that anti-hypertensive treatment will lower the risk of vascular dementia. An observational, longitudinal study of anti-hypertensive use did reveal reduced risk of incident dementia in particular with diuretic treatment (Guo, 1999). More importantly in one of the first intervention trials, anti-hypertensive treatment was associated with a lower incidence of treatment (Forette, 1998).

In the majority of *post-stroke* cross-sectional studies (Barba, 2000; Censori, 1996; Loeb, 1992; Tatemichi, 1990) and a few poorly designed community surveys (Boston, 1999; Kokmen, 1996) (cross-sectional selected sample and retrospective data collection) indicate hypertension is not a risk factor. In one post-stroke sample, hypertension lost its significance after controlling for age (Gorelick, 1993). Furthermore, some groups report that relative *hypo*tension i.e. systolic BP < 135-140 mm Hg is more prevalent in vascular dementia in cross-sectional post-stroke (Gorelick, 1993; Pohjasvaara, 1998) and community groups (Guo, 1996). A key finding in the longitudinal study in Gothenberg is that individuals who develop dementia have a higher, pathological blood pressure before the onset of dementia and blood pressure declined more rapidly in those who developed dementia (Skoog, 1996). This fits in well with the small vessel disease hypothesis. Hypertension can cause arteriosclerosis and lipohyalinosis but if hypotension supervenes, white matter ischaemia may follow.

2.4.1.2 Age

Community longitudinal (Fratiglioni, 1997; Hebert, 2000; Ross, 1999; Yamada, 1999; Yoshitake, 1995) and cross-sectional studies (Kokmen, 1996; Ueda, 1992) conclude that increasing age is an independent risk factor for VaD. Post-stroke cross-sectional studies repeat this finding (Barba, 2000; Desmond, 2000; Gorelick, 1993; House, 1990; Tatemichi, 1992; Tatemichi, 1993; Tatemichi, 1990). Since age will predispose to neurodegenerative processes, the older the subject the more likely a critical threshold for dementia is attained.

2.4.1.3 Stroke features

Stroke is usually included in the definition for vascular dementia, thus will obviously appear as a risk factor. Community studies have shown that more than one stroke further increases dementia risk (Boston, 1999; Yoshitake, 1995). Cross-sectional prevalence studies conducted up to 3 months post-stroke report large hemisphere (i.e. large cortical anterior circulation syndromes) as an independent risk factor for dementia (Barba, 2000; Censori, 1996; Desmond, 2000; Pohjasvaara, 1998; Tatemichi, 1993; Tatemichi, 1990). However longitudinal data indicates lacunar stroke (i.e. small subcortical infarcts) is the most common stroke subtype in post-stroke dementia (Ross, 1999; Yoshitake, 1995). This is consistent with

the different pathophysiology and prognosis of stroke subtypes based on the Oxford Community Stroke Project classification scheme (Bamford, 1991). Cortical hemisphere infarcts are related to large artery atherosclerotic disease resulting in large infarct volumes and high mortality in the first year, in contrast to lacunar stroke which is related to small vessel disease and low mortality rates.

2.4.1.4 Diabetes mellitus

Diabetes is an independent determinant for vascular dementia in both community cohorts (Boston, 1999; Fujishima and Tsuchihashi, 1999; Hebert, 2000) and post-stroke samples (Censori, 1996; Desmond, 2000; Tatemichi, 1993). The odds ratios quoted tend to lie in the range 1.4-2.6. Diabetes is also a risk factor for subtle cognitive deficits in abstract reasoning and visuospatial dysfunction (Desmond, 1993) whilst impaired glucose tolerance increases the risk of asymptomatic lacunar infarction (Kase, 1989) and vascular dementia (Curb, 1999). Some post-stroke studies concluded that diabetes was not a risk factor for VaD (Barba, 2000; Loeb, 1992; Tatemichi, 1993; Tatemichi, 1990). Diabetes can exert its adverse effect on stroke risk by several mechanisms. The risk of large vessel atheroma is increased which in turn may lead to anterior circulation infarction. There may be an indirect effect from cardiac disease increasing the risk of cardio-embolic disease due to atrial fibrillation and myocardial infarction. There is a theoretical risk of small vessel disease as a result of microvascular injury (as occurs in retinopathy and renal disease) or from proximal small vessel atheroma.

2.4.1.5 Cholesterol

Hyperlipidaemia and elevated low-density lipoprotein cholesterol are risk factors for vascular dementia according to longitudinal data (Meyer, 2000; Moroney, 1999) but cross-sectional data has not revealed an association (Barba, 2000; Boston, 1999; Carantoni, 2000). The mechanisms of cholesterol increasing dementia risk are probably similar to those mentioned above for diabetes.

2.4.1.6 Ischaemic heart disease

Longitudinal community studies report ischaemic heart disease is a significant risk factor for VaD (Fujishima and Tsuchihashi, 1999; Hebert, 2000; Ross, 1999) as well as post-stroke surveys (Gorelick, 1993; Tatemichi, 1990). Atherosclerotic disease results in an increase in the proportion of cognitively impaired subjects in the population (Breteler, 1994a; Hofman, 1997). Atherosclerosis, defined by the presence of carotid artery disease and abnormal ankle-brachial pressure index, was predictably associated with VaD but also increased the risk of Alzheimer's disease with odds ratios in the ranges 1.9-3.2 and 1.3-1.9 respectively (Breteler, 1994a; Hofman, 1997). Some post-stroke cross-sectional studies did not find an association of ischaemic heart disease with VaD (Barba, 2000; Censori, 1996; Kokmen, 1996).

2.4.1.7 Education

Higher educational level is consistently reported as a protective factor against vascular dementia in both community and post-stroke studies (Desmond, 2000; Gorelick, 1993; Lindsay, 1997; Pohjasvaara, 1998; Pohjasvaara, 2000; Tatemichi, 1992; Tatemichi, 1993). Education also protects against Alzheimer's disease (Ott, 1995).

2.4.1.8 Apolipoprotein E genotype

Apolipoprotein E plays a vital role in lipid transport. The ε4 allele is a recognised risk factor for coronary artery disease and Alzheimer's disease, probably due to alterations in cholesterol handling (Frikke-Schmidt, 2001). Apolipoprotein ε4 allele frequency can increase in vascular dementia with the association being even stronger in homozygotes compared with heterozygotes (Hebert, 2000; Kalman, 1998; Katzman, 1997; Slooter, 1997). Some investigations have found an interaction between Apo ε4 and atherosclerotic disease. Firstly the strength of the association with vascular dementia is increased in the presence of carotid artery and peripheral vessel atherosclerosis (Hofman, 1997). Secondly the apolipoprotein ε4 genotype is associated with the dementia phenotype only in the presence of atherosclerotic disease as indicated by white matter lesions (Skoog, 1998b). It has been suggested that the interaction between apolipoprotein ε4 and peripheral vascular disease attenuates the adaptability of the cerebral vasculature during ageing (Kalaria, 1997a).

In contrast there are a number of studies which have not found an association between apolipoprotein ε4 and VaD (Barba, 2000; Bergem, 1997; Bergem and Lannfelt, 1997; Burlinson, 1998; Chapman, 1998; Frikke-Schmidt, 2001). These reports include concordance studies in twins. Therefore status of the apolipoprotein ε4 allele as a risk factor for VaD is controversial.

2.4.1.9 Smoking

Several studies report cigarette smoking as a risk factor for VaD (Fujishima and Tsuchihashi, 1999; Gorelick, 1993; Shaji, 1996) and a further noted a non-significant trend towards increased risk (Pohjasvaara, 1998). Some have not found an association but again these are post-stroke samples with methodological weaknesses (Censori, 1996; Kokmen, 1996; Loeb, 1992).

It is interesting that some of the traditional atherosclerotic risk factors (smoking, hyperlipidaemia, apolipoprotein ε4) are not consistently associated with vascular dementia in some of these studies. This may reflect the concept of different pathophysiology in vascular dementia compared with, for example, coronary artery disease and peripheral arterial disease, as previously discussed.

2.4.1.10 Atrial fibrillation

Several cross-sectional post-stroke studies indicate atrial fibrillation is associated with vascular dementia or cognitive impairment (Barba, 2000; Censori, 1996; Ott, 1997). In one report the association was not significant after multiple regression analysis (Censori, 1996) but a larger community study demonstrated how atrial fibrillation is an independent risk factor not only for vascular dementia but Alzheimer's disease as well, even after adjusting for stroke (Ott, 1997). Atrial fibrillation increased the risk of poor cognitive performance in a study comparing lone atrial fibrillation subjects with sinus rhythm, supporting the hypothesis of atrial fibrillation causing silent cerebral infarction and hence cognitive impairment (O'Connell, 1998).

2.4.1.11 Race

Studies in North America show that VaD risk is increased in non-Caucasian subjects (Desmond, 2000; Tatemichi, 1992; Tatemichi, 1993).

2.4.1.12 Alcohol

Excess alcohol consumption is an independent risk factor for vascular dementia according to longitudinal community and cross-sectional studies (Fujishima and Tsuchihashi, 1999; Lindsay, 1997; Yoshitake, 1995).

2.4.1.13 Aspirin

A community prevalence study reported aspirin use as an independent risk factor for VaD (Lindsay, 1997) which is surprising given the proven benefits of aspirin in primary and secondary prevention of cerebrovascular disease (Antiplatelet Trialists Collaboration, 1994; Collaborative Group of the Primary Prevention Project (PPP), 2001). The authors suggested this finding may be due to Neymans bias, with aspirin prolonging the survival of subjects with VaD but a longitudinal survey also found increased risk of VaD with aspirin use (Hebert, 2000). Both studies used ICD-10 (good specificity but poor sensitivity) to diagnose VaD.

2.4.1.14 Homocysteine

Elevated plasma homocysteine is now a recognised independent risk factor for vascular disease as reviewed by Diaz-Arrastia (Diaz-Arrastia, 2000). The pathophysiological mechanism remaining unknown but is possibly related to homocysteine metabolites producing free radicals and hence endothelial damage. Increased homocysteine level correlates inversely with cognitive function (Lehmann, 1999) and is risk factor for small vessel vascular dementia (Fassbender, 1999) and Alzheimer's disease (Clarke, 1998).

2.4.1.15 *Miscellaneous risk factors*

A variety of other risk factors occasionally appear in studies. Positive correlations with vascular dementia have been found for renal disease (proteinuria or nephropathy) (Barba, 2000; Gorelick, 1993), a family history of dementia (Gorelick, 1993), pesticide exposure (Hebert, 2000; Lindsay, 1997) rural location (Hebert, 2000) and residing in an area of socio-economic deprivation (Whalley, 1995). Protective factors against VaD include western diet and vitamin E consumption (Ross, 1999) and shellfish consumption and exercise for women (Hebert, 2000).

2.4.1.16 *Orthostatic hypotension*

A prospective study of non-demented stroke subjects found an increased incidence of dementia associated with 'hypoxic-ischaemic disorders' after controlling for demographic factors, recurrent stroke and baseline cognitive function (Moroney, 1996). This supported the hypothesis that cerebral hypoperfusion might be a contributing pathogenetic mechanism in the pathogenesis of dementia. Furthermore the association was stronger in older subjects. Orthostatic hypotension is more prevalent in post-stroke dementia compared with non-demented subjects 3 months post-stroke (Pohjasvaara, 1998). Orthostatic hypotension has been reported as an independent risk factor for stroke in a prospective community study (Eigenbrodt, 2000). The concept of abnormal blood pressure and heart rate control, or Neurocardiovascular Instability, is discussed in more detail below.

2.4.2 Conclusion

Age and hypertension are established risk factors for vascular dementia. Diabetes and atherosclerotic disease are probably strong risk factors. A number of stroke characteristics predisposing to dementia have been suggested. A variety of other risk factors have only been intermittently identified, usually in prospective community cohorts. The same risk factors' effects appear to be diluted in post-stroke samples. Blood pressure homeostasis has led to useful insights in to the pathophysiology of vascular dementia.

2.5 White matter lesions and neuroimaging

Neuroimaging plays a vital role in the diagnosis of dementia. Cerebrovascular disease on CT or MRI is usually a requirement in the diagnostic criteria for VaD as previously discussed. CT scanning is the the more common form of neuroimaging but the advent of MRI has enabled more detailed analysis of in vivo neuropathological studies of VaD. Interest often focuses on the role of white matter lesions. This finding has been referred to as 'leukoaraiosis' (Hachinski, 1987) and overlaps with the term 'white matter hyperintensities' as seen on MRI. In this discussion the term white matter lesions (WML) will be used for consistency.

2.5.1 Neuropathology

Post-mortem studies have identified the pathology of white matter lesions of neuroimaging. White matter lesions reveal demyelination, gliosis, dilated perivascular spaces, lacunar infarction and arteriosclerosis (Awad, 1986; Chimowitz, 1992; Fazekas, 1993; Grafton, 1991; Gupta, 1988; van Swieten, 1991b). There are differences in the pathology of periventricular and deep white matter hyperintensities on MRI (Fazekas, 1993). Neuropathological studies indicate that periventricular hyperintensities may have different underlying pathology when compared with deep white matter lesions (Roman, 2002). Periventricular lesions are often non-ischaemic, with only larger (>5 mm) periventricular hyperintensities having an ischaemic basis (Thomas, 2003). Deep white matter lesions are more frequently related to cerebral ischaemia (Thomas, 2002). One study suggested that arteriosclerosis was the key initial change followed by demyelination, axonal loss and finally perivascular space dilatation as a late change (van Swieten, 1991b).

2.5.2 Risk factors

Systolic blood pressure, heart disease and peripheral vascular disease were associated with WML on CT in a selected group of referrals to a memory clinic (Amar, 1995). Age, heart failure and systolic blood pressure below 130 mm Hg were associated with CT WML in a sample of geriatric medical patients (Raiha, 1993).

MRI studies have found that blood pressure, age and stroke history in dementia (Almkvist, 1992; Inzitari, 1987) and age and diabetes in a post-stroke sample (Schmidt, 1992) were predictors of WML compared with normal controls. A history of hypertension (Schmidt, 1995; van Swieten, 1991a) and a substantial increase *or* decrease in diastolic blood pressure over 20 years (de Leeuw, 1999) are associated with WML in non-demented individuals. Left ventricular hypertrophy, an accurate marker of hypertension, is an independent correlate of asymptomatic WML (Kohara, 1999). Community based cross-sectional studies concluded that age, prior stroke, hypertension (Breteler, 1994c; Longstreth, 1996), lower forced expiratory volume (Longstreth, 1996), history of myocardial infarction, factor VIIc and fibrinogen levels were significant and independent risk factors for WML on MRI (Breteler, 1994c).

2.5.3 Clinical manifestations

Healthy elderly with WML have subtle cognitive deficits compared with individuals without WML (Boone, 1992; Breteler, 1994b; Schmidt, 1993; Steingart, 1987). These cognitive deficits are principally in executive control and attentional tasks that are typical of subcortical VaD. In contrast others have found no association between WML and cognition in healthy elderly but did note that WML were preceded by cognitive decline in old age (Garde, 2000;

Hunt, 1989). WML are increased in dementia and are more common in VaD than Alzheimer's disease (Aharon-Peretz, 1988; Amar, 1995; Barber, 1999; Charletta, 1995). In post-stroke samples severity of WML is a correlate of dementia (Liu, 1992; Pohjasvaara, 2000). However some have found no increase in WML in Alzheimer's disease compared with normal controls (Erkinjuntti, 1994; Raiha, 1993).

A case controlled study has confirmed that gait disorder and urinary incontinence are more common in individuals with WML (Tarvonen-Schroder, 1996) as suggested in a review article (Roman, 1987).

2.5.4 Conclusions

WML have similar risk factors compared with stroke indicating a vascular aetiology. This is supported by neuropathological correlates. WML are associated with poorer cognitive performance in healthy elderly and vascular dementia. However WML can be asymptomatic indicating that other factors influence the expression of cognitive impairment.

2.6 Neurocardiovascular instability

2.6.1 Normal ageing

The cardiovascular system is regulated by the autonomic nervous system to maintain an appropriate level of perfusion to all organs. There are afferent neural connections from the heart and blood vessels to the central nervous system. Central processing leads to an efferent outflow of parasympathetic and sympathetic activity back to the heart and vessels (Spyer, 1992). The system is designed to increase heart rate and peripheral resistance if blood pressure falls and decrease these parameters when blood pressure rises. Peripheral baroreceptors play a key role in detecting blood pressure changes in the major vessels and relay this information to the central nervous system. The speed of this response is known as the baroreceptor sensitivity, which may be expressed as the unit change in heart rate per unit change in blood pressure. The autonomic nervous system control of the cardiovascular system is not thought to qualitatively change in healthy ageing, and this is discussed in more detail in Chapter 3. However it is recognised that there are quantitative changes with normal ageing. Baroreceptor sensitivity is reduced in healthy aged individuals and the affinity of beta-adrenoreceptors for agonists decreases with age (Collins, 1980; Feldman, 1984; Kenny, 1987; Lipsitz, 1989; Piccirillo, 2001; Shimada, 1986; Spyer, 1992; Veerman, 1994). Despite reduced baroreflex sensitivity, it appears that cerebral autoregulation may not deteriorate with age (Carey, 2000).

2.6.2 The syndrome of Neurocardiovascular Instability

Abnormal heart rate and blood pressure control is common in the elderly. These abnormalities have a number of recognised clinical presentations which include orthostatic hypotension, carotid sinus hypersensitivity, vasovagal syncope and supine hypertension. They frequently co-exist. This syndrome is referred to as Neurocardiovascular Instability (Kenny and Dey, 1998).

Orthostatic hypotension is defined as a reduction in systolic blood pressure of more than 20 mm Hg or diastolic blood pressure of 10 mmHg on standing (Anonymous, 1996). Carotid sinus hypersensitivity (CSH) is an abnormal response to carotid sinus massage. This has 3 forms, cardioinhibitory (CICSH) with asystole for greater than 3 seconds, vasodepressor (VDCSH) with a greater than 50 mmHg decrease in systolic blood pressure or mixed where CICSH and VDCSH co-exist. Vasovagal syncope is a clinical diagnosis of syncope usually induced by a precipitating factor or situation which provokes hypotension and/or bradycardia sufficiently profound to provoke cerebral ischaemia and loss of neural function. Vasovagal syncope is also known as neurocardiogenic syncope and also can be separated into cardioinhibitory, vasodepressor and mixed forms (Kenny and Dey, 1998). These hypotensive, bradycardic disorders are common causes of dizziness, falls and syncope in the elderly (McIntosh, 1993).

Primary autonomic failure, Multiple System Atrophy and other causes of autonomic failure can also lead to orthostatic hypotension and impaired baroreflex sensitivity. Thus Neurocardiovascular Instability and autonomic failure, or dysautonomias, are a potential cause of hypotensive, bradycardic challenges to the ageing brain and hence ischaemic damage to white matter.

2.6.3 Dysautonomia and stroke

There are several case series published on transient ischaemic attacks and stroke occurring in individuals who suffered from orthostatic hypotension on a background of severe ipsilateral carotid stenosis (Dobkin, 1989; Hankey and Gubbay, 1987; Ruff, 1981). In these cases severe hypotensive episodes were temporally connected to cerebral ischaemic damage. A clinicopathological series of 145 stroke victims found that 37 cases had no evidence of macro-infarction but did have a clear history of hypotensive disorders at the time of stroke presentation (Mitchinson, 1980). White matter lesions of small vessel disease have been documented in autopsies of patients with severe orthostatic hypotension (Brun and Englund, 1986; De Reuck and Eecken, 1978; Ginsberg, 1976).

Small case series have reported a high prevalence of white matter disease on CT and MRI studies of individuals with marked orthostatic hypotension (Harrison and Marshall, 1984;

McQuinn and O'Leary, 1987). A risk factor analysis for CT white matter lesions in 251 geriatric patients revealed that orthostatic hypotension was associated with (and supine hypotension was an independent correlate for) WML (Raiha, 1993). A case controlled study found that systolic hypotension was significantly associated with leukoaraiosis compared with normal CT scans (Tarvonen-Schroder, 1996). Orthostatic hypotension in healthy community dwelling elderly is associated with severity of lacunes and periventricular hyperintensities on MRI (Matsubayashi, 1997). Absence of normal nocturnal dipping in blood pressure and excessive dipping in nocturnal blood pressure are both risk factors for white matter disease on MRI (Kario, 1997; Tohgi, 1991). White matter disease is worse in hypertensives (and associated with poor cognitive performance) treated with calcium channel antagonists and diuretics compared with beta-blocker therapy possibly related to their greater hypotensive effect (Heckbert, 1997). Deep white matter disease on MRI correlates with the degree of hypotension from CSH or orthostatic hypotension (Ballard, 2000). A study testing the hypothesis that borderzone infarcts of differing aetiology would have characteristic patterns on diffusion and perfusion weighted MRI did identify a sub-group with normal perfusion scans and no evidence of large artery or cardio-embolic disease immediately post-stroke who did have evidence of peri-infarct hypotension (Chaves, 2000). This lends support to the concept of hypoperfusion causing ischaemia in sclerosed small vessels.

2.6.4 Dysautonomia and dementia

Small case controlled studies have demonstrated abnormal parasympathetic function on standard autonomic tests in Alzheimer's disease compared with healthy elderly controls (Algotsson, 1995; Elmstahl, 1992; Idiaquez and Sandoval, 1999; Wang, 1994). Impairment of sympathetic autonomic function during sleep studies (Aharon-Peretz, 1992; Ferini-Strambi and Smirne, 1997; Franceschi, 1986) and on standing (Borson, 1989; Burke, 1994a; Burke, 1994b; Vitiello, 1993) has also been demonstrated in Alzheimer's disease subjects. Abnormal heart rate variability (perhaps reflecting abnormal parasympathetic function) occurs in Alzheimer's disease (Aharon-Peretz, 1992). There are very few reports on dysautonomia in vascular dementia. A study comparing VaD, Alzheimer's disease and a control group found the Alzheimer's disease and control groups had similar autonomic function but the VaD had abnormal parasympathetic and preserved sympathetic function (Yamamoto, 1990).

Orthostatic hypotension is very common in Alzheimer's disease (32-39%), VaD (52%) and dementia with Lewy bodies (40%) (Ballard, 1998; Passant, 1997). Orthostatic hypotension correlates with middle cerebral artery blood flow and cognitive performance in non-demented elderly and the degree of blood pressure reduction in orthostatic hypotension in healthy elderly is associated with subsequent cognitive impairment (Elmstahl and Rosen, 1997; Stout

and Kenny, 2000). Healthy elderly with orthostatic hypotension have poorer MMSE and visuospatial skills than subjects without orthostatic hypotension (Matsubayashi, 1997).

Cardio-inhibitory carotid sinus hypersensitivity is markedly increased in Alzheimer's disease (28%) and dementia with Lewy bodies (41%) whereas rates in healthy elderly are approximately 4% (Ballard, 1998; Wentink, 1993). Vasodepressor CSH is also high in Alzheimer's disease (16%) and dementia with Lewy bodies (10%). Elderly subjects with CSH have impaired attention and working memory compared with healthy controls (Parry, 1999). Hypertension is a known risk factor for VaD and baroreflex sensitivity is impaired in hypertension, this relationship being independent of the age (Shimada, 1986). A group with VaD due to white matter disease were 'non-dippers' (i.e. absence of normal reduction in blood pressure during nocturnal period/sleeping) on 24 hour blood pressure recordings compared with controls (Tohgi, 1991) and abnormal circadian rhythm of systolic blood pressure is an independent risk factor for MRI white matter lesions (Sander, 2000b). A small case series of 6 VaD subjects where evolution of dementia was temporally connected with cerebral hypoperfusion revealed marked white matter lesions on CT in 5 out of 6 cases and on post-mortem in 3 out of 3 cases (Sulkava and Erkinjuntti, 1987). There are very few correlative clinicopathological studies but one investigation has shown central noradrenergic neurons of the sympathetic system are significantly reduced in Alzheimer's disease but unchanged in vascular dementia (Mann, 1982).

2.6.5 Cerebral blood flow and autoregulation

During a hypotensive challenge, cerebral blood flow is normally protected by autoregulation. Impaired autoregulation may be an integral part of the pathogenesis of white matter disease. The results from cerebral blood flow and autoregulation studies over the last 30 years are not entirely consistent but do provide some interesting results. Cerebral oxygenation in the frontal cortex significantly decreases on standing in healthy elderly (and correlates with attenuated diastolic blood pressure rise) but does not change in young controls (Mehagnoul-Schipper, 2000). However cerebral autoregulation is not impaired in healthy elderly subjects compared with young controls (Carey, 2000). Although cerebral blood flow is reduced in hypotension due to CSH, this is normally compensated for by intact cerebral autoregulation (Leftheriotis, 2000). One study found an age related decrease in regional cerebral blood flow in the limbic and association cortices and hypothesised this may contribute to normal changes in memory in healthy ageing (Martin, 1991).

There are contrasting reports on the cerebral blood flow response during orthostatic hypotension in individuals with autonomic failure. Some have demonstrated impaired cerebral blood flow or a non-significant trend towards impaired autoregulation (Bondar,

1997; Lagi, 1994). Others have shown preserved cerebral blood flow and cerebral autoregulation (Brooks, 1989; Thomas and Bannister, 1980). One group (Novak, 1998) concluded there are three patterns of cerebral autoregulation in orthostatic hypotension – normal, extended (i.e. broadened plateau of constant cerebral blood flow for a range of cerebral perfusion pressures) and failure of autoregulation. This variety of response is again shown in a small series of eleven subjects with orthostatic hypotension where the seven cases with symptomatic orthostatic hypotension had abnormal autoregulation in contrast to preserved autoregulation in four asymptomatic cases (Wollner, 1979).

Animal studies have indicated differential autoregulatory behaviour between white and grey matter: induced hypotension in newborn dogs produced a selective reduction in cerebral blood flow to white matter, initially sparing grey matter (Young, 1982). This is reflected in human studies showing cerebral autoregulation is impaired in white matter disease as seen on neuroimaging (Matsushita, 1994; Young, 1982). Cerebral blood flow is reduced in vascular dementia when compared with both healthy elderly controls and Alzheimer's disease (Abe, 1996; Hachinski, 1987; Kawamura, 1991b; Scheel, 1999; Starkstein, 1996). Cerebral blood flow is even reduced in asymptomatic subjects with white matter disease on MRI (Meguro, 1990). Fluctuations in cognitive performance correlate with cerebral blood flow in VaD (Meyer, 1988). A small case series reported that white matter cerebral blood flow and oxygen extraction fraction was substantially decreased in dementia with small vessel disease but white matter cerebral blood flow was more modestly reduced and oxygen extraction fraction *increased* in non-demented individuals who went on to develop dementia 1 year later (Yao, 1992). This supports the hypothesis of critically impaired perfusion in small vessel disease, or 'misery perfusion', as a prelude to white matter damage and dementia.

Abnormal cerebral blood flow and autoregulation is potentially modifiable. A case series of 7 individuals with chronic atrioventricular block of 6-36 months duration had symptoms of fatigue, poor concentration and memory impairment. Following pacemaker insertion, cerebral blood flow and cognitive function improved (Sulg, 1969). Cerebral autoregulation is impaired in hypertension but treated hypertensives have intermediate abnormalities compared with untreated hypertensives and controls (Strandgaard, 1976).

2.6.6 Conclusions

Disorders of blood pressure, heart rate and baroreflex sensitivity are common in dementia. Observational studies support the hypothesis that these abnormalities may play a role in the pathogenesis of white matter disease and cognitive decline.

2.7 *Aims and objectives*

This MD thesis will aim to fulfill the following objectives, using a sample of elderly subjects with a history of cerebrovascular disease:

- To determine the prevalence of autonomic dysfunction in elderly stroke survivors compared with healthy controls.
- To determine if autonomic dysfunction and blood pressure variability in elderly stroke survivors correlates with white matter disease in a cross-sectional study.
- To determine if autonomic dysfunction and blood pressure variability in elderly stroke survivors are risk factors for cognitive decline in a longitudinal study.

3 The assessment of cardiovascular autonomic function and blood pressure variability

This chapter will review the physiological basis and utility of cardiovascular autonomic function tests employed in this research study. Techniques have evolved over a period of 40 years, and I will attempt to describe what we can deduce about cardiovascular autonomic function from the results of reflex tests and power spectral analysis of heart rate variability. The clinical value of these investigations is also discussed.

I will also review the influence of blood pressure variability on clinical outcomes, in particular the relationship between ambulatory blood pressure variability and cerebrovascular disease.

Autonomic research frequently utilises drugs acting on the cardiovascular system. action of the drugs quoted in this section are summarised below.

Beta-adrenoceptor blocking agents (e.g. propranolol): beta-blockers antagonise the beta-adrenoceptors in the heart and peripheral vasculature, thus blocking the action of the sympathetic nervous system on the cardiovascular system.

Atropine: a natural alkaloid that is a highly selective antagonist of acetylcholine at muscarinic receptors. The main cardiac effect is increase in heart rate by blocking parasympathetic activity at the sinoatrial and atrioventricular nodes.

Adrenaline, noradrenaline, and phenylephrine: adrenaline and noradrenaline are also known as epinephrine and norepinephrine. These are sympathomimetics that act on peripheral α-receptors to produce vasoconstriction, cardiac β_1 receptors leading to increase in heart rate and contractility, and peripheral plus cardiac β_2 receptors that stimulate vasodilatation. Adrenaline predominantly acts on α and β_1 receptors. Noradrenaline mainly acts on α-receptors. Phenylephrine has similar vasopressor action with a longer duration of action than noradrenaline.

Guanethidine: this is an adrenergic blocking agent. It is taken up by adrenergic nerve terminals and depletes noradrenaline from nerve terminals causing hypotension.

Phentolamine: this blocks α-receptors at both post-synaptic α_1 and pre-synaptic α_2 receptors. This leads to postural hypotension and reflex tachycardia.

Gallamine: a muscle relaxant that leads to muscle paralysis by competitively blocking the motor end-plate cholinergic receptor.

The basic structure of the cardiovascular autonomic nervous system is outlined in Figure 3.1. The sympathetic nervous system acts by releasing noradrenaline that leads to peripheral

vasoconstriction and increases myocardial contractility and heart rate. The parasympathetic nervous system acts by releasing acetylcholine at the cardiac sino-atrial node to increase pulse interval i.e. to slow heart rate.

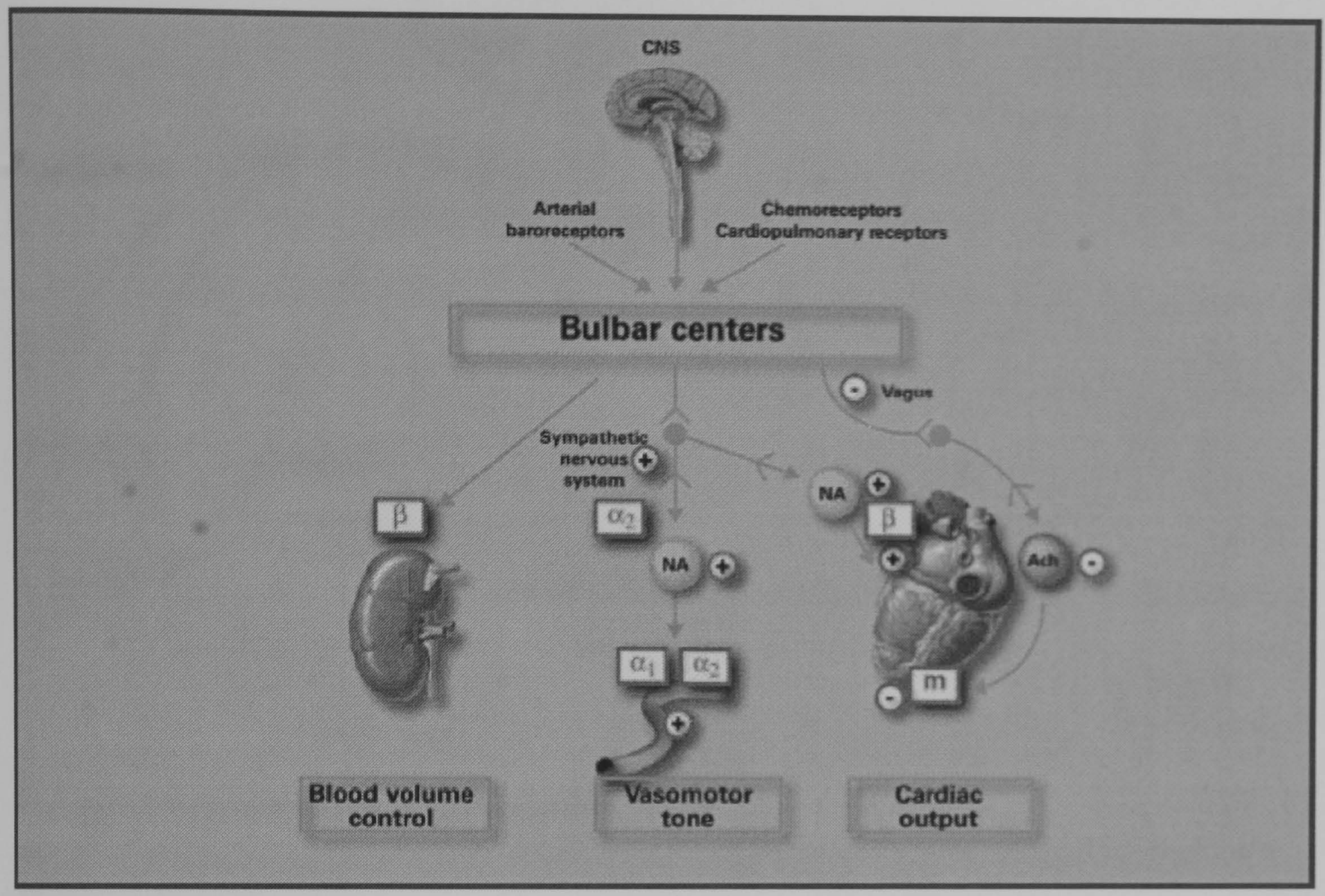

Figure 3-1 Cardiovascular autonomic nervous system

NA, noradrnaline: Ach, acetylcholine.

The brainstem centres involved in regulation of cardiovascular autonomic action are illustrated in Figure 3.2. Cardiovascular regulatory areas are located within the brainstem, primarily the pons and medulla. Afferent information is supplied from the carotid body and aortic arch baroreceptors to the nucleus tractus solitarius. Second order neurones project to two sites in the medulla. Firstly cardiac preganglionic neurones which project to the vagus. Secondly, GABA-containing neurones (adjacent to the nucleus ambiguus) that project to the ventrolateral medulla and then onto the intermediolateral cell column that forms part of the sympathetic system, and hence projects to the blood vessels and heart.

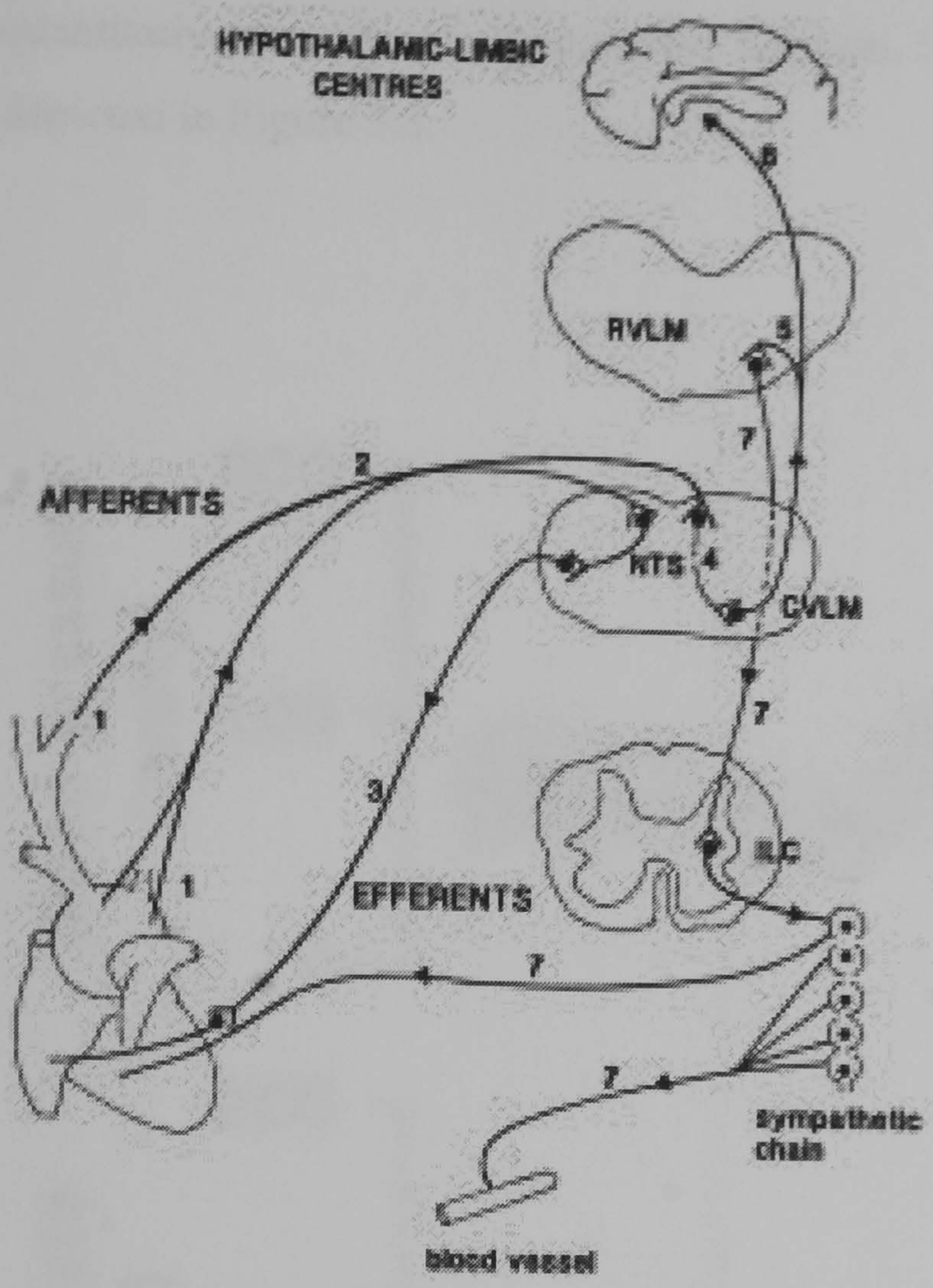

Figure 3-2 Neural circuitry of the baroreflex

RVLM, rostroventrolateral medulla: NTS, nucleus tractus solitarius: CVLM, caudoventrolateral medulla: ILC, intermediolateral cell column

3.1 Cardiovascular autonomic reflex testing

3.1.1 RR interval and blood pressure response to orthostasis

Rising from the supine to upright posture elicits a series of cardiovascular reflexes in healthy humans. These reflexes are designed to maintain cerebral and systemic perfusion pressure. The typical pattern of response is a transient fall in systemic blood pressure, which precipitates a rise in heart rate creating a compensatory rise in cardiac output. The subsequent increase in blood pressure leads to an overshoot in systemic blood pressure. Finally the heart rate slows to allow the cardiac output to decrease; consequently systemic blood pressure attains equilibrium.

This series of haemodynamic changes is controlled by the autonomic nervous system. The initial fall in blood pressure is due to peripheral vasodilatation and there is some evidence to suggest this may be a cardiopulmonary reflex: local metabolic induced vasodilatation or central command mediated cholinergic vasodilatation are other possible contributing effectors (Sprangers). The rise in heart rate results from withdrawal of cardiac vagal tone. The rise in blood pressure is a sympathetic mediated response, countered by a surge in cardiac vagal activity to reduce heart rate. The haemodynamic changes can be measured to give a

quantitative assessment of autonomic function. The cardiovascular response to standing is depicted in Figure 3.3.

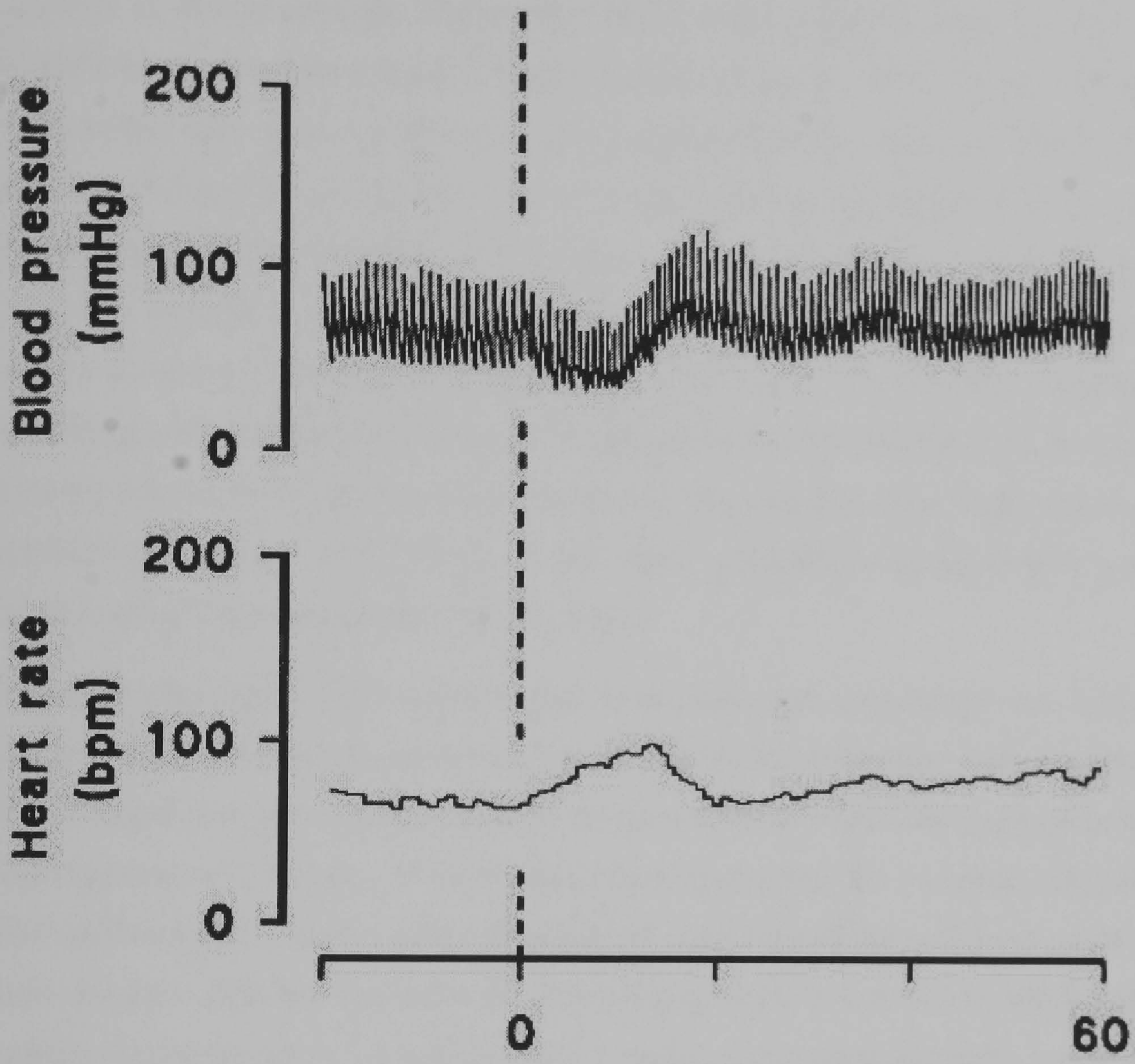

Figure 3-3 Blood pressure and heart rate changes on rising from supine to standing position

The dotted line indicates the moment of rising from supine to standing position. Blood pressure then drops, followed by a rise in heart rate then blood pressure rises which leads to decrease in heart rate.

3.1.1.1 Neural mechanism

In the course of studying cardiovascular response to isometric exercise, Borst et al observed almost instantaneous cardiac acceleration at the start of muscular contraction lasting less than one second. The cardiac acceleration was detectable about 0.4 – 0.6 seconds after start of muscle contraction, and in conjunction with the work of Freyschuss et al (Freyschuss, 1970) they conclude this very rapid response is generated by inhibition of vagal activity (Borst, 1972).

The same group explored the mechanisms of initial heart rate response in the first 30 seconds of standing. A clear bimodal heart rate response to active standing was observed. Active

standing evoked an immediate and rapid heart rate increase, which exactly mirrored heart rate change with brief 3 seconds of isometric exercise but was absent during passive tilt (passive tilt refers to the patient moving from the supine to upright position whilst resting and secured to a tilting table, hence a standing posture is achieved without the subject activating the usual sequence of muscle activity). The exercise reflex mediates this increase. This reflex is blocked by atropine hence is due to vagal inhibition (Ewing, 1978a; Ewing, 1980b). Afferent mechanisms were unclear at that stage (see Isometric Exercise section). The sharp peak in heart rate within 3 seconds is followed by another similar magnitude but more gradual peak at 12 seconds post-stand (the nadir within five seconds does not attain control levels). The secondary increase in heart rate leading to the 12 second peak was also witnessed during passive tilt and is ascribed to the baroreceptor reflex since it is preceded by a temporary fall in arterial pressure. Again the efferent limb appeared to be mediated by vagal mechanisms. The final rapid decrease in heart rate between 12 and 20 seconds is most likely due to reloading of arterial baroreceptors. Clearly the heart rate reflex to standing is complex with a number of stimuli and afferent mechanisms (Borst, 1982).

Sprangers (Sprangers, 1991) concentrated on the cardiovascular changes occurring in the first 5 to 10 seconds on standing up. It was known that the blood pressure falls abruptly at the start of the manoeuvre. Intra-arterial pressure was measured in 10 healthy young adults during three manoeuvres; standing, brief strenuous cycling exercise for 3 seconds and passive tilting. The cardiovascular changes during standing closely mirrored the cycling exercise transients and were quite different to passive tilt. On standing, there was an immediate fall in stroke volume (nadir 8% below control level at 2 seconds), an increase in cardiac output (peak of 24% above control levels at 6 seconds) and a fall in mean arterial pressure (nadir of 25% below control levels at 8 seconds). Therefore the calculated total peripheral resistance promptly fell by 41% at 8 seconds after the start of standing. Because the circulatory transients resembled the changes seen after cycling but not passive tilt, it was hypothesised the active part of standing triggers the fall in peripheral resistance. Two subjects also had right atrial and oesophageal pressure monitored that demonstrated abrupt rises in right atrial pressure of 10 mmHg on standing prior to the fall in peripheral resistance. This was consistent with a rise in cardiac filling pressure as a result of muscle contractions displacing blood to the thorax. The authors hypothesised the fall in peripheral resistance was most likely to be due to the cardiopulmonary reflex, in order to accommodate the shift in circulating volume to the thorax.

The plots of pressure against time in the Sprangers paper depicts a very brief rise in diastolic (and to a lesser extent mean arterial) pressure but not systolic pressure that peaks within 2-3 seconds before decreasing to the nadir levels around 8 seconds. In a study of orthostatic

circulatory control in the elderly using the Finapres, this initial very brief rise in pressure is found in both systolic and diastolic arterial traces before also decreasing to nadirs at around 8-10 seconds (Imholz, 1990a).

Muscle and baroreceptor afferent nerve activity generated by standing is coordinated in the nucleus tractus solitarius. Consequently vagal and sympathetic efferent outflow is adjusted. The immediate adjustment, the exercise reflex, generates a very brief cardioacceleration at 3 seconds and is not part of routine measurement of autonomic function (i.e. is not in the Ewing protocol). However subsequent changes from approximately five seconds onwards are baroreflex mediated and do form one of the baroreflex tests. In an early description by Ewing, the measurement was known as the 30:15 ratio (Low, 1993a). Ewing et al proposed the 30:15 ratio as a test of cardiovagal autonomic function. They studied a group of 22 healthy male adults and 25 diabetic patients, 15 of whom had autonomic neuropathy. Subjects were supine for three minutes then stood within 5 seconds. In healthy controls and diabetics, RR interval changes tend to reach a trough then peak at a similar number of heart beats following the posture change. The trough RR usually occurs at or around the 15^{th} beat, the peak RR interval at or around the 30^{th} beat, hence the 30:15 ratio was used to quantify the response. The ratio was higher in younger compared with older controls, similar between non-neuropathic diabetics and controls but significantly diminished in diabetic patients with autonomic neuropathy. The heart rate response was abolished with atropine alone or in combination with atropine and propranolol but not with propranolol alone. Therefore the 30:15 ratio is dependent on vagal nerve function (Ewing, 1978b).

In younger normal controls, the peak and trough heart rates occur around 12 and 22 seconds after standing (Wieling). Hence the '30:15' ratio quantifies the heart rate changes during change in posture. The ratio reflects the integrity of cardiovagal function. Ewing proposed using the 30:15 ratio as part of a battery of bedside autonomic function tests (Ewing and Clarke, 1982)

Ewing et al also investigated the autonomic mechanisms underlying the initial heart rate changes after standing. Essentially these tests demonstrate that the RR changes after standing are under vagal control, with increased sympathetic activity occurring only if the vagus is blocked. Atropine has a dual action on the RR changes on standing. Firstly the RR shortening decreases and occurs later than the 15^{th} beat when compared with non-atropinised controls. Secondly the rebound RR lengthening is abolished. There is no dose effect with atropine. Addition of propranolol to atropine further minimises the RR changes. Propranolol alone does not alter the pattern of changes but does result in an earlier RR peak lengthening. Therefore (1) the heart rate changes on standing are predominantly controlled by vagal activity, with initial vagal withdrawal followed by a surge in vagal activity and (2) in the situation of vagal

blocking, sympathetic activity can produce a moderate increase in heart rate on standing (Ewing, 1980b). Others have also shown atropine significantly reduces but does not abolish the RR responses during orthostasis (Julu, 1992). This demonstrates the 30:15 ratio is highly dependent on but not entirely dictated by cardiovagal function.

There are other influences on the 30:15 ratio, and a decreased ratio can occur from causes other than vagal damage. This pitfall is reflected by a case with isolated sympathetic vasomotor failure but intact vagal function. This individual was not able to mount a blood pressure overshoot during orthostasis, therefore did not produce a compensatory decrease in RR interval: the 30:15 ratio was greatly diminished but this was not due to vagal damage (van Lieshout, 1989).

Burke et al analysed muscle sympathetic nerve activity (MSNA) in eight healthy adults during recumbency, sitting and standing. Note that measurements were usually taken a matter of minutes after the change in posture to the sitting or standing position since activity involved usually necessitated repositioning the peroneal nerve electrode recording MSNA (usually within 2-15 minutes). MSNA was fairly consistent within individuals in the same position from one 3 minute period to the next but there were large differences in burst incidence (number of bursts/number of heart beats) between individuals. Bursts per minute consistently increased from supine to sitting and from sitting to standing positions. Heart rate increased on both occasions, with on average a greater increase during sit to stand. Burst incidence (bursts per heart beat) also increased from supine to standing position but there was no overall change in burst incidence from sitting to standing position. The magnitude of the change in burst incidence from supine to sitting was inversely related to the former positions' burst incidence ($r = -0.76$). The results also infer that the increase in sympathetic activity from sitting to stable standing position is mainly reflected by tachycardia. For either positional change, change in burst incidence was inversely related to change in heart rate (linear regression $r = -0.81$) thus heart rate and MSNA increase would complement respective activity to generate the desired sympathetic response on postural change. Postural compensating mechanisms are discussed and the authors make the point that the large variation in MSNA when supine (10 to 83 bursts/100 heart beats) raises questions about how those with the lowest MSNA can decrease MSNA and those with the highest activity can further increase activity. However sitting and standing are the true resting physiological postures and the minimal burst incidence in the sitting position was 42 bursts/100 heart beats. Therefore there is scope to up- and down-regulate MSNA from the midrange but those individuals with a high sitting MSNA are probably reliant on heart rate increase to generate the required blood pressure response on standing (Burke, 1977).

3.1.1.2 Age

Imholz et al (Imholz, 1990a) observed the heart rate and blood pressure changes occurring in 40 healthy elderly adults over 70 years of age. There was no younger control group but they demonstrated that *qualitative* changes in orthostatic circulatory responses in the elderly study group were no different from previous studies of younger adults. Prolonged standing for ten minutes to assess steady state responses indicated that, excluding the initial nadir within 15 seconds, the lowest blood pressure values occurred at one minute standing then blood pressure gradually increased whilst heart rate remained stationary.

Age has a strong quantitative effect on the 30:15 ratio. There is a reduction in the heart rate response on standing (Collins, 1980). There is a negative linear influence on the 30:15 ratio with increasing age (Ewing, 1978a; Ewing, 1985; O'Brien, 1986; Vita, 1986; Wieling, 1982). Age appears to account for 30% of the variance of the 30:15 ratio in normal subjects (O'Brien, 1986). One study did not find a relationship for age and log-transformed values of the 30:15 ratio in 85 subjects aged from 31-92 years (Clark and Mapstone, 1986). The peak heart rate occurred slightly later in a study comparing young and elderly (70-86 years old): the younger and older groups' peak RR intervals were recorded at 12 and 14 seconds respectively after standing but this difference may have been largely influenced by the increased time to attain standing posture (3 ± 1 vs. 6 ± 1 seconds) in the elderly. The same study found the magnitude of initial decrease in BP was not different between healthy young and elderly, since in the older group the smaller increase in cardiac output, achieved by heart rate increase, was offset by a smaller decrease in peripheral resistance on standing (Wieling, 1992). Thus the young adapt to standing by a greater RR shortening to counter peripheral blood pooling, whereas the attenuated RR shortening in the elderly is compensated by greater peripheral resistance.

Ziegler et al found postural blood response did not vary with age in subjects aged 15-67 years (Ziegler, 1992b). Piha measured systolic blood pressure 30 seconds after standing and this index did decline with age (Piha, 1993).

3.1.1.3 Other influences

There is no gender effect on the 30:15 ratio (Ewing, 1985; Gerritsen, 2003; Netten, 1992; Wieling, 1982).

3.1.1.4 Repeatability

Some investigators advocate adhering to the RR values close to the 15^{th} and 30^{th} beats for the 30:15 ratio. (Ewing, 1978b). However there may be difficulties in achieving a prompt standing manoeuvre in individuals with joint disease, muscle weakness, frailty etc. There may also be a delayed vagal action with trough and peak RR intervals occurring outside these expected times. This drawback leads to considerable individual differences in the RR30:RR15

ratio, which underestimates the true maximum and minimum RR values (O'Brien, 1986; Wieling, 1982). Thus the RRmax: RRmin is a superior value to assess vagal activity. Other research confirms this approach, with the finding that the shortest RR interval occurs in beats 5 – 25, and the longest RR interval between beats 20 – 40 (Ziegler, 1992b). There is no significant relationship between resting heart rate and the 30:15 ratio (Ewing)

Some investigators take the view that the test range for RRmax: RRmin is too low in older individuals aged more than 65 years to allow differentiation between normal and abnormal. The difference in heart rate between baseline and peak heart rate within 15 seconds of standing provided a greater test range of results and is thought to be a better value for vagal investigation (Piha, 1993; Wieling, 1997). Another group (Gerritsen, 2003) have extended this concept by taking the difference between mean RR interval 1 minute prior to standing and the minimum RR interval within 15 seconds of standing, and this measure does perform better than the RRmax/min in terms of reproducibility and correlation or concordance with other autonomic tests. Co-efficient of variation for the RR min was 14% compared with 39% (transformed) for the RRmax/min.

Investigations of the repeatability of the 30:15 ratio have found conflicting results. In the original Ewing paper, co-efficient of variation (CV) for 30:15 ratio was 5.3% in five young controls tested on different days (interval not stated) (Ewing, 1978b). Ewing et al reported the individual differences in 18 adults tested on three occasions were only significantly different on three out of 52 occasions. However they do not provide any statistical summary of results (Ewing, 1980b). Wieling reports repeatability to be high (Wieling, 1997). Another study found the between subject variation to be the same as within subject variation in a series of repeat tests over four weeks. They used a window of ten beats around the 15^{th} and 30^{th} beats. In practice this suggests the test is not suited to follow-up testing to track progress and raises doubts on the accuracy of the measurement (Lawrence, 1992b). The within subject SD/between subject SD ratio of the 30:15 ratio is smaller in diabetic patients compared with normal controls, and this property improves the tests ability to identify meaningful differences between cases (Lawrence, 1992a). Furthermore the 'windowed' 30:15 ratio had a better (lower) within/between subject SD than the exact 30:15 ratio.

When describing repeatability, an 'adapted coefficient of variation' is a more appropriate method. The adapted CV is obtained by subtracting 1 from the mean CV, so that the relationship between the standard deviation of repeat measurements and the mean of repeat measurements is maintained as results approach the no-response unity value (Murray and Lawrence, 1993). This technique was not used in earlier studies of repeatability within small groups of normal cases but nonetheless they both found the CV was approximately 9% (Netten, 1992; O'Brien, 1986).

A large group of 177 diabetic patients had repeat autonomic tests separated by at least 3 months. The authors report the mean 30:15 ratio remained unchanged. Mean period of time separating sequential testing is not clear, therefore it is difficult to say if the 30:15 ratio has good repeatability or conversely if it did not respond to any change that may have occurred in this particular group. One suspects the latter since the authors infer the follow-up period was several years, and secondly many of the subjects already had abnormal responses at their first visit (Ewing).

3.1.1.5 Test methodology

There is some debate on the ideal test conditions and protocol for assessing the RRmax: RRmin. Although the degree of drop in blood pressure may theoretically vary with the time of testing, one group found no diurnal variation in the RRmax: RRmin (Wieling). Normal practice requires change in posture is from supine to standing upright but the cardiovascular responses are comparable when starting from either supine or sitting positions (Ten Harkel, 1990). However the responses are clearly dependent on the length of the preceding rest period before posture change: HRmax can differ by 20% - 32% when comparing rest periods of 1 and 20 minutes (Borst, 1982; Ten Harkel, 1990). Thus test conditions need standardisation. Five minutes rest would appear to be sufficient. Imholz compared Finapres performance to intra-arterial pressure and found the Finapres overestimated trough systolic pressure by 3 mmHg and diastolic by 2 mmHg, neither of which were significant. Values at 30 and 120 seconds tended to be significantly lower in each case, more so for diastolic pressure (Imholz, 1990b).

3.1.1.6 Relationship with other cardiovascular autonomic tests

The 30:15 ratio appears to be closely associated with total, high and low frequency powers of heart rate variability from power spectral analysis (Freeman, 1991). There is poor correlation between RRmax/min and the I:E ratio derived from heart rate variability during metronomic breathing, despite their shared vagal mechanisms. This underlines the different afferent mechanisms of the heart rate changes (Wieling). Others have demonstrated weak but significant correlations of the 30:15 ratio with RR interval variation, heart rate response to deep breathing and the Valsalva ratio (Vita, 1986). The relative change in heart rate for standing is less than that seen during the Valsalva manoeuvre and more than that seen during metronomic respiration (O'Brien, 1986).

3.1.1.7 Clinical application

The heart rate response to standing is a simple bedside test of autonomic function. In an early clinical study of a small number of 21 adult diabetic patients, Bennett found three patterns of response on standing, using data from other cardiac autonomic tests.

- Normal response with a tachycardia and little or no fall in systolic BP.
- An initial fall in systolic BP which recovered without further increase in heart rate; this group had normal overshoot with Valsalva indicating intact vasoconstrictor response.
- A third group with a fall in systolic BP which was subsequently partially attenuated by a marked tachycardia; this group lacked overshoot for the Valsalva indicating loss of vasoconstrictor response and therefore the tachycardia/partial BP recovery was achieved via cardiac sympathetic drive.

One case had profound hypotension and no tachycardia, indicating severe combined sympathetic and vagal failure (Bennett, 1975).

Ewings' original 30:15 paper described the value of the test in identifying diabetic patients with autonomic neuropathy. All 15 diabetic patients with neuropathy had a 30:15 ratio less than 1.03, less than both the healthy controls and non-neuropathic diabetic groups (Ewing, 1978b). One of the more common clinical uses is in diabetic patients where the 30:15 ratio is a sensitive test for neuropathy (Ziegler, 1992a). The American Diabetes Association do not mention the 30:15 ratio in their report of Standardized Measures in Diabetic Neuropathy (Anonymous, 1995). The American Academy of Neurology do recommend the heart rate response to standing as a test of cardiovagal function (AAN, 1996a). They summarise the test as being sensitive, specific and reproducible (in contrast to Lawrence et al (Lawrence, 1992a; Lawrence, 1992b)), as well as simple, safe and cost-effective. One author does not advocate the 30:15 ratio because of its complex physiology and ill-defined confounding variables (Low, 1993a). There are studies indicating the 30:15 ratio is not a sensitive test for diabetic autonomic neuropathy, and certainly not as sensitive as heart rate variation during deep breathing. Mackay et al (Mackay, 1980) assessed 287 diabetic patients; the 30:15 ratio equivalent was abnormal in 57% cases with autonomic symptoms but sensitivity improved to 90% when detecting the combined groups of borderline abnormal (between 1.5 and 2 SD of control values) and frankly abnormal (less than 2 SD control). However repeat tests remained abnormal in 90% cases when repeated within a year.

Dyrberg et al (Dyrberg, 1981) also report poor sensitivity for detecting autonomic neuropathy in a cohort of 75 diabetic patients compared to 28 controls. The diabetic patients were split into 3 groups according to duration of diabetes. Other autonomic tests demonstrated increasing frequency of abnormal results with increased length of diabetes but the 30:15 ratio did not show any differences when compared with control values (1.14) or within the diabetic groups (diabetes up to 9 years 30:15 = 1.14, 10-19 years 30:15 = 1.15, 20-40 years 30:15 = 1.10).

Normal blood pressure response to standing remains a matter for debate. A consensus statement on definition of orthostatic hypotension in 1996 stated a cut-off of >20 mmHg systolic and >10 mmHg diastolic drop in blood pressure as abnormal. No reference was made to associated symptoms (AAN, 1996b). Wieling et al report a systolic fall of > 40 mmHg and diastolic fall of > 25 mmHg as abnormally large changes on standing (Wieling, 1992).

3.1.1.8 Conclusion

In the first 30 seconds after standing, sympathetic and parasympathetic activity produce distinctive patterns of RR and blood pressure response. Peripheral resistance falls immediately to accommodate sudden thoracic filling therefore blood pressure falls. There is a bimodal peak in heart rate due to vagal withdrawal, with the second peak the larger and more important of the two. Combined cardioacceleration and MSNA output rescue the falling blood pressure, creating a blood pressure overshoot. This is buffered by vagally driven heart rate slowing. The RRmax/RRmin ratio (otherwise known as the 30:15 ratio) reflects vagal integrity. Blood pressure drop is dependent on a number of factors. RRmax/RRmin declines with age. Clinical utility is debateable and reproducibility is moderate at best.

3.1.2 The Valsalva Manoeuvre

The Valsalva manoeuvre is a respiratory manoeuvre that tests baroreflex sensitivity. It is named after Antonio Maria Valsalva (1666-1723). The Valsalva manoeuvre is performed by forced expiration at a constant pressure for a fixed time whilst at rest. By ensuring a small air leak in the expiratory apparatus, the glottis remains open during expiration to ensure that the connection between mouth and airway remains continuous. The physiological changes required for forced expiration increases intrathoracic and intra-abdominal pressure.

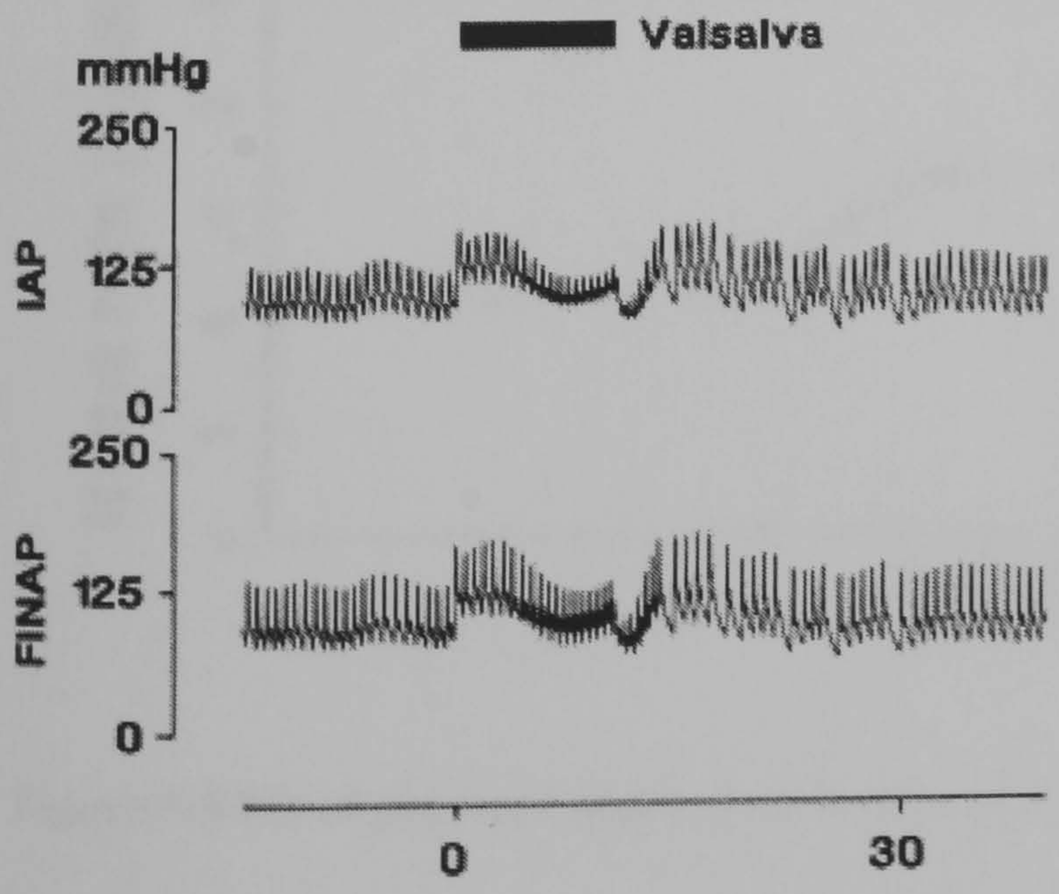

Figure 3-4 The haemodynamic consequnces of the Valsalva manoeuvre

'Valsalva' block represents the duration of forced expiration with an open glottis. The tachogram depicts the pattern of blood pressure and heart rate changes.

This produces a sequence of changes in heart rate and blood pressure dictated by the autonomic system reacting to the cardiovascular stress that results from the pressure increase. The haemodynamic response to the Valsalva manoeuvre is depicted in Figure 3.4.

3.1.2.1 Heart rate response

As the blood pressure falls during forced expiration, the heart rate quickens in order to improve cardiac output. This tachycardia reaches a maximum just after the end of forced expiration. This may be expressed by the minimum RR interval. When blood pressure rebounds shortly after the end of forced expiration, heart rate slows leading to a maximum RR interval. This maximum usually occurs within 30 seconds of the start of the procedure. Heart rate then moderately increases to settle back to a rate similar to that at baseline. The ratio of maximum: minimum RR intervals during the manoeuvre is known as the Valsalva ratio (Levin, 1966). The heart rate changes are shown in the lower panel of Figure 3.5.

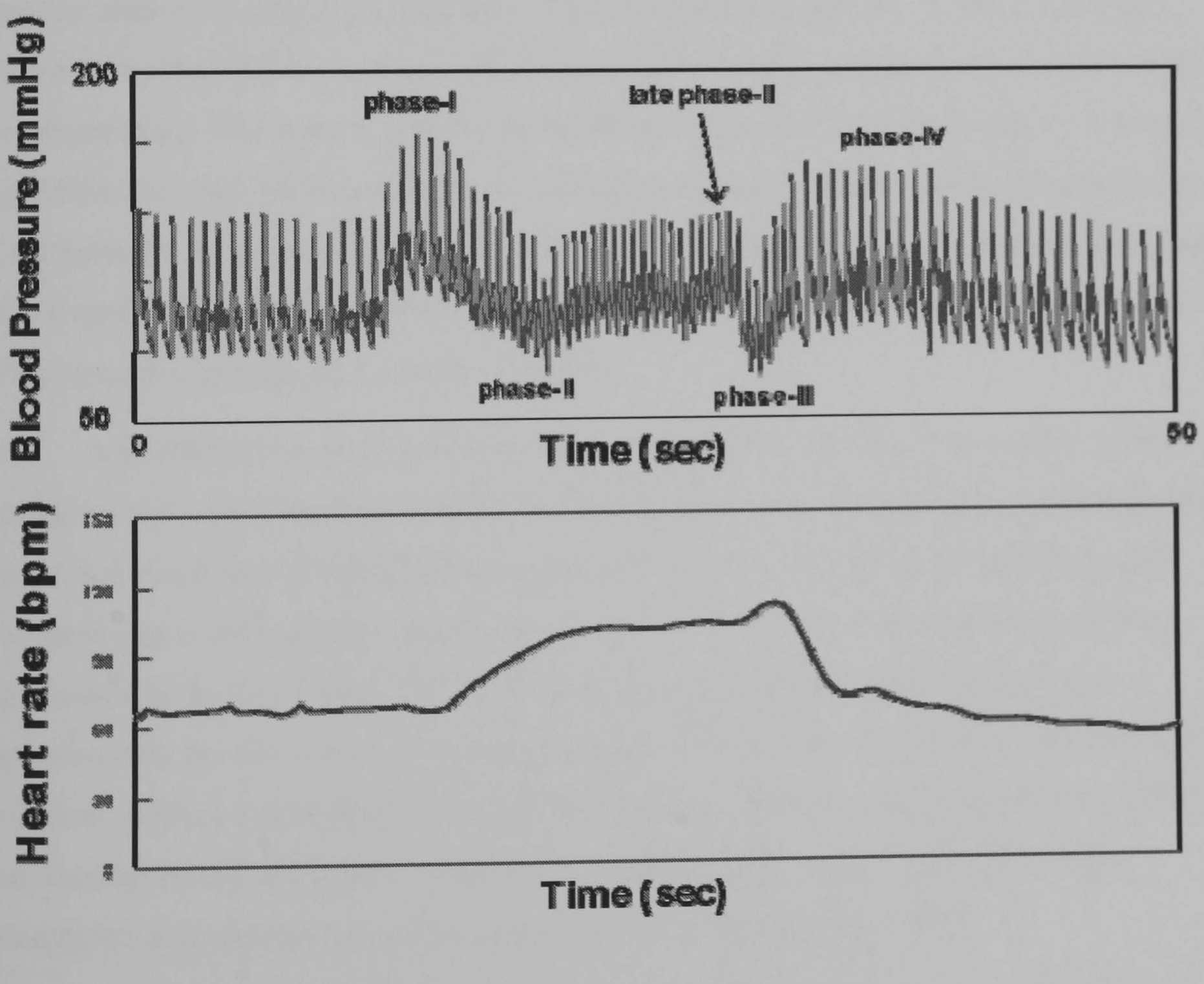

Figure 3-5 Blood pressure phases and heart rate response to the Valsalva manoeuvre

3.1.2.2 *Blood pressure response*

The blood pressure changes follow a pattern split into 4 phases (Hamilton, 1936) that are shown in the upper panel of Figure 3.5. The following description includes physiological observations made by Greenfield et al (Greenfield, 1967) from direct recordings of arterial pressures in 8 normal subjects who underwent renal angiography. Valsalva manoeuvre was performed with expiratory pressure of 40 mmHg for 25 seconds.

I A very transient rise in blood pressure within 2-3 seconds, a mechanical effect due to the rise in intrathoracic and intra-abdominal pressures that compresses the aorta. Stroke volume (SV) and aortic cross-sectional area do not change (Greenfield, 1967). Total autonomic blockade does not alter arterial pressure transients of phase I, confirming its mechanical basis (Korner, 1976).

II*e* A fall in blood pressure, early in phase II. The rise in intrathoracic pressure impedes venous return, affecting cardiac output therefore blood pressure gradually falls in II*e*. Wexler et al (Wexler, 1968) recorded abrupt decrease to near zero in inferior vena cava (IVC) flow from the start of straining until the end of straining in four subjects. In this experiment, straining was for 20-30 s, and on some occasions IVC flow moderately increased towards the end of straining. The drop in systolic blood pressure was partially cushioned by a tachycardia dependent on vagal withdrawal. There is another reaction to the fall in blood pressure in terms of sympathetic function which creates a biphasic pattern of early and late phase II (II*e* and II*l*). Greenfield (Greenfield, 1967) found a rapid decrease in SV and arterial pressure but a 25% increase in peripheral vascular resistance

II*l* A moderate rise in blood pressure, late in phase II. An intact autonomic system recognises the preceding deterioration in blood pressure and the ensuing sympathetic outflow produces a small rise in blood pressure predominantly by increasing peripheral arterial resistance (up to 50% greater than control values at end strain) and also acting on the capacitance beds (Greenfield, 1967). This will usually return the blood pressure to approximately baseline levels in normal subjects. Wexler recorded a brief period of increased IVC flow velocity immediately after release of strain (Wexler, 1968). In a review, Eckberg described increases in efferent sympathetic neural outflow to limb muscles, plasma epinephrine and total peripheral resistance in phase II*l* (Eckberg, 1980).

III A drop in blood pressure. This is the sudden fall in blood pressure, which is a mechanical effect due to fall in intrathoracic pressure that occurs immediately after cessation of forced expiration. This only lasts 1-2 seconds. Sudden augmentation of left ventricular afterload may contribute to the drop in blood pressure that principally results from sudden expansion of the great vessels (Eckberg, 1980). Greenfield noted a reduction in peripheral

resistance but stroke volume did not change. Total autonomic blockade does not alter arterial pressure transients of phase III, confirming its mechanical basis (Korner, 1976). There is an additional burst of sympathetic activity causing a further increment in heart rate (Eckberg, 1980).

IV The final rise in blood pressure. This is the peak blood pressure attained during the blood pressure overshoot which normally occurs within the 15 seconds after termination of forced expiration. During phase IV, venous return, stroke volume and cardiac output return to normal, against a backdrop of elevated peripheral arterial pressure. Hence blood pressure rises above control values in phase IV. Greenfield found stroke volume and arterial pressure rose rapidly to control values after 6-9 beats, peripheral vascular resistance fell to control values within 3 beats of release and aortic area increased. Maximal SV was achieved in the range of 6-15 beats post-release (Greenfield, 1967).

The calculation of blood pressure changes for each of these phases is depicted in Figure 3.6.

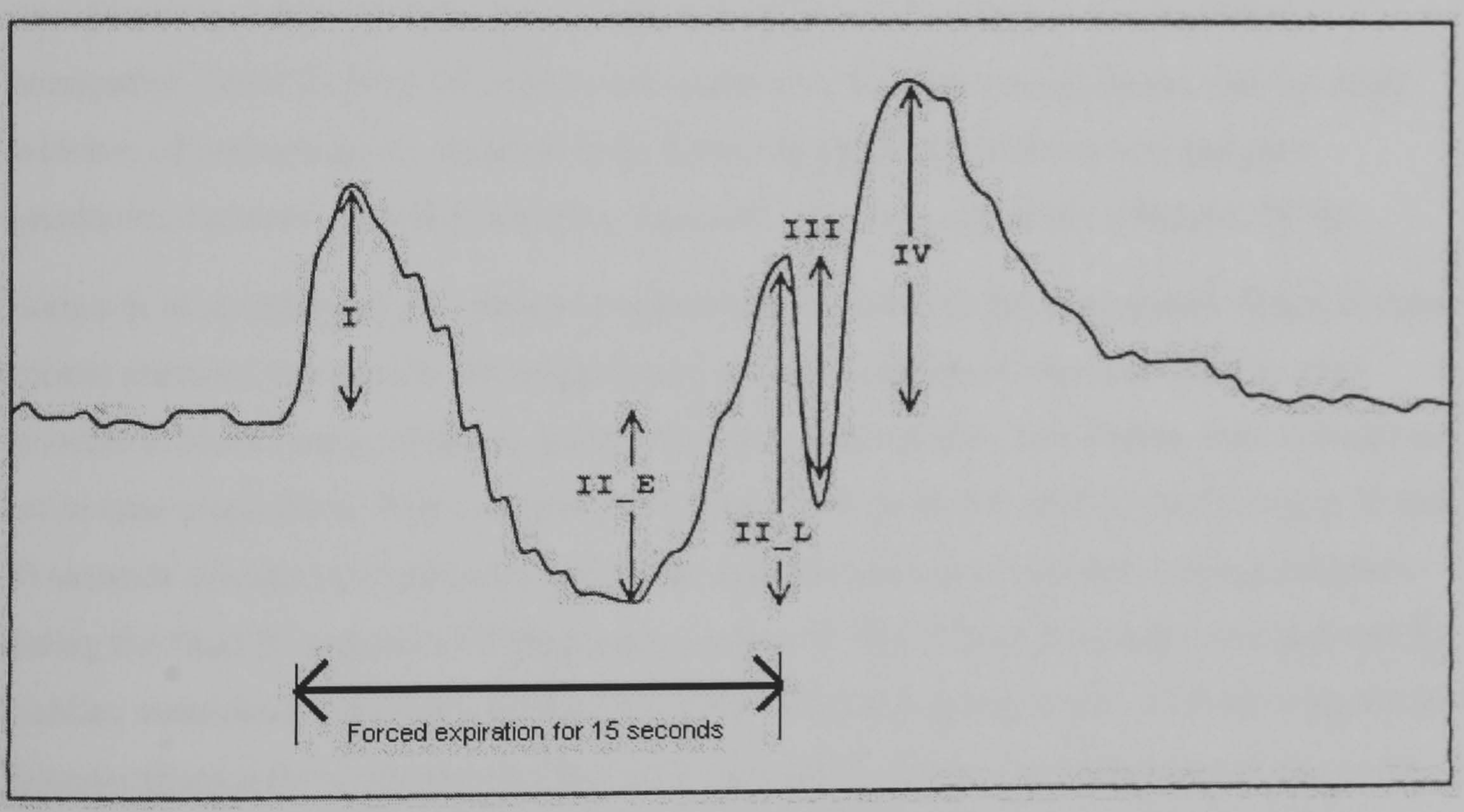

Figure 3-6 Blood pressure measurements for phase I to phase IV of the Valsalva manoeuvre

3.1.2.3 Neural mechanism

The bradycardia occurring during the post-expiratory phase is mediated by parasympathetic neural activity via the vagus. However it is not clear if the tachycardia is predominantly due to sympathetic drive or vagal withdrawal (Bennett, 1976). Spodick et al (Spodick, 1974) demonstrated a significant beta-adrenergic component to fluctuation in heart rate in the first three phases. Beta-blockade slowed the heart rate during early strain, end strain and early in

phase IV. However there was no difference in the post-release bradycardia. Overall the RR interval changes followed the same qualitative course despite beta-blockade.

One group found the Valsalva ratio did diminish with atropine but was not abolished: furthermore there was no significant response to low-dose atropine, unlike the RR variability during deep breathing (Rothschild, 1987). Another report noted the Valsalva ratio is non-significantly reduced after atropinisation, adding weight to the view that the Valsalva ratio is not purely reliant on cardiac vagal function (Julu, 1992).

Bennett et al (Bennett, 1976) described the Valsalva manoeuvre as a test of baroreflex function: individuals with abnormal RR and blood pressure response also had decreased baroreflex sensitivity using the phenylephrine technique. Lack of normal BP overshoot indicated an efferent lesion with loss of vasomotor function.

The sympathetic system is thought to mediate the increase in systemic blood pressure in phase 4. It is generally assumed to result from vasoconstriction but a group of healthy normal controls demonstrated responses in two groups, either forearm vasoconstriction or forearm vaso*dilatation*, whilst all had normal Valsalva ratios i.e. no evidence of autonomic neuropathy. Some diabetics also had post-manoeuvre forearm vasodilatation with no other evidence of neuropathy. In contrast those with orthostatic hypotension also had post-manoeuvre forearm vasodilatation plus abnormal heart rate variability (Bennett, 1979).

Korner et al investigated the impact of autonomic blockade on the four phases. Phase II mean arterial pressure was greatly diminished, and phase IV overshoot was abolished by total autonomic block (using atropine, propranolol and guanethidine), confirming their reliance on autonomic modulation. Physiological parameters were studied in greater detail during 30 and 45 seconds of expiratory pressure, and these measurements concentrated on the parameters during the final 10 seconds of expiration i.e. phase II. The impact of atropine and propranolol (cardiac autonomic blockade) and then with the addition of guanethidine to block sympathetic vasoconstriction (total autonomic blockade) was assessed. Mean arterial pressure increased with increasing expiratory pressure (10 to 30 mmHg), but this was largely abolished by cardiac autonomic blockade (atropine and propranolol), and additional sympathetic vasoconstrictor block with guanethidine led to a large decrease in arterial pressure. Heart rate increase was severely restricted to the same extent with atropine and propranolol alone or with the addition of guanethidine. Rise in total peripheral resistance increased with greater expiratory pressure, this change was not affected by atropine and propranolol but was abolished by guanethidine. Overall the experiment indicated the complex afferent input and the variable role of pathways according to expiratory pressure (Korner, 1976).

Sandroni et al conducted a detailed investigation of the components of the Valsalva manoeuvre and came to several conclusions regarding blood pressure changes (Sandroni, 1991).

1) The BP changes in the Valsalva manoeuvre detect adrenergic vasoconstrictor failure with more sensitivity than orthostatic BP recordings: adrenergic failure leads to a reduction in II*l* in patient groups with orthostatic hypotension (established and borderline) and sympathetic sudomotor failure. Those worst affected patients had more severe falls of BP at II*l* plus absent phase IV.

2) Alpha-adrenergic blockade led to a reduction in II but increased IV blood pressure. The former suggests II*l* is due to alpha-adrenergic activation but IV reflects the effect of cardioacceleration in response to preceding fall in BP.

3) Beta-blockade with propranolol markedly reduced blood pressure at IV, with a smaller reduction in phase II again consistent with the theory that IV is more dependent on cardioacceleration and II is more dependent on peripheral adrenergic function.

4) In summary, II*l* is more dependent on peripheral sympathetic innervation whereas IV is produced by cardiac sympathetic innervation.

Another detailed investigation provided further insight to the complex blood pressure changes of the Valsalva manoeuvre, this time using healthy controls and examining muscle sympathetic nerve activity (MSNA) during the test phases (Smith, 1996).

1) There was a highly significant correlation between straining and increases in MSNA during straining, and with the phase IV BP elevation

2) There was a highly significant correlation between peak MSNA and the change in blood pressure from phase III to IV, suggesting this increase in BP is secondary to MSNA activity (seemingly at odds with Sandroni et al (Sandroni, 1991))

3) The times to return to baseline systolic blood pressure, sympathetic activity and the occurrence of the first post-straining sympathetic burst correlated significantly with the intensity of straining (measured using different expiratory pressures of 10, 20 and 30 mmHg)

4) There was no significant RR variation using an expiratory force of 10 mmHg; RR variation became progressively larger with pressures of 20 and 30 mmHg

5) Aortic cross-sectional area, which was highly reproducible within the 4 cases examined, tended to decrease during straining below control levels and then increased above control levels after the forced expiration

They conclude that (i) MSNA is proportional to straining level and is a function of the arterial baroreflex, and (ii) arterial blood pressure elevations estimate sympathetic nerve activity and reflect integrity of the arterial baroreflex. They hypothesise that changes in baroreceptor activity are more important than absolute levels, and that baroreceptor outflow may reset to preceding pressure transients that last only seconds.

There are some pathological studies indicating the type of neural damage underlying abnormal Valsalva ratio response. Diabetics (who unfortunately did not have ante-mortem autonomic function testing) were found to suffer loss of autonomic nerve fibre density with demyelination and occasional axonal degeneration (Low, 1975). Abnormal blood pressure responses (progressive fall in BP throughout phase II and lack of overshoot in phase IV) were found to have different substrates according to the underlying clinical syndrome. Cases with pure autonomic failure or parkinsonism with sympathetic neurocirculatory failure had evidence of loss of myocardial sympathetic nerve terminals whereas multiple-system atrophy cases had evidence of intact sympathetic terminals and absent nerve traffic (Goldstein, 1997).

A normal VR taken alone should not be viewed as a marker of baroreflex integrity, since RR variation can occur in patients with complete but isolated sympathetic failure (van Lieshout. 1989).

Some investigators have commented on 'square wave responders' referring to the abnormal BP pattern in a small number of cases: the BP remains high throughout the straining phase and the overshoot is absent (Zema, 1980). But this square wave response can change to a normal sinusoidal response by changing from the recumbent to sitting position (van Lieshout, 1989). Robertson et al reported reductions in left ventricular end-diastolic and end-systolic volumes of around 10% and left atrial diameter by 30% but in cardiac failure there was proportionately less decrease in left atrial dimension (Robertson, 1977). In keeping with Levin's hypothesis, the square wave response may be a result of larger intra-cardiac blood volume in certain individuals (Levin, 1966). Square wave response is mostly reported in patients with congestive cardiac failure (Eckberg, 1980).

3.1.2.4 Age

As Low has discussed, there are conflicting results with regard to the influence of age on the VR (Low, 1990). Studies of healthy normal controls have not find any relationship between age and VR (Benarroch, 1991; Ewing, 1985; Vita, 1986). Levin found a small non-significant decrease in VR with age but only assessed middle-aged groups (Levin, 1966). A larger study

of 425 normal controls aged 9-79 did show a significant negative correlation between age and VR (plus a weaker clinically irrelevant association with BMI) (Gelber, 1997). Generally though the majority view is that the Valsalva ratio does decline with age (Clark and Mapstone, 1986; Smith, 1984; Smith and Smith, 1981). Low's discussion and summary of published studies on the effect of age on VR suggests the inconsistencies are due to lack of a standardised protocol for the manoeuvre. The studies with negative findings tended to be studies with smaller numbers, more compacted age range or using the supine position. From his own cohort of 155 subjects, there was a significant inverse relationship between age *x* and VR *y* (Low, 1990).

$$y = 2.27 - 0.01x \qquad R = 0.5 \qquad p < 0.001$$

Smith et al's (Smith and Smith, 1981) healthy cohort of 174 adults aged 16 – 89 years gave the following best-fit relationship, which explained 18% of the total VR variance

Valsalva ratio = 2.156 – (0.009125 x age in years)

Gender and smoking did not influence VR. Subsequent analysis showed that a curvilinear model gave a better fit for variation in VR with age and another linear regression analysis was performed on the natural logarithm of the ratio minus one (Smith, 1984)

Ln (ratio – 1) = 0.2188 – (0.0145 x age in years).

Some authors cast doubt on the usefulness of the Valsalva ratio in older patients. One author concluded the test range for the VR in cases aged over 65 years is too small to differentiate normal from abnormal heart rate control (Piha, 1993). Others feel that elderly and those with neurological disorders cannot perform the Valsalva manoeuvre adequately well to make the test valid (de Jong-de Vos van Steenwijk, 1997).

The change in systolic blood pressure to phase II*e* becomes increasingly negative with age but this does not affect phase IV (Denq, 1998).

3.1.2.5 Other influences

Gender has no influence on the VR (Baldwa and Ewing, 1977; Benarroch, 1991; Gelber, 1997; Smith and Smith, 1981). In a review, Low summarised other important influences as position of the subject, expiratory pressure, duration of effort, inspiratory volume and medication (Low, 1993a). The height of the systolic BP overshoot in phase IV is proportional to left ventricular ejection fraction (Zema, 1980). Gender has no effect on the phase II*e* or IV systolic blood pressure responses but higher resting blood pressure exaggerates the trough of II*e* (Denq, 1998).

3.1.2.6 *Repeatability*

The literature contains a variety of methods of calculating the Valsalva ratio. Unfortunately there is no consensus on the ideal method of calculating the Valsalva ratio. The more common methods for ascertaining the Valsalva ratio are as follows:

1) Mean ratio of three successive Valsalva manoeuvres (Baldwa and Ewing, 1977; Ewing, 1985).

2) The largest value from two (Levin, 1966) or three Valsalva manoeuvres (Low, 1975; Sandroni, 1991; Vita, 1993)

3) Mean of a pair of Valsalva ratios (Bennett, 1976; Lawrence, 1992b)

4) Until two reproducible responses are obtained (Low, 1993a)

5) One VR is required in the clinical practice, according to Mustonon et al (Mustonen, 1989). This report noted the difference between the first VR and the mean VR of 3 efforts was small and not statistically significant in a group of 75 diabetics and 48 controls

6) The mean of the maximum and minimum values, calculated from the mean of each extreme value plus the 2 intervals either side, are used for the ratio (Schumer, 1988).

The Valsalva ratio was not significantly related to the resting heart rate in a study of 135 subjects aged 16-69 and this finding has been replicated elsewhere (Ewing, 1985; Smith and Smith, 1981; Ziegler, 1992b).

Different techniques have been used to measure repeatability. Levin (Levin, 1966) simply measured the mean difference between ratios, which was 0.10 for the VR or 5%. He also noted that increasing the expiratory pressure increases variation in VR. Baldwa et al (Baldwa and Ewing, 1977) investigated 12 normal young adults (on 10 occasions over 2 months) and 7 middle-aged adults (5 occasions over one week). The mean, within-subject SD and between-subject SD variation of the Valsalva ratio was 1.49, 0.17 and 0.18. The mean was lower, within-subject SD the same and between-subject SD results smaller in the middle aged group compared with the young adult group. In other words, repeatability was similar between the two groups but the test range did not allow as good a discrimination between subjects in the older group. The authors felt this represented adequate reproducibility although one could argue these results do not confer very good reproducibility. Smith et al (Smith and Smith, 1981) performed Fisher's intrapair correlation coefficient in assessing repeatability of the VR in 20 healthy subjects aged 20-87 years mean age 40 years. Valsalva ratio was repeated after intervals of 3-8 months. There was a moderate level of correlation; $r = +0.61$, $p<0.01$. Smith

performed another study on repeatability, this time using a group of 19 healthy adults and 19 diabetic patients on two clinic visits (interval not stated). Coefficient of variation for healthy subjects was 15.4% but 10.5% in the diabetic group (Smith and Smith, 1981).

The Valsalva ratio had satisfactory repeat variability (subject mean 1.92. between subject SD 0.38, within subject SD 0.17), and also had the best 'within subject/between subject' ratio (0.45) compared with the 30:15 ratio and deep breathing tests of RR variation in a group of 10 normal controls (Lawrence, 1992b). There were similar results in a group of 11 diabetic subjects but with a small deterioration in within subject/between subject ratio (subject mean 1.74, between subject SD 0.31, within subject SD 0.18, ratio 0.57) (Lawrence, 1992a). The same group of investigators argue the *adapted* co-efficient of variation should be quoted in studies and report this measure demonstrates a strong relationship with the 'within subject/between subject' SD ratio (Murray and Lawrence, 1993). The issue is that co-efficient of variation of the test should have a direct relationship between the SD of repeat measurements and the mean of measurements, which does not hold true for the 'unadapted' CV for the Valsalva ratio. Using a similar mathematical approach but adding log transformation, one group felt that day-to-day reproducibility was poor for the Valsalva ratio and was certainly the worst of a range of autonomic tests (Ziegler, 1992b).

This ideal method (adapted co-efficient of variation) is not widely used but if we do take the opinion that some value can still be derived from the basic CV, some worthwhile results were reported on autonomic testing at different centres over three test dates within 18 months. This report indicated, by virtue of similar CV results, that the Valsalva ratio is reliable and reproducible and benefited from a large number of cases (Schumer, 1988).

The tachycardia ratio was investigated by Baldwa et al. This is the shortest RR interval during the manoeuvre divided by the longest RR interval before the manoeuvre starts. Reproducibility (within-subject SD = 0.043) was actually better than the traditional Valsalva ratio, even allowing for the smaller mean tachycardia ratio of 0.75, compared to 1.49 for the Valsalva ratio. Between-subject variation was 0.05 for the tachycardia ratio (Baldwa and Ewing, 1977).

3.1.2.7 Test methodology

Eckberg (Eckberg, 1980) reviewed methods employed in Valsalva manoeuvre studies and highlighted the lack of standardisation. Straining is initiated after maximal, full or normal inspiration. Mouth pressure is usually 40 mmHg but varies from 30 to 60 mmHg. Duration of straining is often 15 seconds but also varies from 10 to 30 seconds and occasionally longer. Post-straining respiration is rarely controlled but can have a critical effect on reflex changes. Eckberg comments on the multiple regulatory mechanisms and receptors contributing to a the

reflex changes. Net heart rate changes are primarily influenced by central reactions between arterial baroreceptors, chemoreceptors and cardiopulmonary receptors. The prolonged nature of the reflex means that late pressure changes depend on 'peri-reflex' events and not just the 'pre-reflex' stimulus itself.

Position has an important influence on the Valsalva ratio. Most authors recommend performing the manoeuvre in the sitting position and blowing into a mouthpiece for 15 seconds aiming to maintain a pressure of 40 mmHg (Baldwa and Ewing, 1977; Denq, 1998; Ewing, 1985; Low, 1975; Ten Harkel, 1990; Weston, 1996a; Ziegler, 1992b). Twenty seconds (Gelber, 1997; Parati, 1989; Schumer, 1988) or ten second periods (Bennett, 1976; Levin, 1966; Smith and Smith, 1981; Vita, 1993; Vita, 1986; Zema, 1980) have been used elsewhere. One review suggests the subject should be recumbent and also recommends that the maximum RR value used should occur within 30 seconds of commencing forced expiration (Low, 1993a). However the magnitude of the BP fall during straining and subsequent overshoot of phase IV are smallest in the recumbent position and increase with the change to sitting and standing position (Ten Harkel, 1990). In those cases who lack BP overshoot in phase IV, it has been recommended to use the highest BP in the first 15 seconds after completion of expiration (Wieling and Karemaker, 1999). The Valsalva ratio is significantly increased in the standing position compared with the supine and sitting positions because of the increase in maximum heart rate. This study also showed that the period of preceding rest (from 1 to 20 minutes) did not influence the Valsalva ratio or blood pressure changes. The greatest source of poor repeatability is variation in respiratory pattern and cases should be instructed on breathing pattern before and after forced expiration (Lawrence, 1992b). Normal tidal breathing pattern pre-manoeuvre will enhance the Valsalva ratio which is reduced if the subject takes a maximum inspiration pre-strain, but in practice this can be difficult to control (Levin, 1966).

The expiratory pressure has a critical influence on the Valsalva ratio. A wide ranging exploration of the factors affecting Valsalva manoeuvre variables formed the following conclusions (Benarroch, 1991).

- Maximal changes in II*l* and IV correlated with duration of expiration at low (20 mmHg) and high (50 mmHg) expiratory pressures (consistent with the theory these responses are sympathetically mediated) (Korner, 1971). Similar results were produced by Levin but without a significant correlation (Levin, 1966).
- Valsalva ratio correlated with duration of expiration only at the higher 50 mmHg pressure, probably because at lower pressures intrathoracic blood volume can buffer arterial pressure flux

- In general there were stronger correlations between the magnitude of expiratory pressure and blood pressure/heart rate changes than with duration of expiration (e.g. Valsalva ratio correlation r = +0.63).

Overall the literature suggests that expiration for 10 seconds is effective and 15 seconds is optimal. Heart rate and blood pressure changes should always be viewed in tandem. For example there may still be a normal Valsalva ratio in the presence of cardiovagal damage if sympathetic activity produces cardioacceleration in phase II.

Levin also found a significant positive correlation between duration of strain and VR, from 8 to 14 seconds (Levin, 1966). Low's group, in a study setting normative data (Denq, 1998), set the following useful inclusion criteria when evaluating the shape of the blood pressure curve:

1) Expiratory pressure at least 30 mmHg and 10 seconds
2) Reproducible Valsalva manoeuvre BP curve, defined as maximal and minimal systolic blood pressures that differ by less than 10 mmHg between recordings
3) Absence of flat top blood pressure curve (in other words absence of a curve resembling the expiratory waveform)

The blood pressure is best measured using a continuous non-invasive device such as the Finapres. The difference between intra-arterial and Finapres blood pressures tends to be small: in a study of 15 hypertensive adults with a mean age of 50 years, for systolic, mean and diastolic blood pressures the mean differences were 1 ± 9.6, 9 ± 6.8 and 4 ± 6.1 mmHg respectively. Furthermore the Finapres faithfully reproduced patterns in the change of blood pressure during the Valsalva. The device tended to underestimate intra-arterial pressure during the control phase and over-estimate during phase 2, probably related to the changes in flow and diameter of vessels during the procedure (Imholz, 1988). The good correspondence between finger and intra-arterial BP during resting and active phases of the Valsalva manoeuvre was also shown in a study of 17 subjects (Parati, 1989). This study calculated baseline BP from a 20 second before the manoeuvre.

The interaction between the Valsalva manoeuvre and isometric exercise was examined in a small study of 5 males with mild untreated hypertension and 4 normal subjects. Essentially the blood pressure changes remain unchanged if sustained muscular exercise occurs during VM but heart rate changes are attenuated. Although there were similar qualitative changes. the quantitative changes indicate that strenuous muscular activity should be avoided during testing (Ewing, 1976b).

3.1.2.8 Relationship with other cardiovascular autonomic tests

Like other RR measures in the course of developing autonomic neuropathy, the vagally mediated Valsalva ratio tends to deteriorate before the sympathetic-mediated blood pressure measures of isometric exercise and orthostatic hypotension (Ewing, 1985). The Valsalva ratio may not detect subtle early autonomic dysfunction: a group of insulin dependent diabetics had normal bedside tests of autonomic function but definite decline in heart rate variability on spectral analysis (Weston, 1996a). The same group did reveal a reduction in baroreflex sensitivity from both Valsalva manoeuvre and spectral analysis techniques (Weston, 1996a). In another study, the Valsalva ratio did correlate strongly with total, high and low power of short term power spectral analysis of heart rate variability (Freeman, 1991) but others have found poor or absent correlation between the Valsalva ratio and spectral analysis or bedside autonomic tests (Vita, 1986; Ziegler, 1992b). There was a weak correlation with the 30:15 ratio ($r = +0.32$, $p < 0.01$) (Vita, 1986). Some studies have shown good agreement between the Valsalva ratio and expiration/inspiration (E/I) ratio of metronomic respiration in detecting autonomic neuropathy but repeatability of the Valsalva ratio is not as good as the E/I ratio (Smith, 1984; Smith and Smith, 1981).

Parasympathetic damage according to the Valsalva ratio and other RR measures is associated with loss of normal blood pressure diurnal rhythm. Mechanisms may differ according to absence or presence of sympathetic damage, with attenuation of the nocturnal decline or decrease in daytime blood pressure respectively (Carvalho, 2000).

3.1.2.9 Clinical application

In repeat tests at least three months apart in 237 diabetic subjects, the Valsalva ratio did show a moderate but significant decrease from 1.50 to 1.43. This is in contrast to no change seen in heart rate variation during deep breathing and standing, and may reflect the greater test range of the Valsalva ratio in diabetes (Ewing, 1985). A study of 75 diabetic patients at varying stages of neuropathy indicated the Valsalva ratio can detect neuropathy but is not as sensitive as the heart rate variation during metronomic respiration (Dyrberg, 1981). In general, diabetic patients have reduced Valsalva ratio (Low, 1975). Smith (Smith, 1984) observed the Valsalva ratio to perform well in detection of diabetic neuropathy. In 18 patients with abnormal Valsalva ratios, 16 had abnormal 'gold standard' metronomic respiration heart rate variation but 5 patients with normal or borderline Valsalva ratio had abnormal metronomic respiration sinus arrhythmia. In diabetic subjects, those with symptomatic autonomic neuropathy had a significant reduction in Valsalva ratio compared with non-diabetics but there was no difference between asymptomatic diabetics and normal controls (Rothschild, 1987). They also comment that the Valsalva ratio retains its quantitative properties with more severe autonomic dysfunction. The Valsalva ratio ranked as one of the best Ewing battery tests in detecting

cardiovascular autonomic neuropathy in diabetics with peripheral neuropathy (Ziegler, 1992a). Correlation coefficients with resting heart rate variation in 100 diabetic patients, who had varying degrees of autonomic neuropathy, were +0.659 ($p<0.001$) for the Valsalva ratio and +0.727 ($p<0.001$) for the tachycardia ratio (the tachycardia ratio is the shortest RR interval during the manoeuvre divided by the longest RR interval preceding the manoeuvre) (Baldwa and Ewing, 1977).

The Valsalva ratio is used in both the Ewing autonomic battery of cardiovascular tests and Low's Composite Autonomic Scoring Scale (Ewing and Clarke, 1982; Low, 1993b). Both of these batteries are essentially a series of autonomic reflex tests with each test contributing to a summative score that reflects overall autonomic function. Ewing's battery and Low's scale are used in clinical practice in autonomic laboratories.

The Valsalva manoeuvre can also be used to assess baroreceptor reflex sensitivity, by using the ratio of the change in blood pressure to RR interval in phase 4 i.e. the slope of the linear regression between systolic blood pressure and subsequent RR interval. This value bore a good correlation with the phenylephrine method ($r = +0.91$) whilst avoiding the difficulties of cannulation and drug administration (the phenylephrine method is the gold standard for measuring baroreceptor sensitivity, and measures unit change in RR interval as blood pressure is increased by phenylephrine injection) (Palmero, 1981). The slope method was compared to a new baroreflex sensitivity index method where the difference between maximal and minimal RR intervals and systolic blood pressure was used to calculate a ratio. This technique showed good agreement with the slope method (Bland Altman method, $p<0.05$) and acceptable test-retest repeatability (between 11 and 24% intra-subject variation) that was the same as the slope method (Kautzner, 1996).

Other information on cardiovascular function can be deducted from the Valsalva manoeuvre. One report found the phase IV systolic blood pressure overshoot measured using a bedside sphygmomanometer can provide a semi-quantitative estimate of left ventricular function and in so doing, is better than traditional clinical signs and chest radiograph (Zema, 1980). Valsalva ratio also changes in heart failure: Valsalva ratio was lower in cases with higher left ventricular end-diastolic pressure and radiographic evidence of heart failure (Levin, 1966).

3.1.2.10 Conclusion

The Valsalva ratio is a well established test of cardiac vagal and sympathetic function. The mechanically mediated changes in blood pressure at initiation and termination of forced expiration provoke changes in autonomic neural activity. Subsequent blood pressure changes are largely due to sympathetic nerve firing. Heart rate changes, particularly the increase in RR interval during blood pressure overshoot are mainly influenced by vagal activity. However the

responses from the two autonomic limbs are closely intertwined. The Valsalva ratio is reasonably sensitive in detecting vagal damage. Correct technique is critical in obtaining valid results and the ratio is sensitive to the duration and pressure of forced expiration: expiration at 40 mmHg for 15 seconds in an upright position is likely to yield satisfactory results. However repeatability may be moderate at best. The majority of research has used diabetic patients, and the literature provides sufficient evidence to show the Valsalva ratio can reliably detect autonomic neuropathy.

3.1.3 Heart rate response to deep and metronomic respiration

Variation in heart rate with respiration is one of the simplest cardiovascular autonomic tests. The fluctuation in RR interval is closely tied to respiratory movement. RR intervals vary at high frequency, usually mirroring respiratory frequency. The cardiovagal nerve forms the efferent limb of the reflex. The fluctuation of heart rate with respiration is depicted in Figure 3.7.

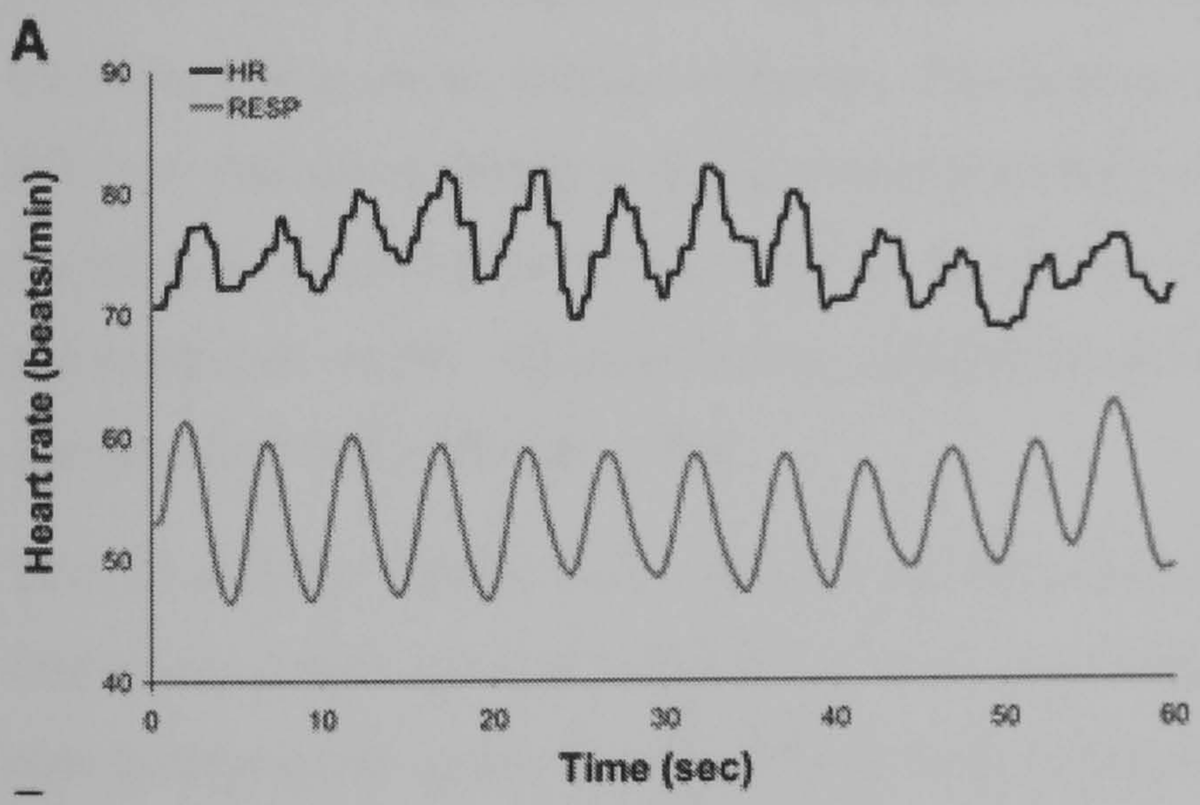

Figure 3-7 Variation in heart rate with respiration

HR, heart rate: Resp, respiration

3.1.3.1 Neural mechanism

Animal models demonstrate a proportional relationship between cardiac vagal efferent activity and respiratory heart period variations in experimental conditions (Katona and Jih, 1975). In other words, fluctuations in cardiovagal activity are mirrored by changes in the magnitude of sinus arrhythmia. A near linear relationship has been shown in humans (Eckberg, 1983; Pfeifer, 1982) but some conclude that linear coupling is only true for vagal traffic below baseline levels (Eckberg, 1988).

There are three potential mechanisms behind the variation in heart rate with respiration.

- Pulmonary stretch receptors, in the lungs or chest wall, initiating a reflex response on lung inflation
- An indirect effect from blood pressure flux (secondary to respiratory movements) stimulating arterial baroreceptors which influence heart rate
- There appears to be a direct interaction between respiratory and cardiovascular centres in the medulla.

The importance of each component has remained controversial (Low, 1990).

Inspiration leads to activation of pulmonary stretch receptors, impulses then travel via the vagus to inhibit the respiratory centre: this is the Hering Breuer respiratory reflex. The respiratory centre has a net inhibitory effect on the cardioinhibitory centre, and vagal activity changes to allow an increase in heart rate. Pulmonary receptors may be less important in humans though.

Increased venous return during inspiration appears to have two effects. Firstly the Bainbridge reflex is a cycle originating in the right atrium due to distension, which triggers vagal afferents acting on the nucleus solitarius. This brainstem centre then influences vagal efferents and alters sinoatrial firing to accommodate increase in venous return. Secondly atrial stretch acts on mechanoreceptors near the sinoatrial node, sending vagal afferent messages to the vasomotor centre which activates sympathetic efferents supplying the sinoatrial node to increase heart rate (Brooks, 1966).

Levy et al (Levy, 1966) used an animal model to demonstrate the probability of direct respiratory neuron activity influencing cardiac autonomic centres. Venous return did not vary during respiratory cycle, the right atrium was continuously drained by gravity, the arterial pressure was held constant and the lungs were collapsed with hili ligated. Oscillation in heart rate was still observed. This was predominantly vagal in origin but there was a residual oscillation after vagal nerve section, about one-third of that seen with intact vagi. With intact vagi, cardiac acceleration commenced towards the end of (but just within) the expiratory phase, i.e. prior to the onset of detectable muscle activity. Overall, appreciable cardiac acceleration only began with the onset of inspiration. Peak heart rate was attained at one-third of the interval toward the next inspiratory effort.

Other observations on the relationship between heart rate and respiration include those of Mecher et al indicating a baroreflex component (Melcher, 1976). During inspiration, vagal efferent activity is curtailed but this relationship is lost during artificial ventilation (Joels and Samueloff, 1956; Katona, 1970).

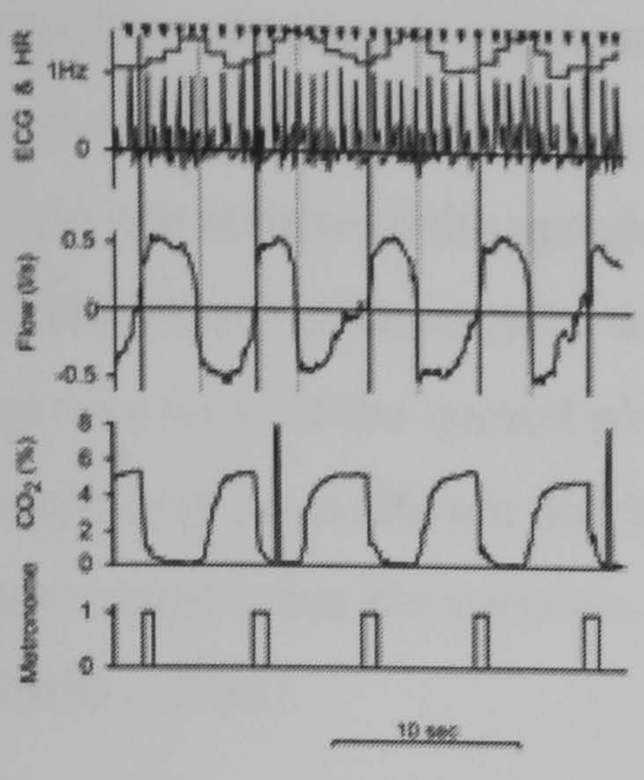

Figure 3-8 Respiratory activity and RR interval variation during metronomic respiration

CO_2 and flow panels indicate respiratory activity. Inspiration leads to increase in heart rate then expiration leads to decrease in heart rate.

Wheeler and Watkins (Wheeler and Watkins, 1973) are widely credited for first advocating heart rate variation during deep metronomic breathing as a test of cardiovascular autonomic neuropathy. The phenomenon of RR variation with respiration was known within the physiology literature, as discussed above. Wheeler and Watkins compared heart rate variation during deep breathing at six to eight breaths per minute whilst supine after 20 minutes rest in nine diabetics with symptoms of advanced autonomic neuropathy, 15 insulin treated diabetics without complications and 25 healthy subjects. There was a striking reduction in heart rate variation in the neuropathy group, all had ≤6 beats per minute variation whilst the other two groups all had much greater beat-to-beat variation. Atropine was given to one healthy subject which abolished the normal respiratory sinus arrhythmia but propranolol had no clear effect, so from this n = 1 test, loss of vagal tone was proposed as the cause of reduced heart rate variation in autonomic neuropathy.

Further work confirmed RR variation is virtually abolished after atropine but there is no additional effect with beta-adrenergic blockade using propranolol. There is no significant effect on RR variation with alpha-adrenergic blockade but there is a significant reduction in RR variation with sympathetic stimulation using isoproterenol (Pfeifer, 1982). There is a significant atropine dose response on RR variation (Rothschild, 1987). Therefore in the resting supine individual, parasympathetic activity is the driving mechanism behind RR variation.

The pattern of respiration imparts a strong influence on the quantity and timing of cardiovagal firing. RR intervals increase as respiratory interval increases and, to a lesser extent, increasing tidal volume increases RR interval. The fluctuation in RR interval is achieved by both prolongation of maximum and (less marked) shortening of minimum RR interval. The phase angle of RR and respiratory interval is a strictly linear relationship – in other words, the

difference in onset of inspiration and RR interval change is closely correlated. Cardioacceleration is synchronous with inspiration at typical respiratory frequencies between 0.15 to 0.25 Hz, but there is a phase lag for very long breathing intervals when supine: this trend can reverse in the upright position with a phase lead at slow respiratory rates (Saul, 1989). Hirsch demonstrated the linear relationship between RR and respiratory intervals holds true for most of the normal physiological range but there is a 'corner frequency' at very slow respiratory rates (Hirsch and Bishop, 1981). There is a 'system resonance' at 5-6 respirations per minute when the variation in RR interval is at its greatest (Angelone and Coulter, 1964; Pfeifer, 1982).

Cardiodeceleration begins in expiration at all breathing intervals, but late in expiration at normal respiratory frequencies of around 0.2 Hz and progressively earlier in expiration as frequency decreases (Eckberg, 1983). Respiratory pattern and depth will therefore influence heart rate variation. In particular tidal volume, can adversely affect repeatability (Lawrence, 1992b). Position is also important when recording heart rate variation. Bennett et al reported a significant difference in RR variation in diabetics with and without intact vagal function during metronomic respiration; this disparity weakened with subjects in supine breathing at normal rates and lost significance in a standing position (Bennett, 1977).

The relationship between respiration and heart rate variation is discussed in more detail in the section on Heart Rate Variability (3.2.4.1).

3.1.3.2 Repeatability

Studies of heart rate variation during metronomic respiration use either the ratio of maximum to minimum RR intervals or the difference in maximum to minimum heart rate. As a result of the physiological link with expiration and inspiration, these are often labelled the E/I ratio (or I/E ratio) and E-I difference.

Ewing and colleagues (Ewing, 1981) compared the predictive value and reproducibility of different methods of quantifying RR interval variation in three groups of diabetic patients; normal, parasympathetic neuropathy and combined sympathetic and parasympathetic neuropathy. During metronomic respiration for one minute, the heart rate difference between maximum and minimum heart rates and the standard deviation of RR intervals over five minutes of quiet breathing performed best at demarcating between different severities of autonomic dysfunction. Max-min heart rate differences did not show any significant differences in repeat tests in 6 tests per individuals (54 subjects) over two days testing. Max-min heart rate and max/min heart rate ratio strongly correlated ($r = +0.97$, p not stated). Max-min heart rate differentiated with the highest significance during sitting and standing compared with lying but the analysis does not constitute a true inter-method comparison and

in effect the performance is very similar. Max-min heart rate was also independent of resting heart rate. The authors recommend the max-min heart rate difference (ideally whilst sitting or standing) as the ideal routine clinical test of RR variation. The standard deviation of RR intervals during five minutes quiet breathing was of similar value and suitable for research purposes.

Max-min heart rate difference had good repeatability in a cohort of ten young adults and diabetic patients, with no significant difference in the mean of max-min heart rate differences from 5 respiratory cycles. In addition, a method of maximal respiration gave equivalent results to timed inspiration-expiration of five seconds each (Hilsted and Jensen, 1979).

In the course of establishing databases for RR variation in large groups of healthy normal cases, heart rate variation during deep breathing produced a skewed distribution. Therefore logarithmic transformation was performed for further analysis (O'Brien, 1986; Wieling, 1982). Ziegler et al (Ziegler, 1992b) also suggested that to obtain a log normal distribution for the E/I ratio, the calculation log (x-1) should be performed.

Many reports comment on the relationship between heart rate during rest and deep, metronomic respiration. There may be a weak but significant correlation between resting heart rate and E/I ratio in metronomic respiration (Wieling, 1982; Ziegler, 1992b), other investigators have not found a relationship (Smith, 1982). There are contrasting results for the relationship between resting heart rate and E-I difference: significant inverse according to Ziegler et al and Smith et al (Smith and Smith, 1981; Ziegler, 1992b) and none according to Ewing et al (Ewing, 1985). Multiple correlation analysis has shown a negligible contribution to heart rate variation in metronomic respiration from basal heart rate (O'Brien, 1986).

The co-efficient of variation during a solitary deep breath was 6 % (range 2-9 %) for the ratio of maximum/minimum RR intervals but 16 % (range 7-28 %) for the heart rate difference in a group of six healthy 20-35 year old males (O'Brien, 1986). Another study results indicate a similar CV of 12.9 % for the heart rate difference in a series of 10 tests over a 2 month period in 13 normal subjects (Ewing, 1985). Using the log(x-1) transformation, Ziegler found a standard deviation factor of 1.19 and 1.32-1.33 for controls and diabetics respectively (E-I difference or E/I ratio) (Ziegler, 1992b).

Although the difference in results in a group of 34 healthy normal controls (aged 19-85) for E-I difference over a mean retest interval of 0.96 years was small (2.12 ± 4.08), it was still a significant difference. This infers that single tests may not be suitable for serial or follow-up observations (Braune, 1996). But within-subject variability is lower than between-subject variability in both normal controls and diabetic patients, for both the E-I difference and E/I

ratio, confirming that the test has value (Lawrence, 1992a; Lawrence, 1992b). The co-efficient of variation for E-I difference in these studies is shown in the following table.

Table 3-1 Co-efficient of variation for heart rate variation during metronomic respiration

Group	Co-efficient of variation for E-I difference	
	Supine	Sitting
Normal (n=10)	28.6%*	16.1%
Diabetic (n=25)	19.6%	17.7%

A Murray 1993 (Murray and Lawrence, 1993). (Data derived from published figures for standard deviation and mean, differs from above figure*: CV = SD/mean)

The 'raw' values for the E/I ratio have a much lower co-efficient of variation. But the same group have highlighted the disadvantage of calculating a CV when the test does not show proportional relationship between the standard deviation and the mean as the response decreases, as is the case for the E/I ratio (and other ratios of RR intervals). They advocate the use of the adapted CV, which is derived by subtracting 1 from the actual values for RR interval ratios – this results in a proportional relationship between the mean and standard deviation as the response diminishes. There is a strong relationship between the adapted CV and the SD ratio i.e. the within subject SD divided by the population SD (normal subjects r = +0.63, diabetic subjects r = +0.91) (Murray and Lawrence, 1993).

Reproducibility for the E-I difference, measured by RRmax – RRmin, was considered moderate at 40% (Gerritsen, 2003). Smith et al's study used a different technique - the Fishers intrapair correlation co-efficient. Reproducibility of E-I difference for deep breathing, repeated at intervals of 3 - 8 months in 20 healthy subjects, was highly significant: r = +0.68, $p<0.01$ (Smith and Smith, 1981).

3.1.3.3 Age

There is a significant decline in RR variation during metronomic respiration with increasing age (Clark and Mapstone, 1986; Ewing, 1985; Lawrence, 1992b; Masaoka, 1985; Smith and Smith, 1981; Vita, 1986; Wieling, 1982; Ziegler, 1992b). Multiple regression analysis indicated age contributed around 30 % of the variation in heart rate variability (O'Brien, 1986). Low supplies a univariate regression model documenting the significant decline in E/I ratio y (beats per minute) for each year of age x (Low, 1990)

$$y = 37.17 - 0.36x \qquad R = 0.7 \qquad p < 0.001$$

Smith and Smith (Smith and Smith, 1981) performed multiple regression on logarithmic-transformed metronomic respiration max-min heart rate differences (ln y), to obtain the following best fit relationship

$$ln\ y = 4.764 - (0.01918 \times age\ in\ years) + (0.0173 \times mean\ RR\ interval)$$

These two variables accounted for 47 % of ln *y* variance.

3.1.3.4 Other influences

Two studies show no gender effect on RR variation in metronomic respiration (Low, 1990; Smith and Smith, 1981; Ziegler, 1992b) but one study has shown a gender effect in the E-I difference (maximum RR – minimum RR) with a male and female medians of 207 and 163 ms respectively (Gerritsen, 2003). There does not appear to be any diurnal variation (Bennett, 1978; Wieling, 1982), although RR variation is more reproducible in the morning: coefficient of variation was 11-12% in the morning but 17-21% in the afternoon for normal and diabetic adults (Bennett, 1978).

One study has demonstrated moking history does not affect metronomic respiration max-min heart rate difference but variation in the max-min heart rate difference significantly increases with decreasing resting heart rate (Smith and Smith, 1981). Masouka observed duration of diabetes significantly decreased max-min HR variation in insulin-treated but not non-insulin dependent diabetic patients. This may have been related to the shorter duration of diabetes in the NIDDM cohort (Masaoka, 1985).

3.1.3.5 Test methodology

Heart rate variation during metronomic respiration is usually assessed by instructing the subject to take six deep breaths, one every ten seconds, for one minute. Inspiratory and expiratory phases are five seconds each. This protocol is quoted in the Ewing battery (Ewing and Clarke, 1982) and has been widely used elsewhere (Clark and Mapstone, 1986; Netten, 1992; Staessen, 2001; Ten Harkel, 1990; Vita, 1986; Wieling, 1982; Ziegler, 1992b). Mackay et al demonstrated maximal heart rate variation during metronomic respiration occured at a respiratory frequency of 6.3 ± 0.6 breaths per minute in adults aged 20-29 years and 5.5 ± 0.3 for age range 30-43 years. The resonant frequency (i.e. respiratory frequency producing the largest variation in RR interval variation) was significantly lower for diabetic patients with autonomic neuropathy. There was a two to three-fold increase in heart rate variation as breathing frequency decreased to the resonant frequency. The relationship was linear after logarithmic transformation of respiratory frequency. At very low breathing rates slower than the resonant frequency, heart rate variation decreased in a non-linear fashion. Diabetic patients with autonomic neuropathy had a clinically small but significant reduction in resonant frequency (Mackay, 1983). These observations are similar to the work of Angelone et al (Angelone and Coulter, 1964).

Sundkvist et al (Sundkvist, 1979) were the first to modify the variation in heart rate to the E I ratio, where E/I = mean value for longest RR intervals during expiration / mean value for shortest RR intervals during inspiration.

Variations on respiratory cycle number and frequency include:

1) A *solitary* deep breath (O'Brien, 1986; Smith, 1982). Bennett and colleagues (Bennett, 1978) reported that there was no difference in heart rate variation for a single deep breath and six breaths over a minute (40.2 vs. 43.8 bpm) for normal adults but for diabetic patients the single deep breath evoked a significantly larger change in heart rate than six repeated deep breaths (20.1 vs. 13.5, $p < 0.001$).

2) Smith reported an age-related E/I ratio normal range for a solitary deep breath, which outperformed a mean E/I ratio because (a) it is not affected by resting heart rate and (b) in practice reduced the false-positive and false-negative results (Smith, 1982).

3) *Six* deep breaths per minute on two occasions; the higher of the two max-min HR difference averages was used for analysis (Masaoka, 1985).

4) *Five* breaths per minute for *six minutes*, using the last five minutes for analysis (Rothschild, 1987).

5) *Six* respiratory cycles per minute for about *two* minutes with the mean heart rate variation of 10 cycles (Mackay, 1980)

6) The mean max-min heart rate difference from *five* cycles performed at *six* cycles per minute (Smith and Smith, 1981)

7) *Eight* cycles, the mean of the five largest responses used for analysis (Low, 1990)

8) *Six* respiration cycles over *one minute* with the average of breathing cycles two to four used for analysis. This study also demonstrated that reproducibility improves in the sitting position compared with the supine position, probably because of more variable respiratory behaviour when supine (Lawrence, 1992b).

Lawrence et al have shown that within-subject variability (and within/between subject standard deviation) improves using multiple respiratory cycles than one solitary breath. Mustonen et al (Mustonen, 1989) have reported no significant difference in the E/I ratio when comparing the means of the 1st to 3rd and 1st to 6th respiratory cycles in both controls and diabetic subjects, which may be useful in subjects who are unable to complete the normal protocol.

There is no influence of preceding rest duration upon RR variation (Ten Harkel, 1990). RR variation is less apparent during standing and improves in the supine position (Bennett, 1977)

but others found a non-significant reduction in RR variation in the sitting and standing position compared with the supine position (Ten Harkel, 1990). Ewing's observations favoured sitting or standing position for optimising performance of the max-min heart rate but without any clinically meaningful differences between results in the three positions (Ewing, 1981).

Netten et al devised a software program for autonomic analysis, and found no difference in results between testing with an computerised program or using a conventional ECG (Netten, 1992).

3.1.3.6 Relationship with other cardiovascular autonomic tests

The correlations between E-I difference and orthostatic changes in heart rate (r = +0.17) or RRmax/RRmin (r = +0.14) are poor (Wieling, 1982). This emphasises the differing physiological mechanisms that determine each response. Ewing et al examined correlation between reflex cardiovascular autonomic tests in 61 diabetic patients, with normal and abnormal autonomic function. Max-min heart rate difference gave the following correlation coefficients in the lying position: Valsalva ratio r = +0.41 ($p<0.001$), 30:15 ratio r = +0.51 ($p<0.001$), isometric handgrip change in DBP r = + 0.36 ($p<0.01$), postural blood pressure fall in SBP r = -0.32 ($p<0.01$) (Ewing, 1981). RR variation in deep breathing is more sensitive than the Valsalva ratio in detecting diabetic autonomic neuropathy (Dyrberg, 1981; Rothschild, 1987). It is also superior to the 30:15 ratio (Mackay, 1980). However heart rate variation from a solitary deep breath tends to be less than that seen during standing or the Valsalva manoeuvre (O'Brien, 1986). Amongst the autonomic tests of heart rate variation, the E-I difference or E/I ratio appears to have the best reproducibility (Smith and Smith, 1981; Ziegler, 1992b).

The E-I difference of maximum- minimum RR intervals agreed moderately well with spectral analysis low and high frequency powers, baroreflex sensitivity (correlation co-efficients +0.49 to +0.52) and standard deviation of normal RR intervals (SD NN, percentage concordance 63%) (Gerritsen, 2003). Bennett and colleagues reported the correlation between the post-Valsalva manoeuvre bradycardia and heart rate variation (single breath) was significant in diabetics but not significant in normal adults (Bennett, 1978).

3.1.3.7 Clinical application

The relationship between respiration and vagal activity is used as a tool to investigate the integrity of parasympathetic function. The deficit in RR variation in diabetics is due to decreased parasympathetic activity and not increase in sympathetic activity (Rothschild, 1987). Dyrberg et al in a comparison of detection of diabetic cardiac neuropathy with tests of

RR variation, found metronomic respiration to be the most sensitive test compared with the Valsalva ratio, 30:15 ratio and heart rate response to exercise (Dyrberg, 1981).

In a study of 261 diabetic patients aged 11-76 years, the rates of abnormal heart rate variation during metronomic respiration were as follows:

Table 3-2 Peripheral neuropathy and autonomic neuropathy in diabetes

Stage of peripheral neuropathy in Type I and II diabetic patients	Abnormal E-I difference (%)	Abnormal E/I ratio (%)
0 none	9.6	8.7
1 subclinical	25.0	26.7
2a symptomatic, no autonomic symptoms	47.2	52.8
2b symptomatic, + autonomic symptoms	100	100

D Ziegler 1992 (Ziegler, 1992a) (%) = percentage of abnormal cases in each stage

Similar high sensitivity was shown in a study of 287 diabetics aged 20-49 years. E-I difference was abnormal in 62 out of 64 cases with the following autonomic symptoms: postural hypotension, diarrhoea, gustatory sweating, upper gastrointestinal atony, bladder atony, unexplained cardiorespiratory arrest. Of the 62 cases, 54 were clearly abnormal (>2 SD of control values) and 8 were borderline abnormal (between 1.5 and 2 SD control values). Serial tests less than 12 months apart were consistently abnormal in 97% cases. Thirty percent of subjects with peripheral neuropathy had abnormal E-I difference (Mackay, 1980). Earlier Sundkvist et al demonstrated the value of E/I ratio in detection of autonomic neuropathy, one of the first papers to use the ratio as opposed to the heart rate difference. The paper could be criticised for making the assumption that those with sensory neuropathy had autonomic neuropathy without using a proven diagnostic test for the latter. Ten out of 18 diabetics with sensory neuropathy and nine of 11 patients with absent ankle reflexes had E/I ratios less than 1.10, in comparison to only two of 25 controls (Sundkvist, 1979).

Diabetic patients with symptoms of autonomic neuropathy have reduced RR variation during deep breathing compared to normal controls but similar (less exaggerated) changes occur in asymptomatic diabetics (Dyrberg, 1981; Mackay, 1980; Pfeifer, 1982; Rothschild, 1987). Ewing et al have also shown a progressive deterioration in max-min heart rate difference in diabetic groups without autonomic neuropathy (19.0 bpm, standard error of the mean (SEM) 1.7), to isolated parasympathetic neuropathy (13.5 bpm, SEM 2.1) to combined sympathetic and parasympathetic neuropathy (11.0 bpm, SEM 1.6, group difference $p<0.01$) (Ewing, 1981). A similar pattern across diabetic subgroups was shown by Smith (Smith, 1982) but on this occasion using a single deep breath. Sundkvist reported the E/I ratio outperformed the Valsalva ratio in detection of detection of autonomic neuropathy in diabetic patients.

Abnormal E/I ratio (<1.09) occurred in 77 % of diabetics with symptomatic autonomic neuropathy (based on questionnaire) but in the same group, only 40 % had an abnormal Valsalva ratio (<1.18). The same tests were abnormal in 54 % and 29 % of diabetics with peripheral neuropathy only. These results were somewhat surprising since subgroup mean values showed incremental decrease from control to peripheral neuropathy to autonomic neuropathy for both E/I ratio and Valsalva ratio (Sundkvist, 1982).

The heart rate response to metronomic respiration was recommended as a measure of cardiovagal function by the American Academy of Neurology, with satisfactory specificity, sensitivity and reproducibility (AAN, 1996a).

Impaired parasympathetic function may have an impact on circadian blood pressure control. Parasympathetic failure preceded sympathetic failure in a group of patients with familial amyloid polyneuropathy. In those with combined failure, diurnal blood pressure variation was absent by virtue of diminished daytime blood pressure levels: however in those with isolated cardiac parasympathetic damage, there was an intermediate degree of diminished diurnal variation (Carvalho, 2000). Mechanisms may include disruption of cardiac baroreceptor afferents.

3.1.3.8 Conclusion

Respiration modulates heart rate via baroreceptors and mechanoreceptors sending afferent traffic to the brainstem and via direct central neural connections between respiratory and cardiac centres. Efferent signals reach the heart via the vagus nerve. Metronomic respiration can provide a measure of cardiovagal function. Heart rate variation during respiration is quantified using the difference between or ratio of maximum and minimum heart rates. The heart rate difference may be advantageous in terms of ability to differentiate patient groups and independence of resting heart rate. Depth and rate of respiration need to be controlled. Age and position of the subject also influence heart rate variation. Repeatability is generally moderate but will deteriorate in poorly controlled conditions. Heart rate variation during metronomic respiration is a useful test in identifying and quantifying cardiovascular autonomic neuropathy.

3.1.4 Isometric exercise

Isometric exercise tests cardiovascular sympathetic function. The individual performs isometric activity, such as handgrip, for a short period (usually three minutes). The change in blood pressure from baseline (before activity) to the end of activity is measured. Blood pressure increases in healthy individuals.

3.1.4.1 Neural mechanism

Donald and colleagues (Donald, 1967) published a review of literature describing the physiology of isometric exercise. Much research had been carried out on dynamic exercise but they commented on the importance of static, or isometric exercise, in older humans. Elderly adults may have curtailed dynamic exercise through general frailty but continually perform isometric exercise throughout the lifespan on a daily basis. The haemodynamic response to static exercise was first investigated by Lindhard in 1920 (Lindhard, 1920) who tested subjects hanging by their hands, with their arms bent, from an overhead beam. He found that oxygen uptake increased after exercise and hypothesised that during isometric exercise the blood flow through muscles was occluded by the mechanical compression of the tensed muscle fibres. In the 1930's experiments by Asmussen and Hansen (Asmussen and Hansen, 1938) found that cardiac output, heart rate and blood pressure all substantially increased during isometric exercise but after cessation of strain, blood pressure and heart rate declined rapidly whilst cardiac output and oxygen uptake underwent further rises before a gradual return to baseline. A series of experiments throughout the 1950s and 1960s concentrated on the observation of Gaskell in 1877 (Gaskell, 1877) that blood supply to a contracting muscle is a compromise between two forces. Firstly, the increase in blood flow to exercising muscle that triggers dilatation of local vessels. Secondly, the occlusive force of contracting muscle mechanically compressing the same local vessels.

Blood flow to isometrically contracting muscle may actually be cut off at sub-maximal muscle capacity, probably by a nipping effect between shortening and stationary fibres. Early experiments suggested the effort provoking this effect may be as little as 20% of maximal voluntary contraction (MVC) but later reports indicate the figure is nearer 70% for the forearm. Muscle tissue benefits from increased blood flow at around 33% MVC.

The increase in flow is directed exclusively at active muscle. Inactive muscle does not attract higher flow rates, nor do muscles in other resting limbs. Therefore Donald hypothesised that the increase in blood pressure during isometric exercise infers vasoconstriction in inactive limbs (Donald, 1967). Even relatively small muscle masses experiencing isometric exercise can provoke marked increases in blood flow and it appears the fatigue encountered is due to insufficient blood flow to contracting muscle. The increase in blood pressure therefore has the physiological benefit of maintaining blood flow in active (contracting) muscle by overcoming resistance in local vascular beds.

Lind et al reported the rise in blood pressure was due to increased cardiac output generated by an increase in heart rate (Lind, 1964). Unexpectedly Lind found no substantial change in peripheral vascular resistance with isometric exercise. Stroke volume did not change at 10-20% MVC but was consistently reduced at 50% MVC, and the latter was associated with a

pronounced tachycardia. Subsequently it was found that in older subjects who were less able to mount an increase in heart rate, the rise in blood pressure was similar to younger adults but generated by *either* increase in stroke volume or peripheral vascular resistance. At low muscle contraction forces, blood influx to isometrically contracting muscle is sufficient for metabolic requirements. Beyond approximately 15% MVC, demand will outstrip supply leading to the sensation of fatigue during prolonged contraction.

The pressor response generated by muscle mass is largely dictated by the MVC specific for that muscle group, i.e. the same response is obtained by 30% MVC for either forearm or leg contractions (Freyschuss, 1970). Pressor response will still occur during isometric exercise even if performed simultaneously with dynamic exercise, despite substantial cardiovascular commitment to dynamic requirements. Pain is a common feature of isometric exercise. However this develops after the pressor response is initiated and the rate of blood pressure rise is not greatly influenced by the severity of discomfort. Therefore pain is not the primary stimulus for increment in blood pressure (Donald, 1967).

In his 1967 review, Donald (Donald, 1967) identified the three potential stimuli for the cardiovascular reflexes observed during isometric exercise: humoral (metabolic substances in the systemic circulation), central autonomic drive or neural reflex. At that point, experimental evidence was lacking but early indications were against humoral factors. The debate continued in the 1970s and 80s.

Freyschuss (Freyschuss, 1970) conducted a series of extensive investigations on isometric exercise in a small number of adult subjects. Heart rate and blood pressure response occurred rapidly at around 10-15 seconds after start of contraction (and were independent of respiratory events and right atrial pressures), suggestive of a neurally mediated mechanism. Both heart rate and blood pressure increase in isometric exercise, in contrast to the normal inverse relationship between heart rate and blood pressure (Figure 3.9). Such dissociation between the usual counter-regulatory forces suggested separate control centres and pathways taking part in the response. Overall normal baroreflex function appeared to be inhibited. Beta-adrenergic blockade did not affect these observed reactions but atropine diminished cardioacceleration, indicating a contribution from vagal withdrawal towards the tachycardia. Phentolamine, a moderate adrenergic blocking drug which has a greater action on circulating adrenergic mediators and lesser action on sympathetic nerve impulses, only resulted in a modest attenuation of the normal blood pressure rise when given alone. This indicates peripheral sympathetic activity contributes to the pressor response. Following blinded neuromuscular blockade to prevent volitional muscle contraction, increases in heart rate of 47-76 % (average 64 %) control values and blood pressure at 37-75 % (average 55 %) control values were still obtained. Therefore central command was suggested to play a role in the normal responses.

An increase in venomotor tone was also observed, and could contribute to the increase in perfusion pressure by cardioacceleration and rise in systemic vascular resistance. Magnitude of pressor response was not correlated to MVC.

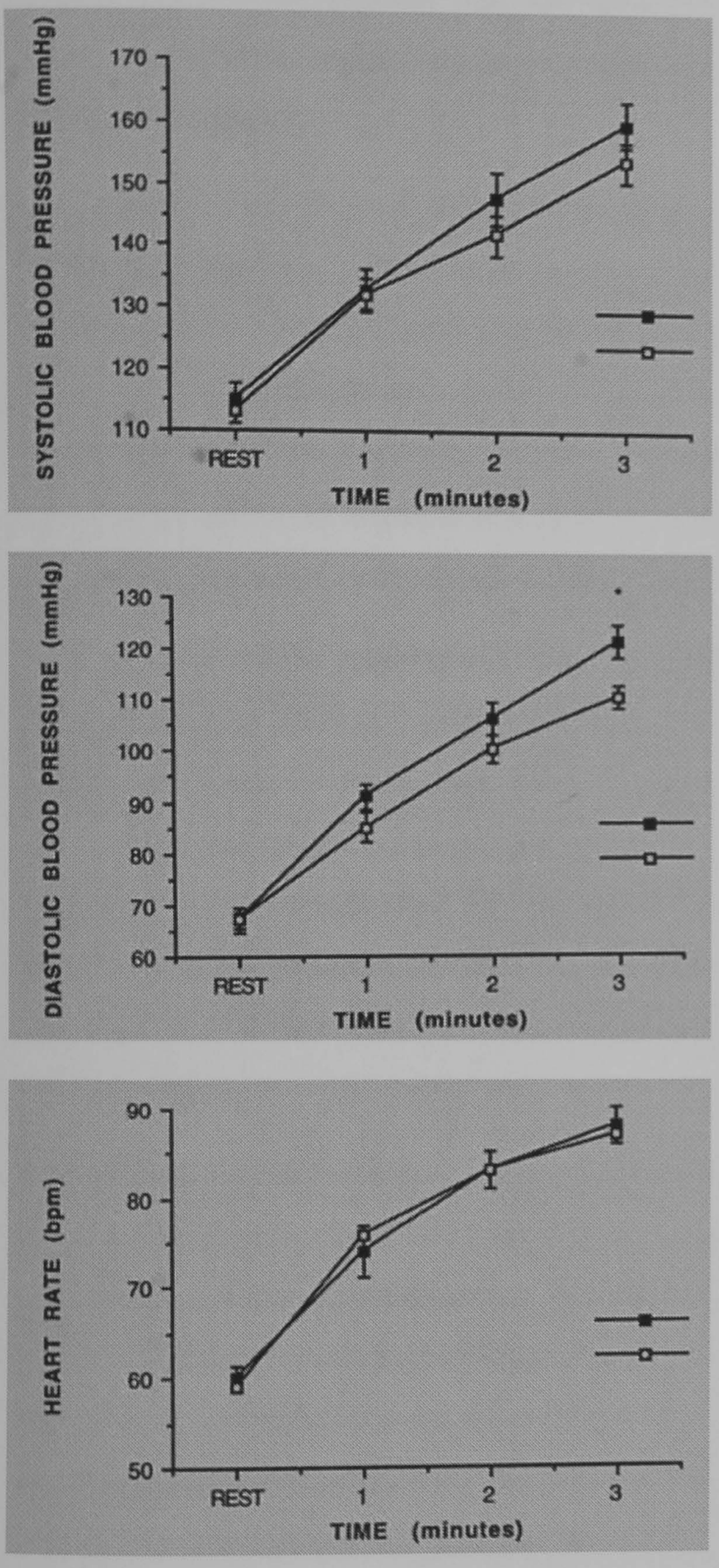

Figure 3-9 Isometric exercise for three minutes: systolic, diastolic and heart rate response

Animal models were used by Coote et al (Coote, 1971) with nerve root stimulation of animal hind-limb muscles in four situations: different stimulation intensity, with gallamine to abolish muscle contraction, following limb afferent nerve section and finally under ischaemic conditions by vascular occlusion. Increasing stimulus intensity led to increasing blood

pressure rise, with a lag of 2-3 seconds. Abolition of muscle contraction with gallamine resulted in no pressor response despite stimulation, indicating the pressor response is a neural reflex initiated in the contracting limb. Non-muscle (articular nerves, vagus) afferent nerve section did not affect the pressor response, indicating the reflex originates in the contracting muscle. The pressor response was potentiated by occlusion of the arterial supply to the contracting muscle, suggesting the response is in some way influenced or generated by metabolic receptors.

In an extension of this work using animal models, arterial and venous occlusion of exercising muscle prolonged the pressor response beyond the period of exercise, providing further support for some form of ischaemic metabolite stimulating the pressor response from within exercising muscle. Complete section of dorsal roots receiving muscle afferents abolished the pressor and heart rate response. Selective nerve blocking experiments provided evidence that the isometric exercise response is mediated by fibres within groups III and IV (i.e. small myelinated fibres and unmyelinated fibres) (McCloskey and Mitchell, 1972).

In contrast, Goodwin (Goodwin, 1972) supported the role of central command from the results on human studies. Vibration stimulus to muscle afferents was used to provide reflex stimulation of muscle contraction at lower levels of central command. Muscle contraction force (controlled with tension gauges visible to the subject) was kept constant before and after addition of vibration stimulus. They observed smaller pressor response and heart rate increases with the addition of vibration stimulus at a set contraction force. In other words, lower levels of central command were associated with reduced blood pressure and heart rate increases during isometric exercise.

Hultman and Sjoholm (Hultman and Sjoholm, 1982) observed cardiovascular response to leg isometric exercise in six young male adults, firstly under 20% maximum voluntary contraction and then non-voluntarily using percutaneous electrical stimulation (such that central command was apparently not involved). The cardiovascular responses were not significantly different between experiments: heart rate increased by 40% and systolic and diastolic blood pressure by 30% and 50% on both occasions. They conclude that muscular activity alone is sufficient to generate the cardiovascular response to isometric exercise, whilst adding that central command may not be without importance.

In a comparison of 61 normal and 126 age matched diabetic subjects, the systolic blood pressure increase was similar but the mean diastolic increase was significantly less in the diabetic group. There was a significant correlation between the MVC and rise in blood pressure both in the normal and diabetic subjects (Ewing, 1974).

In a complex series of experiments, Eldridge et al (Eldridge, 1985) used animal models in various conditions of anaesthesia, decortication and decerebrate conditions, with electrical or pharmacological movement stimulation and under spontaneous actual motion or fictive locomotion (locomotor activity in the motor nerves during paralysis). Among the findings, the pressor response to exercise was no different during fictive locomotion despite the absence of muscular contraction or limb movement and the lack of change in metabolic rate. This provided some support for central command driving the cardiovascular response to exercise , and the hypothalamus appeared to be the signal source.

Activation of chemically sensitive muscle afferents during isometric exercise stimulates muscle sympathetic nerve activity (MSNA) but this effect is only significant during the second minute of exercise. MSNA is peripheral nerve traffic producing vasoconstriction - for a review of this topic see Wallin 1988* (Wallin and Fagius, 1988). Additionally, MSNA further increases during post-handgrip muscle ischaemic whereas heart rate returns to normal. During isolated biceps *in*voluntary muscle contraction, MSNA does increase but heart rate does not. In contrast biceps voluntary muscle contraction does increase heart rate but, in the early phase prior to established muscle ischaemia, does not increase MSNA (and in fact MNSA actually drops in the first minute of handgrip). This series of observations indicates that central command causes tachycardia but does not increase, and instead appears to inhibit MSNA. Secondly, that chemically sensitive muscle afferents are responsible for the rise in MSNA. In sustained handgrip at 30% MVC, the excitatory influence on MSNA prevails in the mid to latter stages and significantly increases. The dissociation of heart rate and blood pressure trends during these experiments underlines the likelihood of the two responses being governed by different mechanisms (Mark, 1985). Figure 3.10 depicts the changes in blood pressure, heart rate and MSNA during isometric exercise.

These findings have been replicated with further evidence of the differential effects of the muscle chemoreflexes and central command on sympathetic and parasympathetic response (Victor, 1987). Mild non-ischaemic exercise stimulates withdrawal of vagal tone and the resultant rise in heart rate increases blood pressure: central command is responsible for this reflex. The arterial pressure increases in proportion to exercise intensity and rise in heart rate. Sustained strenuous or ischaemic exercise stimulates muscle chemoreflexes but this response is relatively delayed until the ischaemic exercise has created the metabolites that excite the afferents. Therefore there is a lag before the metabolite-induced increase in MSNA that augments blood pressure increase already created by heart rate changes. The sympathetic outflow will be directed at non-exercising skeletal muscle and the heart (Victor, 1987).

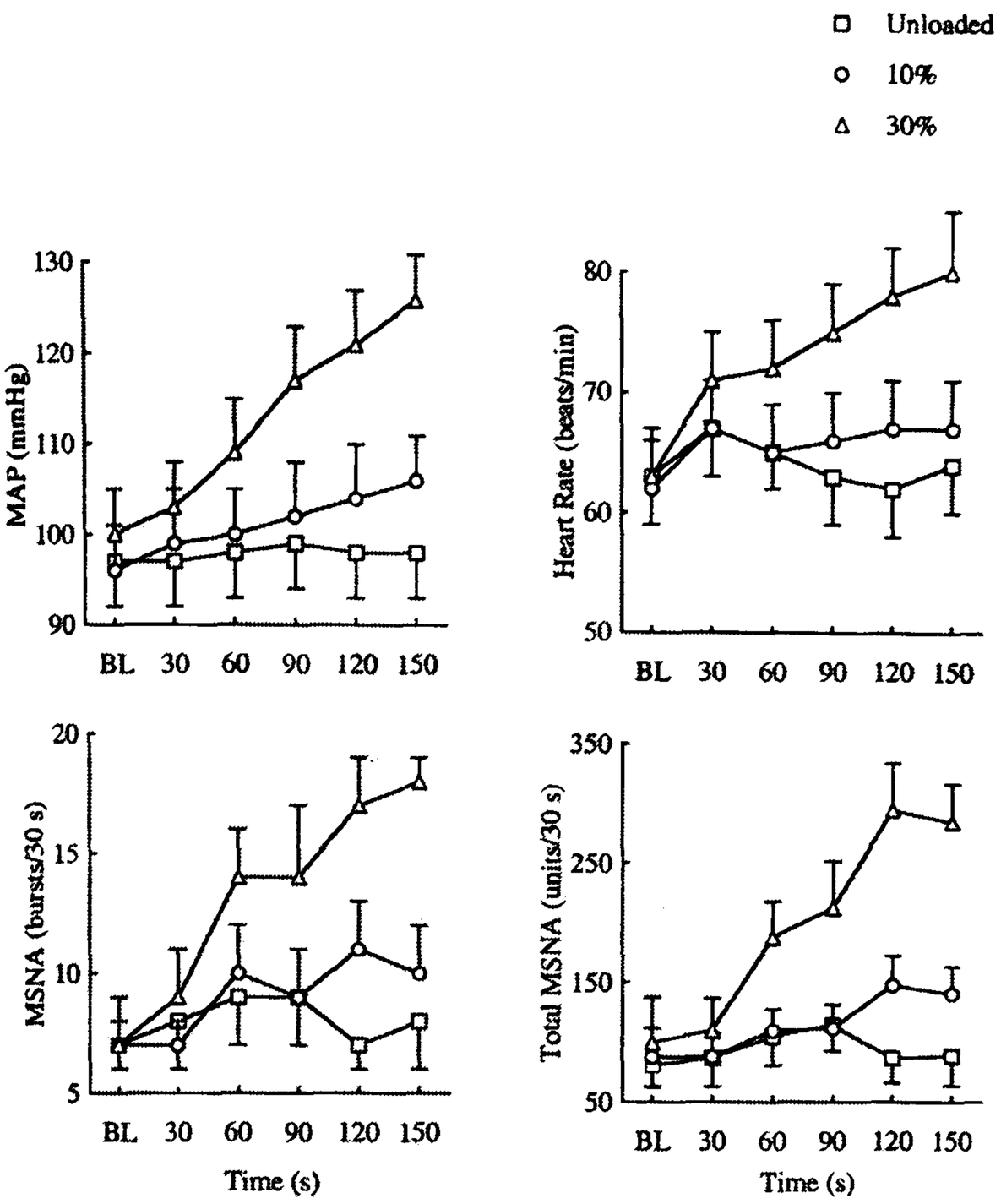

Figure 3-10 isometric exercise: blood pressure, heart rate and MSNA changes.

Heart rate increases rapidly early during exercise then blood pressure steadily increases, mirrored by increasing MSNA. The three dataplots are for three different levels of isometric exercise: baseline activity (squares), 10% mean voluntary contraction (circles) and 30 mean voluntary contraction (triangles)

MAP, mean arterial pressure: MSNA, muscle sympathetic nerve activity: BL, baseline

Victor et al (Wallin, 1987) examined the relationship between MSNA and venous noradrenaline levels during isometric exercise in 16 normotensive and 15 untreated hypertensive patients. There was a small but non-significant elevation of MSNA in hypertensive patients compared with normotensives when expressed as MSNA bursts per minute but no difference when expressed as the product of bursts per minute and burst amplitude. Both groups showed minor elevations of MSNA in the first minute then similar and significant increases in the second minute. Hypertensive patients had a significant increase in noradrenaline after 2 minutes exercise which was significant but without subsequent change. Normotensive patients only showed increase in noradrenaline in recovery period but this was not significant. Individual changes in MSNA were correlated to changes in plasma noradrenaline during the first minute ($r = +0.61$, $p<0.05$) and second minute of handgrip ($r = +0.44$, $p<0.05$) despite the difference in the shape of the curve, with noradrenaline lagging MSNA. The increase in noradrenaline was relatively small but it's likely the plasma level may underestimate true noradrenaline response due to pharmacokinetic properties.

Other workers found MSNA response proportional to the level of muscle contraction force. Seals et al (Seals, 1988) examined heart rate, mean arterial pressure and MSNA responses to

isometric handgrip in 8 young adults at 15%, 25% and 35% MVC over 2.5 minutes. Pressor response was proportional to exercise intensity. The non-fatiguing 15% MVC produced a small but significant increase in pressure and heart rate but no increase in MSNA. They conclude at such light loads, the arterial pressure response is mainly due to vagal withdrawal and hypothesise that muscle perfusion in exercising muscle is adequate such that substantial rise in pressure from sympatho-excitation is not necessary. At 25% and 35% MVC, the first response was again increase in heart rate in proportion to intensity of exercise but reaching maximal level after 1.5 minutes without further rise in the final minute. The second contribution to arterial pressure rise came from increase in MSNA level which did not change significantly until the first minute of exercise but the continued to increase markedly in the final 1.5 minutes in an exercise-intensity dependent fashion. Thus at fatiguing exercise levels, the pressor response is generated by first by cardioacceleration from vagal withdrawal and then later by vasoconstriction from peripheral sympathetic traffic. In the final minute, increase in arterial pressure was very closely correlated with MSNA ($r = +0.99$, $p<0.01$).

This concept was refined by Seals et al (Seals, 1993) by measuring the response until the maximum of endurance time. Handgrip contraction at 20%, 40% and 60% maximum voluntary contraction force was sustained until exhaustion, which occurred at 495, 140 and 73 seconds respectively, with 20 to 25 minutes between tests. Perceived effort was similar in the 12 healthy young adults assessed. When measured as a percentage of maximum endurance time, systolic pressure was no different at the three contraction intensities. Diastolic pressure was lower only at the latter phase of 20% contraction force when compared with 40 % and 60 % contraction force experiments ($P<0.05$). The rate of cardioacceleration rose in proportion to contraction force but then after 20% of maximum endurance time the rate of heart rate increase was similar for the three contraction intensities. Final peak heart rates were significantly different and related to contraction force. MSNA were similar at 40 % and 60 % contraction force throughout endurance time. The rate of MSNA rise and final peak level obtained were lower at 20% contraction force ($p<0.05$). In summary, findings indicated that at sub-maximal isometric contraction, MSNA is influenced by contraction force at low forces. But contractions *above* 20% MVC stimulate peak levels of sympathetic outflow.

Ewing and Borst reported strong voluntary muscle contraction causes cardiac acceleration very quickly after onset of isometric exercise - within 0.4 to 0.6 seconds. They also reported that firstly cardioacceleration is due to sudden vagal inhibition, secondly is largely independent of heart rate and contracting muscle mass and thirdly is only mildly influenced by the strength of muscle contraction. There is a large degree of cardioacceleration variation between subjects (Borst, 1972; Ewing, 1974). Smith et al (Smith, 1976) in a review of the integrated mechanisms of cardiovascular response during exercise, summarised the origins of

tachycardia during exercise. They hypothesised on additional factors contributing to the tachycardia in a time-dependent manner. The increase in heart rate starts very promptly within a few seconds and this initial response is due to vagal withdrawal. After 8-10 seconds sympathetic impulses to the heart can contribute to tachycardia. Continued exercise may increase circulating catecholamines which act as a final contributor to cardioacceleration, probably at least 30 seconds after exercise initiation.

A recent study has shown that during isometric exercise, there is a significant increase in the (absolute and normalised) low frequency component of HRV as well as a significant decrease in the (normalised) high frequency component, suggesting a combined sympathetic and parasympathetic mechanism to the cardio-acceleration. The authors appear to make an assumption that the sympathetic component is 'muscle metabaloreflex' driven, since this reflex stimulus was continued post-exercise by occluding circulation to the exercising limb and the low frequency component remained elevated in this phase, only returning to baseline levels when the occlusion was removed. In contrast, the high frequency component returned to normal immediately post-exercise (and during circulatory occlusion) when heart rate also recovered to baseline levels – this infers an arterial baroreflex response mediated by vagal efferents which has a greater net effect on heart rate than sympathetic activity (Iellamo, 1999).

Diastolic arterial pressure correlates more closely with MSNA than systolic pressure (Sundlof and Wallin, 1978). Watson et al (Watson, 1980) observed a significant correlation between diastolic pressure and plasma norepinephrine ($r = +0.62$, $p<0.05$) but not for systolic pressure during exercise ($r = +0.28$, ns). At rest sympathetic traffic is very low, hence capacity to reduce sympathetic activity in response to rising pressure is much less than ability to increase sympathetic activity when arterial pressure falls (Eckberg, 1988).

*MSNA occurs in bursts, normally increases when blood pressure drops and in normal conditions is almost absent when blood pressure rises. MSNA usually occurs homogeneously without significant regional differences within individuals but activity differs markedly at rest between individuals. MSNA is mostly determined by diastolic pressure and often features a respiratory periodicity. MSNA is mostly involved in the instantaneous stabilization of blood pressure and not the control of mean resting blood pressure (Wallin and Fagius, 1988).

3.1.4.2 Age

Following linear regression, there was a significant relationship between age and isometric exercise diastolic blood pressure response, but this was a positive correlation suggesting increasing diastolic blood pressure with age (Freeman, 1991). Another study tested 124 healthy normal subjects aged 20-90 years and found that age did not influence the diastolic

response to handgrip (Mustonen, 1989). The lack of a relationship between age and the diastolic blood pressure response to handgrip has been shown in other studies using correlation techniques (Vita, 1986; Ziegler, 1992b).

Another study confirmed that age does not influence the blood pressure response during handgrip (note this investigation used the mean arterial blood pressure). The underlying absolute increase in MSNA was the same for older and younger subjects but since there was a higher basal rate of MSNA, the percentage increase in MSNA was significantly smaller in older subjects. The increase in heart rate was significantly smaller in older subjects from an early stage during handgrip. Plasma noradrenaline levels increased by a similar percentage value in young and old but the absolute basal and peak values were higher in the older group (Ng, 1994).

3.1.4.3 Other influences

This is one test of autonomic function where there is a clear sex difference. Smaller diastolic blood pressure response occurs in females (Ewing, 1974; Mustonen, 1989; Ng, 1994; Vita, 1986). This is related to typically lower force of MVC in females which leads to a smaller blood pressure rise than males (Ewing, 1974).

3.1.4.4 Repeatability

There is substantial retest within-subject variation in blood pressure results for isometric exercise in healthy individuals over 1 year, calling into question its value as a means to monitor the course of autonomic dysfunction. The heart rate response is more consistent (Braune, 1996).

From a study of same day reproducibility, the coefficient of variation was 18.6 % in 21 subjects tested using the Finapres and 36.9 % in a smaller group of 8 tested using manual sphygmomanometry (Mustonen, 1989). Parati et al also indicate that reproducibility is poor. In a group of adults aged 20-61 years, handgrip was performed on six occasions at intervals of 30 minutes with intra-arterial pressure measurement. The group average mean arterial pressure coefficient of variation was 22.2%, individual range 12.8 to 32.3% (heart rate CV 24.6%, individual range 11.9% to 49.1%) (Parati, 1985).

Another study gave equivocal results. Firstly they quote a range of reproducibility in 5 normal cases, from mean diastolic increase and SD of 37 ± 4 to 25 ± 10 mm Hg respectively, which indicates poor reproducibility. In contrast, in 25 normal and diabetic cases, the square of the deviation of the difference in diastolic blood pressure rise between subjects was approximately seven times that within subjects ($F = 7.39$, $p < 0.01$), indicating good

reproducibility (Ewing, 1974). In his review however, Low states the blood pressure change during isometric exercise is of limited sensitivity and specificity (Low, 1993a).

There was no significant difference between control and diabetic cases, with standard deviation factors of intra-individual variation of 1.46 and 1.37 respectively (Ziegler, 1992b).

3.1.4.5 Test methodology

The following methods for isometric exercise, using handgrip for exertion, have been used in studies. Diastolic blood pressure response is the preferred outcome except where indicated.

1) 50% of maximum voluntary contraction for 2 minutes (Braune, 1996)

2) 40% MVC sustained until exhaustion, mean arterial pressure quoted (Ng, 1994)

3) 40% MVC for 90 seconds (Parati, 1989; Parati, 1985) with average blood pressure measured in the last 10 seconds of the test and 10 seconds immediately preceding the test.

4) 30% MVC for as long as possible up to a maximum of 5 minutes (with BP measured using manual sphygmomanometry) (Ewing, 1974; Ziegler, 1992b). Interestingly the maximum blood pressure was similar no matter how long subjects maintained MVC, with times ranging from 2 to 5 minutes. Blood pressure was measured 3 to 4 times per minute during testing, the change in blood pressure taken as the difference between the last reading and the means of 3 baseline readings.

5) 30% MVC for 4 minutes, assessing the diastolic blood pressure increase (Freeman, 1991).

6) 30% MVC for 3 minutes, suggested in a review (Low, 1993a) and used in some studies (Mustonen, 1989; Netten, 1992).

7) 30 % MVC for 5 minutes, or at least 3 minutes (Vita, 1986).

8) When manual sphygmomanometry is used, blood pressure is usually recorded at minute intervals, and one group suggested to only record at the third minute since the response is significantly higher at this stage compared to the first and second minutes (Mustonen, 1989).

In a comparison of young and old subjects, mean ages 25 and 64 years respectively, endurance times for 40% MVC and perceived effort as well as post-exercise perceived pain were no different between the two groups (Ng, 1994). In other words the test was equally well-tolerated in both these age groups.

Two groups report on Finapres performance during isometric exercise testing. Thirteen cases had simultaneous intra-arterial (radial) and Finapres blood pressure monitoring during 90 second handgrip: this study indicated the Finapres provides a reasonably accurate estimate of the quantitative change in blood pressure during isometric exercise. The average discrepancy in systolic and diastolic pressure change was respectively 4.6 ± 4.1 and 2.1 ± 1.2 mmHg. Finapres always reproduced the qualitative changes resulting from handgrip (Parati, 1989). Ziegler et al found the Finapres offers clear benefits compared with results obtained using sphygmomanometry; the latter was inconsistent because of difficulties in obtaining the final blood pressure during exercise (Ziegler, 1992b).

The interaction between handgrip isometric exercise and Valsalva was examined by Ewing and colleagues (Ewing, 1976b) by performing concurrent tests in a small group. Essentially neither test had an over-riding influence on the other. But if a Valsalva manoeuvre took place towards the end of exercise, blood pressure response to exercise was exaggerated and heart rate was moderately reduced, therefore it is advised to avoid a Valsalva manoeuvre during isometric exercise testing.

3.1.4.6 Relationship with other cardiovascular autonomic tests

In the Ewing battery, there is a weak but significant correlation with heart rate response to deep breathing only, with no significant correlation with the Valsalva ratio, 30:15 ratio or blood pressure response to standing (Vita, 1986). The change in low frequency power spectral analysis on moving from supine to an upright position is a poor predictor of diastolic blood pressure increase during isometric exercise (Freeman, 1991).

3.1.4.7 Clinical application

Isometric exercise is often used as part of a battery of clinical autonomic function tests. The test was included by Ewing in his protocol and was also advocated by a consensus conference of the American Diabetes Association on diabetic neuropathy (Anonymous, 1988; Ewing and Clarke, 1982). A repeat consensus conference involving the American Diabetes Association and American Academy of Neurology did not include isometric exercise in their suggested cardiovascular autonomic tests and other groups have not recommended the test for routine clinical use (AAN, 1996a; Anonymous, 1995).

Nonetheless, there is a considerable body of literature on the clinical value of isometric exercise in evaluation of sympathetic function. In one study, 22/124 diabetic subjects had an abnormally low (<2 s.d. for normal mean value) diastolic blood pressure change: the presence of abnormal blood pressure response was not related to duration of disorder, method of treatment, control or the presence of microvascular disease. This study also found no significant differences in heart rate response between normal and diabetic subjects. They

concluded the test was useful for the detection of autonomic dysfunction in diabetics (Ewing, 1974). In a review, Low (Low, 1993a) suggested defining a normal response as an increment in diastolic blood pressure of 16 mmHg or more, with 11-15 mmHg as a borderline response.

Although isometric exercise has been shown to be a useful test of efferent adrenergic arterial vasoconstriction, care is required in the interpretation of results. There is one unusual example of a moderate increment in blood pressure in an individual known to have sympathetic failure with intact vagal control: the presumed mechanism was vagal withdrawal leading to cardioacceleration and hence increase in cardiac output (van Lieshout, 1989).

3.1.4.8 Conclusion

Isometric exercise is a test of cardiovascular autonomic reflexes that normally causes a tachycardia and rise in blood pressure. The tachycardia is multifactorial in origin. Early vagal withdrawal is an important component: increases in efferent cardiac sympathetic impulses and circulating catecholamines probably also contribute. The blood pressure rise has dual origin – early cardioacceleration-related rise in cardiac output and most importantly a delayed increase in peripheral muscle sympathetic nerve activity to non-exercising muscle that produces vasoconstriction. MSNA correlates with the force of contracting muscle i.e. is effort dependent. The whole purpose of this haemodynamic adjustment is to maintain and increase perfusion to isometrically-exercising muscle. The reflex is triggered by a combination of afferent neural traffic from ischaemic skeletal muscle and signals from central command areas that control voluntary muscle movement.

Opinion is divided on the value of isometric exercise as a clinical tool for evaluation of cardiovascular autonomic function, with generally a smaller body of experience in the literature. There is no agreement on ideal test protocol and repeatability can be poor. However the test can show differences in autonomic function between groups.

3.1.5 Cold pressor test

The cold pressor test measures the blood pressure response to cold sensory stimulus. The hand is immersed in an ice water bath for up to 2 minutes. The rise in blood pressure from baseline to the final period of immersion is recorded. The normal response is a rise in systolic or diastolic blood pressure of 10-15 mmHg and 10 mmHg respectively. Systolic and diastolic pressure rises of more than 20 and 15 mmHg are abnormal. Subnormal responses may be seen in cases with damage to efferent sympathetic vasoconstrictor function (Wieling and Karemaker, 1999).

3.1.5.1 Neural mechanism

Victor et al (Victor, 1987) examined effects of the cold pressor test in 25 healthy young adults. Arterial pressure, heart rate and MSNA all significantly increased during cold pressor test. Similar to isometric exercise, normal cold pressor response results in the absence of the usual inhibitory effect of increasing blood pressure on sympathetic outflow. Heart rate rise started early within 30 seconds, peaked in the first minute, then declined in the second minute and the response was blocked by propranolol, indicating a cardiosympathetic basis. There was no correlation between MSNA and heart rate. MSNA was significantly higher at the end of the first minute and peaked in the second minute. MSNA strongly correlated with mean arterial pressure increase ($r = +0.86$, $p<0.01$). Mean arterial pressure was maximal in the second minute of immersion and the increase was not affected by propranolol. Peak norepinephrine level did not occur until the end of the first minute of the recovery period: there was a significant correlation between MSNA and norepinephrine levels but the large change in MSNA compared with small differences in norepinephrine levels. Therefore cold pressor elicits an early increase in cardiac sympathetic drive (possibly due to painful stimuli) and a later increase in peripheral muscle sympathetic activity which dictates the blood pressure response (possibly driven by cutaneous afferents).

Stancak et al (Stancak, 1996) observed cardiovascular changes during a 4 minute cold pressor test in 20 young male healthy adults. Systolic and diastolic blood pressure rises were evident in the first minute, maximal at the end of the second minute, and then stabilised with a marginal decrease in the third and fourth minutes. Heart rate rise was maximal at the end of the first minute but then attenuated from second to fourth minute such that third to fourth minute heart rate was not significantly higher than basal heart rate. In the rest phase immediately following cold pressor, heart rate actually decreased to a level below that of pre-test control period but systolic and diastolic pressure remained at a level intermediate to test and pre-test control phases.

Kregel et al and Fagius et al (Fagius, 1989; Kregel, 1992) confirmed the cold pressor test is a powerful activator of muscle nerve sympathetic activity (MSNA). The cold pressor test usually leads to a prompt rise in bursts of MSNA during immersion followed by a slow decrease in bursts during emersion. The level of MSNA correlates well with the rise in blood pressure with a stronger correlation for systolic than diastolic changes. The test itself produces a reversal of normal baroreflex function since an increase in blood pressure usually reduces MSNA. The blood pressure response is proportional to the level of reported discomfort but is not entirely governed by pain since an equivalent electrical painful stimulus (although of a different nature) does not elicit the same changes (Fagius, 1989). Another study also reports only weak correlations between pain and cardiovascular changes during

cold pressor test (Stancak, 1996). This suggests the response is likely to be dependent upon combined input from peripheral pain and cold receptors.

The lack of response in peroneal nerve skin sympathetic nerve activity (SSNA) indicates different regulatory mechanisms controlling MSNA and SSNA. Sundlof et al reported diastolic arterial pressure correlates more closely with MSNA than systolic pressure, in contrast with studies mentioned above (Sundlof and Wallin, 1978).

Ng et al (Ng, 1994) reported the cold pressor test only produces an increase in MSNA with noxious skin cooling. At seven degrees centigrade there was an initial dip in MSNA followed by a steady increase, whereas at zero degrees centigrade the rise in MSNA was instantaneous. In both cases peak MSNA occurs after 2.0 – 2.5 minutes immersion, and is 2-3 x basal levels in the case of zero degrees C. Non-noxious skin cooling at temperatures between 14-28 degrees C actually decreases or has no net effect on MSNA. There is a small immediate tachycardia on immersion at all temperatures but twice as great at zero degrees C: the mechanism is not clear but may be due to arterial baroreflex inhibition (Kregel, 1992).

The previous studies indicate that in the last 20 years many investigators have examined the role of MSNA in cold pressor response. But as early as 1965, Greene et al demonstrated that cold pressor response can have differing mechanisms between individuals. Eight normotensive and 10 hypertensive adults had direct arterial pressure, pulmonary artery pressure and cardiac output measured during cold pressor at 4°C. Most patients responded with a significant increase in blood pressure. Although most subjects produced an increase in total peripheral resistance, an increase in cardiac output generated the pressure rise in 5 cases. In another three cases, there was a combined cardiac output and peripheral resistance change behind the pressor response. Increase in pulmonary artery pressure was a third observed mechanism behind the pressor response (Greene, 1965).

3.1.5.2 Age

In a group of 15 insulin dependent diabetics with autonomic neuropathy, age just failed to reach the level of significance for a negative correlation with cold pressor response (Freeman, 1991).

Cold pressor induced changes in mean arterial pressure did not differ between 15 old and young subjects (mean ages 64 and 25 years respectively). The older group had higher baseline and peak MSNA levels but at one minute the percentage increase in MSNA was not significantly different between young and old groups. Then at 1 ½ and 2 minutes, the percentage increase in MSNA was greater in the younger group. The baseline and peak noradrenaline levels were significantly higher in the older group but the percentage increase in noradrenaline was not different between the groups (young 69%, old 65%). The modest

rise in heart rate was not influenced by age (Ng, 1994). Overall the study indicated sympathetic neural reactivity to stress does not increase with age in healthy humans.

This question was addressed by Pascualy et al (Pascualy, 1999) who compared plasma catecholamine responses to cold pressor test in three healthy groups; 19 young adults mean age 25 years, 28 early aging adults mean age 70 years and 8 advanced age adults mean age 84 years. Catecholamines were sampled at every 5 minutes after immersion in ice water for 1 minute. Blood pressure was measured by automated arm sphygmomanometer after every 5 minute blood sample, unfortunately not utilising continuous blood pressure measurement. Basal noradrenaline was higher, increased to a greater degree but returned to basal level at a similar time point in the oldest age group compared to early aged and young adults. Noradrenaline levels were much higher in the advanced aged group compared with early aging group. Basal epinephrine levels displayed a trend towards elevation in younger adults compared with early aging adults but were significantly higher in the advanced aging compared with early aging group. However advanced aging adults were the only group to demonstrate an epinephrine response to cold pressor test (increase at 5 minutes, normalising at 10 minutes). In summary advanced aging exaggerates the modest effects of early aging on basal sympathetic nervous system activity and greatly enhances the norepinephrine response to cold pressor stress. Advanced aging appears to result in epinephrine rise during cold pressor test not seen in younger people. The authors indicate sampling times may not capture early rise and clearance of catecholamines in the younger groups.

3.1.5.3 Other influences

Hypertensive individuals appear to have an exaggerated pressor response during cold stimulus compared with normotensives. Greene et al made this observation but noted that although the absolute change in pressure was greater, the percentage change relative to control values was exactly the same between groups (Greene, 1965).

3.1.5.4 Repeatability

Reproducibility was examined in a group of 48 healthy normal subjects by Fagius et al (Fagius, 1989). Group results were very similar, but there were up to seven-fold intra-individual differences on tests repeated the same day and up to five-fold on tests separated by up to 3 days. Thus the cold pressor test may not be reliable as a quantitative clinical test. Parati et al (Parati, 1985) also conclude reproducibility is not good. Eight individuals repeated 60 second ice water immersion for six occasions at intervals of 30 minutes, measuring mean intra-arterial pressure. Mean arterial pressure coefficient of variation averaged 17.2% (range 8.2 to 34.7%) for the group (heart rate CV 44.2%, range 18.1 to 158.1%). They hypothesised

variability is a reflection of the complexity behind central processing that governs the pressor response.

3.1.5.5 Test methodology

Period of immersion has ranged from one (Fagius, 1989; Freeman, 1991; Parati, 1989; Parati, 1985), 2 ½ (Ng, 1994) and three minutes. Significant blood pressure changes are evident after one minute and continue, peaking around the 2.0-2.5 minute mark (Kregel, 1992). The quoted temperature has ranged from 0-1°C (Ng, 1994), two (Fagius, 1989) to four (Freeman, 1991) degrees Celsius. Although many investigations use diastolic blood pressure change, some investigators use the systolic pressure response or mean arterial pressure (Freeman, 1991; Ng. 1994). A limited number of studies quantify time period for obtaining blood pressure readings: one study calculated changes using a 20 second period in the one minute prior to the test for baseline values and then the final 10 seconds of the immersion phase and another suggested similar 10 seconds pre-test and final test phase averages (Parati, 1989; Parati, 1985).

The Finapres is a suitable device to record blood pressure during the cold pressor test. In a group of 14 individuals, the Finapres blood pressure values gave an accurate estimate of blood pressure values when compared to the gold standard of radial intra-arterial values. The mean discrepancy between methods for systolic and diastolic pressures were 4.6 ± 4.1 and 2.1 ± 1.2 mmHg respectively. When considering the sign, there was a significant difference in the discrepancy for systolic pressure, +4.3 ± 4.5 mmHg ($p<0.01$) but not diastolic pressure, +0.1 ± 2.5 mmHg (Parati, 1989).

Although the cold pressor test is viewed as a test of efferent sympathetic function, caution may be required in drawing this conclusion . One case report of a patient with known sympathetic failure but intact vagal control still produced a normal rise in blood pressure during the cold pressor test, probably by a mechanism of vagal withdrawal. This enabled an increase in heart rate, producing a rise in cardiac output unopposed by reflex vasodilatation (van Lieshout, 1989).

3.1.5.6 Relationship with other cardiovascular autonomic tests

The postural change in low frequency spectral power, seen as a marker of sympathetic function, did not bear any relationship to cold pressor systolic pressure changes (Freeman, 1991).

3.1.5.7 Clinical application

Wieling et al comment that, in their experience, the cold pressor is the most useful test of efferent sympathetic pathways, since isometric tests are hampered by limited sensitivity and specificity (Wieling, 1997).

3.1.5.8 Conclusion

Cold cutaneous stress provokes increase in blood pressure and heart rate. The blood pressure rise is created from increase in MSNA activity, circulating catecholamines and tachycardia. The proportional contribution can vary according to age. The tachycardia is a combination of cardiosympathetic activity and vagal withdrawal. There is no conclusive evidence of the impact of ageing on numerical blood pressure response to cold cutaneous response. Individual reproducibility is moderate at best, limiting clinical value of cold cutaneous stress in evaluation of sympathetic efferent function.

3.2 Heart rate variability

3.2.1 Terminology

Heart rate variability is a term applied to a number of techniques in the literature. Broadly speaking, these fall into three groups. Firstly the classic 'bedside' cardiovascular autonomic reflexes describe heart rate variability over very short intervals in response to metronomic respiration, orthostasis and the Valsalva manoeuvre. In the last two decades, computerised analysis of heart rate variability led to development of two other techniques, time domain and frequency domain analysis of heart rate variation. These two methods quantify oscillation in heart rate over longer periods, usually minutes to hours. The following discussion focuses on heart rate variability in the frequency domain, a method used in this investigation.

3.2.1.1 History

Beat to beat variability in blood pressure was first documented in the 18th century by Stephen Hales during the first measurement of arterial pressure (Hales, 1733). In the 1960's Hon and Lee reported reduced heart rate variability was a marker of foetal death (Hon and Lee, 1965). Value as an adverse prognostic marker in adults was first shown in the 1970s when Wolf et al (Wolf, 1978) reported reduced variability associated with long-term mortality after myocardial infarction.

3.2.2 Measurement

Beat-to-beat variability in cardiovascular variables reflects the interplay between perturbation to cardiovascular homeostasis and the stimulated response of the cardiovascular regulatory

systems (Appel, 1989). Cardiac autonomic control loops are subject to many influences, which can interact amongst themselves. Such interaction of the influencing components is termed coupling and can lead heart rate, peripheral vascular resistance and blood pressure to demonstrate non-linear dynamic responses. Features of such non-linear systems include (a) small changes creating striking, non-proportional effects and (b) the non-linear system cannot be understood by individual component analysis, because of the coupling phenomenon. Breakdown of this complex array of mechanisms is a feature of pathological states (Goldberger, 1996a). Despite these difficulties, a mathematical model of the interplay between RR interval, blood pressure and peripheral resistance was proposed by de Boer (deBoer, 1987). This model does reproduce the characteristics of RR and blood pressure variability and its accuracy was improved by modifications by Whittam et al (Whittam, 2000).

3.2.3 Frequency domain

Spectral analysis is the process of segregating a signal into its constituent sine waves, by mapping out the different frequencies to represent the strength of each sine wave, or harmonic component (long term heart rate variability studies). The power spectrum presents the squared amplitude of the sine waves as a function of frequency i.e. *x* axis is the frequency, *y* axis is square of the sine wave amplitude. This is the power spectral density for the heart rate variability power spectrum.

For heart rate variation, these frequencies are less than 1 Hz in humans. The spectrum is divided into bands of interest. These bands are not arbitrary: research over the last 25 years indicates the bands reflect activity in distinct limbs of the cardiovascular autonomic system and other feedback loops in response to haemodynamic perturbation. The power of a frequency band is represented by the area under the curve. The number of bands of interest depends on the length of heart rate recording. Longer recordings improve ability of spectral analysis to identify slower heart rate variation. In other words prolonged heart rate recordings are required to detect very low frequency bands.

Stationarity is theoretically an ideal requirement for frequency domain analysis. Stationarity means obtaining a state where all other biological systems, bar those under investigation, are held constant. In effect stationarity is not obtainable in normal research conditions, even during fairly short periods of heart rate assessment (Malliani, 1994). Nonetheless it is an important principle of heart rate variability study to minimise variation in conditions.

In a five minute recording, the two bands of interest are the high frequency and low frequency spectral powers (HF and LF). The high frequency is mediated by the parasympathetic nervous system which is able to rapidly react to cardiovascular perturbation within a few seconds, thus

generating the most rapid fluctuations in human heart rate activity. Sympathetic autonomic response is more sluggish, requiring around 10 to 20 seconds to generate a measurable response to cardiovascular perturbation (Rosenblueth, 1934). Even slower frequencies exist and require prolonged heart rate recording for accurate delineation (Malik, 1996). These slower frequencies are grouped into very low and ultra low frequencies (VLF and ULF). In prolonged heart rate variability studies, VLF and ULF powers dwarf high and low frequency powers. In spite of this disproportionate importance, physiological correlates of ULF and VLF are surprisingly less well understood than high and low frequency powers. Nonetheless ULF and VLF power are still useful when considering clinical applications of heart rate variability. ULF and VLF have been referred to as non-respiratory powers (less than 0.04 Hz) since they do not appear to be altered by changes in respiratory pattern; they are said to be persistent during short term recording (Novak, 1993). This thesis concentrates on obtaining low and high frequency individual power bands as well as the 'total' power band. It is recognised that total power is influenced by VLF and ULF power bands, despite the short five minute recording chosen for study. Understanding the components of the low and high frequency power bands is particularly useful when assessing the neural reflexes governing short term heart rate and blood pressure variability. Mechanisms governing low and high frequency powers are discussed in greater detail below.

The transition from a five minute recording of ECG and blood pressure data to the production of RR interval and systolic blood pressure spectral powers is a highly complex mathematical process which is beyond the scope of this review.

In brief, the concept is based on identifying harmonic components of heart rate variations, which yield peaks in the Fast Fourier Transformation (FFT) power spectrum. In practice, the FFT spectra are often a highly split pattern with power scattered over a broad band with no clearly discernible peaks. In situations where there is a lack of distinct harmonic components after FFT, results can still be comparable to other methods of spectral analysis (Adelmann, 1999). If 24 hour recordings are analysed by spectral methods, taking the mean values of multiple five minute segments over 24 hours yields the same results as continuous spectral analysis over 24 hours whilst offering a simpler processing method (Rottman, 1990).

Limitations of Fast Fourier Transformation to calculate power spectra are the (a) deterministic nature of algorithm used, (b) applicability only to periodical phenomena, not strictly true in this situation, (c) need to window and (d) difficulty in defining with certainty the relative component power (Pagani, 1986).

3.2.3.1 Editing

Ectopic data produce non-sinus R waves that require removal from the recording before power spectral analysis. This is necessary to remove the noise in a recording and allow more accurate reflection of true autonomic activity acting on the sinus node. Artefacts often appear in ECG recordings due to change in body position, poor ECG electrode-skin contact or muscle movement. Despite the very best recording and training techniques, a proportion of records from a study will not be suitable for processing due to artefacts (Liao, 1996). Even with only very small amounts of ectopic data (less than 1 %), heart rate variability measurement can be significantly hampered (Storck, 2001). Furthermore variation in editing methodology can lead to substantially different frequency domain results (Salo, 2001). Some groups stipulate minimum percentage of RR intervals are normal sinus beats(NN intervals), for example 80% for Rottman et al (Rottman, 1990). The cut-off figure needs to yield consistent results whilst permitting a large residual fraction of RR intervals to allow meaningful analysis.

3.2.3.2 Repeatability

Some studies are optimistic, reporting consistent results for low frequency and high frequency over 2 months with reliability co-efficients of 0.68 to 0.77 under free respiration and 0.75 to 0.82 during metronomic respiration (Sinnreich, 1998). In a comprehensive report, Pitzalis demonstrated short term frequency domain measurements possess different degrees of reproducibility (over 2 weeks and 7 months) according to experimental conditions. Total power was reproducible only at rest (Intraclass correlation coefficient, ICC 0.75), high frequency power only during metronomic respiration (ICC 0.65) but low frequency power was reproducible during rest, metronomic respiration and tilt (ICC 0.60 to 0.77) (Pitzalis, 1996). Intraclass correlation co-efficients for three concurrent ECG recordings (i.e. one experiment with simultaneous triplicate ECG recording) yielded intraclass correlation co-efficients of 0.63 to 0.78 for low frequency, 0.72 to 0.91 for high frequency but only poor to fair results for fractional power and LF:HF. These latter results are alarmingly poor for a situation that should exclude natural variation over time and suggests either poor recording quality or poor interobserver agreement in editing technique (Ahmed, 1994). Other reports indicate reproducibility of frequency domain measures of heart rate variability is not good because inter-individual variation is high (Lord, 1997; Malik, 1996; Whittam, 2000).

Patient groups with impaired autonomic function such as post-myocardial infarction exhibit less heart rate variability therefore more reproducibility (Kautzner, 1995; Stein, 1995). Longer-term measures of frequency domain (ULF and VLF) demonstrate better reproducibility. This may be related to varying conditions over 24 hours with a larger number of situations, thereby including a larger number of modifications and thus homogenising this

spectral power. This could viewed as analogous to 'regression to the mean' for that individuals' heart rate variability (Pitzalis, 1996).

3.2.4 Physiological correlates

3.2.4.1 High frequency heart rate variability

The most rapid fluctuation in heart rate is mediated by the vagus nerve with a periodicity that matches the respiratory rate. In normal conditions this is around 0.20 Hz with a range between 0.15 and 0.40 Hz. Synchronicity between respiratory and heart rate variation was addressed in section 3.1.3.1 (Metronomic respiration as an autonomic reflex test) and additional issues are now discussed.

Respiratory activity has a mechanical effect on the intra-thoracic vessels and heart which stimulates vagal outflow to moderate cardiac output. Respiratory activity mechanically modifies arterial blood pressure which then leads to similar high frequency heart rate fluctuation, and not vice versa (Bernardi, 1989; deBoer, 1987; Peters, 1988a; Peters, 1988b; Saul, 1991). Under 'pure vagal conditions', the phase lag (the difference between respiratory activity 'input' and heart rate 'output' frequencies) is close to zero at all frequencies, such that heart rate increases during inspiration (suppression of cardiac vagal activity) and decreases during expiration (Eckberg, 1983; Saul, 1991; Saul, 1989). In addition to the mechanical link, there may be direct neuronal connections between high level respiratory and cardiac autonomic centres (Saul, 1991).

The vagus provides the efferent arm of this circuit (Eckberg, 1983; Katona and Jih, 1975). Katona et al (Katona, 1970) isolated single and multiple cardiovagal efferent nerve fibres in anaesthetised dogs, and described the following outcomes.

- Vagal firing substantially diminished or was abolished during natural inspiration 0.5 seconds before the respiratory nerve commenced firing, and resumed activity immediately after cessation of inspiratory nerve firing (a similar conclusion was reached by Saul et al (Saul, 1991) who also observed that inspiration inhibits sympathetic activity). Vagal firing decreases to increase heart rate as a response to the fall in left ventricular output during inspiration (Peters, 1988b).
- Vagal firing increased as blood pressure rose and vice versa: time interval was usually 60 to 80 ms but with a range of 55 – 140 ms
- Rapid blood pressure changes were followed by more gradual changes in vagal firing, with a longer latency to respond to drops in blood pressure compared with a rise in pressure

- RR interval was closely related to vagal firing

Katona and Jih isolated the vagus nerve of anaesthetised dogs. The nerve strands were manipulated by cooling and re-warming in the presence of autonomic pharmacologic blockade. Results demonstrated how the respiratory sinus arrhythmia, in other words the high frequency variation, is very closely proportional to vagal activity in animals (Katona and Jih, 1975). The sino-atrial node responds as a low pass filter to fluctuations in either sympathetic or parasympathetic tone (Berger, 1989).

Other animal models provide further proof of change in vagal firing with the respiratory cycle. These experiments entailed direct vagal firing measurement in decerebrate cats or conscious dogs, and utilised autonomic blockade to characterise the response. Atropine virtually abolishes high frequency power (Akselrod, 1985; Akselrod, 1981; Billman and Dujardin, 1990; Chess, 1975; Rimoldi, 1990). In the presence of hypotensive drugs that stimulate sympathetic reactivity, low frequency is enhanced and high frequency decreases (Pagani, 1986).

Katona et al (Katona and Jih, 1975) reported high frequency variation in heart rate variability is proportional to cardiovagal impulses in conscious dogs. Pomeranz et al (Pomeranz, 1985) replicated these results in humans. Pomeranz demonstrated that atropine administration greatly diminishes the high frequency component of power spectral analysis in humans. High frequency peak was practically abolished by atropine in both the supine and standing positions with no impact after beta blockade. Standing or passive tilt leads to diminution of HF power (Pagani, 1986; Pagani, 1991; Pitzalis, 1996; Pomeranz, 1985; Sanderson, 1996; Yokoi and Aoki, 1999). Propranolol alone has no effect on HF but adding atropine reduces HF power (Ahmed, 1994). Others have also shown near complete abolition of HF with atropine with minimal additive effect after propranolol (Jokkel, 1995). Beta-blockade alone in the supine state can increase high frequency power (Jokkel, 1995; Pagani, 1986).

Ahmed et al investigated the impact of different sympathetic stimuli on frequency domain power spectral analysis. Exercise and isoproterenol significantly reduced HF, tilt produced a non-significant decrease and epinephrine had no effect (Ahmed, 1994). Fouad (Fouad, 1984) used atropine in human subjects to show how parasympathetic activity is proportional to RR interval variation in quiet resting conditions, and this relationship was not affected by beta-blockade. Psychological stress from mental arithmetic or interview decreases high frequency whereas isometric exercise surprisingly may not have any effect (Pagani, 1991).

There clearly is a relationship between respiratory activity and RR variation and the studies discussed above indicate this is a very close linear relationship. This stance is contested by other workers who have obtained results indicating vagal cardiac nerve firing is not directly

proportional to the (high frequency) respiratory sinus arrhythmia (Eckberg. 1988; Kollai and Mizsei, 1990). Kollai and Mizsei (Kollai and Mizsei, 1990) investigated the respiratory sinus arrhythmia in 29 healthy young adults. They found that parasympathetic cardiac activity could be further reduced by atropine during inspiration i.e. vagal firing was not zero during normal inspiration. They termed the difference between maximum and minimum RR intervals as the respiratory sinus arrhythmia (RSA) and the parasympathetic control (PC) as the change in RR interval after complete parasympathetic blockade by atropine. There was a significant but only moderate correlation between PC and RSA ($r = +0.61$, $p < 0.001$). They also found close associations between RSA and respiratory cycle length ($r = +0.69$, $p < 0.001$) and RSA and tidal volume ($r = +0.89$, $p < 0.001$). Parasympathetic control and respiratory cycle length were not significantly correlated ($r = +0.24$), PC and tidal volume were loosely related ($r = +0.46$). Following multiple linear regression, the strength of correlation for RSA improved when including three variables: PC, respiratory cycle length and tidal volume ($R = 0.93$, adjusted $r^2 = 0.84$). Saul et al (Saul, 1989) found proportionality between the respiratory and heart rate frequencies is seen in the normal operating range but does not hold true at very low frequencies.

Controlled or metronomic respiration is used to augment parasympathetic activity and stimulate the high frequency power. The effect tends to plateau at 6 to 10 breaths per minute (Brown, 1993; Novak, 1993; Pagani, 1986; Pagani, 1991; Sanderson, 1996). The relationship is mostly dependent on rate of respiration but larger tidal volumes also increase high frequency power (Brown, 1993). High frequency power is sometimes termed the respiratory frequency in view of the physiological link to respiratory rate but clearly at low respiratory rates the 'high' frequency will move into the low frequency band when respiratory rate is less than 0.15 Hz. The gain in high or respiratory frequency power spectra is significantly greater at the lowest breathing frequencies i.e. around 6 to 10 breaths per minute compared with 15 to 24 breaths per minute (Brown, 1993). Metronomic respiration concentrates the respiratory high frequency power at the same frequency but during spontaneous breathing rates, the respiratory power can be spread over a wide frequency band (Saul, 1989). If the rate is similar to spontaneous respiration, the effect on HF and LF can be small or negligible (Pitzalis, 1996) and higher respiratory rates actually diminish both powers (Bernardi, 2000; Sanderson, 1996). One report states very slow respiratory rates (less than 0.15 Hz) can also diminish vagal activity (Saul, 1991). Thus, the body of evidence indicates respiratory rate is clearly important in modulating high frequency but there is also evidence demonstrating no significant differences in the heart rate variability results whether the respiratory rate was fixed to a metronome at a physiological rate or not (Goldberger, 1996b; Kim, 1997). In states such as

heart failure where heart rate variation is generally diminished, metronomic respiration can still increase high frequency power (Arrowood, 1995; Sanderson, 1996).

Heart transplant recipients are thought not to develop cardiovagal re-innervation. Indeed atropine does not alter high frequency after transplantation but metronomic respiration does increase low and high frequencies post-transplant (Lord, 1997). Oscillations in RR interval with respiratory manoeuvres in heart transplant patients are usually approximately 5% of that seen in controls, with a larger relative influence of tidal volume (Bernardi, 1989). This effect may be mediated by sinus node/myocardial wall stretch or possibly even persistent intracardiac ganglia.

High frequency power is more of a representation of the *variation* in modulating activity of the cardiovagal nerves rather than a direct measure of cardiovagal impulses. An increase of high frequency power is reflecting an increase in modulations of cardiovagal activity. For example in resting conditions when vagal activity can fluctuate normally, high frequency power will be present and will increase during vagal manoeuvres such as deep breathing (Pagani, 1986). However, completely opposing physiological conditions will both lead to an absence of HF components. Saturated vagal outflow, such as occurs when increasing blood pressure with phenylephrine, will lead to a slow heart rate that cannot vary at all at high frequencies under these physiological conditions (Cerati and Schwartz, 1991). Therefore despite very high vagal activity, high frequency power is reduced (Goldberger, 1994). With the opposite condition of parasympathetic blockade, the heart rate will be fast with no vagal activity but again, the ability for high frequency fluctuation is absent and high frequency power is abolished. Thus completely different levels of vagal activity produce the same absence of high frequency power (Malik and Camm, 1993). Saul et al reported lack of change in high frequency power despite assumed changes in vagal activity during baroreflex stimuli in humans (pressor and hypotensive drug infusions). This report hypothesises high frequency power is unlikely to correlate with mean vagal activity (Saul, 1990). Animal models lend support to this theory: high frequency component increased greatly during modulation of vagal activity by *oscillating* stimulation frequency or magnitude compared to *constant* frequency vagal stimulation (Hedman, 1995).

Goldberger's (Goldberger, 2001) investigation results informed a model whereby heart rate variability increases as parasympathetic activity increases until a plateau or saturation point is attained. Thereafter heart rate variability decreases as parasympathetic activity further increases. There is marked inter-individual variation in the relationship between heart rate variability, perhaps explaining the inconsistencies in some studies on heart rate variation physiology. This also creates a situation where we may not fully appreciate if observed inter-individual differences in HRV are due to true differences in autonomic effects or alternatively

a different relationship between HRV and autonomic effects, depending on where the individual is studied on the ascending-descending response limb of heart rate variability. Kollai's (Kollai and Mizsei, 1990) work has also shown inter-individual variation for the relationship between respiratory cycle length and RSA. Slow breathing usually increases RR interval but can decrease the interval in others. In the majority of subjects, reduced minimum and increased maximum RR contributed to the increase in RSA amplitude caused by slow breathing (type A/Z). In some subjects, the same procedure induced large decreases in minimum RR and very small/negligible changes in maximum RR (type A). For another small group, changes were exactly the opposite (type Z). Kollai hypothesises the variation in reaction is due to different levels of resting parasympathetic activity. For type A, the average cardiovagal activity was likely to be high and near saturation, thus there was little scope for increased firing during expiration but further vagal withdrawal during inspiration could yield a large change in RR interval. In type Z, the vagal firing could be near its floor level during inspiration at basal respiratory rates but there was a large potential for increased vagal firing during expiration at slower respiratory rates. Two hypotheses regarding the saturation effect are (a) that intense acetylcholine release into the sinus node means that enzymatic degradation is outstripped or alternatively (b) there is loss of phasic respiratory changes in vagal nerve firing during the experimentally increased blood pressure.

It can be seen that many of the mechanisms behind heart rate variability are still contested with the conflicting nature of some results. Sleight suggests inconsistent experimental evidence may relate to derangement in baroreflex sensitivity in certain subjects, which in itself is be an important component of RR variability (Sleight, 1995).

There is further evidence that high frequency spectral power is not solely dictated by vagal activity. Indeed, some have clearly shown dissociation between heart rate variability and parasympathetic activity. Goldberger et al used phenylephrine as a vagal stimulant as a result of baroreflex stimulation: the bradycardia reflected increasing vagal activity but there was a simultaneous decrease in high frequency power (Goldberger, 1994). Denervated hearts of transplant patients still display some high frequency power although this is less than 8% control subject levels, with a greater influence from tidal volume (Bernardi, 1989). Heart transplant patients' respiration-related oscillations in heart rate are hypothetically triggered by myocardial wall stretch. In normal subjects, atropine has a strong suppressive effect but does not abolish high frequency power (Eckberg, 1983; Katona and Jih, 1975; Saul, 1991) and this non-autonomic component of high frequency variability is closely related to the rate of change of lung volume or respiratory airflow (Saul, 1991). Exercise is known to reduce vagal activity and at moderate exercise rates, high frequency power decreases but at very high exercise rates the proportion of high frequency power increases, consistent with the theory of

a non-autonomic mechanism contributing to this power (Bernardi, 1990). This non-neural component of high frequency accounts for a small fraction of high frequency power in healthy subjects but the proportional representation can increase in states such as heart failure (El-Omar, 2001).

3.2.4.2 Low frequency heart rate variability

The components of low frequency heart rate variability have been extensively debated in the literature. Some workers argue that low frequency predominantly represents sympathetic effects on the heart (Malliani, 1994; Malliani, 1991) but the consensus view is that low frequency probably represents both vagal and sympathetic input to heart rate variability (Akselrod, 1985; Stein and Kleiger, 1999), and the relative sympathetic/parasympathetic contribution will vary according to physiological conditions. A model has been proposed which incorporates dual parasympathetic and sympathetic action generating low frequency RR variation as a result of feedback (Bernardi, 1994; deBoer, 1987; Sleight, 1995; Whittam, 2000).

The origin of the low frequency variability in heart rate appears to be due to the delay in sympathetic feedback loops. The numerous factors influencing heart rate variability lead to noise but feedback from fluctuation in peripheral vasomotor tone amplifies the noise around 0.1 Hz, creating the low frequency peak (deBoer, 1987). Step by step, this theory starts with the assertion that respiratory movement alters venous return to the heart which leads to arterial blood pressure flux. Consequently baroreceptor stimulation leads to efferent autonomic activity: fast vagal firing within 1 second and slower sympathetic firing over 3 to 10 seconds. The slower sympathetic response is out of phase with the former fast oscillations. This is not buffered by the system. Instead a new oscillation is generated which is also sensed by the baroreceptors, producing a slow i.e. low frequency oscillation around 0.1 Hz. This model is supported by experimental evidence demonstrating how both vagal and sympathetic limbs are required to produce low frequency waves (Bernardi, 1994). The product of this sequence of interactions is sympathetic-mediated low frequency RR variation (Sleight, 1995).

Another hypothesis is that a central neural oscillator creates the low frequency peak, since there are oscillations in central cardiorespiratory neurons, low frequency fluctuation persists during apnoeic states and opposite effects on baroreceptors produce the same increase in low frequency (Janig, 1985; Malliani, 1991). Overall, the consensus opinion is probably that low frequency heart period oscillation is generated by baroreceptor-sensed blood pressure fluctuation (Bernardi, 1994).

The low frequency oscillation is normally centred on 0.1 Hz. It is not fixed to a certain frequency and can vary between approximately 0.04 to 0.13 Hz. Animal experiments

demonstrate an increase in low frequency after sympathetic stimulation through pharmacological hypotension but this increase is abolished by pharmacological ganglionic blockade or arterial baroreceptor denervation (Furlan, 1990; Pagani, 1986; Rimoldi, 1990). However the blood pressure low frequency spectral density is maintained in spite of bilateral stellectomy (Pagani, 1986). This indicates the role of cardiac sympathetic nerves in the generation of low frequency.

In Akselrods' original description of heart rate variability in conscious dogs (Akselrod, 1981). autonomic manipulation revealed the following results:

- Combined parasympathetic and beta blockade abolished all powers with no heart rate variation
- Sympathetic blockade alone partially diminished LF power
- Parasympathetic blockade plus vasodilatation to stimulate sympathetic activity increased LF power
- Beta-sympathetic blockade plus increasing arterial pressure with a vasoconstrictor to reflexly increase parasympathetic activity increased low frequency power, confirming the parasympathetic input to this frequency domain.

Pomeranz (Pomeranz, 1985) found similar results in humans. At rest atropine reduced low frequency spectral power by approximately 84% with no additional affect from propranolol. The act of standing greatly increased low frequency around tenfold. Whilst standing, atropine followed by propranolol produced incremental decreases in low frequency. Propranolol alone also reduced low frequency power. This investigation concluded parasympathetic activity alone produces low frequency at rest but both parasympathetic and sympathetic limbs influence low frequency when upright.

Ahmed et al (Ahmed, 1994) investigated the impact of different sympathetic stimuli on low frequency. Only tilt produced a significant increase in low frequency. Epinephrine led to a modest rise whilst isoproterenol and exercise led to non-significant reductions in low frequency (the latter two with significant decreases in the fractional low frequency power): the sinus node also reacts differently to sympathetic action mediated by neural effects and circulating beta-agonists. These findings underline the pitfalls in drawing conclusions on sympathetic activity based on the low frequency power. Others have also shown increase in low frequency during passive tilt (Pagani, 1986; Pagani, 1991; Pitzalis, 1996). Low frequency: high frequency ratio was not altered by cardiac sympathectomy during supine rest (Lipsitz, 1990) but the ratio was significantly reduced during tilt testing (Tygesen, 1997).

Metronomic breathing, compared with spontaneous respiratory rate, tends to reduce low frequency power (Ahmed, 1994; Novak, 1993; Pagani, 1986; Pagani, 1991; Pitzalis, 1996). There is a small reduction in low frequency power when respiratory rate increases from intermediate to fast rates (Brown, 1993). Where slow metronomic respiration has increased low frequency power, some authors suggest this is the result of respiratory frequency moving into the low frequency range, rather than a simple rise in the localised 0.1 Hz peak (Brown. 1993; Novak, 1993; Sanderson, 1996). In the resting state, MSNA varied with respiration in ten normal subjects. Maximum activity occurred at end expiration and minimum activity usually at end inspiration (Eckberg, 1988). Respiratory related fluctuation in MSNA has been shown elsewhere (Saul, 1990).

Low frequency power may be greater in males (Sinnreich, 1998). High frequency component in absolute terms does not show any gender effect but when expressed as a percentage of total power may be greater in females (Sinnreich, 1998).

One may expect that studies examining muscle sympathetic nerve activity could clarify the debate on sympathetic activity's role in the low frequency peak. In fact they reveal contrasting outcomes. One reports a significant correlation between low frequency and MSNA amplitude using *normalised units* but no association between bursts of MSNA per minute and low frequency (Pagani, 1997). Another has failed to show a positive correlation between MSNA and low frequency power (or LF: HF) in either control or heart failure patients. In fact low frequency power was inversely related to MSNA in heart failure patients (Notarius, 1999). But a revealing study by Saul et al (Saul, 1990) reported that in the resting supine state, MSNA or norepinephrine levels did not correlate with low frequency power but there were weak correlations during sympatho-excitatory states, i.e. falling arterial pressure using the vasodilator nitroprusside. There were no correlations when sympathetic activity decreased during rising arterial pressure. Generally there were marked inter-individual variations in the association between MSNA and low frequency heart rate variability.

Conditions that increase sympathetic activity such as orthostatic stress and stressful interview increase low frequency power (Pagani, 1986; Pagani, 1991; Sanderson, 1996). However results from studies using exercise as a sympathetic stimulus provide conflicting results; low frequency power can substantially diminish during exercise (Bernardi, 1990; Perini, 1990) or modestly (and non-significantly) increase during isometric exercise (Pagani, 1991). Furthermore in heart failure states, which is known to cause sympathetic arousal, low frequency power is actually reduced (Mortara, 1994; Sanderson, 1996; Saul, 1988) with failure to increase on standing (Sanderson, 1996). Reduction of sympathetic activity during for example sleep is associated with reduced low frequency. At rest cardiac norepinephrine

spillover in heart failure patients does not correlate with low frequency (nor LF: HF ratio) (Tygesen, 2001).

Heart transplant patients are thought not to develop vagal re-innervation but may sprout sympathetic cardiac neural connections therefore provide a potential model of pure sympathetic cardiac innervation (Bernardi, 1995). In the post-transplant setting, intracoronary tyramine (releases noradrenaline only from intact pre-synaptic nerve terminals) produced RR interval changes proportional to low frequency component. These results do provide further evidence that low frequency power is directly related to sympathetic activity (Lord, 1997).

Jokkel et al (Jokkel, 1995), using the autoregressive technique for power spectral analysis, demonstrated similar near-abolition of low frequency with atropine with minimal additive effect from beta blockade but propranolol alone actually increased low frequency perhaps due to cardiac vagal nerve stimulation (Kollai, 1994). Other studies report reduced low frequency power with chronic beta blocker use (Ahmed, 1994; Pagani, 1986).

Caution is required when making assumptions about correlating low frequency power to autonomic nerve activity, in the same manner that applies to high frequency power. The low frequency power is expressing the ability of the autonomic nervous system to modulate heart rate variation. If the sympathetic nerves to the heart are at saturation level, there will be little scope for the sympathetic nerves to cause low frequency fluctuation and low frequency power will be diminished. Therefore sympathetic activity is high but low frequency power is low. This underlines the need to interpret findings in the context of the physiological setting (Malik and Camm, 1993).

3.2.4.3 Sympathovagal balance

Expressing the balance of sympathetic and parasympathetic activity using the ratio of low to high frequency power has been an attractive tool in the field of cardiovascular autonomic research (Malliani, 1991). Theoretically, activities such as exercise which lead to increased sympathetic activity increase the value of the LF: HF ratio and the converse applies during vagal stimulation such as metronomic respiration. Some reports do indicate the ratio can describe changes in autonomic activity that absolute values do not fully reflect but also report poor reproducibility of the ratio (Pitzalis, 1996). Unfortunately experimental results have not always validated the concept of 'autonomic balance' using the LF:HF ratio (Lord, 1997; Saul, 1990; Tygesen, 2001). One review adopted a sceptical tone regarding the physiological basis of LF:HF ratio as a marker of sympathovagal balance (Eckberg, 1997).

Another method of expressing the relative modulating activity of autonomic limbs is reporting the low or high frequency in normalised units (Malik, 1996; Malliani, 1994; Pagani, 1986; Pagani, 1991). This is the individual power spectral density (low or high) divided by

the sum of low and high frequency powers. The values can indicate a change in the apparent sympathovagal balance during reflex tests in the absence of change in the absolute values for low or high frequency (Pagani, 1986; Pagani, 1991). There may be some benefit in normalised units reflecting the proportional changes in autonomic balance during baroreflex stimuli since there is a better correlation with MSNA and normalised low frequency power than with absolute low frequency power (Pagani, 1997). However normalised units do not actually offer any more information than the LF:HF ratio (Hojgaard, 1998) and naturally suffer the same weaknesses as LF:HF in not honestly portraying neural activity (Lord, 1997). For example, in the process of adjusting sympathetic tone to lower levels, the LF:HF did not decrease with prolonged supine rest but did decrease with beta blockade. Therefore the ratio does not always reflect changes in sympathovagal balance and LF: HF does not correlate well with MSNA (Hojgaard, 1998; Saul, 1990). Additionally, the LF:HF ratio may behave differently according to the nature of the sympathetic stimulus. Tilt and isoproterenol significantly increased the ratio whereas exercise and epinephrine led to small non-significant increases (Ahmed, 1994).

3.2.4.4 Measurement and recording

Stationary conditions are vital for frequency domain analysis. Stable environment and physiology will produce a steady state autonomic state which will enhance the interpretation of power spectra. Stationarity does not prevent the subject from performing any manoeuvre but requires remaining in the same condition throughout the short term recording – for example resting in quiet conditions, subjected to tilt, or a drug infusion.

Activities such as reading, mental arithmetic or manipulation of respiratory rate will affect heart rate variability results. Reading silently or aloud decreases RR variability and increases low frequency, with similar but larger shifts towards low frequency during silent and aloud mental arithmetic (Bernardi, 2000). The respiratory high frequency power is lower in the upright position than supine (Saul, 1989). Ideally autonomic stimulation by physiological challenge should be incorporated into clinical HRV studies, since one recording in the supine position will not reveal the system's ability to show regulatory capacity (Karemaker, 1997).

3.2.4.5 Conclusion

Power spectral analysis of heart rate variability over five minutes can provide data reflecting cardiovascular autonomic modulation. The high frequency power spectra predominantly arises from vagal activity. Both sympathetic and parasympathetic activity modulate low frequency power spectra. Power spectral data are easily affected by environmental factors and also demonstrate potential for inconsistent responses between individuals.

3.2.5 Short term blood pressure variability

Beat-to-beat variation in blood pressure can be recorded by intra-arterial monitors or non-invasively using the Finapres or the ambulatory version, Portapres, which records measurements over 24 hours. These devices employ Penaz's volume clamp technique; this is a pressure measuring device that fixes finger volume at a preset level by using a fast servosystem and autocalibration procedure. Combined with an infrared photoplethysmograph, the system can measure digital intra-arterial pressure. The non-invasive devices can overestimate intra-arterial systolic pressure at brachial artery level of the arterial tree but this is a relatively minor aberration (Omboni, 1993).

Like heart rate, there are similar variations in blood pressure oscillations with respiration (Figure 3.11). These variations are linked with the heart rate variations in terms of underlying physiology. The high frequency component of blood pressure variability depends on the neurally-mediated beat-to-beat RR changes (deBoer, 1987). As respiratory frequency slows, systolic (and diastolic) frequency slows with the maximum frequency power at the same frequency as respiratory and RR interval maximal powers (Novak, 1993). Vagal activity provides the neural driving mechanism with no significant contribution from sympathetic activity (Saul, 1991). Abolition of RR variability by ganglionic blockade does not remove all high frequency blood pressure variability indicating that mechanical factors are also important determinants on this component (Rimoldi, 1990).

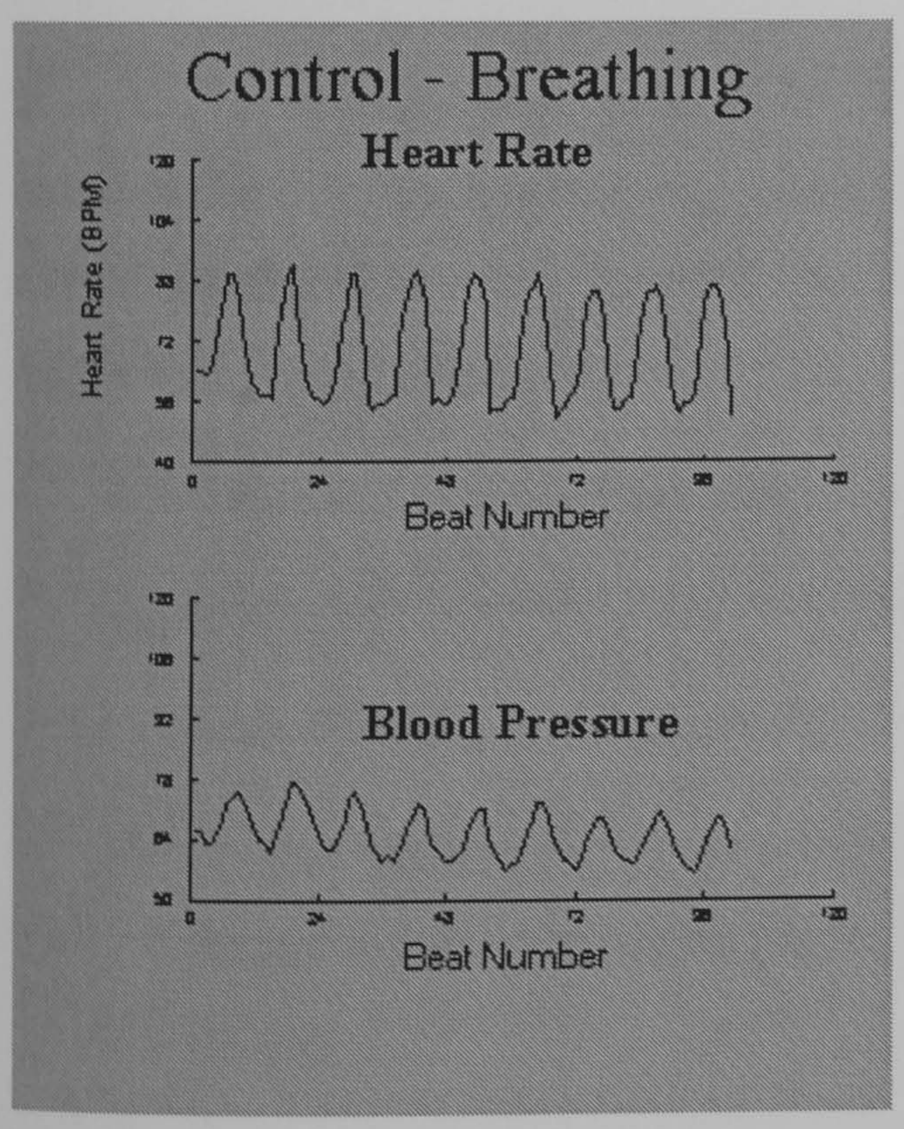

Figure 3-11 Heart rate and blood pressure variation during metronomic respiration

3.2.5.1 *Low frequency blood pressure variability*

This frequency range may be generated by a system resonance in the baroreflex control of peripheral vascular tone implying that peripheral vasomotor activity, and not heart rate, governs low frequency blood pressure variability: the delay in baroreflex feedback amplifies frequencies around the LF 0.1 Hz band (Akselrod, 1985; Bernardi, 1994; deBoer, 1987). The low frequency fluctuation is generated by variation in sympathetic discharge to peripheral vasculature, produced by resonant interaction between fast vagal and slow sympathetic response baroreceptor stimulation (Sleight, 1995). Both parasympathetic and sympathetic activity is involved in the generation of low frequency blood pressure waves. Simultaneous sympathetic stimulation with peripheral sympathetic blockade to the vasculature abolished the low frequency response, but similar provocation with intact neural network led to increased low frequency component indicating the role of peripheral sympathetic activity in generating the low frequency (Rimoldi, 1990). Although bilateral ablation of the stellate ganglion in animals reduces RR low frequency, it does not affect blood pressure spectra (Pagani, 1986).

3.2.5.2 *Ageing, gender and frequency domain analysis*

The qualitative nature of heart rate variability persists in older age, but there is evidence of a quantitative decline in frequency domain measures of heart rate variability with increasing age (Sinnreich, 1998). Low frequency power may be greater in males (Sinnreich, 1998). High frequency component in absolute terms does not show any gender effect but when expressed as a percentage of total power may be greater in females (Sinnreich, 1998).

3.2.6 Clinical applications

It is clear that power spectral analysis of RR and BP variation offers a useful tool to assess cardiovascular autonomic function. However this has not yet translated into clinical practice. The problems are (Wieling, 1997):

- Unproven value in assessment of the individual patient, despite ability to differentiate autonomic function between large groups
- Lack of long term reproducibility
- Lack of clinical utility: power spectral analysis is a very sensitive for detecting early autonomic impairment but at present there is no effective treatment for autonomic failure.

Reduction in vagal activity provides a therapeutic target for high risk groups. Angiotension converting enzyme (ACE) inhibitors actions include an increase in parasympathetic activity after myocardial infarction (Bonaduce, 1994) and chronic cardiac failure (Binkley, 1993), and there is some evidence that benefits include a reduction in sudden death (Cohn, 1991).

Angiotensin II appears to decrease vagal activity (Townend, 1995; Vaile, 1998). Therefore ACE inhibitors (which inhibit production of angiotensin II) may mediate improved outcome via alteration in autonomic activity. Beta adrenergic blockers' benefits are thought to be the product of peripheral action but lipophilic versions may also modulate vagal activity via central beta-1 receptors (Coats, 1992). Physical training up-regulates cardiovagal and reduces sympathetic activity in patients with cardiac failure, which may be the basis for its beneficial effects (Coats, 1992).

3.3 Ambulatory blood pressure variability

Blood pressure is constantly fluctuating. Behavioral factors and arterial baroreflexes are two of the most important influences on blood pressure variability (Parati, 1996; Watson, 1980). Three methods exist for recording fluctuation. The invasive intra-arterial technique and non-invasive finger arterial pressure measuring device provide continuous recordings of beat-to-beat blood pressure. Ambulatory oscillometric blood pressure monitors provide non-continuous recordings, taking intermittent readings on a regular basis, usually at intervals ranging from 10 minutes to one hour. The continuous methods benefit from good accuracy whereas the latter method is more acceptable to patients and is the standard technique in routine clinical settings.

There are three important themes arising from blood pressure variability studies in the last 20 years (Parati and Mancia, 2001).

- Knowledge of the physiological mechanisms underlying fluctuation in blood pressure (from continuous recordings)
- How certain pathological states can alter blood pressure variability
- Data regarding the importance of blood pressure variability as a risk factor for end-organ damage and cardiovascular outcomes

Some aspects of physiological mechanisms in beat-to-beat variability are covered in the section on Heart Rate Variability above. The following discussion concentrates on the role of abnormal blood pressure variability in end-organ damage, with emphasis on cerebrovascular disease.

The literature uses a variety of terms to describe different modes of blood pressure variability with occasionally some inconsistencies. Unfortunately there is no statement regarding definitions and nomenclature of the various techniques. Here blood pressure variability will be discussed in terms of:

- Long term variability: the standard deviation of non-continuous ambulatory blood pressure recording
- Short term variability: the beat-to-beat variability of continuous blood pressure recording, using either time domain or frequency domain techniques
- Pulse pressure: the difference between systolic and diastolic blood pressure
- Diurnal variation: the difference between means of day and night blood pressure from 24 hour recordings

3.3.1 General characteristics of blood pressure and its 24 hour variation

There is a well-recognised alerting response when blood pressure is measured in a clinical setting by a doctor (Mancia, 1983a). Ambulatory blood pressure results are lower than office recordings. The difference varies from 5/3 mmHg in normotensives to around 26/16 in untreated hypertensive patients. There is an age related rise in mean ambulatory blood pressure results. Men have elevated mean ambulatory recordings, with systolic pressure approximately 4 to 6 mmHg and diastolic 5 to 6 mmHg higher than women.

Difficulties in teasing out the relative strengths of systolic, diastolic and mean blood pressure plus pulse pressure are highlighted by their inter-dependence. In the PIUMA study (Verdecchia, 2001), mean ambulatory blood pressure correlated with pulse pressure and diastolic pressure ($r = +0.28$ and $+0.95$ respectively). However diastolic pressure did not correlate with pulse pressure ($r = -0.06$). Using only office blood pressure in 9431 subjects aged 65 years and older, systolic blood pressure correlated significantly with diastolic pressure ($r = +0.50$, $p < 0.01$) but the relationship was modest compared to middle-aged populations. Pulse pressure had a weak and slightly U-shaped correlation with diastolic pressure: less than 80 mmHg DBP, $r = -0.21$ and above 80 mmHg $r = +0.10$ (both $p < 0.001$). There was a strong correlation ($r = +0.82$) between systolic and pulse pressure (Glynn, 2000).

Long term variability is greater than short term blood pressure variability. Absolute values of blood pressure variability increase as mean blood pressure increases but the percentage variability (as a proportion of the mean) can be similar between different blood pressure categories. Blood pressure variability reduces during sleep, presumably due to reduction in activity level (Mancia, 1983b; Veerman, 1996).

Majahalme et al (Majahalme, 1996) noted that in terms of predicting future blood pressure, continuous invasive blood pressure recording can only explain a fraction of the variance of future blood pressure (generally less than 50%), whether measured with ambulatory or repeated casual readings. Despite this, invasive 24 hour ambulatory recordings correlate

reasonably well with follow-up blood pressure and are clearly better than casual office readings in this respect.

3.3.2 Choice of blood pressure parameter

Continuous and non-continuous blood pressure measurement can yield data for systolic, diastolic and mean blood pressure. All of these values have been used in variability studies. Unfortunately there does not appear to be any consensus statement or position view indicating a preference. A theme emerging from the literature in the last 10 years is that stroke and coronary events differ in how they are best predicted by different parameters of blood pressure. Furthermore, lowering of systolic and diastolic pressure or pulse pressure may have a discordant effect on cerebrovascular and cardiac outcomes (Millar and Lever, 2000).

It is well established that isolated systolic hypertension is common in the elderly: up to 30% of people over 60 year's age have elevated systolic pressure over 160 mmHg (Psaty, 1992; Staessen, 1990). Even 'borderline' isolated systolic hypertension with values between 140 to 160 mmHg has a strikingly high prevalence of 18% in people aged over 65 years and carries significantly increased long term risk of cardiovascular disease (HR 1.47, 95% CI 1.24 to 1.74) and death from cardiovascular disease (HR 1.57, 95% 1.24 to 2.00) (Sagie, 1993). Diastolic pressure tends to fall from the seventh decade onwards, in contrast to systolic pressure which displays a consistent linear rise throughout the ages (Franklin, 1997a). Systolic hypertension is probably now regarded as a more important determinant of cardiovascular risk than diastolic pressure (Neaton and Wentworth, 1992). The literature emphasises the importance of systolic pressure above that of diastolic pressure and when compared directly, higher systolic pressure usually carries greater risk than diastolic values (Anonymous, 1997; Kannel, 1996; Khattar, 1999; Psaty, 2001).

Mean arterial pressure is seen as an estimate of vascular resistance but there are three reasons why this assumption can be unreliable in older people (Franklin and Weber, 1994). Firstly, the plateau in mean pressure after age 50 to 60 years can lead to an underestimate in mean arterial pressure, since vascular resistance continues to rise with age. Secondly mean arterial pressure is usually calculated using a factor of 0.33 of the pulse pressure but the pulse wave contour changes with age such that a factor of approximately 0.5 is more appropriate. Thirdly as diastolic pressure declines in older age, the calculated mean pressure will further underestimate the true vascular resistance.

The rise in systolic pressure with age has two origins. Increased vascular resistance contributes to the change but the more important component is probably increased input impedance, which is generated by large artery stiffness and early pulse wave reflection (James, 1995; Messerli, 1983).

It could be argued that since systolic blood pressure is such an important determinant of outcome in the elderly, systolic variability is likely to be a more useful parameter than mean or diastolic blood pressure variability. The literature contains reports using any combination of systolic, diastolic or mean pressure which can make comparison problematic. Systolic, and to a lesser degree, mean blood pressure appear to be more frequently quoted in the literature than diastolic. One could speculate this is a result of diastolic pressure producing fewer significant results than systolic blood pressure.

3.3.3 Long term variability

3.3.3.1 Definition

This is the standard deviation of intermittent mean levels, usually from non-continuous recordings over prolonged periods (typically 24 hours). The frequency of sampling is important, since less frequent measurements reduce power to accurately reflect variability. If the sampling interval is 15 minutes or less, the standard deviation is not significantly different to continuous recordings. Intervals greater than 20 minutes will result in loss of ability to define short term blood pressure changes (Bevan, 1969; di Rienzo, 1983). It is important to bear in mind that similar standard deviation values can arise from radically different types of blood pressure variation (deBoer, 1987).

The long term variability may be summarised as a total, 24 hour figure. However blood pressure fluctuation can differ between daytime active periods to resting nocturnal periods, and it is often helpful to quote separate figures for the two phases. Further refinement by calculating the coefficient of variation (standard deviation / mean blood pressure) adjusts for the increasing variation in blood pressure with higher mean values. One group advocate their index of variation which is the standard deviation of 24 hour blood pressure/square root of mean blood pressure, and this measure is said to have the advantage of being independent of mean blood pressure (Prattichizzo and Galetta, 2002).

Often studies investigate the role of blood pressure variability using correlation or regression but in terms of defining abnormal levels of variability, more than 15 mmHg (daytime systolic variability) is an appropriate cut-off value based on population studies (Sander, 2000a). Similar values are used in other studies (Mancia, 1983b; Verdecchia, 1996).

3.3.3.2 Mechanism

Watson et al (Watson, 1980) used continuous intra-arterial BP recording to investigate factors determining arterial pressure in 26 mild-moderately hypertensive patients under controlled physical activity levels as hospital inpatients. Systolic pressure and its variability were significantly correlated at all times (sleep $r = +0.56$, $p < 0.05$; bed rest $r = +0.73$, $p < 0.01$;

waking r = +0.64, p <0.001) but diastolic pressure and variability were only correlated during bed rest (r = +0.71, p <0.01). Systolic variability increased with age (r = +0.42, p < 0.05) but this association was no longer significant when allowance was made for increase of pressure with age. There was a non-significant tendency for variability to decrease with the leanness index (height^3/weight). In other words, variability tended to increase with obesity. No relationship was observed between variability and circulating catecholamines.

In subjects with untreated hypertension of mild to moderate degree, long term variability has an inverse correlation with baroreflex sensitivity (Mancia, 1986; Parati, 1995a; Watson, 1980). Thus loss of the buffering mechanism of baroreflex sensitivity leads to greater swings in blood pressure. Baroreflex sensitivity can fluctuate in the course of a day, and therefore blood pressure variability can increase not only with the ageing effect but also from hour to hour.

3.3.3.3 End organ damage

Increasing long term variability is associated with organ damage, independent to the mean blood pressure level. Two early studies indicated end-organ damage correlates with the standard deviation of 24 hour blood pressure (Parati, 1987; Pessina, 1985). Frattola et al (Frattola, 1993) assessed the comparative value of short and long term variability from 24 hour beat-to-beat intra-arterial recordings in 73 patients mean age 53 years, over 7 years follow-up. Scoring criteria for end-organ damage were based on left ventricular hypertrophy changes on ECG, cardiac enlargement on chest radiograph, retinal changes of hypertension and left ventricular mass index from echocardiogram. End-organ damage was significantly associated with long-term variability but was not influenced by short term variability. Pickering et al (Pickering and James, 1994) found that daytime diastolic BP variability is an independent risk factor for cardiovascular morbidity in mild hypertension. Palatini et al (Palatini, 1992) have also shown greater degrees of target organ damage (left ventricular hypertrophy and retinal damage) in patients with higher daytime blood pressure variability in addition to the known risk with increasing pressure.

Reasons for long term variability having greater impact on outcomes than short term ('within half hour') variability include (a) they represent a larger proportion of overall variability than short fluctuations (Mancia, 1983b) and (b) they represent the historical blood pressure values more closely (antihypertensives may lower mean blood pressure but do not greatly alter long term BP variability) (Mancia, 1989).

Blood pressure variability has been linked with arterial wall damage. Carotid intima thickness was significantly related to 24 hour non-invasive ambulatory systolic blood pressure variability (Mancia, 2001; Mancia, 1996). Longitudinal studies indicate increasing systolic

blood pressure variability independently predicts progression of intima media thickness (Sander, 2000a). From an initial cohort of 424 patients aged over 55 years who had neurological disorder excluded during hospital admission, 286 cases were followed up with repeat carotid Duplex ultrasonography. A significant and linear relationship was found between intima media wall thickness (IMT) progression and initial systolic blood pressure variability ($r = +0.52$, $p < 0.01$). Following multivariate linear regression, daytime systolic variability was the best predictor of IMT progression, followed by mean systolic pressure, age and pack years of smoking. There was an interaction with diurnal variation: those with excessive daytime systolic variability and reverse of diurnal variation had the worst IMT progression, and those with low daytime variability and normal diurnal variation experienced the least IMT progression

Most of the studies indicating an association between increasing blood pressure variability and cardiovascular morbidity or mortality have used non-invasive techniques (Pickering and James, 1994; Verdecchia, 1996). Early data tended to be cross-sectional. More recently longitudinal studies provide evidence that increasing long term variability is a true independent predictor not only for organ damage, but cardiovascular mortality as well. There were 67 cardiovascular deaths amongst a group of 1542 patients followed over a mean period of 8.5 years (Kikuya, 2000). Daytime systolic blood pressure variability was a significant and independent predictor of cardiovascular mortality. Night-time systolic variability carried a similar but less significant risk. Sander et al (Sander, 2000a) also found increased risk of fatal and non-fatal cardiovascular events in patients with raised blood pressure variability, defined as systolic SD more than 15 mmHg (RR 1.87, 95% CI 1.08 to 3.20, $p < 0.01$).

Until recently, most studies had concentrated on studying blood pressure variability in patient cohorts, in particular hypertensive groups. Sega et al (Sega, 2002) investigated blood pressure variability and end organ damage in the general population. In 1648 people, left ventricular mass index positively correlated with 24 hour systolic and diastolic variability, with regression coefficients 0.57 (95% CI 0.29 to 0.85, $p <0.001$) and 0.70 (95% CI 0.36 to 1.04, $p <0.001$) respectively. However in multivariate analysis, the basic standard deviation of systolic and diastolic BP were no longer significantly correlated with left ventricular mass index (LVMI) but the group used another measure of variability, the individual residual variability. Fast Fourier Transformation of the 72 measurements over 24 hours revealed two cyclic components accounting for most of the variability. These components were tested for their ability to fit the systolic blood pressure profile in each subject. Then the sum squared of the differences between the observed and fitted profile was taken as reflecting the variability unexplained by the cyclic components in each case i.e. the individuals' residual variability. This measure did positively correlate with LVMI in multivariate analysis, and persisted after

adjustment for gender, age and 24 hour average blood pressure values. The study also demonstrated a positive correlation with 24 hour mean blood pressure and LVMI. The individual residual variability may represent the erratic component of blood pressure variability, which may be pivotal in the effect on target organs.

Palatini has argued that ambulatory blood pressure does not hold that great an advantage over carefully performed serial 'office' readings (Palatini, 2002). However, there are studies confirming that target organ damage correlates more closely with mean 24 hour blood pressure and its variability than with office blood pressure measurement (Appel and Stason, 1993; Khattar, 1999; Ohkubo, 1997; Parati, 1987; Perloff, 1983; Verdecchia, 1996; Verdecchia, 1998). In hypertensive patients, left ventricular hypertrophy is more closely correlated with 24 hour ambulatory readings than by clinic blood pressure. Secondly, treatment induced regression of left ventricular hypertrophy is significantly related to reduction in ambulatory readings but does not correlate with office blood pressure readings (Mancia, 1997).

It would seem fair to say that ambulatory blood pressure parameters are able to predict outcomes more accurately than serial office readings. It is widely acknowledged that ambulatory blood pressure recording has cost, time and technical implications in comparison to office blood pressure measurement. Presently it is recommended the advantages of ambulatory measurement are best applied (1) in research settings where the extra degree of accuracy is particularly important and (2) in clinical settings only in certain selected patients (Prasad, 1996). White coat hypertension is the obvious example, but other common uses include investigation of anti-hypertensive drug side-effects, analysing 24 hour blood pressure control and assessing behavioural influences on blood pressure where this affects treatment e.g. employment related hypertension (O'Brien, 2000; Prasad, 1996; Prasad, 1995; Staessen, 1999a). Selective use of ambulatory blood pressure recording may change in the future as we learn more about its value in managing blood pressure in patient groups.

3.3.4 Short term variability

This variable can only be obtained from continuous blood pressure recordings. Short term variability was given prominence by Mancia in the early 1980's (Mancia, 1983b). It refers to the mean variation in beat-to-beat blood pressure over short periods. Mancia et al's form of reporting is the average of 48 standard deviations taken from each 30 minute mean blood pressure in a 24 hour recording. However, short term variability appears to have less clinical value in predicting cardiovascular outcomes in comparison to long term variability (Frattola, 1993).

Frequency domain analysis of short term continuous blood pressure recording is covered in the section on Heart Rate Variability. Hypotheses regarding the autonomic neural control of blood pressure have developed from experimental manipulation of blood pressure variation. These responses alter the blood pressure variation power spectrum which is known to display certain characteristics according to autonomic activity (Parati, 1995b).

Short 5 minute non-invasive continuous blood pressure measurement yielded data of prognostic value in post-stroke patients (Dawson, 2000). Mean arterial pressure variability gave an odds ratio for death/dependency at 30 days after stroke of 1.32 ($p < 0.03$) for every 1 mmHg increase in variability measured within 3 days of cerebral infarction. In a logistic regression model, mean and diastolic blood pressure variability were significant independent predictors of death/dependency at 30 days post-stroke. The highest quartile of mean and diastolic blood pressure variability had worse outcomes than the lower groups.

When measuring a broad band of frequencies, blood pressure variation follows a 1/f distribution: the lower the frequency of blood pressure variability, the greater its power and contribution to greater variance (Parati, 1996). Hence slow frequency variation is the predominant fluctuation in longer blood pressure recordings. Some consider the very slow day-to-night fluctuation as one of the most important blood pressure variabilities. Circadian variation in blood pressure is now discussed in more detail.

3.3.5 Diurnal variation

3.3.5.1 Definition

A circadian variation in blood pressure was first noted by Bevan in the late 1960s and Millar-Craig in 1978 (Bevan, 1969; Millar-Craig, 1978). The presence of circadian variation was debated in the following years (Watson, 1980) but O'Briens' work published in 1988 (O'Brien, 1988) appears to have confirmed the presence of a diurnal variation and provided further stimulus for continuing investigation of this phenomenon.

Normal individuals experience a reduction in blood pressure during sleep. Blood pressure follows a circadian pattern due to variation in physical and mental activity and possibly autonomic and hormonal mechanisms (Pickering, 1982; Ziegler, 1976).

Diurnal variation may be expressed in three ways.

- Absolute change: awake – asleep values
- Percentage change: night/day x 100%
- Percentage dip: day-night/day x 100%

The latter two methods are considered preferable since they account for the increase in diurnal variation with increasing blood pressure between subjects.

To differentiate between abnormal and normal circadian variation in blood pressure, the terms 'non-dipper' and 'dipper' were coined (O'Brien, 1988). A cutoff value of 10% gained popularity. Dippers had a greater than 10% reduction from day to night period whereas non-dippers blood pressure varied by less than 10%. This is a somewhat arbitrary definition. In some early reports, diurnal variation was thought to be normally distributed (Staessen, 1992) but a much larger study indicates that the distribution does deviate from normality, particularly for normotensive subjects but not for hypertensive cases (Staessen, 1997a). Reversal of the normal diurnal rhythm i.e. absence of any reduction in nocturnal blood pressure may be a more useful definition than assigning an arbitrary cutoff value. This was borne out in Staessen et al's (Staessen, 1997a) assessment of ambulatory recordings from an international database of 4765 normotensive and 2555 hypertensive patients aged 10 to 99 years. In normotensive subjects, the 95th percentiles for systolic and diastolic pressure were -0.3/-1.1 mmHg for the nocturnal fall and 99.7%/98.3% for the night-day ratio. In all subjects the systolic/diastolic nocturnal fall and corresponding ratios are shown in Table 3.3. Of all subjects, 3.2% had systolic and diastolic ratios of 100% or more.

Table 3-3 Normal values for diurnal variation in ambulatory blood pressure

	Nocturnal change, mean (mmHg)	Ratio, mean (%)
Systolic pressure	-16.7 ± 11.0	87.2 ± 8.0
Diastolic pressure	-13.6 ± 8.1	83.1 ± 9.6

From Staessen 1997 (Staessen, 1997a)

There are two prominent difficulties in assessing diurnal variation. Firstly reproducibility is poor (Mancia, 1997; Palatini, 1994). Secondly a variety of times have been used to define day and night (or awake and asleep) periods (Staessen, 1997a; Verdecchia, 1994). There is also the issue of non-continuous monitors affecting sleep pattern due to intermittent cuff inflation. Cuff activation overnight disturbs sleep in up to ⅔ of patients: consequently systolic pressure can increase momentarily up to 10 mmHg as a result of cuff activation (Davies, 1994; Palatini, 1992). Conversely actual activity in the night especially nocturnal urination can 'falsely' elevate the night-time blood pressure and result in the misclassification of people as non-dippers. Modest increases in night-time blood pressure associated with waking to micturate effectively increased the systolic non-dipping status by 25% and diastolic non-dipping status by 50% (Perk, 2001). Therefore accounting for participants' nocturnal activity improves the accuracy of results.

3.3.5.2 *Time interval for diurnal variability*

Many studies use fixed time periods based on the complete 24 hour period, for example 07:00 to 22:00 and 22:00 to 07:00. The principal difficulty is that blood pressure substantially and rapidly changes between the hours of 06:00 to 09:59 and 20:00 to 23:59 (Fagard, 1996; Staessen, 1991; Van Hoof, 1989). Studies using the 10:00 to 20:00 and 00:00 to 06:00 fixed interval avoid these transitional periods influencing the awake and sleep blood pressure levels. Therefore they are closer to diary derived values (see Reproducibility). Staessen and colleagues have adopted these narrow fixed time periods in many of their studies (Staessen, 1992; Staessen, 1997a; Staessen, 1996; Staessen, 1999b; Verdecchia, 1998).

3.3.5.3 *Aetiology of abnormal circadian rhythm*

Certain secondary causes of hypertension are more likely to lead to reversal of diurnal rhythm, for example renal artery stenosis, glomerulonephritis, diabetic nephropathy and phaechromocytoma (Middeke and Schrader, 1994). Advanced cardiovascular autonomic neuropathy, for example in primary autonomic failure, diabetes and Shy Drager syndrome, are also associated with non-dipping status (Pickering, 1990; Vagaonescu, 2000). Heart rate variability markers of autonomic function are diminished in hypertensive non-dippers (Kohara, 1995). A report by Kario (Kario, 1997) gives some indication of normal autonomic diurnal variation becoming disturbed, and possibly underlying the loss of normal blood pressure diurnal variation. The study uses unconventional markers of heart rate variability and performs multiple correlations but indicates that sympathetic activity may be lower at night in extreme dippers, and lower during the day in non-dippers. Another report suggested disordered autonomic function alters nocturnal decrease in blood pressure, but in this case found a rise in sympathetic tone overnight (Dodt, 1997).

Kario's group continued their work with an interesting insight into dipping status from passive tilting (Kario, 1998). Extreme dippers experienced a significant increase in blood pressure with tilt i.e. orthostatic *hyper*tension, in contrast to non-dippers who suffered a drop in blood pressure during tilt i.e. orthostatic *hypo*tension. They hypothesise that the upright position during the day may drive the abnormal circadian rhythm, by virtue of increasing blood pressure in extreme dippers and decreasing blood pressure in non-dippers. Unfortunately this cross-sectional study is unable to determine cause and effect in the sequence of changes.

3.3.5.4 *End organ damage*

Non-dippers have more end-organ damage in terms of left ventricular hypertrophy, small vessel cerebrovascular disease and renal damage (Kario, 1996; O'Brien, 1988; Palatini, 1992; Sander, 2000b; Verdecchia, 1994; Verdecchia, 1990). Stroke is more common in non-dipping

hypertensive patients (O'Brien, 1991b). There are some negative studies, but meta-analysis provides further evidence that reduced diurnal variation is independently associated with cardiovascular events (Staessen, 1997a).

In placebo treated participants of the Syst-Eur trial, the night-time systolic blood pressure (00:00-06:00) more accurately predicted cardiovascular end-points than the daytime level, and ambulatory parameters predicted outcomes over and above office blood pressure levels. However, ambulatory levels were non-significant predictors of outcomes in the treatment group. Most importantly they found the relative hazard ratio with a 10% higher systolic night-to-day ratio was 1.41 (95% CI 1.03 to 1.94, p = 0.03) (Staessen, 1999b).

An important finding in the study of diurnal variation is the risk of increased lacunar or white matter cerebrovascular disease in patients with an excessive fall in blood pressure overnight, the so-called 'extreme dippers' (Kario, 1996; Shimada, 1992). Such findings support the hypoperfusion theory of small vessel cerebrovascular disease. Extreme dippers also have greater long term BP variability (Kario, 1996).

Data from a longitudinal study following intra-arterial ambulatory blood pressure recordings reported a significantly higher incidence of cardiovascular events or death in patients with less than 10% dip in nocturnal blood pressure and in patients with smaller nocturnal decreases in both systolic and diastolic pressure (Khattar, 1999).

3.3.6 Pulse pressure

This is often included in discussing blood pressure variability since pulse pressure informs about the blood pressure variation occurring within the cardiac cycle, from systole to diastole (Parati and Mancia, 2001). Stroke volume, major artery wall stiffness and the speed of reflected waves are the variables that determine pulse pressure. Studies mostly of hypertensive patients with concomitant coronary artery disease, conclude pulse pressure can increase end-organ damage and affect cardiovascular outcomes (Parati and Mancia, 2001).

There is a linear increase in systolic blood pressure from 30 years old through to the middle of the ninth decade but diastolic pressure declines after the age of 60. Hence pulse pressure rises steeply in old age, and the rate of increase in pulse pressure significantly increases with higher midlife systolic pressure (Franklin, 1997a). A possible scenario of a synergistic relationship between hypertension and large artery stiffness emerges. The age related decrease in diastolic pressure is a result of increasing major artery stiffness. The decline in capacity of the elastic reservoir results in greater dispersion of stroke volume in systole, a situation compounded by reduced elastic recoil and these factors combine to the fall in diastolic pressure. Pulse pressure is more closely correlated with end-organ damage when

measured by ambulatory methods than with office blood pressure (James, 1995; Khattar, 1997; Verdecchia, 1998).

3.3.6.1 End-organ damage

James et al (James, 1995) reported a significant correlation between media-lumen ratio and pulse pressure that was independent of age, systolic and mean pressure and BP variability ($r = +0.56$, $p = 0.001$). This indicates a role for pulse pressure in alteration of resistance vessels' structure.

Franklin et al (Franklin, 1999) reported coronary artery disease (CAD) risk is positively associated with increasing pulse pressure, and neither systolic nor diastolic pressures were superior to pulse pressure in predicting CAD risk. There was a far greater increase in CAD risk with increments in pulse pressure and static systolic blood pressure than with increments in systolic pressure but stable pulse pressure. Therefore in older age there was an inverse relationship between CAD risk and increasing diastolic pressure. These observations support the hypothesis that CAD risk is more related to the pulsatile stress caused by large artery stiffness during systole than the steady-state stress due to small-vessel resistance during diastole (Nichols, 1986). Other studies provide further evidence that elevated pulse pressure increases the risk of myocardial infarction (Madhavan, 1994). Cross-sectional studies indicate an association between pulse pressure and left ventricular mass (Pannier, 1989).

Cross-sectional studies found an association between pulse pressure and carotid intima media thickness (a marker of atherosclerosis), which was actually greater than that for systolic blood pressure (Franklin, 1997b; Zanchetti, 1998). In a longitudinal study following intra-arterial ambulatory blood pressure recording, mean pulse pressure and systolic (but not diastolic) pressure were significantly correlated with left ventricular mass index and markers of carotid artery atherosclerosis. After stepwise regression, the mean pulse pressure was the strongest independent predictor of left ventricular mass index and carotid intima media thickness (Khattar, 1997). Carotid intima thickness is not only significantly related to pulse pressure, but also to fluctuation in pulse pressure as measured by its standard deviation (Mancia, 2001; Mancia, 1996).

Other studies report that the cardiovascular morbidity associated with increasing pulse pressure is independent of systolic and diastolic pressure (Benetos, 1997; Verdecchia, 1998). Data from the PIUMA study in Italy (Verdecchia, 1998), a cohort of 2010 untreated hypertensive patients followed up for a mean of 3.8 years, demonstrated ambulatory pulse pressure was a potent independent predictor of cardiovascular risk. Across the tertiles of mean 24 hour pulse pressure, the rates of cardiovascular events were 1.19, 1.81 and 4.92 per 100 person years, and 0.11, 0.17 and 1.23 for cardiovascular mortality ($p < 0.01$). The authors did

note the additional predictive value of ambulatory compared to office blood pressure was not clear-cut in the category of cardiovascular mortality. Further PIUMA data analysis revealed that for each 10 mmHg increase in 24 hour pulse pressure, there was an independent 35% increase in cardiac event risk (95% CI 17 to 55%) but mean blood pressure did not contribute to risk (Verdecchia, 2001). Another longitudinal study using intra-arterial ambulatory blood pressure recording found a significantly higher pulse pressure in time to first cardiovascular event or death in 688 patients with hypertension (Khattar, 1999).

3.3.7 Repeatability

Blood pressure variability tends to be only moderately reproducible at best. This is probably due to variation in activities from day to day, and reproducibility does improve when conditions are closely controlled. Gerin et al (Gerin, 1993) performed one 4 hour recording of ambulatory BP on consecutive days (daylight hours), with measurements at 5 minute intervals. They note better results than other reproducibility studies but correlation coefficients are still only moderate.

Table 3-4 Reproducibility of blood pressure variability (Gerin et al 1993)

	Systolic blood pressure	Diastolic blood pressure
Mean level	0.86**	0.76**
Standard deviation	0.52*	0.63**
RMSSD	0.60**	0.49*

From Gerin et al 1993 (Gerin, 1993). RMSSD = root mean square of successive differences

*P < 0.01 **P < 0.001

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3.3.7.1 Mean ambulatory blood pressure

Frequency of sampling influences reproducibility. A minimum frequency of twenty minutes in the day period results in satisfactory reproducibility (Palatini, 1994). Mean ambulatory blood pressure is significantly more reproducible than office blood pressure measurement in older patients both with and without hypertension (Fotherby and Potter, 1993). Coefficient of variation for mean pressure in tests separated by 4-10 weeks is approximately 4 – 6% (Robinson, 1995; Weston, 1996b).

Duration of monitoring influences results from intra-arterial recording, with marked differences between short periods of several hours and the standard 24 hour period. Some reports conclude 6 hours is adequate to describe the mean value (di Rienzo, 1983) whereas others state at least 12 hours recording are required to achieve results similar to the 24 hour mean (Di Rienzo, 1985). Clearly the inclusion of the sleep period will decide how closely short recordings mirror 24 hour averages.

3.3.7.2 *Day-night variation*

Reproducibility of the circadian variation in blood pressure is poor (Staessen, 1992). Fixed time technique that uses all hours in a 24 hour period has two disadvantages. Firstly it can lead to large swings in non-dipper status by broadening the duration of the night period. Secondly it will reduce week-to-week reproducibility (Wong Chung, 1991).

Three statistical techniques for calculating the circadian variation were evaluated using data from 32 young healthy volunteers on two occasions at least 4 weeks apart (Weston, 1996b). Mean blood pressure was reproducible with a coefficient of variation of 4.7%. Fixed times (07:00 to 22:00 and 22:00 to 07:00) returned systolic and diastolic day-night differences CVs of 52 and 59% respectively. This only marginally improved using diary based day-night periods to 40% and 41% but there was a moderate improvement using cusum analysis, with CVs of 24.6% and 28.1%.

A similar examination of performance but using additional 'narrow' fixed time periods was performed in older hypertensive patients. The results for the gold standard diary technique and the narrow fixed time period are shown in Tables 3.5 and 3.6.

Table 3-5 Comparison and reproducibility of day and night mean systolic blood pressure assessed by fixed time (10-20:00 and 00-06:00) and diary method (Robinson et al 1995) (Robinson, 1995)

	Day				Night			
	Visit 1	Visit 2	Sdd	CV (%)	Visit 1	Visit 2	Sdd	CV (%)
Fixed time	132 (17)	133 (14)	10.3	7.8	113 (19)	113 (19)	8.2	7.2
Diary	132 (17)	131 (15)	8.1	6.2	114 (19)	112 (18)	7.1	6.3

Sdd, standard deviation of the within subject differences: CV, coefficient of variation

Table 3-6 Comparison and reproducibility of diurnal variation in systolic blood pressure assessed by fixed time (10-20:00 and 00-06:00) and diary methods (Robinson et al 1995) (Robinson, 1995)

		Visit 1	Visit 2	Sdd	CV (%)
Diurnal SBP change (mmHg)	Fixed time	18.8 (9.9)	19.7 (13.8)	10.3	53.2
	Diary	17.6 (8.4)	18.7 (10.8)	7.0	38.6
Diurnal SBP change (%)	Fixed time	14.4 (7.0)	14.9 (10.0)	7.2	49.0
	Diary	13.5 (5.9)	14.4 (7.8)	5.2	37.0

Adapted from Robinson T 1995(Robinson, 1995). Values are mean (standard deviation) in mmHg unless otherwise stated. Sdd, standard deviation of the within subject differences: CV, coefficient of variation

It can be seen from Table 3.5 that the two methods gave comparable average systolic values for the day and night period. Whilst CV was not as quite as good as diary-derived time period.

degree of reproducibility was similar for the two methods. From Table 3.6, both fixed time and diary methods for reporting diurnal systolic variation had poor reproducibility. Reproducibility was not substantially improved by cusum-derived techniques (Robinson, 1995).

3.3.7.3 Long term variability

One report indicates that for blood pressure variability i.e. standard deviation of mean to be reproducible, sampling frequency in daytime needs to be a minimum of 20 minutes (Palatini, 1994).

3.3.8 Age and blood pressure variability

Variability tends to increase with age (Bertinieri, 2002; Mancia, 1983b; Parati, 1997a; Parati, 1995a). In a small study of eight elderly subjects (mean age 64 years) and eight young subjects (mean age 24 years), 24 hour long term variability was significantly higher in old than young subjects, but there was no difference for diastolic blood pressure variability. Other studies clearly shown a decrease in baroreflex sensitivity with age, and one in particular found the marked increase in baroreflex sensitivity at night in young subjects was not observed in older subjects (Parati, 1995a). This effect is likely to be related to firstly, increasing large artery stiffness that results in larger pressure fluctuation at a set stroke volume, and secondly the reduction in baroreflex sensitivity with age (Parati, 1997b; Parati, 1995a).

Parati et al (Parati, 1997b) examined the pattern of changes affecting systolic blood pressure variability in the elderly compared with middle aged controls. Continuous non-invasive finger pressure monitoring was used to produce spectral bands for heart rate and blood pressure variability. In contrast to the global decrease in heart rate variability affecting all power bands in the elderly group, their blood pressure power spectra changes were concentrated in the very low frequency range with little age difference for the low and high frequency bands.

Franklin hypothesises there are three phases in the linear increase of systolic blood pressure with age (Franklin, 1997a). A gradual increase in diastolic pressure in early to middle age suggests a key role for increasing vascular resistance. Diastolic pressure is steady during the sixth decade and mean arterial pressure displays an asymptotic leveling but pulse pressure has an increased slope: these factors point towards increased vascular resistance and large artery stiffness gaining in a parallel fashion. Finally the drop-off in diastolic pressure in older age indicates the predominance of large artery stiffness driving the associated rise in systolic pressure.

There is a weak but significant inverse correlation between the nocturnal decrease in diastolic blood pressure and age ($r = -0.14$, $p = 0.006$) from a population study of 399 subjects

(Staessen, 1992). The probability of being a non-dipper increased 2.8 times (95% 2.0 - 4.0) from 30 to 60 years and 5.7 times (95% CI 4.4 – 7.4) from 60 to 80 years (Staessen, 1997a). These findings may be due to age-related changes in the circadian sleep-wake rhythm (e.g. increase in night-time wakefulness) and autonomic changes with age (Prinz, 1990).

3.3.9 Blood pressure variability and stroke

Increasing blood pressure is clearly established as a risk factor for stroke disease and other cardiovascular events (Kannel, 1996; MacMahon, 1990). Higher blood pressures are particularly associated with increasing incidence of small vessel disease, i.e. lacunar infarction and white matter hyperintensity, when comparing hypertensive patients with normotensive control subjects (Breteler, 1994c; Liao, 1996; Longstreth, 1996). This association extends to subjects who have normal range blood pressures (DeCarli, 1995).

Historically the main interest in addressing stroke risk has concentrated on average blood pressure but in the last decade, blood pressure variability has grown in importance as a predictor of outcomes in cerebrovascular disease. Ambulatory blood pressure appears to have advantages over clinic blood pressure in stroke disease, not only in predicting stroke incidence but in the evaluation of secondary risks encountered by stroke survivors.

MRI abnormalities in the elderly are associated with elevated mean ambulatory blood pressure and diminished circadian variation (Kawamoto, 1991). In a cross-sectional study, Sander et al (Sander, 2000b) found a significant correlation between systolic (but not diastolic) circadian blood pressure variation and the extent of white matter lesions. Multivariate linear regression found systolic circadian blood pressure variation and age as the only significant factors contributing to white matter lesion burden. This study indicated blood pressure variation was more important than mean level.

Kukla et al (Kukla, 1998) studied 118 patients older over 55 years old, 61 with a history of lacunar infarction and 57 controls. Thirty-three patients had MRI and 85 CT imaging, with number of lacunae recorded by visual inspection. Non-invasive ambulatory blood pressure monitoring was performed at 15 minute intervals over 24 hours. Daytime and nighttime were classed as 06:00 to 22:00 and 22:00 to 06:00 respectively. Circadian variation was defined as average percentage change of nighttime/daytime blood pressure. Patients with lacunar infarction had higher mean blood pressures, an increase in daytime systolic blood pressure variability and a decrease in diurnal variation. Following logistic regression, age, reduced systolic diurnal variation, mean daytime systolic pressure and history of hypertension were significantly related to the presence of lacunar infarction.

Goldstein et al (Goldstein, 1998) studied 144 healthy individuals aged 55 to 79 years old. Non-invasive ambulatory blood pressure monitoring obtained measurements randomly three

times per hour in waking hours and hourly overnight. MRI images were rated visually using an in-house scale of zero to four. Significant associations with increasing white matter hyperintensity burden were found for awake and asleep systolic levels, awake diastolic levels, awake systolic variability (long term i.e. standard deviation) and for reduction in circadian rhythm for both systolic and diastolic pressure.

One study did not find a significant correlation between blood pressure variability and small vessel disease but suffers from small numbers (12 and 13 in each group) and use of a non-validated white matter rating scale (Marti-Fabregas, 2001). Diurnal variation is diminished in the acute post-stroke phase (Kario and Shimada, 1994).

A study by Kario et al (Kario, 1996) included 131 hypertensive patients older than 60 years, with non-invasive blood pressure measurement sampling every 30 minutes for 24 hours and MRI films rated by number of lacunae and severity of periventricular hyperintensities. Both measures of 'silent' cerebrovascular disease were substantially greater in the extreme dippers and non-dippers compared with patients with normal diurnal variation.

The Atherosclerosis Risk in Communities is the only study that has examined the relationship between pulse pressure and cerebrovascular small vessel disease (Liao, 1997). Blood pressure values were obtained from the mean of the second and third office readings in 1920 adults aged 55 to 72 years. White matter lesions on a 1.5T MRI scan were rated on a scale from 0 (none) to 9 (severe) but the authors did perform some post-hoc reclassification into 4 groups, since ratings of 4 or more were rare, and a binary system (0-3, >3) was used for calculating odds ratios. White matter lesion severity was significantly and positively associated with systolic blood pressure and pulse pressure, and weakly associated with diastolic pressure. Following subgroup analysis, these trends were most evident in African Americans (49% of the total cohort) compared with European Americans.

However, one large epidemiological study (Benetos, 1997) measuring office pulse pressure in over 19,000 men aged 40 to 69 years reported slightly contrasting results, using a different cerebrovascular outcome measure. In subjects with normal or elevated mean arterial pressure, increased pulse pressure was a strong predictor of general and *cardiovascular* mortality affecting in particular the coronary circulation, but no significant risk occurred with respect to *stroke* mortality. Furthermore, the interaction between mean pressure and pulse pressure was usually additive but in older subjects this changed to a significant negative interaction. The main conclusion with respect to cerebrovascular disease was that mean blood pressure was a significant predictor of cerebrovascular mortality but increased pulse pressure did not carry significant risk for the cerebral circulation.

From the Systolic Hypertension in the Elderly Program (SHEP) study of 4736 elderly patients with isolated systolic hypertension (Domanski, 1999), higher pulse pressure was associated with an increase in systolic and decrease in diastolic pressure. In a multivariate analysis for each 10 mmHg increase in pulse pressure there was an 11% (95% CI 1% - 22%, p = 0.028) increase in stroke risk. The authors discuss how pulse pressure is a surrogate for conduit vessel stiffness, which mechanistically is linked to stroke by potentiating carotid artery disease and contributing to small vessel disease.

Results from ambulatory blood pressure studies have led to the idea of a J curve relationship between blood pressure level and stroke risk in elderly people (Kario and Pickering, 2000a). A sub-analysis from the Systolic Hypertension in the Elderly Program and an observational study of treated hypertensives found increased cardiovascular risk with lower diastolic blood pressure (Somes, 1999). Kario (Kario and Pickering, 2000b) links these results and another study reporting lower hazard ratio of stroke with higher blood pressure in those patients with tight carotid stenoses (0.52, 95% CI 0.19 – 1.40 (Silvestrini, 2000)), to support the hypothesis that in some individuals, a higher blood pressure is protective by maintaining cerebral perfusion.

Another strand in this argument is that silent myocardial ischaemia can increase in extreme dippers when treated with anti-hypertensive drugs (Pierdomenico, 1998). Such patients may benefit from ambulatory blood pressure monitoring to guide anti-hypertensive use (Kario and Pickering, 2000b). At this point, it is worth re-iterating meta-analysis results to confirm the value of intervention in the majority of patients with high blood pressure. Anti-hypertensive treatment clearly reduces stroke, major cardiovascular events and heart failure in the elderly, but the same study also highlighted no treatment benefit for cardiovascular death and a non-significant excess of all-cause death in the treatment group (Gueyffier, 1999).

Most recently Kario et al (Kario, 2001) report increased stroke incidence with abnormal diurnal variation in a large, prospective study. This study followed 575 Japanese patients for a mean of 41 months. Non-invasive blood pressure monitoring sampled every 30 minutes for 24 hours, and 361 patients had MRI scans which provided data on silent cerebral ischaemic lesions (lacunes < 15 mm). Again they demonstrate that reverse dippers (i.e. day-to-night systolic pressure <0%) and extreme dippers (day-to-night systolic pressure >20%) had a significant excess of silent cerebrovascular disease on MRI. Furthermore abnormal circadian rhythm displayed a J-shaped curve for stroke incidence. Reverse dippers had the worst stroke incidence (22%), and extreme dippers the second worst incidence (12%) when compared with normal dippers (6%). Haemorrhagic stroke was significantly higher in reverse dippers whereas there was a tendency for more ischaemic stroke as percentage dip increased. Dipping status was revealed as an independent predictor of stroke incidence following Cox regression

analysis. Their discussion suggests excessively low nocturnal blood pressure could be particularly important with respect to the hypothesis of hypoperfusion and cerebral damage. However they also note that 55% of strokes in the extreme dippers occurred between in the morning period from 06:00 to 12:00 hours. Other prospective studies demonstrate non-dipping pattern is an independent predictor for cardiovascular disease (Verdecchia, 1994).

Kario's cut-off of > 20% diurnal variation in blood pressure constituting 'extreme dipping' is an arbitrary choice. In the international database study (Staessen, 1997a), the 5th systolic and diastolic percentiles in the normotensive population were -30.2 and -25.2 mmHg respectively, which translated to percentage falls of 23.4% and 31.3%. These values are logical choice, based on a very large study of normal adults.

3.3.10 Pulse pressure, diastolic pressure and cardiovascular risk – a J shape curve?

There is a continuing debate in the literature on the nature of the curve describing the association between the principal blood pressure measures i.e. systolic and diastolic values and various cardiovascular outcomes. Some of these papers are described below, with most of the data obtained from office blood pressure measurement and not ambulatory recordings.

Data from the Medical Research Council (MRC) Mild Hypertension Trial was examined using an empirical linear model (office blood pressure measurement) (Millar and Lever, 2000). Results suggest lower diastolic pressure has different effects on the cerebral and coronary circulation. Stroke risk increased progressively with increasing diastolic pressure but a J shape curve was obtained for coronary events, with perhaps the lowest risk at 70 to 90 mmHg below which coronary risk increases. The authors suggest coronary risk is best predicted by pulse pressure whereas stroke risk is best predicted by mean pressure.

There is further evidence that pulse pressure may not be the optimal predictor for cerebrovascular events. In a large population study, pulse pressure predicted cardiovascular but not stroke mortality (Benetos, 1997). Similar data comes from the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) (Verdecchia, 2001) longitudinal study of 2311 hypertensive subjects, using 24 hour ambulatory blood pressure. Pulse pressure but not mean pressure independently predicted coronary events, whereas mean pressure (and not pulse pressure) independently predicted stroke events. For each 10 mmHg increase in mean pressure, cerebrovascular event risk increased by 42% (95% CI 19 to 69%). An intervention study, the European Working Party on Hypertension in the Elderly (EWPHE) (Amery, 1985), demonstrated a similar pattern. Stroke was independently predicted by mean BP (HR 1.91, 95% CI 1.05 to 2.18) but not pulse pressure (HR 1.10 95% CI 0.90 to 1.36). Coronary events were not predicted by mean BP (HR 1.09 95% CI 0.77 to 1.35). A Japanese cohort had a

similar significant risk of stroke with increase in mean blood pressure but not with increasing pulse pressure (Ohkubo, 2001).

In contrast, a meta-analysis of EWPHE, Syst-Eur and Syst-China studies found that pulse pressure did predict both cerebral and coronary events after adjusting for mean pressure. However this effect was mainly confined to the two studies that recruited patients with isolated systolic hypertension (Blacher, 2000). In the SHEP study, both mean pressure and pulse pressure were significant predictors of stroke, but the risk was proportionately higher for a set increase in mean pressure compared with pulse pressure (20% and 11 % respectively. for each 10 mmHg pressure increase) (Domanski, 1999).

Large scale population based studies have commented on the possibility of a J-shaped curve for the relationship between blood pressure and adverse outcomes. An Italian study recruited 3858 people aged 65 years or older who presented to the GP for any reason. They found that systolic but not diastolic pressure was associated with total and cardiovascular mortality: impact on stroke was not addressed (Alli, 1999). A population based cohort of 6927 inhabitants of Rotterdam found the risk of stroke was linearly related to both increasing systolic and diastolic pressure. However in treated hypertensives, a J-shaped curve existed for both systolic blood pressure and diastolic blood pressure for stroke events with a statistically significant increase in risk in the lowest diastolic pressure category (Voko, 1999). A meta-analysis of eight studies investigating isolated systolic hypertension (treated or untreated) inpatients older than 60 years was performed by Staessen at al (Staessen, 2000). They revealed risk of death was directly associated with systolic blood pressure and inversely associated with diastolic blood pressure (but the latter finding did not apply to stroke incidence in isolation). Glynn et al (Glynn, 2000) did not examine stroke outcome but do report that both systolic and diastolic pressure provide independent prognostic information on risk of cardiovascular and total mortality, but the association of diastolic pressure is largely explained by the confounding effects of frailty and co-morbidity. Additionally pulse pressure appeared to be the best single blood pressure parameter as a marker of mortality risk. Ohkubo et al (Ohkubo, 1997) also found that the association of the lowest quintile of ambulatory blood pressure values with increased mortality was related to deaths due to malignancy i.e. that concurrent illness lowered the blood pressure and not that the low blood pressure was the primary event with regard to cause of death.

There are consistencies and contrasting results from large-scale longitudinal studies. Khattar et al (Khattar, 1999) reported a linear relationship between diastolic pressure and stroke events but a plateau effect was observed in lower diastolic pressures with respect to coronary events. Intervention trial data has been used to discount the theory of a J-shaped curve (Rodgers, 1996). A definitive study from the Cardiovascular Health Study in North America

highlighted the influence of cohort selection on the relationship between systolic, diastolic and pulse pressure and cardiovascular events (Psaty, 2001). They examined a population cohort of 4902 men and women aged 65 years and above. In this cohort there was no suggestion of a J-shaped relationship for any blood pressure parameter and cardiovascular disease. All three measures alone were directly associated with the risk of incident myocardial infarction and stroke. For stroke the hazard ratios were 1.34 for systolic (95% CI 1.21 to 1.47), 1.29 for diastolic (1.17 to 1.42) and 1.21 for pulse pressure (1.10 to 1.34). Systolic pressure was the best predictor for stroke but any two of the three parameters improved the model fit: for myocardial infarction, pulse pressure did not improve the fit of the model including systolic pressure alone. Psaty comments that pulse pressure is not commonly employed by clinicians and holds no advantage over the traditional systolic and diastolic measures of blood pressure (Psaty, 2001). Countering this assertion, Glynn argues that systolic and diastolic contribute independent information and the best single measure of blood pressure to predict mortality is pulse pressure (Glynn, 2000).

Aortic stiffness, the main determinant of pulse pressure, is an independent predictor of fatal stroke in hypertension. In 1715 hypertensive patients, pulse wave velocity remained a significant predictor for fatal stroke after controlling for other cardiovascular risk factors: pulse pressure was a significant predictor in univariate analysis but lost significance after adjusting for age (Laurent, 2003). The authors hypothesise aortic stiffness may mediate its adverse effect by increasing pulse pressure which generates stressful cyclic stretching. Subsequent arterial re-modeling is associated with carotid atherosclerosis, risk of plaque rupture and white matter lesion burden.

Age is an important factor in the interplay between blood pressure parameters and stroke risk. A cohort of 126 hypertensive Japanese patients who suffered a stroke had ambulatory blood pressure data available from one year preceding stroke incidence (Makino, 2000). Blood pressure data was compared with age- and sex-matched hypertensive patients who remained free of stroke. Mean blood pressure and pulse pressure was higher in the stroke group aged less than 70 years but there was no difference in these values for the stroke patients aged over 70 years. The authors note this is an unusual finding and discuss how the older stroke patients may not be representative of more typical patients.

Bilateral severe carotid stenosis constitutes a subgroup of stroke patients for whom blood pressure lowering can have adverse consequences. The linear risk for stroke from increasing systolic or diastolic pressure held true for the UK-TIA, European Carotid Surgery and North American Symptomatic Carotid Endarterectomy Trials when considered as one group (Rothwell, 2003). Increasing pulse pressure only held a significant positive linear relationship in the UK-TIA trial. However the group with bilateral > 70% stenosis changed the association

to a significant negative relationship between stroke risk and pulse pressure (HR 0.43, 95% CI 0.20 to 0.91, p = 0.02), systolic pressure (HR 0.41, p = 0.02) or diastolic pressure (HR 0.58 p=0.19). Compared with patients with both stenoses < 70% , the relative hazard of stroke in patients with bilateral > 70% stenoses was 2.54 at systolic pressures of 130 to 149 mmHg and 5.97 at systolic pressures less than 130 mmHg (both $p \leq 0.001$). The mechanism is probably linked to impaired cerebral perfusion and loss of normal cerebral autoregulation with tight bilateral stenoses (Kistler, 1984). Rothwell et al (Rothwell, 2003) also highlighted that patients with unilateral stenosis are not exposed to the same risk (in line with their much lower prevalence of impaired cerebral perfusion, 5%) and secondly that severe bilateral stenosis is uncommon with a prevalence of approximately 1 to 2%.

Another issue in this debate is the timing and vigour of blood pressure lowering after stroke. In spite of the strength of the overall association for hypertension and stroke risk, blood pressure reduction should proceed gradually. Rapid systemic blood pressure reduction may challenge the already compromised cerebral autoregulation beyond its limits. Transient ischaemia or infarction could follow, leading to worse outcomes in those treated cases (Staessen and Wang, 2001).

3.3.11 Blood pressure variability and cognition

Belleli et al (Bellelli, 2002) stratified 34 elderly (mean age approximately 75 years) into three groups according to MMSE score and found that increasing values of 24 hour BP variability were significantly associated with lower cognitive performance. Systolic Hypertension in Europe (Syst-Eur) Trial patients' blood pressure variability was measured by the standard deviation of six office BP readings over three visits (Birkenhager, 2002). From 2902 patients, 64 developed dementia. Blood pressure variability tended to be positively correlated with the dementia risk. Each 5 mmHg increase in systolic BP variability was associated with a 15 % higher risk of dementia (95% CI 0.90 to 1.47) and each 2 mmHg increase in diastolic BP variability was associated with a 17% higher risk (95% CI 0.96 to 1.42).

An uncontrolled study of 88 Japanese patients mean age 71 found an association between blood pressure variability and the Ravens Coloured Matrices Test which assesses judgment through visual information processing (Kanemaru, 2001).

3.3.12 Blood pressure variability in atrial fibrillation

There are few studies examining the impact of atrial fibrillation (AF) on ambulatory blood pressure. Some question the validity of using ambulatory blood pressure monitoring in atrial fibrillation because of technical factors that make measurement problematic and introduce larger margins of error compared with sinus rhythm (Stewart, 1995). But office sphygmomanometry-based blood pressure readings can also be inconsistent in AF due to the

erratic nature of Korotkov sounds in AF (Sykes, 1990). Ambulatory monitoring should provide an advantage by producing an average of multiple measurements. A study by Lip et al (Lip, 1996) proved the feasibility of ambulatory recording in AF. There was a high percentage of successful recordings at 80%, a figure not too different to sinus rhythm. Furthermore the first measurement on the ambulatory recorders matched the mean of two manual blood pressure measurements for systolic (but not diastolic) pressure. Another report suggests that validated monitors have a satisfactory frequency response to provide an adequate measurement (O'Brien, 1990).

There is evidence that accurate information can be obtained by ambulatory blood pressure monitoring in atrial fibrillation. From 42 patients undergoing cardioversion for atrial fibrillation, 22 completed before and after 24 hour ambulatory blood pressure recordings (Olsen, 2002). In the group of 22, 12 reverted to sinus rhythm whilst 10 remained in atrial fibrillation. There were no significant differences in mean systolic and diastolic levels for both sinus rhythm and atrial fibrillation groups before and after cardioversion. Blood pressure variability and repeatability attained similar levels in both in both groups (SD/mean).

3.3.13 Conclusions

Blood pressure is an important risk factor for cardiovascular outcomes. Ambulatory blood pressure monitoring provides information about the variation in blood pressure (i) between measurements over a period of 24 hours and (ii) between day and night periods. Long-term variability and circadian variation are independent prognostic markers for cerebrovascular disease. Increasing long-term variability significantly increases white matter lesions, atherosclerotic disease and mortality. Reversal of the normal dip in blood pressure from day to night period is associated with cerebrovascular disease; some investigators have found an association between exaggerated drop in nocturnal blood pressure and white matter disease. One could hypothesise these markers could affect cognitive function in elderly stroke survivors.

Increasing blood pressure is firmly established as a risk factor for stroke. Systolic blood pressure bears the strongest relationship for adverse outcome. Increasing diastolic blood pressure is usually associated with increasing cardiovascular disease. Some studies indicate low diastolic blood pressure carries higher risk than intermediate levels, but this is not a universal finding and may be explained by severe co-morbidity associated with the lowest levels of diastolic blood pressure. However in patients with severe bilateral carotid stenosis, decreasing blood pressure does exacerbate cerebral damage. A similar mechanism could drive the white matter disease since cerebral white matter flow may be critically impaired in the arteriosclerotic conditions.

4 Methodology

4.1 Case selection

The purpose of case selection was to find elderly patients living in the local area that had suffered a stroke and were able to attend for a series of hospital-based investigations. Cases were selected from the Cognitive Function After Stroke (COGFAST) Study. COGFAST is an observational and longitudinal study of cognitive function in older stroke patients. The key purpose of COGFAST is to determine the neuropathological substrate of post-stroke dementia. Patients have an annual neuropsychometric assessment and in those cases who give consent for brain tissue donation, key neuropathological changes will be interpreted in the light of emerging post-stroke cognitive deficits.

Cases were recruited from patients presenting to hospital. Collaborative links were established with consultants in Geriatric Medicine with special interest in Stroke Medicine. Systems to screen hospital stroke admissions were established: in most cases hospitals had existing stroke registers that provided patient demographic details. Cases aged 75 years and over were identified and approached to determine willingness to participate in COGFAST. At a later date, those cases consenting to COGFAST were asked to participate in the cardiovascular and MRI investigations. This secondary process was enabled to a large degree by study personnel obtaining further information on patient suitability for the study from the initial screen that provided cognitive function and physical health data. Thus information influencing inclusion criteria was obtained in a series of stages. There were minor variations in the process according to hospital site owing to differences in content of different stroke registers and information available to study investigators. The main study inclusion and exclusion criteria will be described. Then a summary of the selection process according to each hospital stroke register will be discussed.

4.1.1 Inclusion criteria

4.1.1.1 Age 75 years old and above

The minimum age for incident stroke was set at 75 years. The primary aim of COGFAST is to investigate the neuropathology of late-onset dementia; therefore by definition the study required older persons who have survived a stroke. Cut-off age was influenced by the aim of obtaining tissue donation for neuropathological studies. An older cohort meant that a smaller number of patients were required to obtain the required number of brain tissue donations for the main study purpose.

4.1.1.2 Stroke

The subject has suffered a stroke according to World Health Organisation criteria as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin" (Hatano, 1976). The diagnosis was confirmed by a consultant physician (with an interest in stroke medicine) responsible for the local hospital's stroke register. Both infarcts and haemorrhagic strokes were included. Cases with subarachnoid haemorrhage were excluded from the study. The presence of a patient on the stroke register was relied on as a measure of certainty of stroke diagnosis. In other words it was assumed that a person had suffered a stroke, according to the WHO definition, if the collaborating hospital had assigned a diagnosis of stroke. A large number of individuals were involved in case selection. Inter-rater reliability analysis was not feasible. However all patients were assessed at some stage by a consultants with a special interest in stroke. All hospitals used the Oxfordshire Community Stroke Project (OSCP) classification scheme (Bamford, 1991). This is a purely clinically based classification system, relying entirely on stroke symptoms and signs at the time of maximal deficit from the presenting event. Using the OCSP system, the stroke is assigned to one of four groups.

Lacunar stroke (LACS)

- Pure motor stroke, pure sensory stroke, sensori-motor stroke or ataxic hemiparesis.

Total anterior circulation infarcts (TACS)

The following signs in combination

- New higher cerebral dysfunction e.g., dysphasia, dyscalculia, visuospatial disorder
- Homonymous visual field defect
- Ipsilateral motor and/or sensory deficit of at least two areas of the face, arm and leg. If the conscious level is impaired and formal testing of visual fields or higher cerebral function is not possible, a deficit should be assumed

Partial anterior circulation infarcts (PACS)

Patients could fulfil any one of the following three criteria

- Only two of the three components of the TACI syndrome
- Higher cerebral dysfunction alone
- A motor or sensory deficit more restricted than those classified as LACI e.g. confined to one limb or to face and hand but not involving the whole limb

Posterior circulation infarcts (POCS)

Any of the following criteria.

- Ipsilateral cranial nerve palsy with contralateral motor and/or sensory deficit
- Bilateral motor and/or sensory deficit
- Disorder of conjugate eye movements
- Cerebellar dysfunction without ipsilateral long tract deficit
- Isolated homonymous visual field defect

The classification scheme is quick and practical, simple to apply in trained operators and does not require any formal investigations. It has moderate interobserver agreement ($\kappa = 0.54$, 95% confidence interval 0.39 to 0.68) (Lindley, 1993). The four groups have distinctive features, most importantly in natural history (Bamford, 1991). Each group has theoretically distinct pathophysiological mechanisms and does in practice provide an indication of stroke cause (Lindgren, 1994; Mead, 2000b; Tei, 1999). Total anterior circulation infarcts result from occlusion of the middle cerebral artery, either by embolism or thrombosis. Partial anterior circulation infarcts are usually due to embolic occlusion of branches of the middle cerebral artery. Lacunar infarction is usually due to thrombotic occlusion of the deep perforators (arising from the middle cerebral artery) that supply deep hemispheric structures. The OCSP classification can successfully predict the site of infarct in those with a visible lesion in approximately three quarters of patients (Mead, 2000a). The OCSP classification does have limitations. If the ischaemic lesion changes in the initial post-stroke phase, the clinical signs can change leading to a change in classification category. For example a large vessel partial occlusion may initially present with only limb weakness and appear as a lacunar stroke but if the occlusion becomes complete, the full cortical deficit becomes apparent and is revealed as a total anterior circulation infarct. Conversely partial anterior circulation infarcts that rapidly improve may only present as a lacunar stroke if clinical assessment is delayed. Accurate categorisation is highly dependent on clinical skills – inter-rater reliability for signs such as visual field assessment can be poor.

The OSCP classification was obtained from original hospital case notes. The opinion of the most senior doctor trained in stroke medicine was used to classify each case. If there was a discrepancy between two or more senior clinicians' classification, the judgement of the author was used to classify the case: training in stroke assessment took place during clinical training including certification in National Institute of Health stroke scale (Brott, 1989). The judgement decision was based on degree of effort to all ascertain all possible stroke deficits, optimal timing (usually early during admission at time of maximum deficit) and the individuals' experience in stroke medicine.

4.1.1.3 Physical health

The subject had to be in good physical health. They had to be independently mobile and physically capable of completing cardiovascular autonomic tests and the MRI scan. If a

Barthel Index score was available, a score of 10 and above was required: initially this was set as an inclusion criterion but not all hospital stroke registers were able to provide this information. In most cases, a pragmatic decision on individual's suitability was taken during either during case screening at the initial COGFAST assessment or at the second phase of consent by the author.

4.1.2 Exclusion criteria

4.1.2.1 Co-morbidity

Any cases with significant cardiac, respiratory, renal or liver failure were excluded. Similarly any cases with significant chronic illness that resulted in shortened life-expectancy were excluded. Cases with any active malignant disease were excluded. Significant visual impairment led to exclusion from the study, since poor vision would interfere with cognitive assessment.

4.1.2.2 Cognitive function

All subjects with dementia were excluded. Some cases were excluded on this basis on the initial hospital case register screen where there was sufficient information available on cognitive function. Otherwise the subject was interviewed usually with a next of kin and the Mini-Mental State Examination (MMSE) completed during screening for COGFAST at the subjects home (Folstein, 1975). The subject was excluded if the MMSE was less than 24 and had dementia according to DSM IV criteria (American Psychiatric Association, 1994).

The MMSE is a 30 point test of cognitive function conducted by interview. The MMSE was originally devised to differentiate organic from functional organic disorder and was able to quantify serial change in cognitive performance. It is now widely used as a screening tool and measure of cognitive change. The scale has been comprehensively reviewed: test-retest reliability co-efficient was 0.89, and an amalgamation of test-retest and inter-rater reliability co-efficients was 0.83 (Tombaugh and McIntyre, 1992). The questions mostly require verbal responses with some written responses. The MMSE assesses orientation to time and place (10 points), registration of three words (3 points), attention and calculation (5 points), recall of three words (3 points) language (8 points) and visual construction (1 point). It is a simple and relatively quick assessment of cognitive function originally intended to differentiate organic from functional disorders and to monitor clinical change. It is widely used as a general screening tool for cognitive function but may not detect frontal lobe dysfunction. As previously discussed, it was devised at a time when the concept of dementia was modelled on Alzheimer's disease and is not ideally suited to detecting some of the neuropsychological deficits typical of vascular dementia.

DSM IV criteria for vascular dementia are as follows (American Psychiatric Association. 1994).

- Memory loss
- Impairment in at least one other higher cortical function e.g. aphasia, apraxia, agnosia, executive function
- Decline in social or occupational functioning
- Focal neurological signs and symptoms or laboratory evidence of cerebrovascular disease
- Exclusion of delirium

These criteria were checked at the initial phase of case screening by trained interviewers.

4.1.2.3 Trial participation

At the request of collaborating physicians, any patients who were actively engaged in other drug trials or clinical studies were not approached for COGFAST, at the request of collaborating clinicians and in order not to burden patients with numerous clinical study commitments.

4.1.3 Hospital stroke registers and screening

The information held on each stroke register slightly varied between hospitals, as did the protocol (as agreed with local investigators) for contacting cases to obtain consent for the study. However, once basic contact information was obtained, the case selection process did follow a common format. The investigators contacted all candidates by telephone or letter to explain the study and request an appointment to visit the person's home with a next of kin present. At the home interview, an MMSE was performed and the study was explained in detail. Written information on the study was provided. At this stage, consent was sought to recruit to COGFAST. The neuropsychometric schedule was performed at subsequent domiciliary visits. The protocol of neuropsychological tests is described in detail below (section 4.4). The cases consenting to COGFAST were contacted by telephone at a later date to discuss the cardiovascular and MRI investigations. Those cases willing to discuss these investigations were seen at home to explain the cardiovascular and MRI tests in detail. Candidates were assessed regarding suitability for investigations, with respect to physical health and ability to complete the schedule. Safety regarding the MRI scan was checked using the standard protocol used by the Nuffield Hospital where scanning took place. This was a series of questions to ask the candidate if there was any history of accidental injury with metallic objects to soft tissue or surgical procedures for implantation of soft tissue metallic prostheses e.g. cardiac valves, arterial clips or stents, cochlear implants. If any such injury or implantation could not be confidently excluded, then the candidate was excluded from the

MRI scan study. If there were no contra-indications to MRI and consent was obtained, the subject was given dates for firstly the cardiovascular autonomic assessment and secondly the MRI brain scan. The MRI scanning protocol is discussed in detail in below (section 4.3). Subjects were given written information regarding the tests with contact details for study personnel.

4.1.3.1 Freeman Hospital

This hospital site had a computerised stroke register. COGFAST investigators received a list of all stroke cases from research staff at Freeman Road Hospital on a monthly basis. The only information available at this stage was basic demographic data. The cases were all contacted by telephone to arrange an initial COGFAST screening.

4.1.3.2 Queen Elizabeth Hospital, Gateshead

The computerised stroke register was screened by the author to identify cases. This register held information on cognitive function in some instances, which enabled some cases to be excluded from COGFAST at this stage. The register also included a Barthel index score for some individuals, therefore some cases were excluded from the cardiovascular study at this stage by virtue of impaired physical function. All other potential cases were initially contacted by letter signed by the local hospital consultant staff. This letter briefly introduced the study and stated COGFAST investigators would contact the person at home. This served as a means of introducing the third party of the COGFAST investigators and helped to satisfy Caldicott guidance (Department of Health, 2000). These regulations cover a number of uses of patient data. With regard to research, one of the primary aims was to ensure that access to patient data was controlled by the consultant responsible for their care and to ensure that the patient had the opportunity to refuse any contact from third party agencies. The letter briefly describes the nature of the study and gave the support of the consultant staff. Patients were asked to respond only if they did not wish to be contacted.

4.1.3.3 North Tyneside General Hospital

Local research staff screened their computerised hospital stroke register to supply details of cases. They excluded any people who were or had taken part in local research trials. A letter signed by the individual's hospital consultant introduced the study prior to contact from COGFAST.

4.1.3.4 South Tyneside Hospital

The stroke register consisted of paper copies of all stroke cases. When the site was enlisted to the study, the previous year's stroke admissions were screened by me to identify candidates. Subsequently candidates were prospectively identified by the stroke specialist nurse based on

site. The information available included discharge destination and co-morbidity. Therefore some cases were excluded from consideration for the cardiovascular study at this stage. A letter from the local hospital consultant introduced the study to candidates who were then contacted by the COGFAST team.

4.1.4 Consent process

Ethical approval was sought for the study via the Local Research Ethics Committee for Newcastle Hospital Trust and Gateshead Hospital Trust. The first stage of approval in Newcastle regarded recruiting individuals to the neuropsychological and MRI part of the study. Chairman's action was sought for the cardiovascular protocol – this was obtained at a later stage, after recruitment for COGFAST had commenced.

For the Gateshead area, alterations were made to the screening and recruitment process at the request of the hospital stroke physicians. The major change was a letter of introduction to each candidate from the hospital consultant and author. The letter briefly explained the nature of the study and gave the candidate an opportunity to decline participation.

Consent for the cardiovascular and MRI study occurred in two phases. The first phase was consent for the neuropsychological tests. Candidates were contacted by telephone to arrange an appointment at the individual's home address. Some candidates refused to participate during this initial discussion. Where unable to make telephone contact, a letter was sent to the candidate with a request for a meeting. A next-of-kin was also present at this initial meeting with the COGFAST investigator at the candidate's home. The purpose of the study was explained and the nature of the tests involved. Candidates were given written information about the study and given an opportunity to consider participation, with a second interview arranged if more time was required to make a decision. The consent forms required the signature of participant and next-of-kin. The 'assent' obtained from the next-of-kin was required to satisfy ethical standards in a study where over the course of time some cases would develop significant cognitive impairment whilst participating in research tests . The next contact with consenting candidates occurred when the clinical assistant performed the neuropsychometric schedule in the subject's home. The schedule could be performed over two days if required due to subject fatigue. The schedule is described below (section 4.4).

The second phase of consent was my responsibility. Subjects were contacted by telephone to ask if they were interested in participating with cardiovascular and MRI investigations. If so, a meeting was arranged at their home address to discuss these tests in more detail. At this meeting the nature and purpose of the tests were explained, and written information provided. The subject's ability to complete the cardiovascular schedule was assessed. An outline of the investigation protocol was explained to the candidate. General points covered were the

problems with memory decline in stroke survivors and the need to investigate what causes the underlying brain damage mechanisms that lead to dementia. The risk of post-stroke dementia was discussed in broad terms in order not to create distress but that made it clear this was a significant problem requiring investigation.

Safety of the investigation protocol was discussed. All of the autonomic and blood pressure tests did not pose any health risk, except for carotid sinus massage. It was made clear that participation would entail a number of time-consuming hospital visits.

The consent process included an explanation of carotid sinus massage in the investigation of carotid sinus hypersensitivity. The subject was informed of the studies' role in examining how abnormalities of heart rate and blood pressure control may play a part in worsening cognitive function; such changes may be amenable to treatment. Carotid sinus hypersensitivity was explained as a potential treatment target. Carotid sinus massage was therefore a useful investigation but this test entailed a risk of TIA or stroke, quoted as occurring in approximately 1 in 1000 cases according to published data (Davies and Kenny, 1998). Carotid sinus massage would only be performed in cases where any carotid artery stenosis was less than 50%. This information could be obtained from any previous carotid Doppler scan. If this was not available then a carotid scan would be arranged as part of the study protocol. It was made clear to candidates they may wish to participate in the study of autonomic function tests and MRI scanning without performing the carotid sinus massage study. The requirement for a blood test as part of the study was discussed.

The MRI safety checklist was completed to exclude any cases from MRI scanning if there was a history of soft tissue metallic foreign body or prosthesis. As part of the consent process, it was explained that the MRI scan lasted for approximately 22 minutes and was a different process to the CT brain scan that subjects would have experienced at the time of the stroke. In particular the noise and claustrophobic nature of the scanner was discussed, noting that the radiographers were in constant communication with patients and the scan could be stopped at any time at the request of the patient if too distressed to continue.

The following points were emphasised during the consent process

- The cardiovascular tests were purely for the purpose of research. The information obtained would not be routinely relayed to primary or secondary care physicians, unless a previously unidentified and treatable problem was observed. It was noted that, apart from the carotid sinus massage and ambulatory blood pressure tests, in general the autonomic function tests were unlikely to influence routine clinical care.
- The decision to participate or not would in no way influence their health care.

- The only risk entailed carotid sinus massage as previously discussed. In terms of any discomfort involved, we were requesting a blood sample. The ambulatory blood pressure recording required wearing a blood pressure cuff around the upper arm for 24 hours including overnight. It was explained that the automatic cuff inflation may interfere with sleep in around half of users and can cause some discomfort during inflation.

- All travel would be arranged by the COGFAST research team, at no expense for the candidate. Individual taxis were arranged for each study investigation.

- The individual was free to withdraw from the study at any stage, without having to provide any explanation.

If consent was obtained, the subject was asked to sign individual consent forms for the cardiovascular and MRI tests.

4.1.5 Carotid duplex scanning

Carotid duplex scan results were required in those individuals who consented to CSM. The hospital radiography department was contacted to provide any information on previous carotid scanning and if available a copy of the full report was obtained. If there was no previous carotid scan, a duplex scan was arranged as part of the research study. A trained and experienced ultrasonographer performed a standard duplex scan of the carotid arteries to provide information on flow and plaque characteristics in the same manner as a routine clinical investigation. The scan report documented severity of any stenosis and the nature of atheromatous plaque in the extracranial carotid arteries plus flow characteristics in the vertebral arteries. If carotid scanning revealed evidence of carotid artery stenosis greater than 50 % or unstable plaque (soft, mixed, ulcerated, thrombosis) then carotid sinus massage was not performed.

4.2 Clinical research tests

Subjects attended the Falls and Syncope Service at the Royal Victoria Infirmary in Newcastle upon Tyne for the cardiovascular investigations. There were four sections.

- clinical history and examination
- heart rate variability
- autonomic function tests
- ambulatory blood pressure monitor

These sections will be described in detail below, and these methods apply to the investigations described in chapters 5 to 13.

4.2.1 Clinical history and examination

The subject was interviewed to check current health status and document medical history. Any relatives or carers in attendance were given the opportunity to assist with the history with the permission of the subject. The purpose was to record significant cardiovascular co-morbidity. The presence of conditions was rated as yes, no or possible. Efforts were made to record the response as yes or no; possible was only used if there was a strong indication of a clinical condition that was not proven by subsequent events. If the subject's response suggested a possible condition but on further inquiry cast doubt on the likelihood of a condition, then the response was rated as 'no'. For example if the subject thought they had angina but the chest pain did not fit the typical pattern of pain, there were no confirmatory investigations and they were not on appropriate medication, then this was rated as no. The following information was sought, with a brief description of the main question.

- Hypertension. Do you take tablets for blood pressure? Have you ever had high blood pressure?
- Myocardial infraction. Have you ever suffered a heart attack? Have you ever had severe chest pain and had clot-buster treatment in hospital?
- Angina. Have you been told you have angina, or ever suffered chest pains on exertion?
- Atrial fibrillation. Have you had problems with an irregular heart rhythm, needed treatment with digoxin or been told that you have atrial fibrillation?
- Cardiac failure. Have you been told you have heart failure or needed treatment with water tablets?
- Peripheral vascular disease. Have you suffered leg pain/cramp on exercise? Does this come on at a typical distance and get better with rest?
- Diabetes mellitus. Are you diabetic?
- Asthma. Do you have asthma? Do you use inhalers?
- Chronic obstructive pulmonary disease. Do you have chronic bronchitis or emphysema?
- Rheumatic fever. Did you have rheumatic fever as a youngster?
- Hypothyroidism. Do you have an underactive thyroid, or take thyroxine tablets? Have you ever required treatment for thyroid problems?
- Hyperthyroidism. Have you ever had an overactive thyroid?
- Stroke. How many strokes have you suffered?
- Transient ischaemic attack. Have you ever suffered a mini-stroke or TIA?

An enquiry was made for current fitness or any recent illness, and other significant chronic illness not previously covered. Current drug treatment and dose was noted.

4.2.1.1 Examination

Height and weight were recorded in metres and kilograms respectively. A brief cardiovascular examination recorded presence of elevated jugular venous pressure, added heart sounds or murmurs and ankle oedema. Added sounds on chest auscultation were noted. Blood pressure was measured using a sphygmomanometry with the subject semi-supine after a resting period. Pressures were recorded to nearest 2 mmHg and the disappearance of Korotkov V was taken as the diastolic pressure.

4.2.1.2 Clinical scales, reliability and validity

Clinical scales or instruments require assessment to ensure they perform in a reliable manner and produce results that accurately reflect the variable being measured. This process of testing reliability and validity is psychometric assessment. These concepts are summarised below (Bowling, 1998).

Reliability equates to the reproducibility and consistency of the tool. Reproducibility and repeatability seem to be used in an almost interchangeable manner in the literature. Reproducibility is the ability to produce a copy of or representation of the original form and repeatability is the ability to say or do over again, recite or to form an imitation of a previous action. Reliability informs the observer on the homogeneity of the tool and its resistance to random error. Reliability takes several forms; test-retest reliability, inter-rater reliability and internal consistency. Validity similarly has a number of facets.

4.2.1.2.1 Test-retest reliability

Assessing the stability of a measure over a period of time. Repeated measures are performed over a time period where one would not expect the tool to change. Different statistical tests are required according to the nature of data. Cohen's kappa may be used for nominal data, weighted kappa for ordinal data and Pearson's correlation coefficient for continuous data (Altman, 1991). However it has been argued that correlation is not appropriate for assessing agreement over time for a tool where intuitively one expects a good degree of association (Altman, 1991). Therefore the Bland and Altman method of confidence intervals to assess the size of the difference between scores is often preferred to correlation (Bland and Altman, 1986). Correlation could still be strong without giving an acceptably good reproduction of score. Difference between methods is plotted against the mean of each paired measurement to give a visual representation of the difference between methods. The mean and standard deviation of differences can then be plotted and a one sample t-test of the differences against zero will inform if the mean difference is significantly different from zero. The mean difference is a representation of the average bias when comparing methods. The standard deviation shows how well the methods agree for an individual and this value should give a

range of values which cover the agreement between methods i.e. ± 2 SD gives the 95% limits of agreement.

4.2.1.2.2 Inter-rater reliability

The ability of a tool to give the same score when applied by two or more raters testing the same population sample. Again kappa tests can be used. Kappa values less than 0.40 indicate poor agreement, 0.40-0.59 fair, 0.60-0.74 good and 0.75-1.00 excellent agreement. Correlation techniques have also been used but with the same weaknesses as above, so again the Bland and Altman method may be a better choice.

4.2.1.2.3 Internal consistency

A scale usually consists of a series of items that focus on a particular facet. Internal consistency is the ability of particular items to reflect only their intended facet and not the other dimensions of the object being measured. Methods include certain forms of correlation analysis and Cronbachs alpha. Cronbachs alpha is an estimate of reliability based on all possible correlations between all items within the scale. It depends on average correlation between items and the number of items in the tool. A minimum level for Cronbachs alpha has been suggested as 0.70, which implies that 70% of the measured variance is reliable and 30% is due to random error.

4.2.1.2.4 Factor structure

Questions within a tool may be aimed at different dimensions and may not necessarily correlate with each other or the total score. Factor analysis is a process of measuring how questions or items group together in measuring the dimensions (i.e. which items best account for the variance), and how consistently they do so. It will inform how items influence each other. Factor analysis is utilised in scale development to identify items that do or do not correlate with the object under assessment. Factor analysis is also used to confirm that questions tap into the intended factor and are not associated with other factors. Thresholds exist for factor analysis to suggest which factor should be retained in the tool; these are called eigenvalues and >1.5 is commonly used.

4.2.1.2.5 Validity

Validity takes different forms described below and they are not mutually exclusive. Internal validity is that which applies to the population under study, external validity is how applicable the tool is to the general population.

4.2.1.2.6 Face validity

A common-sense verdict of a scale: do the questions appear relevant and clear, according to subjective opinion?

4.2.1.2.7 Content validity

Again subjective but usually a judgement by expert panel if the tool is considered to assess all relevant dimensions of what is being measured in a balanced manner.

4.2.1.2.8 Criterion validity

Criterion validity is the satisfactory accuracy of a measure in comparison with a gold standard, or proxy measure if a gold standard does not exist. It is the correlation between the tool under assessment and the gold standard criterion measure. It can take two forms, confirmation of correct measurement of criteria which is concurrent validity, and ability to correctly predict longitudinal developments of criteria which is predictive validity.

4.2.1.2.9 Construct validity

Construct validity is a comparison between old and new measures to demonstrate measurement of the same construct, particularly useful when there is no gold standard. There are two parts; convergent validity informs about agreement with related variables and discriminant validity requires that the construct is not associated with unrelated variables.

4.2.1.2.10 Sensitivity

The ability of a tool to correctly partition the objects it sets out to identify, i.e. the ability to detect all true positives. Sensitivity describes the proportion of positives that are correctly identified by the test. Also the ability to reflect true change in the object under measurement.

4.2.1.2.11 Specificity

The ability of the tool to refute objects under assessment that do not meet the criteria to be considered a true positive i.e. the ability to minimise false positives. Specificity describes the proportion of negatives that are correctly identified by the test.

The neurological deficit was rated with the Scandinavian Neurological Stroke Scale (Scandinavian Stroke Study Group, 1985). This scale was chosen (a) for relevance in assessing functional recovery in stroke survivors months after the incident event (b) reasonable validity and (c) prior training in use by the author at the Stroke Research Unit at Gateshead Hospital, therefore familiarity was useful.

The scale was devised by the Scandinavian Stroke Study Group with the aim of being easy to use and to be of functional significance to patients (Scandinavian Stroke Study Group, 1985). Therefore it avoided items like visual field and sensory function which are difficult to assess, and chose items which are relevant to those patients making a long term recovery from stroke. It is a nine item scale with an ordinal scoring system for each item, with four items selected in the initial stage as a prognostic index, and a seven item long term score for rating recovery. The Scandinavian Neurological Stroke Scale has good interobserver reliability, with one

study reporting weighted kappa values between 0.688 to 0.912, and high correlation between 2 observers (r = +0.954) (Lindenstrom, 1991). The scale does have high predictive value for neurological status and mortality 3 months post-stroke (Scandinavian Stroke Study Group, 1987; Scandinavian Stroke Study Group, 1988). The scale does have limitations, for example inter-rater reliability can dip to moderate levels as previously mentioned. One item is based on gait and is thus a measure of disability and not impairment.

Extrapyramidal signs are frequent in old age, common causes being Parkinson's disease and associated Parkinsonian syndromes, dementia with Lewy bodies and cerebrovascular disease. Parkinsonian signs were recorded using a modified version of the Unified Parkinson's Disease Rating Scale (MUPDRS) (Fahn S, 1987). Inclusion of the MUPDRS was an attempt to quantify extrapyramidal signs in the cohort, in consideration of potential neuropathological investigations. Secondly it could highlight clinical changes of importance in subjects who cognitively decline. The MUPDRS does not have any proven validity in this context but pragmatically seems a logical tool. It contains the following parts from the motor section of the UPDRS – facial expression, tremor at rest, action tremor, rigidity, arising from chair, posture, gait and bradykinesia. To the author's knowledge, there is no tool specifically designed to measure extrapyramidal signs in cerebrovascular disease.

The electrocardiogram was recorded using the standard 12 leads. The ECG was calibrated such that 1 millivolt led to a 10 mm deflection. The paper speed was 25 mm/s.

4.2.2 Cardiovascular autonomic tests

For the purpose of the autonomic function tests, all subjects were asked to refrain from smoking and caffeine ingestion on the day of the investigations and to avoid food on the morning of the tests. Caffeine within tea and coffee potentially has a sympathomimetic effect which may confound autonomic function testing. All investigations were performed between the hours of 09:00 and 13:00 since there may be some diurnal variability in postural autonomic test results. All testing took place in a warm room. Environmental noise was minimized and subjects were left alone in quiet surroundings during heart rate variability recording since extraneous noise that leads to auditory stimulation may influence heart rate variability, probably by increased sympathetic activity.

A ten minute rest phase was allowed before investigations commenced with the subject supine and not engaged in conversation. This achieved optimal resting state condition for heart rate variability studies. The same sequence was followed for all the clinical autonomic tests. All sections of the investigation protocol were interspersed with a 2 minute rest phase. This was a practical compromise to allow completion of a lengthy testing schedule and avoid subject fatigue. Preceding rest time does not significantly affect response to Valsalva

manoeuvre or metronomic respiration but may influence the RR and blood pressure response to standing (Ten Harkel, 1990).

The testing equipment was explained to the patient. The model is schematically shown in Figure 4.1. The subject was in the supine position with the arm comfortably supported by pillows. Finapres™ (for FINger Arterial PRESsure) and its portable equivalent, Portapres™, machine were used to acquire instantaneous blood pressure (Ohmeda Finapres, Wisconsin, USA and Portapres, TNO-Biomedical Instrumentation, Amsterdam). This is a non-invasive blood pressure monitoring device to record beat-to-beat changes in arterial pressure. It has clear advantages over the intra-arterial method which is invasive and affects autonomic tone (Wieling and Karemaker, 1999).

The system was designed by Penaz in the early 1970s (Penaz, 1973). The principle is a cuff pressure controlled by a feedback system so that the vascular volume is 'clamped' to a preset value. The feedback is provided by a plethysmograph signal in a closed loop system. The inflatable finger cuff pressure is controlled by an electropneumatic system acting on a signal from the integral photoelectric plethysmograph. During the start procedure, the cuff pressure is raised to a level whereby the arterial compartment is compressed to about one-third of its normal volume. This represents the clamped value. Fluctuation in vascular volume (from changes in intravascular pressure) triggers automatic adjustment in cuff pressure. Therefore the intravascular pressure is continuously tracked. The principle was further developed by Wesseling and coworkers to produce the Finapres™ device. One of the most important improvements was the Physiocal procedure. This checks and adjusts the set-point of the volume clamp during operation. The equipment package includes the finger-cuff attached to a small box on the wrist that contains the fast-acting pressure device to adjust cuff pressure, and a main unit containing air-pump, electronics and computer hardware.

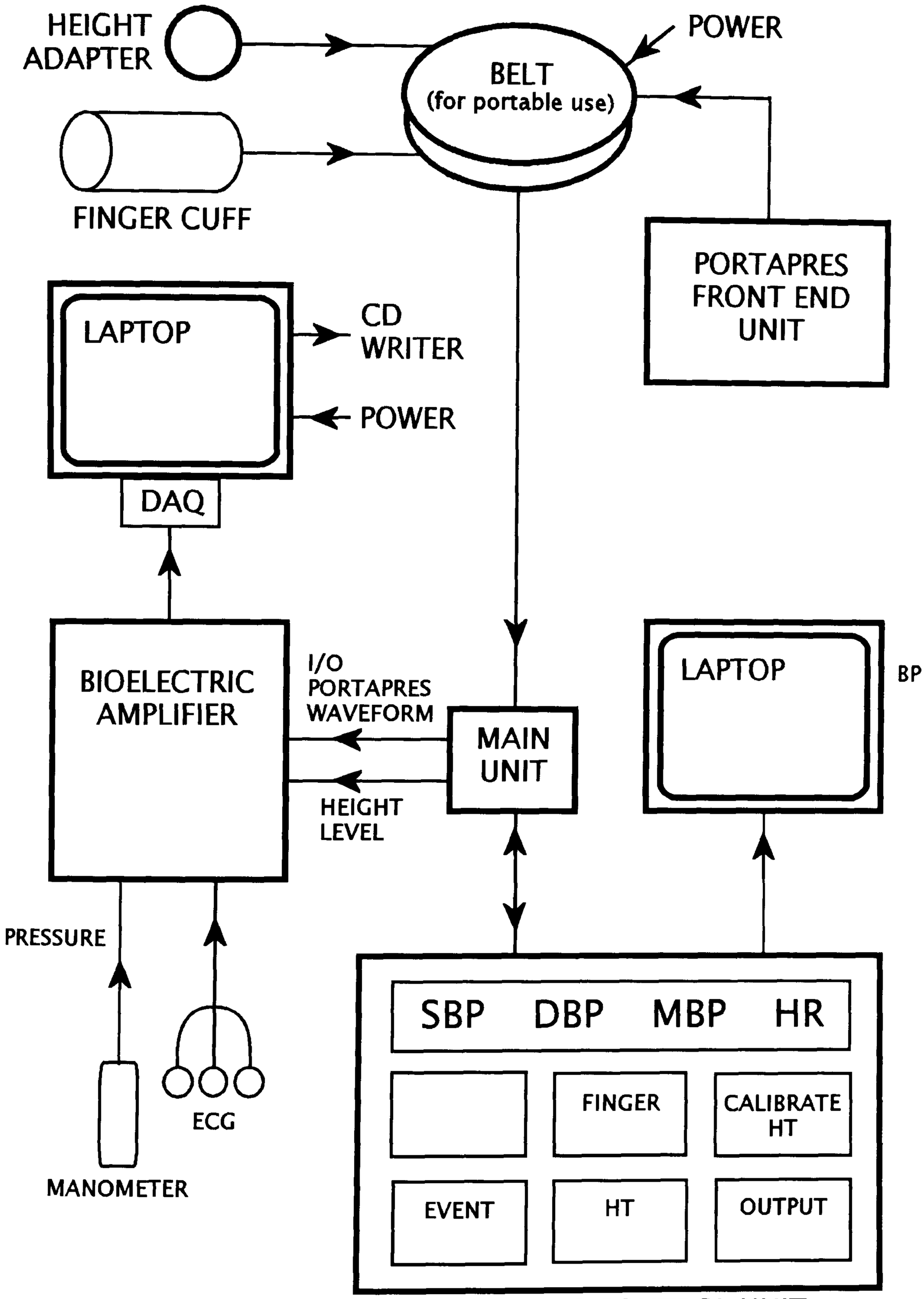

Figure 4-1 RR interval and blood pressure acquisition equipment

The performance of finger arterial pressure monitoring has been extensively reviewed by Imholz and colleagues who collated data from 15 years experience and 43 research articles (Imholz, 1998). These articles compared performance of finger arterial pressure monitoring (Finapres or Portapres) with intra-arterial (or non-invasive but intermittent) blood pressure measurement. Accuracy of the finger devices ranged from -48 to +30 mmHg for systolic, -20 to +18 mmHg for diastolic and -13 to +25 mmHg for mean arterial pressure. However when weighted for the number of subjects, pooled accuracies were -0.8 mmHg (SD 11.9) for systolic, -1.6 mmHg (SD 8.3 mmHg) for diastolic and -1.6 mmHg (SD 7.6 mmHg) for mean arterial pressure. Weighted accuracy of finger blood pressure measurement was within the 5 mmHg limit of the American Association for the Advancement of Medical Instruments (AAMI) but precision was too low for systolic and mean pressure and did not meet the AAMI limit of within 8 mmHg SD (AAMI, 1992). Subdivision of studies according to reference blood pressure, setting and device revealed no significant differences in mean or standard deviation. For measuring beat-to-beat differences, Portapres™ is able to duplicate qualitative changes accurately. There is usually no significant quantitative difference in diastolic or mean pressure when compared with invasive techniques. There are significant differences in systolic pressures but these are usually not clinically relevant i.e. they are small in comparison to the magnitude of response. Omboni has found that for systolic pressures, there are no significant differences between finger and intra-arterial frequency domain power spectral analysis, but there were significant over-estimation of low and mid-frequency bands in the finger trace (Omboni, 1993; Veerman, 1994).

The appropriate finger cuff size was selected to achieve a close fit around the digit. Attention was paid to maintaining a warm environment and occasionally local warming techniques were used to improve capture of the systolic pressure wave (Imholz, 1998). The middle finger was the default choice but in the event of poor systolic waveform, the index or ring finger was tried. Right hand digits were used for testing but if no satisfactory waveform was achieved on the right hand, the left hand was tested. Attempts to keep the finger device at heart level were made as far as possible during changes in posture for the cardiovascular reflex tests (Imholz, 1998).

4.2.2.1 Heart rate variability

Heart rate variability studies were performed using a 5 minute ECG recording using limb lead I with the subject supine in quiet resting conditions. The subject was left to breathe spontaneously at their own free rate and depth. The ECG was recorded using commercial software, sampling at 1000 Hz. Systolic blood pressure was recorded using the Finapres™ or Portapres™, with the physiocal operating to provide intermittent calibration. Five minutes of ECG and systolic pressure waveform data were digitized and stored on computer for

subsequent offline analysis (Labview and DAQ 1200, National Instruments. Newbury). The program identified R wave peaks to produce RR interval data, and the peak systolic pressure following each sinus beat. Non-sinus and ectopic beats were removed automatically then manually if necessary, using an R wave detection software package. The programme inserted an R wave by linear interpolation for missed or ectopic beats (Clayton, 1995).

From the ECG recording, a fixed point for comparative purposes is identified on the R wave, termed the fiducial point. Good quality recordings with high signal-to-noise ratios are required for reliable fiducial point identification. The software program recognises each sequential R wave and generates a series of inter-beat intervals. If there is an unexpected variation in the R wave interval, set at >30%, the software generates an interpolated R wave based on the variation observed in the preceding four R waves. This automatic process is augmented by visual inspection of the softwares' R wave identification: if there is a mis-identification of R wave activity the observer can generate additional or remove inappropriate R wave intervals on a 'best fit' basis. The options available on the software menu are shown in Table 4.1.

Table 4-1 R wave interpolation in CRISP

R wave change required	Interpolated R wave label
From normal to ectopic	Ectopic
From ectopic to normal	Not ectopic
From normal to incorrectly detected	Not R wave
Incorrectly detected to normal	R wave

Reproduced from CRISP User Manual (Whittam, 1998b).

Manually interpolated beats are only possible within boundaries set by software algorithms (Whittam, 1998b). The final edited sequence of RR intervals is saved on disc. The data is fed to another program which applies Fast Fourier Transformation to generate a power spectrum of heart rate variation. A locally developed computer program, ALPHA, was used to produce RR interval power spectra (Whittam, 1998a).

Similarly systolic pressure waveform was inspected to identify any peaks that were not true systolic pressure waves, and these values were replaced by an interpolated value. The program automatically interpolated systolic pressure values during the interruption to recording due to physiocal operation. Power spectral analysis of the edited recording, after fast Fourier transformation, provided values for heart rate and blood pressure variability as a total value and in the low and high frequencies, according to international guidelines (Task Force of the European Society of Cardiology and the North American Society of Pacing and

Electrophysiology, 1996). The boundaries for total, low and high frequency bands were 0 to 0.40, 0.04 to 0.15 and 0.15 to 0.40 Hz respectively. Recordings with significant non-sinus activity (at a level set by the software programme or after review by investigators) were deemed unsuitable for power spectral analysis and excluded from the results.

Combined RR interval and blood pressure measurement can facilitate measurement of baroreflex sensitivity. For this study, baroreflex sensitivity was determined by a validated technique of synchronisation of RR interval and systolic pressure spectral data (Lord, 1998). The program calculated magnitude squared coherence (MSC) between the heart rate and blood pressure variabilities in the 5 minute recording and calculated the alpha index of baroreflex sensitivity where MSC exceeded 0.5 (Clayton, 1995). Spectral index baroreflex sensitivity has been compared with the gold standard phenylephrine technique and demonstrates acceptable agreement, with correlation co-efficients ranging from +0.78 to +0.94 (James, 1998; Piccirillo, 2001; Robbe, 1987).

4.2.2.2 Cardiovascular autonomic reflex tests

Clinical bedside autonomic tests similar to the protocol described by Ewing (Ewing and Clarke, 1982) were performed next with the beat-to-beat RR interval and blood pressure response continuously recorded using an ECG and Finapres™ or Portapres™. RR interval, systolic and diastolic blood pressure data were digitised and stored on computer for off-line analysis. RR interval and blood pressure measurement was performed using a locally developed software programmes based on Labview (Labview and DAQ 1200, National Instruments, Newbury) and software running on the Neuroscope (Medifit Instruments Ltd, UK). The programs displayed RR interval and systolic and diastolic blood pressure on computer screen, along with markers for the start/finish of each autonomic reflex test.

Initially the data was analysed using the TONE program on the Neuroscope™ package. Then a Labview-based program was developed (Regional Medical Physics Department, Royal Victoria Hospital, Newcastle upon Tyne). The Labview programme allowed removal of non-sinus activity from the stored data with interpolations if required for the calculation. Recordings with excessive movement artefact or non-sinus activity were excluded from analysis. Where mean values were used to provide a blood pressure result, the standard number of beats required was set at 20. Where artefact and non-sinus beats affected the 20 beat set of RR intervals, a minimum of ten beats were required for data to be included in analysis.

4.2.2.2.1 Heart rate response to orthostasis

The 30:15 ratio was obtained from RR measurements during a change in posture from the supine to standing position (Ewing, 1978b). Cardio-acceleration due to hypotension on

standing, followed by compensatory cardio-deceleration due to a rebound in blood pressure level led to a trough then peak in RR interval. The shortest and longest RR intervals occur at approximately the 15th beat and 30th beats respectively (Ewing, 1978b): however a technique was employed such that the true minimum and maximum RR intervals occurring in the predicted pattern following standing were selected (Wieling, 1982). Strictly speaking, this value is the RRmax/RRmin and is now the recommended method for quantifying heart rate changes on standing (Wieling, 1997). However throughout this text, the term 30:15 ratio is used for simplicity and to improve differentiation from other RR interval ratios.

4.2.2.2.2 Blood pressure response to orthostasis

Blood pressure change during standing was calculated as the change from the mean blood pressure for the 20 beats immediately prior to standing, to the first blood pressure nadir that was clearly identified in the period immediately after standing. If there was a clear paradoxical rise in blood pressure without any typical nadir, then that peak blood pressure in the same period was used in the analysis.

4.2.2.2.3 Isometric exercise

Isometric exercise was performed by asking the subject to rise from the supine to a sitting position on the couch, and to remain in that sitting position for 3 minutes. The subject had to retain the sitting position without external help, thus producing an isometric exercise stimulus (Donald, 1967). This test replaced the isometric handgrip exercise since it was easier for elderly subjects to follow instructions. The blood pressure response was taken as the difference between the mean diastolic values for the 20 beats immediately prior to sitting and the 20 beats immediately prior to the end of the sitting exercise.

4.2.2.2.4 Valsalva manoeuvre

A Valsalva manoeuvre was performed by the subject blowing into a tube connected to a manometer. The barometric pressure was shown on a visual scale to the subject and they were instructed to blow at approximately 40 mmHg for 15 seconds, then to remain silent and still in the subsequent recovery phase. The Valsalva ratio was taken as longest RR value in the 15 seconds following forced expiration divided by the shortest RR value at the end of the forced expiration phase (Ewing and Clarke, 1982). For inclusion the minimum expiratory time was 10 seconds and there had to be a recognisable expiratory pattern in the respiratory waveform and change in RR interval consistent with a typical response. This procedure was performed on three occasions: the largest ratio (Sandroni, 1991) and the best blood pressure response (shallowest point systolic value of three phase IIe troughs, and highest point systolic value of three phase IV peaks) were used for statistical analysis (Low, 1990).

4.2.2.2.5 Cold pressor test

The cold pressor test was performed in addition to the standard Ewing tests. The subject immersed the left hand in iced cold water for one minute (Fagius, 1989). The diastolic blood pressure response was calculated using the 20 beats prior to immersion and 20 beats during the final phase of immersion. A minimum immersion of 45 seconds was required for inclusion.

4.2.2.2.6 Heart rate response to metronomic respiration

Metronomic respiration was performed by instructing the subject to take six deep timed breaths over one minute. This sequence would produce six pairs of RR interval peaks and troughs. For statistical purposes, the maximum divided by the minimum RR interval produced the E/I ratio (Pfeifer, 1982). Secondly, RR intervals were converted to instantaneous heart rates, which allowed calculation of the absolute change in heart rate from inspiration to expiration, the E-I difference. The mean of E/I ratios and E-I difference were used for analysis, with a minimum of three consecutive cycles of RR data required for inclusion. A typical respiratory pattern in conjunction with typical sinusoidal RR interval change was necessary for correct identification of peaks and troughs.

4.2.2.2.7 Carotid sinus massage

Carotid sinus massage was performed in the supine position for 5 seconds on the right then left carotid sinus (Brignole, 2001). This investigation was only performed if there was no evidence of significant carotid stenosis on Doppler scanning (i.e. less than 50 % stenosis) in the stroke cases and the subject had given informed consent following a discussion of the potential risks of carotid sinus massage. Two values were obtained following carotid sinus massage. The cardio-inhibitory response was the value of the peak RR in the 30 seconds immediately after commencing carotid sinus massage. The vasodepressor response was the difference between the mean systolic blood pressure for 20 beats prior to CSM and the nadir blood pressure in the 30 seconds after carotid sinus massage.

4.2.3 Spirometry

The test procedure was demonstrated to the subject. The subject inspired deeply to maximum capacity then started expiration through the mouthpiece of the spirometer tubing with a tight seal to avoid leakage. The subject was encouraged to continue expiration at maximum force for six seconds or for as long as tolerated. A minimum of two forced expirations were required until a consistent trace was obtained. The forced expiratory volume was recorded at one second post-expiration and the forced vital capacity at six seconds post-expiration. Values were recoded to the nearest 0.05 litre.

4.2.4 Ambulatory blood pressure monitoring

Spacelabs Ambulatory Blood Pressure Monitor 90207 was used for ambulatory blood pressure recording (Spacelabs Medical, Inc, Redmond , Washington, USA). The monitor uses oscillometric techniques to record systolic, diastolic and mean blood pressure plus heart rate. The automatic measurement interval was set at 30 minutes from 07:00 to 22:00 and 60 minutes from 22:00 to 07:00. Cuff size was chosen according to arm circumference. By convention the cuff was attached to the non-dominant arm to minimise normal physical activity affecting blood pressure measurement. It has been shown that the non-dominant arm, which is the left in over 90% of cases, under-estimates blood pressure obtained by simultaneous ambulatory blood pressure recording in the right arm, which is the limb most consistently used for office blood pressure readings (O'Shea and Murphy, 2000). However this was not a predominant concern for this research project where variation within the group was of primary concern, rather than comparison with clinical office measurement. The centre of the inflatable bladder was positioned over the brachial artery using the marker on the cuff. Tubing was placed under clothing to allow a change of clothing without disturbing the recording equipment. A test blood pressure measurement was performed to ensure the equipment was functioning correctly and make the subject aware of the procedure. Consent for the recording to proceed was confirmed after the test reading to ensure the subject was prepared to complete the 24 hour monitoring, in view of the discomfort experienced during cuff inflation. Subjects were instructed not to move the arm during cuff inflation, to keep the arm at heart level during measurement and requested to keep the equipment dry. Subjects were also informed that in the event of a regular automatic recording failing, the monitor would automatically retry measurement within two minutes, but if this failed the next cuff inflation would occur at the pre-programmed interval. Recording commenced between 10:30 and 13:30 i.e. after completing the autonomic test protocol. Subjects were supplied with written instructions regarding the monitor plus a contact number in event of difficulties (O'Brien, 2000). The subject was instructed to inactivate the monitor and remove the equipment after a period of 24 hours.

The Spacelabs 90207 has been validated for use according to the British Hypertension Society Protocol (Fotherby, 1995; O'Brien, 1991a). The monitor achieved grade B for both systolic and diastolic blood pressure according to British Hypertension Society (BHS) criteria (good agreement, recommended for clinical use). It also passed the assessment protocol of the Association for the Advancement of Medical Instrumentation (AAMI) which states that test devices should not differ by more than 5 mmHg from the mercury measurement, with a standard deviation of less than 8 mmHg. O'Brien's validation study (O'Brien, 1991a) included subjects aged from 15 to 86 years. Iqbal et al (Iqbal, 1996) performed a validation

study in older subjects aged 60 to 90 years. Only diastolic blood pressure was satisfactorily recorded in all body positions and at all pressures according to BHS and AAMI standards. At higher blood pressure levels, systolic accuracy deteriorated, especially at blood pressures greater than 200 mmHg.

The machine has the following measurement ranges

- Heart rate 40 to 180 beats per minute
- Systolic pressure 70 to 285 mmHg
- Diastolic pressure 40 to 200 mmHg
- Mean arterial pressure 60 to 240 mmHg

Measurement time is typically 35 to 50 seconds. Cuff pressure will initially inflate to 165 mmHg but if this is not able to capture the systolic pressure, the cuff will inflate to approximately 30 mmHg above the previous systolic pressure, to a maximum of 300 mmHg.

Data from the ambulatory monitor was downloaded via dedicated cable to the hard drive on a computer where data could be accessed using Spacelabs software, the Ambulatory Blood Pressure Report Management System 90121. This program allowed data retrieval, editing and printing. The software program automatically edited measurements. It rejected artifact that resulted in non-physiological blood pressure measurement from, for example, patient movement, heart rate arrhythmia or equipment malfunction. Values outside the following pre-set automatic edit limits were excluded from analysis

- Systolic BP 70 – 240 mmHg
- Diastolic BP 40 – 150 mmHg
- Pulse pressure 20 – 150 mmHg

Manual editing was not performed. Subjects were excluded from further analysis if there were less than 15 readings in the 24 hour period (Staessen, 1997a).

Definition of day and night periods was according to 'narrow' fixed time period (Staessen, 1991). The day period was fixed at 10:00 to 20:00 and night period was 00:00 to 06:00. The morning and evening intervals were excluded from the analysis. This approach allows standardisation of awake and asleep periods which can improve ability to make comparisons between studies (Fagard, 1997). These periods were adopted by Staessen et al (Staessen, 1997a) in the meta-analysis of circadian variation of ambulatory blood pressure. The method does tend to overestimate night blood pressure levels from actual asleep values and underestimate day blood pressure levels compared with awake values. However fixed time methods that exclude the morning and evening phases from the daytime and night-time

periods are closer to the actual asleep and awake pressures than fixed time periods that include the full 24 hours in the two periods (Fagard, 1996). The narrow fixed time period is poorly reproducible but so are the other methods for defining day and night periods. Furthermore using set time period avoids inaccuracy from time diaries which are not always completed by participants (Robinson, 1995). For inclusion in separate day or night analyses, a minimum of 10 measurements in the day or 5 in the night period were required, and both for circadian variation analysis (Staessen, 1997a).

The long term variability of ambulatory blood pressure was defined as the standard deviation of the mean level for either the 24 hour period, day or night period (Mancia, 1983b). The circadian variation was defined as the percentage decrease in blood pressure from the day period: (day pressure – night pressure) / day pressure x 100%. Pulse pressure was the difference between mean values for systolic and diastolic blood pressure.

4.3 Magnetic resonance imaging

MRI brain scans were performed using a 1.5 Tesla GE Signa scanner (General Electric Medical systems, Milwaukee, WI). Imaging was performed on a different day to cardiovascular investigations, with the aim of scanning within 4 weeks of cardiovascular tests. Scanning was performed at the Nuffield Hospital, Newcastle upon Tyne, with a member of the study group on hand to provide assistance regarding check-in and transport. The MRI checklist for contra-indications was repeated on-site by trained and experienced radiographers who briefed the subject on scanning time and conditions: any possibility of contra-indication resulted in exclusion from MRI scanning. Subjects were warned in particular about the noise generated by MRI equipment and possibility of claustrophobia from the enclosed space and head brace. The neck and head were secured in a restraint to reduce the risk of movement during scanning, and all metallic personal objects removed from clothing. Communication to radiography staff was maintained by a two-way transmitter such that instructions and reassurance were provided by radiographers to subjects, and vice versa for any problems encountered by subjects.

4.3.1 MRI scanning protocol

The scanning sequence took 22 minutes. The protocol contained a series of imaging modalities to optimise extraction of structural information on different tissues and pathologies. Information was principally sought on whole and regional brain volumes, discrete cerebrovascular lesions and white matter lesions. The protocol included the intention to repeat MRI after an interval of 2 years to investigate change in structure. The scans are summarised in Table 4.2.

Table 4-2 Summary of MRI protocol in COGFAST

Technique	Technical data	Uses	Examples
T_1 weighted images 3-dimensional Fast Spoiled Gradient (FSPGR), whole brain, coronal plane	TR=12 ms, TEc = 4.2 ms, TI=650 ms, 256x192 matrix, 1.6 mm thickness, flip angle=15°	Cerebral structure, regional volumes and location of white matter, grey matter and (CSF) boundaries	Medial temporal lobe atrophy. Voxel-based morphometry
Fluid-attenuated inversion recovery (FLAIR), whole brain, axial	TR=10,000 ms, TE = 125ms, TI= 2,100ms, 5mm slice thickness, 0.3mm interslice gap	White matter lesions	Visual rating of WMH (Scheltens Scale)
Variable echo, whole brain, axial. Proton density and T_2-weighted	TR= 2,740 ms, TE = 12/84.2 ms, slice thickness = 5mm, interslice gap=0.3mm	White matter lesions	Visual rating of WMH (Scheltens Scale)

TR, repetition time; TI, inversion time; Tec, Echo time; WMH, white matter hyperintensity.

As discussed in Chapter 2, white matter lesions visible on brain imaging are important in the pathophysiology of cognitive impairment. This creates the need for careful evaluation and rating of brain imaging results. Rating scales have evolved to provide uniform interpretation of scans. In this study, we have used established rating scales and developed a novel score to investigate the relationship of cardiovascular variables and cognitive function with imaging abnormalities.

4.3.2 Visual rating of white matter lesions

There are a number of rating scales but none have been validated in morphological studies, (including the Scheltens scale) and most do not rate severity of brain atrophy (Scheltens, 1998). In a comparison of thirteen white matter hyperintensity rating scales, the potential for inconsistency between scales highlighted the risk of contrasting results in the pathological and clinical correlates of the process (Mantyla, 1997). The scales were applied to 395 post-stroke patients in Finland. Agreement was investigated using Goodman-Kruskal measures of association. Agreement for deep white matter hyperintensity was at best 80% but at worst 18%. The Scheltens scale demonstrated the best agreement with two other scales. Agreement between scales was even worse for periventricular hyperintensities, at best 88% received the same grade but at worst only 0.4%. Clearly the probability of obtaining similar grades, order of severity and distribution of lesions can vary widely depending on the scale employed.

The Scheltens scale was used in our study. Scheltens et al designed a rating scale to assess the location and severity of signal hyperintensities on T2-weighted magnetic resonance imaging in the early 1990s (Scheltens, 1993). This formed an extension to a previous scale (by Fazekas) which was disadvantaged by lack of detail on anatomical distribution or extent of

white matter damage. The Scheltens scale has the advantages of taking the number of lesions into account, providing regional information and including separate scores for basal ganglia and infratentorial lesions. The Scheltens score involves a visual rating of the MRI films using T2 weighted images to identify areas of increased signal intensity compared to brain tissue and cerebrospinal fluid. The appearance is then rated on a numerical ordinal scale to provide a semi-quantitative rating of signal hyperintensities, based on size and frequency of hyperintensities. There are four brain regions identified in the system.

Periventricular hyperintensities (PVH): continuous confluent areas of high signal intensity adjacent to anterior or posterior horns of the lateral ventricles ('caps') and along the lateral ventricles ('bands'). A three grade 0-2 scale is used for PVH, maximum score 6.

- 0 = absent
- 1 = ≤ 5 mm
- 2 = > 5mm and < 10 mm
- PVH greater than 10 mm are rated as DWMH.

Deep white matter hyperintensities (DWMH): hyperintensities in the deep and subcortical white matter, divided into frontal, temporal, parietal and occipital regions. If the lesions were directly adjacent to the ventricles, they were rated separately from periventricular hyperintensities. A seven grade 0-6 scale is used for DWMH, maximum score 24.

0. No lesions
1. < 3 mm, n ≤ 5
2. < 3 mm, n > 5
3. 4-10 mm, n ≤ 5
4. 4-10 mm, n > 5
5. 11 mm, n > 1
6. confluent

Basal ganglia hyperintensities. Divided into caudate nucleus, putamen, globus pallidus, thalamus and internal capsule regions. Seven grade 0-6 scale, maximum score 30.

Infra-tentorial hyperintensities. Divided into cerebellum, mesencephalon, pons and medulla regions. Seven grade 0-6 scale, maximum score 24.

Scans were rated blindly and by consensus by three trained and experienced raters.

The scale has been validated in terms of providing good observer reliability. Inter- and intra-observer agreements of the Scheltens scale were compared to the Fazekas scale and the new scale gave good agreements for deep white matter (reproducibility as standard deviation of raters agreement as % of scale range 6.1-5.6%), basal ganglia (4.3-4.4%) and infratentorial (2.9-3.8%) region hyperintensities compared to poor to reasonable agreements for the Fazekas

scale (periventricular 9.5-12.3%, DWM 11.8-15%, kappa 0.34-0.74). There was no advantage over the Fazekas scale when rating periventricular hyperintensities using Scheltens scale (12.6-15.3%) (Scheltens, 1993). This report does use different methods for assessing the two scales. In an overview of visual rating scales, the European Task Force recommended Scheltens scale whilst recognising the lack of knowledge regarding radiological-pathological validation studies (Scheltens, 1998).

4.3.3 White matter volume

The deficiencies of visual rating scales indicate a need for more accurate methods to measure white matter lesion volume. Manual segmentation of white matter lesions will provide an accurate volumetric measure but is subjective and time-consuming requiring a neuroanatomy expert. Automatic segmentation of white matter hyperintensities provides the benefits of accuracy and avoids the lengthy process of manual/semi-automated techniques. Automated image processing software was developed in-house, employing code written with Matlab (the Mathworks Inc, Massuchusetts, USA) and Statistical Parameter Mapping (SPM99, Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK; http://www.fil.ion.ucl.ac.uk) routines. The technique has been validated by comparison with a manual/semi-automated segmentation process of measuring white matter lesion volume. Comparing the automated and semi-automated segmentation techniques, the mean difference in white matter lesion volume was 0.53 SD 3.4 mL (Firbank, 2003). This level of agreement compared favourably with a comparison of two expert humans using a semi-automated contouring technique where a SD of 2.83 ml was obtained (Grimaud, 1996). The method is now described in brief.

FLAIR images were analysed to quantify white matter hyperintensities. Following transfer to computer, images were converted to analyse white matter lesion formation using MRIcro then segmented using code written in Matlab (the Mathworks Inc., Massuchusetts, USA) as previously described (Firbank, 2003). FLAIR images were spatially normalised to the T1-weighted Montreal Neurological Institute template, which approximates Talairach space (Talaraich, 1988), using a 12 parameter affine transformation and non-linear iterations (Ashburner and Friston, 1999). Images were re-sampled to a voxel size of 2x2x2 mm^3 using bi-linear interpolation. Automatic segmentation via cluster analysis produced grey matter, white matter, cerebrospinal fluid and a fourth partition of skull, fat, muscle and voxels with a high degree of partial voluming (Ashburner and Friston, 2000). The brain extract function of SPM99 (Anonymous, 2000) created a brain mask to remove non-brain regions from the original FLAIR images. The masked FLAIR images were then segmented slice-by-slice using a threshold automatically determined from the histogram of pixel intensities. White matter hyperintensities were identified as those pixels with intensity greater than 1.45 times that of

the modal pixel intensity. All segmented white matter hyperintensities were visually inspected and any non-brain structure removed by manual editing. To assess the distribution of white matter hyperintensities in cortical areas, a region of interest map (created in MNI space) was transformed onto the FLAIR image of each subject. The percentage of white matter hyperintensities in the brain was calculated by dividing the volume of segmented WMH by the volume of the brain as determined from the SPM99 segmentation (Ashburner, 1997). Images with movement artifact that precluded volumetric assessment were excluded from this analysis.

4.3.4 Discrete infarcts

Infarct rating used a locally developed scoring system (based on the NINDS-AIREN criteria). Larger cerebrovascular lesions were identified using T_1-weighted images reconstructed in the axial plane. Scans were rated blindly by two raters trained in MRI white matter assessment. The location, size and volume were determined from hard copies of MR images. Side of lesion was recorded in each case. Lesions greater than 3mm in diameter and with signal characteristics similar to that of cerebrospinal fluid were considered as infarct. Cortical and white matter region infarcts were grouped into frontal, parietal, occipital and temporal lobes. For these regions, size was rated on largest diameter in the following four categories: 3-9 mm, 10-29mm, 30-49mm and >50mm. Subcortical regions were putamen, caudate nucleus, globus pallidus, thalamus and internal and external capsules. Two size categories, 3-9 mm and > 10 mm, were employed for subcortical sites.

4.4 Neuropsychometric investigations

The aim of the neuropsychometric protocol is to measure the intellectual and emotional deficits occurring in the study population. The principal aim of COGFAST is to reliably diagnose those individuals who develop dementia and correlate clinical deterioration with neuropathological changes. Dementia is defined as global intellectual impairment, operationalised as memory loss and impairment in at least one other domain of higher cortical function (aphasia, apraxia, agnosia, executive functioning) with decline in social or occupational functioning: delirium and possible non-organic causes of cognitive loss should be excluded (American Psychiatric Association, 1994). It is equally important to characterise more subtle change in intellectual function. One of the key aims of the COGFAST is to describe the intellectual domains that deteriorate in vascular-based cognitive impairment in comparison to dementias of other (neurodegenerative) pathology. The tools chosen need to chart neuropsychometric function over a period. With this in mind, a protocol was devised.

When measuring cognitive impairment in the elderly, several issues should be borne in mind. The test battery should include tools that address a basic minimum of cognitive domains:

attention and orientation, learning and memory, language, motor, visuospatial and executive functions. Intellectual function declines with age in normal elderly individuals. Therefore normative data from a similar age group is required to make comparisons. Intellectual decline may occur in an individual but they remain within normal range of scoring, because of preceding high intellectual capacity. Two methods assist making an adjustment for premorbid ability. Education or occupation reflects the person's intellectual capacity. Standardised reading tests or tests of crystallized intelligence (vocabulary, general knowledge) also inform about previous ability, since they are less affected in earlier stages of organic cognitive decline (Jutagir, 1998). Test results should assist investigators in categorising likely cause of cognitive decline.

Memory function can be divided into primary (short term) and secondary (long-term).

- Primary memory: temporary storage of material in memory for periods up to 30 seconds. This is often referred to as working memory, a central executive system. It utilises and integrates circuits in particular the articulatory loop system and visuospatial scratch pad.

- Secondary memory: the longer term storage of memory. There are different theories on the subdivision of secondary memory, a common one is as follows:

  i. Implicit memory: non-declarative memory. This is the subconscious processing e.g. conditioning, priming, that provides a platform for overall function in the environment

  ii. Explicit memory: declarative memory. This is further subdivided into (a) semantic memory, which is the process of internally representing knowledge of words, facts and concepts of the environment and produce mental models (b) episodic memory, the catalogue of autobiographical experience and the timing of events.

4.4.1 Mini-mental State Examination

This is a widely used measure of cognitive function, applied in everyday practice and clinical research. It is a simple test of cognitive function. The original report suggested three purposes (Folstein, 1975): a means of differentiating organic from functional cognitive disorder, the ability to quantitatively estimate the severity of cognitive impairment and finally to document serial change in function. The MMSE was not intended to be used as a diagnostic test (Folstein, 1975).

4.4.1.1 Requirements

A trained interviewer requires around 5-10 minutes to complete the test, which is based around response to verbal questions with some tests of motor function and language and visuospatial skills involving pen and paper.

4.4.1.2 Scoring system

The maximum score is 30. There are five subsections: orientation, registration, attention/calculation, recall and language. A score of <24 indicates significant cognitive impairment. The threshold has evolved through research findings and is widely accepted in community surveys (Tombaugh and McIntyre, 1992). Traditionally, significant clinical change is suggested from a change of ≥ 4 points, but it is a non-linear rating scale. The influence of educational level has been widely debated in the context of whether educational level represents a psychometric bias or risk factor for cognitive decline (probably through association). It may act in both dimensions.

4.4.1.3 Validity and reliability

The original paper assessed 206 patients with psychiatric disorders. There were correlations of 0.78 with Weschler adult intelligence scales for IQ. Test-retest reliability was 0.89 and a combination of test-retest and inter-rater reliability was 0.83 (Tombaugh and McIntyre, 1992).

Internal consistency has reached Cronbach's alpha levels of 0.96 in medical patients but is lower in community surveys with a score of 0.77 in a community survey of 4917 adults aged 18 to 85+. Test-retest reliability can be poor, for example in patients with delirium where cognition is fluctuating or in control subjects where the ceiling effect and limited test range limits correlation coefficient abilities. Some items in particular are a cause of poor reliability in terms of methodology and scoring system. Generally small longitudinal changes in score should be interpreted with caution (Tombaugh and McIntyre, 1992).

From Tombaugh's review of 25 studies assessing criterion validity, in 70% of studies an MMSE of less than 23 was associated with dementia in at least 79% of subjects but this figure can drop when the mean score is high, for example in community surveys with a higher proportion of normal subjects. Specificity is very dependent on the population characteristics, varying from good specificity for community studies to poor specificity where other types of psychiatric patients are included in the cohort (46-62% specificity).

Correlation co-efficients with a range of representative cognitive screening tests generally range between 0.70 and 0.90 for intelligence and memory tests. In Folsteins' original paper, correlation coefficients with Wechsler Adult Intelligence Scale were +0.78 for the verbal

scale and +0.66 for the Performance Scale (Folstein, 1975). The ceiling effect with the MMSE limits its relationship in some studies.

4.4.1.4 Clinical utility

The MMSE does fulfill its intended aim of a brief screening tool to quantitatively assess severity and measure change in cognitive impairment. As discussed above, there are flaws in its use as a diagnostic tool. Lack of ability to test right hemisphere function and mild language impairment are recognised flaws. However its strengths of simplicity, widespread use and proven validity are clear. Tombaugh and McIntyre (Tombaugh and McIntyre, 1992) recommended the MMSE for use as a screening tool but it should not be used as the sole criterion for diagnosing dementia, or if the individual was of low educational ability or not fluent in English. Scores can be summarised as follows: cognitive impairment, none = 24-30, mild =18-23, severe = 0-17. However weaknesses in assessing serial change have been highlighted in a brief re-examination of test-retest reliability. Cognitive drug studies have used mean improvements of 1.3 as indicating worthwhile drug efficacy but Bowie et al (Bowie, 1999) demonstrated that for an individual, the confidence intervals of a 1 point improvement from 20 to 21 could actually reflect a decrease to 19 or an increase to 23. Forty Old Age Psychiatry specialists administered the MMSE to dummy subjects with a set response but the range of scores obtained was 14 to 27.1, mean 18.5 (95% CI 17.2-19.2).

4.4.2 CAMCOG

This is a test of global cognitive function, derived mainly from section B of the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) (Roth, 1986). Its purpose was the diagnosis and measurement of dementia, although it is worth bearing in mind the original paper concentrates on the tests' ability to separate organic mental disorder i.e. both dementia and delirium, from depressed and healthy normal subjects. It is a pen and paper test completed by trained interviewer and interviewee. There are sub-sections devoted to assessment of particular cognitive domains: memory, executive function, language comprehension, language expression, attention, praxis and abstract thinking.

4.4.2.1 Requirements

The test is lengthy and usually takes 20-30 minutes for completion. A booklet is used which contains images used to assess reading, copying and perceptual ability. It is reasonably well tolerated by patients. In a validation study of 462 community-living people aged 77-96, 44 did not complete the schedule (11 unexplained missing data, 33 frail/refused/too cognitively impaired). In the 418 who completed the test, 46 had missing individual items on CAMCOG (Huppert, 1995).

4.4.2.2 Scoring system

The maximum score was originally 106. A slightly modified version has been reported with a total score of 105 (Leeds, 2001). There are eight subsections: orientation, language, memory, praxis, attention, abstract thinking, perception and calculation. The test incorporates the MMSE for means of comparison but not all MMSE items are included in the CAMCOG total score. The range of scoring for the total score is responsive to high performing individuals and tends to avoid the ceiling effect. Orientation and calculation subscores can be susceptible to ceiling effects (Huppert, 1995).

4.4.2.3 Validity and reliability

In the original paper, inter-rater reliability was quantified using the phi coefficient, where +1.0 is the best possible score. The value was +0.90 (+1.0 to +0.30) for the cognitive section and Pearsons correlation coefficient was +0.97 ($p<0.001$). Test-retest reliability was not assessed. Validity was assessed by comparison with Blessed's dementia score (Blessed, 1968) and a clinical rating of severity of dementia (although it is not clear exactly which clinical rating scale was employed). Both of these other instruments showed close correlation with the eight cognitive subscales (no coefficients quoted, $p<0.000$) (Roth, 1986). There are significant associations of total CAMCOG for age, gender, educational level and social class differences. Age is highly correlated with total score and subscores. For these reasons, cut-off diagnostic points should be treated with caution, depending on the age of subjects under investigation. In Huppert's study (Huppert, 1995), CAMCOG was stated to correlate with MMSE: Spearman`s co-efficient +0.79 with all CAMCOG items, +0.67 excluding MMSE items (no p value quoted).

4.4.2.4 Clinical utility

Cut-off score of 79/80 yielded 92% sensitivity and 96% specificity for the diagnosis of organic disorder (either dementia or delirium).The MMSE section of the CAMCOG yielded sensitivity of 94% and specificity 85% for organic mental disorder. It has been validated in stroke survivors, and elderly populations (Huppert, 1995; Kwa, 1996). Roth's paper (Roth, 1986) indicated CAMCOG is able to discriminate between individuals at the high end of the ability range.

A Dutch study of 129 patients 3 months post-stroke observed the majority (88%) of stroke patients can complete the CAMCOG at this stage of their recovery (Kwa, 1996). The main reason for failure was severe aphasia, indicated by an Aphasia Severity Rating Scale of 0-3 (from the Boston Diagnostic Aphasia Examination). Moderate aphasia (Aphasia Severity Rating Scale 4-5) resulted in only a few missing items and no increased risk of dementia according to total CAMCOG score. Therefore the CAMCOG appears feasible in stroke

patients but the authors note that they were only able to interview 52% of the initial selection of 252 patients. The missing cases included many who were likely to have more severe dysphasic problems. Thus the utility of CAMCOG will be limited in stroke patients.

4.4.3 Cognitive drug Research Assessment System (COGDRAS-D)

This is also known as CDR (Computerised Dementia Research) battery. It was originally intended to examine the effects of drugs on cognitive function (Simpson, 1991). Previously, test batteries measuring reaction time, vigilance and memory required considerable time to complete (> 1½ hours), substantial interviewer training and could lack sensitivity. The CDR battery was designed as a relatively quick and sensitive test that can be applied after minimal training (Ritchie, 1995).

4.4.3.1 Requirements

The protocol takes around 30 minutes to complete. The researcher uses a computer with visible screen to present a series of numerical and pictorial images. Standard instructions are read to the subject. The interviewee responds by pressing YES and NO buttons clearly marked on a separate box. Testing can be suspended if the subject is distracted. Quality of response can be measured by time to react or by proportion of correct responses. The interviewee needs adequate vision and the use of one hand; no keyboard skills are required. In some studies, subjects have refused to perform tests because of poor eyesight (Simpson, 1991).

4.4.3.2 Content and scoring system

The protocol can be refined to suit the clinical aims. The primary value of the CDR battery is to assess the patients' powers of selection, evaluation, quality of working memory and the ability to respond to stimuli or information. There are a number of subsections.

Simple reaction time (SRT). The word YES is displayed intermittently at varying intervals (1.0-2.5 s) in the centre of the screen on 30 occasions. The subject has to press the YES button as soon as the image is displayed. The time taken to respond is recorded. The reaction time can be quantified by the mean, median or standard deviation (the latter a measure of fluctuation). In healthy populations the mean approximates to the median. Results reflect alertness, attention, concentration and the primary stage of memory processing.

Number vigilance. A number is displayed constantly just to right of centre screen. Random digits are displayed just left of centre at the rate of 80 per minute. The subject has to press the YES button every time the digits match. Response is quantified usually by percentage of correct reactions or by the mean of the reaction time: number of false alarms and SD (for

fluctuation) of reaction time may also be used. This tests intensive vigilance, sustained attention and ability to ignore distraction.

Choice reaction time (CRT). YES or NO are displayed at varying intervals (1-2.5 seconds) in the centre of the screen. The subject has to correctly respond with the same YES NO button. Mean (or median) time to react is used, and SD can again reflect fluctuation. This section tests the same domains as SRT plus stimulus discrimination and response organisation.

Memory scanning/numeric working memory. Three digits are presented singly at intervals of 1.2 seconds. The subject has to memorise the digits and then press YES or NO when 18 digits are serially presented if they match one of the original three digits. The sensitivity index (SI) is a dimensionless value of the quantity of correct responses. The index is a non-parametric technique where +1 indicates a perfect score, 0 is by chance alone and -1 indicates all responses were incorrect. In most cases the SI is between 0 and +1. Alternatively mean (or median) time to react can be analysed. NWM assesses the sub-vocal rehearsal of digit sequences and the articulatory loop sub-system of working memory.

Spatial working memory. A simple 2 dimensional picture of a house with 9 windows is presented. Four of the windows are lit. The subject has to memorise this image, then respond with YES or NO when the same house re-appears but with a single, randomly lit window, depending on whether it matches one of the original lit windows. Response can be measured by the percentage of correct answers or percentage accuracy at rejecting incorrect matches, or the sensitivity index which is amalgamated from all responses. Also mean (or median) time to react. This test assesses episodic memory i.e. ability to temporarily retain spatial information using the visuospatial sub-loop of working memory.

Picture recognition. Fourteen pictures are shown for a second time interspersed with 14 new ones in random order. The subject presses YES or NO for each picture depending on a match for the original 14 pictures. Sensitivity index and reaction time are recorded. This assesses episodic secondary non-verbal visual recognition and the ability to discriminate novel from previously presented material.

Face recognition. The same as picture recognition, but using faces instead.

Immediate word recall. Twelve words appear on screen over a set time. Afterwards the subject is asked to recall all 12 words. Score is based on number of correct words, intrusions (words from previous lists) and errors. This tests ability to store and recall verbal information.

There is clearly overlap in the battery and a principal component analysis combined with a Varimax rotation yielded five factors shown in Table 4.3 (Wesnes, 2000).

Table 4-3 Principal component analysis of COGDRAS-D

Factor	CDR value
Speed of memory processes	picture and word recognition speed
	numeric and spatial working memory speed
Quality of episodic memory	immediate and delayed word recall accuracy
	word and picture recognition accuracy
Power of attention	simple and choice reaction time
	digit vigilance detection speed
Continuity of attention	digit vigilance detection accuracy
	choice reaction time accuracy
	digit vigilance false alarms
	tracking error
Quality of working memory	numeric and spatial working memory accuracy

Wesnes KA Psychopharmacology 2000 (Wesnes, 2000).

4.4.3.3 Validity and reliability

The CDR battery was compared with MMSE and Kew cognitive tests in a validation study (Simpson, 1991). Ten patients were unable to perform number vigilance tasks but all other CDR tests could be completed by the 22 controls and 23 patients with dementia. Immediate word recognition, delayed word recognition, delayed picture recognition, memory scanning and choice reaction tasks were significantly impaired in dementia patients and number vigilance was non-significantly lower in dementia. Age was significantly higher in the dementia group so a secondary analysis was performed by excluding younger controls and older dementia cases, and similar differences in performance were obtained. Immediate and delayed word recognition, delayed picture recognition, memory scanning and choice reaction time correlated with the MMSE (sensitivity scores, range of correlation coefficient r +0.53 to +0.65, $p < 0.001$; reaction times r -0.57 to -0.68, $p < 0.001$). There were similar correlations with the Kew Test of memory, aphasia and parietal function. Correlations with a measure of behaviour and everyday skills, the Stockton Rating Scale were weaker for example CRT r = 0.50, $p < 0.05$, but most were still significant. Test-retest reliability was performed in 26 subjects; results are reported using only correlation co-efficients, with values ranging from +0.53 ($p = 0.0079$) for memory scanning sensitivity to +0.93 ($p < 0.0001$) for choice reaction time.

A validation study was performed on 98 subjects split into five groups; 'worried well', depression, dementia, minimal cognitive impairment and other brain disorders (Nicholl, 1995). The dementia group scored least well, 'worried well' the best and the minimal

cognitive impairment group had intermediate scores. Other diagnoses had a wide spread of scores in keeping with the heterogeneous nature of diagnoses. The depressed group performed less well than 'worried well' but to a small non-significant degree and much better than the dementia group. Generally the minimally impaired had accuracy scores at an intermediate level between worried well and dementia but reaction times were similar to the dementia group. This interesting finding points to the importance of speed of memory performance. The authors discuss how this dissociation between accuracy and speed may represent a desire by patients with minimal cognitive impairment to sacrifice speed in the quest for accuracy. Alternatively there may be early deficits in speed of recognition and processing which may herald cognitive decline. The CDR battery has established sensitivity in assessing cognitive change in the elderly and inpatients with dementia (Simpson, 1991). In a criterion validity study, MMSE score correlated with CRT ($r = -0.542$, $p = 0.04$), spatial working memory ($r = 0.938$, $p = 0.01$) and word recognition ($r = -0.949$, $p = 0.01$). However this was a small study of 15 young patients and the test schedule had to be modified due to patient fatigue. There were no significant associations between CDR battery and IQ test but the latter was taken from historical records of only six patients and not at the time of testing (Keith, 1998).

CDR battery was able to measure change in cognition during oxygen administration in healthy young adults but this study did not run other gold standard tests of cognition, so cannot be viewed as a validation study (Moss, 1998). Similar weaknesses are found in a comparison of cognitive function in young and old dentists with regard to the effects of mercury exposure but the CDR battery was shown to be straightforward to use (Ritchie, 1995). CDR was able to detect an improvement in working and long-term memory in healthy middle aged volunteers in response to Gingko biloba (Wesnes, 2000).

4.4.4 Cornell scale for depression in dementia

The scale measures depressive symptomatology in depression. Its value lies in obtaining information from an informant since the patient is often not able to provide reliable answers to traditional depressive scale questionnaires (Alexopoulos, 1988).

4.4.4.1 Requirements

The scale is completed by an interviewer questioning both carer (20 minutes) and patient (10 minutes). The carer needs to have a reasonable knowledge of the patients' day-to-day behaviour and changes that may have occurred. Response is based on the week prior to interview.

4.4.4.2 Scoring system

A 19 item scale, divided into five subsections: mood related signs, behavioural disturbance, physical signs, alteration in circadian function and ideational disturbance. Each of the 19 items can score 0 for absence of positive signs, 1 if mild or intermittent and 2 for severe symptoms. Greater than 10 points indicates significant depression.

4.4.4.3 Reliability and validity

The original paper examined 26 subjects. Inter-rater reliability studies yielded a kappa statistic of 0.67 i.e. good. Internal consistency yielded a Cronbach's alpha score of 0.84. Validity was satisfactory when compared with research diagnostic criteria.

5 Characteristics of the cohort

5.1.1 Demographic data

The assessment period ran from July 2000 to March 2002. All COGFAST participants from Freeman Hospital, Queen Elizabeth Hospital, North Tyneside Hospital and South Tyneside General Hospital were screened. During this period, the total number of candidates recruited to COGFAST was 190. Ninety-seven people consented to participation in the cardiovascular study. Fifty-four (56%) were female and 43 male. The average age was 80.7 SD 4.2 years. range 75.0 to 93.0 years (interquartile range 77.5 to 83.0 years). Further clinical characteristics are described below.

5.1.2 Cerebrovascular lesion

All stroke subtypes were included in the study, with the exception of subarachnoid haemorrhage. Table 5.1 records the numbers in each group according to the Oxford Community Stroke Project (Bamford, 1991). The difference between OCSP population data and this cohort regarding stroke subtype are discussed in a later section.

Table 5-1 Oxfordshire Community Stroke Project stroke classification

Stroke subtype	Hemisphere			Total
	left	right	unknown	
Total Anterior Circulation	2	2	0	4
Partial Anterior Circulation	25	14	1	40
Lacunar	11	17	1	29
Posterior Circulation	4	3	11	18
Unclassified	4	0	2	6
Total	46	36	15	97

Allocation of stroke subtype and affected hemisphere was based on retrospective review of clinical findings in combination with clinical CT brain report. The left hemisphere partial anterior circulation stroke group contains two cases with dysphasia with no CT lesion, assumed to have suffered left hemisphere events. The 'unknown' column contains stroke cases labelled as partial anterior, lacunar stroke and unclassified cases but casenote review did not allow identification of side of either clinical symptoms or acute CT lesion. The 'unclassified' group had insufficient clinical data in casenotes to confidently allocate stroke subtype. Four of these had CT evidence of an acute left hemisphere lesion.

There were 18 cases with clinical evidence of posterior circulation stroke: three had right-sided limb symptoms, four had left-sided limb symptoms and ten did not have lateralising

signs. A minority of the posterior circulation stroke group had visible acute lateralising CT lesions.

Table 5.2 contains data on CT appearance (from casenote radiology reports) in relation to the OSCP stroke subtype.

Table 5-2 CT appearance and stroke subtype

CT abnormality	OSCP subtype				
	TACS	PACS	LACS	POCS	Unclassified
Large cortical/subcortical	3				
Medium/small cortical/subcortical	1	22	2	2	1
Deep hemisphere		6	11		1
Brainstem or cerebellar		1		7	
None		10	16	9	3

Large cortical/subcortical; involves large area or more than one cortical lobe. Medium/small cortical/subcortical; involves only one lobe (frontal, parietal, temporal, occipital) or specifically described as small. Deep hemisphere; basal ganglia, internal capsule, periventricular lesions, thalamus, subthalamus, hypothalamus.

Thirty-nine percent of cases did not have a visible CT lesion that was consistent with the acute cerebrovascular syndrome. All of the total anterior circulation syndrome cases had a visible lesion which was usually a large cortical infarct. Just over half the partial anterior circulation stroke cases had an appropriate medium or small CT lesion involving the cerebral cortex whilst a quarter did not have a visible acute lesion. The vast majority of the lacunar syndromes either had an acute deep hemisphere lesion or no visible acute lesion. Similarly most of the posterior circulation stroke syndromes had an appropriate lesion in the brainstem or cerebellum, or no visible lesion. Of the two posterior circulation stroke cases with a medium/small cortical/subcortical lesion, one was an occipital infarct. There were no primary intracerebral haemorrhages seen on CT scanning. Two cases were reported as haemorrhagic infarction. The first had a partial anterior circulation stroke, with a left hemisphere medium-sized cortical lesion. The second haemorrhagic infarction case had a lacunar-type stroke with a right deep hemisphere lesion on CT scan. The total number of cases in Table 5.2 is 95: two are excluded because they had a delayed MRI scan instead of CT imaging.

5.1.3 Clinical characteristics

The median interval from stroke to cardiovascular assessment was 251 days, interquartile range 197 to 321 days. This interval was more than a year in 18 cases.

Table 5-3 Clinical characteristics of study population

History	Percentage
Congestive cardiac failure	19
Myocardial infarction	19
Angina	21
Myocardial infarction or angina	27
Hypertension	68
Atrial fibrillation	24
Peripheral vascular disease	12
Asthma	16
Chronic obstructive pulmonary disease	17
Asthma or Chronic obstructive pulmonary disease	24
Diabetes mellitus (type II)	6
Hypercholesterolaemia	23
Hypothyroidism	12
Hyperthyroidism	4

Table 5-4 Smoking and alcohol habits of study population

Habit	Usage	Percentage
Smoking	Never	28
	Ex-smoker	14
	Current	58
Alcohol/units per week	None	49
	1-10	37
	11-20	9
	21-50	4
	>50	1

Additional data on cholesterol status were available from fasting blood tests performed on 91 of the participants. Sixty-six percent had fasting total cholesterol levels $\geq$ 5.2 mmol/l, six percent did not have blood cholesterol levels performed. Therefore a total of 76% cases had a prior history of hypercholesterolaemia or elevated fasting cholesterol.

Cardiorespiratory abnormality on examination was infrequent. Only two percent had elevated jugular venous pressure, ankle oedema was present in 22% and on chest auscultation nine percent had expiratory wheeze and five percent had inspiratory crackles. Cardiac murmurs were present in 16% and carotid artery bruits in 14%. Inter-rater reliability of such clinical

Table 5-5 Frequency of previous cerebrovascular events

Event	Total number	Percentage
TIA	0	83
	1	10
	2	1
	3	3
	4	3
Stroke	0	80
	1	14
	2	2
	3	1

Table 5-6 Drug prescription rates in study population

Drug	Percentage
Beta blocker	28
Thiazide diuretic	39
Loop diuretic	17
ACE inhibitor or Angiotensin II blocker	21
Calcium channel blocker	22
Diltiazem	6
Other hypertensive agent	1
Nitrate	13
Nicorandil	2
Amiodarone	1
Digoxin	12
Warfarin	21
Antiplatelet	70
SSRI	9
Tricyclic agent	5
Other sedative drugs	6
Oral corticosteroid	4
Inhaled beta-2 agonist	6
Anti-epileptic	6
Insulin	2
Oral hypoglycaemic	1
Thyroxine	11
Statin	21
Oxybutynin	6

signs is usually very poor – these data are presented for the sake of completeness but cannot be used in data analysis.

Table 5-7 Examination findings of study population

Parameter	Mean ± standard deviation (n=97)	Range
Height / m	1.62 ± 0.10	1.43, 1.96
Weight / kg	67.0 ± 12.7	38.7, 108
Body mass index	25.4 ± 3.7	17.6, 36.9
Systolic blood pressure / mmHg	150.2 ± 24.0	106, 220
Diastolic blood pressure / mmHg	83.2 ± 11.8	56, 110
Forced expiratory volume / litre*	1.71 ± 0.66	0.35, 3.15
Forced vital capacity / litre*	2.37 ± 0.83	0.70, 4.00
FEV/FVC	0.71 ± 0.09	0.50, 0.97
MUPDRS (range 0-32)	2.40 ± 3.08	0, 13
Total SNSS (range 0-58)	55.7 ± 3.3	45, 58
Long-term SNSS (range 0-48)	45.7 ± 3.3	35, 48

MUPDRS, Modified Unified Parkinson's Disease Rating Scale: SNSS, Scandinavian Neurological Stroke Score:
*n = 92 for lung volumes

Eighty-four percent of cases had a modified UPDRS score ≤ 5, only four percent scored 10 or more and the interquartile range was 0 to 3 (maximum score 32). Interquartile range for the total and long-term Scandinavian Neurological Stroke Scale scores were 54 to 58 (maximum score 58) and 44 to 48 (maximum score 48) respectively.

5.1.4 Investigations

Table 5-8 ECG characteristics

ECG abnormality		Number
Sinus rhythm		76
Atrial fibrillation		21
Q waves	None	70
	Inferior	19
	Anterior	5
	Not applicable (BBB)	3
Left ventricular hypertrophy	None	83
	Present	11
	Not applicable (BBB)	3

BBB, bundle branch block

Table 5-9 Neuropsychological parameters

Cognitive test	Number	Mean ± SD or median (IQR)	Range
MMSE	97	27.8 ± 3.1	23, 30
CAMCOG total	97	85.0 ± 8.5	61, 99
GDS	93	4.0 ± 3.1	0, 14
Cornell	94	3.9 ± 3.4	1, 6
CDR Number scanning/ seconds	94	516 (478, 603)	0, 759
CDR choice reaction/ seconds	94	632 (537, 836)	434, 3149
CDR choice reaction SD/ seconds	94	163 (110, 253)	56, 2442
CDR memory/ seconds	93	1126 (962, 1415)	654, 10017

MMSE, Mini-Mental State Examination: CAMCOG, Cambridge Cognitive Examination: GDS, Geriatric Depression Scale: CDR, Computerised Dementia Research battery

Table 5-10 Ambulatory blood pressure recording

Ambulatory blood pressure		Mean ± SD	Range
24 hour	Number of measurements	34.0 ± 6.5	9, 47
	Systolic (mmHg)	137.6 ± 14.8	104, 182
	Diastolic (mmHg)	72.9 ± 8.1	58, 94
	Pulse pressure (mmHg)	64.7 ± 12.8	38, 106
Day	Number of measurements	17.6 ± 3.8	6, 29
	Systolic (mmHg)	141.3 ± 16.0	103, 181
	Diastolic (mmHg)	75.6 ± 9.1	53, 99
	Pulse pressure (mmHg)	65.7 ± 13.7	37, 104
Night	Number of measurements	5.7 ± 1.2	0, 7
	Systolic (mmHg)	129.4 ± 16.1	99, 181
	Diastolic (mmHg)	66.8 ± 8.9	48, 86
	Pulse pressure (mmHg)	62.5 ± 13.0	36, 102

5.1.5 Discussion

Exact figures for number screened for the study are not available since candidates for the study were initially assessed by local hospital staff. Therefore the total number of stroke admissions who were candidates for COGFAST was not collated by investigators. This arrangement was part of the initial agreement with local COGFAST collaborators. Caldicott guidelines also influenced the procedure since potential candidates for the study were not revealed to the COGFAST team without permission from the patient. It is clear the cases identified for the study are a highly selected group, even without the benefit of accurate numbers of candidates at each stage of the selection process.

Allocation of cases to OCSP stroke subtype was performed retrospectively. All stroke patients were assessed by a consultant with expertise in Stroke Medicine during their admission and usually allocated a subtype. However these data were occasionally missing and therefore subtype allocation was performed on a 'best estimate' basis using available clinical data. In some cases senior assessment occurred after improvement of clinical signs; consequently opinion between clinicians on subtype could differ. The OSCP classification should be based on 'the clinical pattern at the time of maximum deficit' (Bamford, 1991). It has been shown that interobserver agreement for the OCSP classification using neurology-trained clinicians can vary from moderate to good (Lindley, 1993). All these factors suggest that whilst the data on stroke classification is based on best available information, they may lack a degree of accuracy.

From Table 5.2 it can be seen that CT appearance does match the stroke subtype for the majority of cases. This is in line with published data indicating the OSCP classification system predicts the site of infarct in approximately three-quarters of patients (Mead, 2000a). From the data in Table 5.2, 56 patients had a visible acute infarct: 43 (77%) had a CT lesion appropriate to the clinical syndrome in keeping with Mead et al's results (Mead, 2000a). Three total anterior circulation stroke cases had a large cortical, 22 partial anterior circulation stroke had a medium/small cortical/subcortical, 11 lacunar stroke cases had a deep hemisphere and 7 posterior circulation stroke cases had a brainstem/cerebellar lesion. It should be borne in mind that rating of OCSP subtype and CT lesion were performed by the author and not blinded. It is likely this favourably influenced the match between clinical and radiological reports. However, the important message is that retrospective data collection was reasonably accurate in indicating the infarct type and size. Furthermore the absence of an acute lesion on CT in 39% of cases is similar to the much larger study of Mead et al. Thirty-five percent of stroke patients did not have a visible acute lesion on imaging in this series of 1012 patients (Mead, 2000a). This radiological comparison seems favourable when it is considered that Mead's study had the benefits of expert neuroradiology opinion and a small proportion of patients had more sensitive MRI scans.

Clearly there were high rates of co-morbidity within the study group. Some of these conditions are known to affect autonomic function – diabetes, myocardial infarction and congestive cardiac failure are more obvious examples. However exclusion of all significant co-morbidity was not a viable option. In order to obtain a reasonable number of candidates, there had to be some allowance for other pathology. Furthermore the aim of the study is to examine the natural history of post-stroke patients and the evolution of cognitive decline. Exclusion of all co-morbidity would lead to a study group not representative of the typical population at risk. Thus results would be hampered by the caveat of highly selected cases.

There is some confirmation from stroke incidence surveys that the cohort here is representative in terms of co-morbidity rates. The German cohort in Erlangen (100,330 inhabitants studied over 4 years) had a history of hypertension in 57%, diabetes in 25%, current smoking habit in 13% and history of cardiac disease 54% (Kolominsky-Rabas, 2001). Diagnostic criteria for hypertension included self-reported history or levels above 160 mmHg for systolic and 95 mmHg for diastolic blood pressure. Diabetes prevalence was probably higher because they included elevated fasting blood glucose but it is not clear if all cases had such a test. Cardiac disease included history of coronary artery disease, congestive cardiac failure, arrhythmia or valvular heart disease as one category. For the Italian cohort in the Belluno province (211,389 inhabitants studied over 1 year), 54% had a history of hypertension, 24% atrial fibrillation, 21% coronary artery disease, 18% diabetes mellitus, 18% current smokers, 9% hypercholesterolaemia, 14% peripheral vascular disease and 13% previous TIA (Lauria, 1995). The Russian cohort in Novosibirsk (158,234 inhabitants over 1 year) had a history of hypertension in 82%, angina in 46%, atrial flutter/fibrillation in 16%, cigarette smoking in 35%, TIA in 11%, diabetes in 9% and congestive heart failure in 5% for cerebral infarction and haemorrhage of all ages (Feigin, 1995). In the Oxfordshire Community Stroke project, 52% had a history of hypertension, 38% ischaemic heart disease, 25% peripheral vascular disease, 13% atrial fibrillation and 10% diabetes mellitus (244 cases of first ever stroke) (Sandercock, 1989). Thirteen percent of patients with stroke were diabetic in the community prevalence survey by O'Mahony et al (O'Mahony, 1999). In the Greek Arcadia Stroke Registry incidence study, risk factors were hypertension in 81%, diabetes mellitus in 29%, coronary artery disease in 20%, atrial fibrillation in 34%% and TIA in 17% (Vemmos, 1999).

The best comparison of co-morbidity for this cohort of stroke patients comes from a study by Rodgers et al (Rodgers, 2004). A total of 329 first-ever strokes occurred in a cohort of 4440 subjects aged over 65 years followed for five years. Crude prevalence rates for stroke risk factors from this study are shown in Table 5.11

When compared with Rodgers study, the stroke patients in our study had very similar prevalence rates for gender, congestive cardiac failure, coronary artery disease (MI or angina), peripheral vascular disease, left ventricular hypertrophy, diabetes and regular alcohol consumption. History of hypertension and presence of atrial fibrillation were much higher, and TIA slightly higher in our cohort. This is unlikely to be solely related to the slight age difference between the studies, 80.7 ± 4.2 years in our study vs. 78.6 ± 6.3 years in Rodgers et al (Rodgers, 2004). Reasons for the discrepancy probably could include ascertainment method and selection bias in our study but it is difficult to say with certainty what led to these differences. Smoking was almost twice as common in our study, and this may be related to

our method of direct questioning compared with reference to GP records for the Rodgers study.

Table 5-11 Crude prevalence rates of risk factors and adjusted hazard ratios for first-ever stroke, Rodgers et al

Risk factor	Stroke		Hazard ratio (95% CI)
	% Yes (n=329)	% No (n=4022)	
Male	46.8	45.8	1.28 (1.02-1.59)
Transient ischaemic attack	12.5	5.5	2.18 (1.57-3.04)
Hypertension (history/treatment)	45.6	33.0	1.68 (1.35-2.09)
Atrial fibrillation (ECG)	9.7	4.6	2.12 (1.47-3.06)
Current smoker	28.2	20.7	1.79 (1.39-2.32)
Left ventricular hypertrophy (ECG)	13.7	9.9	1.38 (1.01-1.90)
Regular alcohol	53.0	45.2	0.77 (0.59-1.02)
Diabetes	7.3	5.7	1.27 (0.84-1.93)
Angina	24.6	20.2	1.34 (1.04-1.72)
Myocardial infarction	11.6	10.7	1.16 (0.82-1.62)
Peripheral vascular disease	10.6	6.3	1.86 (1.31-2.65)
Ischaemic heart disease	28.6	23.4	1.32 (1.05-1.67)
Heart failure	16.1	12.1	1.42 (1.05-1.92)

From Rodgers et al Stroke 2004 (Rodgers, 2004)

For the purposes of analysis requiring binomial data, ex-smoker and never smoked status were subsequently collapsed into one category of 'not current smoker'. This was because many of the ex-smokers had stopped smoking more than 5 years prior to the study and a pragmatic decision to group these cases in the lower risk group seemed appropriate. Otherwise this group may have attained a disproportionate risk if classified the same as current smokers, since smoking risk declines significantly if several years have elapsed since discontinuation. Estimating pack years of smoking would have provided a more useful continuous marker of smoking risk

There were high rates of smoking, both current and ex-smokers in the study group. Smoking can affect autonomic function. Decreased vagal indices, increased sympathetic activity during Valsalva manoeuvre and reduced Valsalva ratio and increased diastolic blood pressure response to isometric exercise have been reported as consequences of smoking (Kotamaki, 1995; Niedermaier, 1993; Poulsen, 1998). Vagal dysfunction is common in alcohol-dependent adults (particularly those with liver disease) and 24 hour heart rate variability demonstrates autonomic damage in those with normal autonomic reflex tests (Duncan, 1980; Hendrickse, 1992; Malpas, 1991).

The cohort had a low mean Modified Unified Parkinson's Disease Rating Scale. This modified version of motor section from the UPDRS contains 5 key items that are independent of cognitive impairment and is specific for neuropathological changes of idiopathic Parkinson's disease (Ballard, 1997). The observed low scores indicate absence of clinically significant extrapyramidal disease in this cohort.

Scandinavian Neurological Stroke Scale scores were generally high, consistent with the selection of a post-stroke cohort alive and independent more than three months post-stroke. The index does not have any relevance for the autonomic investigations but does indicate the majority of the cases were free of disabling neurological deficit.

There was a wide variation in interval between stroke and autonomic testing. The delays arose for two main reasons. Firstly most of the delay occurred early in the course of the study because patients had consented to COGFAST including MRI investigations but ethical approval for the cardiovascular investigations was outstanding. MRI investigations had to proceed in a timely manner and it was not practical to defer until cardiovascular investigation was permissible. Secondly, duration of identification, screening and recruitment process simply meant that several months had elapsed before individuals could be investigated.

The mean office systolic blood pressure was 150 mmHg. Ten cases had an office systolic blood pressure of $\geq$ 180 mmHg and the highest value was 220 mmHg. Therefore accuracy of the Spacelabs 90207 monitor may have been below BHS limits in this small proportion of cases with very elevated blood pressure (Iqbal, 1996). However the office blood pressure value is from one measurement only and therefore may itself exaggerate actual blood pressure. The Spacelabs 90207 device was chosen because of familiarity with use with hospital staff. Overall this ambulatory blood pressure monitor was a satisfactory choice since few subjects had the very high pressures associated with diminishing accuracy of the device in the elderly.

5.1.6 Conclusions

Most of the patients in the cohort had a history of partial anterior, lacunar and posterior circulation stroke. Stroke risk factors were usually similar in prevalence to previous large scale epidemiological surveys. Two risk factors had high prevalence rates when compared to a recent incidence study in the UK. History of hypertension was particularly common affecting 66% of patients. Prevalence of atrial fibrillation was higher than might be expected at 22%. Generally the cohort is representative of an older post-stroke cohort. Comorbidity and medication use has implications for autonomic testing.

6 Cardiovascular autonomic reflex tests

6.1 Introduction

Tests of heart rate and blood pressure variation during cardiovascular provocation are established methods of measuring autonomic function. Autonomic function declines with age. Autonomic reflexes may be impaired in diabetes and other chronic diseases. The study cohort had high rates of co-morbidity. Co-existent chronic disease and medication may have impaired cardiovascular autonomic reflexes. This chapter investigates the impact of cardiovascular risk factors and medication on autonomic tests.

6.2 Method

A series of autonomic reflex tests were performed as described in Chapter 4. Only cases in sinus rhythm on the day of investigation were included. Mean, median and interquartile range values were calculated. Heart rate variation for the metronomic respiration was assessed for both mean ratio and difference in maximum and minimum heart rate for the purpose of comparison with previous studies.

The relationship between co-morbid conditions and drug treatment was examined using correlation and then regression. Correlation was performed using Spearman's rank correlation coefficient between reflex test and co-morbid condition or drug. Multiple linear regression was performed using each cardiovascular autonomic reflex test as the dependent variable. The following were entered as independent co-morbid variables from clinical history: cardiac failure, ischaemic heart disease (history of myocardial infarction or angina), hypertension, peripheral vascular disease, respiratory disease (chronic obstructive pulmonary disease and/or asthma), diabetes mellitus, current smoker and current alcohol consumption. At the same time, use of the following drugs were also entered as independent variables: beta-blocker, thiazide diuretic, loop diuretic, ACE inhibitor or angiotensin II receptor antagonist, dihydropyridine calcium channel antagonist, nitrate, selective-serotonin receptor inhibitor, tricyclics and oxybutynin. Presence of co-morbid condition or drug use was assigned a value of one and absence a value of zero. Regression was performed by entering all candidate predictor variables, then excluding the least significant predictor variable in a stepwise manner. This was repeated until only predictor variables with a significance of $p < 0.05$ remained or there were no significant predictor variables. The regression model for each autonomic test was confirmed by forward regression.

One sample Kolmogorov-Smirnov test revealed that RR ratio tests did not follow a normal distribution. To achieve a normal distribution for regression analysis, logarithmic transformation after subtraction of one was performed i.e. $\log_{10}(x - 1)$. For the heart rate

difference during metronomic respiration, only logarithmic transformation was required to achieve a normal distribution (Ziegler, 1992b).

6.3 Results

Summary of the results is shown in Table 6.1.

Table 6-1 Cardiovascular autonomic reflex test results

Cardiovascular reflex test	Number	Mean ± SD	Median (IQR)	Range
30:15 ratio	71	1.14 ± 0.14	1.10 (1.04, 1.18)	0.96, 1.76
Δsystolic BP orthostasis (mmHg)	72	31.6 ± 31.3	26.0 (9.0, 48.8)	-18, 116
Δdiastolic BP isometric exercise (mmHg)	74	18.4 ± 15.1	17.5 (7.8, 30.3)	-17, 58
Δdiastolic BP cold pressor (mmHg)	72	10.7 ± 13.3	8.5 (4, 14.8)	-24, 74
Valsalva ratio	69	1.25 ± 0.17	1.23 (1.15, 1.33)	1.03, 2.17
Δsystolic BP Valsalva overshoot (mmHg)	68	16.4 ± 17.1	16.5 (4.8, 30.0)	-25, 52
E/I ratio metronomic respiration	73	1.11 ± 0.08	1.08 (1.06, 1.13)	1.02, 1.41
ΔHR metronomic respiration (bpm)	73	6.3 ± 4.5	4.8 (3.3, 7.9)	1.7, 21.8
Δsystolic BP CSM (mmHg)	31	-28.3 ± 12.0	-30.0 (-38.0, -17.0)	-51, -9
ΔRR CSM (ms)	31	1191 ± 269	1122 (978, 1418)	635, 1662

SD, standard deviation: IQR, interquartile range: BP, blood pressure: E/I, ratio of expiratory/inspiratory RR interval during metronomic respiration: HR, heart rate: CSM, carotid sinus massage

Successful completion rates ranged from 89% for blood pressure overshoot during Valsalva manoeuvre to 97% for blood pressure change during isometric exercise. Fifty-one cases (67% of sinus rhythm cases) completed all heart rate response tests satisfactorily. This value excludes carotid sinus massage where number of completed tests was much lower due to exclusions from test contra-indication and test refusal. The number of cases completing all cardiovascular reflex tests was 47, representing 62% of sinus rhythm cases (i.e. all heart rate and blood pressure results available).

For simple correlation tests, the only significant associations occurred between (i) cold pressor and ischaemic heart disease, (ii) Valsalva blood pressure overshoot and beta-blockers, loop diuretic or nitrate, and (iii) systolic blood pressure change from CSM and ACE inhibitors/AIIR antagonists or oxybutynin. There were trends towards correlation for other analyses. For the sake of completeness, all rank correlations with a significance value of less than 0.10 are shown in Table 6.2.

Table 6-2 Correlation between autonomic reflex and co-morbid history or drugs: correlation co-efficient <0.10

Cardiovascular reflex test	Co-morbidity/drug	No.	Spearman's rho	Significance
Log_{10} (30:15 ratio – 1)	Cardiac failure	67	-0.24	0.052
	Respiratory disease	67	-0.22	0.069
Δsystolic BP orthostasis	Tricyclic	67	-0.21	0.080
Δdiastolic BP isometric exercise	ACEi/AIIRA	74	-0.20	0.082
Δdiastolic BP cold pressor	IHD	70	+0.38	0.001
Log_{10} (VR ratio – 1)	Loop diuretic	69	-0.22	0.069
Δsystolic BP Valsalva overshoot	Beta-blocker	68	-0.28	0.019
	Loop diuretic	68	-0.25	0.038
	Nitrate	68	-0.27	0.025
Log_{10} (E/I ratio – 1)	None			
Log_e ΔHR metronomic respiration	Hypertension	73	-0.23	0.051
Δsystolic BP CSM	ACEi/AIIRA	31	-0.41	0.021
	Oxybutynin	31	-0.36	0.046
ΔRR CSM	None			

BP, blood pressure: ACEi/AIIRA, ACE inhibitor or angiotensin II receptor antagonist: IHD, ischaemic heart disease: VR, Valsalva ratio: E/I, ratio of expiratory/inspiratory RR interval during metronomic respiration: HR, heart rate: CSM, carotid sinus massage

Results from multiple linear regression of co-morbid conditions and drugs on autonomic tests are shown in Table 6.3. History of ischaemic heart disease significantly increased the diastolic blood pressure response to cold cutaneous stress by 8.8 mmHg (95% CI 4.2 to 13.4 mmHg). History of hypertension was a significant and negative predictor of the transformed value for the change in heart rate with metronomic respiration. Prescription of angiotensin system blocking drugs significantly decreased the systolic blood pressure reaction to carotid sinus massage by -13.5 mmHg (95% CI -24.6 to -2.4 mmHg). For the RR interval tests, transformed values for the 30:15 ratio, Valsalva ratio and E/I ratio were not significantly predicted by co-morbid conditions or drug therapy.

Drop in blood pressure on standing produced contradictory predictor variables using entry method for regression. Drop in blood pressure on standing was reduced by history of diabetes and cardiac failure and worsened by the presence of loop diuretic and calcium channel blocker. However using *forward* regression technique, there were no significant predictor variables for drop in blood pressure on standing. Blood pressure response to isometric exercise and Valsalva manoeuvre were not significantly predicted by any co-morbid conditions or drugs.

Table 6-3 Predictor variables for cardiovascular autonomic reflex tests

Autonomic test	Significant predictor variable	Regression coefficient	
		B	95% CI
Log_{10} (30:15 ratio -1)	None		
Δsystolic BP orthostasis	None*		
Δdiastolic BP isometric exercise	None		
Δdiastolic cold pressor	Ischaemic heart disease	+8.8	+4.2, 13.4
Log_{10} (VR – 1)	None		
Δsystolic BP Valsalva overshoot	None		
Log_{10} (E/I ratio – 1)	None		
Log_e ΔHR metronomic respiration	Hypertension	-0.31	-0.61, -0.02
Δsystolic BP CSM	ACEi/AIIRA	-13.5	-24.6, -2.4
Δsystolic RR CSM	None		

BP, blood pressure: ACEi/AIIRA, ACE inhibitor or angiotensin II receptor antagonist: VR, Valsalva ratio: E/I, ratio of expiratory/inspiratory RR interval during metronomic respiration: HR, heart rate: CSM, carotid sinus massage: *, see text.

6.4 Discussion

The majority of cases were able to complete cardiovascular autonomic reflex tests. This appears to be a reasonable yield of results considering the nature of the cohort. Many authors comment on difficulties in performing reflex tests in older subjects but rates of successful test completion are rarely quoted. In a review of 10 years performing clinical autonomic investigations, Ewing et al (Ewing, 1985) report 70% of 774 diabetic patients completed all five of his autonomic test battery. It would appear our completion rate compares favourably with this series, particularly as the Ewing patients were probably a younger group than our cohort. The overall completion rate of 62% includes two additional blood pressure tests compared with the Ewing protocol (systolic overshoot during Valsalva and cold pressor tests). In our study, the completion rate for the Ewing battery alone is 66%.

Values from this cohort can be compared with published reports of normal values for autonomic reflex tests summarised in Table 6.4. There are few studies focussing on older adults comparable in age to our cohort. The closest match is probably Clark's study that included patients up to 92 years age (Clark and Mapstone, 1986). Most of the summarised studies in the table do not accurately state the proportion of older subjects; instead the older adults are included in the total number of subjects in each study.

Table 6-4 Normal values for cardiovascular autonomic reflex tests in older adults

Autonomic test	Mathias. Age ≥70. Mean ± SEM (95% CI)	Wieling. Age 75-80. Abnormal values	Ziegler. Age 65. Lower 97.7 centile value	O'Brien. Age 75. Lower 95% (90%) confidence limit	Clark. Age 80. Lower 90% confidence limit (est.)	Ewing. Age max. 69. Abnormal values
30:15 ratio		<1.00	1.06	1.01 (1.03)	1.01	≤1.00
ΔHR standing (beats/min)		<11		3 (4)		
Valsalva ratio	1.33 ± 0.06 (1.21 to 1.45)	< 1.00	1.16	1.09 (1.13)	1.02	≤1.10
E/I ratio respiration			1.10	1.00 (1.02)		
ΔHR respiration (beats/min)	9 ± 1 (8 to 10)	< 7		2 (3)	8	≤10
ΔSBP standing/ mmHg			-27		-30	≤30
ΔDBP isometric/ mmHg	11 ± 1 (9 to 13)		+5			≤10
ΔDBP cold pressor/ mmHg	12 ± 4 (5 to 19)	< 10, > 15				
ΔSBP Valsalva/ mmHg						

Mathias 1999 (Mathias and Bannister, 1999). Subjects aged >70, number not stated, estimate ≤ 10 subjects. Automated sphygmomanometer. Heart rate recording method not known.

Wieling 1999 (Wieling and Karemaker, 1999). Subjects aged 75-80. Adapted from age group 70-74 where no values given for 30:15 and Valsalva ratios for 75-80 year olds. Number not known. Possible combination of Finapres and sphygmomanometer, and combination of pen recorder and computerised heart rate recording.

Ziegler et al 1992 (Ziegler, 1992b). Subjects aged up to 67. Blood pressure changes during handgrip and posture change independent of age. Quoted 2.3 centile values for RR interval ratios. Automated sphygmomanometer. Computerised heart rate recording.

O'Brien 1986 (O'Brien, 1986). 36 subjects aged 70-85 years. Computerised heart rate recording

Clark 1986 (Clark and Mapstone, 1986). 85 subjects aged 31-92, mean age 66 years. Values estimated from plots of mean, 90% and 95% confidence limits. Pen recorder for heart rate and manual sphygmomanometer for blood pressure recordings.

Ewing 1985 (Ewing, 1985). Subjects aged 16-69, variable number for each autonomic test from 71 to 139. Values for VR, postural blood pressure response and isometric exercise not age dependent. Standard sphygmomanometer, probable combination of pen recorder and computerised heart rate recording.

None of the studies provide a robust comparison for this cohort's results. The average age of subjects is usually younger than our cohort. Each report only includes a selection of the tests performed in this study. Finally the techniques used in the studies in Table 6.4 differ substantially from this cohort – only a few use computerised methods of measuring RR interval and most use intermittent blood pressure recording using manual/automated sphygmomanometers. Our computerised method is clearly different from intermittent sphygmomanometric methods of blood pressure measurement, in particular beat-to-beat blood pressure monitoring using Finapres and averaging short sections of blood pressure values.

Consensus of opinion indicates heart rate variation diminution with age reduces the test range of RR ratio tests. This impairs ability to define abnormal from normal. Using change in heart rate during cardiovascular provocation may be more useful in older subjects, particularly for heart rate variation during metronomic respiration as suggested by Piha et al (Piha, 1993). Piha reported the tests of choice in the elderly were (i) from the Valsalva manoeuvre, heart rate difference and tachycardia difference and (ii) from the orthostatic test, max-rest difference, immediate and later change in systolic blood pressure. Usefulness of metronomic respiration heart rate change was limited, and Valsalva ratio and 30:15 ratios were reported as not useful in the elderly. His test group included 26 adults aged over 70 years. The conclusions regarding test limitations were based on regression equation data that indicated 'critical ages', where the tolerance limit (outer confidence limit of normal) reach a value where no response occurs. Our cohort does appear to display a reasonable range of results for the RR intervals, but as discussed no valid reference range is currently available to confidently define normal from abnormal autonomic function in this elderly stroke group.

An inherent disadvantage of the study design was inclusion of subjects with conditions and drugs that may affect cardiovascular autonomic function. Correlation results indicated there were some associations with drugs or co-morbidity with reflex tests of heart rate and blood pressure. However multiple linear regression only found significant effects for ischaemic heart disease on cold pressor response, angiotensin-blocking drugs on blood pressure drop during carotid sinus massage and hypertension on heart rate difference during metronomic respiration. These results are somewhat surprising; one would anticipate more marked effects on autonomic indices from the multitude of confounding factors. Absence of such effects could be related to reduced test range of autonomic response in the elderly and the number of subjects in the dependent variable group (approximately 70) in relation to the number of independent variables entered in to the regression model (17). Multiple regression results provide some reassurance for lack of interference from confounding factors but cannot be taken as evidence of absence of effect for these reasons.

Unfortunately one participant suffered a cerebrovascular event during carotid sinus massage requiring hospital admission. There was rapid onset of dysphasia and mild right limb weakness within minutes of left carotid sinus massage. Symptoms persisted beyond 24 hours but the patient did recover and was discharged home. There was minimal blood pressure fall and heart rate slowing during the carotid sinus massage, therefore it is assumed the event was embolic from carotid vessel atheroma. This is despite absence of significant carotid artery disease on prior carotid Duplex scanning. Carotid sinus massage was performed on a total of 41 participants (42 %). This one case within such a small cohort suggests it may be necessary to re-evaluate the risk involved in carotid sinus massage following stroke. All participants gave informed and written consent to the procedure, with a risk of stroke estimated at one in one thousand people undergoing carotid sinus massage (Davies and Kenny, 1998). In view of the event in this small sample, the risk may now appear to be larger for stroke patients.

6.5 Conclusions

Successful acquisition of individual autonomic reflex data was achieved in 89-97% of cases. Sixty-two percent of cases completed all the intended reflex tests, similar to a previously reported large study. In spite of the large number of confounding factors, few autonomic reflexes were significantly predicted by co-morbidity or cardiovascular drug prescription. There are limited resources available for defining a normal reference range to define abnormal autonomic function in this cohort.

7 A comparison of cardiovascular autonomic function between stroke patients and control cases

7.1 Introduction

Cardiovascular autonomic function controls blood pressure and heart rate homeostasis. Large studies indicate autonomic dysfunction is an independent risk factor for cardiovascular and all cause mortality - Framingham data, the Atherosclerosis Risk in Communities (ARIC) study (which used ECG RR variation) and the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) study (which analysed time domain heart rate variability during 24 ECG recordings) (Dekker, 2000; La Rovere, 1998; Tsuji, 1994). Stroke prevalence in the UK is approximately 17.5/1000 population and the age standardised prevalence rate of stroke in people aged 55 years and over has been estimated at 47 per 1000 population (Geddes, 1996; O'Mahony, 1999). Autonomic dysfunction is potentially a common sequelae of stroke through damage to central autonomic regulatory structures.

Studies of middle-aged stroke cases indicate that autonomic function is impaired in the early stages of stroke but may improve in the first six months post-stroke (Korpelainen, 1996a; Korpelainen, 1996b; Korpelainen, 1994). However, half of all strokes occur in older patients over the age of 75 years; to our knowledge this age category has not been studied in terms of autonomic reflexes and heart rate variability. Autonomic damage could have adverse consequences in stroke survivors. Firstly impaired baroreflex sensitivity could leave individuals vulnerable to fluctuations in systemic blood pressure, which may lead to progression of white matter lesions through transient hypotension (Skoog, 1998a). This may have particular relevance in older patients where baroreflex sensitivity is already diminished as a natural phenomenon of the ageing process (Collins, 1980; Gerritsen, 2000; Kardos, 2001; Piccirillo, 2001; Shimada, 1986; Veerman, 1994). It has also been clearly established that impaired heart rate variability is a marker for sudden cardiac death following myocardial infarction (Kleiger, 1987; La Rovere, 1998; Piccirillo, 2001; Shimada, 1986; Veerman, 1994; Zuanetti, 1996). The risk of sudden death may be mediated by sympathovagal imbalance, in particular reduced vagal activity which creates susceptibility to malignant arrhythmias. This risk is likely to extend to patients with cerebrovascular disease who clearly share similar risk factor profiles and vascular pathology with coronary artery disease populations. Summary data from three trials collecting data over nearly 20,000 patient years suggested incidence of sudden death following TIA/minor stroke was approximately 1.0 % per annum (95 % CI 0.8 to 1.1) (Algra, 2003). However there are surprisingly few reports in the literature concerning any association between arrhythmic mortality and stroke: one study of 62 ischaemic stroke

patients indicated a link between insular cortex stroke, impaired heart rate variability and arrhythmic death and more recently it has been shown that impaired baroreflex sensitivity is associated with significantly increased mortality 4 years post-stroke (Robinson, 2003; Tokgozoglu, 1999).

One single test in isolation is considered inadequate for assessment of cardiovascular autonomic function. A series of autonomic tests is required to describe the site and severity of autonomic dysfunction. The Ewing battery is an established and simple bedside series of tests measuring the heart rate and blood pressure response to autonomic stimuli (Ewing and Clarke, 1982). Sympathetic and parasympathetic damage produce a typical pattern of abnormal response, providing a qualitative picture of function.

Heart rate variability is an alternative method of assessing autonomic behaviour based on computerized measures of RR variability from prolonged ECG recordings. The variability can be expressed in a number of methods, one of the more common techniques being power spectral analysis of RR frequencies. For short term power spectral analysis of heart rate variability, a five minute recording is recognised as being suitable for assessment of autonomic function (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Combined with blood pressure measurement, this technique can also measure baroreflex sensitivity.

These two approaches to autonomic assessment have contrasting benefits. The Ewing battery has the advantages of being relatively simple and straightforward at the cost of requiring good subject compliance. Power spectral analysis of heart rate variability has the advantage of being independent of subject compliance at the cost of procedural complexity. The Ewing battery is in widespread clinical use whereas HRV is predominantly a research technique.

We set out to investigate if autonomic function is impaired in a group of elderly stroke survivors, a group not previously reported in the literature, in comparison with community-living, age-matched controls with no history of cerebrovascular disease.

7.2 Method

Methodology is described in detail in Chapter 4. Controls were recruited from a community study of autonomic function in older people and from the relatives of persons taking part in another related study in Newcastle upon Tyne. Controls were matched for age and were community living subjects without any history of stroke. All subjects gave informed written and signed consent to the study.

The investigation sequence was the same for cases and controls. All subjects were asked to refrain from smoking and caffeine ingestion on the day of the investigations and to eat a light

breakfast only. All investigations were performed between the hours of 09:00 and 13:00, and took place in a warm and quiet room. A ten minute rest phase was allowed before investigations commenced with the subject supine and not engaged in conversation. All sections of the investigation protocol were interspersed with a 2 minute rest phase.

Cardiovascular autonomic reflex tests and power spectral analysis of heart rate variability were compared between stroke patients and controls. The following cardiovascular reflex tests were measured: 30:15 ratio and postural change in blood pressure during standing, diastolic blood pressure change during isometric exercise, Valsalva ratio and systolic blood pressure overshoot during Valsalva manoeuvre and heart rate variation during metronomic respiration. Five minutes of resting RR interval and systolic blood pressure variation were analysed by power spectral analysis to obtain total, low and high frequency heart rate variability power and baroreflex sensitivity in low and high frequency bands.

Carotid sinus massage was performed in the supine position for 5 seconds on the right then left carotid sinus (Brignole, 2001). Two values were obtained following carotid sinus massage. The cardio-inhibitory response was the value of the peak RR in the 30 seconds immediately after commencing carotid sinus massage. The vasodepressor response was the difference between the mean systolic blood pressure for 20 beats prior to carotid sinus massage and the nadir blood pressure in the 30 seconds post-carotid sinus massage.

Autonomic data may vary with laboratory techniques, and it is recommended that centres use their own reference data. Since our group of cases were investigated using a locally developed software, we used the results from the control group to provide reference values. RR interval and power spectral analysis data were not normally distributed and group comparisons were calculated using Mann Whitney Signed Ranks Test. Interquartile ranges are provided to compare the distribution of data. Skewed data were transformed using the natural logarithm, and then group means were compared using the t test to provide a confidence interval for the observed differences. Blood pressure may influence autonomic function (Harrington, 2000a). Since there was a difference in systolic ambulatory blood pressure, linear regression using systolic ambulatory blood pressure was performed on transformed data. Blood pressure data from the Ewing and cold pressor tests were normally distributed; group comparisons were made using the students t test. A result was considered statistically significant if the p value was less than 0.05.

7.3 *Results*

Seventy-six cases and seventy controls consented to the study. There was no difference in age between the controls and cases (80.5 ± 4.3 vs. 80.1 ± 4.0, t-test $p = 0.592$). There was a preponderance of males in controls and females in the cases that was not statistically

significant (54% male controls vs. 42% cases, Pearson Chi-Square p = 0.141). Cases had a significantly higher mean systolic ambulatory blood pressure than controls (130 ± 13 vs. 138 ± 15 mmHg, p = 0.001) but there was no significant difference between diastolic levels (70 ± 8 vs. 72 ± 8 mmHg, p = 0.143). Three cases did not tolerate the ambulatory blood pressure measurement.

Table 7-1 Clinical characteristics of stroke patients and control cases

	Controls (n=70) ± SD	Cases (n=76) ± SD
Mean age	80.5 ± 4.3	80.1 ± 4.0
Male:female (% male)	38:32 (54%)	32:44 (42%)
Mean systolic ambulatory blood pressure (mmHg)	130 ± 13	138 ± 15 *
Mean diastolic ambulatory blood pressure (mmHg)	70 ± 8	72 ± 8
Body mass index (kg/m²)	25.08 ± 3.93	25.24 ± 3.90

* t test: p = 0.001: n = 73 for ambulatory blood pressure

Table 7-2 Co-morbidity in stroke patients and control cases

Self reported history	Control Number (%)	Stroke Number (%)	RR	95% confidence intervals	P value
Cardiac failure	6 (9)	9 (12)	1.38	0.52 - 3.69	0.592
Myocardial infarction	9 (13)	13 (17)	1.33	0.61 - 2.92	0.498
Angina	17 (24)	13 (17)	0.70	0.37 - 1.34	0.311
MI or angina	22 (31)	19 (25)	1.17	0.64 - 2.11	0.696
Hypertension	21 (30)	51 (67)	2.24	1.51 - 3.31	<0.001
PVD	6 (9)	9 (12)	1.38	0.52 - 3.69	0.592
Asthma	2 (3)	13 (17)	5.98	1.40 - 25.64	0.005
COPD	5 (7)	15 (20)	2.76	1.06 - 7.19	0.031
Asthma or COPD	7 (10)	20 (26)	2.63	1.19 -5.85	0.018
Diabetes mellitus	6 (9)	4 (5)	0.61	0.18 - 2.09	0.521
Hypercholesterolaemia	15 (21)	19 (25)	1.17	0.64 - 2.11	0.696
Current smoker	33 (47)	43 (57)	1.20	0.87 -1.65	0.320
Current alcohol	47 (67)	36 (47)	0.71	0.53 – 0.94	0.019

P value, Pearson Chi Square exact significance (2 sided): RR, Relative Risk: PVD, peripheral vascular disease: COPD, chronic obstructive pulmonary disease

The mean interval between stroke and autonomic assessment was 0.75 ± 0.34 years. The stroke group had a mean Scandinavian Neurological Stroke Scale score of 55.6 s.d. 3.3. (median score 57, range 45 – 58). Thirty two cases had a partial anterior stroke. 26 lacunar stroke, 13 posterior, 2 total anterior circulation stroke and three were not classifiable. On brain imaging 53 cases had a visible cerebrovascular lesion (relevant or old) whilst 23 had no visible lesion on CT. In the cases where there was a visible cerebrovascular lesion, 30 cases had a relevant lesion alone, of which 28 were ischaemic infarctions and 2 were haemorrhagic infarctions. Another thirteen cases had a combination of relevant plus old lesions which were all ischaemic infarcts, and finally nine had only old lesions which were all ischaemic infarcts.

Co-morbid conditions are shown in Table 7.2.There was a significantly higher number of cases in the stroke group with a history of hypertension (relative risk (RR) 2.24, $p < 0.001$) and asthma or COPD (RR 2.63, $p = 0.018$). There was a significantly smaller number of cases in the stroke group who did not currently consume alcohol (RR 0.71, $p = 0.019$).

Table 7-3 **Current drug use in stroke patients and control cases**

Drug class	Control Number (%)	Stroke Number (%)	RR	95% CI	P value
Beta-blocker	13 (19)	20 (26)	1.42	0.76 – 2.63	0.323
Thiazide	8 (11)	32 (42)	3.69	1.82 – 7.46	<0.001
Loop diuretic	9 (13)	8 (11)	0.82	0.33 – 2.00	0.797
ACE inhibitor or AIIR blocker	14 (20)	16 (21)	1.05	0.56 – 2.00	1.00
Dihydropyridine CCB	7 (10)	19 (25)	2.50	1.12 – 5.59	0.029
Nitrate	7 (10)	10 (13)	1.32	0.53 – 3.27	0.613
SSRI	3 (4)	7 (9)	2.15	0.58 – 8.00	0.331
Tricyclic antidepressant	2 (3)	4 (5)	1.84	0.35 – 9.71	0.683
Oxybutynin	0 (0)	5 (7)	-	-	0.059

P value, Pearson Chi-Square exact 2-sided test: RR, Relative risk: ACE, angiotensin-converting enzyme inhibitor: AIIR blocker, angiotensin-II receptor antagonist: SSRI, selective serotonin re-uptake inhibitor

There was significantly higher prevalence of drug use in the stroke group for thiazide diuretics (RR =3.69, $p < 0.001$) and dihydropyridine calcium channel antagonists (RR 2.50, $p = 0.029$). There were no significant differences in the use of other cardiovascular-acting drugs between cases and controls (Table 7.3)

Table 7-4 Cardiovascular reflex tests: RR interval changes

Test	Group	Number	Median	Interquartile range	P value
30:15 ratio	Control	65	1.11	1.05, 1.19	0.549
	Stroke	71	1.10	1.04, 1.18	
Valsalva ratio	Control	66	1.36	1.23, 1.56	<0.001
	Stroke	69	1.23	1.15, 1.33	
E/I ratio	Control	68	1.11	1.08, 1.17	0.005
	stroke	73	1.08	1.06, 1.13	
ΔHR/ bpm (respiration)	Control	68	-6.87	-10.76, -5.05	0.002
	Stroke	73	-4.85	-7.87, -3.31	

P value, Mann Whitney test, exact significance (2-tailed): Interquartile range, Weighted average: E/I, expiratory/inspiratory ratio during metronomic respiration: ΔHR, difference in heart rate during metronomic respiration

Regarding outliers, 30:15 ratio results revealed 1 case and 2 controls with values > 1.60 and the Valsalva ratio revealed 1 case with a value greater than 2.0 but removal of these values

did not have a significant bearing upon results. There were no outlying values for metronomic respiration.

7.3.1 Heart rate changes

There were significant differences in the RR interval measures (Table 7.4). Stroke cases had smaller Valsalva ratio (medians: control 1.36, stroke 1.23, $p < 0.001$) and less heart rate variation during metronomic respiration (medians: control -6.87 beats per minute, stroke -4.85 beats per minute, $p = 0.002$). There was no difference between groups for the 30:15 ratio (median: controls 1.11, stroke 1.10, $p = 0.549$).

Table 7-5 Cardiovascular reflex tests: Transformed data and linear regression for systolic blood pressure

Test	Group	Log_e mean ± s.d.	Log_emean difference (95% CI)	P value (1)	SBP regression mean diff	P value (2)
30:15 ratio	Control	0.12 ± 0.10	0.00	0.956	0.01	0.710
	Stroke	0.11 ± 0.10	(-0.04,0.04)		(-0.05, 0.03)	
Valsalva ratio	Control	0.32 ± 0.15	0.11	0.000	0.11	0.000
	Stroke	0.22 ± 0.13	(0.06, 0.15)		(0.06, 0.16)	
E/I ratio	Control	0.12 ± 0.07	0.02	0.042	0.02	0.060
	stroke	0.10 ± 0.07	(0.00, 0.05)		(0.00, 0.05)	
ΔHR/bpm (respiration)	Control	1.95 ± 0.57	0.30	0.003	0.30	0.005
	Stroke	1.65 ± 0.61	(0.11, 0.50)		(0.09, 0.51)	

Log_e mean ± s.d., Log_e transformed values ± standard deviation:

P value (1), t-test for the log_e transformed mean difference: SBP regression mean diff, mean log_e transformed differences after linear regression for ambulatory systolic blood pressure

P value (2), t-test for the log_e transformed differences after linear regression for ambulatory systolic blood pressure

E/I ratio, mean of maximum/minimum RR intervals during metronomic respiration: ΔHR/bpm, mean of variation in heart rate (maximum – minimum) during metronomic respiration

Natural log transformation of the RR variables produced more normally distributed data. T-tests of the transformed mean differences yielded a similar pattern of results in that the stroke group had statistically significant smaller Valsalva ratios and less heart rate variation during metronomic respiration than controls, with the change in heart rate during metronomic respiration more significant than the E/I ratio. Following linear regression for ambulatory systolic blood pressure, the pattern of differences did not change but the strength of the significance values did alter and the difference in E/I ratio lost significance (Table 7.5).

Table 7-6 Cardiovascular reflex tests: blood pressure changes and linear regression for systolic blood pressure

Test	Group (number)	Mean ± s.d. / mmHg	Mean difference/ mmHg (95% CI)	P value (1)	Post- regression difference (95%CI)	P value (2)
ΔSBP standing	Control (65)	24.5 ± 14.7	-7.1	0.096	-6.7	0.133
	Stroke (72)	31.6 ± 31.3	(-15.5, 1.2)		(-15.5, 2.1)	
ΔDBP Isometric exercise	Control (64)	12.0 ± 11.4	-6.4	0.007	-5.1	0.033
	Stroke (74)	18.4 ± 15.1	(-10.9, -1.8)		(-9.7, -0.4)	
ΔDBP cold pressor	Control (64)	8.6 ± 10.0	-2.2	0.289	-1.57	0.471
	Stroke (72)	10.7 ± 13.3	(-6.2, 1.9)		(-5.86, 2.73)	
ΔSBP phase IV Valsalva	Control (66)	24.3 ± 23.2	7.9	0.027	9.6	0.011
	Stroke (68)	16.4 ±17.1	(0.9, 14.8)		(2.3, 16.9)	

P value (1), t test: P value (2), t test for mean differences following linear regression for ambulatory systolic blood pressure: ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure

Table 7.6 displays the results of reflex blood pressure responses. In the stroke group, there was a significantly smaller rise in systolic pressure from baseline to peak phase IV of the Valsalva manoeuvre (control 24.3 ± 23.2 mmHg, stroke 16.4 ± 17.1, p = 0.027).

The stroke group did display different responses to the control group for the baroreflex challenges of the isometric exercise and cold pressor tests. There were exaggerated increases in blood pressure following these autonomic stimuli. The increase in diastolic pressure during isometric exercise was significantly higher in stroke cases (control 12.0 ± 11.4 mmHg vs. stroke 18.4 ± 15.1 mmHg, p = 0.007). The moderately larger increase in diastolic blood pressure in the stroke group during the cold pressor test did not reach significance (control 8.6 ± 10.0 mmHg, stroke 10.7 13.3 mmHg, p = 0.289). Although there was a tendency for a larger fall in systolic blood pressure during change to standing posture in the stroke group, this was not statistically significant (control 24.46 mmHg, stroke 31.60 mmHg, p = 0.096).

After linear regression for ambulatory systolic blood pressure, the differences in diastolic change during isometric exercise and systolic change to phase IV Valsalva remained significant (Table 7.6). The differences in systolic blood pressure change on standing and diastolic change during cold pressor were reduced after the regression for systolic ambulatory blood pressure.

Table 7-7 **Power spectral analysis of heart rate variability**

Test	Group	Number	Median	Interquartile range	P value
SD RR interval, ms	Control	64	29.82	19.53, 42.99	0.049
	Stroke	68	24.26	18.82, 34.54	
Total power, ms^2	Control	64	649	243, 1151	0.037
	Stroke	68	327	212, 739	
LF power, ms^2	Control	64	191	75, 438	0.008
	Stroke	68	94	62, 225	
HF power, ms^2	Control	64	93	39. 252	0.102
	Stroke	68	72	33, 161	
LF/HF	Control	64	1.71	0.79, 3.40	0.316
	Stroke	68	1.39	0.69, 3.00	
Alpha LF BRS, ms/ mmHg	Control	41	6.02	3.99, 9.70	0.004
	Stroke	39	3.72	2.92, 6.05	
Alpha HF BRS, ms/ mmHg	Control	50	6.53	3.95, 12.74	0.107
	Stroke	59	5.13	2.97, 8.80	

P value, Mann Whitney test, exact significance (2-tailed): Interquartile range, Weighted average: SD RR, standard deviation RR intervals: LF, low frequency: HF, high frequency: BRS, baroreflex sensitivity

Table 7.7 displays results of power spectral analysis of heart rate variability and baroreflex sensitivity. Five minute heart rate variability was significantly worse in the stroke group for total power ($p = 0.037$) and low frequency power spectral densities ($p = 0.008$). There was a trend towards impaired high frequency heart rate variability for stroke cases in the high frequency range but this did not attain statistical significance ($p = 0.102$). Baroreflex sensitivity was reduced in the stroke group. The alpha co-efficient of baroreflex sensitivity was significantly worse in the low frequency range in stroke cases ($p = 0.004$) and non-significantly worse in the high frequency range ($p = 0.107$).

Table 7-8 **Power spectral analysis of heart rate variability: transformed data and linear regression for systolic blood pressure**

Test	Group	Log_e mean ± s.d.	Log_emean difference (95% CI)	P value (1)	SBP regression mean diff	P value (2)
SD RR , ms	Control	3.38 ± 0.54	0.17	0.044	0.16	0.085
	Stroke	3.21 ± 0.44	(0.00, 0.34)		(-.02, 0.33)	
Total power, ms^2	Control	6.31 ± 1.17	0.40	0.032	0.34	0.084
	Stroke	5.90 ± 0.96	(0.04, 0.77)		(-0.05, 0.72)	
LF power, ms^2	Control	5.16 ± 1.28	0.51	0.014	0.42	0.052
	Stroke	4.65 ± 1.05	(0.10, 0.91)		(-0.00, 0.84)	
HF power, ms^2	Control	4.70 ± 1.44	0.37	0.111	0.39	0.113
	Stroke	4.33 ± 1.23	(-0.09, 0.83)		(-0.09, 0.88)	
LF/HF ratio	Control	0.46 ± 0.98	0.13	0.422	0.03	0.882
	Stroke	0.33 ± 0.91	(-0.19, 0.46)		(-0.31, 0.36)	
Alpha LF, ms/ mmHg	Control	1.80 ± 0.67	0.40	0.006	0.38	0.012
	Stroke	1.40 ± 0.57	(0.12, 0.68)		(0.09, 0.68)	
Alpha HF, ms/ mmHg	Control	1.89 ± 0.85	0.20	0.189	0.18	0.270
	Stroke	1.68 ± 0.10	(-0.10, 0.51)		(-0.14, 0.50)	

Log_e mean ± s.d., Log_e transformed values ± standard deviation: P value (1), t-test for the log_e transformed mean difference: SBP regression mean diff., mean log_e transformed differences after linear regression for ambulatory systolic blood pressure: P value (2), t-test for the log_e transformed differences after linear regression for ambulatory systolic blood pressure: SD RR, standard deviation RR intervals: LF, low frequency: HF, high frequency: BRS, baroreflex sensitivity

Linear regression for ambulatory systolic blood pressure did affect the significance of observations regarding 5 minute heart rate variability and baroreflex sensitivity (Table 7.8).

7.4 Discussion

This study has demonstrated abnormal cardiovascular autonomic function in elderly stroke survivors. Heart rate changes during metronomic respiration and the Valsalva ratio were significantly decreased in stroke cases. There was no difference in the mean 30:15 ratio between stroke cases and controls. For the blood pressure measures, systolic response at phase IV of the Valsalva manoeuvre was significantly lower in stroke cases. Change in

diastolic pressure was significantly increased for isometric exercise in stroke cases compared with controls. There was a non-significant difference for the cold pressor change in diastolic pressure, with a trend towards a greater increase in stroke cases. There was also a trend towards an exaggerated fall in systolic pressure during orthostasis which did not reach statistical significance. Heart rate variability studies confirmed a picture of autonomic dysfunction in post-stroke cases with significantly reduced total and low power heart rate variability, but these differences may be related to systolic blood pressure. There was a trend towards reduced high frequency heart rate variability in the stroke group. Baroreflex sensitivity was significantly reduced in the low frequency range, and non-significantly lower in the high frequency range in the stroke group.

The lower variation in heart rate with metronomic respiration and Valsalva ratio in stroke cases is consistent with impaired cardiovagal function. The lower peak systolic pressure at phase IV of the Valsalva manoeuvre indicates impaired sympathetic function. There were significantly different diastolic responses to the isometric exercise and cold pressor stimuli, but with *higher* diastolic pressure rises in the stroke group compared to the control group. The exaggerated rise in blood pressure during isometric exercise and cold pressor tests is somewhat surprising in that one may expect impaired responses to these tests in the context of reduced sympathetic function. However, it is consistent with the theory of impaired baroreflex function. This conclusion is supported by the finding of reduced baroreflex sensitivity from the power spectral analysis of heart rate and blood pressure variability. The tendency towards a greater drop in systolic pressure during orthostasis suggest how abnormal baroreflex function may have a clinical impact on patients.

Lower peak systolic blood pressure during overshoot in phase IV of the Valsalva manoeuvre indicates impaired sympathetic response to a sudden hypotensive challenge. This is a result of reduced peripheral efferent sympathetic traffic and probably a reduced cardiac sympathetic response as well (Sandroni, 1991; Smith, 1996). The normal increase in blood pressure during isometric exercise is attributed to sympathetic efferent activity (Mark, 1985; Seals, 1988; Sundlof and Wallin, 1978) but in this study the stroke group had 'supranormal' increases during isometric exercise and cold pressor tests. Thus Valsalva and isometric/cold pressor results appear contradictory. There are a number of potential explanations. One hypothesis to explain the discrepancy is that sympathetic damage means that immediate blood pressure increase is impaired in the face of rapid hypotension due to neural damage but there is residual sympathetic efferent activity to drive a blood pressure over a more prolonged stress such as the three minute isometric exercise test. Even though this activity is subnormal, its effect will be compounded by absence of the damping effect from impaired baroreflex activity.

Furthermore the stimulus and afferent limb may differ between phase IV Valsalva blood pressure overshoot and isometric diastolic blood pressure responses. There are three proposed stimuli for the isometric exercise blood pressure response: central command, neural afferent reflex from the contracting muscle and ischaemic metabolite (Coote, 1971; Goodwin, 1972; McCloskey and Mitchell, 1972). In contrast the stimulus for the Valsalva phase IV response is principally dependent on reduced peripheral resistance (Greenfield, 1967). Therefore there are a range of stimuli for the isometric response which may have an additive and compensatory action, whereas valsalva blood pressure overshoot is reliant on other immediate neural mechanisms. Isometric blood pressure response mechanisms are additive over the exercise time period (Seals, 1993; Seals, 1988; Smith, 1976).

Vasopressin release can contribute to isometric blood pressure increase in hypotensive individuals under autonomic blockade but occurs later than the normal sympathetic mediated response (Jordan, 2000b). McAllister demonstrated augmented pressor response in isometric exercise pressure response in hypertensive patients on long-term propranolol therapy (McAllister, 1979). Jordan et al describe a family with monogenic hypertension related to central vascular anomaly where sympathetic stimuli produced excessive increases in blood pressure during autonomic testing, and the mechanism appeared to be failure of baroreflex buffering (Jordan, 2000c). Pressor response to simple water ingestion is augmented in healthy elderly compared with controls and this seems to be due to increased sympathetic activity that is not adequately buffered by baroreflex mechanisms (Jordan, 2000a).

7.4.1 Previous studies of post-stroke autonomic function

In general, studies of stroke cohorts younger than that described in our results have shown evidence of impaired autonomic dysfunction based on either Ewing battery or heart rate variability measures (Barron, 1994; Korpelainen, 1996a; Korpelainen, 1996b; Korpelainen, 1994; Tokgozoglu, 1999). There appears to be a time dependent effect with improvement of autonomic function 6 months post stroke (Korpelainen, 1996b; Korpelainen, 1994). Stroke locality can influence autonomic function and insular cortex stroke in particular has been implicated in affecting autonomic control (Korpelainen, 1996a; Sander and Klingelhofer, 1996; Tokgozoglu, 1999). Small post-stroke studies have indicated basal sympathetic nerve activity may be upregulated but with an attenuated response during cold pressor stimulation and impaired orthostatic blood pressure control (Mizushima, 1998; Robinson and Potter, 1995).

7.4.2 Dysautonomia and white matter lesions

We have shown that in older stroke patients blood pressure may not respond appropriately to changes in systemic pressure. Others have shown disorders of baroreflex function post-stroke

in younger post-stroke cohorts (Robinson and Potter, 1997; Robinson and Potter, 1995). Our findings in this age group carry particular importance. Cognitive impairment is common in stroke survivors with a prevalence rates quoted at 17 - 29 % with the highest rates in the older age categories, such as the patients investigated in this study (Barba, 2000; Henon, 2001; Inzitari, 1998; Lowery, 2002). One of the key mechanisms in cognitive decline appears to be small vessel cerebrovascular disease in the white matter (Aharon-Peretz, 1988; Barber. 1999; Charletta, 1995; Liu, 1992; Pohjasvaara, 2000). These white matter lesions are associated with the typical neuropsychological profile of impaired executive function and attentional deficits in post-stroke dementia (Desmond, 1999; Looi and Sachdev, 1999). The sequence of events driving the progression of white matter lesions is not clear. Key neuropathological features in white matter disease include sclerotic hypertrophied arterioles feeding the deep white matter (Fisher, 1969). It would seem plausible that transient drops in perfusion pressure cause white matter ischaemia in these 'at risk' areas (De Reuck, 1971). It could also be envisaged that an abnormally high surge in perfusion pressure could exacerbate hypertensive damage to the arteriolar tree or precipitate microscopic luminal wall damage that results in micro-haemorrhage. This cycle of damage could accumulate over a period, resulting in extensive white matter damage and clinically leading to cognitive decline. The elderly post-stoke patient who has apparently made a good recovery from stroke has a 'brain at risk'. Impaired autonomic function is potentially an important part in the pathophysiology leading to subcortical dementia.

The clinical significance of this hypothesis relates to the current interest in blood pressure control and stroke. Treatment of hypertension is clearly of benefit in primary prevention of stroke (Staessen, 2001). More recently secondary prevention of stroke and other cardiovascular events by blood pressure lowering has gained prominence (Collaborative Group of the Primary Prevention Project (PPP), 2001). The optimal method of blood pressure management in the immediate aftermath of stroke is currently the subject of a large multicentre randomised prospective trial (Anonymous, 2004). In daily clinical practice, the decision to institute blood pressure lowering treatment is made in the context of the individual, some of whom clearly do not tolerate aggressive blood pressure reduction. There are reports that relative *hypo*tension is more prevalent in vascular dementia in cross-sectional post-stroke and community groups (Gorelick, 1993; Guo, 1996; Pohjasvaara, 1998). The Gothenberg community study group described how individuals who develop dementia have a higher, pathological blood pressure before the onset of dementia but that blood pressure declined more rapidly in those who developed dementia (Skoog, 1996). This raises two questions. Firstly, on the timing and degree to which blood pressure should be lowered,

particularly in the elderly. Secondly, does unforeseen autonomic impairment negate the benefits of anti-hypertensives?

7.4.3 Dysautonomia and sudden death

The second clinically relevant point regarding our results relates to the reduction in heart rate variability. Abnormal autonomic tone is an independent predictor of increased cardiovascular mortality following myocardial infarction (Kleiger, 1987; La Rovere, 1998; Zuanetti, 1996). The risk is most evident in those with other cardiac risk factors such as reduced left ventricular function (Bailey, 2001; Hartikainen, 1996). The postulated mechanism is a reduction in vagal activity, increasing vulnerability to malignant cardiac arrhythmias that lead to sudden death. Fei et al reported a decrease in the high frequency preceding ventricular tachycardia in ambulatory recordings (Fei, 1994), Chiladakis et al reported an increase in low frequency and decrease in high frequency spectral component of heart rate variability preceding accelerated idioventricular rhythm following thrombolysis for acute myocardial infarction (Chiladakis, 2001) and Huikiri found reduced long term RR variability with abnormal beat-to-beat heart rate dynamics preceding onset of ventricular tachycardia in ambulatory ECGs of 15 post-myocardial infarction patients (Huikuri, 1996). We have shown similar abnormalities in heart rate variability in elderly stroke survivors.

From this investigation, the so-called sympathovagal balance reflected by the LF/HF ratio was no different between stroke cases and controls and this might be seen as a mitigating factor against the hypothetical increased risk of malignant arrhythmia. However the clinical usefulness of this measure is not consistent in published reports (Lampert, 2003; Lanza, 1998) and caution is required when making assumptions on actual autonomic nerve activity from heart rate variability measures (Karemaker, 1997; Malik and Camm, 1993). In general, results from our investigations do indicate that elderly stroke survivors have a disordered cardiovascular autonomic system with reduced vagal activity and impaired baroreflexes.

Another issue in our hypothesis is that we have investigated frequency domain heart rate variability in 5 minute recordings, whereas many of the important post-myocardial infarction findings are derived from time domain measures of 24 hour heart rate variability recordings (Kleiger, 1987; La Rovere, 1998; Zuanetti, 1996). From the CAPS study group, it has been shown that *24 hour* spectral analysis of heart rate variability is a significant and independent risk for mortality when measured early post-myocardial infarction (Bigger, 1992b). Furthermore the same group has demonstrated frequency domain measures from randomly selected 2 to 15 minute recordings late post-myocardial infarction do have significant predictive value for mortality (Bigger, 1993). The Zutphen community study has reported that time domain variables from short term 2 minute ECG recordings are significantly associated

with increased mortality (Dekker, 2000). Additionally there does appear to be a close association between 5 minute and 24 hour heart rate variability: low frequency and SDNN are very closely linked, and high frequency and pNN50 and rMSSD are also tightly correlated (Bigger, 1992a). One group has shown that a stepwise approach to identifying high risk patients using first short term 5 minute heart rate variability to screen for abnormal heart rate variability index before refining the risk stratification with 24 hour heart rate variability studies is an effective technique (Faber, 1996). Thus there is some validity in postulating the short term heart rate recording abnormalities from our study carry an adverse prognosis. Furthermore, we have shown that one time domain measure is reduced in the stroke group i.e. the standard deviation of all normal RR intervals (SDNN). This is significantly correlated ($r = 0.51$, $p < .001$) with another time domain measure, the Heart Rate Variability index (HRVi), used by Camm's group and both SDNN and HRVi have predictive power for cardiovascular events post-myocardial infraction (Fei, 1996).

Although reduced heart rate variability does indicate increased risk of mortality, it may be viewed as a treatment target. There are reports of propranolol, amiodarone and thrombolysis being associated with improvement in autonomic tone and a reduction in mortality (Lampert, 2003; Lind, 2001; Malik, 2000).

We are drawing comparisons between coronary artery disease and cerebrovascular disease in terms of a hypothetical shared risk for sudden death. Whilst recognising some of the differences between these patient groups, there are many similarities and it seems fair to hypothesise that stroke patients bear similar risks of sudden death related to autonomic dysfunction to that seen in post-myocardial infarction patients. However there is a surprising dearth of literature on the risk of sudden cardiac or arrhythmic death post-stroke. Recently Robinson et al demonstrated significantly increased all-cause mortality in a group of stroke survivors with impaired baroreflex sensitivity (Robinson, 2003). To our knowledge, only one study has quantified the risk of sudden death in stroke survivors (Pop, 1994). Prolonged QTc independently predicts cardiac death (RR 2.8) and all cause mortality (RR 2.9) in stroke survivors (Wong, 2003) and there are reports linking QTc prolongation with cardiovascular autonomic neuropathy in diabetic patients (Jermendy, 1991).

Stroke victims have high rates of cardiac arrhythmia in the acute post-stroke phase (Mikolich, 1981). Sudden cardiac death accounted for 43% of cardiac deaths in 3021 patients with minor stroke or TIA followed for more than 2 years post-stroke and older age was an independent predictor of sudden death (Pop, 1994). The high prevalence of deranged autonomic function in older stroke patients may explain this observation.

7.4.4 Study limitations

Some of the weaknesses of this study are addressed in the previous discussion of heart rate variability investigation techniques. We have investigated a representative section of stroke survivors who were able to participate in the study protocol. These patients are involved in the longitudinal observational study on the natural history of cognitive function in elderly stroke survivors. Therefore we felt it was appropriate not to exclude patients with co-morbid conditions or requiring treatment with cardiovascular medication. This resulted in a heterogeneous group of stroke patients with a number of factors which may influence autonomic function tests. Higher systolic blood pressure level in the stroke group was the most important difference, and hypertension is known to affect autonomic function (Harrington, 2000a). Indeed linear regression for 24 hour ambulatory systolic blood pressure did alter the strength of the significance for some differences, but did not completely eradicate the observed differences. Linear regression for a history of asthma or COPD did not negate the significance of the differences (see section 7.6). It should be noted that some of the subjects with a history of asthma or COPD were taking inhaled beta-agonists or antimuscarinic agents which may have influenced autonomic function. This was not examined in the data analysis.

Regarding drug therapy, the only significant difference between stroke case and controls for cardiovascular medication use was for thiazide diuretics and dihydropyridine calcium channel blockers. Linear regression for each of these factors did not negate the significance our findings (see section 7.6). The literature suggests that some cardiovascular drugs have a neutral or even beneficial effect on autonomic function. Beta-adrenergic blocking drugs can improve autonomic function, in particular high frequency of heart rate variability (Goldsmith, 1997; Lampert, 2003; Salo, 1999). ACE inhibitor drug studies indicate variable effect on autonomic function but mostly a neutral effect (Guasti, 2001; Licker, 2000; Okabayashi, 1997; Salo, 1999). Dihydropyridine calcium channel blocking drugs tend to improve baroreflex sensitivity but can increase low frequency heart rate variability (James, 1999; Lefrandt, 2001; Lucini, 1997).

Therefore we may argue the (non-significant) higher use of beta blockers and ACE inhibitors may potentially have masked further impairment in autonomic indices. In general, it would appear the deterioration in autonomic function in this cohort of stroke survivors was not the outcome of cardiovascular drug use.

7.5 Conclusions

In summary we have compared autonomic function in a group of elderly ambulant stroke survivors with a group of community living controls. Stroke cases demonstrated evidence of

impaired cardiovagal function. There were contrasting results with respect to sympathetic function tests. Blood pressure response in phase IV of the Valsalva manoeuvre was impaired but there was a 'supernormal' increase in diastolic blood pressure during isometric exercise. These findings may be related to reduced baroreceptor function observed from short term spectral analysis of heart rate and blood pressure variability. Some of the abnormalities in autonomic function, especially short term heart rate variability, may be related to the higher blood pressure autonomic reflexes seen in stroke survivors. Potentially important clinical implications of disordered autonomic function are the progression of white matter disease and risk of sudden death.

7.6 Appendix

Data from this chapter was accepted for publication in article form to the journal 'Stroke' (McLaren, 2005). Referees' comments included the suggestion that data should be amalgamated in one table and multiple regression performed for all potential confounding factors. Multiple linear regression was performed on logarithmic transformed RR indices and spectral powers of heart rate variability, and on raw blood pressure reflex data. Each autonomic variable was entered as a dependent variable and the following as independent variables: hypertension, asthma/COPD, myocardial infarction, peripheral vascular disease, diabetes mellitus, cardiac failure, alcohol habit, thiazide, calcium channel blocker, beta-blocker, age and systolic blood pressure from ambulatory blood pressure. Stepwise multiple linear regression was performed until only significant terms remained. Then case or control status was entered as an independent variable. The confidence interval and significance value of the regression coefficient for case/control status for each autonomic test are shown in Table 7.9. It can be seen that significant differences persist after multiple linear regression.

Table 7-9 Summary data for difference between control and stroke cases: blood pressure variability and logarithmic transformed RR data with regression analyses

Variable (control number, stroke number)	Mean ± standard deviation		Difference in means Students t test		Difference in means adjusted for ambulatory SBP		Difference in means adjusted for all confounding factors	
	Control	Stroke	95% CI	P	95% CI	P	95% CI	P
Log 30:15(65, 71)	0.12 ± 0.10	0.12 ± 0.12	-0.04, 0.04	0.956	-0.05, 0.03	0.710	-0.04, 0.03	0.732
Log VR (66, 69)	0.32 ± 0.15	0.22 ± 0.13	0.06, 0.15	<0.001	0.06, 0.16	<0.001	0.06, 0.15	<0.001
Log E-I (68, 73)	1.95 ± 0.57	1.65 ± 0.61	0.11, 0.50	0.003	0.09, 0.51	0.005	0.00, 0.42	0.049
Log SD RR (64, 68)	3.38 ± 0.54	3.21 ± 0.44	0.00, 0.34	0.044	-0.02, 0.33	0.085	0.03, 0.36	0.025
Log Tot. power (64, 68)	6.31 ± 1.17	5.90 ± 0.96	0.04, 0.77	0.032	-0.05, 0.72	0.084	0.04, 0.77	0.032
Log LF (64, 68)	5.16 ± 1.28	4.65 ± 1.05	0.10, 0.91	0.014	-0.00, 0.84	0.052	0.10, 0.91	0.014
Log HF (64, 68)	4.70 ± 1.44	4.33 ± 1.23	-0.09, 0.83	0.111	-0.09, 0.88	0.113	-0.14, 0.77	0.174
Log αBRS LF (41, 39)	1.80 ± 0.67	1.40 ± 0.57	0.12, 0.68	0.006	0.09, 0.68	0.012	0.23, 0.39	<0.001
Log α BRSHF (50,59)	1.89 ± 0.85	1.68 ± 0.10	-0.10, 0.51	0.189	-0.14, 0.50	0.270	-0.06, 0.51	0.124
Δ SBP stand (65,72)	24.5 ± 14.7	31.6 ± 31.3	-15.5, 1.2	0.096	-15.5, 2.1	0.133	-14.5, 2.3	0.153
Δ DBP isometric (64, 74)	12.0 ± 11.4	18.4 ± 15.1	-10.9, -1.8	0.007	-9.7, -0.4	0.033	-17.0, -1.1	0.027
Δ DBP coldpress (64, 72)	8.6 ± 10.0	10.7 ± 13.3	-6.2, 1.9	0.289	-5.86, 2.73	0.471	-5.6, 2.2	0.398
Δ SBP Valsalva (66, 68)	24.3 ± 23.2	16.4 ± 17.1	0.9, 14.8	0.027	2.3, 16.9	0.011	-0.1, 13.3	0.052

Log, logarithmic transformed value: 30:15, 30:15 ratio: VR, Valsalva ratio: E-I, heart rate difference during metronomic respiration:sd RR, standard deviation of RR intervals over 5 minutes: Tot. power, total spectral power of 5 minutes heart rate variability: LF, low frequency spectral power: HF, high frequency spectral power: αBRS. Alpha baroreflex sensitivity in high and low frequency ranges: ΔSBP stand, systolic blood pressure drop during orthostasis: ΔDBP isometric, diastolic blood pressure change during isometric exercise: ΔDBP coldpress, change in diastolic blood pressure during cold pressor: ΔSBP Valsalva, change in systolic blood pressure during Valsalva manoeuvre

8 The association between autonomic function and white matter lesions

8.1 Introduction

White matter lesions (WML) are common abnormalities on brain imaging in older people. WML are independently associated with cognitive impairment in otherwise normal subjects (Breteler, 1994b; Schmidt, 1993). White matter lesions are also closely associated with vascular dementia and Alzheimer's disease (Liu, 1992; Starkstein, 1997). Subcortical vascular dementia includes extensive white matter lesions as one of its diagnostic criteria (Erkinjuntti, 2000b; Roman, 1993).

Neuropathological features of white matter lesions are demyelination, neuronal loss and gliosis (Erkinjuntti, 1996). In older age, the deep penetrating vessels feeding white matter are altered by hyalinisation and sclerosis (Fisher, 1969). Cerebral perfusion is maintained by a combination of systemic arterial pressure buffered by cerebral autoregulation in order to maintain a constant smooth flow to cerebral tissue. Mechanisms for white matter lesion formation are likely to include hypoperfusion of the small vessels supplying the deep white matter (De Reuck, 1971). Arteriolar damage within the white matter renders tissue vulnerable to changes in blood flow.

There is evidence pointing to a role for fluctuation in systemic perfusion pressure increasing white matter lesion burden (Pantoni and Garcia, 1997). Cardiovascular autonomic function is crucial in the control of systemic arterial pressure, in particular smoothing short term fluctuations in perfusion pressure. Therefore, impaired cardiovascular autonomic function could potentially exacerbate white matter lesions by failing to control arterial pressure. The role of autonomic dysfunction in white matter lesion progression has not been extensively reported in the literature. We hypothesised that cardiovascular autonomic dysfunction is associated with white matter lesions in older stroke survivors.

There are a number of bedside cardiovascular autonomic reflex tests to assess sympathetic and parasympathetic function. The results of several tests can be summarised as a score (Ewing and Clarke, 1982; Low, 1993b). Such scores are preferred to individual tests since they provide a more complete picture of autonomic function (Ewing, 1985; Mathias and Bannister, 1999). We hypothesised there is an association between aggregate scores of autonomic function and MRI white matter lesions.

8.2 Method

Methodology is described in detail in Chapter 4. Stroke patients of 75 years of age or older were recruited from consecutive patients on representative hospital based stroke registers in

Tyneside and Wearside. These patients, who had been discharged from hospital, were free of dementia and free of any disabilities which would preclude comprehension of and compliance with autonomic function tests. The patient cohort was comprehensively assessed at 3 months post stroke using a standardized neuropsychological battery to exclude dementia, allowing time for the resolution of post-stroke delirium and stabilization of physical impairment. The investigation sequence was the same for all patients.

The following cardiovascular reflex tests were measured: 30:15 ratio and postural change in blood pressure during standing, diastolic blood pressure change during isometric exercise, Valsalva ratio and systolic blood pressure overshoot during Valsalva manoeuvre and heart rate variation during metronomic respiration. Five minutes of resting RR interval and systolic blood pressure variation were analysed by power spectral analysis to obtain total, low and high frequency heart rate variability spectral powers, and baroreflex sensitivity in low and high frequency bands. Carotid sinus massage was performed in the supine position for 5 seconds on the right then left carotid sinus. Cardio-inhibitory and vasodepressor responses were recorded. Standard deviation of RR interval over five minute's supine rest was also included in the assessment. Only patients in sinus rhythm were included in the analysis. Technically poor quality data that made RR interval and blood pressure measurement unreliable were excluded from analysis.

8.2.1 MRI scanning protocol

A 1.5 GE Signa scanner (General Electric, Milwaukee, WI, USA) was used to acquire MR images. T2 images were acquired to perform visual ratings of white matter hyperintensities. Structural T1 weighted 3D FSPGR (Fast Spoiled Gradient Echo) images were acquired for visual rating of medial temporal atrophy. Fluid Attenuated In version Recovery (FLAIR) images acquired axially were used to determine the volume of white matter hyperintensities using an automated measuring process.

Hard copies of FLAIR and T2 images were used to visually rate the severity of white matter lesions, known as hyperintensities for the purpose of the scale. For this analysis, Scheltens regional sub-score for deep white matter hyperintensities and the total Schelten's score (see section 7.3.2) were correlated with autonomic function. All scans were rated blindly and consensus achieved between three trained raters.

8.2.2 Statistical analysis

White matter lesions were correlated with individual autonomic indices (Tables 8.2 and 8.3). Spearman's rank correlation was used since the majority of the autonomic indices appeared to have a skewed distribution. Scheltens visual rating scale provides ordinal data, and the automated estimate of cortical white matter lesion percentage volume had a trend towards a

skewed distribution (one-sample Kolmogorov-Smirnov Z score 1.337, p = 0.056). A result was considered statistically significant if the p value was less than 0.05.

Chapter 7 discussed the issues behind forming normal values from which to create an autonomic scoring system. Three different scoring systems were explored.

8.2.2.1 Ewing score

The five autonomic tests described by Ewing (Ewing and Clarke, 1982) were selected for this score – 30:15 ratio, Valsalva ratio, change in heart rate with metronomic respiration, drop in blood pressure on standing and change in diastolic blood pressure during isometric exercise. The 30:15 ratio is the ratio of the longest RR interval at around the 30^{th} beat to the shortest RR interval at around the 15^{th} beat after standing. The Valsalva ratio is the ratio of the longest RR interval after the manoeuvre to the shortest RR interval during the Valsalva manoeuvre. Change in heart rate during metronomic respiration is the mean difference in maxima and minima heart rates during six cycles of timed, deep respiration. The drop in blood pressure during standing is the difference in systolic blood pressure from the supine baseline reading to the nadir occuring after standing. The change in diastolic blood pressure during isometric sitting exercise is the difference between the final peak value after 3 minutes exercise and resting pre-exercise value.

Ewing's normal values were substituted by values derived from age-matched controls from the study in Chapter 7. Each autonomic test was considered abnormal if below the fifth percentile and allocated a score of 2, borderline if between the fifth and tenth percentile with a score of one and normal tests above the tenth percentile scored zero. Hence a normal subject scored zero and increasing autonomic impairment led to higher scores up to a maximum abnormal score of ten. Only cases completing all 5 Ewing tests were included in the correlation with cortical white matter lesion percentage volume, which was assessed using Spearman's rank correlation.

8.2.2.2 Composite score A

Since the Ewing test battery contained autonomic indices that did not differentiate between normal and abnormal autonomic function in stroke patients, a composite battery was selected that only contained autonomic indices that were significantly different between stroke patients and control cases (see Chapter 7). Therefore diastolic blood pressure change during isometric exercise, Valsalva ratio, Valsalva systolic blood pressure overshoot, heart rate variation during metronomic respiration and spectral power of total heart rate variability were included. Each autonomic test was considered abnormal if below the fifth percentile and allocated a score of one. Normal tests above the fifth percentile scored zero. Normal subjects scored zero and increasing autonomic impairment led to increasing scores with a maximum score of 5.

The total score was correlated with cortical white matter lesion percentage volume only for cases completing all 5 tests (Spearman's rank correlation).

8.2.2.3 Composite score B

This score was devised to see if (i) a score using all available reflex tests correlated with white matter lesion volume and (ii) how it performed in comparison with the Ewing score and Composite Score A. Table 8.1 lists indices included in composite score B and records the cut-off value for definition of abnormal score. The aim in choosing the thresholds was to identify at least some cases with abnormal scores for each test so that all autonomic indices could be utilised and contribute to the total score. Thus thresholds were chosen, not because they particularly represent the true abnormal value for the cohort but for their ability to discriminate 'good' versus 'poor' scores.

One-way analysis of covariance was performed to investigate if there was a difference in white matter lesion volume between total scores within each scoring system.

Table 8-1 Threshold for abnormal scores for composite score B

Autonomic index	Abnormal result	Reference
30:15 ratio	<1.01	Clark 1986 (Clark and Mapstone, 1986)
Valsalva ratio	<1.09	O'Brien 1986 (O'Brien, 1986)
ΔHR metronomic respiration	<4 bpm	O'Brien 1986 (O'Brien, 1986)
SD RR interval	<20 ms	Dekker JM 1997 (Dekker, 1997)
Δsystolic BP orthostasis	>30 mmHg	Clark 1986 (Clark and Mapstone, 1986)
Δdiastolic BP isometric exercise	<5 or >15 mmHg	Ziegler 1992, Wieling 1999 (Wieling and Karemaker, 1999; Ziegler, 1992b)
Δdiastolic BP cold pressor	<5 or >15 mmHg	Mathias, Wieling (Mathias and Bannister, 1999; Wieling and Karemaker, 1999)
Systolic BP overshoot Valsalva	<0 mmHg	Low PA 1993 (Low, 1993a)

8.3 Results

There were no significant correlations between Scheltens scale score and individual indices of autonomic function (Table 8.2).

Table 8-2 Correlation between cardiovascular autonomic function and Scheltens rating scale

Autonomic test	Number	White matter hyperintensities		Total hyperintensities	
		r_s	P	r_s	P
30:15 ratio	62	-0.057	0.661	-0.050	0.699
Valsalva ratio	61	+0.001	0.996	-0.066	0.614
E/I ratio	65	-0.081	0.522	-0.095	0.451
ΔHR respiration	65	+0.017	0.891	-0.018	0.888
ΔSBP standing	63	-0.142	0.266	-0.133	0.298
ΔDBP Isometric exercise	66	+0.042	0.737	+0.091	0.468
ΔDBP cold pressor	65	-0.083	0.510	-0.046	0.714
ΔSBP Valsalva	60	-0.181	0.167	-0.179	0.170
ΔRR CSM	26	+0.071	0.729	+0.132	0.521
ΔSBP CSM	26	-0.180	0.380	-0.317	0.115
Total HRV	61	-0.107	0.413	-0.133	0.307
LF HRV	61	+0.007	0.958	-0.089	0.496
HF HRV	61	-0.055	0.675	-0.050	0.703
BRS low frequency	38	+0.056	0.738	+0.085	0.613
BRS high frequency	55	-0.040	0.773	-0.027	0.847

E/I, expiratory/inspiratory ratio during metronomic respiration: ΔHR respiration, mean change in heart rate during metronomic respiration: ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: ΔRR, change in RR interval: CSM, carotid sinus massage: Total HRV, spectral power of heart rate variability: LF, low frequency spectral power: HF, high frequency spectral power: BRS, baroreflex sensitivity

Automated cortical WML percentage volume measurement demonstrated some trends towards correlation with two measures of blood pressure changes (Table 8.3). Increasing diastolic blood pressure increase during isometric exercise had a trend towards association with increasing white matter lesion damage. Larger blood pressure drop during carotid sinus massage indicated a trend towards association with increasing white matter lesion burden (blood pressure drop during carotid sinus massage measured as negative value).

The automated measure of white matter lesion volume produced results for lesions within frontal, parietal, temporal and occipital lobes. The only significant correlation between white matter volume and autonomic function was for frontal white matter volume and high frequency range baroreflex sensitivity with a correlation co-efficient -0.276 (95% CI -0.009 to -0.506, p = 0.044).

Between 50 and 55 cases completed all parts of the autonomic scores for the correlation with white matter lesion volume. White matter lesion volume increased with higher composite A scores but this was not significant (Figure 8.2, Spearman's rank correlation coefficient r_s = +0.127, p = 0.358). There was no association between higher scores of autonomic impairment and white matter lesion using the Ewing score (Figure 8.1, r_s = -0.004, p = 0.975) or

composite score B (Figure 8.3, r_s = 0.012, p = 0.935). Few cases scored highly on any of the three scoring systems.

Table 8-3 Correlation between cardiovascular autonomic function and cortical white matter lesion percentage volume

Autonomic test	Number	Cortical white matter lesion % volume	
		r_s	P
30:15 ratio	61	-0.051	0.695
Valsalva ratio	60	+0.031	0.814
E/I ratio	64	-0.036	0.776
E-I difference	64	0.033	0.798
SBP standing	62	-0.081	0.530
DBP Isometric exercise	65	+0.208	0.097
DBP cold pressor	64	+0.030	0.816
SBP Valsalva	59	-0.201	0.126
RR CSM	26	+0.043	0.833
SBP CSM	26	-0.336	0.094
Total HRV	60	-0.214	0.101
LF HRV	60	-0.071	0.591
HF HRV	60	-0.120	0.361
BRS low frequency	38	-0.017	0.918
BRS high frequency	54	-0.159	0.249

E/I, expiratory/inspiratory ratio during metronomic respiration: ΔHR respiration, mean change in heart rate during metronomic respiration: ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: ΔRR, change in RR interval: CSM, carotid sinus massage: Total HRV, spectral power of heart rate variability: LF, low frequency spectral power: HF, high frequency spectral power: BRS, baroreflex sensitivity

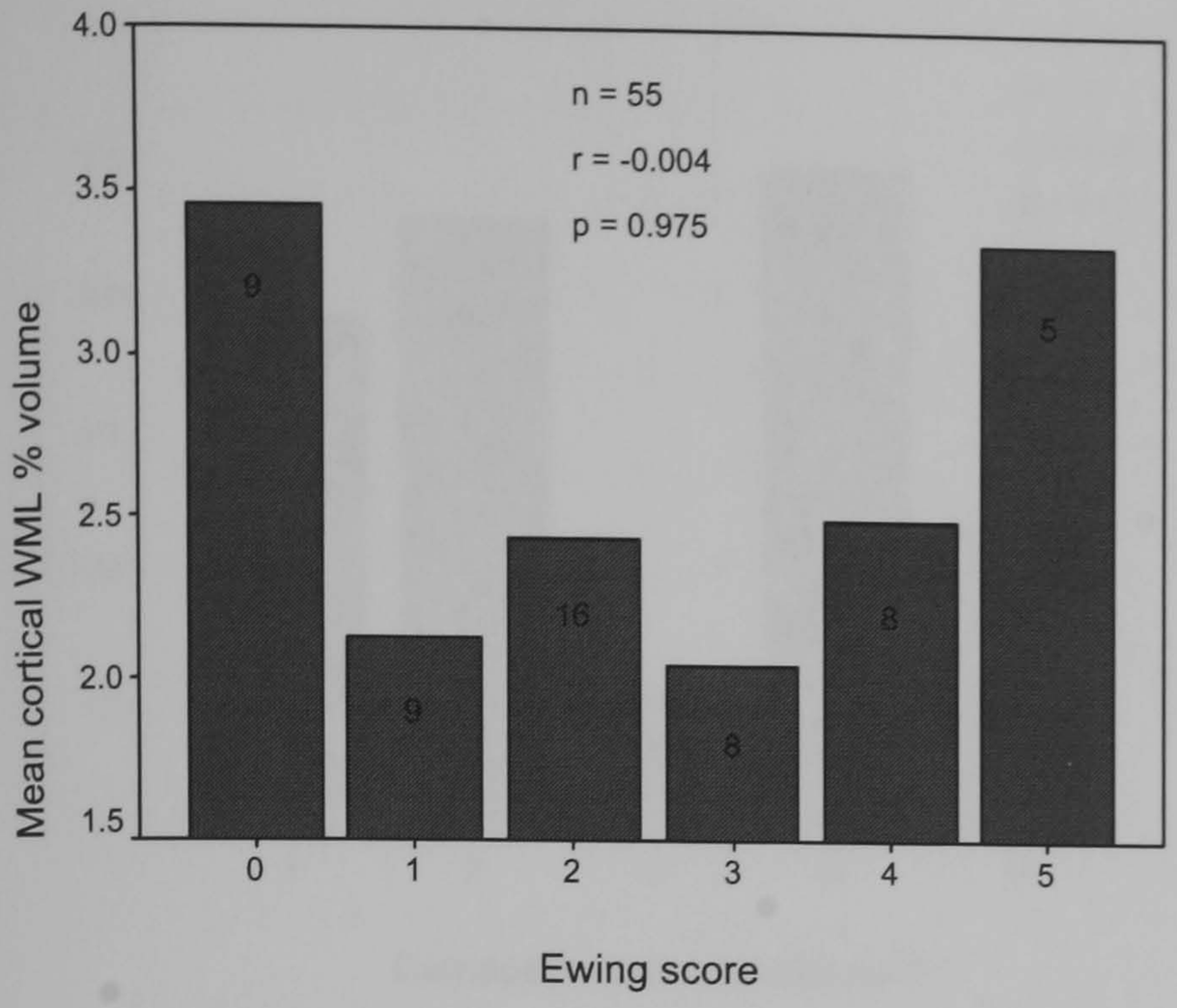

Figure 8-1 Cortical white matter lesion percentage volume according to Ewing score

Numbers in bars = number of cases for each Ewing score: r, Spearman's rank correlation.

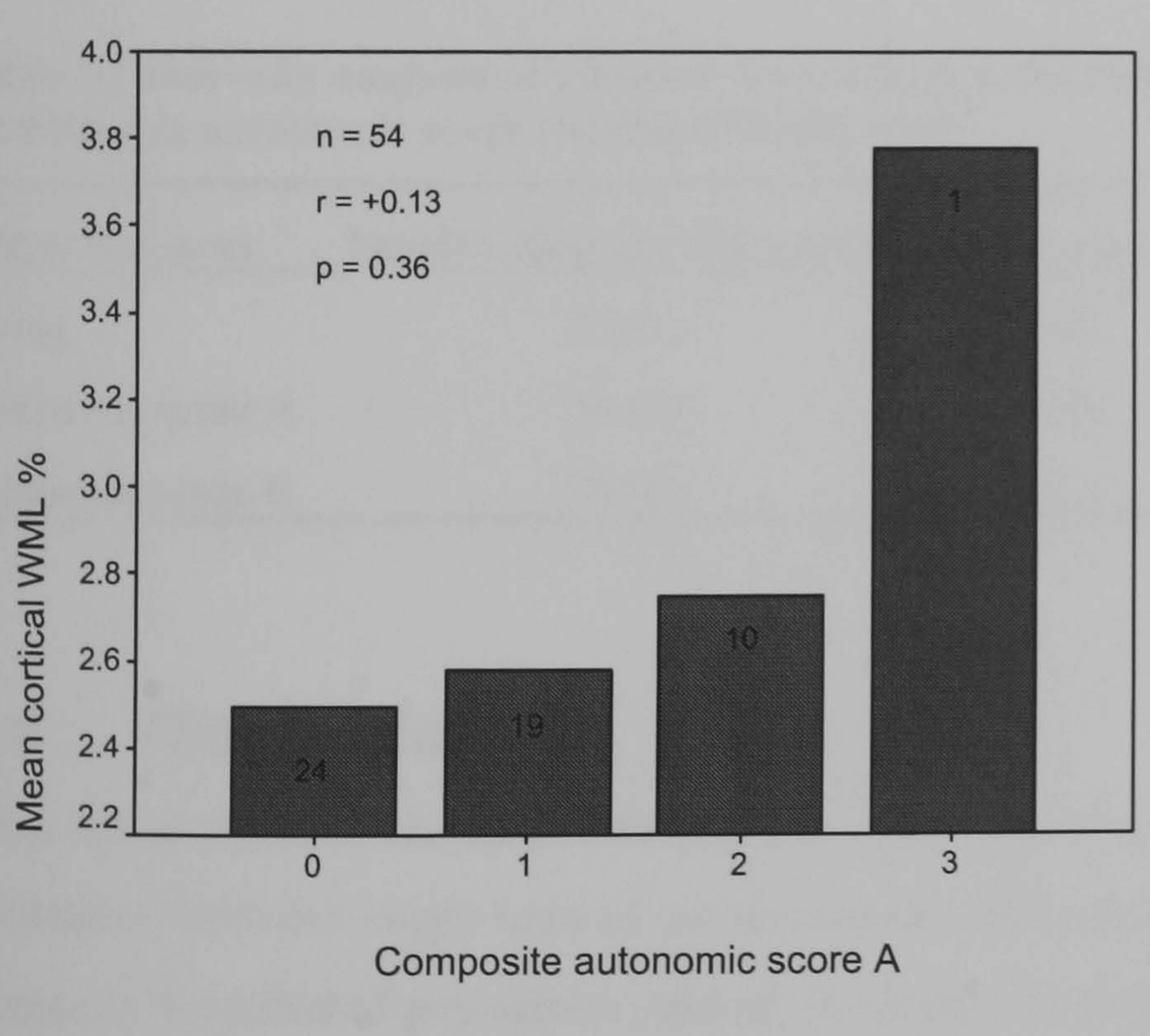

Figure 8-2 Cortical white matter lesion percentage volume according to Composite score A

Numbers in bars = number of cases for each autonomic score: r, Spearman's rank correlation.

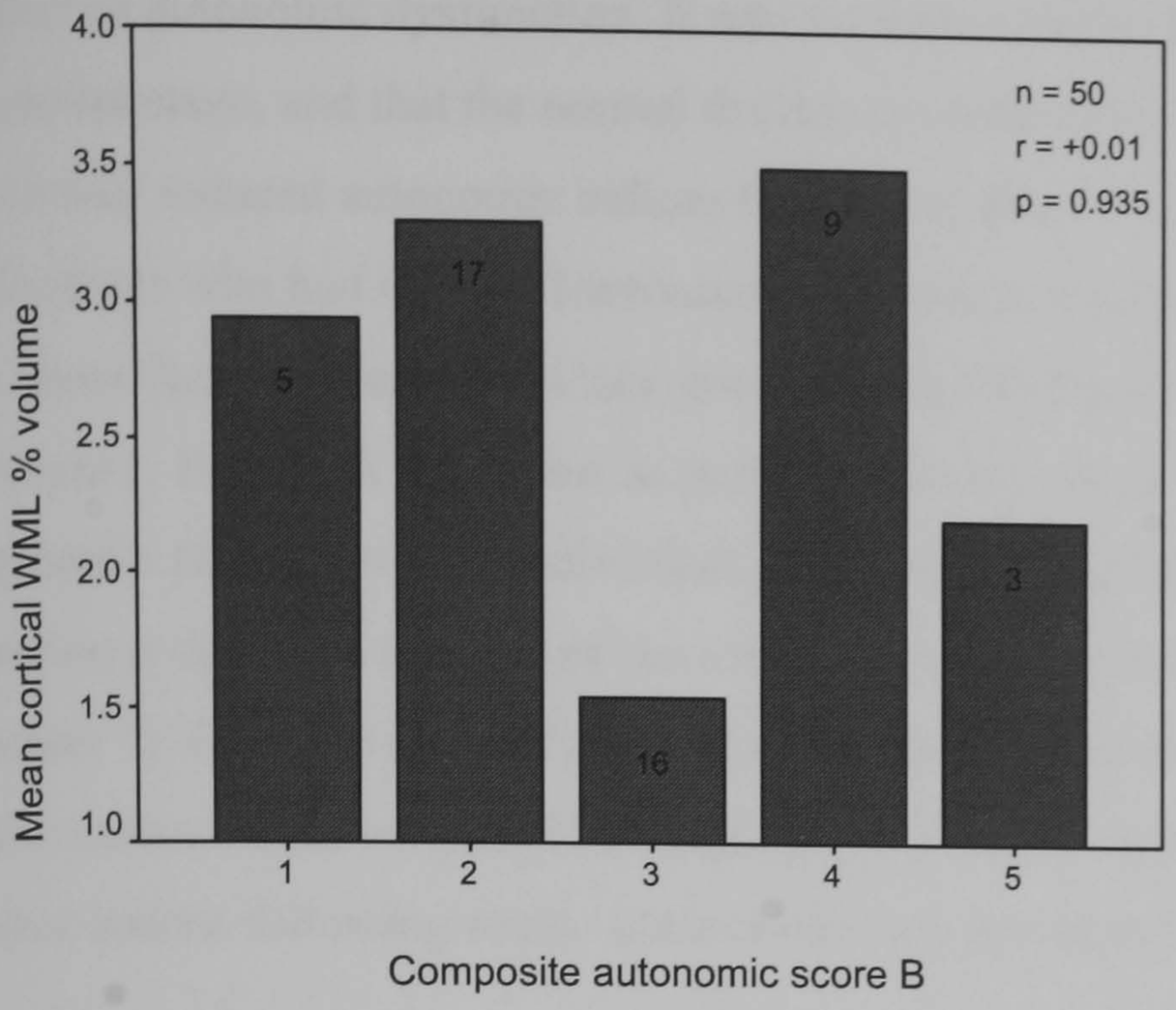

Figure 8-3 Cortical white matter lesion percentage volume according to Composite score B

Numbers in bars = number of cases for each autonomic score: r, Spearman's rank correlation.

There were no differences between scores within each of the scoring systems on one-way analysis of variance (Table 8.4).

Table 8-4 One-way analysis of variance for cortical white matter lesion percentage volume according to autonomic score (Kruskal-Wallis test)

Autonomic score	Number (degrees of freedom)	Chi square	Significance
Ewing	55 (5)	2.661	0.752
Composite score A	54 (3)	1.308	0.727
Composite score B	50 (4)	5.402	0.248

8.4 Discussion

From this analysis of autonomic function in stroke survivors, there is no significant association between single tests of cardiovascular autonomic function and white matter disease in the selected population studied. A composite score based on abnormal cardiovascular autonomic function indicated increasing cortical white matter damage in the presence of higher scores but this was not significant. Overall there is no evidence to support the hypothesis that autonomic damage will predispose to white matter ischaemia. Potential reasons for the lack of association with individual autonomic tests will be discussed first.

Firstly this may be a false negative result. The study design may reduce the chance of detecting a pathophysiological link between autonomic damage and cerebral hypoperfusion. The cross-sectional nature of the analysis does not provide scope to detect a longitudinal

impact of autonomic dysfunction. It may be such a mechanism has already expressed itself at an earlier stage, and that the normal decline in autonomic function with age will lead to uniformly reduced autonomic indices that do not allow for differentiation between those individuals who had impaired autonomic function at a young age (Piha, 1993). Furthermore we know that autonomic tests lack good reproducibility (Braune, 1996; Gerritsen, 2003; Lawrence, 1992b). A single test as performed in this study may not accurately reflect autonomic function within individuals, even though we know that as a group, autonomic function is deranged in older stroke survivors compared with age matched controls (see Chapter 7). Even the summary scores of autonomic function did not show any linkage with white matter lesion volume. The longitudinal effect of impaired autonomic function on white matter lesions following stroke is examined in a subsequent chapter.

The study may lack power to demonstrate a significant association between autonomic tests and white matter damage. The intended study size of 100 subjects was not attained and further loss of subjects occurred for a number of reasons. Principal reason for exclusion was atrial fibrillation. Nearly a quarter of the study group was in atrial fibrillation. Lack of sinus rhythm means that beat to beat RR variation is not under the same autonomic control as occurs during sinus rhythm, invalidating the cardiovascular reflex tests and power spectral analysis. The more prolonged blood pressure autonomic reflex tests (cold pressor and isometric exercise) are theoretically not so dependent on beat-to-beat control mechanisms. Therefore patients in atrial fibrillation could potentially be included in the analysis of cold pressor and isometric exercise tests to improve the power. However it is arguable whether this is valid since there is loss of normal beat-to-beat sympathovagal influence on RR interval.

8.4.1.1 Cerebral autoregulation

The absence of significant association between autonomic function and white matter damage suggests there is not a pathophysiological link between the two processes. Cerebral perfusion is dependent on systemic perfusion pressure but as discussed in section 2.6, cerebral autoregulation offers a protective mechanism. There is evidence that cerebral autoregulation can protect cerebral blood flow despite sharp drops in systemic perfusion pressure (Leftheriotis, 2000). Therefore we could support the hypothesis that despite transient systemic hypoperfusion from autonomic failure, normal cerebral perfusion will continue in those with normal cerebral autoregulation and protect the white matter from ischaemic insult (Novak, 1998). The study did not include measures of cerebral autoregulation. Clearly this would be an interesting issue for future studies. One potential study design would be a between group comparison: a healthy control group, groups with isolated autonomic failure or isolated cerebral dysautoregulation, and a fourth group with combined autonomic and cerebral autoregulation failure. The hypothesis is that the combined failure group would have the most

extensive white matter disease, with intermediate levels of white matter disease in the other two abnormal groups.

8.4.1.2 Stroke subtype

Inclusion of all different stroke subtype may have diluted the study's ability to find a significant association between autonomic dysfunction and white matter disease. The lacunar hypothesis suggests that individuals with small vessel disease strokes are the ones most at risk of subsequent white matter disease progression (Bamford and Warlow, 1988; Esiri, 1997; Yao, 1992). In this study of 76 cases in sinus rhythm, 26 (34 %) cases had a history of lacunar stroke, a fraction of the total number. Thirteen cases (17 %) had a history of posterior circulation stroke, amongst whom there will have been a proportion of lacunar strokes as well as embolic and atherothrombotic events (Warlow, 2001). Therefore less than half the group could be considered as high risk cases for the study hypothesis. However the overlap of cerebrovascular pathology in stroke patients suggest that many of the anterior circulation stroke cases will have concomitant small vessel disease, and thus are at some sort of intermediate risk compared with those presenting with lacunar infarction (Brun, 1994).

8.4.2 Composite scores

It is interesting that a combination of autonomic results rated normal or abnormal using the composite score indicated increasing white matter lesion volume is associated with deterioration in overall autonomic function. Collating autonomic function results into a score theoretically provides a more balanced estimation of overall autonomic function (Bellavere, 1983; Ewing and Clarke, 1982). Ewing's description of his autonomic test battery and other authorities have emphasised the importance of performing a variety of autonomic tests, rather than making assumptions based on one single autonomic test (Ewing and Clarke, 1982; Ewing, 1985; Low, 1993c; Mathias and Bannister, 1999). However a score requiring completion of a series of autonomic tests will introduce some bias, since it will select patients of an ability to satisfactorily perform all parts of the scoring system. More disabled individuals may have the worst autonomic function, therefore loss of cases may affect ability to detect an association with white matter lesion.

Three scores were examined in this investigation. The Ewing based score was chosen since it corresponds to an established series of tests. It has the advantage of many years clinical experience and is useful in assisting prognostication of diabetic patients (Ewing, 1976a; Ewing, 1980a). It is recognised that there are differences between Ewings' original description and our methodology. The main differences are sitting instead of handgrip for isometric exercise, and the use of RR maxima and minima with wider 'beat number' windows rather than close adherence to the 30:15 ratio. The drawbacks with the Ewing based score are

that the original normal values are clearly less appropriate for the age group under study in this investigation and it uses autonomic indices that do not appear to be any different in the stroke group compared with values in a control population (Chapter 7). Two of the tests did not indicate any difference between control and stroke groups – 30:15 ratio, blood pressure response to standing. Therefore, normal and abnormal function in the stroke group could not be defined from 'control' data.

8.4.2.1 Composite score A

Therefore composite score A was devised on the basis of tests that were significantly abnormal in the stroke group when compared with the community living controls (see Chapter 7). In other words it concentrated on tests that were known to assist in differentiation of impaired from normal autonomic function. It is acknowledged this part of the analysis was not significant, and furthermore there was only one case in the highest scoring group (total composite score 3). The composite score A only included one measure from power spectral analysis of heart rate variability, the total power spectrum. Low frequency power spectra are closely correlated with the total value and would not offer any extra dimension to composite score A. Low frequency range baroreflex sensitivity was significantly lower in the stroke group compared with control cases but this item was not included since the lower number of individuals with baroreflex sensitivity data could have further limited number available for study in the composite score. There are no grounds for supporting the use of composite score A beyond this investigation but it provides an illustration of a possible link between impaired autonomic function and white matter lesions.

The composite score was derived using autonomic function data from community living controls, as described in Chapter 7. This group comprised community living elderly adults and did include patients with conditions that could affect cardiovascular autonomic function. Therefore it does not form a group suitable for defining normality data for cardiovascular autonomic function in this age group. However it does form a control group who were similar in most respects to the stroke group, bar the presence of cerebrovascular disease. Therefore the group provides appropriate normality data to examine the affects of impaired autonomic function in the presence of stroke disease. Relatively small numbers in the control group may have limited ability to accurately define the outer limits of normality using the standard deviation. In other words the smaller the number of cases means the study is less likely to represent the true population mean and distribution. Secondly normal autonomic test results generally diminish with age thus reduced test range may have further compromised ability to reliably identify abnormal autonomic function from the control group in Chapter 7 (O'Brien, 1986; Piha, 1993).

8.4.2.2 Composite score B

Composite score B was devised to utilise all recorded cardiovascular autonomic reflex tests. Standard deviation of RR interval (from five minutes of resting heart rate data) was added since this is a relatively straightforward measure of heart rate variation that is known to be associated with adverse outcomes (Dekker, 2000). There were potential benefits to sensitivity of a scoring system by increasing the number of items, compared with the five items from Ewing score and composite score A. The method of defining normal and abnormal scores for B was far from robust, but the selection of thresholds from seven sources was a pragmatic solution and was achieved in some instances by a consensus method where there was no clear answer to the ideal normal value. In effect, composite score B did not show any association with white matter lesion volume.

In addition to cerebral autoregulation, there may be other important influences on white matter disease progression; these other factors could have a more powerful effect than autonomic impairment. This is examined in the following chapter.

8.5 Conclusion

Individual and composite measures of cardiovascular autonomic function do not show an association with white matter lesion volume in selected older stroke survivors.

9 Blood pressure variability and white matter lesion volume

9.1 Introduction

Older stroke survivors are at high risk of cognitive decline and dementia. At three months post-stroke, dementia will affect up to one-quarter of patients (Desmond, 2000; Pohjasvaara, 1997). In those who are not diagnosed with dementia at three months, the risks of incident dementia in the following 12 months remains greatly elevated compared with stroke-free controls, with estimated odds ratio between 6 and 9 (Kokmen, 1996; Tatemichi, 1994b). Trigger factors for conversion to cognitive impairment and dementia are not clear. White matter lesions may be an important mediator. White matter lesions are a substrate of cognitive deficit in older people both with and without dementia (Breteler, 1994c; CFAS, 2001). Determinants of white matter lesions are not completely understood. Age is the strongest risk factor for white matter lesions but hypertension is the most important treatable risk factor yet identified (Breteler, 1994c; van Swieten, 1991a). Historically, interest has centred on average clinic blood pressure to predict target organ damage. With the advent of ambulatory blood pressure monitoring, blood pressure variability is now recognised as an independent predictor of target organ damage (Sander, 2000a). This is particularly important for white matter lesions since brief but recurrent hypoperfusion may drive white matter lesion progression (Pantoni and Garcia, 1997). Abnormal blood pressure variability could potentiate white matter lesions by increasing ischaemic damage in the cerebral circulation from episodic low flow (Skoog, 1998a). Severity of white matter hyperintensities was significantly associated with transient drops in blood pressure in patients with neurodegenerative dementia (Ballard, 2000). Ambulatory blood pressure studies indicate that increased blood pressure variability and loss of diurnal variation are associated with small vessel disease (Goldstein, 1998; Kario, 2001; Kukla, 1998). Office blood pressure measurement has also shown that higher pulse pressure is related to small vessel disease (Liao, 1997). These studies utilised semi-quantitative visual rating scales of white matter lesion burden and the majority of patients were less than 75 years old. Automated MRI analysis can now provide more reliable white matter lesion quantification than visual rating scales (Firbank, 2003).

We hypothesised that blood pressure variability is a risk factor for white matter lesions in older stroke survivors without dementia, using a novel automated measure of white matter lesion volume.

9.2 Methods

Stroke patients aged 75 years and over were recruited from consecutive patients on representative hospital based stroke registers in Tyneside, UK. Selection and initial assessment has been previously described in detail in Chapter 4. A nested sample of patients who were in sinus rhythm and able to comply with ambulatory blood pressure monitoring and head MRI scan were included in this substudy. Exclusion criteria were dementia (American Psychiatric Association, 1994) and contra-indication to MRI scan.

History of ischaemic heart disease (angina or myocardial infarction), hypertension, peripheral vascular disease, hypercholesterolaemia and diabetes were recorded. Patients were classified as smokers if a current smoker. Presence of risk factors was allocated a score of one, absence a score of zero.

9.2.1 Ambulatory blood pressure monitoring

Sinus rhythm was confirmed from twelve-lead ECG. Spacelabs Ambulatory Blood Pressure Monitor 90207 was used for ambulatory blood pressure recording (Spacelabs Medical, Inc, Redmond, Washington, USA). This oscillometric device has been validated by the British Hypertension Society (O'Brien, 1991a) and is also validated for clinical use in the elderly (Iqbal, 1996). The automatic measurement interval was set at 30 minutes from 07:00 to 22:00 and 60 minutes from 22:00 to 07:00. Cuff size was chosen according to arm circumference and attached to the non-dominant arm, with the cuff bladder positioned over the brachial artery. Subjects were instructed to follow a normal daily routine but to keep the arm alongside the trunk during daytime measurement (Staessen, 1995). Recording commenced between 10:30 and 13:30. The device was inactivated by the subject after a period of 24 hours.

Data was downloaded from the monitor to a computer and a Spacelabs program automatically edited measurements (Ambulatory Blood Pressure Report Management System 90121, Spacelabs Medical inc 1996). Artifact that resulted in non-physiological blood pressure measurement was rejected. Values outside the following pre-set automatic edit limits were excluded from analysis: systolic range 70 – 240 mmHg, diastolic 40 – 150 mmHg, pulse pressure 20 – 150 mmHg. Manual editing was not performed.

A minimum of 16 measurements over 24 hours were required for inclusion in analysis. For the purpose of analysis, the day period was fixed at 10:00 to 20:00 and night period 00:00 to 06:00 (Staessen, 1997a; van Ittersum, 1995). Variability of ambulatory blood pressure was defined as the standard deviation of the average systolic or diastolic level (systolic or diastolic SD) (Mancia, 1983b). Pulse pressure (PP) was the difference between mean systolic and diastolic blood pressure. Diurnal variation was the percentage change in blood pressure from day to night-time, calculated as ((day BP– night BP) /day BP) x 100%. For inclusion in

circadian variation analyses, a minimum of 10 measurements in the day and 5 in the night period were required (Staessen, 1997a).

9.2.2 MRI

Subjects were scanned with a 1.5T GE Signa system (General Electric, Milwaukee, WI, USA). Axial FLAIR (Fluid Attenuated Inversion Recovery) images were acquired to visualise and determine the volume of white matter hyperintensities, as described in Chapter 4. Data were transferred to a computer for analysis. The percentage of white matter hyperintensities in the brain was calculated by dividing the volume of segmented white matter hyperintensities by the volume of the brain as determined from the SPM99 segmentation (Ashburner and Friston, 2000).

9.2.3 Statistical analysis

The association between ambulatory blood pressure data and white matter lesion volume was analysed in the three periods - 24 hours, day and night. Average blood pressure, variability, diurnal variation and pulse pressure were examined for each period. For the former three variables, both systolic and diastolic blood pressure data were included in analysis.

For each period, the level of confounding among the exploratory blood pressure variables was explored using the Pearson correlation coefficients. The association between white matter lesions and the explanatory variables (blood pressure variables and cardiovascular risk factors) was assessed using linear regression. Initially the explanatory variables were considered individually in a simple regression model. Multiple regression was then used to investigate the relative importance of the explanatory variables taking into account confounding and collinearity. Cardiovascular risk factors and one of the three time period ambulatory blood pressure variables were jointly entered into regression models. Initially explanatory variables were selected using a forward selection procedure; robustness of the results was checked by using backward elimination. Results are presented in the form of regression coefficients with 95% confidence intervals. The proportion of variance explained by the model (adjusted R^2) is given. Statistical tests were performed using SPSS release 11 (Statistical Package for the Social Sciences Inc, Chicago, Illinois) and tests with p value <0.05 were considered statistically significant.

9.3 Results

Ninety seven patients were assessed in the substudy. Twenty-one patients were excluded from ambulatory blood pressure analysis due to atrial fibrillation, 10 patients lacked MRI data (MRI contra-indication or movement artifact on images) and four cases had incomplete ambulatory data (intolerance of monitor in 3, <16 readings in 1). Clinical characteristics of

the 62 patients are shown in Table 9.1. The mean number of ambulatory blood pressure measurements was 34.5 ± 5.6.

Table 9-1 Clinical characteristics (n = 62)

Demographic/clinical feature	Mean ± s.d. or number (%)
Mean age, years	80 ± 4
Female	37 (60%)
Mean weight, kg	66 ± 13
Mean body mass index, kg/m²	25 ± 4
IHD	15 (24%)
Hypertension	45 (73%)
LVH on ECG	3 (5%)
PVD	7 (11%)
Diabetes	4 (7%)
Hypercholesterolaemia	17 (27%)
Current smoker	36 (58%)

IHD, ischaemic heart disease: LVH, left ventricular hypertrophy: PVD, peripheral vascular disease.

9.3.1 Correlations

Correlations for 24 hour, day and night ambulatory blood pressure variables are shown in Table 9.4. As expected, there were strong correlations between systolic and diastolic measurements of the same variables (average pressure, variability, diurnal variation), and between the same systolic or diastolic variables of different time periods. There were only weak correlations between systolic average pressure and variability and no significant correlation between diastolic average pressure and variability. The correlation between systolic and diastolic variability was at least as strong as the association between average systolic and diastolic pressure. Pulse pressure strongly correlated with systolic but not diastolic pressure.

9.3.2 Regression

Regression coefficients for cardiovascular risk factors and ambulatory blood pressure variables on white matter lesion volume are shown in Table 9.2.

Table 9-2 Univariate regression of ambulatory blood pressure on white matter lesion volume

Independent variable		Regression coefficient B		P
		B	95% CI	
Age, years		+0.158	+0.015, +0.302	0.031
Gender[1]		-0.714	-1.927, +0.499	0.244
Smoking[2]		-0.755	-1.959, +0.449	0.215
IHD[3]		-0.947	-2.331, +0.437	0.176
Diabetes[3]		+1.813	-0.592, +4.218	0.137
Cholesterol[3]		-0.600	-1.940, +0.740	0.374
LVH[3]		-0.952	-3.747, +1.842	0.498
24 hour (n=62)	SBP	+0.037	-0.002, +0.077	0.062
	DBP	+0.096	+0.030, +0.163	0.005
	SSD	+0.208	+0.066, +0.350	0.005
	DSD	+0.282	+0.074, +0.490	0.009
	PP	+0.009	-0.041, +0.058	0.724
Day (n=60)	SBP	+0.039	+0.000, +0.079	0.052
	DBP	+0.086	+0.024, +0.148	0.007
	SSD	+0.201	+0.086, +0.316	0.001
	DSD	+0.272	+0.101, +0.443	0.002
	PP	+0.008	-0.040, +0.057	0.729
Night (n=59)	SBP	+0.027	-0.008, +0.063	0.131
	DBP	+0.083	+0.019, +0.148	0.012
	SSD	+0.046	-0.074, +0.165	0.445
	DSD	+0.004	-0.172, +0.179	0.967
	PP	+0.006	-0.041, +0.053	0.801
Day-night (n=57)	SDV	-0.012	-0.093, +0.070	0.778
	DDV	-0.008	-0.078, +0.062	0.824

[1]male =1, female=0

[2]current smoker=1, never or ex-smoker=0

[3]positive history=1, negative=0

24 hour, day and night values in mmHg. Day-night values are percentage change from day to night.

IHD, ischaemic heart disease: LVH, left ventricular hypertrophy: PVD, peripheral vascular disease: SBP, DBP, average systolic and diastolic blood pressure: SSD, DSD, systolic or diastolic variability: PP, pulse pressure: SDV, DDV, systolic and diastolic diurnal variation

9.3.2.1 Cardiovascular risk factors

When white matter lesion volume was regressed on all cardiovascular risk factors, age was the only risk factor with a significant regression coefficient with white matter lesion volume ($p=0.031$) and explained approximately 6% of the variance.

9.3.2.2 24 hour ABP and cardiovascular risk factors

Systolic variability and average diastolic pressure remained significant in the final model and diabetes was the only cardiovascular risk factor with a significant contribution (Table 9.3). This model explained approximately 22% of the variance ($p=0.001$). The same variables were produced in the final model using either forward or backward regression on white matter lesion volume.

9.3.2.3 Daytime ABP and cardiovascular risk factors

Mean number of daytime measurements was 18.1 ± 3.3. The final regression model of cardiovascular risk factors and daytime ambulatory blood pressure variables on white matter lesion volume was similar to the 24 hour model (Table 9.3). Systolic variability, average diastolic pressure and diabetes were the significant terms in the final regression model and explained approximately 24% of white matter lesion volume variance ($p<0.001$). Forward and backward regression produced the same results.

9.3.2.4 Nighttime ambulatory blood pressure and cardiovascular risk factors

Mean number of nighttime measurements was 5.9 s.d. 0.4. In the regression of cardiovascular risk factors and nighttime ambulatory blood pressure variables on white matter lesion volume, gender, diabetes and average diastolic pressure were the final significant terms but only explained approximately 20% of the white matter lesion volume variance ($p=0.001$, Table 9.3).

Table 9-3 Ambulatory blood pressure regression models on white matter lesion volume: 24 hour, day and night periods

Phase	Significant terms	Regression coefficient		Stand. coefficient	P value
		B	95% CI		
24 hr	Constant	-6.333	-11.104, -1.562		0.010
(n=62)	DBP	+0.086	+0.022, +0.151	0.318	0.009
	SSD	+0.174	+0.039, +0.308	0.300	0.012
	Diabetes	+2.699	+0.554, +4.844	0.293	0.015
Day	Constant	-5.214	-9.582, -0.846		0.020
(n=60)	SSD	+0.160	+0.049, +0.271	0.339	0.005
	DBP	+0.072	+0.013, +0.130	0.291	0.017
	Diabetes	+2.419	+0.282, +4.556	0.263	0.027
Night	Constant	-3.693	-7.768, +0.383		0.075
(n=59)	DBP	+0.100	+0.039, +0.162	0.390	0.002
	Diabetes	+2.900	+0.753, +5.047	0.328	0.009
	Gender	-1.204	-2.304, -0.104	-0.264	0.033

[1]male =1, female=0

[2]current smoker=1, never or ex-smoker=0

[3]positive history=1, negative=0

24 hour, day and night values in mmHg. Day-night values are percentage change from day to night.

SBP, DBP, average systolic and diastolic blood pressure: SSD, DSD, systolic or diastolic variability: PP, pulse pressure: SDV, DDV, systolic and diastolic diurnal variation

See Table 2 for abbreviations.

Table 9-4 Pearson correlation co-efficients for ambulatory blood pressure

		24 hr (n=62)				Day (n=62)					Night (n=61)					Diurnal (n=61)	
		DBP	SSD	DSD	PP	SBP	DBP	SSD	DSD	PP	SBP	DBP	SSD	DSD	PP	SDV	DDV
24 hr	SBP	+0.57‡	+0.37†	+0.15	+0.82‡	+0.95‡	+0.50‡	+0.27*	+0.07	+0.77‡	+0.89‡	+0.49‡	+0.19	+0.01	+0.81‡	-0.12	-0.02
	DBP		+0.21	+0.22	-0.01	+0.54‡	+0.96‡	+0.20	+0.12	-0.06	+0.52‡	+0.86‡	+0.11	+0.05	+0.09	-0.11	+0.09
	SSD			+0.74‡	+0.31*	+0.44‡	+0.25*	+0.84‡	+0.70‡	+0.34†	+0.13	-0.02	+0.33*	+0.05	+0.18	+0.34†	+0.33†
	DSD				+0.03	+0.27*	+0.32*	+0.59‡	+0.87‡	+0.09	-0.05	-0.06	+0.32*	+0.12	-0.02	+0.39†	+0.44‡
	PP					+0.78‡	-0.06	+0.19	+0.00	+0.97‡	+0.71‡	+0.00	+0.16	-0.03	+0.91‡	-0.07	-0.08
Day	SBP						+0.54‡	+0.28*	+0.16	+0.80‡	+0.75‡	+0.39†	+0.14	-0.03	+0.70‡	+0.16	+0.18
	DBP							+0.21	+0.19	-0.08	+0.39†	+0.72‡	+0.09	-0.00	+0.02	+0.09	+0.33†
	SSD								+0.74‡	+0.18	+0.17	+0.09	+0.21	-0.04	+0.16	+0.07	+0.12
	DSD									+0.05	-0.03	-0.01	+0.27*	-0.04	-0.04	+0.24	+0.22
	PP										+0.60‡	-0.06	+0.10	-0.03	+0.81‡	+0.12	-0.02
Night	SBP											+0.64‡	+0.26*	+0.09	+0.86‡	-0.54‡	-0.33†
	DBP												+0.14	+0.14	+0.15	-0.45‡	-0.40†
	SSD													+0.54‡	+0.25	-0.21	-0.07
	DSD														+0.02	-0.16	-0.20
	PP															-0.39†	-0.16
Diurnal	SDV																+0.72‡

SBP, systolic blood pressure: DBP, diastolic blood pressure: SSD, systolic blood pressure variability: DSD, diastolic blood pressure variability: PP, pulse pressure: SDV, diurnal variation in systolic blood pressure: DDV, diurnal variation in diastolic blood pressure.

* $p<0.05$, † $p<0.01$, ‡ $p<0.001$)

9.4 Discussion

We have shown that white matter lesion volume in older non-demented stroke survivors is associated with systolic blood pressure variability over 24 hour ambulatory monitoring. The other significant associations with white matter lesion volume were for increase in average diastolic blood pressure and diabetes. Systolic blood pressure variability was of similar importance to these other classic risk factors. The clinical relevance of our findings is further support for the hypothesis of episodic cerebral hypoperfusion causing white matter lesions (Pantoni and Garcia, 1997). While we cannot prove causation from this cross-sectional study, results are in keeping with our hypothesis that these changes will lead to cognitive decline. The importance of our work lies firstly in the use of an accurate white matter lesion volume measurement tool and secondly in focusing on elderly stroke survivors who are at particularly high risk of developing dementia late after stroke (Desmond, 2002; Kokmen, 1996).

9.4.1 Systolic variability

There is growing interest in the value of blood pressure variability markers from ambulatory recording. Average ambulatory blood pressure over 24 hours is a superior marker of end-organ damage and cardiovascular events than office blood pressure in both untreated and treated hypertensive patients (Clement, 2003; Staessen, 1999b). There is further evidence that ambulatory blood pressure variability is a predictor of end-organ damage and cardiovascular events, independent of average blood pressure (Kikuya, 2000; Sander, 2000a). Previous studies observe that daytime variability is more predictive of target organ damage than nighttime variability (Goldstein, 1998; Kikuya, 2000; Palatini, 1992) and systolic variability is more predictive than diastolic variability (Palatini, 1992; Sander, 2000a; Sander, 2000b). Our results mirror these findings because daytime systolic variability was more closely associated with white matter lesion volume than either nighttime or diastolic values. The greater number of measurements imparts greater statistical power to daytime values but there may be important pathophysiological reasons for the stronger association between daytime variability and end-organ damage. The erratic component of blood pressure variability for example orthostatic hypotension, post-prandial hypotension, stress-related hypertension is most likely to occur during daytime periods and these events may be a principal cause of end-organ hypoperfusion and endothelial stress (Sega, 2002). It has previously been shown that orthostatic hypotension is associated with white matter lesions (Ballard, 2000; Longstreth, 1996; Raiha, 1993).

It is postulated that blood pressure variability exerts adverse effects directly through physical means i.e. intermittent low cerebral perfusion (Skoog, 1998a). Other cellular or neurodegenerative mechanisms could also play a role. There is evidence that increased oscillatory shear stress enhances surface endothelial levels of cell adhesion molecules and alters cell oxidative mechanisms, changes that are likely to play a part in the atherosclerotic process (Chappell, 1998). In animal models, small decreases in cerebral blood flow over days or more prolonged hypoxic states enhance amyloid precursor protein generation which in turn may increase the burden of toxic amyloid beta-peptide (Green, 2002; Shi, 2000). Therefore frequent falls in cerebral blood flow in the already compromised ischaemic penumbra or deep white matter of stroke patients could trigger neurodegenerative processes (Kobari, 1990).

9.4.2 Average diastolic blood pressure

Average diastolic blood pressure demonstrated a positive relationship with white matter lesion volume. Hypertension is an established risk factor for white matter lesions (Lewington, 2002; Skoog, 1998a). It is interesting that the correlation between diastolic blood pressure and white matter lesion volume was stronger than the correlation between systolic blood pressure and white matter lesion volume since systolic pressure is usually the stronger predictor for end-organ damage (Lewington, 2002). There is a debate on the relative merits of systolic, diastolic and pulse pressure as risk markers of vascular events: a prominent opinion is that systolic blood pressure and diastolic blood pressure remain the most valuable markers and pulse pressure is less informative (Lewington, 2002). The positive linear relationship between blood pressure and stroke risk persists in older adults; the proportional change in stroke risk per unit change in blood pressure is less powerful compared to middle age but annual absolute differences in risk are greater in old age (Lewington, 2002). We should be wary regarding the role of our findings within the debate in view of our relatively small and selected cohort, and therefore we judge that the stronger correlation between diastolic blood pressure and white matter lesion volume than systolic blood pressure and white matter lesion volume is of uncertain significance. In general, our findings do match the expected increase in white matter lesions with high blood pressure. The other component in considering the role of blood pressure in white matter lesions and cognitive function is the time course of events. There is some evidence regarding individuals with a history of hypertension who experience a decline in blood pressure with age later suffer progression of white matter lesions and cognitive impairment (Skoog, 1996). This question needs to be addressed in longitudinal studies.

9.4.3 Diabetes

Our final regression model contained diabetes as a term that was significantly associated with white matter lesion volume. Diabetes has previously been linked with white matter lesions (Schmidt, 1992). Our findings are in keeping with the proposed vascular mechanisms behind white matter lesions, since diabetes is known to be a powerful predictor of microvascular pathology.

9.4.4 Age

Increasing age is a well established risk factor for white matter lesions (Breteler, 1994c; Longstreth, 1996). Our study found a significant positive association between age and WML volume based on the univariate regression between the two variables. When using backward elimination, age was the final non-significant term to be eliminated from each regression model. Age was closely associated with blood pressure variability; when this term was included in the model the improvement in fit obtained by adding age was not statistically significant. The small number in this cohort and the use of an older population with restricted age range may have limited the power to confirm the importance of age in the final regression model.

9.4.5 Ambulatory blood pressure time period

The results for 24 hour and daytime ambulatory blood pressure regression models were very similar, with marginally greater variance of white matter lesion volume explained by the daytime model. One would expect daytime ambulatory variables to display stronger associations than 24 hour variables since the latter included nighttime values.

The contrast between day and night-time results in our study will have been influenced by the small number of ambulatory blood pressure measurements made over the night-time period, therefore affecting accuracy. We have utilised narrow fixed time windows for the diurnal variation in ambulatory blood pressure, since the narrow six hour sleeping period is valid for accurate estimates of diary-based blood pressure during sleep without resorting to more complex analysis (van Ittersum, 1995). Furthermore the chosen time periods were recommended by the Working Group of the International Database, to facilitate comparison between studies (Staessen, 1997a). Within our cohort, diurnal blood pressure variation did not show any correlation with white matter lesion volume.

9.4.6 Study limitations

We have shown significant associations for white matter lesions with blood pressure markers and diabetes but the relatively small number of patients studied may have compromised power to detect weaker associations. Frequency of ambulatory blood pressure sampling was low, especially for nighttime period, and may have affected the accuracy of blood pressure variability but any greater frequency of ambulatory blood pressure sampling would reduce tolerability in this older and moderately frail cohort. The ambulatory monitor used in the study has been validated for use in the elderly but is less accurate in measuring systolic blood pressure, underestimating systolic blood pressure at higher pressures (Iqbal, 1996). This cross-sectional study is not able to determine whether increased systolic variability is a cause of white matter lesion volume. Indeed subcortical ischaemia may lead to central autonomic control damage and itself cause increases in blood pressure variability. However there is strong circumstantial evidence that increasing systolic variability will drive progression of small vessel cerebrovascular disease.

9.5 Conclusion

White matter lesion volume was significantly associated with systolic and diastolic blood pressure variability ($p<0.01$), average diastolic blood pressure ($p<0.05$) and age ($p=0.031$) on simple regression. Following multiple regression on white matter lesion volume, systolic blood pressure variability, average diastolic pressure and diabetes were the final terms in a model that explained 24% of white matter lesion variance ($p<0.001$). The low variance of the model indicates poor predictive value. In conclusion, we have shown that systolic blood pressure variability, average diastolic blood pressure and diabetes are significantly associated with white matter lesion volume in older stroke survivors. We hypothesise that individuals with greater blood pressure variability at risk of cognitive decline due to intermittent hypoperfusion of white matter regions. In addition to established vascular risk factors, increased ambulatory blood pressure variability may place older stroke survivors at increased risk of dementia.

10 Correlation of white matter lesion volume and pulmonary function

10.1 Introduction

MRI is an important step in the investigation of dementia. Dementia groups have characteristic structural changes on brain imaging studies (Scheltens, 2002). Cerebral atrophy is a predictor of Alzheimer's disease in healthy individuals from large scale community studies and in high risk individuals with a family history (Fox, 2001; Kuller, 2003). Longitudinal studies report the rate of brain atrophy is significantly increased in dementia (Cardenas, 2003; Chan, 2001). Subcortical ischaemic vascular dementia is characterised by extensive deep white matter hyperintensities, best seen on MRI (Roman, 2002).

Hypoxic or hypotensive disorders have been suggested as a mechanism for neurodegeneration leading to dementia, particularly in stroke survivors (Kokmen, 1996; Moroney, 1996; Tatemichi, 1994b). Such events may result in vascular dementia but it is now clear that Alzheimer's disease shares risk factors and imaging abnormalities with vascular dementia (Barber, 1999; Skoog, 1999). Vascular factors that potentiate hypoxic and ischaemic damage to the ageing brain may be important not only in prevention and treatment of the vascular dementia, but for Alzheimer's disease as well.

Respiratory disease will produce hypoxic episodes and could prove to be an important substrate in the pathogenesis of dementia. Hypoxic states are certainly a risk factor for neurodegenerative damage at a cellular level (Green, 2002). Some reports link respiratory disease with dementia but they are based on heterogeneous diseases or do not include imaging studies (Kokmen, 1996; Moroney, 1996; Schaub, 2000; Tatemichi, 1994b). To our knowledge, there are no reports examining the impact of pulmonary function on brain imaging indices. We set out to determine if there is an association between pulmonary function tests and MRI risk factors for dementia.

10.2 Method

The test procedure was explained and demonstrated to the subject. The subject was seated in an upright position after a prolonged rest period. Forced expiration for six seconds was performed up to a maximum of three attempts until a consistent trace was obtained. Forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were recorded from the paper trace to the nearest 0.05 litre. Forced vital capacity was the maximum expired volume at six seconds. The largest of the three volume recordings was used for analysis.

10.2.1 Magnetic Resonance Imaging

Whole brain volume and cortical white matter lesion volume were measured as described in section 4.3. All cases with pulmonary function and whole brain volume data available were included. Cases were excluded if pulmonary function data was unreliable due to inadequate expiratory effort or technical errors with spirometry.

10.2.2 Statistics

10.2.2.1 Whole brain volume

The association between pulmonary function and MRI whole brain volume was first examined by bivariate correlation. Next, in a multiple linear regression analysis, whole brain volume was entered as the dependent variable. Gender, age, history of cardiac failure, ischaemic heart disease, hypertension, respiratory disease or hypercholesterolaemia, current smoker or alcohol consumer status, forced expiratory volume, forced vital capacity, FEV_1/FVC, height, weight and body mass index were entered as independent variables. Risk factors in binary data were assigned a value of 1 if present e.g. cardiac failure present = 1, cardiac failure absent = 0.

10.2.2.2 Cortical white matter lesion volume

To consider all potential predictor variables that may influence cortical white matter damage, multiple linear regression was performed using baseline cortical white matter lesion percentage volume as the dependent variable and all clinical features that achieved a significance level of less than 0.10 from single linear regression on cortical white matter lesion volume. The following explanatory variables were considered. Clinical features: gender, age, ECG rhythm, cardiac failure, ischaemic heart disease, hypertension, peripheral vascular disease, asthma/COPD, diabetes, hypercholesterolaemia, current smoker, current alcohol drinker, number of strokes, body mass index. Drugs: prescription of beta-blocker, thiazide, loop diuretic, ACE inhibitor or angiotensin II receptor blocker, calcium channel blocker, nitrate, anticoagulant, antiplatelet, neuroleptic, statin and oxybutynin were entered as an independent variable. Blood parameters: haematocrit, erythrocyte sedimentation rate, fibrinogen, homocysteine, C-reactive protein, fasting glucose, haemoglobin A1c, total cholesterol, high density cholesterol, low density cholesterol, triglyceride, vitamin B12 and folate level were entered as independent variables.

Other variables considered for entry to the multiple regression model were cardiovascular autonomic reflex tests, heart rate variability, baroreflex sensitivity and ambulatory blood pressure. From results seen in Chapters 8, no reflex tests achieved the <0.1 significance level. From ambulatory blood pressure results in Chapter 9, only daytime and night-time variables were

considered for inclusion. Daytime mean systolic and diastolic levels, daytime systolic and diastolic variability and night-time mean diastolic blood pressure level met the inclusion significance level. From results in this chapter, FEV_1 and FVC met the criteria. Since cardiac failure status was strongly correlated with loop diuretic, the latter was excluded from the multiple regression analysis. HDL cholesterol was also excluded from multiple regression since the total number of cases with HDL data fell substantially below the number of cases with results for other independent variables in the multiple regression analysis.

10.3 Results

10.3.1 Whole brain volume

Seventy five cases were analysed in the correlation between whole brain volume and pulmonary function, with results shown in Table 10.1.

Table 10-1 Correlation between pulmonary function and whole brain volume (n = 75)

Pulmonary function	Correlation coefficient	P value
FEV_1	+0.438	<0.001
FVC	+0.454	<0.001
FEV_1/FVC	+0.183	0.115

P values of Pearson correlation coefficient: FEV_1, forced expiratory volume in one second: forced vital capacity

There was a significant correlation between whole brain volume and FEV or FVC. However on multiple regression analysis, the only significant terms predicting whole brain volume were height and current smoker status.

Whole brain volume = (535798 x height) – (41154 x current smoker) + 158040

Height (metres): current smoker = 1, never/ex-smoker = 0.

Increasing height had a significant linear association with whole brain volume. Smokers had a significant reduction in whole brain volume.

10.3.2 White matter lesion volume

Coefficients for simple regression of pulmonary function on cortical white matter lesion percentage volume at baseline are shown in Table 10.2. Following simple regression of potential risk factors on white matter lesion volume, only those factors with significance values less than 0.10 are summarised in Table 10.3.

Table 10-2 Regression of pulmonary function on cortical white matter lesion volume

White matter lesion variable	Pulmonary function	Regression coefficient	95% confidence interval	P value
Baseline (n=81)	FEV_1	-0.701	-1.419, +0.080	0.056
	FVC	-0.681	-1.254, -0.108	0.020
	FEV_1/FVC	+0.183	-5.027, +5.393	0.944

FEV_1, forced expiratory volume in one second: FVC, forced vital capacity

Table 10-3 Significant independent variables from simple linear regression on cortical white matter lesion volume

Variable	Total degrees freedom	Regression coefficient		p
		B	95% confidence interval	
Age	82	+0.135	+0.024, +0.246	0.017
Cardiac failure	82	+1.442	+0.258, +2.625	0.018
Diabetes	82	+1.679	-0.151, +3.509	0.072
Loop diuretic	82	+1.360	+0.064, +2.656	0.040
Haemoglobin A1c	73	+0.793	-0.007, +1.594	0.052
HDL cholesterol	54	+1.263	-0.093, +2.620	0.067

All variables from Table 10.3, daytime systolic and diastolic levels and daytime systolic and diastolic variability (Table 9.3) and FEV_1 and FVC (Table 10.2) were entered into a multiple linear regression model. The least significant term was removed in a stepwise manner, until only terms with a significance level of less than 0.05 remained. The following model was obtained.

White matter lesion % volume at baseline = (1.65 x CCF) + (2.04 x DM) – (0.64 x FVC) + (0.13 x daytime diastolic variability) + (0.07 x night mean diastolic) – 3.11

Total degrees of freedom = 66, adjusted $R^2 = 0.318$, $p < 0.001$

The significant predictor variables from this equation are summarized in Table 10.4.

Table 10-4 Significant independent variables from multiple linear regression on white matter lesion volume at baseline MRI

Independent variable	Regression co-efficient		Significance
	B	95% confidence interval	
CCF	+1.66	+0.50, +2.81	0.006
Diabetes	+2.04	+0.32, +3.76	0.021
FVC	-0.64	-1.17, -0.11	0.020
ABP Systolic SD (day)	+0.13	+0.04, +0.22	0.005
ABP Diastolic mean (night)	+0.07	+0.02, +0.13	0.022

CCF, Congestive cardiac failure: FVC, Forced vital capacity: ABP systolic SD (day), Ambulatory systolic blood pressure variability 10:00-20:00: ABP diastolic mean (night), Ambulatory diastolic blood pressure mean 00:00-06:00.

10.4 Discussion

This chapter reports firstly the association between pulmonary function and MRI variables at baseline, and secondly reports the significant independent variables that predict cortical white matter lesion volume when including all independent variables from clinical records, cardiovascular autonomic reflex tests, heart rate variability, baroreflex sensitivity, pulmonary function, haematological and biochemical indices.

10.4.1 Whole brain volume and pulmonary function

There was a close correlation between lung volumes and whole brain volume. Results show decreasing FEV_1 and FVC are associated with smaller whole brain volumes. The correlation is highly significant. However on multiple linear regression the only significant independent variables predicting whole brain volume were height and smoking status. The significance of height in the regression equation suggests the correlation between lung volumes and whole brain volume could be interpreted as a normal finding, reflecting the proportional variation in organ size. Pulmonary function variables were not significant independent predictors of whole brain volume but smoking did negatively predict whole brain volume. This association could be mediated by direct toxic effects of smoking, associated vascular disease or by smokings' harmful effects on respiratory function. Smoking is associated with a doubling of risk of dementia, and this link is traditionally thought to be a result of smoking-related vascular disease (Ott, 1998). Smoking is a potential causative link between reduced lung volume and structural brain changes, but these data only indicate an association. No firm conclusions can be drawn regarding a pathogenetic link between smoking and reduced whole brain volume.

There is large amount of evidence indicating whole brain atrophy cannot be regarded as a benign phenomenon. Whole brain atrophy on CT is associated with Alzheimer's disease and a controlled study found atrophy rates of 0.5 % in controls and greater than 2% per year in Alzheimer's disease (Fox, 1996; Freeborough and Fox, 1997; Smith and Jobst, 1996). Cardenas et al found increased rate of brain atrophy in patients with Alzheimer's disease or mixed vascular and Alzheimer's disease and there is a correlation between atrophy and cognitive decline on MMSE (Cardenas, 2003; Fox, 1999).

From this basic cross-sectional study, bivariate correlation analysis indicates an association between reduced lung volume and whole brain volume. This does not provide evidence for reduced pulmonary function providing a cause for reduction in whole brain volume. After multiple regression on whole brain volume, smoking was a significant predictor variable whereas lung volumes were not. Thus smoking provides a potential pathological link between reduced respiratory function and decreasing brain volume. Longitudinal studies are required to further test the hypothesis, in particular to examine if there are increased rates of cerebral atrophy in smokers. If so, is smokings' effect dependent on decline in respiratory function and therefore related to hypoxia or is it independent of respiratory function and therefore more likely to be related to vascular factors and neurodegeneration? Mechanisms related to deteriorating respiratory function could offer a novel therapeutic target in patients suffering cognitive decline.

10.4.2 White matter lesion volume and pulmonary function

Forced vital capacity was significantly associated with cortical white matter lesion volume at baseline MRI. There was a trend towards forced expiratory volume being associated with white matter lesion volume. These results support the hypothesis of hypoxic-hypotensive disorders potentiating cognitive decline. Reduced pulmonary function will enhance the risk of hypoxia, especially during systemic illness in those with respiratory reserve. Moroney et al (Moroney, 1996) found that out of 185 stroke survivors (with a mean age of 70 years), those suffering a broad collection of so-called 'hypoxic-ischaemic' disorders were significantly more likely to develop dementia. The damage to white matter could be mediated by simple ischaemic damage from reduced oxygen tension. Other mechanisms may play a role, and we could hypothesise for a role in reduced respiratory function leading to alterations in metabolic conditions that may lead to a more insidious effect on brain structure and function, compared to transient short term hypoxia.

Hypoxic states affect regulation of gene expression. Ischaemia generates amyloid precursor protein as a protective action (Kogure and Kato, 1993; Koistinaho, 1996). Amyloid beta peptide is then produced as a derivative of amyloid precursor protein. Amyloid beta peptide is toxic to

neuronal cells and is an important neuropathological manifestation of Alzheimer's disease. There are reports confirming increased amyloid beta peptide formation after exposure to ischaemic conditions (Jendroska, 1997; Yokota, 1996). In particular, chronic hypoxia models suffer high levels of amyloid beta peptide, which in conjunction with reactive oxygen species leads to pathological remodeling of cell function (Green, 2002). Calcium handling by cortical astrocytes, crucial to neuronal activity, is severely disturbed in conditions of prolonged hypoxia and amyloid beta peptide can play a role in generation of pathological calcium channels (Smith, 2003; Taylor, 1999). These findings provide important biochemical evidence of a link between hypoxic conditions and neurodegenerative changes associated with dementia.

Aforementioned studies are largely experimental models. Clinical data from humans provides limited support of respiratory disease exacerbating cognitive decline. A small controlled study indicated that cerebral metabolism will change to anaerobic pathways in patients with relatively moderate COPD. Ten patients (aged 65 years) with stable COPD revealed evidence of significantly altered cerebral metabolism. There were marked differences in phosphorus containing metabolites in the presence of respiratory disease, indicating much greater anaerobic metabolism within cerebral neurones in those with COPD (Mathur, 1999).

There are reports linking COPD and cognitive impairment. Schaub et al performed a cross-sectional study of ventilatory capacity and dementia prevalence in 437 participants older than 69 years. FEV_1 was one of four lung volume variables with significantly increased odds ratio of dementia in the group with the worst compared with best respiratory function (Schaub, 2000). An uncontrolled study of 18 patients with COPD reported neuropsychometric deficits in individuals with poorer respiratory function. Memory, attention and speed of information processing were closely associated with both arterial carbon dioxide partial pressure, and less strongly correlated with oxygen partial pressure (Stuss, 1997).

The Rotterdam study, an epidemiological survey of 1077 community-living elderly, reported periventricular white matter lesions were significantly and independently associated with lower oxygen saturation on pulse oximetry, and non-significantly associated with history of COPD (van Dijk, 2004).

Our data shows that FEV_1 and FVC were associated with white matter lesion volume but the FEV_1/FVC ratio was not associated with white matter lesion. This is not in keeping with the hypothesis that reduced respiratory function is associated with white matter lesions, since reduced FEV_1/FVC is essential for the diagnosis of obstructive airways disease. However, a much larger

study also failed to find a correlation between COPD (or oxygen saturation) and subcortical white matter lesions or lacunar infarcts (van Dijk, 2004).

10.4.3 Multiple linear regression of all variables on white matter lesion volume

Simple linear regression was performed to select a group of independent variables that demonstrate a strong association with white matter lesion volume. Thus more than 70 variables were narrowed down to a candidate list of 12 predictor variables (blood pressure, pulmonary function and blood indices). Multiple linear regression of the 12 clinical indices produced a model with five independent variables that were significantly associated with white matter lesion volume. The presence of cardiac failure or diabetes mellitus was associated with a 1.54 or 2.07 percent increase in percentage white matter lesion volume compared with individuals without these conditions. For every 100 ml fall in FVC, percentage white matter lesion volume increased by 0.06%. From ambulatory blood pressure monitoring, each one mmHg increase in daytime systolic BP variability or night-time mean diastolic blood pressure was associated with 0.20 and 0.08 percent increase in cortical white matter lesion percentage volume respectively.

Methodology for obtaining this regression model bears some similarities with that previously described in Chapter 9, and may appear to be repetitious. The Chapter 9 analysis is based on a limited number of established vascular risk factors and includes cases in sinus rhythm only. The regression analysis performed in this chapter is more wide ranging and carries with it a larger element of speculation. It also includes cases in sinus rhythm and atrial fibrillation. In view of these different conditions, it appears pertinent to pose the same question. The final regression model in this chapter may be less robust than Chapter 9 regression model for two reasons. Firstly a very large number of potential independent variables are considered and secondly some cases lack data for some of the variables. In spite of these drawbacks, the regression model indicates a number of interesting associations with baseline cortical white matter lesions.

There is little information in the literature relating to an association between cardiac failure and white matter lesions. Raiha et al (Raiha, 1993) divided 204 hospitalised patients with CT scan data into those with and without cardiac failure. White matter low attenuation was more common in those with heart failure compared with those free of heart failure (34% vs. 14%, $p = 0.0012$), and the authors felt this supported the hypothesis of hypoperfusion driving white matter disease. More recently Zuccala et al (Zuccala, 2001) identified impaired cognitive function in heart failure patients in conjunction with hypotension. Cardioembolic disease or hypotension are both potential mechanisms for white matter damage in the presence of left ventricular impairment

(Pullicino and Hart, 2001). Cardiac failure presents difficulties with respect to defining disease presence, particularly in large community studies. Definition could be based on clinical criteria which is time consuming and probably prone to inter-observer error. Alternatively definition could be based on echocardiographic assessment of left ventricular function. Definition in this study was not ideal: the participant was asked about history of heart failure or use of diuretic drug therapy. The response was almost invariably qualified by use of loop diuretic, which does not always equate to left ventricular failure in community living individuals. Therefore the association of cardiac failure in this regression model may relate more to an association between loop diuretic (and not left ventricular failure) with white matter lesions.

Diabetes was a significant independent predictor variable for white matter lesions. This replicates the findings of Schmidt et al who found age and diabetes to be the only independent predictors of white matter lesions in a study of stroke patients and healthy volunteers. In Schmidt's study, mean age of both stroke and control groups was 54 years, and there was a surprisingly similar burden of white matter disease in the two groups (Schmidt, 1992). Another unusual finding in this report is history of cerebrovascular events did not appear to be an independent predictor of white matter lesion. Presence of diabetes as a significant explanatory variable of white matter lesions supports a role for microvascular disease in the aetiology of white matter lesions.

These findings may provide a therapeutic target for prevention of white matter disease progression: there was a trend towards significance for an positive association between baseline white matter lesions and haemoglobin A1c (R = +0.793, 95% CI -0.01 to +1.59, p = 0.052) that indicates the association is not just the presence of diabetes but linearly related to the degree of glycaemic control. One could hypothesise that good diabetic control may reduce small vessel disease progression, and also emphasizes the importance of identifying vascular risk factors in patients at risk of cognitive decline. The hypothesis would require a randomized controlled trial to investigate the value and safety of tight diabetic control, particularly since this approach can run the risk of hypoglycaemic episodes in older, frail patients with all its attendant risks.

Forced vital capacity was independently associated with white matter lesion volume in the regression model. This provides further support for the hypoxic-ischaemic model of white matter disease. However the link remains purely speculative. It is quite possible that both white matter lesions and reduced lung volume are due to some unrelated process, but a number of candidate factors were considered in the regression model and did not significantly assist prediction of white matter disease. The association could be a reflection of proportional variation in organ size but (i) white matter lesions cannot be considered a normal process (ii) cortical white matter lesion

volume was measured in percentage terms and (iii) addition of height to the regression model did not alter the final and significant terms. The hypothesis could be tested by examining the risk to white matter from arterial oxygen saturation and partial pressure. Prolonged oxygen saturation monitoring is an even more attractive risk factor for investigation, as a means of investigating dynamic oxygen levels in the aetiology of white matter disease. A large community study has recently revealed an association between pulse oximetry and white matter lesions (van Dijk, 2004).

In Chapter 9, the focus was on ambulatory blood pressure variables and association with white matter lesion volume. Mean diastolic blood pressure was significantly associated with white matter lesion volume. Systolic blood pressure variability was also an independent predictor of white matter lesion volume in the cross-sectional study. These two predictor variables have retained their significance in the regression model in this more extensive examination of white matter disease. These risk factors were discussed in Chapter 9. The fact they remain significant in this second analysis confirms their importance. However the regression slope for these ambulatory blood pressure variables is shallower than slopes for cardiac failure, diabetes and forced vital capacity.

10.5 Conclusions

Smoking is associated with a significant reduction in whole brain volume. No other cardiovascular or pulmonary risk factors independently predicted baseline whole brain volume. In a repeat of the regression model for cortical white matter lesion volume, the following significant predictors for white matter damage were identified: cardiac failure, diabetes, forced vital capacity, daytime systolic blood pressure variability and diastolic blood pressure level at night. This supports the 'hypoxic-ischaemic' theory of white matter lesions.

11 Correlation of autonomic function and ambulatory blood pressure with baseline neuropsychological function

11.1 Introduction

As discussed in Chapter 2, our hypothesis is that abnormal autonomic function will lead to cognitive impairment due to recurrent white matter ischaemia in older stroke survivors. Therefore cardiovascular autonomic reflexes may demonstrate an association with cognitive function tests. In this chapter the relationships between cognitive tests and cardiovascular indices are examined.

11.2 Method

Cardiovascular autonomic reflex tests, power spectral analysis of heart rate variability and baroreflex sensitivity and 24 hour ambulatory blood pressure were performed as described in Chapter 4. Subjects in atrial fibrillation were excluded from analysis. Neuropsychological tests were applied as described in Chapter 4. From the CAMCOG test, total score was used as a marker of global cognitive function and the executive and attentional subscores were used as a marker of subcortical function (Roth, 1986). A key mechanism in the hypothesis is that recurrent white matter ischaemia can lead to attentional and executive deficits. Numerical vigilance, choice reaction time and memory scanning were selected from the CDR battery as additional markers of subcortical circuit damage. Mean reaction time for number vigilance, choice reaction time and memory scanning tasks were recorded as well as the standard deviation from choice reaction time. These CDR indices assess alertness, attention, concentration and the standard deviation of choice reaction time reflects fluctuation in attention (Simpson, 1991).

Correlation co-efficients were determined between each autonomic or blood pressure variable and neuropsychological test. Spearman's rank correlation was calculated in view of the skewed distribution of most RR interval tests, attentional and memory scores from CAMCOG and all CDR tests.

11.3 Results

11.3.1 CAMCOG Total and sub-scores

There were inverse correlations between Δdiastolic blood pressure during isometric exercise and both attention and executive subscores on the CAMCOG total score ($p = 0.011$ and 0.017

respectively). Attention subscore significantly and inversely correlated with total and high frequency power spectra of heart rate variability (p = 0.037 and 0.004 respectively, Table 11.1).

Table 11-1 Correlation between CAMCOG subscores, CAMCOG total score with cardiovascular reflex tests and power spectral analysis

Autonomic variable	Number	Attention subscore		Executive subscore		Memory subscore		Total CAMCOG	
		R	P	R	P	R	P	R	P
Reflex tests									
30:15 ratio	71	+0.179	0.135	-0.068	0.571	+0.068	0.572	-0.006	0.959
Valsalva ratio	69	+0.164	0.178	+0.011	0.929	+0.100	0.415	+0.058	0.637
dHR respiration	73	-0.094	0.431	-0.071	0.553	+0.127	0.283	+0.000	0.999
ΔSBP orthostasis	72	+0.044	0.711	+0.139	0.245	+0.069	0.564	+0.090	0.454
ΔDBP isometric	74	-0.293	**0.011**	-0.276	**0.017**	-0.178	0.130	-0.174	0.139
ΔDBP cold press	72	-0.180	0.131	+0.018	0.883	-0.124	0.298	-0.77	0.519
ΔSBP Valsalva	68	+0.140	0.254	+0.117	0.340	+0.031	0.801	+0.133	0.279
RR post-CSM	31	-0.022	0.906	+0.070	0.710	-0.129	0.488	-0.025	0.893
ΔSBP post-CSM	31	-0.151	0.419	-0.068	0.715	+0.141	0.450	-0.008	0.964
PSA									
sdRR	68	-0.278	**0.022**	-0.001	0.993	+0.002	0.987	-0.066	0.291
Total HRV	68	-0.253	**0.037**	+0.061	0.622	+0.048	0.699	-0.012	0.920
Low freq. HRV	68	-0.215	0.078	+0.122	0.320	+0.136	0.268	+0.069	0.578
High freq. HRV	68	-0.344	**0.004**	+0.033	0.787	+0.064	0.605	-0.081	0.509
Low freq. BRS	39	-0.278	0.087	+0.158	0.337	+0.057	0.732	+0.087	0.598
High freq. BRS	59	-0.162	0.220	+0.158	0.231	+0.023	0.860	+0.091	0.492

dHR respiration, change in heart rate during metronomic respiration: ΔSBP, change in systolic blood pressure during: orthostasis, active standing: Valsalva, blood pressure overshoot during Valsalva: post-CSM, during carotid sinus massage: ΔDBP, change in diastolic blood pressure during isometric, isometric exercise: cold press., cold cutaneous stress: PSA, power spectral analysis: sdRR, standard deviation of mean RR interval: HRV, heart rate variability: BRS, baroreflex sensitivity.

Executive subscore inversely correlated with mean diastolic and mean arterial daytime blood pressure level (p = 0.004 and 0.034, Table 11.2). Results indicated an inverse association between diurnal blood pressure variation and cognitive function according to CAMCOG. Significant correlations were observed between larger night-time diastolic blood pressure dip and lower executive, memory and total CAMCOG scores (p = 0.011, 0.004 and 0.012 respectively, Table 11.2). There was a similar but slightly weaker pattern of correlation using systolic nocturnal dip.

Top and bottom quartiles of diastolic percentage nocturnal dippers were compared. The bottom quartile of dipping status had a daytime mean diastolic blood pressure of 69 ± 9 mmHg and night-time mean of 69 ± 8 mmHg (0 % nocturnal dip, 95 % CI for diastolic blood pressure dip -2.5 to + 2.5 mmHg, p = 1.00). The top quartile of dipping status had a daytime diastolic mean of 77 ± 10 mmHg and night-time mean of 60 ± 9 mmHg, an average 22 % nocturnal dip in diastolic blood pressure (95 % CI for diastolic blood pressure dip +14.6 to +19.0 mmHg, p < 0.001). There was a significant difference between total CAMCOG scores for top and bottom quartiles of dippers of 8.7 points (95 % CI 1.5 to 15.8, p = 0.020)

Table 11-2 Correlation between CAMCOG subscores, total CAMCOG score and ambulatory blood pressure

ABP variable	Number	Attention subscore		Executive subscore		Memory subscore		Total CAMCOG	
		R	P	R	P	R	P	R	P
Day									
Mean systolic	69	-0.088	0.474	-0.141	0.248	-0.066	0.592	-0.087	0.479
Mean diastolic	69	-0.176	0.149	-0.345	**0.004**	-0.150	0.218	-0.228	0.059
Mean arterial	69	-0.145	0.236	-0.256	**0.034**	-0.076	0.536	-0.151	0.215
Systolic SD	69	-0.079	0.517	-0.050	0.682	+0.009	0.938	-0.029	0.813
Diastolic SD	69	-0.193	0.112	-0.171	0.159	-0.123	0.314	-0.108	0.376
Mean arterial SD	69	-0.086	0.482	-0.187	0.124	-0.079	0.518	-0.166	0.173
Pulse pressure	69	+0.060	0.622	+0.067	0.585	+0.085	0.489	+0.086	0.482
Night									
Mean systolic	67	+0.077	0.537	-0.020	0.874	+0.078	0.531	+0.043	0.727
Mean diastolic	67	-0.028	0.820	-0.095	0.445	+0.052	0.675	-0.029	0.818
Mean arterial	67	+0.067	0.591	-0.077	0.536	+0.101	0.416	+0.026	0.835
Systolic SD	67	+0.033	0.793	-0.027	0.829	-0.131	0.292	-0.083	0.502
Diastolic SD	67	+0.017	0.894	-0.035	0.780	+0.008	0.949	-0.056	0.650
Mean arterial SD	67	+0.019	0.881	-0.133	0.284	-0.147	0.235	-0.193	0.119
Pulse pressure	67	+0.060	0.632	+0.014	0.907	+0.076	0.544	+0.045	0.719
Diurnal variation									
Systolic	65	-0.220	0.078	-0.168	0.182	-0.275	**0.026**	-0.264	**0.034**
Diastolic	65	-0.165	0.190	-0.313	**0.011**	-0.349	**0.004**	-0.310	**0.012**

ABP, ambulatory blood pressure: SD, standard deviation of blood pressure

11.3.2 CDR results

Table 11-3 Correlation between CDR scores with cardiovascular reflex tests and power spectral analysis

Autonomic variable	N	Number vigilance		Choice reaction time		Choice reaction time s.d.		Memory scanning	
		R	P	R	P	R	P	R	P
Reflex tests									
30:15 ratio	68	+0.09	0.486	-0.07	0.585	-0.05	0.705	-0.10	0.431
Valsalva ratio	67	-0.17	0.183	-0.20	0.102	-0.11	0.394	**-0.25**	**0.044**
dHR resp.	70	+0.03	0.778	-0.16	0.190	-0.03	0.793	+0.03	0.784
ΔSBP stand	69	-0.10	0.400	-0.02	0.905	-0.19	0.121	-0.08	0.495
ΔDBP isom.	71	**+0.26**	**0.027**	+0.18	0.145	+0.19	0.107	+0.20	0.102
ΔDBP cold p.	69	+0.12	0.337	-0.06	0.600	+0.01	0.925	+0.01	0.909
ΔSBP Valsal.	66	-0.13	0.296	-0.09	0.464	+0.03	0.828	-0.11	0.397
RR post-CSM	29	-0.20	0.293	-0.14	0.471	-0.10	0.595	-0.30	0.126
ΔSBP CSM	29	-0.04	0.848	+0.03	0.887	+0.13	0.505	+0.26	0.186
PSA									
sdRR	65	+0.03	0.835	-0.08	0.517	-0.16	0.210	-0.05	0.684
Total HRV	65	-0.02	0.857	-0.11	0.388	-0.18	0.157	-0.02	0.893
LF HRV	65	+0.02	0.899	**-0.25**	**0.048**	**-0.24**	**0.050**	-0.11	0.678
HF HRV	65	-0.00	0.983	-0.14	0.261	-0.16	0.192	-0.08	0.542
LF BRS	37	+0.15	0.362	-0.06	0.727	-0.17	0.315	-0.11	0.512
HF BRS	56	-0.05	0.735	**-0.30**	**0.027**	**-0.40**	**0.002**	-0.11	0.446

dHR resp., change in heart rate during metronomic respiration: ΔSBP, change in systolic blood pressure during: stand, active standing: Valsal., blood pressure overshoot during Valsalva: post-CSM, during carotid sinus massage: ΔDBP, change in diastolic blood pressure during: isom., isometric exercise: cold p., cold cutaneous stress: PSA, power spectral analysis: sdRR, standard deviation of mean RR interval: HRV, heart rate variability: LF, low frequency: HF, high frequency: BRS. Baroreflex sensitivity.

Table 11-4 Correlation between CDR scores and ambulatory blood pressure

ABP variable	N	Number vigilance		Choice reaction time		Choice reaction time s.d.		Memory scanning	
		r	P	R	P	R	P	R	P
Day									
Mean systolic	66	+0.043	0.732	+0.062	0.621	+0.110	0.381	+0.118	0.351
Mean diastolic	66	+0.109	0.385	+0.177	0.154	**+0.266**	**0.031**	+0.195	0.119
Mean arterial	66	+0.040	0.752	+0.118	0.344	+0.195	0.117	+0.152	0.226
Systolic SD	66	+0.132	0.290	+0.042	0.738	-0.021	0.867	+0.148	0.238
Diastolic SD	66	+0.145	0.245	+0.021	0.865	+0.040	0.750	+0.218	0.082
Mean arterial SD	66	+0.184	0.140	+0.139	0.266	+0.109	0.384	+0.238	0.056
Pulse pressure	66	+0.006	0.962	-0.005	0.967	-0.052	0.680	+0.009	0.944
Night									
Mean systolic	64	-0.075	0.554	+0.072	0.573	+0.060	0.635	+0.097	0.447
Mean diastolic	64	-0.118	0.353	+0.003	0.982	+0.080	0.531	+0.024	0.851
Mean arterial	64	-0.103	0.418	+0.040	0.753	+0.063	0.622	+0.084	0.510
Systolic SD	64	-0.118	0.354	-0.008	0.953	-0.074	0.559	-0.170	0.179
Diastolic SD	64	+0.023	0.855	+0.051	0.691	+0.157	0.216	+0.055	0.669
Mean arterial SD	64	+0.001	0.993	+0.038	0.766	+0.083	0.513	+0.010	0.937
Pulse pressure	64	-0.017	0.893	+0.101	0.426	+0.051	0.692	+0.145	0.254
Diurnal variation									
Systolic	62	+0.043	0.738	-0.034	0.791	+0.081	0.532	+0.009	0.946
Diastolic	62	+0.184	0.152	+0.176	0.170	+0.220	0.086	+0.102	0.430

ABP, ambulatory blood pressure: SD, standard deviation of blood pressure

11.4 Discussion

11.4.1 Autonomic function and CAMCOG

Table 11.1 indicates a negative association between diastolic blood pressure increase during isometric exercise and attention and executive subscores. In view of most of the other correlations being non-significant on a background of multiple significance testing, this may be a Type I error. The difference in isometric exercise diastolic blood pressure response between stroke cases and controls was an exaggerated increase in blood pressure following stroke (Chapter 7). A potential mechanism is impaired baroreflex control of sympathetic driven vasoconstriction during isometric stress (section 7.4.1). If we extrapolate results on exaggerated blood pressure during

isometric stress and impaired baroreflex sensitivity in stroke patients (section 7.3) to findings in this section regarding the association between isometric exercise and CAMCOG subscores, we could hypothesis that defective blood pressure control could lead to surges in cerebral perfusion pressure and microvascular damage of subcortical neuronal circuits. Alternatively some other unknown neurodegenerative process could simultaneously damage baroreflex centres as well as centres controlling cognitive processes.

The heart rate variability correlations record a negative significant association between total and high frequency heart rate variability and attention CAMCOG subscore only. Again one suspects there is a substantial chance this is a Type I error, since there was multiple significance testing and none of the other hypothesised associations were significant.

11.4.2 Ambulatory blood pressure and CAMCOG

We have shown an inverse relationship between increasing circadian diastolic blood pressure variation and CAMCOG score. Larger drop in blood pressure overnight was associated with more impaired cognitive function in this cohort of older stroke survivors.

Low blood pressure overnight will increase the risk of hypotensive episodes, possibly leading to cerebral hypoperfusion. This has special relevance in older stroke patients where cerebral autoregulation can shift rightwards following midlife hypertension, leaving the brain vulnerable to low perfusion pressure (Choi, 1998; Mehagnoul-Schipper, 2000; Wollner, 1979). Ensuing ischaemic damage, especially in the white matter, may result in cognitive decline, providing a likely mechanism for the observed association.

These findings also raise the question of optimal blood pressure-lowering strategy in older stroke patients. The benefits of blood pressure lowering in primary and secondary prevention of stroke are beyond doubt (MacMahon, 1990; Progress Collaborative Group, 2001). Recently randomised controlled trials have shown anti-hypertensive treatment reduces risk of cognitive decline in patients of any age with cerebrovascular disease but no benefit in cognitive outcome for ≥70 year old hypertensive patients (Lithell, 2003; Tzourio, 2003). We hypothesise that excessive blood pressure reduction, particularly for night-time blood pressure, may have a detrimental impact on cognitive function in older stroke survivors. Twenty four hour ambulatory blood pressure monitoring could assist such decision making.

11.4.3 Autonomic indices and CDR

Table 11.3 reveals a small number of associations between CDR indices and cardiovascular autonomic reflex tests or heart rate variability. Larger Valsalva ratio was associated with faster

memory scanning time. Larger exercise-induced rises in blood pressure were associated with prolongation in number vigilance reaction time. Higher levels of low frequency heart rate variability were weakly associated with faster choice reaction time and reduced fluctuation in choice reaction time. Better high frequency baroreflex sensitivity was strongly associated with faster choice reaction time and reduced fluctuation in choice reaction time. These results provide some indication of an association between impaired autonomic function and impairments in alertness, attention and concentration. The cardiovascular reflex test and power spectral analysis results indicate changes in cardiovagal activity, sympathetic function and baroreflex sensitivity. Damage to vagal mechanisms will result in loss of rapid heart rate adjustment during alterations in systemic pressure, and the isometric test indicates that during exercise, systemic blood pressure rises excessively perhaps through loss of baroreflex damping. The negative association between high frequency baroreflex sensitivity and mean choice reaction time and standard deviation is the most important finding. It suggests baroreflex damage could lead to deranged subcortical function. This could be mediated by fluctuation in systemic pressure resulting in ischaemic damage to central white matter. However in Chapter 10, the trend towards a negative association between baroreflex sensitivity and cortical white matter lesion volume was in the correct direction but not statistically significant ($r = -0.159$, $p = 0.249$). In other words we have not demonstrated the hypothesised link between autonomic function and brain substrate of cognitive impairment.

11.4.4 Ambulatory blood pressure and CDR

There was only one significant correlation between ambulatory blood pressure indices and CDR results, namely an association between higher daytime diastolic blood pressure and increased fluctuation in choices reaction time.

11.5 Conclusions

To our knowledge, this is the first study to extensively study autonomic function and cognitive function in older stroke patients. Results do confirm a small number of associations between dysautonomia and cognitive deficit. It provides limited support for the hypothesis of autonomic dysfunction leading to ischaemic-hypoxic cerebral damage and hence post-stroke cognitive decline. These results do not provide evidence of cause, and this will be explored in the following chapters.

12 Correlation between cardiovascular function and white matter disease progression on serial MR imaging

12.1 Introduction

The principal hypothesis, that increased RR interval and blood pressure variability increases white matter ischaemia, is examined in this chapter by performing sequential brain imaging. White matter lesion progression will lead to decline in cognitive function. Older stroke patients are at high risk of dementia, and decrements in cognition because of white matter disease could lead to dementia. This will have serious implications on the health and independence of patients.

Most data describing risk factors for white matter lesions are derived from cross-sectional studies (section 2.5.2). Only a few studies have performed serial brain imaging on older patients to examine rate of change in white matter lesions and risk factors for lesion progression. Some studies were disadvantaged by use of insensitive CT or relatively small numbers of subjects (Veldink, 1998; Wahlund, 1996; Wohl, 1994). MRI scanning is the optimal method for quantifying white matter disease with its greater sensitivity for white matter lesion compared with CT scanning (van Swieten, 1990). Visual rating scales have reasonable inter-observer agreement but semi-automated methods offer the prospect of better accuracy and repeatability (Scheltens, 1998). It also remains to be seen if progression in white matter lesions actually correlates with cognitive change, with some studies failing to establish such a connection (Schmidt, 1999; Wahlund, 1996).

The aim was to examine risk factors for progression of white matter lesion volume. Candidate risk factors considered in correlation analysis were autonomic function indices, ambulatory blood pressure level and variability, clinical risk factors and haemorrheological factors. Brain MRI scanning was repeated after an interval of two years and cortical white matter lesion volume measured by a semi-automated method.

12.2 Method

Autonomic function and blood pressure variability were assessed as described in Chapter 3. These tests were performed once at baseline following study recruitment. Standard cardiovascular autonomic reflex tests were performed: postural blood pressure change and 30:15 ratio during orthostasis, diastolic blood pressure change during three minute isometric exercise and one minute cold pressor tests, heart rate variation and systolic blood pressure change at overshoot of

the Valsalva manoeuvre, and heart rate variation during metronomic respiration over one minute. Baseline MRI scans was performed at approximately the same time as cardiovascular assessment. Study participants were invited to attend for repeat MRI scanning. Some candidates had deceased in the two year interim. Other exclusions included refusal to attend, poor physical health, pacemaker implantation and refusal.

White matter lesion volume was measured by pixel volume and converted to a percentage of the total brain pixel volume. Only cortical white matter lesion volume was included. Cortical white matter volume is the most important of white matter lesion volume and is the key location for lesion that will lead to cognitive deficit. It is accepted that ischaemic white matter lesions do not improve and tend to increase over time in older subjects (Schmidt, 1999; Wahlund, 1996; Yamauchi, 2002). Therefore any individuals with a decrease in white matter lesion volume were excluded on the assumption this was due to technical factors or movement artifact from baseline scan.

12.2.1 Statistical analysis

Risk factors for white matter lesion progression were investigated using regression analysis. Regression of cardiovascular variables on white matter lesion volume was evaluated using the rate of cortical white matter lesion increase as the dependent variable. Second year cortical white matter lesion volume was subtracted from the baseline volume. The difference in volume was divided by time interval to obtain the rate of white matter change ((2^{nd} year WML – baseline WML)/2).

Autonomic and blood pressure indices were correlated with rate of white matter lesion volume increase using Spearman's rank correlation. Only cases in sinus rhythm were selected for the correlation with autonomic indices. As a next step, all potential contributory factors were examined by simple linear regression. Rate of white matter lesion volume was entered as explanatory variable. The following were entered as predictor variables: gender, age, cardiac failure, ischaemic heart disease, hypertension, peripheral vascular disease, COPD/asthma, diabetes, hypercholesterolaemia, smoking history, alcohol consumption, FEV_1, FVC, FEV_1/FVC, prescription of beta-blocker, ACE inhibitor, calcium channel blocker or nitrate, haemorrhologic factors, all cardiovascular reflex tests and power spectral analysis variables and all ambulatory blood pressure variables from day and night phases.

Explanatory variables with a p value less than 0.10 were entered into a multiple linear regression model. Backward elimination was performed to eliminate the least significant explanatory

variable in a stepwise method until a model containing only significant explanatory variables was obtained.

As a supplementary study, basic cortical white matter lesion volume on *second* year MRI was entered as the dependent variable. The same process of simple linear regression to identify important candidate predictors followed by multiple linear regression was performed but using the second year cortical white matter lesion volume as the explanatory variable.

12.3 Results

There were a total of 83 cases with valid white matter lesion volume data from baseline scan and 45 attended for repeat MRI scanning. Of these 45 cases, four were excluded because the automated measurement effectively produced outliers i.e. large increases in white matter lesion volume out of keeping with most cases and probably due to movement artifact. Only one patient was excluded on the basis of spurious decrease in white matter lesion increase from baseline to second year.

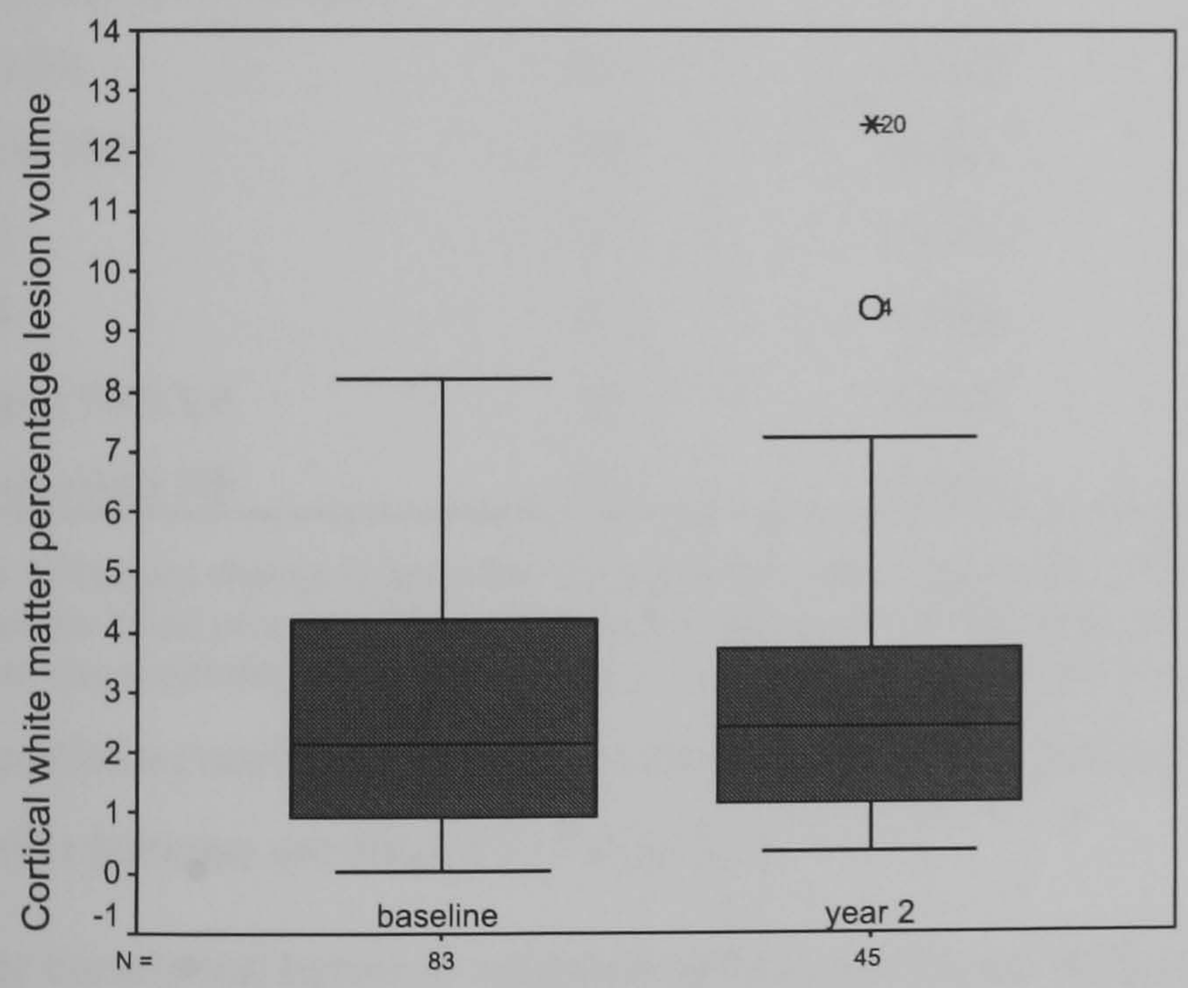

Figure 12-1 Boxplot for cortical white matter lesion volume for all cases (sinus rhythm and AF)

The mean cortical white matter lesion percentage volume was 2.0 ± 1.4 % at baseline and 2.6 ± 1.9 % on second year scan, a significant mean difference of 0.6 % (95% CI 0.3 to 0.8 %, $p < 0.001$). A maximum of 30 patients were available for correlation between autonomic indices and rate of white matter lesion volume increase (mean age 79.1 ± 2.9 years, 17 male), and 27 for the ambulatory blood pressure analysis (sinus rhythm only, mean age 79.1 ± 3.1 years, 15 male).

Correlation coefficients between autonomic indices and rate of white matter lesion increase are shown in Table 12.1.

Table 12-1 Cardiovascular autonomic function and rate of cortical white matter lesion increase

Variable	Number	Correlation coefficient	P
Reflex tests			
30:15 ratio	27	+0.321	0.102
Valsalva ratio	29	+0.160	0.408
E-I difference	29	+0.033	0.863
Active stand ΔSBP	29	-0.029	0.833
ΔDBP Isometric exercise	30	-0.047	0.803
ΔDBP cold pressor	30	+0.079	0.678
ΔSBP Valsalva manoeuvre	29	-0.120	0.535
ΔRR CSM	9	+0.300	0.433
ΔSBP CSM	9	-0.200	0.606
Power spectral analysis			
SD RR	27	+0.025	0.901
Total HRV	27	+0.017	0.933
LF	27	+0.079	0.696
HF	27	-0.076	0.707
Alpha BRS LF	19	-0.082	0.737
Alpha BRS HF	22	-0.027	0.907

E-I difference, change in heart rate during metronomic respiration: ΔSBP, systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: SD RR, standard deviation of 5 minutes RR intervals: HRV, heart rate variability power spectrum: LF, low frequency: HF, high frequency: BRS, baroreflex sensitivity.

Correlation coefficients between ambulatory blood pressure indices and rate of white matter lesion increase are shown in Table 12.2.

The correlation between ambulatory blood pressure indices and rate of white matter lesion increase was repeated using patients in both sinus rhythm and atrial fibrillation cases. These results are shown in Table 12.3.

Table 12-2 Ambulatory blood pressure and rate of cortical white matter lesion volume increase: sinus rhythm only

BP variable	Correlation coefficient	p
Day (n=27)		
SBP mean	-0.263	0.185
DBP mean	-0.201	0.185
MAP mean	-0.271	0.171
SBP variability	+0.147	0.464
DBP variability	+0.046	0.821
MAP variability	+0.000	0.999
Pulse pressure	-0.210	0.294
Night (n=26)		
SBP mean	-0.301	0.136
DBP mean	-0.254	0.210
MAP mean	-0.332	0.098
SBP variability	+0.000	0.999
DBP variability	-0.080	0.699
MAP variability	-0.177	0.388
Pulse pressure	-0.190	0.353
Diurnal variation		
SBP mean	+0.137	0.504
DBP mean	-0.001	0.996

SBP, systolic blood pressure: DBP, diastolic blood pressure: MAP, mean arterial pressure

The strength of correlation between mean ambulatory pressure at night appeared to increase with the addition of cases in atrial fibrillation.

Table 12-3 Ambulatory blood pressure and rate of cortical white matter lesion volume increase: sinus rhythm and atrial fibrillation

BP variable	Correlation coefficient	p
Day (n=34)		
SBP mean	-0.114	0.523
DBP mean	-0.302	0.083
MAP mean	-0.173	0.327
SBP variability	+0.301	0.084
DBP variability	+0.000	0.999
MAP variability	+0.120	0.498
Pulse pressure	+0.018	0.921
Night (n=33)		
SBP mean	-0.255	0.151
DBP mean	**-0.408**	**0.018**
MAP mean	**-0.367**	**0.036**
SBP variability	+0.025	0.891
DBP variability	+0.043	0.813
MAP variability	-0.075	0.677
Pulse pressure	-0.021	0.906
Diurnal variation (n=32)		
SBP mean	+0.117	0.523
DBP mean	+0.045	0.805

SBP, systolic blood pressure: DBP, diastolic blood pressure: MAP, mean arterial pressure

12.3.1.1 Multiple linear regression on rate of cortical white matter lesion volume increase

Only those predictor variables with a significance value less than 0.10 are shown in Table 12.4.

All variables shown in Table 12.4 were considered for inclusion in multiple linear regression on rate of white matter lesion increase. All cases with valid repeat MRI scans were included. Inclusion of all the variables in Table 12.4 in led to the following equation for predicting rate of increase of white matter lesion volume

$$\textit{Rate of cortical white matter lesion increase} = (0.393 \times IHD) + (0.001 \times folate) - 0.320$$

(IHD, ischaemic heart disease)

Table 12-4 Independent predictor variables for rate of white matter lesion increase: all variables with p < 0.10

Explanatory variable	Degrees of freedom	Regression coefficient		P
		B	95% confidence interval	
Cardiac failure	39	+0.417	+0.097, +0.737	0.012
Ischaemic heart disease	39	+0.314	+0.069, +0.558	0.013
Diabetes	39	+0.379	-0.076, +0.834	0.100
Thiazide	39	-0.321	-0.547, -0.096	0.006
ACEi or AIIRA	39	+0.300	+0.006, +0.595	0.046
Nitrate	39	+0.338	-0.021, +0.697	0.064
MAP mean (day)	33	-0.012	-0.025, +0.002	0.100
SBP mean (night)	32	-0.008	-0.016, +0.000	0.050
MAP mean (night)	32	-0.0136	-0.026, -0.001	0.038
DBP mean (night)	32	-0.017	-0.033, 0.000	0.050
Haemoglobin A1c	34	+0.246	+0.062, +0.430	0.010
Glucose	35	+0.132	+0.004, +0.261	0.044
Red cell folate	33	+0.001	+0.000, +0.003	0.021
Vitamin B12	34	+0.001	-0.000, +0.002	0.073

ACEi or AIIRA, ACE inhibitor or angiotensin-II receptor antagonist: MAP, mean arterial pressure: SBP, systolic blood pressure: DBP, diastolic blood pressure.

Table 12-5 Regression coefficients for pulmonary function regression on rate of cortical white matter lesion volume increase and total second year white matter lesion percentage volume

White matter lesion variable	Pulmonary function	Regression coefficient	95% confidence interval	P value
Rate of change (n=42)	FEV_1	-0.022	-0.267, +0.223	0.854
	FVC	-0.026	-0.227, +0.174	0.792
	FEV_1/FVC	+0.558	-1.347, +2.464	0.557
Year 2 (n=42)	FEV_1	-0.681	-1.801, +0.439	0.227
	FVC	-0.698	-1.605, +0.209	0.128
	FEV_1/FVC	+0.186	-8.723, +9.095	0.967

FEV_1, forced expiratory volume in one second: FVC, forced vital capacity.

Therefore cases with a history of ischaemic heart disease had a higher rate of white matter lesion increase than those without ischaemic heart disease in the order of 0.4% per year. The equation

also predicts a 0.01% annual increase in white matter lesion volume for each 10 microgram per litre increase in red cell folate level. The final model predicts 32% of the variance of white matter lesion increase and the ANOVA model has 33 total degrees of freedom and a significance value of 0.003.

The regression analysis was repeated on two occasions for the purpose of assessing impact of different inclusion criteria. The principle was to assess impact of tightening restrictions on predictor variables entered in the multiple regression analysis. Numbers available for analysis were relatively small and there was a proportionately higher number of predictor variables.

The first repeat regression analysis was in two steps. The first step was to only include cases where the scatter plots were consistent with a potential association with white matter lesion increase. For example, cardiac failure was excluded since there were only a small number of positive cases with relatively high levels of white matter lesion increase, with the impression of a skewed effect. ACE inhibitor and nitrate use, and mean night-time systolic blood pressure level were excluded for similar reasons. Additional selection criteria of cases meeting the 24 hour ambulatory inclusion criteria were applied i.e. number of day and night blood pressure readings more than 9 and 4 respectively. The ensuing low numbers of cases with haemoglobin A1c, glucose, red cell folate and vitamin B12 data led to the second step of excluding these variables, since numbers were deemed inadequate for inclusion in the regression equation. The remaining variables were then regressed on white matter lesion increase: ischaemic heart disease, thiazide prescription, daytime mean MAP, night-time mean MAP and diastolic blood pressure, with the result.

$$\textit{Rate of white matter lesion increase} = (+0.285 \times \textit{IHD}) - (0.294 \times \textit{thiazide}) + 0.337$$

This equation predicts 29% of white matter lesion increase variance, with 31 degrees of freedom and significance level of 0.003 in the ANOVA model.

The second repeat in regression analysis was to only include predictor variables where results were available for all cases selected. Thus cardiac failure, ischaemic heart disease, diabetes, thiazide, ACE inhibitor and nitrate use were included in the regression equation. This produced the following equation (see Table 12.6):

$$\textit{Rate of white matter lesion increase} = (0.321 \times \textit{CCF}) + (0.307 \times \textit{IHD}) - (0.256 \times \textit{thiazide}) + 0.264$$

CCF, congestive cardiac failure: IHD, ischaemic heart disease

This model predicts 36% of white matter lesion increase variance, and the ANOVA model has 39 degrees of freedom with a significance level of < 0.001. It indicates that presence of cardiac failure or ischaemic heart disease is associated with 0.32 and 0.31 percent higher annual increase in white matter lesion burden compared with individuals without these risk factors. In contrast prescription of thiazide is associated with a reduction in white matter lesion progression, around 0.26 percent annual reduction compared with those not taking thiazide drugs.

Table 12-6 Regression co-efficients for predictor variables of cortical white matter lesion volume progression

Predictor variable	Regression coefficient	95 % CI for coefficient	Significance
Congestive cardiac failure	+0.312	+0.036, +0.606	0.029
Ischaemic heart disease	+0.307	+0.097, +0.517	0.005
Thiazide	-0.256	-0.460, -0.052	0.015

12.3.1.2 Total volume of cortical white matter lesions on year 2 MRI scan

Regression coefficients for those predictor variables with significance value < 0.10 for regression on total white matter lesion percentage are shown in Table 12.7.

The variables shown in Table 12.7 were considered for inclusion in multiple linear regression on second year white matter lesion volume. Scatter plots indicated the association between white matter lesion volume and C-reactive protein and glucose was not robust due to outliers. The association with loop diuretic appeared less robust due to small number in the positive group. Two other variables - HDL cholesterol and homocysteine - were excluded on the basis of low numbers of cases with results. Therefore the following variables were entered into multiple linear regression on second year white matter lesion volume: cardiac failure, thiazide, ACE inhibitor, nitrate, haemoglobin A1c and folate. This produced the following model:

Second year white matter lesion percentage volume = (1.099 x haemoglobin A1c) + (0.008 x folate) – 6.243

This model predicted 48% of white matter lesion variance, and the ANOVA model had a total of 29 degrees of freedom and a significance value of < 0.001. The model predicts that every one percent increase in haemoglobin A1c was associated with approximately 1.1 % increase in white matter lesion.

Table 12-7 Significant predictor variables on regression on white matter lesion volume at year 2 (p < 0.10)

Explanatory variable	Degrees of freedom	Regression coefficient		P
		B	95% confidence interval	
Cardiac failure	39	+2.484	+0.918, +4.050	0.003
Diabetes	39	+2.316	+0.046, -4.586	0.046
Thiazide	39	-1.034	-2.250, +0.183	0.093
ACEi/AIIRA	39	+1.417	-0.089, +2.924	0.064
Nitrate	39	+1.976	+0.184, +3.769	0.032
Loop diuretic	39	+2.198	-0.085, +4.481	0.059
C-reactive protein	38	+0.023	-0.001, +0.047	0.061
Glucose	35	+0.654	-0.018, +1.326	0.056
Haemoglobin A1c	34	+1.264	+0.232, +2.297	0.018
HDL cholesterol	28	-1.719	-3.287, -0.152	0.033
Red cell folate	33	+0.010	+0.001, +0.015	0.001
Homocysteine	21	-0.136	-0.295, +0.022	0.088

ACEi/AIIRA, ACE inhibitor or angiotensin-II receptor antagonist: HDL, high density lipoprotein

A second regression analysis was performed to examine the effect of loosening inclusion criteria for entry in the regression model. This second regression analysis included all variables in Table 12.7 in regression on second year white matter lesion volume. This produced the following model

Second year white matter lesion percentage volume = (1.851 x haemoglobin A1c) – (1.875 x HDL cholesterol) – 5.862

This model predicted 44% of white matter lesion volume variance, and the ANOVA model had a total 26 degrees of freedom and a significance value of < 0.001. The model predicts that every one percent increase in haemoglobin A1c is associated with an increase in 1.8% increase in white matter lesion volume but each 1 mmol/l increase in HDL cholesterol is associated with a 1.9% decrease in second year white matter lesion volume.

12.4 Discussion

Multiple linear regression of individual independent variables on rate of white matter lesion increase identified ischaemic heart disease and cardiac failure as significant risk factors for progression of white matter disease, with thiazide prescription being associated with a protective action against white matter lesion volume progression. When regressing multiple independent

variables on MRI white matter lesion on the scan performed two years after cardiovascular assessment, haemoglobin A1c had a positive and HDL cholesterol an inverse relationship with total cortical white matter lesion volume.

12.4.1 Rate of white matter lesion increase from baseline to year 2: correlation

Taking the correlation results first, there is an interesting association between higher nocturnal diastolic blood pressure and reduced rate of white matter lesion progression. This correlation was only significant when including both sinus rhythm and atrial fibrillation in the correlation. Thus strength of correlation between mean ambulatory pressure at night appeared to increase with the addition of cases in atrial fibrillation but regression analysis indicated this association was independent of atrial fibrillation.

Therefore baseline nighttime blood pressure was inversely associated with rate of white matter lesion volume increase. Higher nocturnal blood pressure at baseline led to slower progression in white matter lesion damage. It suggests that older stroke patients with higher diastolic blood pressure in the first year after stroke suffer less white matter ischaemia in the subsequent two years. Conversely those with lower systemic perfusion pressure overnight will suffer higher rates of central neuronal damage.

This needs to be put in context of section 11.4.2 results where nocturnal blood pressure dip was inversely associated with total CAMCOG score. One could hypothesise that a combination of large nocturnal diastolic blood pressure dip in the first year after stroke and low overnight mean diastolic blood pressure could have an synergistic and detrimental effect. Large nocturnal pressure dips will be associated with poorer cognitive function at the outset and if the same individuals are subjected to low mean diastolic pressure overnight in the years following stroke, they will suffer the highest rates of white matter lesion progression, further worsening cognitive performance.

These findings make for interesting comparison with the results from the Gothenberg and Kungsholmen cohorts. Guo et al (Guo, 1996) report blood pressure was lower in patients with vascular dementia in the Kungsholmen community cross-sectional study. Both low and high levels of baseline systolic blood pressures were associated with follow-up MMSE score (Guo, 1997). Further longitudinal studies revealed that low diastolic and high systolic pressure were significantly associated with incidence of all-cause dementia (Qiu, 2003). For low diastolic blood pressure (≤65 mmHg vs. 66-90 mmHg), the adjusted relative risk of Alzheimer disease was 1.7 (95% CI 1.1 to 2.4) and 1.5 (95% CI 1.0 to 2.1) for all-cause dementia. Results from our study

provide further evidence that low baseline diastolic blood pressure could lead to white matter hypoperfusion and potentiate cognitive decline in post-stroke patients.

Although diastolic blood pressure significantly correlated with rate of white matter lesion increase, it did not remain a significant predictor variable in the regression models. Multiple regression is discussed below.

None of the autonomic indices predicted rate of white matter lesion increase. Lack of association with white matter lesion volume at baseline was reported in Chapter 9. Therefore it is not surprising that autonomic function did not significantly predict deterioration in ischaemic white matter damage. Reasons for the lack of association as discussed in Chapter 9 may also apply for this longitudinal study. Despite the inclusion of 76 sinus rhythm cases in the baseline study, reduced number available for study at 2 years will have impaired power to detect significant associations. The largest number for correlation with rate of white matter lesion increase was 30 for blood pressure increase during isometric exercise and the smallest number just 9 cases for carotid sinus massage results.

Cerebral autoregulation may be a more important factor in maintaining white matter blood flow and avoiding ischaemic lesions. Repeatability of the reflex autonomic tests is suboptimal, thus results from our single assessment may have lacked accuracy. The reflex tests describe short term RR and blood pressure variability in response to cardiovascular stress over a period of seconds to minutes. The ischaemic conditions required to cause white matter lesions may be a more severe and prolonged alteration in cerebral perfusion over several minutes or even hours. Other mechanisms may be more important determinants of white matter lesion progression.

12.4.2 Rate of white matter lesion increase from baseline to year 2: regression models

Multiple linear regression of risk factors on *rate* of white matter lesion progression identified ischaemic heart disease and cardiac failure as predictors of white matter lesion volume. This is the first study to report these conditions as risk factors for white matter lesion progression. Findings provide further evidence for the ischaemic nature of white matter lesions in older adults (Ferro and Madureira, 2002; Jeerakathil, 2004; Roman, 2002). Ischaemic heart disease could act as a mediator by two mechanisms. The presence of classic vascular risk factors – hypertension, diabetes, smoking, hypercholesterolaemia – may be best summarised by presence of ischaemic heart disease, and indicate similar biological mechanisms driving both coronary artery disease and small vessel cerebrovascular disease. Alternatively those with ischaemic heart disease may be

individuals who suffer dysrhythmias, hypotensive episodes etc that are central to the 'hypoxic-ischaemic' hypothesis of white matter disease.

Presence of cardiac failure in the regression model was dependent on data analysis technique. This highlights a weakness of the study: results were susceptible to modelling technique which raises questions on robustness. Inclusion of the different models allows one to judge their strengths and weaknesses. Larger numbers and obtaining data for all variables in all individuals would allow confident selection of regression technique. These difficulties stem from the nature of the cohort; an older group of volunteers who were not able to tolerate all investigations and suffer high mortality rates, thus limiting the scope for successful completion of longitudinal studies.

The protective value against white matter lesion volume progression obtained from thiazide use seems very pertinent in the light of the PROGRESS group results. Not only did thiazide prescription (as part of a blood pressure lowering regimens) reduce risk of stroke and cerebrovascular events, there was also evidence of decreased risk of cognitive decline in stroke patients (Lithell, 2003; Progress Collaborative Group, 2001). Our findings are in keeping with the PROGRESS group. Thiazide use benefited patients by association with reduced progression of white matter lesion volume. Commentators on the PROGRESS study attribute thiazide benefits to blood pressure lowering and perhaps additional metabolic actions of thiazides especially with indapamide.

12.4.3 Previous studies

There are a few studies examining risk factors for white matter lesion progression. A very small study of 16 healthy elderly subjects assessed at an interval of three years found no difference in white matter lesions on MRI and no overt cognitive change (Wohl, 1994). Wahlund et al (Wahlund, 1996) performed MRI scans on three occasions over five years on 13 healthy adults aged over 70 years. Different MRI scanners were used over the three investigations, and the higher field strength on repeat MRI scanning may have increased lesion detection by virtue of greater sensitivity. The study was further weakened by small number of participants. Cerebral white matter hyperintensities significantly increased but periventricular hyperintensities did not significantly change. Neuropsychometric performance did not deteriorate over the five year assessment period.

The largest serial imaging study was part of the Austrian Stroke Prevention Study (Schmidt, 1999). Two hundred and seventy three community living adult volunteers, mean age 60 ± 6 years

and free of cerebrovascular disease, were examined with serial 1.5 Tesla MR imaging three years apart. Using the visual rating scale of Fazekas, 65% of subjects had white matter hyperintensities, and 18 % percent suffered progression in white matter hyperintensty grade over three years. Individuals with lesion progression were older, had higher diastolic blood pressure, higher fibrinogen levels and had higher grades of white matter hyperintensity on baseline imaging. Following logistic regression, diastolic blood pressure and moderately severe white matter hyperintensity at baseline remained the only significant and independent predictors of lesion progression. This contrasts with our study where diastolic blood pressure did not predict progression. It is known that diastolic blood pressure increases in middle age, plateaus and can then fall after the seventh decade (Franklin, 1997a); hence diastolic blood pressure may have lost its predictive strength in our older cohort. Weaknesses of the Schmidt study (Schmidt, 1999) included poor interrater reliability for rating minor white matter disease and use of the 4-grade Fazekas scale. This white matter lesion rating scale probably lacked sensitivity for accurate quantification of white matter lesion progression. It is interesting to note this study did not find any association between lesion progression and cognitive decline. Short follow-up period, relatively young cohort and neuropsychometric test repeatability were probably factors contributing to these negative findings.

Similar to Schmidt et al (Schmidt, 1999), Veldink's study (Veldink, 1998) in a community study in Amsterdam also reported higher diastolic blood pressure was a significant predictor using multiple linear regression on progression of white matter hyperintensity rating. But again this study only assessed a small number of subjects (14) and used a visual rating scale (Scheltens, 1993). In contrast our study benefited from larger number of participants and more accurate and sensitive semi-automated white matter lesion volume measurement. Mean age in Veldink et al's study (Veldink, 1998) at 76 ± 5 years was more comparable to our study.

Yamauchi et al (Yamauchi, 2002) performed serial MRI on 89 Japanese patients mean age 66 ± 9 years at an interval of 51 ± 19 months. The 79 patients without severe WML at baseline were analysed by multiple linear regression to determine independent risk factors for increase in white matter lesion score. The following significant predictor variables were identified: moderate hypertension ($p = 0.0016$), uncontrolled diabetes ($p = 0.0038$) and current smoking ($p = 0.04$).

It is notable that age did not predict white matter lesion progression in our study. This is in keeping with results from the studies of Schmidt and Veldink (Schmidt, 1999; Veldink, 1998). Although age is closely associated with accumulation of white matter lesions, it is not necessarily a risk factor for progression.

Baseline folate level was a significant and positive predictor of white matter lesion progression in the first regression model. However high folate levels have no hypothetical relationship to white matter lesions and this seems very likely to be a chance finding. Furthermore the regression slope for the relationship between folate and white matter lesion increase was shallow.

To the best of our knowledge, this also appears to be one of a few studies reporting white matter lesion progression using semi-automated measurement of white matter lesions quantified as a continuous variable. The studies of Schmidt, Wahlund, Veldink and Yamauchi (Schmidt, 1999; Veldink, 1998; Wahlund, 1996; Yamauchi, 2002) all used visual rating scales, which are reasonably sensitive but lack the accuracy and absence of bias obtained with automated measurement. Taylor et al used a semi-automated white matter lesion volume measurement to obtain actual volumes. Following multiple logistic regression, age (p =0.0117) and diabetes (0.0215) were significant predictors of increase in lesion volume in Taylor's study (Taylor, 2003).

12.4.4 Total volume of cortical white matter lesions on year 2 MRI scan

In contrast to the regression model for rate of white matter progression, only haemoglobin A1c had significant predictive value for the cortical white matter lesion volumes at two years after study recruitment. Haemoglobin A1c reflects long-term glycaemic control which is a strong risk factor for arteriosclerotic disease. Passare et al (Passare, 2004) found that loop diuretic use was significantly and independently associated with elevated haemoglobin A1c in older community living subjects. They suggest diuretics may lead to hyperglycaemia. If this was correct, it could be hypothesised that since cardiac failure was as an independent predictor of white matter lesion progression, these patients were all treated with loop diuretics and may have led to higher haemoglobin A1c levels. An inverse association with HDL cholesterol level is again consistent with the ischaemic basis of white matter lesions in older age – elevated HDL indicates favourable cholesterol metabolism with lower risk of coronary artery disease. Glycaemic control and cholesterol metabolism offer plausible treatment targets for white matter lesion progression.

One suspects total white matter lesion volume at second year MRI is an inferior outcome measure to the 'repeat-baseline MRI difference' method. The latter measures the real change in white matter lesion volume over two years and means that each patient acts as their own control. Simply using the total white matter lesion volume at two years does not provide a dynamic measure of white matter variation, and is more akin to a cross-sectional study. But it does allow an estimation of future white matter lesion burden from baseline risk factors. This analysis did

identify some interesting risk factors which are even more consistent with the ischaemic hypothesis of white matter lesions than predictor variables for rate of white matter lesion change.

12.4.5 Blood pressure variability in atrial fibrillation

There are few studies examining the impact of atrial fibrillation on ambulatory blood pressure. Some question the validity of using ambulatory blood pressure monitoring in atrial fibrillation because of technical factors that make measurement problematic and introduce larger margins of error compared with sinus rhythm (Stewart, 1995). But office sphygmomanometry-based blood pressure readings can also be inconsistent in atrial fibrillation due to the erratic nature of Korotkov sounds in atrial fibrillation (Sykes, 1990). Ambulatory monitoring should provide an advantage by producing an average of multiple measurements. A study by Lip et al (Lip, 1996) proved the feasibility of ambulatory recording in atrial fibrillation. There were a high percentage of successful recordings at 80%, a figure not too different to sinus rhythm. Furthermore the first measurement on the ambulatory recorders matched the mean of two manual blood pressure measurements for systolic (but not diastolic) pressure. Another report suggests that validated monitors have a satisfactory frequency response to provide an adequate measurement (O'Brien, 1990).

There is evidence that accurate information can be obtained by ambulatory blood pressure monitoring in atrial fibrillation. From 42 patients undergoing cardioversion for AF, 22 completed before and after 24 hour ambulatory blood pressure recordings (Olsen, 2002). In the group of 22, 12 reverted to sinus rhythm whilst 10 remained in atrial fibrillation. There were no significant differences in mean systolic and diastolic levels for both sinus rhythm and atrial fibrillation groups before and after cardioversion. Blood pressure variability and repeatability attained similar levels in both groups (SD/mean).

12.4.6 Study limitations

Interval between imaging was chosen to balance the need to allow time for significant progression of lesions against the risk of high rates of participant attrition. One study found no change over one year but significant white matter lesion progression after two years on MRI scanning (Veldink, 1998). Our study did suffer from a high attrition rate of participants over two years, due to further clinical events rendering subjects unfit for imaging, refusal for repeat scanning and deaths. Larger interval would certainly provided greater change in white matter lesion burden and improve ability to define risk factors but at the cost of higher attrition rates. The substantial loss of individuals at second scan may have hindered the study's power. Severe

white matter lesion at baseline is reported as an independent predictor of stroke risk, and individuals with mild white matter lesions that progress also have high stroke rates (Yamauchi, 2002). Therefore selection bias may have affected our study, since those not attending for repeat scan are more likely to suffer the highest rates of cerebrovascular disease (Ferro and Madureira, 2002). Performance bias is less likely to have influenced outcome since most patients secondary prevention treatment was optimized following presentation with stroke at secondary care services.

Five individuals with repeat MRI data were excluded from data analysis. Four recorded large increases in white matter lesion volume that suggested movement artifact leading to falsely elevated increases. This assumption is difficult to disprove and may have led to loss of individuals with true and severe white matter lesion progression. This could weaken ability to detect predictor variables.

Identifying individuals with progression in white matter lesion volume could benefit targeting treatment at those with high rates of lesion progression. Such patients are theoretically at greatest risk of cognitive decline. A similar approach has been suggested for hippocampal and whole brain atrophy in Alzheimer's disease (Fox and Schott, 2004; Scheltens, 2002). Reduction in rate of progression could provide evidence that a treatment is effective in preventing ischaemic white matter damage and provide a useful diagnostic tool. However it remains to be seen whether progression in white matter lesions correlates with deterioration in cognitive function. Clinical relevance extends beyond cognitive function. A recent report has found an association between white matter lesion progression and both gait disorder and bladder dysfunction in older adults (Whitman, 2001).

12.5 Conclusions

Autonomic and ambulatory blood pressure indices do not predict rate of white matter lesion progression. Ischaemic heart disease and cardiac failure are associated with higher rates of white matter lesion progression, whereas thiazide diuretic prescription is associated with reduced rate of white matter lesion progression. Mechanisms for these associations deserve further study. This study does not provide supportive evidence that autonomic dysfunction increases damage to subcortical circuits in the white matter.

13 Correlation between cardiovascular autonomic tests and blood pressure variability with longitudinal CAMCOG and CDR

13.1 Introduction

Loss of cardiovascular autonomic function can produce large fluctuations in systemic perfusion pressure. White matter damage may follow where cerebral autoregulation is unable to maintain cerebral perfusion pressure. Therefore hypoxic and ischaemic injury can lead to decline in cognitive function. In this chapter, the relationship between baseline cardiovascular autonomic function and cognitive function after one and two years is explored.

13.2 Method

Cardiovascular investigations were performed at baseline as described in Chapter 4. Autonomic reflex tests, heart rate variability and ambulatory blood pressure were performed. The following variables were obtained for analysis. From reflex tests, 30:15 ratio and change in systolic blood pressure on standing, change in diastolic blood pressure during isometric exercise and cold cutaneous stress, Valsalva ratio and change in systolic blood pressure from baseline to overshoot during Valsalva manoeuvre and change in heart rate during metronomic respiration for one minute. From heart rate variability, total, low and high frequency spectral powers and baroreflex sensitivity for low and high frequencies. From 24 hour ambulatory blood pressure recording, mean systolic and diastolic levels, variability of systolic and diastolic blood pressure (standard deviation of the mean), pulse pressure and difference between day and night levels of systolic and diastolic blood pressure. Only cases with more than 15 readings over 24 hours were included. Cases in non-sinus rhythm were excluded from analysis.

Serial cognitive function was performed at baseline, then one and two years later. The full neuropsychometric schedule is described in section 4.4. CAMCOG variables were total score and attention and executive subscores. CDR battery variables were choice reaction time, memory scanning and number vigilance. Total CAMCOG is an indicator of global cognitive function. The other cognitive variables reflect executive and attentional performance which are impaired following white matter damage.

13.2.1 Statistical analysis

The difference in CAMCOG score and CDR mean times were compared within subjects using the Friedman Test for the change between three points, baseline to year 1 to year 2. Differences

between each separate interval were compared within subjects using Wilcoxon Signed Ranks Test: baseline to year 1, year 1 to year 2 and baseline to year 2.

Association was explored using linear regression. To obtain a sense of how much baseline cognitive function predicts performance at years one and two, linear regression was used to describe the variance of follow-up cognitive score determined by baseline function. Thus for each cognitive variable, the score at year 1 and year 2 were treated as explanatory variables and the score at baseline was entered as the predictor variable. The variance of year 1 or 2 according to baseline values and the significance of the ANOVA model are reported.

Cardiovascular predictor variables were then added to the regression model containing baseline neuropsychometric as the other independent variable and neuropsychometric variable at year 1 or 2 as the dependent variable. The candidate independent variables were obtained from cardiovascular reflex tests, spectral analysis of heart rate variability and 24 hour ambulatory blood pressure variability. Each one of the six terms from the neuropsychometric tests was entered as explanatory variable. The regression coefficient and its significance value are reported in Tables 13.3 to 13.8.

13.3 Results

The mean interval between neuropsychometric testing from baseline to year one was 397 ± 61 days, and from year one to year two was 356 ± 57 days.

13.3.1 CAMCOG

There was no significant change in total CAMCOG score and attention subscore from year to year. There was a significant improvement in CAMCOG executive subscore in the period of cognitive follow-up (Friedman Test < 0.001, Table 13.1). Assessing the year-on-year change, executive subscore change improved from baseline to year 1 and from baseline to year 2 ($p < 0.001$) but not from year 1 to year 2 ($p = 0.080$, Table 13.2).

Table 13-1 CAMCOG total score and attention and executive subscores at baseline and year 1-2

	Total (0-107)	Attention (0-7)	Executive (0-28)
Baseline (n=76)	87 (81-92)	6 (5-7)	13 (10-17)
Year 1 (n=62)	89 (84-93)	6 (5-7)	16 (14-19)
Year 2 (n=52)	90 (84-94)	6 (5-7)	16 (14-20)
Chi-square (n=49)	1.319	0.124	24.29
P	0.517	0.940	<0.001

Scores are median (interquartile range): Friedman Test, degrees of freedom = 2.

Table 13-2 CAMCOG total score and attention and executive subscores year-on-year change

CAMCOG	Year on year change	N	Z score	Significance
Total	Baseline to year 1	62	-1.006	0.314
	Year 1 to 2	49	-1.550	0.121
	Baseline to year 2	52	-1.351	0.177
Attention subscore	Baseline to year 1	62	-1.423	0.155
	Year 1 to 2	49	-0.854	0.393
	Baseline to year 2	52	-0.544	0.587
Executive subscore	Baseline to year 1	62	-4.135	<0.001
	Year 1 to 2	49	-1.752	0.080
	Baseline to year 2	52	-4.907	<0.001

Wilcoxon Signed Ranks Test

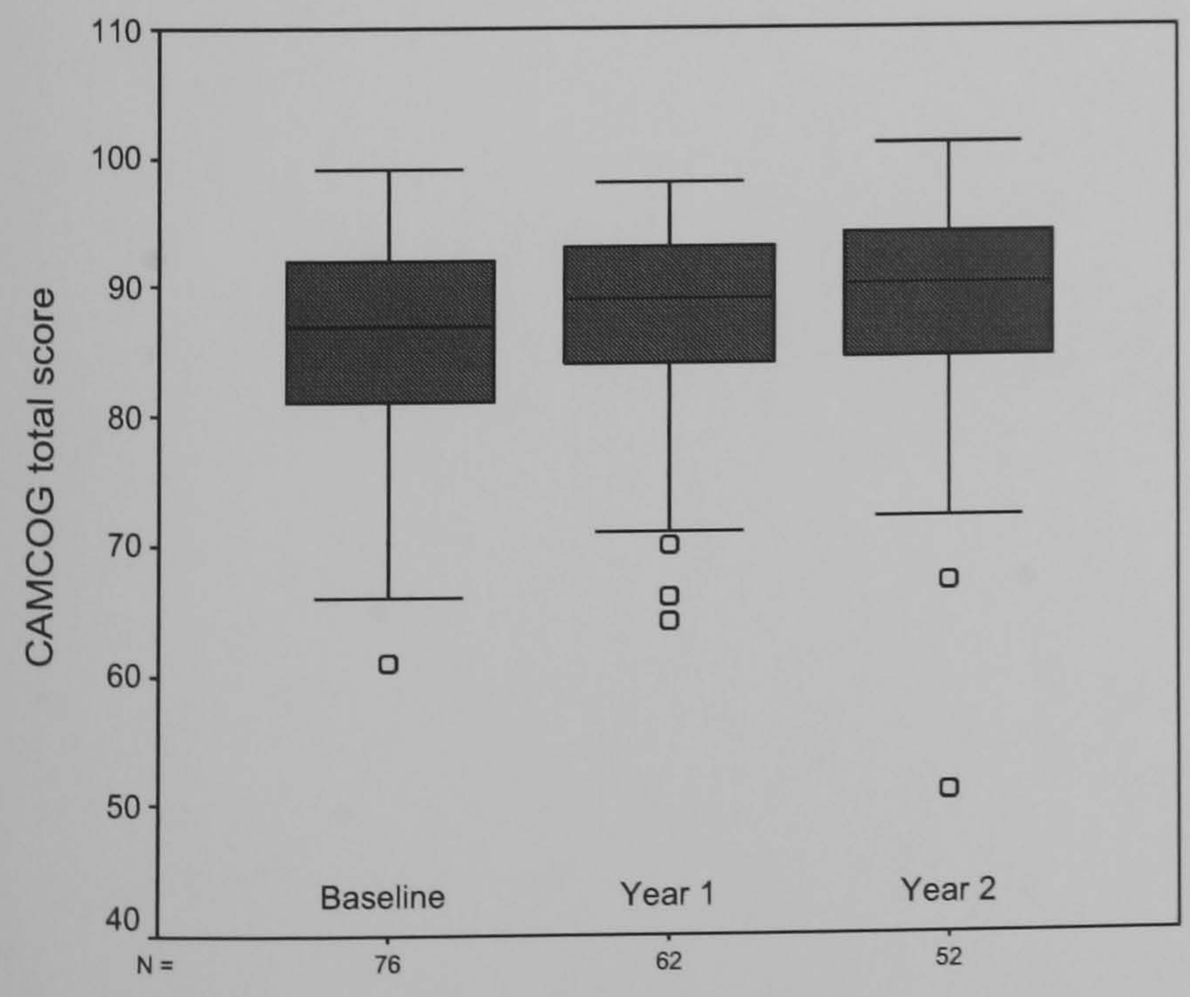

Figure 13-1 Total CAMCOG score for patients in sinus rhythm

13.3.1.1 Total CAMCOG

Scatterplots indicate a trend towards improvement in total CAMCOG score over one and two years but this was not significant (Figure 13.1 and Table 13.2). Baseline CAMCOG score explained 49 % and 43 % of the variance of year 1 and year 2 CAMCOG scores respectively. Valsalva ratio significantly predicted total CAMCOG score after two years (B = +11.4, 95 % CI for B +1.5 to 21.2, p = 0.025) but there were no other significant predictors of total CAMCOG from the cardiovascular assessment (Table 13.3).

CAMCOG year 1 = (CAMCOG baseline x 0.720) + 24.9

Adjusted r^2 =0.491 p<0.001

CAMCOG year 2 = (CAMCOG baseline x 0.698) + 27.6

Adjusted r^2 =0.431 p<0.001

Table 13-3 Correlation between autonomic tests and total CAMCOG score after 1 and 2 years

Autonomic variable	CAMCOG total year 1			CAMCOG total year 2		
	n	Regression coefficient	p	n	Regression coefficient	p
Reflex tests						
30:15 ratio	59	-3.308	0.516	47	-3.123	0.688
Valsalva ratio	56	+0.306	0.941	47	+11.352	0.025
E-I ratio	60	+0.141	0.369	47	-0.169	0.431
ΔSBP orthostasis	60	+0.036	0.130	48	+0.023	0.472
ΔDBP isometric exercise	61	+0.015	0.765	50	+0.029	0.659
ΔDBP cold pressor	59	+0.006	0.912	51	-0.023	0.737
ΔSBP Valsalva	55	+0.033	0.473	45	+0.076	0.238
RR post-CSM	24	-0.009	0.141	21	-0.007	0.206
ΔSBP post-CSM	24	+0.125	0.334	21	-0.004	0.969
Power spectral analysis						
Total HRV	58	+0.000	0.797	35	+0.001	0.577
Low frequency HRV	58	+0.017	0.504	35	+0.003	0.488
High frequency HRV	58	+0.001	0.663	35	+0.002	0.577
Low frequency BRS	36	+0.059	0.889	24	-0.413	0.362
High frequency BRS	50	-0.101	0.523	29	-0.198	0.370
24 hour blood pressure						
Systolic mean BP	59	+0.032	0.500	49	+0.014	0.836
Diastolic mean BP	59	+0.005	0.956	49	+0.191	0.181
Systolic variability	59	+0.128	0.457	49	+0.002	0.995
Diastolic variability	59	-0.118	0.649	49	+0.199	0.641
Pulse pressure	59	+0.046	0.426	49	-0.043	0.601
Systolic diurnal change	57	-0.029	0.778	49	-0.120	0.463
Diastolic diurnal change	57	+0.021	0.809	49	+0.028	0.841

ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: HRV, heart rate variability: BRS, baroreflex sensitivity: BP, blood pressure

13.3.1.2 CAMCOG attention subscore

There was a clear ceiling effect on CAMCOG attention subscore. There was no significant change in attention subscore at one and two years follow-up (Table 13.2 and Figure 13.2). Regression analysis indicates baseline attention subscore explains 12 % and 44 % of the variance of attention subscore at one and two years respectively. There was a trend towards higher mean

24 hour systolic blood pressure reducing attention score at one year (p = 0.079) but no significant predictors from the cardiovascular assessment (Table 13.4).

CAMCOG attention1 = (CAMCOG baseline x 0.445) + 3.1

Adjusted $R^2 = 0.124$, $p = 0.003$

CAMCOG attention 2 = (CAMCOG baseline x 0.683) + 1.8

Adjusted $R^2 = 0.441$, $p < 0.001$

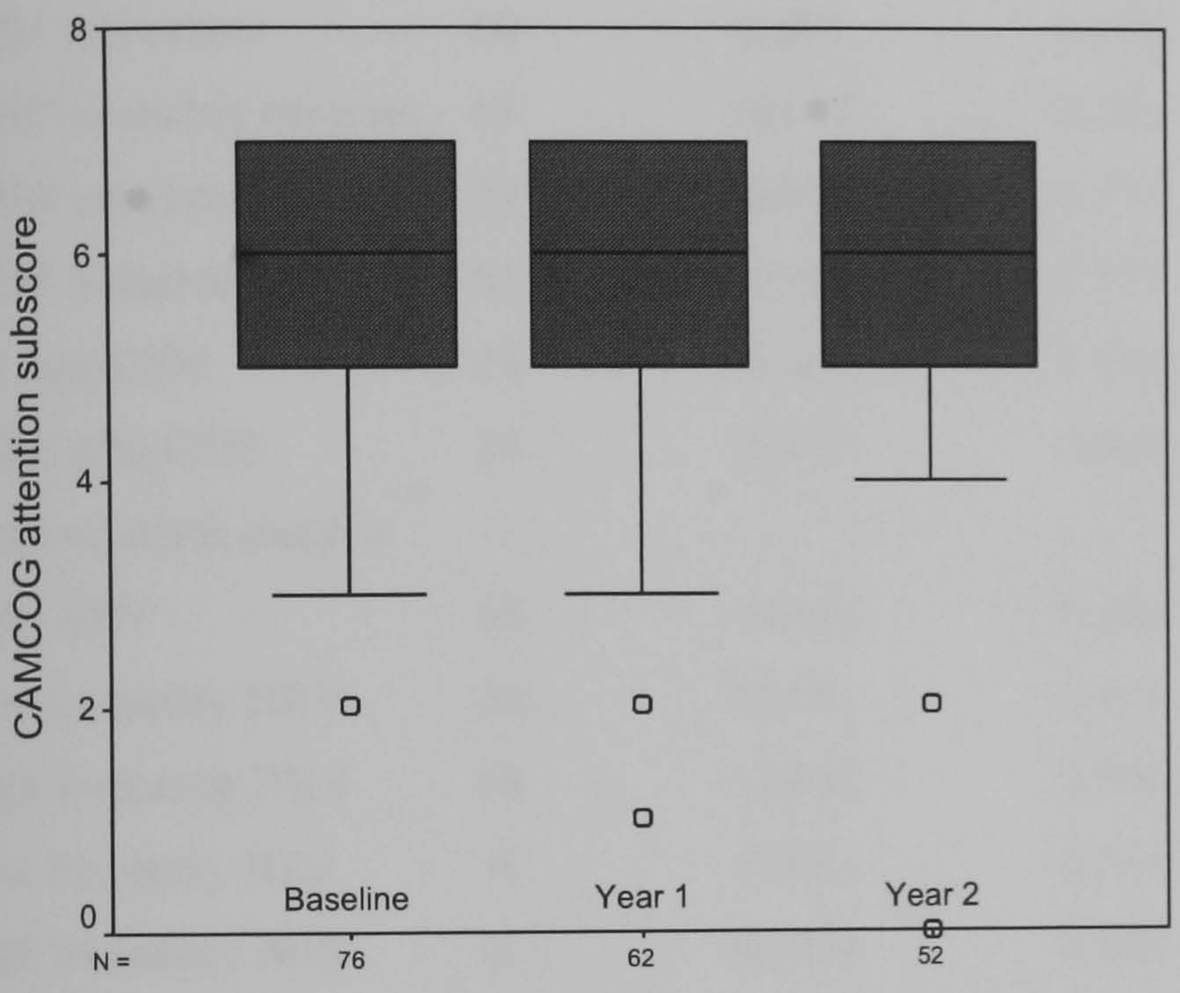

Figure 13-2 Attention subscore for patients in sinus rhythm

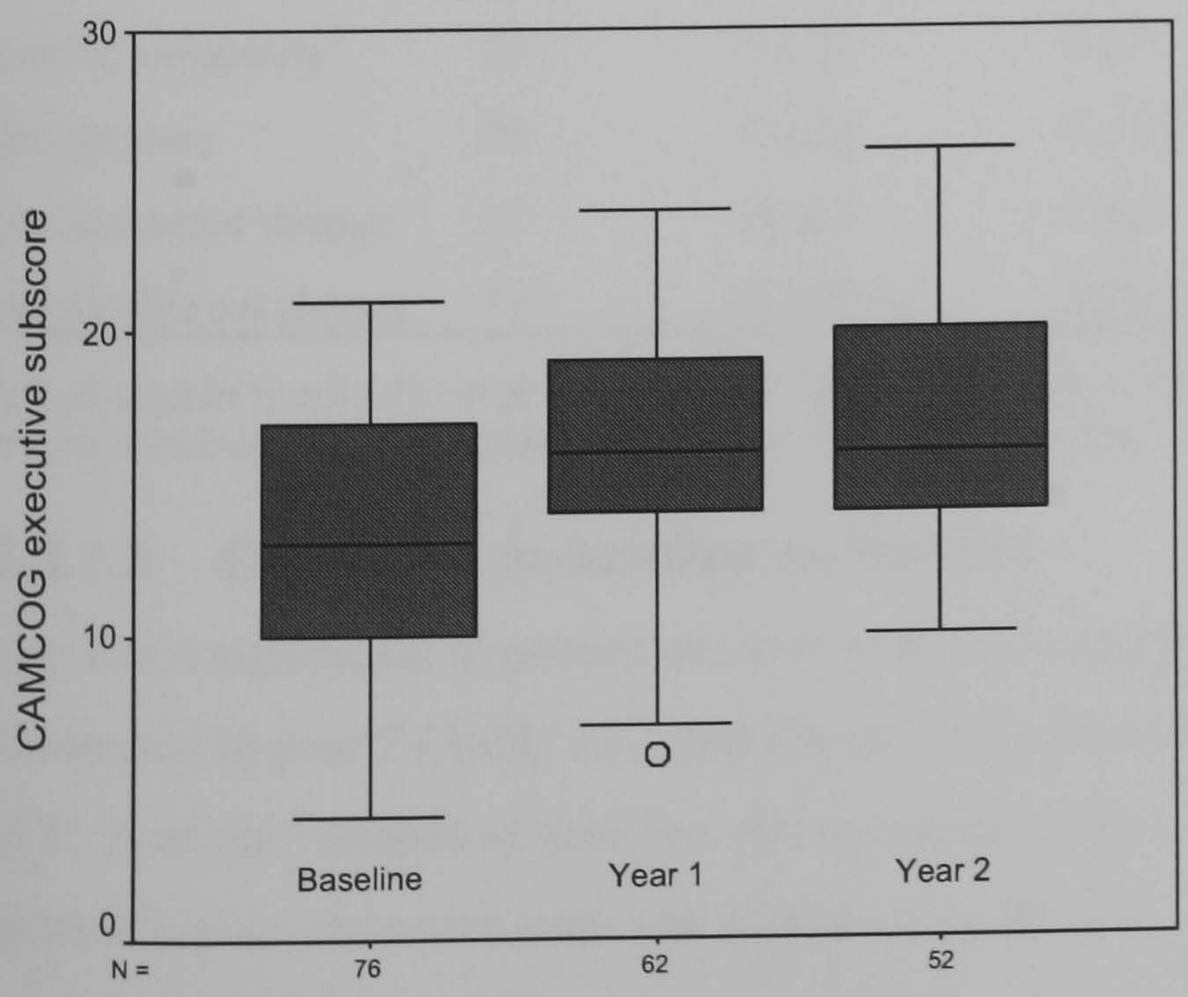

Figure 13-3 Executive subscore for patients in sinus rhythm

Table 13-4 Correlation of autonomic function and ambulatory blood pressure with CAMCOG attention subscore

Autonomic variable	CAMCOG attention year 1			CAMCOG attention year 2		
	n	Regression coefficient	p	n	Regression coefficient	p
Reflex tests						
30:15 ratio	59	+1.184	0.359	47	-0.617	0.540
Valsalva ratio	56	-0.968	0.358	47	-0.096	0.890
E-I ratio	60	+0.023	0.581	47	-0.015	0.612
ΔSBP orthostasis	60	-0.001	0.870	48	+0.002	0.616
ΔDBP isometric exercise	61	+0.012	0.375	50	+0.006	0.565
ΔDBP cold pressor	59	-0.008	0.593	51	-0.010	0.360
ΔSBP Valsalva	55	+0.007	0.577	45	-0.004	0.135
RR post-CSM	24	-0.005	0.136	21	-0.002	0.519
ΔSBP post-CSM	24	+0.065	0.404	21	+0.059	0.338
Power spectral analysis						
Total HRV	58	+0.000	0.184	35	+0.000	0.067
Low frequency HRV	58	+0.001	0.170	35	+0.001	0.047
High frequency HRV	58	+0.001	0.074	35	+0.001	0.025
Low frequency BRS	36	+0.021	0.534	24	+0.067	0.282
High frequency BRS	50	+0.014	0.876	29	+0.001	0.945
24 hr blood pressure						
Systolic mean BP	59	-0.023	0.079	49	-0.026	0.805
Diastolic mean BP	59	-0.036	0.125	49	+0.018	0.389
Systolic variability	59	-0.030	0.518	49	+0.011	0.774
Diastolic variability	59	-0.124	0.071	49	-0.033	0.598
Pulse pressure	59	-0.018	0.259	49	-0.011	0.411
Systolic diurnal change	57	-0.018	0.516	49	+0.001	0.981
Diastolic diurnal change	57	-0.000	0.988	49	+0.008	0.695

ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: HRV, heart rate variability: BRS, baroreflex sensitivity: BP, blood pressure

13.3.1.3 CAMCOG executive subscore

There was a significant improvement in CAMCOG executive subscore from baseline to year 1 and baseline to year 2 (Table 13.2 and Figure 13.3). Baseline executive subscore explained 17 % and 35 % of the variance at year one and two respectively. Higher 24 hour systolic blood pressure predicted higher executive subscore at year one only (B = +0.066, 95% CI +0.006 to +0.126, p =

0.032, Table 13.5). Better systolic blood pressure response to Valsalva manoeuvre predicted higher executive score at year 2 (B = +0.079, 95 % CI +0.023 to +0.135, p = 0.006).

Executive subscore from CAMCOG

CAMCOG executive 1 = (CAMCOG baseline x 0.407) + 10.5

Adjusted $R^2 = 0.171$, $p < 0.001$

CAMCOG executive 2 = (CAMCOG baseline x 0.514) + 9.9

Adjusted $R^2 = 0.346$, $p < 0.001$

Table 13-5 Correlation of autonomic function and ambulatory blood pressure with CAMCOG executive subscore

Autonomic variable	CAMCOG executive year 1			CAMCOG executive year 2		
	n	Regression coefficient	p	n	Regression coefficient	p
Reflex tests						
30:15 ratio	59	-3.452	0.285	47	+1.884	0.594
Valsalva ratio	56	-1.630	0.539	47	+4.004	0.096
E-I ratio	60	+0.081	0.423	47	-0.017	0.860
ΔSBP orthostasis	60	+0.013	0.394	48	-0.010	0.460
ΔDBP isometric exercise	61	+0.007	0.818	50	+0.035	0.235
ΔDBP cold pressor	59	-0.047	0.177	51	-0.043	0.165
ΔSBP Valsalva	55	+0.000	0.991	45	+0.079	0.006
RR post-CSM	24	-0.005	0.136	21	-0.002	0.519
ΔSBP post-CSM	24	+0.065	0.404	21	+0.059	0.338
Power spectral analysis						
Total HRV	58	-0.000	0.522	35	-0.000	0.901
Low frequency HRV	58	-0.000	0.911	35	+0.000	0.789
High frequency HRV	58	-0.001	0.314	35	-0.000	0.971
Low frequency BRS	36	+0.126	0.550	24	-0.092	0.694
High frequency BRS	50	-0.052	0.592	29	-0.030	0.747
24 hr blood pressure						
Systolic mean BP	59	+0.066	0.032	49	+0.000	0.993
Diastolic mean BP	59	+0.080	0.168	49	-0.000	1.000
Systolic variability	59	+0.110	0.328	49	-0.011	0.919
Diastolic variability	59	+0.144	0.410	49	+0.098	0.611
Pulse pressure	59	+0.063	0.092	49	+0.000	0.992
Systolic diurnal change	57	-0.015	0.821	49	-0.016	0.822
Diastolic diurnal change	57	+0.038	0.519	49	-0.001	0.989

ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: HRV, heart rate variability: BRS, baroreflex sensitivity: BP, blood pressure

13.3.2 CDR results

There was no significant change in mean times for number vigilance, choice reaction and memory scanning tests (Table 13.6-7, Figures 13.4-13.6).

Table 13-6 CDR mean times for baseline, year 1 and 2

	Number vigilance	Choice reaction time	Memory scanning
Baseline (n=73)	511 (475-596)	610 (531-834)	1064 (967-1441)
Year 1 (n=58)	519 (459-599)	593 (535-751)	1077 (856-1268)
Year 2 (n=38)	509 (474-581)	602 (528-704)	1028 (866-1374)
Chi-square	0.788	1.697	2.182
P	0.674	0.428	0.336

Scores are median (interquartile range): Friedman Test, degrees of freedom = 2

Table 13-7 CDR mean times: year-on-year change

CDR	Year on year	N	Z score	Significance
Number vigilance	Baseline to year 1	56	-0.057	0.954
	Year 1 to year 2	34	-0.778	0.437
	Baseline to year 2	37	-0.173	0.862
Choice reaction time	Baseline to year 1	56	-0.131	0.896
	Year 1 to year 2	34	-0.966	0.334
	Baseline to year 2	37	-1.079	0.281
Memory scanning	Baseline to year 1	56	-1.785	0.074
	Year 1 to year 2	34	-1.051	0.293
	Baseline to year 2	37	-0.430	0.667

Wilcoxon Signed Ranks Test

13.3.2.1 CDR: number vigilance

There was no change in number vigilance score at one and two years. Number vigilance at baseline predicted 45 % and 14 % of the variance of year 1 and year 2 times respectively. Higher Valsalva ratio predicted better number vigilance performance at year 2 (B = -193.6, 95% CI -382.8 to -4.4, p = 0.045). Higher total and high frequency spectral powers of heart rate variability

predicted longer number vigilance time at year 2 (p = 0.050 and 0.021 respectively). There were no other cardiovascular predictors of CDR number vigilance times (Table 13.8).

Number vigilance 1 = (number vigilance baseline x 0.683) + 165.9

Adjusted $R^2 = 0.452$, $p < 0.001$

Number vigilance 2 = (number vigilance baseline x 0.471) + 277.9

Adjusted $R^2 = 0.144$, $p = 0.012$

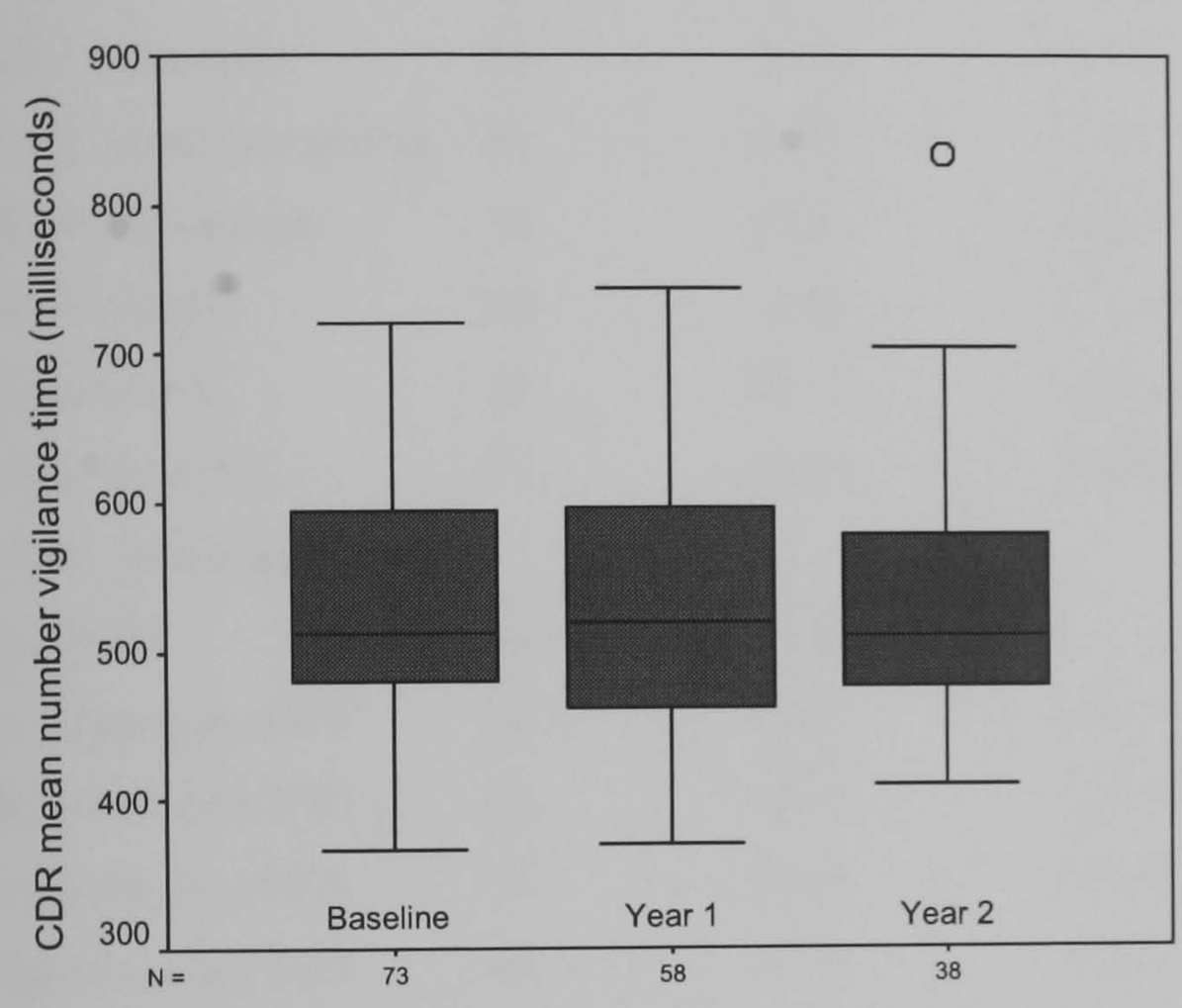

Figure 13-4 Mean number vigilance time for patients in sinus rhythm

Table 13-8 Correlation of autonomic function and ambulatory blood pressure with number vigilance on CDR test

Autonomic variable	Number vigilance year 1			Number vigilance year 2		
	n	Regression coefficient	p	n	Regression coefficient	p
Reflex tests						
30:15 ratio	53	+65.5	0.251	34	+23.34	0.790
Valsalva ratio	51	-63.9	0.184	35	-193.56	0.045
E-I ratio	54	-1.58	0.376	36	+1.46	0.677
ΔSBP orthostasis	54	-0.16	0.571	35	-0.52	0.246
ΔDBP isometric exercise	55	+0.29	0.603	35	+0.45	0.648
ΔDBP cold pressor	54	-0.01	0.988	38	+0.45	0.667
ΔSBP Valsalva	50	-0.85	0.123	35	-0.99	0.308
RR post-CSM	21	+0.01	0.060	19	-0.11	0.212
ΔSBP post-CSM	21	+0.26	0.828	19	-0.04	0.982
Power spectral analysis						
Total HRV	54	+0.02	0.121	35	+0.03	0.050
Low frequency HRV	54	+0.02	0.423	35	+0.06	0.102
High frequency HRV	54	+0.05	0.118	35	+0.10	0.021
Low frequency BRS	34	+1.84	0.690	24	-1.47	0.804
High frequency BRS	46	+1.95	0.238	24	+3.04	0.241
24 hr blood pressure						
Systolic mean BP	55	-0.31	0.573	35	+1.03	0.281
Diastolic mean BP	55	-0.44	0.644	35	+1.11	0.532
Systolic variability	55	-0.36	0.852	35	+0.99	0.817
Diastolic variability	55	+3.47	0.229	35	+7.10	0.292
Pulse pressure	55	-0.26	0.706	35	+0.99	0.383
Systolic diurnal change	53	+0.40	0.719	35	+0.55	0.801
Diastolic diurnal change	53	+0.89	0.344	35	+0.53	0.762

ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: HRV, heart rate variability: BRS, baroreflex sensitivity: BP, blood pressure

13.3.2.2 CDR: choice reaction time

Scatterplots indicate wide variance in choice reaction time with outlying values. Baseline choice reaction time predicted 13 % and 14 % of the variance at year 1 and year 2 respectively. Larger increases in diastolic blood pressure during isometric exercise predicted worse choice reaction time at year 1 (B = +2.9, 95% CI +0.1 to +5.6, p = 0.041). Better blood pressure response during

Valsalva manoeuvre predicted quicker choice reaction time at year 1 (B = -3.1, 95% CI -5.7 to -0.5, p = 0.019). There were no other cardiovascular predictors of choice reaction time at year 1 and 2 (Table 13.9).

CRT 1 = (CRT baseline x 0.306) + 437.5

Adjusted $R^2 = 0.127$, p = 0.006

CRT 2 = (CRT baseline x 0.200) + 477.3

Adjusted $R^2 = 0.137$, p = 0.015

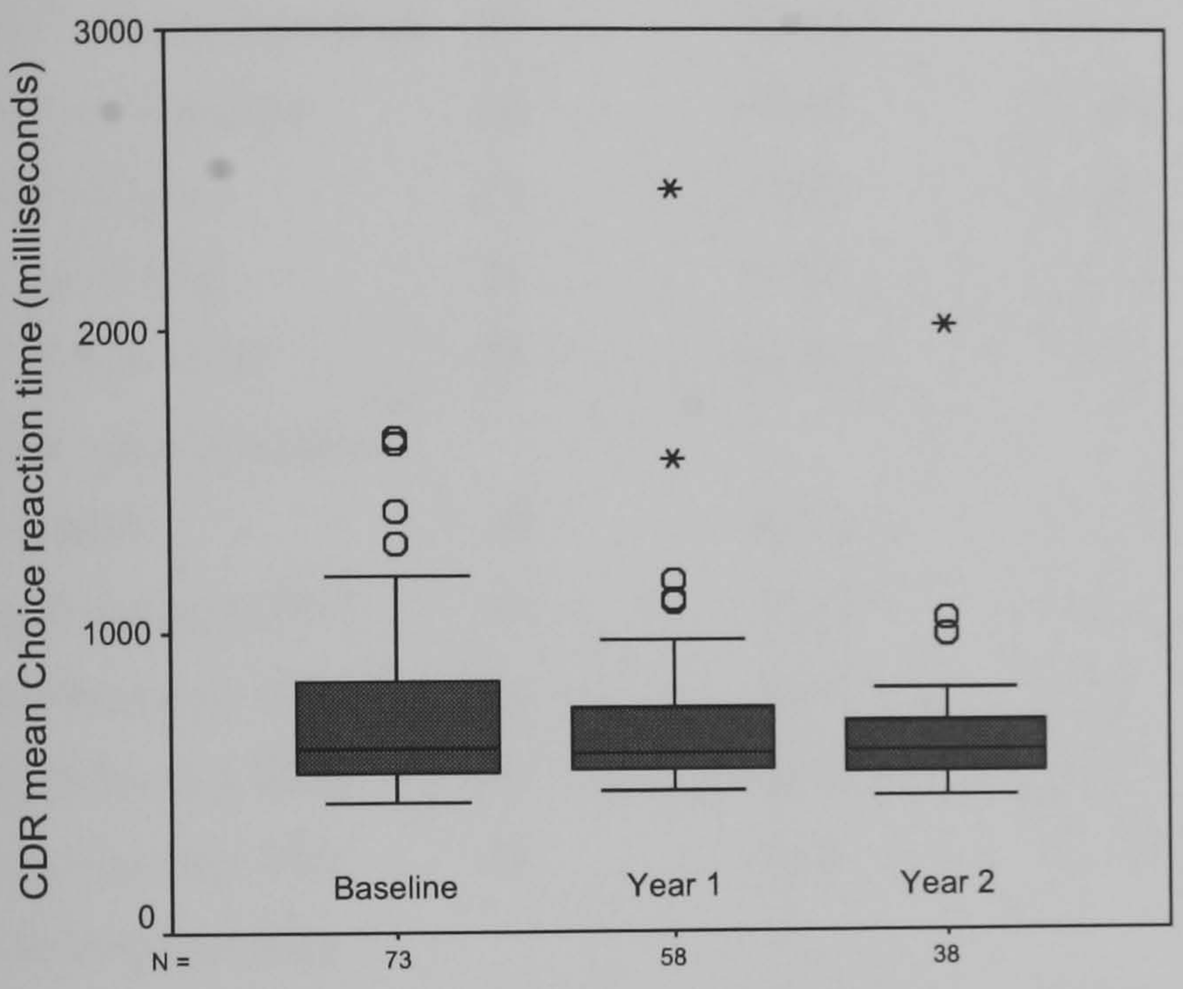

Figure 13-5 Mean choice reaction time for patients in sinus rhythm

Table 13-9 Correlation of autonomic function and ambulatory blood pressure with Choice reaction time on CDR test

Autonomic variable	CRT year 1			CRT year 2		
	n	Regression coefficient	p	n	Regression coefficient	p
Reflex tests						
30:15 ratio	55	-40.85	0.785	34	+145.62	0.260
Valsalva ratio	52	-112.70	0.380	35	-129.6	0.323
E-I ratio	56	-2.70	0.570	36	+2.29	0.622
ΔSBP orthostasis	56	+0.14	0.851	35	-0.051	0.468
ΔDBP isometric exercise	57	+2.88	0.041	38	+0.33	0.794
ΔDBP cold pressor	56	+0.01	0.994	38	+0.36	0.788
ΔSBP Valsalva	51	-3.12	0.019	35	-1.76	0.152
RR post-CSM	23	+0.04	0.796	19	-0.08	0.566
ΔSBP post-CSM	23	+0.064	0.837	19	+4.06	0.107
Power spectral analysis						
Total HRV	54	+0.01	0.756	35	+0.04	0.053
Low frequency HRV	54	-0.00	0.968	35	+0.02	0.588
High frequency HRV	54	-0.01	0.951	35	+0.10	0.034
Low frequency BRS	34	-2.40	0.843	24	-4.16	0.524
High frequency BRS	46	-0.88	0.853	29	+1.83	0.557
24 hr blood pressure						
Systolic mean BP	55	+0.14	0.922	35	-1.07	0.373
Diastolic BP	55	+1.20	0.635	35	+1.03	0.647
Systolic variability	55	+6.51	0.195	35	+3.69	0.497
Diastolic variability	55	+11.09	0.183	35	+7.68	0.385
Pulse pressure	55	-0.39	0.829	35	-1.98	0.166
Systolic diurnal change	53	+4.48	0.104	35	-1.36	0.628
Diastolic diurnal change	53	+3.59	0.133	35	-1.73	0.462

ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: HRV, heart rate variability: BRS, baroreflex sensitivity: BP, blood pressure

13.3.2.3 CDR: memory scanning

There was no significant change in memory scanning time from baseline to year 1 or year 2.

Baseline time predicted 26% and 24 % of memory scanning time at year 1 and 2 respectively.

There were no significant cardiovascular predictors of memory scanning time during cognitive follow-up (Table 13.10).

Memory scanning 1 = (memory scanning baseline x 0.412) + 608.5

Adjusted R = 0.256, p < 0.001

Memory scanning 2 = (memory scanning baseline x 0.472) +

Adjusted R = 0.242, p = 0.002

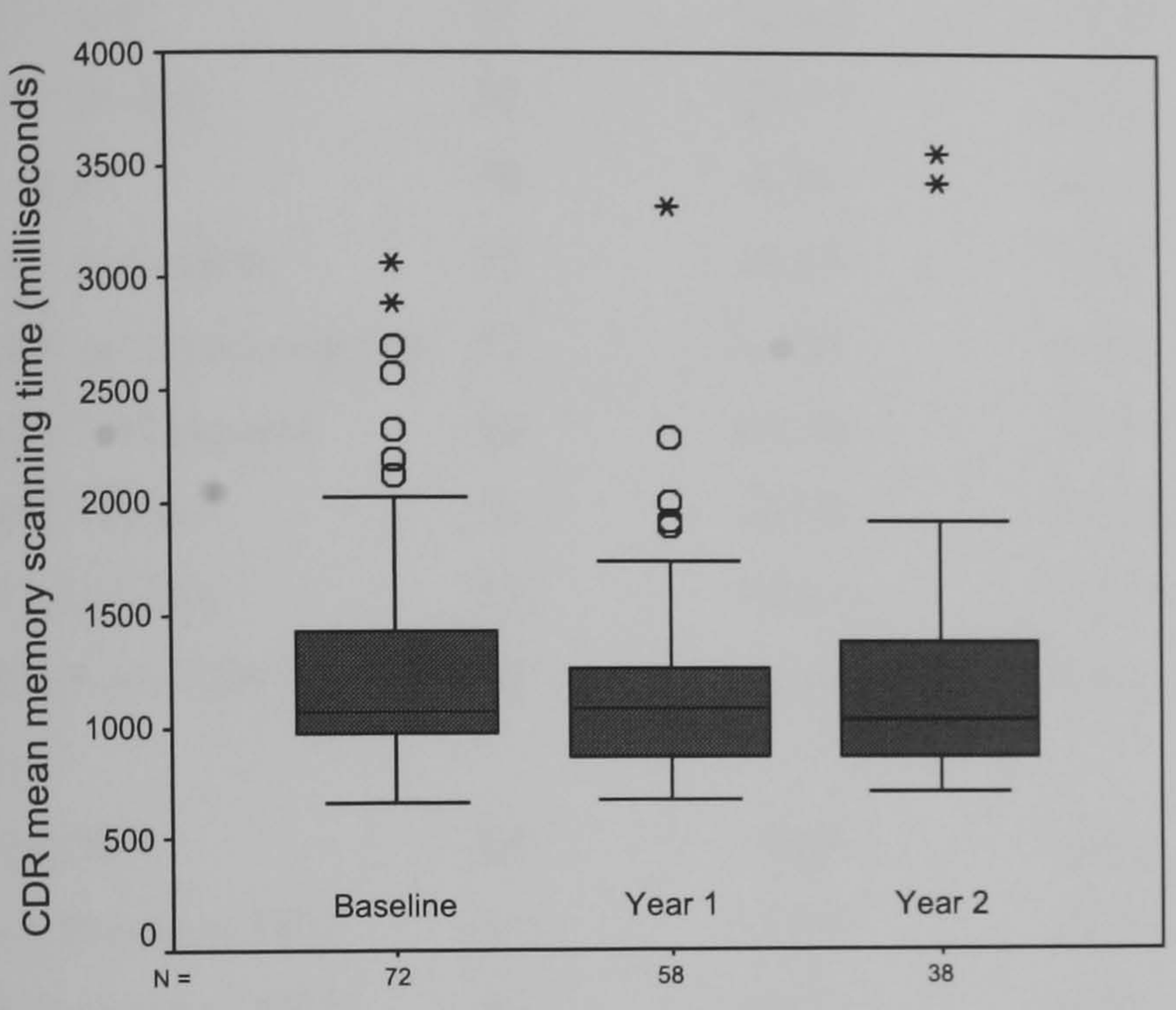

Figure 13-6 Mean memory scanning time for patients in sinus rhythm

Table 13-10 Correlation of autonomic function and ambulatory blood pressure with memory scanning on CDR test

Autonomic variable	Memory scanning year 1			Memory scanning year 2		
	n	Regression coefficient	p	n	Regression coefficient	p
Reflex tests						
30:15 ratio	55	+315.5	0.264	34	-254.1	0.458
Valsalva ratio	52	+324.2	0.158	35	+179.9	0.582
E-I ratio	56	-8.84	0.325	36	-19.17	0.061
ΔSBP orthostasis	55	+0.19	0.895	35		
ΔDBP isometric exercise	57	+1.64	0.548	38	-0.82	0.792
ΔDBP cold pressor	56	+2.58	0.374	38	+3.27	0.290
ΔSBP Valsalva	51	-2.60	0.320	35	-4.45	0.136
RR post-CSM	23	+0.37	0.179	19	+0.40	0.171
ΔSBP post-CSM	23	+2.70	0.665	19	-7.50	0.169
PSA						
Total HRV	54	+0.08	0.201	32	+0.03	0.575
Low frequency HRV	54	+0.04	0.777	32	-0.01	0.911
High frequency HRV	54	+0.17	0.302	32	+0.09	0.500
Low frequency. BRS	34	+5.86	0.794	22	-20.05	0.203
High frequency BRS	46	+12.72	0.142	26	-0.80	0.924
24 hr blood pressure						
Systolic mean	55	-0.04	0.989	35	+0.90	0.741
Diastolic mean	55	-4.96	0.280	35	+1.65	0.746
Systolic variability	55	+15.02	0.094	35	-3.21	0.795
Diastolic variability	55	+17.13	0.253	35	-27.36	0.165
Pulse pressure	55	+2.39	0.460	35	+0.62	0.850
Systolic diurnal change	53	-5.32	0.336	35	-2.04	0.748
Diastolic diurnal change	53	-2.66	0.573	35	-0.36	0.945

ΔSBP, change in systolic blood pressure: ΔDBP, change in diastolic blood pressure: CSM, carotid sinus massage: HRV, heart rate variability: BRS, baroreflex sensitivity: BP, blood pressure

13.4 Discussion

In this group of older stroke survivors, results do not indicate consistent, significant associations between post-stroke autonomic function and subsequent CAMCOG score or measures of attention, vigilance and executive function from the CDR battery. The majority of the regression

analyses do not reach statistical significance. A minority are significant but caution should be adopted for several reasons. Firstly none are highly significant. In view of the large number of statistical tests performed, there is a high chance of a false positive result. This raises the issue of setting a more stringent threshold for statistical significance than the frequently used but arbitrary value of 0.05. None of the significance tests attain significance less than 0.01. Secondly if there were a true effect one would anticipate consistently significant effects on both year 1 and year 2 scores, which did not occur. This underlines the possibility of the few significant results being a chance effect.

13.4.1 Study limitations

The p value is the probability of having observed experimental data when the null hypothesis is true. The null hypothesis is the concept that when an experiment is performed comparing populations, there will be no difference between the two groups. Thus when the p value becomes smaller, the null hypothesis becomes less likely until we reach an arbitrary cutoff point i.e. 0.05 indicating the null hypothesis is rejected and we conclude there is a difference between the groups in the study population. In the results shown above the general lack of significance indicates that there is no effect of cardiovascular autonomic function on subsequent cognitive function, as measured by CAMCOG and CDR tests. However it is important to consider the possibility of errors made in using the significance level. A Type I error is already mentioned above, in that the few significant results seen in this regression study may be chance findings due to multiple significance testing. A Type II error may also have occurred from insufficient sample size. This is an important consideration, with approximately 60-70 subjects tested at year 1 and only 30 or so at year 2.

Other reasons contributing to the possibly false negative findings need consideration. As discussed in Chapter 3, the cardiovascular autonomic tests have weaknesses. Repeatability of reflex tests, power spectral analysis and ambulatory blood pressure is not good and questions the accuracy of each test. Within the cardiovascular reflex tests, heart rate change during metronomic respiration offers the best repeatability. Repeatability of cognitive function is also poor, particularly for the CDR scores (section 4.4.3). Thus the variation in time for CAMCOG and CDR scores may be partly due to natural fluctuation in score over time and not necessarily due to true change in cognition. From the linear regression analysis the variance of CDR scores at year 1 and 2 is surprisingly low, suggesting poor repeatability, even allowing for the time interval between annual tests. One would expect more of the year 1 and 2 scores to be influenced by baseline score. Interobserver differences may influence performance on CAMCOG and CDR

testing. Interobserver differences could also lead to variation in autonomic reflex tests and power spectral analysis (in the latter's editing), and to a lesser degree ambulatory blood pressure although the potential for observer error is probably smaller with the automated nature of power spectral analysis data collection. Inter-rater difference is less relevant for the single assessment of cardiovascular function at baseline but could affect repeated measures of neuropsychometric performance. No data is available on interrater reliability with respect to neuropsychometric testing.

The cohort was not homogenous. For example, stroke type, comorbidity and medication varied quite widely between individuals. These clinical factors may exert far greater influence on cognitive function than the autonomic function. The relatively small number studied at follow-up in conjunction with 'noise' from potent established risk factors probably reduced the power of the study to identify any significant impact of dysautonomia on neuropsychological measures.

13.4.2 Post-stroke dementia rates

Improvement in cognitive function was not expected since the effect of age and high risk of further cerebrovascular events would make cognitive decline much higher than the background community rates. Kokmen et al (Kokmen, 1996) report a nine-fold risk in dementia risk in the first year after stroke and remains twice the expected rate in the following years. Loeb report dementia rates 4-12 times the expected rate in the four years following lacunar stroke (Loeb, 1992). Tatemichi et al (Tatemichi, 1994b) have also shown greatly elevated relative risk of incident dementia for 4 years following stroke. Most of the incident cases of dementia in Henon et al's three year post-stroke study occured within 6 months of stroke (Henon, 2001). Zhu et al (Zhu, 2000) reported significantly elevated 2.4 relative risk of dementia for the three years following stroke, but after three years the relative risk was no longer significant. However there are data indicating how a large proportion of stroke patients do experience improvement in cognitive function in the months and years following stroke. Wade et al (Wade, 1988) noted improvements in certain cognitive domains more than three months after stroke. Desmond et al estimated cognitive improvement in approximately a third of patients and this occurred after the three month post-stroke interval, indicating this change was not likely to be resolving delirium of the acute post-stroke phase (Desmond, 1996). Components increasing the chance of recovery include visual neglect and left hemisphere stroke, suggesting that cortical anterior circulation strokes fare better (Desmond, 1996; Wade, 1988). It's likely that small vessel disease may nullify chance of improvement as a result of progressive white matter ischaemia and associated neurodegeneration (Ballard, 2003a; Desmond, 1996). Data from our own cohort identified 50 %

of older stroke survivors achieving improvement in global cognition whilst 9 % developed incident dementia from assessment 3 and 15 months post-stroke (Ballard, 2003a).

13.4.3 Absence of post-stroke cognitive decline

The absence of cognitive decline, and in some instances improvement in function, in the sinus rhythm cohort may therefore be part of a natural phenomenon in the long-term recovery of older stroke patients. A potentially more important factor is selection bias for follow-up neuropsychmetric testing. Indications for frequent testing to capture incident cognitive decline was discussed in Chapter 2. It is possible those not attending for cognitive assessment at year 1 and year 2 (14 and 24 respectively) were the individuals suffering significant cognitive impairment and dementia. Post-stroke cognitive impairment is associated with higher mortality and adverse outcomes (Desmond, 1998b; Tatemichi, 1994a). Reduced detection of cognitive decline will impair ability to identify the significant predictors of cognitive impairment.

Neuropsychometric tools used in this study are probably reliable in detection of cognitive deficit. CAMCOG has been validated in the detection of dementia in stroke patients (de Koning, 1998). The CDR battery has not been validated for detection of post-stroke dementia but was chosen for its ability to detect abnormal 'central processing speed', particularly relevant in the aetiology of vascular dementia (Erkinjuntti, 2000a; O'Brien, 2003). The body of knowledge is far greater for the CAMCOG than CDR. CDR is a useful and sensitive tool that is able to detect abnormal cognitive function in patients with Alzheimer's disease and dementia with Lewy bodies compared with control values and additionally was strongly related to MMSE score (Ballard, 2001). CDR has also shown responsiveness to cognitive drug enhancer treatment (Wesnes, 2002). Data from the COGFAST cohort indicates CDR is able to define impaired cognitive processing speed, working memory and executive function in post-stroke patients compared with elderly controls (Ballard, 2003b). CDR indices are also related to white matter lesion volume, especially frontal lobe damage, from COGFAST data (Burton, 2004).

13.4.4 Previous studies

There is a dearth of reports investigating the impact of cardiovascular autonomic function on cognitive function in normal individuals. Compared with normal controls, Aharon-Peretz et al (Aharon-Peretz, 1992) identified changes in power spectra of heart rate variability in Alzheimer`s disease indicating relatively higher ratio of low:high frequency power ratio. This study used unconventional definitions for power spectra bands and the low:high frequency ratio measure has proved unreliable as a means of quantifying sympathetic/parasympathetic balance in other work.

Elmstahl et al (Elmstahl, 1992) did not detect any difference in the expiration:inspiration RR interval ratio between elderly patients with Alzheimer's disease and controls but impaired heart rate deceleration with 8 minute tilting suggesting impaired dynamic vagal response in Alzheimer's disease. Franceschi et al (Franceschi, 1986) were unable to reliably perform cardiovascular reflex tests in a cohort of 18 patients with Alzheimer's disease, therefore used a sleep-dependent measure of autonomic activity. Results suggested defective cardiac autonomic control, predominantly from sympathetic dysfunction. Heart rate and blood pressure response to standing were satisfactory in a handful of studied patients. Passant et al (Passant, 1996) recorded asymptomatic orthostatic hypotension in patients with dementia (Alzheimer's disease and vascular) during head-up tilt in association with decrease in frontal lobe cerebral blood flow. Prevalence of carotid sinus hypersensitivity is elevated in dementia with Lewy bodies (Ballard, 1998). Patients with neurodegenerative dementia have greatly increased odds ratio for deep white matter lesions in the presence of vasodepressor carotid sinus hypersensitivity or orthostatic hypotension with drop in blood pressure greater than 30 mmHg (Ballard, 2000). All these studies are cross-sectional.

A handful of studies have investigated the association of blood pressure variability with cognitive parameters. Cicconetti et al's (Cicconetti, 2003) study of 40 hypertensives divided into dippers and non-dippers did not find any difference in MMSE score or other cognitive damage between the two groups. Van Boxtel's analysis (van Boxtel, 1998) of 115 community living adults ambulatory blood pressure data concluded that reduced circadian variation was associated with poorer cognitive measures. The deficits were characterised by impaired memory and reduced processing speed. Belleli et al (Bellelli, 2002) reported an association between 24 hour ambulatory blood pressure variability and MMSE score. Deterioration in visual processing skills was associated with increased daytime short-term blood pressure variability and higher nocturnal blood pressure in a Japanese study of 88 older adults (Kanemaru, 2001).

14 Discussion

This chapter will discuss issues surrounding data acquisition and analysis and address study limitations as a whole. The focus is on the cardiovascular investigations. Cardiovascular autonomic function testing has formed the focus of this thesis, and this discussion will mainly examine issues around cardiovascular autonomic reflex tests, heart rate and blood pressure variability. Limitations of the MRI data and neuropsychometric data are treated as beyond the scope of this discussion for reasons of brevity.

14.1 Recruitment

Recruitment required a longer period than anticipated and just failed to reach the target. Recruitment rates were partially governed by the number recruited to the COGFAST study. Just over half those within COGFAST gave additional consent for the cardiovascular and MRI investigations. The majority of non-responders were simply participants declining to take part in the clinical investigations. A minority were excluded because of potential history of intraocular foreign body. Since there was no permission from the Ethical Committee for orbital radiographs to be performed (from risk of exposure to ionising radiation) all candidates recalling previous ocular injury from metallic objects were automatically excluded from the cardiovascular and MRI investigations. All these injuries occurred in the workplace many years prior to the study and it is almost certain the injuries were superficial corneal abrasions but since orbital radiograph was a prerequisite for MRI scanning they could not be recruited to the study. Inclusion of permission to radiograph orbits in these circumstances could have enhanced recruitment rates.

Case selection should lend caution to the application of results to the larger post-stroke population in the community. Only cases presenting to hospital were screened and recruited to the study. It is likely that patients with milder strokes do not present to secondary care. Thus conclusions on autonomic function in stroke survivors may not hold true in these less severe stroke cases.

One prominent limitation of the data within this thesis is lack of information on the total number of stroke patients screened within each hospital database. This information was not routinely collected within the study and it is impossible to accurately state the total number of patients admitted with stroke, those surviving to three months, those suitable for inclusion and the number who refused to be considered for the study. It is clear the 97 participants in the cardiovascular substudy are a highly selected group of stroke survivors. The information on exclusions would assist interpretation of applicability of results to the wider population. One could assume the candidates who declined and were not suitable for inclusion are probably no fitter than those

willing to commit to hospital-based research investigations and may have higher levels of comorbidity. Section 5.1.5 discusses the similarities in clinical composition of this cohort and much larger post-stroke cohorts in epidemiological surveys.

14.2 Clinical data acquisition

The clinical characteristics were collapsed into binomial groups for the purposes of simplifying analysis. This resulted in some loss of definition of some risk factors. For the most part this was not of consequence, for example the very small numbers of 'not known' entries were collapsed into 'not present' categories for clinical characteristics such as hypertension or myocardial infarction. However identification of risk from alcohol and smoking habit could have been affected by collapsing details into binomial groups. Alcohol status was collapsed into non-drinker or drinker.

Participants' past medical history was obtained by direct interview at the time of cardiovascular testing. Advantages conferred included ensuring completeness of data and avoiding retrospective data collection. Disadvantages were possibility of recall bias and lack of clinical data to corroborate data in the case of uncertainty. However it has been shown that face-to-face interview is a reasonably reliable method of collating clinical data in similar clinical studies (Davis T communication to Northern BGS Meeting 2002 regarding data collection for Rodgers et al (Rodgers, 2004)). For participants from the Queen Elizabeth Hospital, hospital casenotes were viewed as part of the COGFAST screening programme. Past medical history in those people who subsequently agreed to the study was noted and used to clarify any areas of uncertainty regarding clinical history from face-to-face interview. Casenotes were scrutinised for all study participants to ascertain clinical risk factors prior to stroke, to provide data in the same manner for all COGFAST participants. This provided a further source of information if there was uncertainty from interview. However original information recorded in hospital notes was obtained by numerous medical staff, with the absence of a structured questionnaire for all cases: therefore data could be incomplete and interviewee response could lack consistency. Furthermore there is a similar risk of recall bias (perhaps higher risk for elderly patients with an acute illness) and events could have occurred in the intervening period between hospital admission and study participation. The ideal source of past clinical data is probably Primary Care casenotes but the study did not have Ethical Committee approval for such access.

14.3 Clinical study design

Several important issues concern the general approach to our study design. Issues that may have limited the ability of the study to accurately record autonomic function are discussed below. Such limitations may have undermined the ability to detect association between dysautonomia and cognitive decline. As discussed above, study recruitment was slower than anticipated and had to stop just short of the intended target of 100 since the time limit for clinical data acquisition came to a close. Prevalence of atrial fibrillation further reduced the total number whose autonomic function could be fully assessed. It could be argued that sinus rhythm should have been a criterion for study inclusion. This would not have made any difference to the total number of participants in sinus rhythm since strenuous efforts were made to screen all candidates. The overall effect of excluding participants in atrial fibrillation would be to reduce numbers available for MRI scanning. Furthermore, it would have led to further selection of cases for MRI studies, whereas the main COGFAST study required a representative cohort of older stroke survivors.

There are no similar studies on which one could perform a power calculation to inform the study of number of stroke patients required to detect effects on cognitive data and white matter lesion from variation in autunomic function

14.4 Finapres operation

Cardiovascular autonomic investigation is reliant on beat-to-beat blood pressure monitoring. The Finapres™ and Portapres™ systems used in this study are widely used and benefit from a wealth of research experience. Difficulties often appear in practice and this was our experience. In some instances, setting up the finger cuff took 5-10 minutes because the Finapres™ or Portapres™ was unable to measure the finger arterial pressure. Joint disease, cold hands and good cuff fit were important limiting factors. The Finapres may not accurately reflect true blood pressure due to transmission of the pressure pulse along the arm arteries that distorts waveform and lowers mean blood pressure. This is particularly relevant in our cohort with high rates of systolic hypertension that is associated with arterial wall stiffening. Imholz's review underlines how Finapres™ is adequate in recording average pressure across a group but lacks accuracy in individuals (Imholz, 1998), which may have undermined power to match white matter lesion and cognitive change to autonomic function within individuals.

Data quality was easily degraded by movement artifact. This was particularly true for the orthostatic reflex and isometric exercise tests. The frailer patients usually required some assistance during standing. In the course of maintaining balance on standing, upper limb

movement was common. Muscle movement interfered with RR interval and blood pressure data during selection of single data points for the 30:15 ratio and systolic pressure nadir. It was possible to overcome this pitfall for the isometric exercise by editing and appropriate selection of 'clean' data points to obtain a representative mean blood pressure over 20 beats.

14.5 Cardiovascular autonomic reflex tests

Orthostasis was often difficult to perform in a timely manner for some participants. The baseline RR interval and blood pressure trace suggested an alerting response in some participants as well. It is likely such factors may have altered RR and blood response to orthostasis. This could potentially have altered blood pressure drop during standing which is dependent on preceding rest time (Ten Harkel, 1990). Emotional stress or alerting response could upregulate sympathetic activity prior to standing. Most achieved standing balance within a reasonable time frame. Appliance of the RRmax/RRmin principle (as opposed to stricter 15^{th} and 30^{th} RR interval ratios) was essential to allow correct identification of heart rate maxima and minima. The initial intention was to adhere to Ziegler's recommended window of beats 5-25 for the minimum RR and 20-40 for maximum RR interval (Ziegler, 1992b). In fact some cases had RR maxima substantially beyond the 40 beat mark: 3 were between beat numbers 40 and 50, one at beat 53 and one at beat number 64. It seemed valid to still include these examples since there were recognisable RR peaks and troughs and there is little reason to doubt they do not have the same underlying physiological basis as other participants. Secondly there was variance in supine-to-stand time which could not have been fairly represented on the 'start' marker during offline analysis of autonomic data.

Valsalva manoeuvre presented its own set of difficulties. Many subjects did not understand the procedure during initial explanation. Attaining the 40 mmHg expiratory pressure was universally difficult but most subjects did achieve this target. It is difficult to quantify the overall performance of actual expiratory pressure and duration because there was no recording system for these values. In practice the following criteria needed to be met in order to include a subjects' Valsalva manoeuvre data; evidence that satisfactory expiratory pressure was attained, surpassing the 10 second expiration time, absence of excessive artifact at critical data points and most importantly, the presence of the expected pattern of heart rate and blood pressure fluctuation during Valsalva manoeuvre (Denq, 1998). Variation in expiratory pressure and duration can significantly affect Valsalva reflex results. It seems likely that difficulty in adhering to the protocol could have influenced accuracy of Valsalva ratio and systolic blood pressure overshoot data.

Isometric exercise was performed using a three minute sitting exercise. This was chosen over the more usual handgrip exercise which in our experience is not adequately performed by older subjects. The sitting position produced the required isometric stress in a manner that older subjects could understand and usually achieve. It certainly required isometric exercise that led to fatigue, which is the crucial issue in stimulating the autonomic system (Donald, 1967; Hultman and Sjoholm, 1982; Seals, 1988).

Cold pressor test was surprisingly well tolerated with nearly all participants completing one minute of hand immersion in the iced-water bath. Most required some encouragement and guidance regarding remaining time for the test. This seemed only fair to improve completion rates, and is unlikely to substantially affect the pressor response from any emotional stress resulting from verbal intervention. All subjects were reassured they could discontinue the test at any point. The immersion time of one minute was influenced by a desire to maximise compliance. Two minutes of immersion is theoretically advantageous; two minutes allows maximal blood pressure response as a result of MSNA activity (Stancak, 1996; Victor, 1987). Diastolic response may have been submaximal in one minute but conditions were the same for all participants. Fresh ice was used in all cases but ideally temperature should be recorded to ensure standardisation of stimulus, which can vary according to temperature and hence alter intensity of cardiovascular response (Kregel, 1992; Ng, 1994).

Metronomic respiration was successful in nearly all cases. All participants were able to follow instructions. The exclusions applied to individuals where non-sinus activity during metronomic respiration made it impossible to accurately identify peak and trough RR data points. Optimal position for metronomic respiration (supine or sitting) is debatable, supine is deemed satisfactory and sitting position does not offer any known performance advantage for RR interval variation (Ewing, 1981; Ten Harkel, 1990). However sitting position may have improved participants ability to follow instructions through visual cueing and may have assisted maximal inspiration in participants with respiratory muscle weakness: tidal volume is known to alter heart rate variation during metronomic respiration (Brown, 1993).

14.5.1 Carotid sinus massage

Consent to carotid sinus massage was low and there were also a number of exclusions based on carotid Duplex results. Therefore the number available for data analysis was low, affecting power to detect association. The safety of carotid sinus massage for patients with cerebrovascular disease is brought into question following side-effects in one case resulting from carotid sinus massage.

14.6 Heart rate variability

The three principal components of heart rate variability, total, high and low frequency spectral powers did not provide any predictive power with regard to white matter lesion and neuropsychological outcomes. This is somewhat surprising since the technique has distinct advantages over the reflex tests. Five minutes of heart rate variability data theoretically reflects cardiac autonomic modulation over a longer period than the shorter reflex tests. Heart rate variability studies eliminate the need for the subject to understand and comply with correct procedure for reflex tests, which appears a very attractive proposition in frail older patients. The literature contains extensive work on the origins of spectral powers of heart rate variability, and it was anticipated results could inform hypotheses regarding autonomic pathology.

In practice the complexity of heart rate variability analysis in this cohort presented some difficulties. Recording environment was not ideal. Quiet, temperature controlled conditions free of external noise and distraction are the optimal conditions (Bernardi, 2000). Our recording environment was close to a busy clinical area which may have led to minor noise disturbance. The couch was not designed with providing perfect supine resting conditions in mind and some patients clearly indicated they felt a little restless during the recording. There is also the issue of physical comorbidity and drug therapy. It seems likely these confounding factors exerted some influence on results. Whether these effects were sufficient to mask the natural underlying heart rate variation is debatable.

As discussed in Section 4.1, the underlying purpose of COGFAST at large is the study of the natural history of cognitive function and neuropathological correlates thereof in older stroke patients. To eliminate all subjects with any cardiovascular disease or prescribed drugs in the cardiovascular substudy would not accurately represent this group of patients. One could argue that a moderate tightening of exclusion criteria may have improved the situation but case recruitment was sufficiently challenging without further restriction on entrants. Omission of drug therapy for a prescribed period is an attractive concept to eliminate drug confounding effects. One could propose any cardiovascular drug could be stopped a period of days before cardiovascular assessment. However this approach can also present problems. Clear instructions and perhaps extra manpower would be required to ensure the appropriate drugs were omitted at the correct time. A more serious concern is the ethical ramifications of withdrawing cardiovascular drugs for a voluntary research study. For example adequate beta-blocker withdrawal raises the possibility of a rebound syndrome. Wholesale anti-hypertensive withdrawal could lead to rebound hypertension with a risk of further cerebrovascular event (a risk that is difficult to quantify and

probably very small but still present for a group of this size and age). Omission of diuretics could lead to decompensation in heart failure. Overall, inclusion of patients on cardiovascular drugs in the study may raise concerns on their confounding effects but in practice it minimised risk and disruption for participants and improved recruitment. Section 7.3 contains data indicating there were actually few significant differences between the stroke cohort and community control group with regard to prevalence of confounding factors. Secondly, results of multiple regression analysis shown in Table 7.9 indicate differences in autonomic function between these groups still hold true after allowing for potential confounding factors.

Editing heart rate variability data raised further issues on the techniques' usefulness. The concept of power spectral analysis is reliant on sinus rhythm. Ascertaining sinus rhythm from the 12 lead ECG was straightforward but confirming sinus rhythm over five minutes data was often challenging. Non-sinus activity was frequent, usually in the form of a limited number of ectopic beats that were easily removed using CRISP software. Other recordings had more frequent ectopic data that in some instances were time-consuming to eradicate using the software and more rarely led to doubts on the underlying rhythm. Textbook depictions of power spectra describe neat, defined peaks but in practice for this cohort the power spectrum seemed often rather chaotic with no discernable low and high frequency peaks and the majority of the power concentrated in the < 0.04 Hz band. Difficult cases were discussed with senior Medical Physicists to ensure correct application of the technology and exclusion of unsuitable data. Despite these steps, data for total, high and low frequency power spectra were widely dispersed. A threshold value for inclusion (total HRV less than 7000 ms^2) was chosen, partly arbitrarily and partly after further review of individual data, to exclude the more extreme outliers. There appears to be little data in the literature to allow direct comparison of results for a group of this age and clinical description. Comparison with normative value is also hindered by the different hardware and software employed by research groups.

The discussion in section 3.2.4 focused on the value of heart rate variability analysis to inform about sympathetic and parasympathetic mechanisms. Since the low and high frequency spectral power were relatively small compared with the spectral power less than 0.04 Hz (i.e. very low frequency), one could hypothesise the total heart rate variability data is more informative on the non-neural components of heart rate variability (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

It appears that any extrapolation on cardiovascular autonomic function from the five minute heart rate variability study may have been overwhelmed by 'noise' from environmental issues,

confounding effects of drugs and comorbidity and editing process. The technique has clearly proved useful in other 'cleaner' cohorts, mostly as a means of detecting autonomic neuropathy in diabetic patients and mortality risk in ischaemic heart disease (see Section 7.4.3). It should be remembered there were significant differences in total and low frequency spectral powers compared with community living, stroke-free control cases. The absence of predictive power for white matter disease and neuropsychometric outcomes tends to refute the hypothesis that cardiovascular autonomic dysfunction will increase risk of cognitive impairment. Baroreflex sensitivity indices from power spectral analysis offers an attractive risk marker for white matter disease but also lacked any significant association with the outcomes. The smaller number of stroke patients with follow-up data at two years must have affected power to identify predictive value. It would be interesting to examine heart rate variability and baroreflex sensitivity as a predictor of mortality, and sufficient data to inform such a study may become apparent with prolonged follow-up of the cohort. Robinson et al (Robinson, 2003) concluded baroreflex sensitivity was useful in identifying raised mortality risk in stroke patients. Their cohort was slightly younger but larger (124 patients, mean age 70 years) and mortality differences were apparent after 4 ½ years follow-up.

Revisions to heart rate variability methodology could improve the data quality and confidence in relationship to underlying autonomic activity. Inclusion criteria could be made more stringent as discussed above. The recording environment could be optimised by isolation in quiet surroundings with complete absence of distraction (Bernardi, 2000). Prolongation of the recording period to approximately 15 or 20 minutes would allow three or four separate blocks of heart rate variability and baroreflex sensitivity data that could be averaged to provide more accurate data. This approach has been used elsewhere (time constraints did not allow this method for this study) (Harrington, 2000a). Editing criteria could be set to produce strict inclusion and exclusion criteria. Information about dynamic autonomic behaviour could be obtained by incorporating autonomic challenges (Karemaker, 1997). For example five minutes of data at rest and normal respiratory rate could act as a baseline, metronomic respiration for five minutes can provide a vagal stimulus and passive tilt for five minutes could provide stimulus for sympathetic activity. Some may argue that respiration should be controlled in any situation of heart rate variability analysis (Bernardi, 2000). The logistics of adequate respiratory rate control in this type of older cohort remains to be seen. Finally this study has concentrated solely on the frequency domain of heart rate variability for five minutes of data. Time domain analysis of heart rate variability over longer periods (typically 24 hours) offers benefits in terms of robustness and proven clinical value, and also confers the ability to make direct comparison with other data in

the literature (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

14.7 Review of autonomic function after stroke

Frank et al (Frank, 1992) anecdotally noticed reduced heart rate variability was diminished in patients in neurology intensive care and subsequently in an uncontrolled study demonstrated reduced heart rate variability during deep breathing in 27 patients with a variety of focal intracranial lesions. Barron et al (Barron, 1994), in a controlled study, found total heart rate variability significantly diminished in stroke survivors, mean age 69 years, within 11 days of an anterior circulation infarct with CT evidence of infarction. Korpelainen et al (Korpelainen, 1994) investigated parasympathetic mediated responses in 40 stroke patients mean age 51 years and found significantly impaired heart rate responses to normal and deep breathing, Valsalva manoeuvre and tilting in the acute phase post-stroke (2-10 days). However at 6 months parasympathetic function appeared to have recovered since the only significant difference remained in the parasympathetic response to tilt. The same group investigated heart rate variability from prolonged 24 hour ECG recordings in the acute phase, and 1 and 6 months post-stroke and repeated the finding of impaired heart rate variability immediately post-stroke (31 cases with hemispheric infarctions, mean age 52 years, compared with controls) (Korpelainen, 1996b). Results indicate a moderate improvement in heart rate variability and sympathovagal balance at 6 months, but do not indicate if this was a significant change form the acute phase. Heart rate variability was also reduced immediately post-stroke in patients with medullary infarction with subsequent improvement but pontine infarction did not affect heart rate variability at any stage (Korpelainen, 1996a).

Tokgozoglu et al (Tokgozoglu, 1999) performed power spectral analysis of heart rate variability in a controlled study of 62 patients with ischaemic stroke and concluded that stroke patients had significantly reduced low and high frequency HRV. In a small number of patients who died suddenly there was a preponderance of patients with right insula stroke involvement who also had the worst heart rate variability. It was suggested this area may be an important anatomic location in the control of autonomic tone. The autonomic response to insular stroke appears to be influenced by blood pressure: normotensive cases demonstrate more abnormal diurnal blood pressure variation, higher serum norepinephrine levels and higher rates of QTc prolongation and arrhythmias than hypertensive cases (Sander and Klingelhofer, 1996).

A case-controlled study of heart rate variability during deep breathing in 23 stroke patients, mean age 59 years, found a significant reduction in RR variability limited to right sided stroke lesions

(Naver, 1996). Cases with left sided lesions had similar RR variability to controls and there was a significant difference between left and right sided cases. Sympathetic function was assessed by examining blood pressure changes during isometric exercise which did not reveal any difference between cases and controls nor left and right sided strokes.

Symptoms suggestive of unilateral autonomic dysfunction are common following stroke. One study found 43% of cases complained of coldness in the limb contra-lesional to the stroke that was related to skin vasomotor reflex asymmetry (Naver, 1995). A controlled study of changes in muscle sympathetic nerve activity during cold pressor in 8 males, mean age 58 years, found increased MSNA in the basal state: MSNA increased in both groups but the increase was attenuated in the stroke group, suggesting that post-stroke autonomic dysfunction may be caused by central damage leading to tonic activation of MSNA (Mizushima, 1998).

One study indicated development of autonomic dysfunction 5 years after onset of Type 2 diabetes mellitus was associated with increased stroke risk, an effect which persisted after allowing for the influence of known cardiovascular risk factors (Toyry, 1996).

Robinson et al (Robinson and Potter, 1997) demonstrated impaired baroreflex function in a group of 13 stroke patients mean age 64 years. Stroke cases did not match the increase in forearm vascular resistance that occured in controls during lower body negative pressure induced hypotension (but did manage to mount a similar systemic blood pressure response, probably by an increase in cardiac output). They speculated this effect could be due to either abnormal cardiopulmonary/baroreceptor mediated reflexes or impairment of the efferent vasoconstrictor function. In a small group of 9 stroke cases, orthostatic blood pressure control was impaired but there was no evidence of post-prandial hypotension compared with controls (Robinson and Potter, 1995). Cardiac baroreceptor sensitivity measured using the power spectral analysis derived alpha index was found to be significantly lower in a group of 124 ischaemic stroke patients (mean age 70.4 years) than controls. Additionally stroke cases with the more impaired baroreflex sensitivity (less than median value) had significantly poorer long-term outcome, with a mortality rate of 28% compared with 8% over a median follow-up of over 4 years (Robinson, 2003).

14.8 Summary of results

Chapter 7 reported on differences in autonomic function in the stroke patients compared with stroke free control adults. Stroke patients had deficits in RR variation during metronomic respiration and Valsalva manoeuvre, exaggerated diastolic blood response during isometric

exercise and a trend towards impaired blood pressure response to Valsalva manoeuvre. Spectral powers of total and low frequency heart rate variability were reduced in stroke cases, who also had impaired baroreflex sensitivity in the low frequency range. This makes a significant addition to the literature. To the author's knowledge, this is one of the oldest post-stroke groups in whom autonomic function has been investigated and one of very few to be studied at a time distant from incident stroke. The studies detailed above show that autonomic function is impaired in the acute aftermath of stroke. Our results indicate autonomic function is impaired in older stroke patients and these deficits persist following stroke recovery. Mizushima et al's (Mizushima, 1998) identification of abnormal MSNA following stroke could explain the exaggerated blood pressure response we observed during isometric exercise. We have also recorded impaired vagal function similar to Korpelainen et al (Korpelainen, 1996a; Korpelainen, 1996b; Korpelainen, 1994) but in our cohort the deficit persists long after resolution of the stroke.

14.8.1 Cardiovascular autonomic reflex tests

There was little evidence to support the hypothesis that autonomic dysfunction is associated with white matter lesions and cognitive decline. There was no correlation between autonomic function and baseline cortical white matter lesion volume. It is noteworthy that the few weak associations between autonomic function and neuropsychometric tests occurred with the autonomic indices that were abnormal in the stroke group. From results in section 7.3, it is assumed the exaggerated diastolic blood pressure response to isometric exercise is abnormal, as suggested by others i.e. the stroke group had the abnormally elevated diastolic response and it was not a case of the controls having an abnormally suppressed response (Mathias and Bannister, 1999). Higher diastolic response in isometric exercise was associated with poorer attention score and number vigilance time. It is suggested that hypertension is the cause of exaggerated isometric exercise response (Mathias and Bannister, 1999) and previous work has shown that hypertension is associated with deterioration in subcortical function (Harrington, 2000b). However the abnormal isometric exercise response in the stroke group appears to occur independently of elevated blood pressure (Table 7.9). Discussion in section 3.1.4.1 of isometric exercise neural substrate highlights the various components of blood pressure response over three minutes, with sympathetic muscle sympathetic nerve activity playing a dominant role. Baroreflex function will normally control the extent of blood pressure elevation. It is hypothesised that the defective baroreflex sensitivity allows undamped blood pressure rise in older stroke patients. This could lead to small vessel damage in the white matter, and account for the abnormal subcortical cognitive function.

Consistent with this hypothesis is the association of better high frequency baroreflex sensitivity with quicker choice reaction time and fluctuation time.

Longitudinal data did not provide strong evidence of an association between autonomic dysfunction and subsequent white matter lesion increase and cognitive decline. The analyses in Chapters 12 and 13 involve multiple significance testing therefore the few significant associations need to be interpreted with caution. Furthermore there were no autonomic correlates of white matter disease progression, which conflicts with the above hypothesis where autonomic indices were associated with CAMCOG scores and CDR times.

Higher Valsalva ratio was associated with better total CAMCOG score and number vigilance time at year 2. Therefore better vagal responsiveness links with higher global cognitive score and measures of attention. Choice reaction time is one of the more consistent indices on the CDR battery, reflecting attention and concentration skills. Quicker choice reaction time at year 1 of neuropsychometric follow-up was associated with better systolic blood pressure response during Valsalva manoeuvre, which is a measure of intact sympathetic function (with a contribution form vagal activity). Participants with the higher blood pressure overshoot also performed better on the CAMCOG executive subscore at year 2 (but not year 1). RR and blood pressure response to Valsalva manoeuvre seems to be the autonomic test with most evidence of an association with cognitive function from these investigations. Reasons why this may have occurred include the larger test range for Valsalva ratio compared with RR variation during orthostasis and metronomic respiration. Thus the Valsalva ratio has more power to identify normal and abnormal autonomic function. Secondly the Valsalva manoeuvre was performed on three occasions with the largest value of three (for ratio and systolic overshoot) used for data purposes. Hence three tests improved the likelihood of a participant providing the optimal response to the autonomic stimulus, whereas other tests were performed once and therefore prone to interindividual variation.

Returning to the theme of exaggerated isometric blood pressure response in the stroke group, the tests' potential role as a marker of adverse outcome is strengthened by the association with prolonged choice reaction time at year one of follow-up.

14.8.2 Power spectral analysis

There was no strong evidence of spectral powers of heart rate variability acting as marker of white matter lesion and cognitive outcomes. Low and high frequency spectral powers were significantly associated with CAMCOG attention subscore at year 2 only, but the regression

coefficient indicated this relationship was a shallow slope. There were no other significant associations as hypothesised for heart rate variability and baroreflex sensitivity. But there was a significant association between increasing high frequency power and prolonged number vigilance and choice reaction at year 2. This result is contrary to expectations and one suspects this is a chance finding.

14.8.3 Blood pressure variability

One of the main findings from this investigation is the ability for systolic blood pressure variability to independently predict cortical white matter lesion volume. Twenty-four hour and daytime systolic blood pressure variability were both significant predictor variables in a regression model for cross-sectional white matter lesion volume. This is similar to results from previous studies (Mancia, 2001; Mancia, 1996; Sander, 2000a). Our results extend this association to an older post-stroke cohort. The mechanism underlying excessive blood pressure variation may include cardiovascular autonomic dysfunction (Kario, 1997; Kohara, 1995; Pickering, 1990; Vagaonescu, 2000). Increased blood pressure variability is closely linked to impaired baroreflex sensitivity, which has also been demonstrated in our cohort (section 7.3) (Mancia, 1986; Watson, 1980). Another issue linking increased blood pressure variability and end-organ damage is that abnormal blood pressure variability persists even after treatment of hypertension, therefore increased variability may be a surrogate marker of significant midlife hypertension (Mancia, 1989).

The strength of the cross-sectional association meant the lack of longitudinal association for white matter lesion progression and cognitive decline was surprising given the reasonably strong hypothesis. In terms of methodology, there were some limitations in ambulatory blood pressure data acquisition. Sampling frequency was 30 minutes in the daytime and hourly overnight, in order to maximise participant tolerance. However optimal ambulatory blood pressure sampling is thought to be a minimum of every 20 minutes. Slower frequencies diminish repeatability and weaken the association between ambulatory non-continuous recordings and intra-arterial blood pressure variability (Bevan, 1969; di Rienzo, 1983; Palatini, 1994). Therefore our method may have weakened the ability to accurately measure blood pressure variability and reduce power to predict outcomes. The minimum data set required for ambulatory blood pressure recording was relatively low at 15 within 24 hours. This mirrored the minimum requirement for the International Database survey but did not meet more stringent standards from other sources which range from 21 to 72 measurements within 24 hours (O'Brien, 2000; Sega, 2002). In a similar manner the number of measurements obtained in the night-time phase was set at the bare minimum and the

relatively low mean number of night-time blood pressure values may have impaired ability to accurately define circadian variation in ambulatory blood pressure. Pulse pressure did not seem to hold any predictive value for white matter lesions or cognitive outcomes. This concurs with themes emerging from studies in the last 20 years which indicate that pulse pressure is not a consistent risk marker for cerebrovascular events (Amery, 1985; Benetos, 1997).

Simple mean blood pressure levels carried some predictive power for adverse outcomes although not as many as one might anticipate. Mean diastolic blood pressure was an independent predictor of cortical white matter lesion volume on baseline MRI scan. Mean systolic and diastolic blood pressure were associated with reduced executive subscore on the baseline CAMCOG assessment. Harrington et al (Harrington, 2000b) is just one study that identified reduced central processing speed in older hypertensive patients using the same CDR battery featured in this study. Hypertension is a clear risk factor for cerebrovascular disease so these findings are not unexpected.

Diurnal variation in blood pressure did not reveal many significant associations with outcomes apart from the link between increased nocturnal dip and lower CAMCOG score. Consideration is also given to other results - higher mean night-time diastolic pressure was associated with reduced rate of white matter lesion progression, and higher mean systolic pressure was associated with better CAMCOG executive subscores. Therefore in certain situations a higher blood pressure may be preferable to improve cerebral perfusion and avoid white matter ischaemia. This hypothesis is probably most relevant to higher night-time diastolic pressure which will reduce the percentage circadian variation of blood pressure. Large drop in blood pressure overnight is theoretically poorly tolerated by impaired baroreflex sensitivity.

14.9 Further studies

A small number of significant associations between RR interval and blood pressure variability with white matter lesions and cognitive impairment does provide some limited support for the hypotensive-ischaemic theory of subcortical ischaemic vascular dementia. The hypothesis has gathered much attention and further work is required to clarify the role of cardiovascular risk factors in the pathogenesis of white matter disease (Ferro and Madureira, 2002; O'Brien, 2003; Roman, 2002). The cardiovascular autonomic reflex tests and power spectral analysis of heart rate variability have not demonstrated strong and consistent links with the outcomes under investigation. Limitations of the reflex tests in the elderly appear to restrict usefulness but investigation of a younger age group may uncover a role in cerebral ischaemia. Strength of autonomic tests as reliable risk markers could also be enhance by repeated measures of the reflex

tests. Cerebral autoregulation is an attractive risk marker in the hypothesis and this has not been addressed in this study. Future studies combining cardiovascular autonomic reflexes and cerebral autoregulatory function could determine which plays the key role in pathogenesis of white matter disease. Ambulatory blood pressure is clearly an important risk marker that also has relevance for current clinical practice. Studies evaluating treatment of abnormal long term variability of ambulatory blood pressure will prove valuable as this parameter emerges as a potent predictor of adverse outcome. Finally a longer interval for brain imaging and cognitive assessment may uncover more pronounced structural and functional changes in older stroke patients that are related to perturbation of heart rate and blood pressure variability.

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