PATTERNS OF PROTEIN CONSUMPTION THROUGHOUT ADULTHOOD AND PHYSICAL CAPABILITY IN LATER LIFE

A thesis submitted to the Newcastle University for the degree of Doctor of Philosophy

By

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Abstract

Sarcopenia is a geriatric syndrome characterised by low muscle mass and low muscle function, caused by an imbalance between muscle protein synthesis and degradation. It has a multifactorial aetiology, but primary causes include a sedentary lifestyle and poor nutrition. Sarcopenia is associated with an increased risk of disability and mortality. This project aimed to test the hypothesis that adulthood patterns of protein consumption influence physical capability in later life.

Dietary and physical capability data were obtained from the MRC National Survey of Health and Development, a British birth cohort comprising ~5000 individuals born in 1946. Dietary data were collected by 5d food diary in 1982, 1989 and 1999 when participants were 36, 43 and 53 y. Hand grip strength, chair rise time and timed up and go were measured in 2006/10 when participants were 60/64 y. Anthropometric, physical activity and socioeconomic variables were also provided. Using data for those participants who provided dietary information in all years, relationships between adulthood patterns of protein consumption and measures of physical performance were investigated using hierarchical linear regression.

Concurrent measures of height, body composition and abdominal circumference were the strongest determinants of hand grip strength in males. In females, health status was also predictive. Health status, abdominal circumference and physical activity were predictive of chair rise time in males and females. In sensitivity analyses, low protein consumption in males was associated with a significantly poorer performance. Health status was the strongest determinant of timed up and go performance in males and females. In sensitivity analyses, low protein consumption in males was associated with a significantly poorer performance and socioeconomic position became significant.

In this cohort, protein consumption was high. After excluding predicted misreporters, protein intakes averaged 1.2 g/kg/d. Meanwhile rates of obesity/abdominal circumference increased significantly, accompanied by declining levels of physical activity.

This work is dedicated to my daughter, Imogen Charlotte Irene Munro

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List of Abbreviations

aLM	Appendicular lean mass
ABC	(Health) Ageing and body composition (Study)
ADL	Activities of daily living
AGEs	Advanced glycation endproducts
ATP	Adenosine triphosphate
BCAA	Branched-chain amino acid
BMC	Bone mineral content
BMI	Body Mass Index (kg/m^2)
BMR	Basal metabolic rate
BP	Blood pressure
CG	Cytosine-guanine
CHO	Carbohydrate
COPD	Chronic obstructive pulmonary disorder
CRF	Clinical Research Facility
CRP	C-reactive protein
CRT	Chair rise time
CT	Computed tomography
CVD	Cardiovascular disease
CHD	Coronary heart disease
CSA	Cross-sectional area
DALYs	Disability adjusted life years
DEXA/DXA	Dual energy X ray absorptiometry
DIDO	Diet In Data Out
DLW	Doubly (deuterium) labelled water
DoH	Department of health
DNA	Deoxyribonucleic acid
DIY	Do It Yourself
EAA	Essential amino acids
ELSA	English Longitudinal Study of Ageing
ESRC	Economic and Social Research Council
EAR	Estimated average requirement
EI	Energy intake

FSR	Fractional synthetic rate
FFM	Fat free mass
FSA	Food Standards Agency
FFQ	Food frequency questionnaire
GLM	General Linear Model
HCS	Hertfordshire Cohort Study
HAS	Hertfordshire Ageing Study
HDL	High density lipoprotein
HLE	Healthy life expectancy
HGS	Hand grip strength
HSE	Health Survey for England
IL-6	Interleukin-6
IHD	Ischemic heart disease
LE	Life expectancy
LEP	Leg extensor power/Lower extremity performance
LHA	Lifelong health and ageing
MAP	Mean arterial (blood) pressure
MET	Metabolic equivalent
MMSE	Mini-Mental State Examination
MPS	Muscle protein synthesis
MPSS	Muscle protein synthesis score
MRC	Medical Research Council
MetS	Metabolic syndrome
mTOR	Mammalian target of rapamycin
NSDS	National Child Development Study
NIH	National Institutes of Health
NDNS	National Diet and Nutrition Survey
NSHD	MRC National Survey of Health and Development
NHANES	US National Health and Nutrition Survey
NHS	National Health Service
NMJ	Neuromuscular junction
ONS	Office of National Statistics
PA	Physical activity

PAL	Physical activity level
PPTE	Protein as a percentage of daily energy
RE	Resistance exercise
RNI	Reference nutrient intake
RMA	GLM Repeated Measures Analysis
RA	Regression Analysis/es
ROS	Reactive oxygen species
RCT	Randomised controlled trial
SPPB	Short physical performance battery
SOD	Superoxide dismutase
TAG	Triglycerides
TE	Total energy
TEE	Total energy expenditure
TEI	Total energy intake
TUG	Timed up and go test
TFR	Total fertility rate
TNF-α	Tumour Necrosis Factor-α
UAMA	Upper arm muscle area
USDA	United States Department of Agriculture
VIF	Variance Inflation Factor (for multicollinearity)
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist hip ratio

CHAPTER 1

Introduction

1.1 An ageing society

1.1.1 Demography

The term ‘demographic transition’ refers to the societal shift from high birth and death rates to low birth and death rates. In most Western societies, considerable and unique challenges are now posed by the consequence of this relatively recent transition – a rapidly ageing population. The term ‘second demographic transition’ explains the phenomenon of declining *future* fertility and Europe is the continent with the lowest total fertility rate (TFR). In England and Wales the average completed family size for women born in 1966 was 1.91 children per woman compared to 2.36 children per woman born in 1939. The TFR in 2011 was 1.93 children per woman [ONS].

In Biodemography of Human Ageing (Vaupel, 2010) observes that death is being delayed because people are entering older age in better health. Personal behaviour is crucial in achieving a long life (compared with one’s contemporaries) but the general level of population longevity is determined by medicine and prosperity. Postponement of senescence in the future will depend on improving the health of older and younger people.

An ageing population refers to both the increase in average age of the population and an increase in the number and proportion of older people in the population [ONS, 2012]. The average age of the UK population in 1985 was 35.4 years, in 2010 it was 39.7 y and by 2035 it is projected to be 42.2 years. However, in terms of the provision of health, pension and social care resources, it is the increase in the number and proportion of much older people in the population that will present the most considerable challenges to society. Those aged ≥ 65 y (the Young-Old) accounted for 15% of the total UK population in 1985, this increased to 17% in 2010 and by 2035 is projected to reach 23% of the total UK population. Similarly, those aged 85 y or older accounted for only 1% of the UK population in 1985, increasing to 2% in 2010. By 2035 this group – the oldest old – are projected to account for 5% of the total UK population, numbering ~3.5 million.

Drawing upon research in the Newcastle 85+ Study, a cohort of 800 individuals all born in the North East of England in 1921 (Collerton *et al.*, 2007) projections for the next 20 y suggested substantial increases in the number requiring 24 h care due to population ageing and a proportionate increase in demand for care-home places (Jagger *et al.*, 2011). 'Apocalyptic demography' – the portrayal of population ageing as a financial burden – was found to be widespread in the Economist, an influential weekly magazine. The negative portrayal of older people as 'frail non-contributors' rather than as a benefit to society or scientific advance may negatively shape the attitudes of economic and political opinion formers (Martin *et al.*, 2009).

1.1.2 Reasons for population ageing

Population ageing can explained by a combination of factors, including past declines in fertility rates, past improvements in mortality rates among children and young adults and continuing improvements in mortality rates at the oldest ages. Medical and social advancement, sanitation and immunisation have also greatly reduced the impact of most common communicable diseases reducing premature mortality. While antibiotic resistance may pose a serious health risk for the future, the greatest risk posed to the health of society today lie in chronic, non-communicable diseases the single most significant risk factor for which is age.

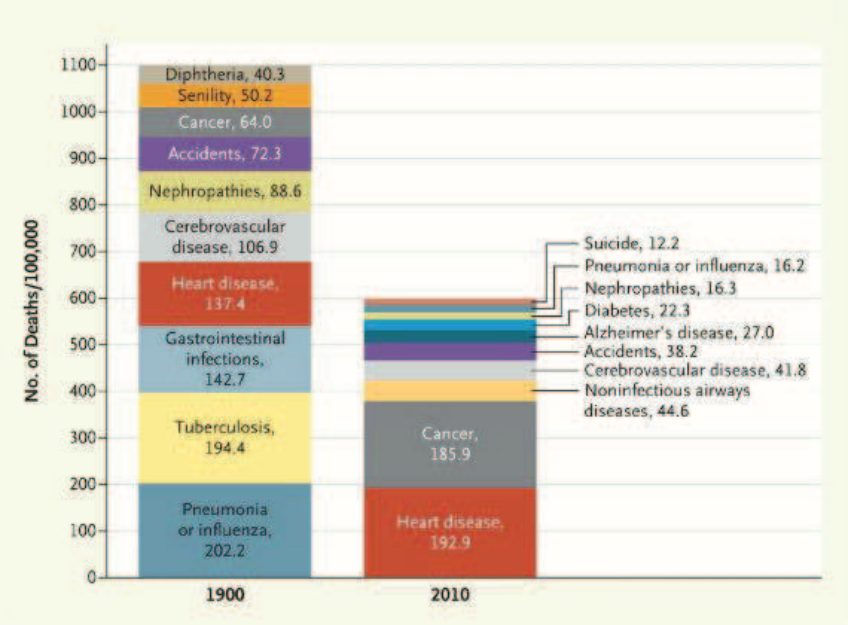
A rapidly ageing population presents considerable challenges to governments and society in terms of public spending and the provision of scarce resources. Pension and health care provision – both NHS and (long term) social care – have recently been amended and will undoubtedly face further necessary structural changes into the future. What was affordable in the past is now no longer seen as affordable and the allocation and provision of societal resources must reflect increasing longevity.

One of the most significant challenges of population ageing is the increase in the number of people with health needs in later life as those over the age of 65 years account for the highest activity and spend across primary and secondary care. [http://www.nhs.uk/NHSEngland/NSF/Pages/Olderpeople.aspx][accessed 29 5 13] This is entirely to be expected as age is the single, greatest risk factor for many (if not all) common chronic diseases. In addition to population ageing, society has also experienced a rapid increase in rates of obesity and its associated conditions, driven by chronic overconsumption of energy and falling levels of physical activity. Diet and physical inactivity accounted for 14.3% of UK disability-adjusted life-years in 2010 (Murray *et al.*, 2013a)

1.1.3 Age-related chronic disease

Over thirty years ago in 1980, James Fries observed (Fries, 1980) that chronic age-related disease had already replaced acute illness and infection as the biggest health threats to society. This 'epidemiologic transition' was graphically depicted by (Jones *et al.*, 2012):

Figure 1.1 Top 10 causes of death, 1900 compared to 2010



In the space of ~100 years, cancer and heart disease, which once accounted for 64 and 137.4 deaths, respectively per 100,000 accounted for 186 and 193/100,000 in 2010. Influenza and pneumonia, once the leading causes of death in 1900 (accounting for 202.0 per 100,000 death) were 9th in 2010, accounting for 16.2 per 100,000.

1.1.4 Healthy life expectancy

The number and proportion of people living into very old age is increasing, but increasing life expectancy is not always accompanied by good health. In 2008/10, in the UK at birth, males and females could expect to spend more than 80% of their lives in good or very good health – this is termed healthy life expectancy (HLE)(ONS). For males and females, life expectancy (LE) – an estimate of average expected life span – was 78.1 and 82.1 years, and healthy life expectancy 63.5 and 65.7 years, respectively. Males and females, therefore, on average could expect to spend 14.6 and 16.4 years of life in *poor* health, respectively.

Table 1.1 Life Expectancy (LE) and Healthy Life Expectancy (HLE) in the UK at birth

	2005/07			2008/10		
	LE (y)	HLE (y)	HLE as a proportion of LE (%)	LE (y)	HLE (y)	HLE as a proportion of LE (%)
Males	77.2	61.4	**79.6**	78.1	63.5	**81.4**
Females	81.5	62.9	**77.2**	82.1	65.7	**80.0**

Notwithstanding that HLE as a proportion of LE increased significantly for males and females over the period 2005/07 – 2008/10, UK performance against comparable societies such as other European countries, Australia and Canada, is poor. In analysis undertaken at the Institute for Health Metrics and Evaluation (Murray *et al.*, 2013a) the UK ranked 12th out of 19 countries of similar affluence (the EU15+). The UK performed significantly worse than the EU15+ for age-standardised death rates, years of life lost rates and life expectancy in 1990 and by 2010 its relative position had worsened. In 2010 cf. the EU15+ the UK had significantly higher rates of age-standardised years of life lost from ischaemic heart disease, COPD, lower respiratory infections, breast cancer, other cardiovascular and circulatory disorders, oesophageal cancer, preterm birth complications, congenital anomalies and aortic aneurysm. The research concluded that as years lived with disability per person, by age and gender had not changed substantially from 1990 to 2010 but age-specific mortality had fallen, the importance of chronic disability was rising.

Major causes of years lived with disability in 2010 were musculoskeletal disorders (30.5% of years lived with disability) and mental/behavioural disorders (21.5%). Tobacco, increased blood pressure and a high BMI (kg/m^2) were the leading risk factors for Disability-Adjusted Life-Years (DALYs). In the United States, poor diet and low levels of physical activity are the leading cause of DALYs with high BMI in third place followed by hypertension and high fasting plasma glucose (Murray, 2013)

In 2008/10 average life expectancy at age 65 y for UK males and females was 17.8 and 20.4 y, respectively. At age 65 y (around the age of retirement) males could expect to enjoy a further 10 years of life in good health and females 11.6 years; conversely, males and females could expect to spend 7.7 and 8.8 years of life in poor health, respectively.

Table 1.2 Life Expectancy and Healthy Life Expectancy in the UK at 65 y

	2005/07			2008/10		
	LE (y)	HLE (y)	HLE as a proportion of LE (%)	LE (y)	HLE (y)	HLE as a proportion of LE
Males	17.2	9.9	**57.5**	17.8	10.1	**56.8**
Females	19.9	10.9	**55.0**	20.4	11.6	**56.8**

Figures suggest that the trend for HLE as a proportion of LE is different for males and females. Over the period 2005/07 – 2008/10 there was an (insignificant) decrease in HLE as a proportion of LE for males at 65 y whereas for females HLE as a proportion of LE increased significantly.

Chronic age-related disease has replaced acute illness and infection as the major health threat to society and the importance of chronic disability is increasing, but what are the causes and origins of age-related chronic disease?

1.1.5 Lifecourse origins of age-related chronic disease

As the word chronic suggests, the most common causes of death have a multifaceted and complex aetiology, characterised by the prolonged presence of multiple/coexisting risk factors and lifecourse insults. Chronic disease originates early in life and develops slowly and insidiously over decades, ultimately resulting in persistent illness, disability and mortality. ONS 2011 statistics confirm that cancers and cardiovascular diseases remain the most common cause of death in England and Wales. Of 484,367 registered deaths in England and Wales, the leading causes were:

	Male	Female
Heart diseases	1 (16.1% of deaths)	1 (10.7% of deaths)
Lung cancer	2 (7.2%)	5 (5.3%)
Stroke	3 (6.1%)	3 (8.7%)
Chronic Resp. diseases	4 (5.8%)	-
Dementia & Alzheimer's	5 (5.1%)	2 (10.3%)
Flu & pneumonia	-	4 (6%)

Cancers were responsible for 30% of all registered deaths (2,023 deaths per million in the male population) and 1,478 deaths per million (in the female population). Cardiovascular (circulatory) disease accounted for 29% of all deaths, respiratory diseases (e.g. pneumonia/ COPD) 14% of deaths and dementia/ Alzheimer's 5.1% of deaths in men and 10.3% in women.

NHS Choices [accessed 21 5 13]
http://www.nhs.uk/news/2012/11November/Pages/Changes-to-trends-in-disease-related-deaths.aspx

Findings from the INTERHEART study suggest that nine modifiable risk factors explain most of the risk of myocardial infarction worldwide *viz.* hypertension, smoking, abdominal obesity, diet, physical activity, diabetes, alcohol intake, psychosocial factors and apolipoproteins (Anand *et al.*, 2008). There is considerable commonality in significant risk factors for all stroke: a history of hypertension, current smoking, abdominal obesity/waist-to-hip ratio[1], diet risk score[2], [3], regular physical activity, diabetes mellitus, alcohol intake[4], psychosocial stress and depression, cardiac causes and ratio of apolipoproteins B to A1. Collectively, these factors accounted for 88% of the population-attributable risks for all stroke (O'Donnell *et al.*, 2010).

Evidence such as this appears to suggest that lifestyle factors operating only in adulthood explain the increasing incidence and prevalence of age-related chronic disease. However, adverse environmental influences that operate in adult life to 'accelerate' normal ageing processes, do not fully explain interindividual variability in longevity (Barker, 2012). In terms of the 'new developmental model for the origins of chronic disease', malnutrition and other adverse influences operating during foetal development alter gene expression and slow growth. Insufficient resources during developmental periods disproportionately affect organs lower down the hierarchy (e.g. kidney and lungs cf. the brain) resulting in reduced function. Ultimately, this confers a vulnerability to later life environmental insults and a programmed predisposition (or greater susceptibility) to age-related disease.

In a systematic review of 18 observational studies including ~150,000 people, the strength and consistency of the observed relationship between birth weight and ischemic heart disease in later life was investigated (Huxley *et al.*, 2007); a 1 kg increase in birth weight was associated with a 10 – 20% lower risk of later life IHD.

[1] BMI was not associated with stroke
[2] Increased consumption of fruit and fish (but not vegetables) was associated with reduced risk
[3] Associated with increased risk, increased consumption of red meat, organ meats, eggs, fried foods, pizza, salty snacks and cooking with lard
[4] Alcohol intake has a J-shaped relation with ischaemic stroke but is associated with a graded increased risk of intracerebral haemorrhagic stroke

In a systematic review and meta-analysis of 57 studies published between 1989 – 2007 (Xue and Michels, 2007) the intrauterine environment was held to contribute to female predisposition to breast cancer; increased risk was associated with increased birth weight and length and higher maternal and paternal age. In a systematic review and meta-analysis (Risnes *et al.*, 2011) a moderate inverse association of birthweight with adult all-cause mortality was found – a 6% lower risk per kilogram increase in birthweight, but there was a stronger inverse association with cardiovascular mortality (a 12% lower risk per kg increase in birth weight). Conversely, a strong association of higher birthweight with increased risk of cancer death was observed in males (13% increased risk per kilogram of birthweight); this association was weaker (4% per kg) for females.

The lifecourse approach to chronic disease epidemiology is defined as the study of the long-term effects on chronic disease risk of physical and social exposures during gestation, childhood, adolescence, young adulthood and later adult life. Biological, social and socio-biological pathways between exposures, intermediaries, confounders and outcomes are temporally interlinked and interrelated; crucially, insults are accumulated across the lifecourse (Ben-Shlomo and Kuh, 2002).

The World Health Organisation differentiates between four conceptual models of the life course [The implications for training of embracing A Life Course Approach to Health. World Health Organisation, 2000. WHO/NMH/HPS/00.2 (Accessed 22 November, 2012)]:

1. A critical period model
2. A critical period model with later effect modifiers
3. Accumulation of risk with independent and uncorrelated insults
4. Accumulation of risk with correlated insults (clustering, chains or pathways of risk)

Intrauterine programming and/or environmental influences during intrauterine life were considered responsible for the significant and positive association between birth weight and DXA-determined adult whole body bone and lean mass in 143 Sheffield-residents at 70 – 75 y. Associations between birth weight and whole body fat were weaker and insignificant – adult lifestyle factors appeared here to be more important (Gale *et al.*, 2001)

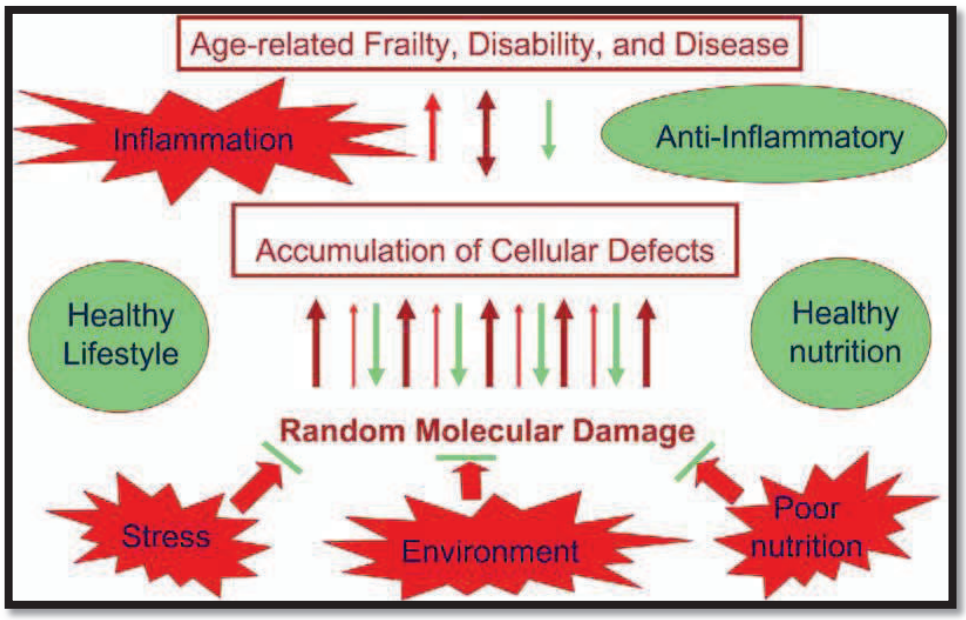
In the Hertfordshire Cohort Study, ~600 participants born 1931 – 1939, size at birth was found to be associated with measured forearm and calf muscle size (Sayer *et al.*, 2008a) and grip strength in men and women (Sayer *et al.*, 2004) after adjustment for adult height and weight. However, adult lifestyle factors, particularly those affecting body weight were thought to be more important than developmental influences on most measures of physical performance and physical activity in this cohort (Martin HJ, 2009). In ~2800 participants of the National Survey of Health and Development (the 1946 British Birth Cohort) birth weight and prepubertal height gain were associated with midlife (53 y) grip strength (Kuh *et al.*, 2006b). Early weight gain (before 7 y) in males only was positively related to their performance at standing balance and chair rise time at 53 y (Kuh *et al.*, 2006a) independently of adult body size, social class, habitual physical activity and health status.

1.2 The ageing individual

1.2.1 The biology of ageing

Multiple theories compete to explain and elucidate the processes underlying human ageing. In Understanding the Odd Science of Ageing, (Kirkwood, 2005) explains that part of the oddity is in dismantling common preconceptions about why ageing occurs, principally that it is a programmed event. Secondly, that ageing remains inherently complex notwithstanding recent scientific advancements in experimental investigative techniques.

Figure 1.2 Damage and ageing (Kirkwood, 2005)



Ageing results from the accumulation of unrepaired cellular damage due to evolved limitations in somatic maintenance and repair – the disposable soma theory ((Kirkwood, 1977). Damage is stochastic, but its rate of accumulation depends on the ability of the organism to eliminate and repair damage. As cellular damage accumulates (often accompanied by inflammation) this eventually manifests as age-related disease, disability and frailty but the ageing process is plastic and amenable to modification – nutrition and lifestyle can either accelerate or slow the accumulation of cellular damage (Kirkwood, 2005).

In DNA damage, aging and cancer the author (Hoeijmakers, 2009) describes how aging and cancer both result from DNA injury – whether by exogenous or endogenous sources. An elaborate genomic maintenance apparatus, comprising multiple repair systems, exists. Defective repair processes have been identified that result in specific (cancer and non-cancer) diseases. When repair processes fail, the result may be cancer or cell death (apoptosis) or senescence, a state of irreversible replicative quiescence.

The cellular consequences of ageing, in particular the accumulation of damage in stem cells may play a critical role in ageing (Jones and Rando, 2011).

The free radical theory (Harman, 1956) describes how ageing and degenerative diseases are attributable to the attack of free radicals on cell constituents and connective tissues. The mitochondria is the cellular organelle responsible for the production of adenosine triphosphate (ATP) from dietary nutrients, a process known as oxidative phosphorylation. Throughout this process there is leakage of free electrons. Unbound and unstable, these reactive oxygen species (ROS) e.g. superoxide, hydrogen peroxide and the hydroxyl radical, indiscriminately cause damage to nearby cellular structures, e.g. lipid membranes, cellular proteins (and amino acids) and nuclear and mitochondrial DNA. Oxidative phosphorylation is responsible for the vast majority of ROS generated, but other sources include chronic inflammation/ infection and exposures to toxins such as cigarette smoke, drugs, alcohol and pollution. Health and lifestyle therefore operate to add to or diminish the oxidative load. Where an imbalance persists between oxidant production and antioxidant activity – i.e. where there is persistent loss of redox homeostasis – damage inflicted at the cellular level accumulates, eventually affecting structure and function at a tissue and organ level and ultimately manifesting as morbidity.

1.2.2 Antioxidant capacity

Endogenous antioxidant mechanisms operate to minimise damage by 'mopping up' excessive ROS, e.g. superoxide dismutase (SOD). This enzyme catalyses the neutralisation/deactivation of superoxide. Other antioxidant enzymes are glutathione peroxidase and catalase. In addition to endogenous antioxidant capability, dietary nutrients may provide supplementary exogenous antioxidants – water and fat soluble vitamins, e.g. vitamins C and E, beta carotene and lycopene provide additional ROS scavenging capacity. Dietary micronutrients i.e. selenium, iron and zinc are required to provide essential cofactors for antioxidant enzymes. A diet, rich in fruit and vegetables may therefore enhance the body's inherent antioxidant capacity, whereas a diet deficient in these vitamins would not. Similarly a diet lacking in essential micronutrients (e.g. selenium and manganese) may operate to impair enzymatic antioxidant processes (e.g. glutathione peroxidase and SOD).

There is an age-related decline in the processes that would ordinarily repair or eliminate oxidative damage (Langie *et al.*, 2012) one consequence of which is that the elderly may need to consume more antioxidants in order to counteract increased oxidative stress and to compensate for a reduced enzymatic antioxidant defence (Pae, 2012). Paradoxically, most studies which have attempted to boost antioxidant defences by supplements of 'antioxidant' micronutrients (e.g. selenium) have not shown health benefits whilst others have shown adverse effects (Bjelakovic G, 2008; Rees K, 2013).

In 643 older (mean age 77.3 y) community dwelling female participants of the Women's Health and Aging Study I, lowest quartile intakes of vitamins B_6 B_{12} and selenium were predictive of incident disability in activities of daily living after 3 years of follow up (Bartali *et al.*, 2006b). The role of low micronutrients (antioxidants and vitamins) as cross-sectional and longitudinal correlates of mobility disability was consistent with a growing number of studies showing that a diet rich in fruit and vegetables has a beneficial role in healthy ageing (Milaneschi Y, 2010).

Weight loss, a reduction in total energy intake and a reduction in the intake of specific nutrients are associated with the age-related changes in body composition and physical function characteristic of the transition from independence to disability in older adults (Inzitari *et al.*, 2011). Undernutrition in the elderly – low intakes of protein, certain vitamins, micronutrients and antioxidants – have all been associated with negative functional outcomes. Intervention studies using nutritional supplementation continue to show inconclusive results in the prevention of functional impairment and disability, however these results are complicated by several factors. Variability in dose, supplementation with mixed nutrients, compensatory reduction of dietary intake during supplementation and ultimately by the fact that people eat meals, not single nutrients or foods. Dietary patterns should be studied and randomised clinical trials should mimic 'real world' situations; objective measures of physical performance should be primary outcomes and not nutritional status or anthropometrics (which are intermediate outcomes) (Inzitari *et al.*, 2011).

1.2.3 Immunosenescence and inflammation

Innate and adaptive (T cell and B cell) systems comprise the human immune function and there are striking age-related defects and decline in T cell function. B cell-mediated humoral immune responses are also believed to be compromised during aging. Innate immune responses are diminished in ageing, while some are unchanged or elevated – the term 'dysregulation' fails to fully describe this phenomenon. While many aspects of immune function decline with aging some become *overactive* e.g. increased autoantibody production or an upregulated inflammation state. There is considerable heterogeneity in immunosenescence owing to the interaction of genetics, environment, lifestyle and nutrition (Pae, 2012). The age-related, chronically upregulated inflammation state, is often denoted by the term *inflammaging*; higher peripheral levels of inflammatory cytokines and acute-phase reaction proteins from the liver e.g. CRP cf. young subjects. This inflammation state has been implicated in the pathogenesis of several common and disabling diseases most of which have a clear connection to advancing age including CVD, type 2 diabetes, Alzheimer's, Parkinson's, osteoporosis and rheumatoid arthritis.

Higher plasma concentrations of IL-6 and TNFα were associated with lower muscle mass and lower muscle strength in 3075 well-functioning older participants of the Health ABC Study. Total body fat (included as a potential confounder) was positively correlated with cytokine levels, especially in women (Visser *et al.*, 2002b). Consistent associations between TNFα and 5 y decline in muscle mass and strength were explained in terms of increased muscle catabolism – by direct stimulation of protein loss and the alteration of muscle protein so as to reduce force production (Schaap *et al.*, 2009).

Higher circulating levels of IL-6 attributable to muscle atrophy and/or its role in disease, predicted disability onset in older persons (Ferrucci L, 1999) and higher circulating levels of IL-6 and CRP were associated with mortality in 1293 healthy nondisabled participants of the Iowa 65+ Rural Health Study, followed prospectively for a mean of 4.6 y (Harris *et al.*, 1999). Human aging was shown to be associated with heightened muscle inflammation susceptibility – a higher basal state of proinflammatory signalling; the authors (Merritt *et al.*, 2013) suggest that this contributes to the impaired regenerative capacity of older skeletal muscle.

In the evolution of the human lifespan (Finch, 2010) observes that the human diet has shifted to increased consumption of animal tissue. These are linked to increased ingestion of trace metals, fat and pathogens and (when cooked), advanced glycation endproducts (AGEs) which are diabetogenic and proatherosclerotic. The apolipoprotein E alleles (*ApoE*) is proposed as a 'meat-adaptive candidate gene' with a range of pleiotropic effects, i.e. clearance of triglyceride-rich lipoproteins from the blood but accelerated degenerative changes in arteries and brain and greater risk of CHD and Alzheimer's disease – all of which are characterised by a heightened immune/inflammatory response. It is suggested that this extends the antagonistic pleiotropy theory of aging (Finch, 2010).

1.2.4 Metabolic stress

Ageing is associated with loss of metabolic homeostasis perhaps best illustrated by a description of the Metabolic Syndrome (MetS) – a cluster of metabolic/ biochemical processes exhibiting various degrees of dysregulation – the risk of which increases with AGE. The presence of three or more of the following warrants a diagnosis of metabolic syndrome: central obesity, elevated TAG (hypertriglyceridemia), reduced HDL cholesterol, hypertension, and elevated fasting glucose/insulin resistance (hyperglycaemia). This group of risk factors increases the risk of heart disease, diabetes and stroke. Chronic low-grade systemic inflammation is also believed to be implicated in the amplification of this condition.

1.2.5 Epigenetics

Epigenetics describes the modification of the genome without changing the underlying genetic DNA code – the modification and maintenance of gene activity states. The most studied epigenetic mechanisms are DNA methylation and histone modification both of which take place in the nucleus. DNA methylation describes the addition of a methyl group to the cytosine molecule of a cytosine-guanine (CG) dinucleotide. High concentrations of repeating CG dinucleotides are known as CpG islands. Hypermethylation of gene promoter areas silence gene transcription as methylation prevents transcription factor binding to the promoter, whereas hypomethylation is associated with gene transcription.

Aging is associated with gene specific hypermethylation (gene silencing) and global (organism-wide) hypomethylation which may cause genomic instability. Reversible histone (protein) modification can be by methylation, acetylation, phosphorylation or ubiquitination. These tags or motifs covalently attach to specific amino acids e.g. arginine methylation. The attachment of these motifs promotes an open chromatin structure which facilitates gene transcription whereas a closed chromatin structure presents a physical barrier to the enzymes and regulatory factors required replication, transcription and repair (Mendez-Acuna L, 2010). Epigenetic modification is plastic and amenable to change by nutrition *in utero* and throughout life. Aberrant epigenetic patterning may switch off genes that protect and repair the genome or switch on genes which operate to facilitate metabolic dysregulation or disease (Sawan and Herceg, 2010).

1.2.6 Healthy ageing

What is apparent from the preceding discussion is that the ageing process is plastic and highly amenable to the influence of lifestyle factors, in particular nutrition and physical activity. What defines healthy ageing is arguably highly subjective, although commonality in factors does exist. It is generally thought of as the maintenance and preservation of functional independence (personal autonomy), vigour, mobility, cognition and social participation, and the absence of disease and disability.

In a meta-analytic review (Holt-Lunstad *et al.*, 2010) including data from 308,849 individuals followed up for ~7.5 y, individuals with adequate social relationships had a 50% greater likelihood of survival compared to those with inadequate or poor social relationships – the effect was comparable with smoking cessation and exceeded the more well-known risk factors for mortality of obesity and physical inactivity. Lowry (Lowry KA, 2012) describes successful ageing as a continuum of functional independence, a multidimensional construct that could be viewed as a continuum of achievement including aspects of mobility and social participation and not only the presence or absence of disease.

Recent physiologic studies on well-characterised groups of old people show the adaptive capacity of various organ-systems with age; along with physical ability, maintenance of cognitive function is considered a key component in the definition of successful ageing. Lifestyle can modify outcomes of ageing – nutrition improves immune status and physical activity, functional performance. Individuals surviving in very good health are not mere examples of passive survival but biological outcomes of the adaptive capacity these systems (Vallejo, 2012).

1.2.7 Physical frailty

As an individual ages, comorbidities may cluster and the individual may become frail. Frailty encompasses physical, physiological, social and psychological aspects previously defined as a clinical syndrome or ageing phenotype in which three or more of the following are present: unintentional weight loss, self-reported exhaustion, weakness (as evidenced by poor grip strength), slow walking speed and low physical activity. In 5317 participants of the Cardiovascular Health Study aged ≥ 65 y, the frailty phenotype was predictive of falls, worsening mobility or ADL disability and death (Fried *et al.*, 2001).

Frailty is commonly characterised by the loss of physiological reserve which is analogous to organ reserve – defined as the ability of the stressed organism to restore homeostasis after perturbation (Fries, 1980). When organ reserve is lost and homeostasis cannot be restored, death is inevitable. Frailty is not a specific medical disease, but is evident over time through an excess vulnerability to stressors with a reduced ability to maintain or regain homeostasis after a destabilising event (Walston *et al.*, 2006). In older adults there is a 'spectrum of resilience' from most frail (in the presence or absence of disease) to robust and highly independent.

Frailty, affecting both musculoskeletal (sarcopenia and osteoporosis) and non-musculoskeletal systems, results from reaching a threshold of decline across multiple organ systems. Purported contributory mechanisms include chronic low-grade inflammation (proinflammatory cytokines and CRP), increased biomarkers of coagulation and fibrinolysis, hormonal changes, vitamin D deficiency and obesity (Gielen *et al.*, 2012).

Using data from the Newcastle 85+ Study the importance of inflammatory markers (IL-6, TNF-α and CRP) previously established in the younger-old were confirmed in the very old (Collerton *et al.*, 2012).

Moderate physical activity can be of substantial benefit to frail older people and regular leisure activities, such as walking and gardening can provide considerable benefits. Increasing physical activity can reduce systemic concentrations of proinflammatory biomarkers, improve sarcopenia, physical and cognitive function and mood (Landi *et al.*, 2010). In 802 participants (mean aged 74.1 y) of the InCHIANTI study (Bartali *et al.*, 2006a) low energy consumption was significantly associated with frailty. Low energy-adjusted intakes of protein, vitamins D, E, C and folate were also significantly and independently associated with frailty, as defined by (Fried *et al.*, 2001)

1.3 Physical capability

Physical capability refers to the muscle strength and functional capacity that enable us to perform the tasks of everyday living. It is a reflection of musculoskeletal and neuromuscular health. Bone health is beyond the ambit of this work and this dissertation focuses on physical capability as a reflection of muscular and neuromuscular structure and function.

1.3.1 Muscle structure

1.3.1.1 Muscle physiology

Skeletal muscle is the largest organ in the human body (Pedersen and Febbraio, 2012). It is striated tissue which attaches to bone by tendons enabling body movement. The myofibre is the smallest 'complete contractile system' – a single multinucleated muscle cell. Myofibres comprise myofibrils – chains of proteins (actin and myosin myofilaments) whose shortening and lengthening movement produce force. Their lattice arrangement, within repeated sarcomere bands, produce the striations characteristic of skeletal muscle. Skeletal muscle comprises bundles of myofibres enveloped first by fascicles into muscle fibres; these fibres are then formed into larger bundles by perimysium and finally into complete and distinct muscles by an outer wrapping (the epimysium) which assumes a variety of shape and size dictated by location and function.

Muscle fibres are classified as slow (type I) and fast (type II) by the type of myosin present and principal type of metabolism; slow (Type I) are characterised by long twitch times (slower contraction velocity), lower peak force and a higher resistance to fatigue. They are high in oxidative enzymes (high oxidative capacity[5]) but low in glycolytic[6] markers. There are 3 types of fast (Type II) fibre; fatigue resistant; fast fatiguable and fast intermediate. Ageing is associated with a net conversion of type II fibres (which tend to be larger) to type I which are smaller – resulting in the observed age-related loss of muscle mass/ muscle CSA (Deschenes *et al.*, 2010).

Substantially smaller type II muscle fibre size in a group of elderly men (mean age 71 y) compared with their younger (mean age 23 y) counterparts, fully explained the group difference in quadriceps cross-sectional area. Prolonged, resistance type exercise over a period of 6 months resulted in an 24% increase in type II fibre size, in these elderly men (Nilwik *et al.*, 2013).

1.3.1.2 Neuromuscular structure

The neuromuscular junction (NMJ) allows communication between motor neurons (neural cells) and muscle fibres – it is the site of the transduction of electrical stimuli generated by the nervous system to the muscle fibre, resulting in muscle action (Deschenes MR, 1994). Age-related denervation of myofibres at the NMJ were found to precede the fibre atrophy characteristic of sarcopenia and this could be delayed with high amounts of neuromuscular activity (Deschenes *et al.*, 2010). Loss of muscle mass and strength is attributable to the progressive atrophy and loss of individual muscle fibres associated with the loss of motor units. This is accompanied by a reduction in muscle quality due to the infiltration of fat and other non-contractile material (Ryall *et al.*, 2008).

[5] Derives energy from fatty acids / dependent on oxygen
[6] Glycolytic – derives energy from glucose either anaerobically (in the cytoplasm) or aerobically (in the mitochondria)

1.3.1.3 Intramuscular lipid

Skeletal muscle fat exists as extramyocellular lipid contained in adipocytes embedded between muscle fibres and intramyocellular lipid, droplets of triglyceride, formed on muscle cell membranes. Fatty infiltration of muscle (myosteatosis) was observed to increase with age and was associated with reduced muscle mass, muscle strength, physical performance (SPPB) and increased risk of hip fracture (Lang *et al.*, 2010). Fat infiltration of muscle was predictive of clinical fracture in older adults (Schafer *et al.*, 2010). In the Health, Aging and Body Composition Study, lower extremity performance (LEP) in men and women (70 – 79 y) was measured by 6 m walk and chair stands. Smaller midthigh muscle area and greater muscle fat infiltration were associated with poorer physical performance. Reduced muscle attenuation (fat infiltration) was associated with poorer LEP independently of total body fat and muscle area (Visser *et al.*, 2002a) and muscle attenuation and muscle strength independently predicted mobility limitation (Visser *et al.*, 2005).

1.3.2 Muscle function

1.3.2.1 Metabolic function

Skeletal muscle is the main target tissue of insulin and the age-associated loss of muscle mass (sarcopenia) is associated with adverse glucose metabolism (insulin resistance and susceptibility to diabetes) (Srikanthan *et al.*, 2010). Many age-related diseases (metabolic syndrome, cancer, Alzheimer's and Parkinson's disease) are associated with the functional status, metabolic demand and mass of skeletal muscle (Demontis *et al.*, 2013). Loss of contractile tissue is associated with increased risk of type 2 diabetes, osteoporosis and obesity (Deschenes *et al.*, 2010) and fat infiltration of muscle was higher in those with diabetes or impaired glucose metabolism cf. those with normal glucose metabolism (Schafer *et al.*, 2010).

Skeletal muscle is an endocrine organ producing and releasing cytokines (referred to as myokines). In relation to exercise, IL-6 is the first cytokine present in the circulation, whereas the classical proinflammatory cytokines (TNF-α and IL-1β) generally do not increase with exercise.

Data suggested that exercise-induced IL-6 exerted inhibitory effects on TNF-α and IL-6 and induced a delay in the increase in C-reactive protein (CRP). Exercise also provoked an increase in circulating levels of anti-inflammatory cytokines & cytokine inhibitors. The authors suggested that regular exercise may offer protection against atherosclerosis (characterised by inflammation), vascular and ultimately systemic low-grade inflammation (Pedersen and Febbraio, 2008).

1.3.2.2 Muscle performance

Muscle strength is a composite term determined by muscle mass (volume, composition, fibre number and size) and structure (e.g. fibre type and pennation angle) which determine force-generating capacity and power. Muscle force is a measure of the load applied to bone, whereas power is a measure of function (Ward, 2012). Age-related effects in calf muscle cross-sectional area (measured by CT) and muscle force and power (by jumping mechanography) were studied in relation to sedentarism (Runge *et al.*, 2004). The non-sedentary population exhibited a >50% peak force and power loss between the age of 20 – 80 without a reduction in calf muscle cross-sectional area.

1.3.2.3 Measuring muscle function

1.3.2.3.1 Composite measures

Physical capability is defined as the muscle strength and functional capacity that enables the performance of Activities of Daily Living (ADL). ADL assessment is often self-reported and subjective, rendering such data problematic when comparisons are required within a research setting over time or across diverse study designs. As such, a need was identified for objective assessments. The National Institutes of Health (NIH) Toolbox is an example of a standardised set of measures (including cognitive, emotional, motor & sensory domains) that can be used across a variety of study designs providing comparability and thus facilitating the monitoring of function over time. Pertinent to this dissertation is motor function, defined as the ability to use and control muscles and movement including dexterity, strength, balance, locomotion and endurance. The motor function component strength, which refers to the muscle's ability to generate force against a physical object, is assessed by the measure of hand grip strength, as this provides an approximation of overall muscle strength.

Another composite measure of physical performance and capability widely used in research settings is the Short Physical Performance Battery (SPPB). The battery is administered to assess lower extremity function in older people and usually comprises standing balance tests (side-by-side, semi-tandem and tandem positions), a test of gait speed and chair rise time (5 repetitions) (Guralnik JM, 1994). This research presented evidence that the SPPB provided information not available from self-reported items, in particular a gradient of risk for mortality and nursing home admission among those highly-functioning individuals who reported almost no disability.

1.3.2.3.2 Individual objective measures – grip strength

Individual objective measures of physical capability such as hand grip strength, gait speed, chair rise and standing balance time were predictive of all-cause mortality and subsequent health and in older community-dwelling populations (Cooper *et al.*, 2010; Cooper *et al.*, 2011b). In a systematic review of prospective longitudinal studies assessing the predictive value of individual physical frailty indicators on ADL disability in those aged ≥ 65 y, indicators including grip strength were found to be predictive of ADL disability in community-dwelling elderly people. Slow gait speed and low physical activity had the greatest predictive power followed by weight loss, lower extremity function, balance and muscle strength (Vermeulen *et al.*, 2011)

Epidemiological studies have demonstrated that low hand grip strength in healthy adults predicts increased risk of functional limitation and disability in older age as well as all-cause mortality. As muscle function reacts early to nutritional deprivation, hand grip strength can also be used as a marker of nutritional status (Norman *et al.*, 2011).

In approximately 600 participants of the Hertfordshire Cohort Study (63 – 73 y) grip strength was found to be a good marker of physical performance (as tested by the SPPB). A 1 kg increase in grip strength was associated with a decrease in 6 m timed up and go, 3 m walk- and chair rise time in males and females. The authors observed that a single, simple measure of muscle strength was more feasible in a clinical setting than completing the short physical performance battery (Stevens *et al.*, 2012).

Isokinetic dynamometry (the gold standard for testing muscle strength) was compared with hand-held dynamometry in a systematic review of 19 studies. Minimal differences between hand-held dynamometry and isokinetic testing were demonstrated and hand-held devices were held to be reliable and valid instruments for the assessment of muscle strength in a clinical setting (Stark *et al.*, 2011). Referring to the European Working Group on Sarcopenia in Older People's endorsement of grip strength as a measure of muscle strength (Cruz-Jentoft *et al.*, 2010) this review of highlighted variability in approach and in the reporting of grip strength and recommended a consistent, standardised approach to enable the better assessment of sarcopenia (Roberts *et al.*, 2011).

In the Hertfordshire Cohort Study lower grip strength was associated with reduced health-related quality of life in older (59 – 73 y) men and women (Sayer *et al.*, 2006) and in a random sample of ~800 individuals aged ≥ 65 y from across the United Kingdom, poorer grip strength was associated with increased all-cause mortality and cardiovascular and cancer mortality in men but not in women (Gale *et al.*, 2007). In 119 moderately to severely disabled women (mean age 78.3 y) of the Women's Health and Aging Study, hand grip strength was a powerful predictor of mortality over 5 y (Rantanen *et al.*, 2003). The presence of 17 chronic diseases, inflammation, poor nutritional status, disuse and depression did not explain this association.

1.3.2.3.3 Chair rise/Sit to stand

Sit to stand requires the forward movement of the body's centre of mass both in the anterior-posterior and vertical plane, push-off and stabilisation once standing is achieved (Herman T, 2011). Four phases are described by (Schenkman M, 1990): flexion-momentum, momentum-transfer, extension and stabilisation. Measures may be strongly influenced *inter alia* by seat height (a lower height associated with a more demanding test), chair type, use of arm and backrests and foot position (Janssen *et al.*, 2002b). In 669 community-dwelling older (mean age 78.9 y) men and women, quadriceps[7] strength was the most important variable in explaining the variance in sit to stand time, however, other variables measures accounted for more than half the explained variability in performance. When measures of vision, peripheral sensation, reaction time, balance and health status were included, the final regression model explained ~35% of the variability in sit to stand performance (Lord *et al.*, 2002).

Leg power has been shown to be significantly associated with physical performance when measured by stair climb, chair stand and gait (tandem, habitual, maximal) tests and the SPPB (Bean *et al.*, 2002) explaining between 12 and 45% of the variability in the outcome. The relationship between chair rise performance (time to rise from a chair 10 times) and standing balance time were assessed against leg extensor power (LEP) as measured by a Nottingham Power Rig in a sub-sample of 174 NSHD participants (53 y). Chair rise performance should not be thought of as purely a proxy measure of leg power as it requires lower limb strength, good balance and coordination (Hardy R, 2010).

1.3.2.3.4 Timed up and go

The timed up and go test is a single, but composite measure of functional mobility including transfer tasks (standing up and sitting down), walking and turning; assessing the neuromuscular components of power, agility and balance. A poor performance has been associated with poor muscle strength, balance, slow gait, fear of falling, physical inactivity and ADL impairments (Schoene *et al.*, 2013). The American and British Geriatrics Societies and the Society of Nordic Geriatricians recommend the TUG as a screening tool to test for fall risk (Herman T, 2011).

[7] Group of 4 muscles located on the front of the thigh

In addition to the requirements of the sit to stand test described above (Janssen *et al.*, 2002b) the timed up and go test also demands appropriate initiation of stepping, once standing is stabilised, acceleration, deceleration and preparation to turn twice (Herman T, 2011). In assessing the properties of the timed up and go test (Herman T, 2011) concluded it was an appropriate tool for the assessment of functional ability even in healthy older adults (mean age 76.4 y). The (TUG) test was compared favourably to the Berg balance test and the Dynamic Gait Index as performance was related to executive function (planning, orientation in space and organisation) not properties of the simpler balance or gait tests. The authors speculated that it was the transferring and turning subtasks of the TUG that tested these cognitive resources.

Performance at timed up and go was influenced not only by lower limb strength and balance, but by reaction time, vision and pain in 280 older (mean age 74.9 y) community dwelling individuals (Kwan MM, 2011). In a systematic review and meta-analysis (Schoene *et al.*, 2013) timed up and go was shown not be predictive of falls in healthy high-functioning older people (≥ 60 y) and had a moderate predictive ability among less healthy, lower-functioning older people.

1.3.3 Maintenance of muscle mass

1.3.3.1 Muscle protein turnover

Protein turnover in the whole body denotes the interconversions (in both directions) between amino acids and proteins. Measurement methods include the precursor method which measures the incorporation of labelled amino acids (typically leucine) into body protein and the end-product method, which measures the excretion of ^{15}N labelled (typically glycine) in urea and ammonia. Whole body protein synthesis in normal adult men (estimated using the end-product average method and [^{15}N]glycine) was ~ 4 g of protein, per kilogram body weight, per day (Waterlow, 1984). When considering individual tissues (in the rat), the fractional synthetic rate for skeletal muscle was 17% i.e. over a period of 6 days, all skeletal muscle was renewed. Skeletal muscle contributes ~25% to whole body protein synthesis, the liver 21%, skin 18% and the small intestine 15% (Waterlow, 1984).

Muscle protein breakdown is a biological process that contributes to the maintenance of intracellular amino acid levels, maintaining muscle protein quality by removing damaged proteins and allowing their constituent amino acids to be used for the synthesis of new functional muscle proteins (Churchward-Venne *et al.*, 2012).

1.3.3.2 Muscle protein synthesis

Only in the postprandial state, when the substrates for muscle protein are available, can new muscle be made. Amino acids, in particular the essential amino acids, comprise the main anabolic signal (Volpi *et al.*, 2003).

When protein is ingested, circulating plasma essential amino acids stimulate the expression of amino acids transporters (LAT1, SNAT2, CD98 and PAT1) which transport amino acids across the cellular membrane from the intestinal lumen and into the bloodstream (Drummond *et al.*, 2010). Amino acid 'sensors', currently unknown, respond to the change in amino acid concentration and activate the protein kinase mTORC1 (mammalian target of rapamycin complex 1). Via the phosphorylation of downstream protein effectors such as p70S6k and 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1) the translational initiation of muscle protein synthesis is affected according to the 'central dogma' of molecular biology *viz.* replication, transcription and translation. Protein synthesis in the cytoplasm is followed by post-translational modification and protein folding into secondary and tertiary structures. Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids (Dickinson *et al.*, 2011). Leucine (a branched-chain (BCAA) amino acid) is a unique and key regulator of the translational initiation of muscle protein synthesis. Unlike the other BCAAs (isoleucine and valine) leucine potently increases the phosphorylation of mTOR and its downstream effectors p70S6k and 4E-BP1. The target of the p70S6 kinase is the S6 ribosomal protein. Phosphorylation induces protein synthesis (Deldicque *et al.*, 2005). Bed rest (inactivity) impairs skeletal muscle amino acid transporter expression, mTORC1 signalling and protein synthesis in response to essential amino acids in older adults. The authors speculated that inactivity contributes to muscle loss in older people (Drummond *et al.*, 2012).

1.3.4 Age-related changes in body composition and muscle

Age is associated with dramatic changes in body composition (Kohara, 2013) decreases in muscle mass are often accompanied by increases in fat mass, especially intra-abdominal fat.

1.3.4.1 Adiposity

Ageing is associated with increasing adiposity. Computed tomography was used to investigate the age-related differences in body composition between middle-aged (mean age 43.6 y) and older men (mean age 69.4 y) (Borkan *et al.*, 1983): in the older men, weight was significantly lower, driven by significantly less lean body weight (49.6 kg cf. 56.2 kg). The older men also had significantly more internal abdominal fat and less upper leg, abdominal and upper arm lean tissue. Fat infiltration in leg muscle, *latissimus dorsi* and deep back muscle was significantly higher in older men compared to their younger counterparts.

1.3.4.2 Sarcopenia

A termed originally coined by Irwin Rosenberg in 1989 derived from the Greek, *sarx* & *penia*: poverty of flesh. There are currently two consensus documents that define sarcopenia; the European Working Group on Sarcopenia in Older People (Cruz-Jentoft *et al.*, 2010) recommended the use of both low muscle mass and muscle function. Three stages were described: presarcopenia, sarcopenia and severe sarcopenia. The International Working Group on Sarcopenia (Fielding RA, 2011) uses gait speed and objectively measured low muscle mass. Dynapenia is the age-associated loss of muscle strength (Clark and Manini, 2012) which may not be as a direct result of age-associated declines in muscle mass.

1.3.4.3 Sarcopenic obesity

As with sarcopenia, there is no standard definition of sarcopenic obesity (Kohara, 2013) and the phenotype describes more than just a combination of the two pathological conditions. Independently, sarcopenia and obesity have an additive, synergistic effect for the development of sarcopenic obesity. In a study of 2943 older (mean age 69 y) participants of the Korean National Health Examination and Nutrition Study (Chung *et al.*, 2013), sarcopenia was defined as appendicular skeletal muscle mass / weight (%) of < 1 standard deviation below the sex-specific mean for young adults and obesity as a BMI ≥ 25 kg/m^2. 42% of men and 42.7% of women were sarcopenic, 26.8% and 39% were obese and 18.4% and 25.8% were sarcopenic obese, respectively. This latter group was most strongly associated with insulin resistance, metabolic syndrome and cardiovascular risk factors than any other group.

1.3.4.4 Anabolic resistance of ageing muscle

Previously thought to be a reduction in basal muscle protein synthesis, it is now known that the nutrient stimulation of muscle protein anabolism is blunted with ageing (Breen and Phillips, 2011) and that this is a key factor in the loss of skeletal muscle mass with ageing (Koopman, 2011).

In 2000 (Volpi *et al.*, 2000) concluded that the response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced endogenous hyperinsulinemia was impaired in healthy elderly subjects due to the unresponsiveness of protein synthesis. Muscle protein synthesis shows less anabolic sensitivity to essential amino acids in the elderly and deficits in signalling proteins (mammalian target of rapamycin (mTOR), p70 S6 kinase and eukaryotic initiation factor) underlie the amino acid resistance of aging muscle (Guillet *et al.*, 2004; Cuthbertson D, 2005). The phosphorylation of mRNA translational signalling proteins (in particular mTOR and its downstream targets) in response to whey protein ingestion after a bout of resistance exercise were investigated in a group of healthy young and older (60 – 75 y) men. Post-training, signalling protein phosphorylation was reduced in older men compared to their younger counterparts indicating a lack of sensitivity to anabolic stimuli in this age group (Farnfield *et al.*, 2011).

Diminished accretion of muscle proteins after ingestion of a small bolus of essential amino acids (Katsanos *et al.*, 2005) can be attenuated in the elderly with a higher proportion of leucine (Katsanos *et al.*, 2006). Postprandial muscle protein accretion was investigated in two groups of elderly (mean age 74.3 y) after ingestion of 20 g phenylalanine-labelled casein protein either with or without additional (2.5 g) crystalline leucine. Muscle-protein bound phenylalanine enrichments were significantly greater in the group ingesting additional leucine, 2 and 6 hours after ingestion; this equated to a 22% greater muscle protein synthetic rate over the whole postprandial period (Wall *et al.*, 2013)

In a similar experiment in 24 males (mean age 75 y) there were no differences in muscle protein-bound labelled phenylalanine enrichments 6 hours after casein protein ingestion, given with or without carbohydrate (Hamer *et al.*, 2013). Protein co-ingestion with carbohydrate did not augment incorporation into muscle in this group of elderly men.

In addition to muscle resistance to the anabolic stimuli of amino acids, elderly muscle may also exhibit resistance to the antiproteolytic effects of insulin. In research by (Wilkes *et al.*, 2009) in groups of young and older (mean age 65 y) men, a low physiologic dose of insulin (equivalent to that expected following a low-glycemic meal) lowered leg protein breakdown by 12% in the older men compared to 47% in the younger group. When the activity of muscle Akt-protein kinase B (considered a proxy of insulin action) and phosphorylation of mTOR signalling proteins were measured, activity of Akt-PKB was diminished, potentially mediating the blunting of insulin inhibition of leg proteolysis.

1.4 Protein needs across the lifecourse

1.4.1 Protein recommendations

The first Food and Agriculture Organization of the United Nations (FAO) Expert Consultation on population protein requirements was in 1955. In 1963 protein was reviewed again, collaboratively with the World Health Organisation. Energy and protein requirements were considered together in 1971 by a Joint FAO/WHO Expert Committee and their report published in 1973. The WHO Technical Report Series No. 724 (published in 1985) reported on the joint FAO/WHO/UNU Expert Consultation on energy and protein requirements held in 1981.

In 2002 a joint WHO/FAO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition was held, culminating in the latest WHO Technical Report Series No. 935 (published in 2007). The (Rand *et al.*, 2003) meta-analysis which indicated a median requirement of 105 mg nitrogen/kg per day or 0.66 g/ kg per day of protein, was accepted as the best estimate of a population average requirement (the Estimated Average Requirement (EAR)) for healthy adults. In the same report, 133 mg nitrogen/ kg per day, or 0.83 g per kg of bodyweight per day of protein was expected to meet the requirements of most (97.5%) of the healthy adult population (the Reference Nutrient Intake (RNI)).

Although not applicable to NSHD participants who provided dietary data in 1982 - 1999, these requirements were used to determine whether participants met protein recommendations.

1.4.1.1 Protein needs in older adults

In 2066 community dwelling black and white participants of the Health, Aging & Body Composition Study (mean age 74.5 y), changes in lean mass and appendicular lean mass (aLM) were assessed (by DEXA) at 3 y follow up in relation to energy-adjusted dietary protein intake (Houston *et al.*, 2008). Female participants who reported energy intakes < 500 or > 3500 kcals/d and males reporting EI < 800 or > 4000 kcals/d, were excluded. Protein intake was associated with 3 y changes in lean and appendicular lean mass; participants in the highest quintile of protein intake lost ~40% less lean mass than those in the lowest quintile. After adjustment for potential confounders (e.g. age, gender, race, physical activity and health status) regression coefficients for changes in total lean mass and aLM per unit of energy-adjusted total protein intake were 6.38 (p=0.02) and 4.10 (p=0.007) respectively. Adjusted regression coefficients remained significant for animal protein but not for vegetable protein. However, when participants were stratified by weight change status, and after adjustment for potential confounding, protein intake was associated changes in aLM in weight gainers and losers, but not in those who were weight stable (Houston *et al.*, 2008).

In a cohort of 740 non-institutionalised participants of the Tasmanian Older Cohort Study (mean age at baseline 62 y) DEXA-measured appendicular lean mass 2.6 y follow up; leg strength knee extension; physical activity by pedometers; those who failed to meet the Australian and New Zealand recommended dietary intake (RDI) for protein had significantly lower appendicular lean mass (aLM) at baseline (0.81 kg) and follow up (0.79 kg) after adjustment for energy intake, age, gender and physical activity (Scott *et al.*, 2010). There was a significant positive association between aLM and energy-protein and intakes were positively predictive of aLM change over 2.6 y. No associations were found between nutrients and muscle strength.

In 862 white Western Australian community-dwelling women (mean age 75 y) nutrient intake and anthropometric measures (e.g. BMI and upper arm muscle area (UAMA)) were taken at baseline. At 5 y follow up anthropometry and DXA-determined body composition were assessed (Meng *et al.*, 2009). After adjusting for age, height, energy intake and physical activity, those in the upper tertile of protein intake (>87 g/d) had significantly higher whole body (5.3%) and appendicular lean mass (6.6%) than subjects in the other two groups.

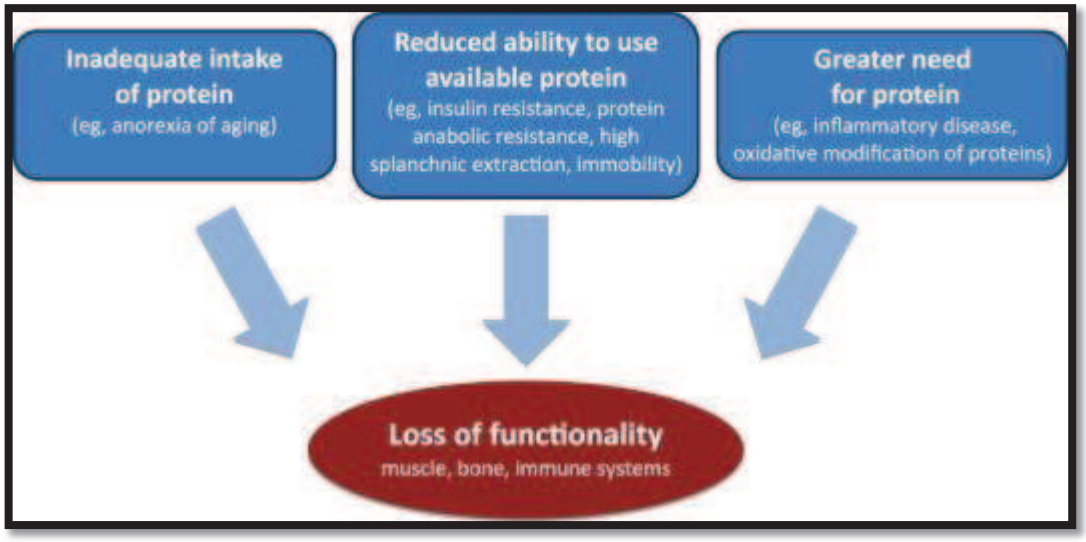
In participants of the InCHIANTI study (mean age 72.9 y) knee extension strength was measured at baseline and at 3 y follow up (using a hand-held dynamometer). The main effect of protein intake in muscle strength was insignificant, however, in persons with high levels of the inflammatory markers CRP, IL-6 and TNF-α lower protein intake was associated with a greater decline in muscle strength (Bartali *et al.*, 2012).

In a subset of 24,417 women of the Women's Health Initiative observational study, (65 – 79 y) with plausible self-reported energy intakes (600 – 5000 kcal/d), measurement error was corrected for by the use of an approach which calibrated energy and protein intake using recovery biomarkers. Estimates were used to investigate protein intake in relation to incident frailty. Frailty was assessed using criteria developed by (Fried *et al.*, 2001). Protein intakes were expressed in grams, as a percentage of total energy intake and as a ratio of grams per kilogram of body weight. A 20% increase in uncalibrated protein intake (as a percentage of total energy) was associated with a 12% lower risk of frailty whereas a 20% increase in calibrated protein intake was associated with a 32% lower risk of frailty (Beasley *et al.*, 2010). Using uncalibrated intakes underestimated the strength of the association.

The existence of a 'leucine threshold' was hypothesised by (Breen and Phillips, 2011) based upon observations by (Katsanos *et al.*, 2006; Koopman *et al.*, 2006; Rieu *et al.*, 2006; Norton *et al.*, 2009; Atherton *et al.*, 2010).

A recent position paper (Bauer *et al.*, 2013) from the PROT-AGE (protein needs with aging) study group was entitled 'Evidence-based recommendations for optimal dietary protein intake in older people'

Figure 1.3 The age-related causes of protein shortfall – impairment of musculoskeletal and immune function (Bauer *et al.*, 2013)



The main points of the position paper were as follows:

1. Older adults need more dietary protein than younger adults, average daily intakes are recommended to be between 1 – 1.2 g/kg/d.
2. Age-related changes in protein metabolism include higher splanchnic extraction of amino acids and a declining anabolic response to ingested amino acids/anabolic resistance.
3. Older adults may need more protein to offset inflammatory and catabolic conditions that accompany age-related chronic and acute disease. In these circumstances, recommended intakes are 1.2 – 1.5 g/kg/day. Severe kidney disease without dialysis is an exception to this rule and protein intakes should be restricted.
4. Endurance exercise (30 minutes/d) and resistance exercise (2 – 3 times/week) is recommended. Higher protein intakes (≥ 1.2 g/kg/d) are recommended for those exercising and active. Protein or amino acid supplementation in close temporal proximity to exercise is also recommended.

1.4.2 Protein quality

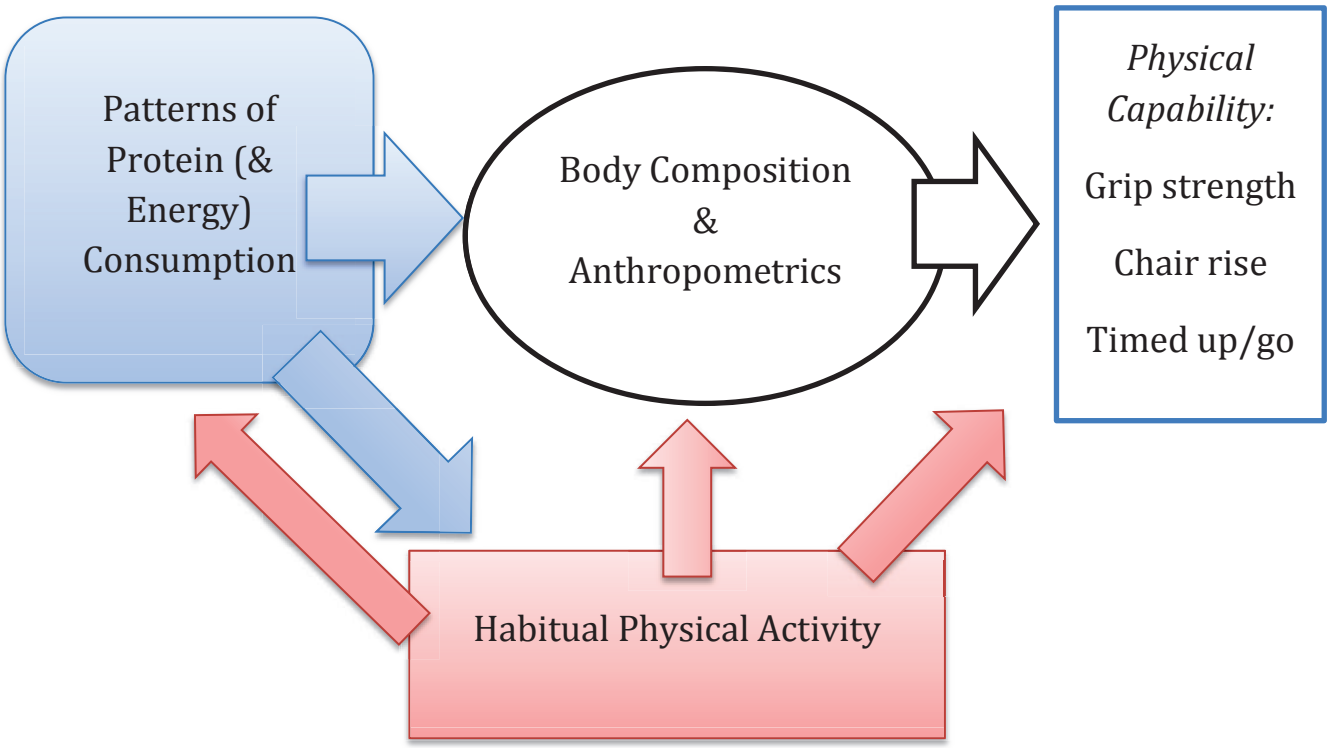
Protein quality is determined according to the Protein Digestibility Corrected Amino Acid Score (PDCAAS) – it is a means of evaluating protein quality by the determination of the protein amino acid profile. In the present research it was not possible to determine protein quality as amino acid data were not available.

In (Beasley et al., 2010) quality of protein was summarised as the sum of essential amino acids, as defined by having a recommended intake assigned by the Joint FAO/WHO/UNU Expert Consultation (histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine). Joint FAO/WHO/UNU/EC Energy and Protein requirements Vol. 2008 1985 and the WHO Protein and amino acid requirements in human nutrition. Report of a FAO/WHO/UNU consultation. WHO Press; 2007. p. 150. WHO Technical Report Series

1.5 Analytical/research strategy

The analytical model and research strategy for this project is depicted in Figure 1.4. In approaching and developing the analytical model, physical capability at 60 – 64 y was expected to be determined primarily by body composition and anthropometry, both of which were hypothesised to be associated with habitual diet and physical activity.

Figure 1.4 Analytical model and Project research strategy



1.6 Hypothesis

Adulthood patterns of protein consumption predict physical capability in older age

1.7 Aims

1. To test the hypothesis that low protein consumption throughout adulthood impairs physical capability in later life;
2. To test the hypothesis that diurnal patterns of protein consumption throughout adulthood influence physical capability in later life

1.8 Objectives

1. To characterise and to quantify patterns of protein consumption (both mean daily intake and diurnal patterns of intake) in a cohort of individuals providing dietary data by 5 d food diary in 1982, 1989 and 1999 when aged 36 y, 43 y and 53 y

2. To determine and to characterise physical capability at age 60 – 64 y using a range of techniques including hand grip strength, timed up and go and chair rise time

3. To determine and to characterise other variables identified *a priori* as potentially mediating (or confounding) the relationship between protein consumption and physical capability. These variables include body composition and anthropometrics, habitual physical activity, socioeconomic status, health status and other related (meta)data

4. To apply a range of statistical techniques, including hierarchical linear regression, to this dataset to determine which variables, including patterns of protein consumption during adulthood, predict physical performance at age 60 – 64 y

CHAPTER 2

Methodology

2.1 Introduction

Birth cohorts – the prospective tracking of individuals from birth – represent the best way to study the ageing trajectory. This is especially so when circumstances of birth (including prenatal exposures) are known and there is information on childhood development and illness since these early life events and exposures may have long-term effects of health and wellbeing and on the ageing process (Hanson *et al.*, 2011). Through repeated contacts/ monitoring, a myriad of exposures throughout childhood, early adulthood and adulthood into retirement and old age may be observed and recorded. Outcomes of choice such as impairments (disease/ disability (morbidity)) and mortality may be investigated in relation to known exposures, while adjusting for potential confounding factors, to provide robust evidence of causative relationships and significant interactions (Power *et al.*, 2013).

2.1.1 British birth cohorts

There are currently four British birth cohort studies: i) the MRC National Survey of Health and Development, or 1946 British birth cohort, ii) the National Child Development Study (NCDS) or 1958 British birth cohort, iii) the 1970 British Cohort Study and iv) the Millennium cohort study, established in 2000. These latter 3 studies are managed by the Centre for Longitudinal Studies and funded by the Economic and Social Research Council (ESRC).

The National Child Development Study is a cohort of ~17000 individuals born in a single week in 1958. To date, participants have been followed up in eight 'sweeps' from age 7 – 50 y. In 1999 when cohort members were aged 45 y they participated in a biomedical survey in which objective measures of ill-health and biomedical risk factors were assessed. In 2013 cohort members will be contacted again, at age 55 y (Power and Elliott, 2006). The 1970 British birth cohort also follows the lives of ~17000 individuals all born in a single week in 1970 (Elliott and Shepherd, 2006). To date, this cohort have been followed up in seven sweeps from age 5 – 34 y in the latest sweep (2004), data were also collected from cohort members' children. The Millennium cohort study was designed specifically to examine child wellbeing e.g. effects of breastfeeding, childhood activity, sleep characteristics, mental health and diabetes, the impact of television and electronic games. Groups living in disadvantaged circumstances, those from minority ethnic backgrounds and those in born outside of England were intentionally over-sampled (http://www.cls.ioe.ac.uk).

Health (including social inequalities and health-related behaviours), educational and social development, major life transitions – education into employment, dependent status within families of origin to independent homemakers and parenthood, lifetime employment to retirement – may be observed.

2.1.2 Other British cohorts

The Cohort and Longitudinal Studies Enhancement Resources (CLOSER) programme, launched in 2012, aims to exploit the value of the UK's largest and longest-running longitudinal studies, creating a collaborative network which (currently) comprises nine participating studies: i) Avon Longitudinal Study of Parents and Children, ii) 1970 BCS, iii) Hertfordshire Cohort Study, iv) Life Study, v) Millennium Cohort Study, vi) 1958 NCDS, vii) NSHD, viii) Southampton Women's Study and ix) Understanding Society http://www.closerprogramme.co.uk/

2.1.3 National Survey of Health and Development

The Medical Research Council's (MRC) National Survey of Health and Development (NSHD) the oldest of the British birth cohorts, is a socially stratified sample of all single births in England, Scotland and Wales, in the week 3 – 9 March, 1946. Funded by the MRC since 1962, the NSHD is now part of the cross-cohort Healthy Ageing across the Lifecourse (HALCyon) programme led by the MRC Unit for Lifelong Health and Ageing (LHA). The HALCyon programme comprises nine cohort studies: Lothian 1921, The Hertfordshire Cohort (HCS) and Ageing (HAS) Studies, 1920 – 39, Boyd Orr 1925 – 37, Aberdeen 1936, The National Child Development Study (NCDS) 1958, The English Longitudinal Study of Ageing (ELSA) and the Caerphilly study [http://www.halcyon.ac.uk/ accessed 11 12 12].

The NSHD, initiated and originally led (for the first 33 years) by Dr James WB Douglas, was tasked to address the issue of falling national fertility and to examine the quality of existing maternity services, pre-NHS (founded in 1948). Health visitors interviewed the mothers of all babies born during one week in March, 1946 at their eight-week check-up (*n*=16,695). By June 1946, 13,687 mothers had been interviewed and results of this survey were published in 1947 as "Maternity in Great Britain" (Wadsworth *et al.*, 2006). Observations of stark health inequalities led to the follow-up of 5,362 of the original maternity survey and this sample became the NSHD. The sample taken for follow-up comprised all single, legitimate births to wives of non-manual and agricultural workers and one in four of all such births to wives of manual workers (Braddon FE, 1988).

NSHD participants have been followed-up extensively. This included; during pre-school and throughout school years, up to age 15 y, from 15 – 30 y and during their 30's, 40's, 50's and 60's. Repeated measures of cognitive development, physical growth, physical and emotional functioning from early life and throughout adulthood have enabled the examination of lifelong development and, more recently, the ageing processes (Kuh *et al.*, 2011).

2.1.4 The lifecourse approach

The lifecourse approach to chronic disease epidemiology, defined as the study long-term effects on chronic disease risk of physical and social exposures during gestation, childhood, adolescence, young adulthood and later life (Ben-Shlomo and Kuh, 2002) is applicable to wider notions of health and wellbeing. In the current context, longitudinal data enabled the study of the long-term effects of dietary and physical activity exposures throughout adulthood on the risk of poor physical capability in older age. Repeat measures of dietary and physical activity exposures enabled the characterisation of *habitual* patterns over a longer period – i.e. adulthood, rather than at a single time point – and this may be valuable in determining causality. The lifecourse approach is particularly valuable where *intra* individual exposures exhibit considerable temporal variability, such as diet or physical activity.

Using self-reported leisure time physical activity data from NSHD participants collected at ages 36, 43 and 53 years (Cooper et al., 2011b) created a 'lifetime physical activity score' to examine the association between physical activity *across adulthood* and physical performance in midlife. Similarly, in the same cohort (Dodds *et al.*, 2013) examined the effect of leisure time physical activity at ages 36, 43, 53 and 60 – 64 y on mid-life grip strength at 60 – 64 y using a 'cumulative score'. This was done to examine whether there was a cumulative effect of physical activity across adulthood on mid-life grip strength. Also in the same cohort, (Murray *et al.*, 2013b) examined the effect of area deprivation across the lifecourse (at 4, 26 and 53 y) and physical capability in midlife.

Of a target sample of 3163, 84% (2661) responded to the latest invitation (from 2006 – 2010) to attend one of six clinical research facilities (CRFs) across the UK. Manual social class, obesity, lower educational attainment, lower childhood cognition and lifelong smoking predicted a lower likelihood of overall response rate to this invitation and poorer CRF cooperation. Of 2661 NSHD participants contacted in the latest round, 79% had provided data at ages 26 y, 36 y, 43 y, 53 y and 60 – 64 y. The occupational social class and unemployment profile of continuing participants appeared to be similar to the England Census, 2001, but participants appear somewhat more advantaged with respect to home ownership and limiting illness (Stafford M, 2013).

2.2 Physical capability data

Physical capability at 53 y and at 60 – 64 y were considered in this project. At age 53 y 3035 NSHD participants provided some physical capability data and of these 2984 were visited at home by a trained nurse (Kuh *et al.*, 2005).

2.2.1 NSHD participants at 53 y

Height was measured with the head in the Frankfort plane and without shoes with a portable stadiometer (CMS, London) to the nearest 0.5 cm. Weight was measured to the nearest 0.5 kg using CMS scales, in light clothing and no shoes. Voluntary isometric hand grip strength was measured using an electronic handheld dynamometer while strong verbal encouragement was given. Two values for each hand were recorded. Chair rise time was measured using a stopwatch, and was taken as the *minimum* amount of time taken to rise from a sitting position to a standing position with straight back and legs and sit down again, ten times. An armless, straight-backed, hard chair was used (the seat ~46 cm from the floor) and participants wore no shoes. A leisure time physical activity questionnaire was completed at the same visit (Kuh *et al.*, 2005).

2.2.2 NSHD participants at 60 – 64 y

A feasibility study was held at the Wellcome Trust Clinical Research Facility (CRF) in Manchester involving a randomly selected 10% sample of NSHD participants closest to this CRF. All traceable participants (n=3116) were then invited to attend one of six Clinical Research Facilities (CRFs) at Manchester, Edinburgh, Birmingham, Cardiff and two in London. One of the weaknesses of this data collection process was its duration, almost 5 y from the start of the feasibility study to the end of the main data collection (Kuh *et al.*, 2011). Clinics were held and attended over a period of FOUR years – 2006 to 2010 when participants were aged 60 – 64 y. For the purpose of the present project, data for this collection period were provided by the MRC as if collected at one time period (i.e. 2006/10) and details of participant actual age at the time of the collection of physical capability data were not known. Consequently, in the present project, all participants were treated as if they were the same age.

Those who were unwilling or unable to attend a Clinical Research Facility were offered a home visit with fewer assessments (e.g. no DEXA body composition). In addition to a postal questionnaire, self-reported health was confirmed by clinical tests and GP reports. Study members were asked to fast from 2000 hours the day preceding attendance. Throughout physical capability tests nurses were trained to give strong verbal encouragement to elicit the best possible performance from each individual. Individuals with severe cardiorespiratory disease, untreated hypertension (≥200 mmHg systolic or ≥ 102 mmHg diastolic), hip/ knee replacements, severe hip/ knee problems or those unable to stand, were excluded from these assessments (Kuh *et al.*, 2011).

Grip strength was measured isometrically using an electronic handgrip dynamometer, custom made by the Medical Physics and Clinical Engineering Department of Queen's Medical Centre, Nottingham and calibrated using a back-loading rig. These dynamometers were accurate to ± 0.5 kg and were available in two sizes to accommodate different hand sizes. Two values were recorded for each hand (Kuh *et al.*, 2011). Chair rise time was measured as the time taken to rise from a sitting to a standing position and to sit down again, 10 times. Timed up-and-go measured the time taken for participants to rise from a chair, walk at a normal pace for 6 metres and sit back in the chair.

Two measures of systolic and diastolic blood pressure were taken using an OMRON HEM-705 with participants sitting down. For the DEXA bone and body composition scans, all CRF sites used QDR 4500 Discovery scanners (Hologic Inc., Bedford, MA)(Kuh *et al.*, 2011).

2.3 Dietary data

Dietary data were collected by research nurses at the participants' home on three occasions in 1982, 1989 and 1999. In 1982 and 1989 a 2 day dietary (retrospective) recall and 5 day food diary were completed. In 1999 there was no 2 day dietary recall. Two day dietary recalls (all consumption in the immediate past 2 days) were completed by the nurse *and* the participant while the 5 day food diary was left with the participant to be completed prospectively and returned by post to the MRC Human Nutrition Research unit at Cambridge. All food and drink consumed by participants was recorded, whether consumed at, or away from home. Portion sizes were estimated with reference to common household measures and guidance notes and photographs were provided (Prynne *et al.*, 2005). As the present project used only those dietary intake data collected by 5 d estimated food diaries, there will be no further reference to the dietary intake data collected by 2 d dietary recall.

2.3.1 Dietary assessment in 1982

Of 3322 diaries issued, 73% (2424) were completed for 4 or more days and returned by post. 1284 diaries (39%) were completed fully. There were no statistically significant differences in gender, social class or education in those who had completed and returned a diary, and those who did not (Braddon FE, 1988). Diary information was manually converted into food codes and weights and the nutrient composition of foods determined with reference to McCance and Widdowson's "The Composition of Foods", in-house communications, manufacturers and individual recipes. Portion sizes were determined with reference to standard household measures and with average portions (with reference to a weighted intake survey conducted in a similar age group)(Braddon FE, 1988).

2.3.2 Dietary assessment in 1989

In 1989, 3262 NSHD participants were contacted successfully and dietary intake data were collected as in 1982 (Price *et al.*, 1997). Diaries were coded and checked using a bespoke direct entry computer programme, Diet In, Data Out (DIDO) which generated a food code and an associated weight/ portion size (g) for each item of food and drink recorded. The output file was exported to a suite of programs based upon McCance and Widdowson's *The Composition of Foods*, for nutrient analysis (Price *et al.*, 1997).

2.3.3 Dietary assessment in 1999

In 1999 3035 participants were contacted and of these 1776 returned 5 day food diaries. Diaries were coded using DIDO as before and nutrient analysis determined with reference to McCance and Widdowson's *The Composition of Foods* fourth edition (Prynne *et al.*, 2005).

2.3.4 The dietary dataset used in the present project

After the application to collaborate with the NSHD was approved by the MRC Unit for Lifelong Health and Ageing, a dietary data dataset (in IBM SPSS version 19.0 format) was received [HNR_030412.sav] comprising 241 813 cases; this included diary and recall data for 1982 (88092 cases), 1989 (100376 cases) and 1999 (53345 cases). The dietary data were organised by meal (or eating occasion) on each of the 5 days in the recording period (see Table 2.1 below, for an example).

Table 2.1 Example of an individual (NSHD ID 2) 1982 diary entry

StudyTitle	DiaryDate	Day	Meal	Energy (kcals)	Protein (g)
NSHD 82 Diary	18 Jun 1982	Friday	Breakfast	**179.75**	**3.40**
			Mid-Morning	5.20	.52
			Lunch	307.19	9.39
			Tea	5.20	.52
			Evening Meal	858.51	39.51
			Late Evening	22.89	.00
	21 Jun 1982	Monday	Breakfast	**179.75**	**3.40**
			Mid-Morning	2.60	.26
			Lunch	385.45	15.92
			Tea	130.78	1.43
			Evening Meal	635.40	19.51
			Late Evening	122.64	.89
	19 Jun 1982	Saturday	Breakfast	**179.75**	**3.40**
			Mid-Morning	2.60	.26
			Lunch	984.49	26.24
			Evening Meal	786.77	20.97
			Late Evening	280.60	2.50
	20 Jun 1982	Sunday	Breakfast	**359.50**	**6.79**
			Mid-Morning	1.90	.19
			Lunch	618.75	25.91
			Tea	2.60	.26
			Evening Meal	775.77	20.17
			Late Evening	163.20	.36
	17 Jun 1982	Thursday	Breakfast	**179.05**	**3.33**
			Mid-Morning	2.60	.26
			Lunch	258.96	15.55
			Tea	205.27	2.47
			Evening Meal	635.43	41.43
			Late Evening	114.78	.62

For each meal (where consumed), the nutrient data provided (where applicable) were energy (kcals) and protein (g) (as shown in Table 2.1 above). Also provided (but not investigated in the present project) were data on intakes of energy (kJ), fat (g), carbohydrate (g), calcium (mg), iron (mg) (haem (mg) and non haem (mg)), vitamin A retinol equivalents (ug), vitamin C (mg), alcohol (g), total NSP (g) and total weight of food consumed.

2.3.5 Preparing the dietary data for analysis

Each year was labelled and copied into separate datasets e.g. data for 1982 to the dataset [1982.sav]. As 1982/1989 datasets contained 5 d food diary and 24 h recall data a numeric code was added to indicate whether dietary data were from a diary (1) or a recall (2) and each was copied into separate datasets.

2.3.5.1 Calculating mean meal intakes (energy and protein)

A numeric code was added to indicate meals, i.e. 1 = first thing; 2 = breakfast; 3 = mid-morning; 4 = lunch; 5 = tea; 6 = evening meal; 7 = late evening and 8 = extras. Each meal was sequentially selected (i.e. meal 1, meal 2, meal 3) and data were aggregated by NSHD ID (i.e. the break variable = NSHD_ID). In summaries of variable(s) Energy_kcals & Protein_g were selected and the aggregate function/ summary statistic selected was **Sum**. Summaries of variables generated were Energy_kcals_**sum** & Protein_g_**sum.**

Sequentially aggregated meal values were copied to a new dataset and renamed i.e. kcals_sum1, kcals_sum2… (for meal energy) and protein_sum1, protein_sum2... (for meal protein). All summed variables (kcals_sum and protein_sum) were divided by 5 to generate 5 d average values of meal energy (kcals) and meal protein (g) intakes for each individual as recorded by the 5 d food diary. The 5 d meal mean was derived from all meals consumed within that 'meal slot', regardless of how many occasions across the 5 recording days a meal was consumed i.e. a given individual may have consumed a meal in that slot on 1 – 5 occasions. In the example above for a randomly-selected individual (Table 2.1) mean daily meal consumption was 216 kcals (at breakfast), 3 kcals (mid-morning), 51 kcals (at lunch), 69 kcals (at tea), 738 kcals (at the evening meal) and 141 kcals (late evening).

2.3.5.2 Mean daily energy and protein

To derive mean daily energy and protein intakes, summed meal values were summed and divided by 5. In the above example (Table 2.1) mean daily energy consumption was 1677 kcals.

2.3.5.3 Meal energy and protein

To calculate meal energy as a percentage of total daily energy, meal energy (kcals) was divided by total daily energy in the relevant year (calculated as described above) and multiplied by 100. To calculate meal protein as a percentage of total daily protein, meal protein (g) was divided by total daily protein in the relevant year (calculated as described above) and multiplied by 100. To calculate meal protein as a percentage of total daily energy, meal protein (g) was multiplied by 4, divided by total daily energy in the relevant year (calculated as described above) and multiplied by 100

2.3.5.4 Identification of the subset of NSHD participants who provided dietary data in all years

In the dataset which comprised NSHD participants who ever provided dietary data (*n*=3019), where energy data was provided in a particular year, a variable was created to reflect that fact, i.e. NutData(year) = 1. This process was repeated for all three years and the three variables summed to indicate the number of occasions each participant had provided dietary data. In 3 measurement years, 817 NSHD participants provided dietary data in only one year, 939 in two years and 1263 in all years. Individuals who provided dietary data in all years were selected and all data were copied into a new dataset.

2.4 Dietary subgroups

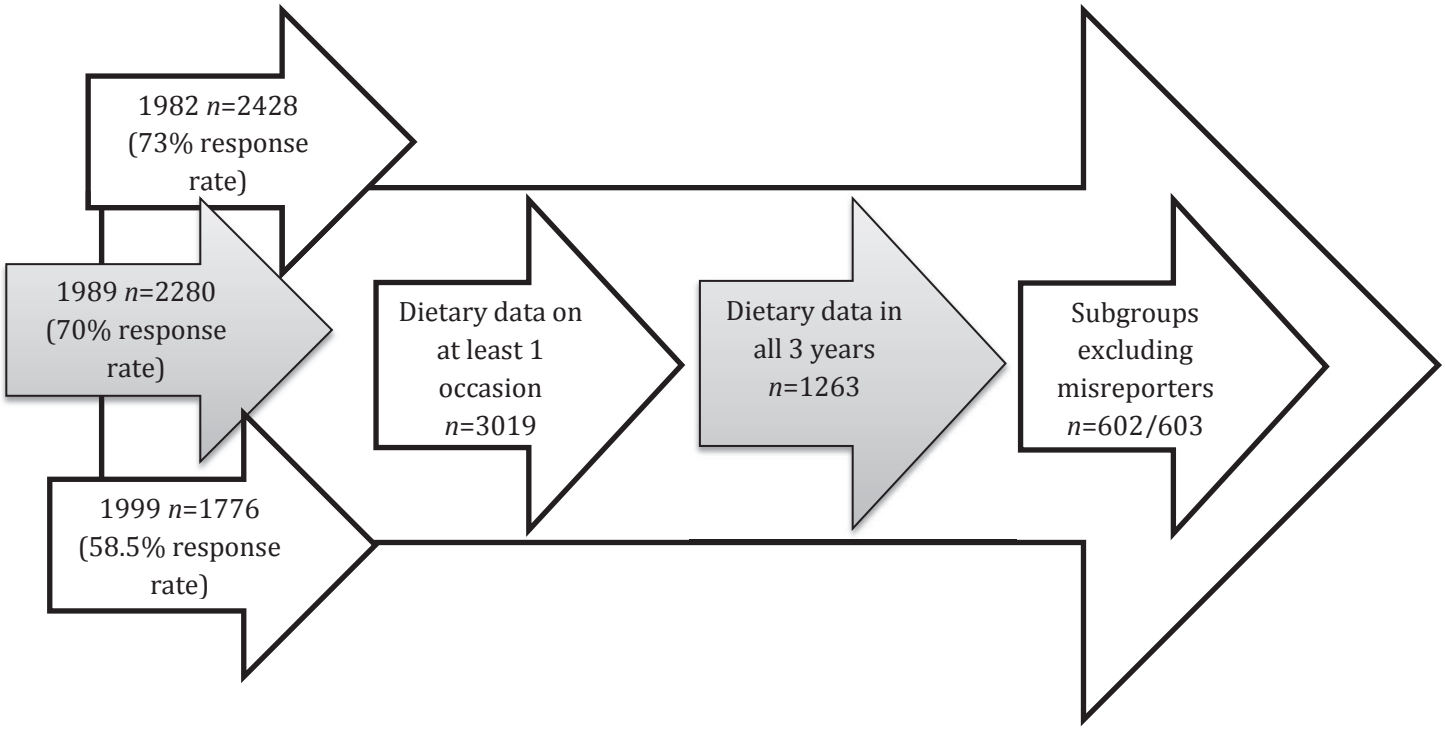
Using dietary data collected on 3 reporting occasions (1982, 1989 and 1999) 3,019 NSHD participants (out of approximately 5,362 in total) provided dietary data on at least one occasion, and dietary reporting was observed to fall over time. Of these individuals, 1,263 NSHD participants provided dietary data in all three reporting years and it is in this group that trends were examined and analyses undertaken. It must be noted that this group represents a rather 'special,' self-selected sample; lifestyle (including dietary), anthropometric and outcome characteristics (i.e. physical capability in older age) of this sample may differ from the NSHD cohort as a whole, and participant self-selection of this kind has implications for what the final analyses will show. For this reason, notwithstanding the overall representativeness of the NSHD cohort (*n*=5,362), results derived from smaller, self-selected subgroups may be incapable of being extrapolated to the entire cohort and to the general UK population.

Table 2.2 Response rates for diaries, 1982 – 1999 in NSHD participants

Year	NSHD participants contacted (*n*)	Dietary data provided (*n*)	Response percentage (%)	Male (*n*)	Female (*n*)
1982	3322	2428	73	1192	1236
1989	3262	2280	70	1125	1155
1999	3035	1776	58.5	827	949

Of the ~3,000 – 3,300 NSHS participants contacted in each measurement sweep, the response rate fell from 73 – 58.5%.

Figure 2.1 Dietary subgroups (*n*) in the analyses



The response rate for 5 d food diaries fell from 73% (*n*=2428) in 1982 to <60% in 1999 (*n*=1776) (Figure 2.1). Providing dietary data on at least one occasion was a subset of 3019 individuals. For the main regression analyses (see Chapter 6), a sub-cohort of individuals who provided dietary data in all years (*n*=1263) was used. For the two sensitivity analyses (see Chapter 6) the smallest sub-cohorts studied (*n*=602/*n*=603) comprised individuals who had provided dietary data in all years and were predicted never to have misreported their energy intake.

2.5 Diurnal patterns of dietary protein intake and calculation of the muscle protein synthesis score (MPSS)

As this research project examined diurnal patterns of protein consumption specifically, it was first necessary to devise a method by which protein intakes across the day could be captured. This was achieved by the implementation of a novel protein scoring system (called here the muscle protein synthesis score (MPSS)) which scored protein consumption of ≥ 20 g at any of eight eating occasions across the day. The implementation and calculation of the score is described below (Section 2.4.4). What follows here (Section 2.4) is an explanation of the rationale underlying the choice of a 20 g protein threshold. This is based on the hypothesis that this is the minimum amount of protein needed in a meal to maintain adequate levels of whole body protein synthesis (including in skeletal muscle) which is important in ensuring that older adults have sufficient physical strength to carry out activities of daily living. The latter is assessed by quantifying physical capability via hand grip strength, chair rise time and timed up and go in the present project.

2.5.1 Overview of evidence for impact of quantity of ingested protein per meal on muscle protein synthesis

The research of Marie-Agnès Arnal (Arnal *et al.*, 1999) was a particular impetus for the current research. In this study, 15 older women (mean age 68 y) were fed either a so-called a "pulse" diet – 79 % of daily protein intake at 12 noon (n=8) – or a so-called "spread" diet in which daily protein intake was spread more evenly over 4 meals (n=7). Daily protein intake was calculated as 1.7 g per kg of fat free mass, per day. Protein accretion (N balance) and daily protein turnover (urinary excretion of [^{15}N] and [^{15}N]ammonia) were measured outcomes. A 15 d adaptive period was used to achieve similar protein status in all women which was equivalent to 0.74 g protein · kg body weight ·d. During the 14 d experimental period protein intake was increased to 1.05 g protein · kg body weight · d (70% animal-derived, 30% plant-derived). Nitrogen balance during the experimental period was 27 ± 6 (mg N· kg FFM · d) in the spread diet group compared with 54 ± 7 (mg N · kg FFM · d) in the pulse group (p<0.001). From the urea data, there was a significantly higher daily protein gain in the pulse diet group (0.61 cf. 0.42 g · kg FFM · d) driven largely by a 19% higher rate of protein synthesis (4.48 cf. 3.75 g · kg FFM · d). In addition, overall protein gain was significantly higher in the pulse group than in the spread diet group (0.92 cf. 0.60 g · kg FFM · 12 h). During the 14 d experimental period there was a slight decrease in FFM among women in the spread diet group whereas there was no detectable change in those on the pulse diet (Arnal *et al.*, 1999). This study demonstrated that although the same quantity of protein was eaten daily, the pattern of intake across the day modulated protein accretion, daily protein turnover and body composition (FFM) in these older women. No effect of diurnal pattern of protein consumption on protein synthesis or protein accretion were observed when the study was repeated in younger (26 y) women (Arnal *et al.*, 2000).

Essential amino acids are primarily responsible for the stimulation of muscle protein anabolism (Volpi *et al.*, 2003) and there is evidence that muscle protein anabolism can be stimulated by increased amino acid availability in the elderly (71 y) (Volpi *et al.*, 1998). However, constant nutrient delivery by intravenous amino acids does not emulate the usual pattern of amino acid supply in meal-eating humans and eliminates the effects of discrete bouts of food ingestion followed by variable rates of gastric emptying and digestion. A more 'meal-like' bolus ingestion of 15 g of essential amino acids stimulated muscle protein synthesis acutely in young (34 y) and in elderly (67 y) subjects, notwithstanding age-related differences in the time course of plasma phenylalanine kinetics (Paddon-Jones *et al.*, 2004).

Rates of muscle protein synthesis in young (28 y) and elderly (mean age 70 ± 6 y) male subjects were compared after ingestion of 0, 2.5, 5, 10, 20 and (for the elderly only) 40 g of essential amino acids (EAA) (Cuthbertson D, 2005). In young men, 2.5 – 10 g EAA stimulated the myofibrillar protein fractional synthetic rate (FSR) in a dose-dependent manner, while 20 g failed to elicit any *additional* stimulation. In elderly men 40 g EAA failed to promote rates of muscle protein synthesis to those seen at 10 g in the young, and ingestion of 10 g EAA raised rates of muscle protein synthesis to the same extent as observed with 5 g in the young. The authors advised that elderly people should eat protein 'effectively' to raise their plasma EAA concentration to trigger the maximum anabolic response; and this could be achieved with 10 g EAA (equivalent to ~113 g of high quality protein) (Cuthbertson D, 2005). Symons (Symons *et al.*, 2009) reported that a 113 g serving of lean beef (a protein rich food) contained sufficient amino acids (30 g in total, ~12 g essential) to increase muscle protein synthesis by 50% in both young and elderly males and females and that there was no further increase with a large serving of 340 g of lean beef.

Whether ingestion of a *small* amount of essential amino acids (EAAs) affects muscle protein accretion differentially in elderly (68 y) compared with young (31 y) adults was examined by (Katsanos *et al.*, 2005) in a study in which muscle protein accretion and synthesis were measured using the femoral arteriovenous phenylalanine net balance technique during a constant infusion of deuterated L-phenylalanine. After a bolus ingestion of ~7 g EAAs, mean net phenylalanine (Phe) uptake into protein was significantly less in the older participants and the mean rate of Phe disappearance (proportional to protein synthesis) was increased above basal levels only in the younger participants. Such findings were posited to indicate the important role of the amount of amino acids ingested in a single eating occasion in stimulating muscle protein synthesis and that smaller intakes spread over the day might fail to stimulate muscle protein synthesis adequately and contribute to age-associated muscle protein loss. The authors suggested that per-meal protein intake may be more important than total daily protein intake for individuals where total daily intake is spread over several meals.

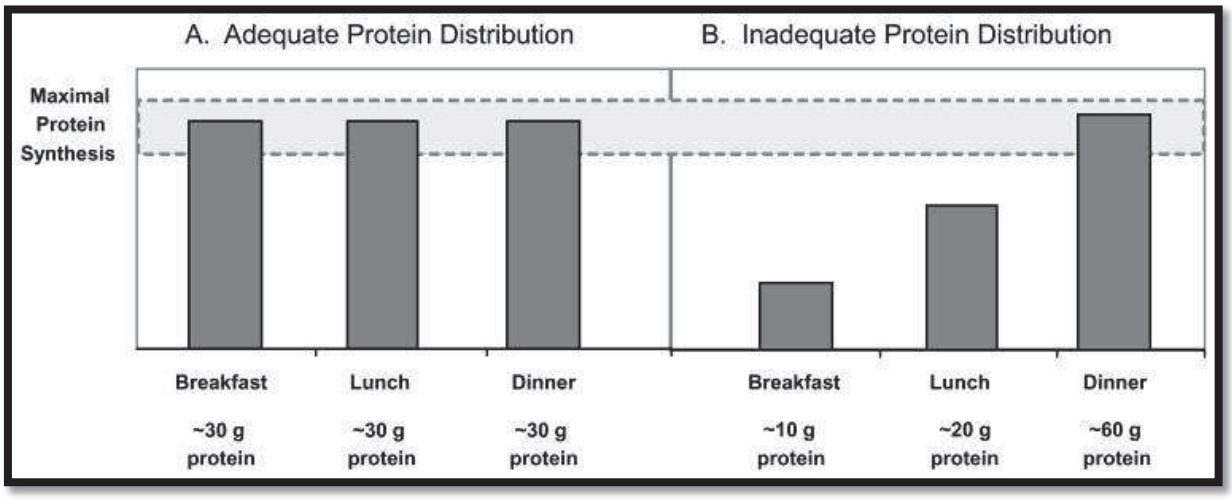
In (Bouillanne *et al.*, 2013) a protein pulse feeding regime was implemented for 6 weeks in 29 older, malnourished/at risk patients, at an inpatient rehabilitation unit, in which 72% of daily (1.31 g/kg) protein was consumed at 1 meal, at noon. DEXA body composition (lean mass, appendicular lean mass and body cell mass)), hand grip strength and ADL scores were determined at baseline and after 6 weeks. Results from patients on the pulse diet were compared to 34 other inpatients who had consumed (1.27 g/kg/d) protein, but in an evenly spread regime, over 4 meals. Lean mass, appendicular skeletal muscle mass and body cell mass indices (kg/m^2) increased significantly in the protein pulse fed group compared with those in the spread diet group. Hand grip strength and ADL scores were not significantly different between the groups. Median body weight in the 2 groups was 52 kg, and protein 67 g/d (providing ~22 g essential amino acids (EAA)). At noon, the pulse diet had provided 16 g of EAA compared with 7 g provided in the spread diet. The optimal dose of EAA required to stimulate muscle protein synthesis (Katsanos *et al.*, 2005) had been reached in the pulse group, but not in the spread group which explained the differences in body composition indices.

2.5.2 Impact of protein quality and of specific amino acids on muscle protein synthesis in older people

As noted above, a relatively small bolus dose of EAA (7g) based on the amino acid composition of whey protein did not stimulate muscle protein synthesis in older people (Katsanos *et al.*, 2005). However, when the proportion of leucine in the EAA mixture was increased from 26% (representing the composition of whey protein) to 41% the attenuated response of muscle protein synthesis in older people was reversed i.e. the muscle protein fractional synthetic rate was increased significantly (Katsanos *et al.*, 2006). Mean leg phenylalanine net balance (a reflection of the balance between muscle protein synthesis and muscle protein degradation) was significantly improved in younger participants when given either the standard or the leucine-enriched EAA mixture, but net balance was increased in older participants only after ingestion of the 41% leucine EAA mixture. The authors noted that the increase in plasma leucine concentration that resulted from ingestion of the 26% leucine mixture was equivalent to that expected following consumption of a meal of average protein content (~15 g) (Katsanos *et al.*, 2006).

In a 2009 review it was observed that muscle protein synthesis was *blunted* in the elderly when protein and carbohydrates were coingested or when the quantity of protein was less than ~20 g per meal (Paddon-Jones D, 2009). As a 20 g serving of most animal/ plant-based proteins contains 5 – 8 g of essential amino acids, and as ageing was associated with an inability of skeletal muscle to respond to low (~7.5 g) doses of essential amino acids (Katsanos *et al.*, 2005), the authors recommended that 25 – 30 g of high quality protein (~10 g EAA) per meal would stimulate skeletal muscle mass maximally providing a useful strategy to help maintain muscle mass in older subjects and in reducing the risk of sarcopenia. The proposed relationship between protein ingestion per meal and the resultant anabolic response, was depicted as pictorial example (Figure 2.2).

Figure 2.2 A concept diagram illustrating the theoretical impact of quantity and distribution of protein intake across the day on muscle protein synthesis (Paddon-Jones D, 2009)



The utility of the (Paddon-Jones D, 2009) recommendation of 25 – 30 g protein per meal was investigated in a cohort of older (68.7 y) Mexicans by (Ruiz Valenzuela RE, 2013). In a cross-sectional study design, the difference in DEXA-determined appendicular skeletal muscle mass was assessed in those who consumed < 25 g at any of 3 main meals with those who consumed > 25 g protein during at least one meal. After adjusting for body weight, gender and height, no significant differences in appendicular lean mass were reported. In reporting meal time protein consumption, the authors used the higher protein threshold of 30 g and reported that 81% and 86% of subjects consumed < 30 g of protein at breakfast and at the evening meal, respectively. At both meal times, protein 'under-consumption' was most evident in females, an observation explained with reference to the significantly lower energy intakes among females compared with males. Physical activity or exercise is a well-recognised stimulus for skeletal muscle protein synthesis and there is good evidence of positive interactions between exercise and nutrient intake in promoting protein synthesis (see (Wackerhage and Rennie, 2006) for review). In a study to examine interactions between exercise and nutrition, 20 g of whey protein resulted in maximal stimulation of muscle protein synthesis in 30 older men (aged 71 ± 5 y) whereas < 20 g was insufficient to mount a robust increase in muscle protein synthesis compared with the fasted state (Yang *et al.*, 2012a).

Changes in myofibrillar protein fractional synthetic rate (FSR) in the same 30 older men was compared after ingestion of 0 g, 20 g or 40 g of soy protein and results compared to those following ingestion of equivalent amounts of whey protein (Yang *et al.*, 2012b). In contrast to whey protein, 20 g and 40 g soy failed to stimulate increased rates of myofibrillar FSR at rest, and only after a bout of resistance exercise did 40 g soy significantly increase myofibrillar FSR. The authors concluded that the relationship between protein intake and muscle protein synthesis was both dose and protein-source dependent with soy exhibiting a reduced ability to stimulate muscle protein synthesis due to its lower leucine content (~8% compared with ~12% in whey). Protein source-dependent differences in rates of leucine oxidation were also observed; a greater proportion of amino acids from 20 g soy (compared to 20 g whey) were diverted towards oxidation and were thus unavailable for protein synthesis.

In 33 healthy older (73 ± 2y) men, graded intakes (10, 20 or 35 g) of labelled whey protein were administered and it was observed that only the highest dose (35 g) increased muscle protein synthesis significantly above basal levels (Pennings *et al.*, 2012). In an earlier study (Pennings *et al.*, 2011) compared the effects of 20 g of whey with the same dose of a more slowly digestible protein (casein) and found that whey protein ingestion stimulated postprandial muscle protein accretion more effectively than casein or casein hydrolysate. This was explained in terms of the difference in digestion and absorption kinetics and amino acid composition (12.5% leucine cf. 8.5% in casein hydrolysate) of whey.

2.5.3 Rationale for the derivation of the muscle protein synthesis score (MPSS)

Since changes in protein accretion/ retention, daily protein turnover and body composition could be achieved by the modulation of protein feeding patterns alone (Arnal *et al.*, 1999) the effects of diurnal patterns of protein consumption on physical capability deserves further examination. There is a paucity of information on diurnal patterns of protein consumption among populations and particularly in longitudinal studies which can address effects on health in later life. From the literature it was also established that in older people a small bolus ingestion of ~7 g of essential amino acids (equivalent to ~15 g of meal protein), was insufficient to stimulate muscle protein synthesis (Katsanos *et al.*, 2005). A dose of 10 g essential amino acids (equivalent to ~25 g of high quality protein) was shown to stimulate muscle protein synthesis in both elderly and young men (Cuthbertson D, 2005), but in comparison (Yang *et al.*, 2012a) showed that doses of (isolated whey) protein < 20 g did not increase MPS above basal, fasting values in older (71 y) men. It has been proposed that the ingestion of 25 – 30 g of high quality protein per meal may be a useful strategy to overcome age-related anabolic deficiency (Paddon-Jones D, 2009).

Notwithstanding some studies were conducted exclusively in one gender (Arnal *et al.*, 1999) (Cuthbertson D, 2005) (Yang *et al.*, 2012a) there was no indication that reported effects of protein quantity on muscle protein synthesis are gender-specific. For the present project, 20 g was chosen as the cut-off for protein intake because of the risk that a higher threshold would affect women disproportionately since daily energy (kcals) and protein intakes (g/d) are higher in males than in females. This latter point is well illustrated in the study by (Ruiz Valenzuela RE, 2013).

2.5.4 Muscle protein synthesis score – implementation and calculation

Where meal protein intake was ≥ 20 g at any of the eight eating occasions in 1982, 1989 and 1999, this was scored one (1). Daily scores (the sum of eight eating occasions) were then calculated for each individual. Thus the lowest and highest possible scores were 0 and 8 respectively in each year of measurement. These yearly variables were merged into the dataset that comprised NSHD participants who had provided dietary data in all years. An adulthood muscle protein synthesis score was calculated by summing the 3 yearly muscle protein synthesis scores. This score was a reflection of the frequency with which protein ≥ 20 g had been consumed across the day during at three measurement periods, 1982 – 1999 and, therefore, the best estimate of potential for muscle protein synthesis across adulthood.

2.6 Identification of predicted under- and over-reporting

Total energy expenditure (TEE) is presumed to be equivalent to total energy intake (TEI) in weight-stable individuals. TEE can be attributed to the sum of energy expended in basal metabolic rate (BMR), in physical activity and in thermogenesis (attributable *inter alia* to food consumption, shivering and drug (caffeine, nicotine and alcohol) intake). This equivalence between TEI and TEE in weight-stable adults provides a simple basis for predicting potential over- and under-reporters of dietary energy intake. On this basis, predicted under-reporters are those in whom reported energy intake is less than that which would be compatible with long-term weight maintenance with the converse for likely over-reporters. To predict TEI for the purposes of identifying predicted under-reporters, TEE was estimated by calculated BMR multiplied by an estimated physical activity factor or level (Physical Activity Level).

2.6.1 Identification of estimated under-reporting

Schofield's age-stratified equations (Schofield, 1985) for the prediction of BMR (from body weight) formed the basis of the equations published in the FAO/WHO/UNU document, Energy and Protein Requirements, 1985. Their universal validity and application was subsequently queried as 47% of the database used to develop the equations comprised Italian (predominantly military) subjects, with very few individuals from tropical regions. In 2005, new equations (now known as the "Oxford" equations) (Henry, 2005) for the estimation of BMR were developed using data from published and measured values (~10500 BMR values) excluding Italian subjects and including many more from tropical regions (Henry, 2005). In the present study, these Henry/ Oxford equations were used to estimate BMR for the NSHD participants who provided dietary data in all years. As no significant advantage was afforded in predicting BMR with the inclusion of height (Henry, 2005), height was not used and the following gender-specific equations were employed:

Males (30 – 60 years) BMR (kcal/ d) = $14.2W^{8} + 593$
Females (30 – 60 years) BMR (kcal/ d) = $9.74W + 694$

[8] Body weight in kg in the relevant year in which dietary intake was recorded

Once BMR was estimated, it was necessary to multiply the resultant energy expenditure by a physical activity level (PAL). The PAL chosen was the 'Goldberg cut-off' of 1.14 (Goldberg GR, 1991) which is appropriate for methods purporting to measure habitual intake among individuals (*n*=1) as in the NSHD. Where reported EI was less than 1.14 * BMR such individuals were identified as predicted (or likely) under-reporters. The use of this cut-off value does not take into account the true TEE of each individual.

Within the general population of the United Kingdom, the range of PAL values for individuals in energy balance and leading sustainable lifestyle is between 1.38 and 2.5 (SACN, 2011). In determining dietary reference values for energy for the UK population in 2011, the Scientific Advisory Committee for Nutrition identified appropriate values for PAL for adults (19 – 65 y) from an analysis of available total energy expenditure (TEE) literature. PAL values for adults (the median, 25th and 75th centiles) were calculated directly from individual TEE values reported in the OPEN and Beltsville data sets. Where previously COMA (DH, 1991) had reported a PAL value 1.4 for adults, the median PAL value of a reference adult population like the UK, where ~60% are overweight or obese, was designated as 1.63 (SACN, 2011). By comparison, the use of 'Goldberg cut-off' (a PAL 1.14) is designed to identify individuals who are reporting energy intakes that are *unsustainable* in the long term and *inconsistent* with long-term survival.

2.6.2 Implications of under-reporting energy vs. protein

There is evidence that protein is better reported than total energy intake (Livingstone and Black, 2003); in double validations that employed doubly labelled water (DLW) and urinary nitrogen to validate energy intake (EI) (Black *et al.*, 1995; Black, 1997; Black *et al.*, 2000) the average reporting bias for protein was –2% compared with –14% for energy, and the proportion of individuals identified as under-reporting was greater by DLW than urinary nitrogen excretion. Macronutrients most likely to be under-reported are those deemed less socially desirable; obese men selectively under-reported fat intake in (Goris *et al.*, 2000) and amongst 38 healthy women (34 overweight and 12 obese) subjects tended to report their intake in a socially desirable way, by eating or reporting less frequently foods considered unhealthful or fattening, like sweets and fried foods (Scagliusi *et al.*, 2003).

In 36 034 subjects of the European Prospective Investigation into Cancer and Nutrition (aged 35 – 74 y), the degree of under-reporting was found to differ by nutrient. The study suggested that under-reporting was greater for fat and alcohol than for protein and carbohydrate intake (Ocke *et al.*, 2009). By comparison, protein intakes reported by self-administered FFQ in the EPIC study (Kroke *et al.*, 1999) were ~23% lower than estimates derived from urinary nitrogen and reported energy intakes 22% less (on average) than TEE measured by DLW.

2.6.3 Identification of estimated over-reporting

The approach to the identification of over-reporters was as reported by (Johansson *et al.*, 1998). In a sample of 3,020 Norwegian subjects (16 – 79 y) mean age 42.7 ± 16.1 (males) and 41.6 ± 16.7 (females), estimates of BMR were calculated from standard equations based on weight, age and sex. EI:BMR was calculated for each individual and compared with cut-off values for EI:BMR of <1.14, 1.14 – 1.34 (under-reporters), 1.35 – 2.39 (normal range) and ≥ 2.4 (over-reporters). Compared to those reporting a normal EI:BMR, over-reporters were younger, had lower BMIs, were more likely to be lean (a BMI < 20 kg/m^2) and to want to increase their weight. In the present research project, Oxford predictive equations (Henry, 2005) were used to determine BMR and individuals were classified as likely over-reporters if their EI:BMR was greater than, or equal to 2.4.

In analysis of 574 measurements of TEE using doubly-labelled water, an EI:BMR above the range 2.0–2.4 was suggested as the maximum which was likely to be sustainable by (Black *et al.*, 1996). When considering upper limits of human energy expenditure, it is important to distinguish between the maximum rate of energy expenditure which is achievable over a short period of time and the maximum sustainable as a long-term way of life. The maximum achieved over short periods, e.g. by competitors in the *Tour de France* or in polar exploration is suggested to be a PAL of 4.0. For serious athletes, the PAL range is 2 – 3.5 and for soldiers on active duty, lumberjacks and colliers an average PAL of ~2.4 is suggested. Estimates of PAL > 2.4 were obtained during periods of rigorous training and are unlikely to be sustained over the long term (Shetty, 2005)(Table 2.3).

Table 2.3 Physical Activity Level (PAL) attributable to lifestyle and level of activity (Shetty, 2005)

Lifestyle and level of activity	**PAL**
Chair/ bed-bound	1.2
Seated work with no option of moving around and little or no strenuous leisure activity	1.4 – 1.5
Seated work with discretion and requirement to move around but little or no strenuous leisure activity	1.6 – 1.7
Standing work (e.g. housework, shop assistant)	1.8 – 1.9
Significant amounts of sport or strenuous leisure activity (30 – 60 minutes, 4 – 5 times a week)	+0.3
Strenuous work or highly active leisure time	2.0 – 2.4

2.7 Identification of low protein consumers

As a prerequisite for testing the hypothesis that relatively low protein intake across adulthood would predict poorer physical capability in middle age (60 – 64 years) – see Chapter 6 – it was necessary to define low protein intake. For this purpose, low protein consumers were identified in six ways:

1. Those in quintile 1 of absolute mean protein intake across all 3 years of measurement (g/d);

2. Those in quintile 1 of mean protein intake across all 3 years of measurement relative to body mass (g/kg/d);

3. Those in quintile 1 of mean protein intake across all 3 years of measurement (expressed as a percentage of total daily energy intake (%TE));

4. Those in quartile 1 of the muscle protein synthesis score (MPSS);

5. Those in quintile 1 of absolute mean protein intake across all 3 years of measurement (g/d) excluding individuals predicted to have ever under- or over-reported their energy intake throughout the period 1982 – 1999.

6. Those in quintile 1 of mean protein intake across all 3 years of measurement relative to body mass (g/kg/d) excluding individuals predicted to have ever under- or over-reported their energy intake throughout the period 1982 – 1999.

These last two calculations (5 and 6 above) were undertaken as sensitivity analyses to determine the effect of predicted under- and over-reporting of energy intakes on the outcomes of hierarchical linear regression analyses used to test the hypothesis.

2.7.1 Quintile 1 of mean protein intake across all 3 years of measurement, relative to body mass (g/kg/d)

To calculate protein intakes relative to body weight, individual body weights (kg) in 1982, 1989 and 1999 were merged into the dietary dataset which comprised NSHD participants who had provided dietary data in all 3 years.

Daily protein, per kg of body weight, per day was calculated in all 3 years, using the expression e.g. daily protein (g) in 1982/body weight (kg) in 1982. A 3 y mean of these values was calculated, split by gender and quintiles calculated separately for males and females.

Table 2.4 Quintiles of mean protein intake across all 3 years of measurement relative to body mass (g/kg/d) in male NSHD participants who provided dietary data in all years

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (g/kg/d)	Frequency (*n*)	Percent
1	≤ 0.91	114	20.1
2	0.92 – 1.03	113	19.9
3	1.04 – 1.12	113	19.9
4	1.13 – 1.26	113	19.9
5	≥ 1.27	114	20.1

Using protein intakes collected over three measurement periods, 1982 – 1999, 114 males in quintile 1 consumed, on average ≤ 0.91 g/kg/d (Table 2.4).

Table 2.5 Quintiles of mean protein intake across all 3 years of measurement relative to body mass (g/kg/d) in female NSHD participants who provided dietary data in all years

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (g/kg/d)	Frequency (*n*)	Percent
1	≤ 0.86	139	20
2	0.87 – 0.99	139	20
3	1.00 – 1.10	139	20
4	1.11 – 1.24	139	20
5	≥ 1.25	139	20

Using protein intakes collected over three measurement periods, 139 females in quintile 1 consumed on average ≤ 0.86 g/kg/d (Table 2.5).

A new categorical variable was created to identify males and females in quintile 1 (=1) and individuals in higher quintiles (=0). The use of this variable in regression analyses compared all individuals in quintile 1 of mean protein (*n*=253) with those in higher quintiles of mean protein (*n*=1009) across 3 years of measurement, relative to body mass (g/kg/d).

2.7.2 Quintile 1 of absolute mean protein intake across all 3 years of measurement (g/d)

The 3 y mean of daily protein consumption (g) in 1982, 1989 and 1999 was calculated using all reported values provided by NSHD participants who provided dietary data in all 3 years (*n*=1263). The mean was split into male and female variables and quintiles calculated separately.

Table 2.6 Quintiles of absolute mean protein intake across all 3 years of measurement (g/d) in male NSHD participants who provided dietary data in all years

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (g/d)	Frequency (*n*)	Percent
1	≤ 71.43	114	20.1
2	71.44 – 79.68	114	20.1
3	79.69 – 86.38	113	19.9
4	86.39 – 95.89	113	19.9
5	≥ 95.9	114	20.1

Using protein intakes collected over three measurement periods, 1982 – 1999, 114 males in quintile 1 consumed on average ≤ 71.43 g/d (Table 2.6).

Table 2.7 Quintiles of absolute mean protein intake across all 3 years of measurement (g/d) in female NSHD participants who provided dietary data in all years

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (g/d)	Frequency (*n*)	Percent
1	≤ 56.85	139	20
2	56.86 – 63.46	139	20
3	63.47 – 69.03	139	20
4	69.04 – 76.78	139	20
5	≥ 76.79	139	20

Using protein intakes collected at all 3 measurement periods, 139 females in quintile 1 consumed on average ≤ 56.85 g/d (Table 2.7).

A new categorical variable was created to identify males and females in quintile 1 (=1) and individuals in higher quintiles (=0). The use of this variable in regression analyses compared all individuals in quintile 1 of absolute mean protein (*n*=253) with those in higher quintiles of absolute mean protein (*n*=1010) across 3 years of measurement.

2.7.3 Quintile 1 of mean protein intake across all 3 years of measurement (expressed as a percentage of total daily energy intake)

Daily protein as a percentage of daily energy was calculated in all 3 years using the expression e.g. ((daily protein (g) in 1982 * 4) / daily energy in 1982) * 100. A mean was calculated from 3 values, split by gender and quintiles calculated separately for males and females.

Table 2.8 Quintiles of mean protein intake across all 3 years of measurement (expressed as a percentage of total daily energy intake) in male NSHD participants who provided dietary data in all years

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (expressed as a percentage of total daily energy)	Frequency (*n*)	Percent
1	≤ 13.14	113	19.9
2	13.15 – 14.1	115	20.2
3	14.11 – 14.94	113	19.9
4	14.95 – 15.99	113	19.9
5	≥ 16.00	114	20.1

Using protein intakes collected over three measurement periods, 1982 – 1999, 113 males in quintile 1 consumed on average ≤ 13.14% of total daily energy, as protein (Table 2.8).

Table 2.9 Quintiles of mean protein intake across all 3 years of measurement (expressed as a percentage of total daily energy intake) in female NSHD participants who provided dietary data in all years

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (expressed as a percentage of total daily energy)	Frequency (*n*)	Percent
1	≤ 13.63	139	20
2	13.64 – 14.76	139	20
3	14.77 – 15.69	139	20
4	15.70 – 17.20	139	20
5	≥ 17.21	139	20

Using protein intakes collected at all 3 measurement periods, 139 females in quintile 1 consumed on average ≤ 13.63% of total daily energy, as protein (Table 2.9).

A new categorical variable was created to identify males and females in quintile 1 (=1) and individuals in higher quintiles (=0). The use of this variable compared all individuals in quintile 1 of mean protein (*n*=252) with those in higher quintiles of mean protein (*n*=1011) across 3 years of measurement (expressed as a percentage of total daily energy intake).

2.7.4 Quartile 1 of the adulthood muscle protein synthesis score

In NSHD participants who had provided dietary data in all years, the adulthood muscle protein synthesis score was split by gender and quartiles calculated.

Table 2.10 Quartiles of adulthood muscle protein synthesis score in NSHD participants who provided dietary data in all years

Quartiles of Muscle Protein Synthesis Score	Males (*n*=568)	Females (*n*=695)
1	≤4 (*n*=153) (26.9%)	≤3 (*n*=261) (37.6%)
2	5 (*n*=160) (28.2%)	4 (*n*=191) (27.5%)
3	6 (*n*=180) (31.7%)	5 (*n*=157) (22.6%)
4	7+ (*n*=75) (13.2%)	6+ (*n*=86) (12.4%)

Using protein intakes collected at all 3 measurement periods, 1982 – 1999, 153 males and 261 females in quartile 1 had a muscle protein synthesis score ≤ 4 and ≤ 3, respectively (Table 2.10). A new categorical variable was created to identify individuals in MPSS score quartile 1 (=1) and individuals in higher quartiles (=0). The use of this variable in regression analyses compared all individuals in quartile 1 of MPSS (*n*=414) with those in higher quartiles of MPSS (*n*=849) across 3 years of measurement.

2.7.5 Quintile 1 of absolute mean protein intake across all 3 years of measurement (g/d) excluding predicted misreporters

For the first sensitivity analysis, in NSHD participants who had provided dietary data in all years (*n*=1263), individuals predicted to have ever under- or over-reported their energy intake were identified (*n*=660) and their values for mean protein (g/d) excluded from the analysis. Gender specific quintiles were calculated as before.

Table 2.11 Quintiles of absolute mean protein intake across all 3 years of measurement (g/d) in male NSHD participants who provided dietary data in all years, excluding predicted under- or over-reporters

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (g/d) excluding predicted under- and over-reporters	Frequency (*n*)	Percent
1	≤ 80.00	57	20
2	80.01 – 85.89	57	20
3	85.90 – 92.78	57	20
4	92.79 – 99.50	57	20
5	≥ 99.51	57	20

After the exclusion of predicted under- or over-reporters and using protein intakes collected over three measurement periods, 57 males in quintile 1 consumed on average ≤ 80 g/d (Table 2.11).

Table 2.12 Quintiles of absolute mean protein intake across all 3 years of measurement (g/d) in female NSHD participants who provided dietary data in all years, excluding predicted under- or over-reporters

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement (g/d) excluding predicted under- and over-reporters	Frequency (*n*)	Percent
1	≤ 63.49	64	20.1
2	63.5 – 69.06	64	20.1
3	69.07 – 74.1	62	19.5
4	74.11 – 80.00	65	20.4
5	≥ 80.01	63	19.8

After the exclusion of predicted under- or over-reporters and using protein intakes collected over three measurement periods, 64 females in quintile 1 consumed on average ≤ 63.49 g/d (Table 2.12).

A new categorical variable was created to identify males and females in quintile 1 (=1) and individuals in higher quintiles (=0). The use of this variable compared all individuals in quintile 1 of absolute mean protein (*n*=121) with those in higher quintiles of absolute mean protein (*n*=482) across 3 years of measurement.

2.7.6 Quintile 1 of mean protein intake across all 3 years of measurement, relative to body mass (g/kg/d) excluding predicted misreporters

For the second sensitivity analysis, in NSHD participants who had provided dietary data in all years (*n*=1263), individuals predicted to have ever under- or over-reported their energy intake were identified (*n*=660) and their values for mean protein, relative to body mass (g/kg/d) excluded from the analysis. Gender specific quintiles were calculated as before.

Table 2.13 Quintiles of mean protein intake across all 3 years of measurement, relative to body mass (g/kg/d) in male NSHD participants who provided dietary data in all years, excluding predicted misreporters

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement relative to body mass (g/kg/d) excluding predicted under- and over-reporters	Frequency (*n*)	Percent
1	≤ 1.05	57	20.1
2	1.06 – 1.12	56	19.7
3	1.13 – 1.21	58	20.4
4	1.22 – 1.32	56	19.7
5	≥ 1.33	57	20.1

After the exclusion of predicted misreporters and using protein intakes collected over three measurement periods, 1982 – 1999, 57 males in quintile 1 consumed ≤ 1.05 g/kg/d (Table 2.13).

Table 2.14 Quintiles of mean protein intake across all 3 years of measurement relative to body mass (g/kg/d) in female NSHD participants who provided dietary data in all years, excluding predicted misreporters

Quintile	Quintile cutpoints of mean protein intake across all 3 y of measurement relative to body mass (g/kg/d) excluding predicted under- and over-reporters	Frequency (*n*)	Percent
1	≤ 1.02	63	19.8
2	1.03 – 1.11	64	20.1
3	1.12 – 1.22	63	19.8
4	1.23 – 1.33	65	20.4
5	≥ 1.34	63	19.8

After the exclusion of predicted under- and over-reporters and using protein intakes collected over three measurement periods, 63 females in quintile 1 consumed on average ≤ 1.02 g/kg/d (Table 2.14).

A new categorical variable was created to identify males and females in quintile 1 (=1) and individuals in higher quintiles (=0). The use of this variable compared all individuals in quintile 1 of mean protein (*n*=120) with those in higher quintiles of mean protein (*n*=482) across 3 years of measurement, relative to body mass (g/kg/d).

2.8 Habitual Physical activity

2.8.1 Habitual Physical Activity in 1982

In 1982 when NSHD cohort members were 36 y they were visited at home by a trained nurse who questioned them on the frequency and duration of their participation in a range of leisure time activities in the preceding month. The questionnaire administered was based on the Minnesota leisure time physical activity questionnaire (Taylor *et al.*, 1978). As 90% of these interviews were conducted between the months of April and September seasonal influences were subsequently investigated. Significant seasonal fluctuations in the frequency of reported activities were identified (Kuh, 1992) and as such questionnaire responses are likely to overestimate average levels of physical activity over a whole year.

Three main areas of activity were identified: cycling and walking, DIY/ heavy gardening and sports and recreational activities. In 1982 for each activity, participants were classified as:

Inactive (reported no participation in the previous month);

Moderately active (reported participation 1 – 4 times in the previous month) or;

Most active (reported participation 5 or more times in the previous month).

The criteria used to classify physical activity into these categories are summarised in (Table 2.15) below (Kuh, 1992).

Table 2.15 Classification of leisure time physical activity of NSHD participants in 1982 (Kuh, 1992)

Type of Physical Activity	MOST active	LESS active	INACTIVE
Cycling and walking	Either 1. Normally rides or walks to work for at least 0.5 h (round trip) or 2. 12 rides/ walks of 0.5 h in leisure time in previous month	Either 1. Normally rides or walks to work for < 0.5 h (round trip) or 2. 1-11 rides/ walks of 0.5 h in leisure time in previous month	Does not normally ride/ walk to work and no reports of riding/ walking in leisure time in previous month
DIY/ Heavy gardening	Five + times in the previous month	1 – 4 times in the previous month	No reported activity in the previous month
Sports and recreational activities (27)	Five + times in the previous month	1 – 4 times in the previous month	No reported activity in the previous month

Heavy gardening comprised any of ten heavy gardening activities, e.g. digging earth, chopping wood, brick laying and moving heavy objects. Sports and recreational activities (from a list of 27 activities) included badminton, swimming, yoga, football, jogging, dancing and exercises at home e.g. press ups (Table 2.15).

Of the NSHD cohort contacted in 1982, 3299 individuals (1639 males and 1660 females) provided data on their participation in cycling and walking in the preceding month when aged 36 y. By the method described above (Table 2.15) they were allocated (by the MRC) to one of 3 categories: inactive (value = 0), less active (value = 1) or most active (value = 2); 23 individuals were classified as unknown and removed from the analysis (Table 2.16).

Table 2.16 Participation in cycling and walking in 1982 by NSHD cohort members

	Frequency (*n*)	Percentage
Inactive	727	22
Less active	1495	45.3
Most active	1077	32.6
Total	3299	100

In 1982 NSHD participants were asked about their participation in DIY activities and heavy gardening. As for cycling/walking, respondents (*n*=3309) were allocated to one of 3 categories; inactive, less active or most active. Thirteen individuals were classified as unknown and removed from the analysis (Table 2.17).

Table 2.17 Participation in DIY and heavy gardening in 1982 by NSHD cohort members

	Frequency (*n*)	Percentage
Inactive	1520	45.9
Less active	1121	33.9
Most active	668	20.2
Total	3309	100

Participation in a range of sporting and recreational activities was also recorded at 36 y and respondents (*n*=3309) classified as either inactive, less active or most active. Thirteen were classified as unknown and were removed from the analysis (Table 2.18).

Table 2.18 Participation in a range of sport/ recreational activities in 1982 by NSHD cohort members

	Frequency (*n*)	Percentage
Inactive	1219	36.8
Less active	837	25.3
Most active	1253	37.9
Total	3309	100

2.8.1.1 Creating a summary value for leisure time physical activity in 1982

As the categorical values were consistent and comparable, i.e. inactive (=0), less active (=1) and most active (=2) across all three physical activity variables in 1982, the three values were added together for all individuals to produce a summary score for leisure time physical activity at age 36 y (Table 2.19).

Table 2.19 summary values for leisure time physical activity in 1982 for NSHD participants who provided data for all three activities

Summary score for leisure time physical activity, 1982	Frequency (*n*)	Percentage
0	191	5.8
1	456	13.8
2	722	21.9
3	780	23.7
4	667	20.2
5	360	10.9
6	121	3.7
Total	3297	100

2.8.1.2 Classification of the 1982 summary value

A summary value for all three physical activities examined in 1982 was available for 3297 individuals (Table 2.19) and values ranged from 0 – 6; i.e. 191 individuals reported no participation (classified inactive) in all of the activities examined in 1982 and 121 individuals were classified as most active in all three activities.

Using the summary value created for 1982, participants were then classified as either inactive (0), moderately active (1) or most active (2); i.e. the classification used in 1989 and 1999.

Individuals with a summary value of 0 were classified as inactive (categorical value = 0) (*n*=191) and those with a summary value of 1 (*n*=456), 2 (*n*=722) or 3 (*n*=780) were classified as moderately active (categorical value = 1) (*n*=1958). See (Table 2.20) below.

A summary value of 4 (*n*=667) could result from a combination of 1, 1, 2 (less active, less active, most active in 3 leisure time activities) or a combination of 2, 2, 0 (most active, most active, inactive in 3 leisure time activities). The former (1, 1, 2 combination) was valid for 388 individuals and the latter (2, 2, 0 combination) for 279 individuals.

Individuals with a 1982 summary value of 4 who had been classified in 3 activities as less active, less active, most active (i.e. the 1, 1, 2 combination) (*n*=338) were classified as moderately active (categorical value = 1). Individuals with a 1982 summary value of 4 who had been classified in 3 activities most active, most active, inactive (i.e. the 2, 2, 0 combination) (*n*=279) were classified most active (categorical value = 2). See (Table 2.20) below.

Individuals with a summary value of 5 (*n*=360) or 6 (*n*=121) were classified as most active (categorical value = 2) (*n*=481) (Table 2.20).

Table 2.20 Classification of 1982 summary value

Summary value	Classification	Categorical value	Frequency (*n*)	Category frequency (*n*)
0	Inactive	0	191	191
1	Moderately active	1	456	2346
2	Moderately active	1	722	
3	Moderately active	1	780	
4 (1,1,2)	Moderately active	1	388	
4 (2,2,0)	Most active	2	279	760
5	Most active	2	360	
6	Most active	2	121	

In 1982, 3297 NSHD participants (aged 36 y) were classified by their participation in a range of leisure time physical activity pursuits into inactive (*n*=191) (5.8%), moderately active (*n*=2346) (71.2%) and most active (*n*=760) (23.1%). This summary variable was merged into the dataset which comprised NSHD participants who provided dietary data in all years.

2.8.2 Habitual Physical Activity in 1989

In 1989 (at age 43 y) NSHD participants participation in sports, vigorous leisure activities or exercises, how many months in the year and the monthly frequency of each activity were reported (Cooper *et al.*, 2011b).

Table 2.21 Sports and recreational activity in 1989 in NSHD participants

	Frequency (*n*)	Percentage
Inactive	1699	52.1
Moderately active	753	23.1
Most active	810	24.8
Total	3262	100

Where participation in any relevant sports/recreational activities was reported as none, individuals were classified as inactive (category value = 0); where participation was recorded as 1 – 4 times a month, individuals were classified as moderately active (categorical value = 1) and where participation was reported as 5 or more times a month, individuals were classified as most active (categorical value = 2), following methodology described by (Cooper *et al.*, 2011b). Where individuals were classified as participation unknown (*n*=2100) these were removed from the analysis. These data were merged into the dataset which comprised NSHD participants who had provided dietary data in all years.

2.8.3 Habitual Physical Activity in 1999

In 1999 (at age 53 years), leisure time participation in any sports, vigorous leisure activities or exercise, not including getting to and from work, in the past 4 weeks, and the number of occasions on which these activities were undertaken, was reported (Cooper *et al.*, 2011b). Participants were categorised as inactive (reported no participation), moderately active (participated in relevant activities one to four times in the previous 4 weeks) or most active (participated in relevant activities five or more times in the previous 4 weeks). The variable generated in 1999 by the MRC specifically *excluded* activity involved in getting to and from work. In this respect it was not comparable with the 1982 summary variable (specifically the cycling and walking component) which differentiated, but included, both cycling and walking to/from work and during leisure time. This issue serves to highlight one of the difficulties in longitudinal cohort studies, namely that of collecting different, non-comparable data at different time points.

A single physical activity variable was available at age 53 y (Table 2.22). Where participants were classified as participation unknown (*n*=2) or not interviewed (*n*=2374) these were removed from the analysis. This variable (available for 2986 individuals) was merged into the dataset which comprised NSHD participants who had provided dietary data in all years.

Table 2.22 Physical activity in 1999 in NSHD participants

	Frequency (*n*)	Percentage
Inactive	1477	49.5
Moderately active	518	17.3
Most active	991	33.2
Total	2986	100

2.8.4 Derivation of an adulthood physical activity score

An adulthood leisure time physical activity score was calculated following methodology described by (Cooper *et al.*, 2011b). Adulthood physical activity scores reflected habitual leisure time activity only as occupational activity was never measured throughout this period.

Table 2.23 Adulthood leisure time physical activity scores for NSHD participants who provided physical activity data in 1982, 1989 and 1999

Adulthood physical activity score	Frequency (*n*)	Percentage
0	84	3.2
1	676	26.1
2	481	18.6
3	493	19.0
4	408	15.8
5	307	11.9
6	140	5.4

Adulthood physical activity scores were available for 2589 individuals and ranged from 0 (classified as inactive over 3 measurement periods) to 6 (classified as most active over 3 measurement periods) (Table 2.23). Using these scores, and following methodology described by (Cooper *et al.*, 2011b) individuals were classified as either inactive at all 3 ages (those scoring 0) (*n*=84), more active (scoring 1 or 2) (*n*=1157), active (scoring 3 or 4) (*n*=901) or most active at all 3 ages (scoring 5 or 6) (*n*=447) (Table 2.24). This categorical variable was merged into the dietary dataset which comprised NSHD participants who provided dietary data in all years.

Table 2.24 Classification of adulthood physical activity scores for NSHD participants who provided physical activity data in 1982, 1989 and 1999

Adulthood leisure time physical activity	Frequency (*n*)	Percentage
Inactive	84	3.2
More active	1157	44.7
Active	901	34.8
Most active	447	17.3
Total	2589	100

2.9 Creation of dummy variables for categorical data

In preparation for hierarchical linear regression analyses, dummy variables were created for the categorical variables: adulthood physical activity, self-reported health status at 60 – 64 y, participant's socioeconomic position (SEP) at 53 y and participant's SEP at 4 y (father's SEP in 1950).

2.9.1 Adulthood physical activity

In NSHD participants who had provided dietary data in all 3 years, those classified as inactive at all 3 ages (*n*=34) and more active (*n*=517) were combined for the analysis into a new group, labelled 'sedentary'. Sedentary was the reference group/category (=0) against which two dummy variables were compared: MoreActive and MostActive (Table 2.25).

Table 2.25 Adulthood physical activity. Creation of a reference category (sedentary) and 2 dummy variables (MoreActive and MostActive) in NSHD participants who provided dietary data in all years

	Frequency (*n*)	Percentage
Sedentary	551	43.8
MoreActive	485	38.5
MostActive	223	17.7
Total	1259	100

2.9.2 Self-reported health status at 60 – 64 y

Among NSHD participants who had provided dietary data in all 3 years, those with a self-reported health status of excellent (*n*=154) and very good (*n*=463) were combined into a new category: excellent/very good. This was the reference category (=0) against which three dummy variables, good, fair and poor, were compared (Table 2.26).

Table 2.26 Self-reported health status at 60 – 64 y. Creation of a reference category (excellent/very good) and 3 dummy variables (good, fair and poor) in NSHD participants who provided dietary data in all years

	Frequency (*n*)	Percentage
Excellent/Very good	617	54.9
Good	370	32.9
Fair	114	10.2
Poor	22	2.0
Total	1123	100

2.9.3 Participants' socioeconomic position (SEP) at 53 y

Among NSHD participants who had provided dietary data in all years, those classified as SEP I (professional) (*n*=97) and II (intermediate) (*n*=476) at 53 y were combined to create the reference category for participants' SEP at 53 y (*n*=573) (Table 2.27).

Table 2.27 SEP at 53 y. Creation of a reference category (Professional/Intermediate) and 4 dummy variables in NSHD participants who provided dietary data in all years

	Frequency (*n*)	Percentage
I Professional /II Intermediate	573	45.5
IIINM Skilled (non-manual)	320	25.4
IIIM Skilled (manual)	180	14.3
IV Partly skilled	131	10.4
V Unskilled	54	4.3
Total	1258	100

2.9.4 Father's socioeconomic position (SEP) in 1950

Among NSHD participants who had provided dietary data in all years, those classified as father's SEP I (professional) (*n*=86) and father's SEP II (intermediate) (*n*=231) were combined to create the reference category for father's SEP when participant was aged 4 y (*n*=317) (Table 2.28).

Table 2.28 SEP at 53 y; creation of a reference category (Professional/Intermediate) and 4 dummy variables in NSHD participants who provided dietary data in all years

	Frequency (*n*)	Percentage
I Professional /II Intermediate	317	27.0
IIINM Skilled (non-manual)	256	21.8
IIIM Skilled (manual)	309	26.3
IV Partly skilled	235	20.0
V Unskilled	57	4.9
Total	1174	100

2.10 Hierarchical linear regression – methodology

Hierarchical linear regression was used to test the hypothesis that relatively low protein intake across adulthood would predict poorer physical capability in middle age (60 – 64 y). In addition to considering the effect of measures of protein intake, this analysis considered anthropometric measures, adulthood physical activity, measures of self-reported health status and socioeconomic position (in 1950 and 1999) as potential predictors of physical capability. Models were split by gender as performance in the physical capability measures differed significantly for males and females.

Four protein variables were created as described above i.e. NSHD participants who had provided dietary data in all 3 measurement years were identified as low protein consumers if they were in quintile 1 of 3 year mean daily protein consumption when expressed as absolute intake (g/d), as a percentage of daily energy intake and in grams per kilogram of body weight. Diurnal protein consumption was captured using the muscle protein synthesis score, and those in quartile 1 were identified as low protein consumers. Each of these protein variables was used in hierarchical linear regression modelling using the subset of individuals who provided dietary data in all years only. The protein variable was always pushed FIRST into the regression model (as independent variable 1) before determining the subsequent order of predictors. In addition two sensitivity analyses were conducted to determine the effect of under- and over-reporting on the outcomes of hierarchical linear regression modelling. Quintiles of 3 y mean daily protein (g/d) and daily protein adjusted for body weight (g/kg/d) were recalculated after excluding all individuals who had ever been predicted to have under- or over-reported their energy intake. These new variables were also pushed first into the model prior to determining the subsequent order of predictors.

Three dependent variables (of physical capability at 60 – 64 y) were examined in turn:

1. Grip strength (in kg) at 60 – 64 y
2. Chair rise time (in seconds) at 60 – 64 y
3. Timed up and go time (seconds) at 60 – 64 y

After the protein variable was specified as independent variable 1, each of the variables shown in Table 2.29 were tested individually (except for the grouped dummy variables) to determine the order of their ability to predict the outcome (dependent variable):

Table 2.29 Variables tested in hierarchical linear regression analyses

Measures
Height (m) at 60 – 64 y Weight (kg) at 60 – 64 y BMI (kg/m^2) at 60 – 64 y Abdominal circumference at 60 – 64 y
Whole body fat mass (kg) at 60 – 64 y Appendicular fat mass (kg) at 60 – 64 y Body fat percentage at 60 – 64 y
Whole body lean mass (kg) at 60 – 64 y/height^2 Appendicular lean mass (kg) at 60 – 64 y/height^2
Adulthood habitual physical activity Reference category = Sedentary vs. _More active _Most active
Self-reported health status at 60 – 64 years Reference category = Excellent/ very good vs. _Good _Fair _Poor
Participant's SEP (at 53 y) and father's SEP (in 1950 (when participant was 4 y)) Reference category = Professional (SEP I)/Intermediate (SEPII) vs. _IIINM (Skilled, Non-manual) _IIIM (Skilled, Manual) _IV Partly skilled _V Unskilled

Dummy (categorical) variables were always put into the regression model as a group, i.e. for self-reported health status at 60 – 64 years, the 3 dummy variables _Good, _Fair and _Poor were entered into the independent(s) box together.

2.10.1 Hierarchical linear regression analysis – an example

Table 2.30 Hierarchical linear regression – an example of methodology

Variable Name	Model Summary Change Statistics	
	R^2 Change	*p*-value
Abdominal circumference (cm)		.450
BMI (kg/m^2)		.509
Body fat percentage	.023	**.006**
Whole body lean mass (kg) *adjusted for height^2*	.017	**.017**
Whole body fat mass (kg)		.605
Self-reported health status at 60 – 64 y	.024	**.020**
Adulthood physical activity		.107
Height (m)	**.089***	**<.001**
Appendicular fat mass (kg)		.406
Appendicular lean mass (kg) *adjusted for height^2*	.034	**.001**
Body weight (kg)	.014	**.013**
Participant's SEP at 53 y		.123
Father's SEP (in 1950/when participant 4 y)		.087

Each variable (or group of dummy variables) was tested in turn after the protein variable had been specified as the first independent variable. From the output generated, the Change Statistics were examined, specifically the Sig. *F* Change (*p*-value) and R Square Change associated with the inclusion of the new variable into the model (see Table 2.30 above). Where the *F* Change was significant, the probability statistic (*p*-value) was emboldened. The variable selected as next in the hierarchy of predictors (independent variables) was marked with a (*) on the R^2 Change statistic (see height, above). In this example, height would be selected as independent variable 2 and the process repeated to identify independent variable 3.

The R^2 Change and the significance of the *F* ratio (*p*-value) associated with adding each new variable to the model indicated the change in the model's ability to predict the dependent variable. Where the *F* ratio was significant ($p<0.001$) for more than one variable, the variable associated with the greatest R^2 Change was selected. Where the *F* ratio was significant (i.e. $p<0.05$) the Variance Inflation Factor was also noted in order to monitor multicollinearity; values in excess of 5 were not tolerated.

For reasons of multicollinearity, where whole body lean mass was included in the model, appendicular lean mass was not tested and where whole body fat mass was included in the model, appendicular fat mass was not tested. Where the addition of a new variable resulted in an insignificant *F* Change (p>0.05) the multicollinearity/ VIF was not quoted as this variable would never be included in the model. This methodology was repeated until all variables tested produced insignificant (p>0.050) *F* ratios, i.e. none of the tested variables produced a significant R^2 change.

2.10.2 Hierarchical linear regression – interpreting the coefficients

All protein intake variables (with the exception of the muscle protein synthesis score) were devised and coded in the same way, i.e. quintile 1 was coded as (= 1) and higher quintiles as (= 0). The muscle protein synthesis score was split into *quartiles* and coded in the same way.

Table 2.31 An example taken from Chapter 6. Predicting hand grip strength in females at 60 – 64 y using quintiles of protein consumption relative to body mass (g/kg/d)

Model	Unstandardized Coefficients	*p*-value
	B	
Quintiles of protein intake (g/kg/d)	-.120	.900
Height (m)	30.298	**.000**
Self-reported health status _Good	-.529	.464
_Fair	-5.610	**.000**
_Poor	-10.597	**.026**
Appendicular LEAN mass (kg)/ht^2	2.586	**.000**
Abdominal circumference (cm)	-.158	**.000**

In this example (Table 2.31) the protein variable compared females in quintile 1 with females in higher quintiles of mean protein, across all 3 years of measurement, relative to body mass (g/kg/d). Dummy variables for self-reported health status at 60 – 64 y were devised as described above i.e. the reference category was excellent/very good and dummy variables (_Good, _Fair and _Poor) were compared to this reference category. Height, appendicular lean mass/ht^2 and abdominal circumference were continuous variables. For continuous variables, every 1 unit increase in their value was either positively (for height and appendicular lean mass) or negatively (for abdominal circumference) associated with the outcome (hand grip strength at 60 – 64 y).

When interpreting dummy variables, the coefficient (B value) attributable to the dummy variable is compared to the reference category. The beta value indicates the change in the dependent variable due to the dummy variable changing from 0 (the reference category excellent/very good) to 1, e.g. _Poor. The change in hand grip strength (kg) associated with the dummy variable changing from excellent/ very good (0) to poor (1) in this example was -10.6 kg (p=0.026).

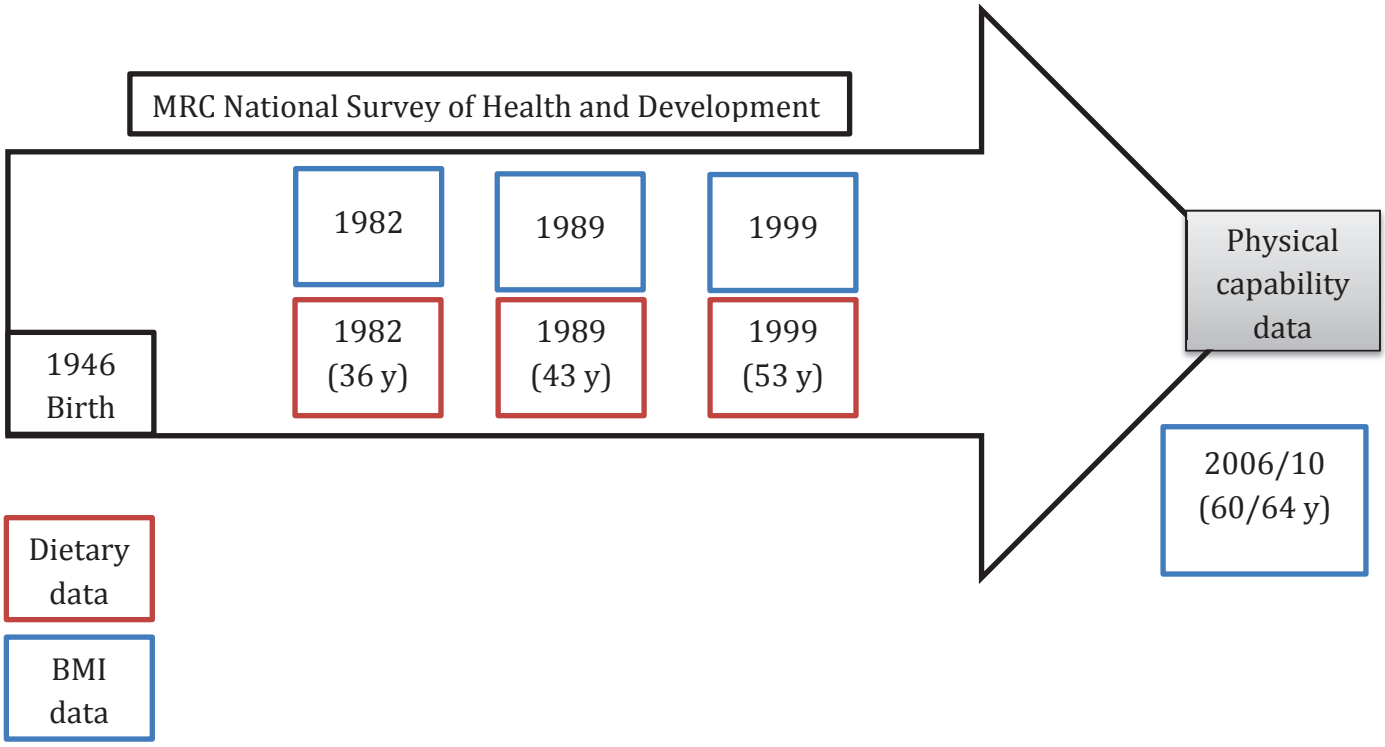
CHAPTER 3

Daily Protein and Energy Consumption

3.1 Introduction

NSHD participants, all born in the first week of March 1946, provided dietary data throughout adulthood at four measurement periods – when they were 36 y, 43 y, 53 y and 60 – 64 y. Anthropometric data was also collected at these measurement periods. Tests of physical capability were conducted at the latest clinical data collection in 2006 – 10 when participants were aged 60 – 64 y (hand grip strength, chair rise time and timed up and go (see Figure 3.1 below). This project has examined whether patterns of protein consumption across adulthood (at 36, 43 and 53 y) can explain or predict physical capability at 60 – 64 y. This chapter examines total daily protein and energy consumption and anthropometry in each year of measurement. Trends are examined in the subset of NSHD participants who provided dietary data in all 3 years.

Figure 3.1 Collection of dietary and other data across adulthood by NSHD participants, 1982 – 2006



3.2 Sample sizes

The numbers of study participants for whom there was usable data on dietary intake, body mass and BMI at each of the measurement periods were identified (Table 3.1)

Table 3.1 Samples sizes across the years, by variable

	All	Males	Females
Dietary intake	(*n*)	(*n*)	(*n*)
1982	2428	1192	1236
1989	2280	1125	1155
1999	1776	827	949
Body weight			
1982	2778	1383	1395
1989	2772	1372	1400
1999	2550	1252	1298
2006-10	1981	950	1031
BMI			
1982	2404	1179	1225
1989	2264	1118	1146
1999	1755	815	940
2006-10	2219	1061	1158

In 1982, when they were aged 36 y, 2428 NSHD participants provided dietary data by 5 d estimated food diary, 1192 males and 1236 females. In 1989 this fell to 2280 and in 1999 the sample size for those with dietary data was 1776. Similarly, sample sizes providing body mass and BMI data fell as the NSHD cohort members aged (Table 3.1). Dietary data were provided on at least one occasion by 3019 NSHD cohort members. Of this group, 817 participants (27.1%) provided dietary data on one occasion only; 939 (31.1%) on two occasions only and 1263 (41.8%) on every occasion, i.e. in all 3 measurement periods. Of the latter, 568 were males and 695 were females.

Unless stated otherwise, all descriptive statistics were calculated from the dataset which included NSHD participants who provided dietary data on at least one occasion (*n*=3019). Where trends over time are examined, this is with reference to the subgroup who provided dietary data in all years (*n*=1263)

3.3 Under- and over-reporting of dietary intake

Under- and over-reporters were identified using the approach described in Chapter 2. To summarise, the Oxford equations (Henry, 2005) were used to determine BMR and under-reporters were defined as those with energy intakes less than BMR * 1.14 (the Goldberg cut-off for *n*=1 and 28 days (for methods purporting to measure habitual intake (Goldberg GR, 1991)). Over-reporters were identified using the methodology described by (Johansson *et al.*, 1998) as outlined in Chapter 2 which is based on the principle that a ratio of EI:BMR > 2.4 is likely to be unsustainable in the long term.

3.3.1 Under-reporting of dietary intake

Table 3.2 Predicted under-reporting by those NSHD participants who provided dietary data on at least one occasion (*n*=3019)

	1982	**1989**	**1999**
n	2418	2269	1758
Not under-reporters (*n*)	1531	1638	1181
Predicted under-reporters (*n*)	887	631	577
Predicted under-reporters (%)	**36.7%**	**27.8%**	**32.8%**

In 1982 data were available to estimate the likely event of under-reporting by 2418 individuals. Of these, 1531 individuals appeared not to under-report their energy intake (EI) but 887 individuals (36.7% of the 1982 cohort) reported an EI < BMI * 1.14 i.e. an implausible EI which is inconsistent with long term survival. Similarly, in 1989 and 1999 the proportions of likely under-reporters were 27.8% and 32.8% respectively (Table 3.2).

Table 3.3 Estimates of likely dietary under-reporting by those who provided dietary data on at least one occasion (*n*=3019), by gender

	1982		**1989**		**1999**	
	M	F	M	F	M	F
n	1187	1231	1118	1151	815	943
Not under-reporters *(n)*	821	710	825	813	547	634
Predicted under-reporters *(n)*	366	521	293	338	268	309
Predicted under-reporters (%)	30.8	**42.3**	26.2	**29.4**	32.9	**32.8**

In 1982 when aged 36 y, estimated under-reporting was much higher (37% greater) in females than in males. However, whilst the proportion of male under-reporters remained relatively constant across the 3 measurement years, the proportion of female under-reporters fell with time and by age of 53 (in 1999) was virtually identical to that of males (Table 3.3). In the smaller subset of 1263 NSHD participants who provided dietary data in all three measurement years, data were available in 1982 to estimate the likely extent of under-reporting by 1260 individuals (Table 3.4).

Table 3.4 Predicted under-reporting by those NSHD participants who provided dietary data in all years (*n*=1263)

	1982	**1989**	**1999**
n	1260	1257	1252
Not under-reporters (*n*)	875	962	894
Predicted under-reporters (*n*)	385	295	358
Predicted under-reporters (%)	**30.6%**	**23.5%**	**28.6%**

In 1982 30.6% of this smaller subset reported an energy intake < BMI * 1.14. Similarly in 1989 and 1999, the proportions of likely under-reporters were 23.5% and 28.6% respectively (Table 3.4). The incidence of estimated under-reporting was lower in all years in the subset of individuals who provided dietary data in all years compared with those who reported intakes data in only some years.

Table 3.5 Estimates of likely dietary under-reporting by those NSHD participants who provided dietary data in all years (*n*=1263) by gender

	1982		**1989**		**1999**	
	M	F	M	F	M	F
n	567	693	565	692	562	690
Not under-reporters *(n)*	423	452	442	520	400	494
Predicted under-reporters *(n)*	144	241	123	172	162	196
Predicted under-reporters (%)	25.4	34.8	21.8	24.9	28.8	28.4

Of the subset who provided dietary data in all years the incidence of likely under-reporting was higher in females than in males in 1982/89 but in 1999 the proportion of female under-reporters had fallen to slightly below that of males (Table 3.5).

3.3.2 Over-reporting of dietary intake

Table 3.6 Predicted over-reporting by those NSHD participants who provided dietary data on at least one occasion (*n*=3019)

	1982	**1989**	**1999**
n	2418	2269	1758
Not over-reporters (*n*)	2396	2241	1755
Predicted over-reporters (*n*)	22	28	3
Predicted over-reporters (%)	**0.9**	**1.2**	**0.2**

Rates of over-reporting (identified by the ratio EI:BMR > 2.4) were very low in all years (Table 3.6).

3.4 Dietary intake and anthropometry in the 1982, 1989 and 1999 reporting cohorts

Gender-stratified anthropometry (weight, height and BMI (kg/m^2)) in the 1982, 1989 and 1999 reporting cohorts (at 36, 43 and 53 y, respectively) are contained in Tables 3.23 – 3.28 in the appendices to this chapter.

Gender-stratified energy (kcals) and protein consumption (expressed in g/d, g/kg/d and as a percentage of total energy) (including and excluding predicted misreporters), and energy consumption stratified by BMI class in the 1982, 1989 and 1999 reporting cohorts are contained in Tables 3.29 – 3.27 also in the appendices to this chapter.

3.5 Distribution of protein intake data

Currently there is no gold standard method to test the normality of data (Kim, 2013); in large samples such as the NSHD dataset, the eyeball test is useful but formal tests such as Shapiro-Wilk and Kolmogorov-Smirnov can be unreliable producing results that are incompatible with the eyeball test.

3.5.1 Using skewness and kurtosis to assess normality

Skewness is a measure of the asymmetry of a variable and kurtosis is a measure of how peaked/flat the distribution appears. The skew and excess kurtosis[9] (cf. proper kurtosis) of a normal, completely symmetrical distribution should both be zero. In determining substantial non-normality in sample sizes greater than *n*=300 (Kim, 2013) recommends reliance on the histogram and *absolute* values of skewness and kurtosis – i.e. for skewness a value > 2 and for kurtosis proper a value > 7 would indicate substantial non-normality.

Table 3.7 Assessing the normality of gender-specific protein intake distributions (g/d) using skewness and excess kurtosis

	Skewness (SE)(*n*)		Excess kurtosis (SE)(*n*)	
Cohort	M	F	M	F
1982	0.23 (.071) *n*=1192	0.33 (.070) *n*=1236	.59 (.142) *n*=1192	2.4 (.139) *n*=1236
1989	0.44 (.073) *n*=1125	0.28 (.072) *n*=1155	2.7 (.146) *n*=1125	1.1 (.144) *n*=1155
1999	0.35 (.085) *n*=827	0.13 (.079) *n*=949	0.9 (.170) *n*=827	0.78 (.159) *n*=949

By this criteria and using protein intake data from the 1982, 1989 and 1999 reporting cohorts, substantial non-normality did not exist, i.e. all values of skewness were < 2 and all values for kurtosis proper (calculated by adding 3 to the value provided by SPSS) were < 7 (Table 3.7).

[9] SPSS provides a figure for 'excess kurtosis' which is calculated by subtracting 3 from kurtosis 'proper'

Table 3.8 Assessing the normality of gender-specific protein intake distributions (g/d) using skewness and excess kurtosis among NSHD participants who provided dietary data in all years (*n*=1263)

	Skewness (SE)		Excess kurtosis (SE)	
	M (*n*=568)	F (*n*=695)	M (*n*=568)	F (*n*=695)
1982	.229 (.103)	.628 (.093)	.826 (.205)	**4.2** (.185)
1989	1.12 (.103)	.496 (.093)	**4.7** (.205)	1.6 (.185)
1999	.298 (.103)	.105 (.093)	.656 (.205)	1.1 (.185)
3 y mean	.685 (.103)	.612 (.093)	2.0 (.205)	2.5 (.185)

By the same criteria and using protein intake data from participants who provided dietary data in all years (*n*=1263) all values of skewness were < 2. Values for kurtosis proper (calculated by adding 3 to the value provided by SPSS) were < 7 in all years with the exception of males in 1989 (kurtosis proper = 7.7) and females in 1982 (kurtosis proper = 7.2) (Table 3.8).

3.5.2 Using Shapiro-Wilk and Kolmogorov-Smirnov to assess normality

Tables 3.9 and 3.10 show the relevant statistic, significance (*p*-value) and degrees of freedom with gender as factor. Stem and leaf and Q – Q plots were also considered.

Table 3.9 Assessing normality of gender-specific protein intake distributions (g/d) with Shapiro-Wilk and Kolmogorov-Smirnov

Reporting cohort	Males		Females	
	K-S	Shapiro-Wilk	K-S	Shapiro-Wilk
1982	.023 *p*=.138 (*n*=1192)	.993 ***p*=.000** (*n*=1192)	.041 ***p*=.000** (*n*=1236)	.983 ***p*=.000** (*n*=1236)
1989	.046 ***p*=.000** (*n*=1125)	.978 ***p*=.000** (*n*=1125)	.036 ***p*=.001** (*n*=1155)	.991 ***p*=.000** (*n*=1155)
1999	.036 ***p*=.013** (*n*=827)	.988 ***p*=.000** (*n*=827)	.034 ***p*=.012** (*n*=949)	.993 ***p*=.000** (*n*=949)

Table 3.10 Assessing normality of gender-specific protein intake distributions (g/d) with Shapiro-Wilk and Kolmogorov-Smirnov among NSHD participants who provided dietary data in all years

	Males (*n*=568)		Females (*n*=695)	
	K-S	Shapiro-Wilk	K-S	Shapiro-Wilk
1982	.037 *p*=.059	.991 ***p*=.002**	.035 ***p*=.045**	.970 ***p*=.000**
1989	.081 ***p*=.000**	.946 ***p*=.000**	.046 ***p*=.001**	.983 ***p*=.000**
1999	.031 *p*=.200	.990 ***p*=.001**	.042 ***p*=.005**	.989 ***p*=.000**
3 y mean protein intake	.043 ***p*=.015**	.974 ***p*=.000**	.040 ***p*=.010**	.978 ***p*=.000**

Field (Field, 2011) suggests that the Shapiro-Wilk and Kolmogorov-Smirnov tests have limitations when applied to large datasets, as they can show significance even when data are only slightly different from a normal distribution. Field recommends that such results should be interpreted in conjunction with histograms, Q – Q plots and values of skew and kurtosis.

Consideration of 'extremes' (values and (*n*)) from stem and leaf plots. Daily reported protein intakes (g/d) with gender as factor.

Table 3.11 Extreme values (from stem and leaf plots). Protein intake (g/d) among NSHD participants who provided dietary data in all years

	Males (*n*=568)			Females (*n*=695)		
	Mean (±SD)	Lower (g/d)	Upper (g/d)	Mean (±SD)	Lower (g/d)	Upper (g/d)
1982	78.6 (20.6)	≤27.0 (*n*=3)	≥131 (*n*=8)	61.2 (15.9)	None	≥104 (*n*=5)
1989	**86.5** (22.8)	≤35 (*n*=3)	≥135 (*n*=12)	68.5 (17.2)	≤23 (*n*=3)	≥110 (*n*=8)
1999	86.4 (18.9)	≤25 (*n*=2)	≥137 (*n*=6)	70.9 (14.9)	≤34 (*n*=4)	≥109 (*n*=12)
3 y mean	83.9 (15.7)	None	≥123 (*n*=8)	66.9 (12.2)	≤32 (*n*=2)	≥98 (*n*=8)

Of the sub-cohort who provided dietary data in all years, males (*n*=568) in 1989 reported a mean protein intake of 86.5 g/d (SD ±22.8). Values for Kolmogorov-Smirnov and Shapiro-Wilk tests were both significant ($p<0.001$) (see Table 3.10). A consideration of the stem and leaf plot indicated that extreme values were reported by 3 males who reported protein consumption of ≤ 35 g/d and 12 males who reported protein consumption of ≥ 135 g/d (Table 3.11).

In SPSS, 'outliers' can be requested using the Analyse/Descriptive statistics/Explore function. The output of this function is the 5 highest and lowest values by case number for males and females separately (where gender is specified as the factor).

Table 3.12 Examination of male outliers (highest 5 values) in the reporting of daily protein intake (g/d) among NSHD participants who provided dietary data in all years

Year	Case number	Protein intake (g/d)	Energy intake (kcals/d)	Protein intake as a % of total daily energy intake
1982	915	168.25	4488.28	15.0
	535	152.71	3194.81	19.1
	543	148.64	3563.51	16.7
	447	140.17	4796.82	11.7
	967	135.29	3440.98	15.7
1989	664	229.30	4554.08	20.1
	535	219.37	5442.43	16.1
	543	173.22	4462.19	15.5
	23	155.01	3316.32	18.7
	1007	154.78	4486.66	13.8
1999	1110	160.42	4242.11	15.1
	712	155.35	3223.13	19.3
	756	145.87	2853.45	20.4
	25	139.47	3767.16	14.8
	966	137.74	2707.15	20.4

Table 3.13 Examination of female outliers (highest 5 values) in the reporting of daily protein intake (g/d) among NSHD participants who provided dietary data in all years

Year	Case number	Protein intake (g/d)	Energy intake (kcals/d)	Protein intake as a % of total daily energy intake
1982	1142	180.93	3833.18	18.9
	1055	112.66	3060.25	14.7
	1086	112.01	2958.41	15.1
	483	105.74	2201.63	19.2
	385	103.63	2912.30	14.2
1989	615	158.41	4030.94	15.7
	1142	143.84	4316.28	13.3
	181	126.74	3140.56	16.1
	1231	120.18	3203.71	15.0
	1245	117.38	2400.56	19.6
1999	466	125.36	1937.55	25.9*
	1119	122.03	2451.77	19.9
	141	115.53	1937.63	23.8*
	296	114.23	2390.19	19.1
	485	112.54	2495.81	18.0

Female cases 466 and 141 warranted further investigation as absolute protein intakes (g) and protein intakes as a percentage of total daily energy intakes (kcal) were high. Diurnal protein intakes (g) across 8 meal slots were examined and BMI in 1999 (at 53 y) noted. In 1999, case number 466 had a BMI of 37.42 kg/m^2 and case number 141 had a BMI of 32.22 kg/m^2. All reported values appeared valid and were not excluded.

Table 3.14 Examination of diurnal protein intakes (g) in 1999 for female case numbers 466 and 141

	Reported protein intake (g) at eight meals across the day in 1999							
	1	2	3	4	5	6	7	8
Case 466	0.1	24.09	3.14	45.21	1.0	41.85	2.89	7.09
Case 141	2.21	11.32	4.57	29.94	1.89	61.82	3.0	0.8

In conclusion, in large samples (e.g. > n=100) parametric tests (which assume a Gaussian/bell-shaped distribution) are 'robust' – the p-value will be substantially correct even if the population deviates from a Gaussian population, i.e. the assumption is somewhat violated (Marusteri, 2010). In the regression analyses, sample sizes were always > n=600 (including the sensitivity analyses). Small deviations from normality result in significant results (i.e. the distribution is non-normal) when Kolmogorov-Smirnov and Shapiro-Wilk are used for large sample sizes, notwithstanding that the deviation will not affect the result of the parametric test (Ghasemi A., 2012).

3.6 Trends in dietary intake and anthropometry, 1982 – 1999

Age-specific patterns of energy and protein consumption were investigated in the subset of NSHD participants who provided dietary data in all measurement years, 568 males and 695 females.

3.6.1 Adulthood energy consumption

Table 3.15 Mean energy intake (kcals/d) in NSHD participants who provided dietary data in all 3 years

	1982	**1989**	**1999**	*p*-value
	Mean (SEM)			
MALES				
Mean energy intake (*n*=568)	2289 (26.2)	2451 (27.4)	2262 (21.7)	<0.001
Mean energy intake excluding predicted misreporters	2528 (21.5) (*n*=420)	2647 (23.3) (*n*=437)	2479 (19.0) (*n*=404)	<0.001 (*n*=285)
FEMALES				
Mean energy intake (*n*=695)	1662 (18.4)	1858 (18.8)	1778 (14.4)	<0.001
Mean energy intake excluding predicted misreporters	1907 (15.4) (*n*=446)	2027 (15.4) (*n*=515)	1942 (13.1) (*n*=499)	<0.001 (*n*=318)

In outcomes of General Linear Model (GLM) repeated measures analyses (with Bonferroni adjustment) mean daily energy intake in 1989 was significantly higher than in 1982 and in 1999 ($p<0.001$) in males, whereas daily energy consumption in 1999 did not differ significantly from that in 1982. In females, all reported energy intakes were significantly different between years ($p<0.001$).

When predicted misreporters were excluded from the analyses, all energy intakes were significantly different between years in males ($p<0.05$). In females, mean daily intake in 1989 was significantly higher than in 1982 and 1989 ($p<0.001$) but 1982 and 1999 were not significantly different.

3.6.2 Trends in BMI (kg/m^2)

Table 3.16 Mean BMI (kg/m^2) of NSHD participants who provided height and weight data at four measurement periods, 1982 – 2006/10

	1982	**1989**	**1999**	**2006/10**	***p*-value**
Males (*n*=883)	24.5	25.5	27.2	27.8	<0.001
Females (*n*=990)	23.3	24.8	27.3	28.1	<0.001

BMI (kg/m^2) data at all four measurement periods (1982, 1989, 1999 and 2006/10) were provided by 1873 NSHD participants. On average male BMI increased by 3.3 kg/m^2 and female BMI by 4.8 kg/m^2 between the ages of 36 y and 60 – 64 years (Table 3.16). On average males were overweight (BMI > 25 kg/m^2) in 1989 at 43 y whereas females were overweight in 1999 at 53 y. In this subset of individuals, in outcomes of General Linear Model (GLM) repeated measures analyses (with Bonferroni adjustment) the increase in BMI was significant at every measurement period in males and females (p<0.001).

3.6.3 Trends in daily protein consumption

Table 3.17 Mean daily protein consumption in male NSHD participants who provided dietary data in all 3 measurement years

MALES	**1982**	**1989**	**1999**	*p*-value	3 y mean (SEM)
	Mean (SEM)				
Daily protein intake (g) (*n*=568)	79 (0.9)	87 (1.0)	86 (0.8)	<0.001	84 (0.66)
Daily protein intake (g/kg/d)	1.06 (0.01) (*n*=567)	1.13 (0.01) (*n*=565)	1.07 (0.01) (*n*=562)	<0.001 (*n*=561)	1.09 (0.01) (*n*=567)
Daily protein intake as a percentage of total daily energy (%) (*n*=568)	14.0 (0.1)	14.3 (0.1)	15.5 (0.1)	<0.001	14.6 (0.1)

Among males who provided dietary data in all years, absolute protein consumption averaged 84 g/d over the period 1982 – 1999. In outcomes of General Linear Model (GLM) repeated measures analyses (with Bonferroni adjustment) protein intakes (g/d) increased significantly in 1989 compared to 1982 ($p<0.001$) whereas consumption in 1999 did not differ significantly from that reported in 1989.

Protein intakes relative to body mass (g/kg/d) in 1989 were significantly higher than in 1982 and 1999 ($p=0.001$) whereas consumption in 1999 did not differ significantly from that reported in 1982. Protein intakes expressed as a percentage of total daily energy increased significantly ($p<0.001$) in every reporting year in this subset of males.

Table 3.18 Average daily protein consumption in female NSHD participants who provided dietary data in all measurement years

FEMALES	**1982**	**1989**	**1999**	*p*-value	3 y mean (SEM)
	Mean (SEM)				
Daily protein intake (g) (*n*=695)	61.2 (0.6)	69 (0.7)	71 (0.6)	<0.001	67 (0.5)
Daily protein intake (g/kg/d)	1.03 (0.01) (*n*=693)	1.1 (0.01) (*n*=692)	1.04 (0.01) (*n*=690)	<0.001 (*n*=685)	1.1 (0.01) (*n*=695)
Daily protein intake as a percentage of total daily energy (%) (*n*=695)	15.2 (0.13)	15.1 (0.1)	16.2 (0.1)	<0.001	15.5 (0.1)

Among female NSHD participants who provided dietary data in all 3 years, absolute protein intake averaged 67 g/d. In outcomes of General Linear Model (GLM) repeated measures analyses (with Bonferroni adjustment) there were significant increases in consumption (g/d) in each reporting year (p=0.001).

Protein intakes relative to body mass (g/kg/d) in 1989 were significantly higher than those reported in 1982 and 1999 (p<0.001) whereas consumption in 1999 did not differ significantly from that reported in 1982. Protein expressed as a percentage of total daily energy fell insignificant in 1989 but was significantly higher in 1999 compared with 1982 and 1989 (p<0.001).

3.7 Characterising low protein consumers

To characterise 'low protein consumers', gender-specific quintiles of absolute daily protein consumption (g/d) were derived, using the mean protein intake across all 3 years of measurement (g/d) as described in Chapter 2 (Section 2.6.2).

Table 3.19 Gender-specific quintile cut points of absolute mean protein intake across 3 years of measurement (g/d) for NSHD participants who provided dietary data in all years

	Q1	Q2	Q3	Q4	Q5
Males (*n*=568)	≤71.43	71.44-79.68	79.69-86.39	86.40-95.89	95.9+
n	114	113	113	115	113
Females (*n*=695)	≤56.85	56.86-63.46	63.47-69.03	69.04-76.78	76.79+
n	139	139	139	139	139

Males in quintile 1 (*n*=114) had mean protein intake ≤ 71.4 g/d whereas those in quintile 5 had a mean intake of ≥ 95.9 g/d. Females in quintile 1 (*n*=139) had a mean protein intake approximately 15 g/d less than men in the equivalent quintile whereas the gender difference was nearly 20 g/d for those in quintile 5 (Table 3.19).

In characterising low protein consumers (those in quintile 1 of protein consumption vs. those in higher quintiles of consumption) differences between group means (for continuous variables) were tested using One-Way ANOVA. Differences between group membership (for categorical variables) were tested using crosstabs/the Chi-square test of association (Pearson Chi-Square) (2-sided) (adjustment for multiple testing was not possible (increased chance of a type 1 error)).

Table 3.20 Characteristics of male NSHD participants who provided dietary data in all years. Low protein consumers (quintile 1) vs. higher quintiles of absolute mean protein intake across 3 years of measurement (g/d)

	Q1 ≤71.43 g/d	Q2 – Q5 71.44 – 95.9+ g/d	*p*-value
	Mean (±SD) (*n*)		
3 y energy intake (kcal/d)	1775 (289)(114)	2475 (397)(454)	**<0.001**
3 y protein intake (g/d)	63.6 (6.24)(114)	89 (12.9)(454)	**<0.001**
3 y protein intake (g/kg/d)	0.84 (0.2)(114)	1.15 (0.2)(453)	**<0.001**
3 y daily protein intake (%TE)	14.7 (2.0)(114)	14.6 (1.7)(454)	0.578
BMI (2006/10)(kg/m^2)	27.6 (4.4)(91)	27.3 (3.8)(373)	0.562
Weight (2006/10)(kg)	83.3 (13.6)(91)	84 (13.0)(373)	0.631
Abdominal circumference (2006/10)(cm)	101 (10.8)(90)	99.3 (11.0)(374)	0.357
Appendicular fat (2006/10)(kg)	9.7 (2.9)(60)	10.1 (2.9)(289)	0.461
Appendicular lean/ht^2 (2006/10)(kg)	7.8 (1.0)(60)	8.0 (0.9)(289)	0.233
Estimated misreporting (%):			**<0.001**[1]
Never	10.5	60	
Once	25.4	29.6	
Twice	36.8	8.2	
All years	27.2	2.2	
Education (26y) (%):			0.548
None	25.2	26.2	
Sub GCE	8.1	4.3	
O Level	17.1	15.5	
A Level	30.6	32.3	
Degree+	18.9	21.6	
Smoking (%):			0.208
Never	30.1	27.6	
Predominantly a non-smoker	47.8	40.1	
Predominantly a smoker	15	21.7	
Lifelong smoker	7.1	10.5	
Physical activity (%):			0.131
Sedentary	45.6	39.8	
MoreActive	41.2	38.7	
MostActive	13.2	21.5	
Health Status (%):			0.057
Excellent/very good	57	57.8	
Good	22	30.6	
Fair	17	8.8	
Poor	4	2.8	

[1]Chi-Square test for estimated misreporting: χ^2 (3) = 177.5, p<0.001. No adjustment for multiple testing

3.7.1 Male low protein consumers

Males in quintile 1 (Q1) (consuming ≤ 71.43 g/d) (Table 3.20) consumed on average 63.6 g/d of protein over the three reporting periods, 25.4 g/d less than males in higher quintiles of protein consumption (p<0.001). Protein intake relative to body mass (g/kg/d) was also significantly less (0.31 g/kg/d) among males in quintile 1. Protein expressed as a percentage of total daily energy (PPTE %)) was not significantly different between the two groups. In terms of anthropometry (including body composition), highest educational attainment at 26 y, smoking behaviour (up to age 53 y), habitual physical activity and health status at 60 – 64 y, there were no significant differences between males in quintile 1 and those in the higher quintiles of protein intake.

Table 3.21 Characteristics of female NSHD participants who provided dietary data in all years. Low protein consumers (quintile 1) vs. higher quintiles of absolute mean protein intake across 3 years of measurement (g/d)

	Q1 ≤56.85 g/d	Q2 – Q5 56.86 – 76.79+ g/d	*p*-value
	Mean (±SD)(*n*)		
3 y energy intake (kcal/d)	1386 (236)(139)	1861 (322)(556)	**<0.001**
3 y protein intake (g/d)	51 (5.1)(139)	71 (1.0)(556)	**<0.001**
3 y protein intake (g/kg/d)	0.79 (0.2)(139)	1.1 (0.2)(556)	**<0.001**
3 y daily protein intake (%TE)	15.1 (2.3)(139)	15.6 (2.2)(556)	**0.018**
BMI (2006/10)(kg/m^2)	28 (5.0)(102)	27.0 (4.8)(472)	0.077
Weight (2006/10)(kg)	72 (12.7)(102)	71 (13.0)(472)	0.614
Abdominal circumference (2006/10)(cm)	91.4 (13)(102)	90.4 (12)(472)	0.475
Appendicular fat (2006/10)(kg)	14.6 (4.2)(67)	13.9 (4.1)(363)	0.178
Appendicular lean/ht^2 (2006/10)(kg)	6.2 (0.9)(67)	6.1 (0.8)(363)	0.404
Estimated misreporting (%)			**<0.001**[1]
Never	9.4	54.9	
Once	28.1	27.3	
Twice	32.4	14.2	
All years	30.2	3.6	
Education (26y) (%)			**0.009**[2]
None	41	26.2	
Sub GCE	7.5	8.4	
O Level	23.9	29.8	
A Level	24.6	27.9	
Degree+	3	7.7	
Smoking (%):			**0.023**[3]
Never	29.7	39.3	
Predominantly a non-smoker	33.3	36	
Predominantly a smoker	21.7	16	
Lifelong smoker	15.2	8.8	
Physical activity (%):			0.361
Sedentary	51.4	44.7	
MoreActive	34.1	38.9	
MostActive	14.5	16.4	
Health Status (%):			0.081
Excellent/very good	50.8	53.3	
Good	36.7	36.1	
Fair	9.2	10.1	
Poor	3.3	0.6	

[1]Chi-Square test for estimated misreporting: χ^2 (3) = 159, p<0.001; [2]Chi-Square test for highest educational attainment at 26 y: χ^2 (4) = 13.4, p<0.05; [3]Chi-Square test for smoking: χ^2 (3) = 9.5, p<0.05. No adjustment for multiple testing

3.7.2 Female low protein consumers

Females in quintile 1 (consuming ≤ 56.85 g/d) (Table 3.21) consumed on average 51 g/d over three reporting periods, 20 g less per day than females in higher quintiles of consumption. Protein intake relative to body mass (g/kg/d) and intake expressed as a percentage of total daily energy (PPTE %) were also significantly less among females in quintile 1. Anthropometric and body composition measures were not significantly different between the groups. In highest educational attainment at 26 y there were proportionately more females in quintile 1 without formal educational qualifications (41 vs. 26.2%) and proportionately less educated to degree level or above (3 vs. 7.7%). Differences in highest educational attainment between the two groups (quintile 1 compared with higher quintiles) were significant (p=0.009). There were proportionately more females in quintile 1 who were lifelong smokers at 53 y (15.2 vs. 8.8%) and less who were never smokers (29.7 vs. 39.3%) compared with females in the higher quintiles (p=0.023).

In predicted misreporting, there were significantly higher levels amongst individuals in quintile 1 and differences in misreporting between the two groups were significant (p<0.001) in males and females.

3.8 Discussion

This chapter examined the daily energy and protein consumption by NSHD participants who provided estimates of dietary intake by 5 day food diary in 1982, 1989 and 1999 when aged 36 y, 43 y and 53 y, respectively. Trends across time in protein and energy consumption and in anthropometry were investigated using the smaller subset of individuals who provided dietary data in all 3 years (*n*=1263).

3.8.1 Estimated under- and over-reporting

Quantitative assessment of habitual dietary intake is challenging and it is well recognised that all dietary assessment methods, including the 5d food diary used in the NSHD, may deliver intake estimates for some individuals which are unlikely to be reliable (Bingham, 1991). The use of the 5d food diary in NSHD, and the Oxford equations (Henry, 2005) (for BMR) and (Goldberg GR, 1991) cut-off (PAL = 1.14) (for *n*=1 and 28 day) to identify individual under-reporters, have resulted in rates of estimated under-reporting in the current study that are difficult to compare to other studies.

Black (Black, 2000) estimated TEE using Schofield equations (for those aged > 64 y) for BMR, and the WHO recommended PAL for light activity (1.55). The ratio EI:EE < 0.76 was used to identify under-reporting using individual data from 21 studies (n=429)(18 – 75+). 37.5% of women and 27.9% of men were identified as under-reporting their energy intake. In the age range 30 – 39 y the rate was 35.2% compared with 36.7% in the present study (at 36 y); and in the age range 40 – 64 y the rate was 40.7% compared with 27.8% (at 43 y) and 32.8% (at 53 y) in the present study. Notwithstanding methodological differences in the present study and less heterogeneity in subject age, rates of under-reporting in the present study were comparable at 36 y but less at 43 y and 53 y. The ratio EI:EE > 1.24 was used to identify over-reporting (Black, 2000). Over-reporting in the age range 30 – 39 was 4.2% and in the age range 40 – 64 y was 3.5%. These rates were much higher than those seen in the present study, however in the present study the ratio EI:BMR > 2.4 was used.

In the Observing Protein and Energy Nutrition (OPEN) Study (Subar *et al.*, 2003) assessed dietary measurement error in the food frequency questionnaire (FFQ) and 24 h recall (5 pass method) against two unbiased biomarkers of protein and energy intake (urinary nitrogen and doubly labelled water) in 484 men and women aged 40 – 69 y. Although not directly comparable to the present study (which used a 5 d food diary) the percentage of respondents classified as under-reporters[10] were 20.8% of men and 22.3% of women (using the 24 h recall) and 49.6% of men and 49% of women (using the FFQ). In the present study, rates were never as high as those seen when using the FFQ but were higher than those identified when using the 24 h recall. It is known that values reported via FFQ are subject to substantial error (Subar *et al.*, 2003) and it was this that led to the use of more expensive assessment instruments, such as food records and a variety of 24 h recall instruments in large epidemiological studies. Under-reporting was highest in those with a BMI ≥ 30 kg/m^2; using the 24 h recall, 33.3% of men and 35% of women; and 66.2% and 46.7% respectively, when the FFQ was used.

Subsequent analysis of OPEN Study data compared the Goldberg method (PAL = 1.55) with a doubly labelled water (criterion method) in 451 men and women reporting dietary data via FFQ and 24 hour recall. TEE and cutpoints were calculated as above (Black, 2000). 10% of men and 13% of women underreported their EI on 24 h recalls and 52% of men and 51% of women on the FFQ (Tooze *et al.*, 2012).

(Huang *et al.*, 2005) screened for implausible reports by comparing reported EI (from 2 non-consecutive 24 h dietary recalls) directly with predicted or measured TEE in the USDA Continuing Survey of Food Intake by Individuals (CSFII) 1994 – 1996 (20 – 90 y) (*n*=6499); all subjects were assigned to a low activity category (a PAL between 1.4 and 1.59). Using a ± 1 SD cut-off (the most stringent) the sample retained was 41% of total reports (*n*=2686).

[10] Values below the 95% CI of the log ratio of reported intakes to biomarker measurements

The incidence of estimated under-reporting in the present study was lower in all years amongst the smaller subset who provided dietary data in all years. Estimated under-reporting was always higher in female participants and tracked adiposity class in all years. This was consistent with the finding of others, e.g. (Johansson *et al.*, 1998) and (Lissner *et al.*, 2007). In this latter study, data was again provided by the OPEN Study (*n*=390) in which 27% of participants were obese. Obese men reported 84% and 69% of their biomarker energy requirement compared with 93% and 76% reported by leaner men, by 24 h recall and FFQ, respectively. Obese women reported 80% of their biomarker energy requirement compared with 92% reported by non-obese women using the 24 h recall and all intakes were significantly different by obesity status. With the FFQ however, obese women reported 71% of their biomarker energy requirement compared to 75% reported by their leaner counterparts and this difference was not statistically significant.

In this project, observed estimated over-reporting was very low in all measurement years, only ever reaching 1.2% (in 1989). There was a tendency for estimated over-reporting to be higher in males and this was expected based on the findings of (Johansson *et al.*, 1998) (7% of men compared with 5% of women) and (Black, 2000) (4.9% of men compared to 3.8% of women).

In the identification of estimated under- and over-reporters, it must be stated that these cut-off values do not take into account the true total energy expenditure (TEE) of each individual – variation in habitual physical activity and lifestyle behaviours such a smoking, are not accounted for in the use of predictive equations for TEE. Only biomarkers such as doubly (deuterium) labelled water (DLW) (Schoeller, 1988) and urinary nitrogen (Bingham and Cummings, 1985; Bingham, 2003) provide an unbiased assessment of true total energy expenditure and protein intake. After deuterium is administered, the labelled hydrogen is eliminated as water and the oxygen isotope as water and carbon dioxide; these represent accurate measures of TEE which are assumed to equate to TEI (in energy balance)/amongst stable weight individuals. These biomarkers are non-invasive and non-restrictive and therefore ideal for free living subjects, however they are expensive to administer in large epidemiological studies.

DLW has dispelled the theories that advocated additional training in the recording of EI and 'metabolic efficiency' and shown that under-reporting is present amongst obese persons, obese adolescents, post-obese persons, athletes, soldiers allowed to eat *ad libitum* and high altitude explorers (Hill and Davies, 2001). In females (18 – 57 y) TEE was determined by doubly labelled water and EI estimated by 3 24 h recalls, a 3 d food diary and a FFQ. Frequent under-reporters had a greater BMI, social desirability and body dissatisfaction score and lower incomes (Scagliusi *et al.*, 2009). Social desirability and social approval were found to distort estimates of EI in a manner that varied by educational status (Hebert *et al.*, 2002) and under-reporting was linked to increased adiposity and body size, dietary restraint and socioeconomic status in (Hill and Davies, 2001).

In a systematic review by (Poslusna *et al.*, 2009) 37 relevant studies of misreporting of dietary intake in adults were identified where EI was assessed by 24 h recall (16 studies) or estimated (11)/ weighed (11) food records. Methods most used to identify misreporting were Goldberg (45% studies) and DLW (24%). The percentage of under-reporters across all studies averaged 30% which is consistent with present observations.

In the NORKOST Study (Johansson *et al.*, 1998) 3144 Norwegian men and women (16 – 79 y) completed a self-administered FFQ. BMR was calculated from standard equations (Schofield, 1985) and the ratio EI:BMR < 1.14 (Goldberg GR, 1991) used to identify the lowest value for EI:BMR that could reflect actual EI over a given period (referred to as severe under-reporters). The range EI:BMR 1.14 – 1.34 was used to define under-reporters and a ratio EI:BMR ≥ 2.4 identified over-reporters. Participant mean age and BMI was 42.7 y (24.6 kg/m^2) in men and 41.6 y (23.4 kg/m^2) in women. In the NORKOST study 20% of men and 25% of women reported an EI < BMR * 1.14 compared with the present study (at age 43 y) in which 21.8% of men and 24.9% of women were predicted to have under-reported their energy intake. Notwithstanding a similar methodology (the use of Schofield and not Henry/Oxford equations) the higher rates of under-reporting in the NSHD could be explained by higher BMIs at 43 y; 25.4 kg/m^2 in men and 24.7 kg/m^2 in women.

The covert inspection of thirty-three obese and non-obese females, restricted within a metabolic unit, found that energy, carbohydrates, added sugar and between-meal snack foods (foods 'less central to the meal') were statistically most likely to be under-reported. Fat and alcohol intakes were under-reported but this was not statistically significant. Protein was slightly over-reported (100.9%); among non-obese, percentage reported protein was 95% and among obese females this was 105.9%. The author hypothesised that under-reporting was a consequence of poor memory as 'healthy' and 'unhealthy' foods were both inaccurately reported (Poppitt SD, 1998).

3.8.2 Overweight and obesity

Among NSHD participants who provided BMI data in all four measurement years, the increase in BMI was significant in every year in males and females (Table 3.16).

Table 3.22 Prevalence of overweight and obesity. A comparison of NSHD participants at 53 and 60 – 64 y with participants of the Health Survey for England, 1999 and 2011

	HSE 1999	NSHD 1999	HSE 2011	NSHD 2006/10
Age	45 – 54 y	53 y	55 – 64 y	60 – 64 y
Overweight (%)				
Males	49	51	44	44
Females	35	36	36	36
Obese (%)				
Males	23	19	31	26
Females	26	22	32	26

The Health Survey for England (HSE) is an annual survey of the adult population (16 – ≥75 y) comprising a representative sample of the general population living in private households in England. It began in 1991 and the latest report was published in 2011 (*n*=8610) (Public Health England). When the HSE was carried out in 1999, among those aged 45 – 54 y, 49% of males and 35% of females were overweight. In NSHD cohort in 1999 (when participants were 53 y) 51% of males and 36% of females were overweight (Table 3.22). Rates of overweight were therefore comparable between the two cohorts, at similar ages.

Rates of obesity at 45 -54 y were 4% higher in HSE survey participants compared with the NSHD cohort. In the latest HSE among those aged 55 – 64 y rates of overweight are identical to those the NSHD cohort at age 60 – 64 y. By comparison, rates of obesity among NSHD participants continue to be substantially (5 – 6%) less than in the HSE cohort.

3.8.3 The National Diet and Nutrition Survey

The National Diet and Nutrition Survey (NDNS) provides yearly data on the dietary habits and nutritional status of a representative sample of the UK population (1000 - 1500 individuals), utilising an estimated (un-weighed) 4 d food diary to collect all consumption, both inside and outside the home. The NDNS became a rolling programme in 2008, and *combined data* is now available for the years 2008/09, 2009/10 and 2010/11 (DoH and FSA). A total of 1491 adults (19 – 64 y) completed diaries from which mean daily intakes of energy and protein form the basis for this comparison. In order to assess the extent of under- and over-reporting, the DLW technique was used to measure TEE in a sub-sample of NDNS participants, however results of these analyses will only be published at a later date.

NDNS mean total energy intake for adults was 2151 kcal/d for males and 1614 kcal/d for females. Notwithstanding greater age heterogeneity in the NDNS, mean daily energy consumption in NSHD participants who provided dietary data in all years was always higher than in the NDNS sample in the equivalent age range (19 – 64 y).

In the NDNS (2008/09 – 2010/11) mean adult protein consumption (g/d) was 86.5 g/d for males and 65 g/d for females. Protein as a percentage of total energy was 16.4% for males and 16.6% for females. Amongst NSHD participants, protein intake as a percentage of total energy was always less than in the NDNS. However, greater age heterogeneity, especially in the younger age groups may explain this observation.

3.9 Appendices

3.9.1 Anthropometry in the 1982 cohort

Of 2428 individuals who provided dietary data in 1982 (when they were 36 y), height and weight data were available to calculate BMI (kg/m^2) for 2404 NSHD participants. The average male BMI was 24.7 kg/m^2 and the average female BMI was 23.4 kg/m^2 (Table 3.23).

Table 3.23 Anthropometry in NSHD participants who provided dietary data in 1982

	Weight	Height	BMI (kg/m^2)
	Mean (SD)		
Males	76.2 (11.2) (*n*=1187)	1.76 (0.07) (*n*=1179)	24.7 (3.17) (*n*=1179)
Females	61.7 (10.7) (*n*=1231)	1.62 (0.06) (*n*=1229)	23.4 (3.91) (*n*=1225)

Table 3.24 Classification of BMI in NSHD participants who provided dietary data in 1982

	All	Males	Females
	n (%)		
Underweight	55 (2.3)	12 (1)	43 (3.5)
Normal range	1553 (64.6)	668 (56.7)	885 (72.2)
Overweight	653 (27.2)	434 (36.8)	219 (17.9)
Obese	143 (5.9)	65 (5.5)	78 (6.4)

Among NSHD participants who provided dietary data in 1982, 2.3% were underweight, 64.6% had a BMI in the normal range, 27.2% were overweight and 5.9% were obese at 36 y. The majority of males and females (64.6%) had a BMI in the normal range although 42.3% of males and 24.3% of females were either overweight or obese. The proportion of males who were overweight was much higher (19% greater) than the proportion of overweight females at this age (Table 3.24).

3.9.2 Anthropometry in the 1989 cohort

In 1989, 2280 NSHD participants provided dietary data, and of these 2264 provided anthropometric data. Mean male BMI was 25.4 kg/m^2 which was overweight according to the WHO international classification and mean female BMI was 24.7 kg/m^2 which was within the normal range (Table 3.25).

Table 3.25 Anthropometry in NSHD participants who provided dietary data in 1989

	Weight	Height	BMI (kg/m^2)
	Mean (SD)		
Males	78.3 (11.6) (*n*=1118)	1.75 (0.07) (*n*=1119)	25.4 (3.3) (*n*=1118)
Females	65.2 (12.0) (*n*=1151)	1.62 (0.06) (*n*=1148)	24.7 (4.5) (*n*=1146)

Table 3.26 Classification of BMI in NSHD participants who provided dietary data in 1989

	All	Males	Females
	n (%)		
Underweight	24 (1.1)	7 (0.6)	17 (1.5)
Normal range	1247 (55.1)	523 (46.8)	724 (63.2)
Overweight	763 (33.7)	492 (44.0)	271 (23.6)
Obese	230 (10.2)	96 (8.6)	134 (11.7)

At age 43 y, the majority (55.1%) of NSHD participants had a BMI in the normal range, 33.7% were overweight and 10.2% were obese. 52.6% of males and 35.3% of females were either overweight or obese (Table 3.26).

3.9.3 Anthropometry in the 1999 cohort

Of the 1776 NSHD cohort members who provided dietary data in 1999, anthropometric data were provided by 1755 individuals. The average male BMI was 27 kg/m^2 and the average female BMI was 26.9 kg/m^2. Both males and females were overweight in terms of the WHO international classification (Table 3.27).

Table 3.27 Anthropometry in NSHD participants who provided dietary data in 1999

	Weight	Height	BMI (kg/m^2)
	Mean (SD)		
Males	82.8 (12.9) (*n*=815)	1.75 (0.07) (*n*=815)	27 (3.82) (*n*=815)
Females	70.5 (14.0) (*n*=943)	1.62 (0.06) (*n*=946)	26.9 (5.3) (*n*=940)

Table 3.28 Classification of BMI in NSHD participants who provided dietary data in 1999

	All	Males	Females
	n (%)		
n	1755	815	940
Underweight	4 (0.2)	1 (0.1)	3 (0.3)
Normal range	639 (36.4)	244 (29.9)	395 (42.0)
Overweight	749 (42.7)	415 (50.9)	334 (35.5)
Obese	363 (20.7)	155 (19.0)	208 (22.1)

In 1999 the largest proportion (42.7%) of individuals were classified as overweight, 36.4% had a BMI in the normal range and 20.7% were obese. At age 53 the majority of males (50.9%) were overweight and 70% of males and 57.6% of females were either overweight or obese (Table 3.28).

3.9.4 Energy and protein consumption in the 1982 cohort

In 1982 when NSHD cohort members were aged 36 y, 2428 individuals provided estimates of protein and energy intake via a 5 d food diary.

Table 3.29 Mean daily consumption of energy and protein by NSHD participants who provided dietary data in 1982

	Males	Excluding predicted misreporters	**Females**	Excluding predicted misreporters
	Mean (SD)			
	n=1192	*n*=810	*n*=1236	*n*=699
Mean energy intake (kcal/d)	2241 (665.6)	2541 (459)	1580 (500.0)	1891 (317)
Mean protein intake (g/d)	78 (22.0)	87 (16.6)	59.2 (16.4)	67 (11.6)
Mean protein intake (g/kg/d)	1.04 (0.32) (*n*=1187)	1.2 (0.24)	0.99 (0.31) (*n*=1231)	1.2 (0.22)
Mean protein intake as a percentage of total daily energy	14.2% (2.4)	13.8% (2.0)	15.5% (3.6)	14.3% (2.1)

Reported mean energy consumption was 2241 kcals/d for males and 1580 kcals/d for females. Mean reported protein consumption was 78 g/d for males and 59.2 g/d for females in those who provided dietary data in 1982. In males, protein consumption averaged 1.04 g/kg/d and in females, 0.99 g/kg/d. After excluding likely misreporters, mean protein consumption increased to 87 g/d and 67 g/d in males and females respectively, equivalent to 1.2g/kg/d (Table 3.29).

3.9.5 Energy consumption by BMI class in the 1982 cohort

Table 3.30 Mean energy consumption (kcal/d) in male NSHD participants who provided dietary data in 1982, by BMI classification

	Underweight	Normal	Overweight	Obese
	Mean (SD)			
Daily energy intake	1750 (619.2) (*n*=12)	2278 (657) (*n*=668)	2229 (659) (*n*=434)	2059 (711) (*n*=65)
Daily energy intake excluding predicted misreporters	2092 (421) (*n*=8)	2510 (457) (*n*=496)	2601 (449) (*n*=274)	2690 (495) (*n*=26)

In 1982 (when participants were 36 y) total daily reported energy intake by overweight and obese men was lower than that reported by normal weight men. However, when predicted misreporters were excluded from the analysis, this pattern was reversed and reported energy intakes increased across all four BMI groups (Table 3.30).

Table 3.31 Mean energy consumption (kcal/d) in female NSHD participants who provided dietary data in 1982, by BMI classification

	Underweight	Normal	Overweight	Obese
	Mean (SD)			
Daily energy intake	1900 (492) (*n*=43)	1627 (478) (*n*=885)	1404 (500) (*n*=219)	1365 (541) (*n*=78)
Daily energy intake excluding predicted misreporters	1866 (358) (*n*=35)	1877 (309) (*n*=569)	1965 (319) (*n*=74)	2130 (385) (*n*=17)

In females in 1982 females classified as underweight reported the highest mean daily energy intake and females classified as obese, the lowest. When estimated misreporters were excluded from the analysis, reported energy intake by females increased with increasing adiposity class (Table 3.31).

3.9.6 Energy and protein consumption in the 1989 cohort

In 1989 when NSHD study members were aged 43 y, 2280 individuals provided estimates of protein and energy intake in a 5 day food diary.

Table 3.32 Average daily consumption of energy and protein by NSHD participants who provided dietary data in 1989

	Males	Excluding predicted misreporters	**Females**	Excluding predicted misreporters
	Mean (SD)			
	n=1125	*n*=809	*n*=1155	*n*=801
Mean energy intake (kcal/d)	2360 (671.2)	2609 (481)	1793 (510)	2010 (342)
Mean protein intake (g/d)	84.3 (23.1)	91 (18.7)	67.1 (17.7)	72.8 (14.2)
Mean protein intake (g/kg/d)	1.1 (0.33) (*n*=1118)	1.2 (0.3)	1.06 (0.33) (*n*=1151)	1.2 (0.25)
Mean protein intake as a percentage of total daily energy	14.5% (2.41)	14% (2.1)	15.3% (3.04)	14.6% (2.2)

Amongst NSHD participants who provided dietary data in 1989, reported mean energy consumption was 2360 kcals/d for males and 1793 kcals/d for females. Mean protein consumption was 84.3 g/d for males and 67.1 g/d for females. After excluding predicted energy misreporters, mean protein consumption was 91 g/d for males and 73 g/d for females, which was equivalent to 1.2 g/kg/d (Table 3.32).

3.9.7 Energy consumption by BMI class in the 1989 cohort

Table 3.33 Mean energy consumption (kcal/d) in male NSHD participants who provided dietary data in 1989, by BMI classification

	Underweight	Normal	Overweight	Obese
	Mean (SD)			
Daily energy intake	2293 (804) (*n*=7)	2393 (612) (*n*=523)	2347 (723) (*n*=492)	2287 (681) (*n*=96)
Daily energy intake excluding predicted misreporters	2309 (552) (*n*=5)	2549 (455) (*n*=424)	2666 (502) (*n*=330)	2774 (461) (*n*=50)

When estimated misreporters were excluded from the analyses, energy intakes among NSHD males who reported dietary data in 1989 increased across all BMI groups (Table 3.33).

Table 3.34 Mean energy consumption (kcal/d) in female NSHD participants who provided dietary data in 1989, by BMI classification

	Underweight	Normal	Overweight	Obese
	Mean (SD)			
Daily energy intake	2223 (366) (*n*=17)	1845 (499) (*n*=724)	1726 (484) (*n*=271)	1603 (551) (*n*=134)
Daily energy intake excluding predicted misreporters	2137 (292) (*n*=15)	1997 (347) (*n*=572)	2019 (338) (*n*=162)	2101 (299) (*n*=48)

In 1989 after predicted misreporters were excluded from the analyses, females classified as underweight still reported the highest energy intake (2137 kcals/d) (Table 3.34).

3.9.8 Energy and protein consumption in the 1999 cohort

In 1999 when NSHD study members were aged 53 y, 1776 individuals provided estimates of protein and energy intake via a 5 day food diary.

Table 3.35 Mean daily consumption of energy and protein in NSHD participants who provided dietary data in 1999

	Males	Excluding predicted misreporters	**Females**	Excluding predicted misreporters
	Mean (SD)			
	n=827	*n*=544	*n*=949	*n*=634
Mean energy intake (kcal/d)	2235 (526)	2486 (372)	1748 (385)	1939 (291)
Mean protein intake (g/d)	85.4 (19.1)	92 (16.7)	70.3 (14.9)	75.3 (13.3)
Mean protein intake (g/kg/d)	1.05 (0.3) (*n*=815)	1.2 (0.2)	1.03 (0.3) (*n*=943)	1.13 (0.2)
Mean protein intake as a percentage of total daily energy	15.6% (2.6)	14.8% (2.2)	16.4% (2.8)	15.6% (2.4)

Reported mean energy consumption in 1999 was 2235 kcals/d for males and 1748 kcals/d for females. Mean reported protein consumption was 85.4 g/d for males and 70.3 g/d for females. After excluding likely misreporters mean daily protein consumption was 92 g/d (1.2 g/kg/d) in males and 75.3 g/d (1.13 g/kg/d) in females (Table 3.35).

3.9.9 Energy consumption by BMI class in the 1999 cohort

Table 3.36 Mean energy consumption (kcal/d) in male NSHD participants who provided dietary data in 1999, by BMI classification

	Underweight	Normal	Overweight	Obese
	Mean (SD)			
Daily energy intake	2332 (*n*=1)	2277 (508) (*n*=244)	2226 (515) (*n*=415)	2210 (574) (*n*=155)
Daily energy intake excluding predicted misreporters	2332 (*n*=1)	2417 (362) (*n*=199)	2474 (341) (*n*=283)	2771 (414) (*n*=61)

Table 3.37 Mean energy consumption (kcal/d) in female NSHD participants who provided dietary data in 1999, by BMI classification

	Underweight	Normal	Overweight	Obese
	Mean (SD)			
Daily energy intake	2003 (486) (*n*=3)	1765 (359) (*n*=395)	1729 (373) (*n*=334)	1746 (445) (*n*=208)
Daily energy intake excluding predicted misreporters	2003 (486) (*n*=3)	1888 (280) (*n*=315)	1939 (269) (*n*=215)	2108 (306) (*n*=99)

CHAPTER 4

Diurnal Patterns of Energy and Protein Consumption

4.1 Introduction

Time of day and proportions of macronutrients consumed are related to total daily food intake (de Castro, 2007). High morning carbohydrate, fat and protein intake was associated with reduced daily carbohydrate, fat and protein intake respectively – the effect was macronutrient-specific. In comparison, high evening intake of either total food energy, carbohydrate or fat was associated with a higher overall daily energy intake. Consumption of low-density food in the morning and avoiding the consumption of high-density foods between 5 pm and the early hours of the morning was associated with a reduction in daily energy consumption (de Castro, 2009).

There is a paucity of research on diurnal patterns of consumption, and in particular protein consumption. In 2012 (Tieland *et al.*, 2012a) described protein intakes across the day in community-dwelling, frail and institutionalised elderly. In community-dwelling individuals (two groups (65 – 74 y) and (75 – 97 y) protein intakes were particularly low (10 ± 10 g) at breakfast. In the frail and the institutionalised, protein intake at breakfast was 8 ± 5 g and 12 ± 6 g, respectively. Although daily protein intakes, relative to body mass (0.8 – 1.1 g/kg/d) were well above the recommendation (0.8 g/kg/d) protein distribution throughout the day was uneven, and provided scope for improvement. The authors, referring to the research of (Paddon-Jones D, 2009) suggested that by increasing protein at breakfast (to at least 20 g) this may represent a dietary strategy for the postponement of sarcopenia in older people.

In a randomised double-blind, placebo-controlled trial (Tieland *et al.*, 2012c) 65 frail (the (Fried *et al.*, 2001) criteria) elderly (mean age 81 y and 78 y) subjects received either 15 g of protein after breakfast and lunch or a placebo, for 24 weeks. Primary outcome was DEXA-measured lean mass and secondary outcomes were muscle fibre CSA, strength (1 maximum repetition leg press), hand grip strength and short physical performance battery (SPPB) (balance, gait speed and chair rise). There was no significant time, treatment or treatment x time interaction effects on any of the body composition parameters; hand grip strength did not improve and leg press improved in both groups. The SPPB score increased significantly in the protein group only, the chair rise component showing the greatest improvement (13.7 ± 1.0 to 11.1 ± 1.1 seconds), the treatment x time interaction (p=0.055). Referring to the (Paddon-Jones D, 2009) protein recommendation of ≥ 20 g per meal, after supplementation the protein group consumed ≥ 25 g at each meal compared with the placebo group who consumed 11 ± 1 g at breakfast and 17 ± 2 g at lunch. In a second (related) randomised double-blind, placebo-controlled trial (Tieland *et al.*, 2012b) two groups of frail, elderly subjects (mean age 79 (placebo) and 78 y (protein)) were further randomised to a 24 week resistance exercise (RE) training programme. Primary and secondary outcomes were as above. In sharp contrast to (Tieland *et al.*, 2012c) there were significant increases in lean mass (1.3 kg) and appendicular lean mass (0.9 kg) in the protein group only; treatment x time interactions, p=0.006; p<0.001. Strength and physical performance improved in both groups with no significant treatment x time interaction effect. The 30 g protein supplementation, which was a prerequisite for the gain muscle mass, increased daily intakes from 1.0 to 1.4 g/kg/d without reducing daily energy intake.

In a cross-sectional pilot study, conducted in 78 older adults (mean age 68.7 y) diet was assessed by 3 non-consecutive 24 h recalls and appendicular skeletal muscle mass by dual-energy X-ray absorptiometry (Ruiz Valenzuela RE, 2013). Subjects were grouped by whether they had consumed > 25 g of protein during at least one (main meal) or not. Appendicular skeletal muscle mass differences between the groups became insignificant after adjusting for body weight, gender and height.

In 17 younger subjects (35 ± 3 y) and 17 older (68 ± 2 y) subjects, changes in muscle protein synthesis in response to 30 g (113 g of 90% lean beef) and 90 g (340 g) servings of protein were examined (Symons *et al.*, 2009). Under resting conditions, protein synthesis after ingestion of both servings increased mixed muscle FSR values in both young and elderly subjects. The authors recommended multiple, moderate-sized servings of high quality protein throughout the day rather than a single large dose to optimise muscle growth.

In 2012 (Volpi *et al.*, 2013) specifically enquired – is the optimal level of protein intake for older adults greater than the current Recommended Dietary Allowance? In the United States this is currently 0.8 g/kg/d (Rand *et al.*, 2003). As ageing is associated with a blunted anabolic response to dietary amino acids, a purported threshold dose of leucine for stimulation of muscle protein synthesis in older adults is suggested to be ~3 g, corresponding to the per meal recommendation of 25 – 30 g by (Paddon-Jones D, 2009). The authors infer that any meal containing < 3 g leucine would be less anabolic for skeletal muscle in older adults, leading to alternative utilisation of dietary protein – oxidation or lipogenesis. As NHANES III data indicate that older American adults have a mean daily intake of ~0.9 g/kg/d but consume ~50% of their daily protein at dinner, average weight individuals (70 kg) are stimulating muscle protein synthesis only at the evening meal.

As discussed, many researchers (Arnal *et al.*, 1999; Cuthbertson D, 2005; Katsanos *et al.*, 2005; Boirie, 2009; Paddon-Jones D, 2009; Symons *et al.*, 2009; Breen and Phillips, 2011; Pennings *et al.*, 2012; Tieland *et al.*, 2012a; Bouillanne *et al.*, 2013; Ruiz Valenzuela RE, 2013; Volpi *et al.*, 2013) have made reference to the fact that *per meal* protein intake may be more important than total daily protein intake, where this is spread out over several meals, especially in relation to the maintenance of muscle mass in older people and the prevention of sarcopenia. Specific protein feeding strategies, suggests (Bauer *et al.*, 2013), represent advancing refinement in our understanding of muscle protein synthesis in older people. With a higher per-meal protein threshold for the stimulation of muscle protein anabolism, evidence suggests that a per meal protein consumption of 25 – 30 g (containing 2.5 – 2.8 g of leucine) and an even distribution across the day may offer benefits.

This chapter will examine trends in diurnal patterns of protein and energy consumption as reported by estimated 5 d food diary by NSHD participants who provided dietary data in all years. The approach to meal identification was as reported by (Almoosawi *et al.*, 2012). This is an examination of how participants consumed energy and protein across the day based upon consumption recorded in estimated 5 d food diaries. The two 24 h recalls, completed by participants in 1982 and 1989, are not discussed here.

4.2 Eating occasions

Meal slots were labelled as follows:

Table 4.1 Labelled meal slots in 5 d food diaries completed by NSHD participants in 3 measurement years, 1982 - 1999

1 Pre breakfast	2 Breakfast	3 Mid-Morning	4 Lunch	5 Tea	6 Evening Meal	7 Late Evening	8 Extras

Extras was a slot provided for participants to record consumption not otherwise allocated to any other eating occasion. Meal slots were specifically labelled (Table 4.1) in the diaries, but eating occasions were subjectively interpreted by individuals completing the food diary. Times were not stated in the diary and were not required to be given by participants.

4.3 Diurnal energy consumption

Energy consumption across the day was examined in order that protein density of mealtime energy could be determined.

4.3.1 Meal energy (kcals)

Table 4.2 Outcomes of GLM repeated measures analysis: Mean meal energy intakes (kcal) amongst NSHD participants who provided dietary data in all years

	Males (*n*=568)			*p*-value	Females (*n*=695)			*p*-value
Meal	1982	1989	1999		1982	1989	1999	
1	23	25	29	0.226	15[a]	16[a]	18[b]	**0.014**
2	316	332	316	0.095	245	244	255	0.066
3	94[a]	110[b]	93[a]	**0.010**	63	69	66	0.127
4	654[a]	699[b]	608[c]	**<0.001**	461[a]	514[b]	481[c]	**<0.001**
5	101[a]	113	122[b]	**0.004**	84[a]	96[b]	104[b]	**<0.001**
6	932[a]	851[b]	798[c]	**<0.001**	679	693[a]	662[b]	**0.012**
7	169[a]	205[b]	189	**0.004**	115[a]	140[b]	124[a]	**<0.001**
8	0.1[a]	116[b]	107[b]	**<0.001**	0.1[a]	86[b]	67[c]	**<0.001**

GLM Repeated measures analysis (time) with Bonferroni adjustment for multiple comparisons. Where Mauchly's Test (of Sphericity) was significant ($p<0.05$) i.e. the assumption of sphericity was violated, the Greenhouse-Geisser corrected probability was reported. Where subscript letters are the same there was no significant difference between values, where subscript letters are different there was a significant difference between values.

In the subset of NSHD participants who reported dietary data in all 3 years there was no significant change in mean energy consumption (kcals) at breakfast (meal 2). Energy consumption at lunch (meal 4) was significantly different in all years for males and females; in males increasing in 1989 and decreasing in 1999 to a level below that reported in 1982. In females, lunchtime consumption also increased in 1989 falling in 1999 but to a level still higher than that reported in 1982. Mean energy consumption at tea (meal 5) increased significantly across the 3 measurement periods in males and females. Mean energy consumption at the evening meal (meal 6) fell significantly across adulthood in males, whereas in females, consumption only fell between 1989 (at 43 y) and 1999 (at 53 y) (p=0.012) (Table 4.2).

4.3.2 Meal energy (as a percentage of total daily energy)

Table 4.3 Outcomes of GLM repeated measures analysis: Mean meal energy intakes (as a percentage of total daily energy) amongst NSHD participants who provided dietary data in all years

	Males (*n*=568)			*p*-value	Females (*n*=695)			*p*-value
Meal	1982	1989	1999		1982	1989	1999	
1	1	1	1	0.172	1	0.8[a]	1[b]	**0.003**
2	14	13	14	0.052	15[a]	13[b]	14[a]	**<0.001**
3	4	4	4	0.040	4	4	4	0.827
4	29[a]	29[a]	27[b]	**<0.001**	28	28	27	0.078
5	4[a]	5[a]	5[b]	**<0.001**	5[a]	5[a]	6[b]	**0.001**
6	41[a]	35[b]	36[b]	**<0.001**	41[a]	38[b]	37[b]	**<0.001**
7	7	8	8	0.048	7	7	7	0.109
8	0.005[a]	4[b]	5[b]	**<0.001**	0.01[a]	4[b]	4[c]	**<0.001**

GLM Repeated measures analysis (time) with Bonferroni adjustment for multiple comparisons. Where Mauchly's Test (of Sphericity) was significant the Greenhouse-Geisser probability was reported. Only where subscript letters are different was there a significant difference between values.

In 1982/89 lunch (meal 4) consumption provided 29% of total daily energy intake (TE) in males; this fell significantly in 1999 to 27% of TE. The evening meal, which provided 41% of TE in 1982 provided significantly less (35 – 36% of TE) in 1989/99. A similar pattern was seen in females (Table 4.3).

4.4 Diurnal protein consumption

4.4.1 Meal protein (g)

Table 4.4 Outcomes of GLM repeated measures analysis: Mean meal protein intakes (g) for NSHD participants who provided dietary data in all years

	Males (*n*=568)			*p*-value	Females (*n*=695)			*p*-value
Meal	1982	1989	1999		1982	1989	1999	
1	0.6[a]	0.8[b]	1[b]	**<0.001**	0.5[a]	0.7[b]	0.9[c]	**<0.001**
2	10[a]	11[b]	11[b]	**0.001**	8[a]	8[a]	9[b]	**<0.001**
3	2.6[a]	3.5[b]	3	**0.001**	1.7[a]	2[b]	2[b]	**0.002**
4	25	26	25	0.064	19[a]	20[b]	20[b]	**<0.001**
5	2.5[a]	3	3.5[b]	**<0.001**	2[a]	2.6[b]	3[c]	**<0.001**
6	35	36	37	0.052	28[a]	30[b]	31[b]	**<0.001**
7	2.6[a]	5[b]	5[b]	**<0.001**	2[a]	4[b]	3.5[c]	**<0.001**
8	0.01[a]	1.5[b]	1.3[b]	**<0.001**	0.01[a]	1[b]	1[b]	**<0.001**

GLM Repeated measures analysis (time) with Bonferroni adjustment for multiple comparisons. Greenhouse-Geisser probability was reported where the assumption of sphericity was violated. Only where letters are different was there is a significant difference between values.

In female NSHD participants who provided dietary data in all years, meal protein consumption (g) at breakfast (meal 2) increased significantly to a mean of 9 g in 1999 from a mean of 8 g reported in 1982/89. Protein consumption at lunch (meal 4) and at the evening meal (meal 6) increased in 1989 to a mean of 20 and 30 g from a mean of 19 and 28 g reported in 1982, respectively. In males protein consumption at breakfast (meal 2) averaged 11 g in 1989/99 up from a mean of 10 g reported in 1982. Protein consumption at lunch (meal 4) and at the evening meal (meal 6) did not differ significantly across the 3 measurement periods in males, always averaging ≥ 25 g and ≥35 g, respectively.

In 1999 when NSHD participants were aged 53 y, at the 3 main eating occasions (breakfast, lunch and the evening meal) males were consuming on average 11 g, 25 g and 37 g of protein, respectively; whereas females were consuming on average 9 g, 20 g and 31 g at the 3 main meals across the day (Table 4.4).

4.4.2 Meal protein (as a percentage of meal energy)

Table 4.5 Outcomes of GLM repeated measures analysis: Mean meal protein intake (as a percentage of meal energy) for NSHD participants who provided dietary data in all years

	Males (n=568)			*p*-value	Females (n=695)			*p*-value
Meal	1982	1989	1999		1982	1989	1999	
1	8[a]	10[b]	12[c]	**<0.001**	11[a]	14[b]	16[c]	**<0.001**
2	13[a]	13[b]	14[c]	**<0.001**	13[a]	14[a]	14[b]	**<0.001**
3	13[a]	15[b]	15[b]	**0.001**	15	16	16	0.294
4	16[a]	15[b]	16[c]	**<0.001**	17[a]	16[b]	17[a]	**<0.001**
5	11[a]	13[b]	13[b]	**<0.001**	12[a]	14[b]	14[b]	**0.001**
6	16[a]	17[b]	19[c]	**<0.001**	17[a]	18[b]	19[c]	**<0.001**
7	6[a]	11[b]	11[b]	**<0.001**	7[a]	13[b]	12[b]	**<0.001**
8	3.4	3.6	3.6	0.750	4	4	3.8	0.595

GLM Repeated measures analysis (time) with Bonferroni adjustment for multiple comparisons. Greenhouse-Geisser probability was reported where the assumption of sphericity was violated. Only where letters are different was there is a significant difference between values.

Protein, as a percentage of meal energy (protein density) increased significantly at all 3 main meals in all measurement years in males. In 1999, the protein density of breakfast, lunch and the evening meal was 14, 16 and 19%. In females, meal protein as a percentage of meal energy at breakfast, lunch and at the evening meal was 14, 17 and 19% in 1999 when they were age 53 y (Table 4.5).

4.4.3 Meal protein (as a percentage of total daily protein)

Table 4.6 Outcomes of GLM repeated measures analysis: Mean meal protein intake (as a percentage of total daily protein) for NSHD participants who reported dietary data in all years

	Males (*n*=568)			*p*-value	Females (*n*=695)			*p*-value
Meal	1982	1989	1999		1982	1989	1999	
1	1[a]	1	1[b]	**0.002**	1[a]	1[b]	1[c]	**<0.001**
2	13	12	13	0.499	13[a]	12[b]	13[a]	**<0.001**
3	3[a]	4[b]	3[a]	**0.002**	3	3	3	0.961
4	32[a]	30[b]	29[c]	**<0.001**	30[a]	29[b]	28[b]	**<0.001**
5	3[a]	3[a]	4[b]	**0.003**	3[a]	4[a]	4b	**<0.001**
6	45[a]	41[b]	43[b]	**<0.001**	46[a]	45[b]	44[b]	**<0.001**
7	3[a]	6[b]	5[b]	**<0.001**	3[a]	6[b]	5[c]	**<0.001**
8	0.01[a]	2[b]	2[b]	**<0.001**	0.01[a]	2[b]	1[c]	**<0.001**

Bonferroni adjustment for multiple comparisons. Where the assumption of sphericity was violated Greenhouse-Geisser probability was reported. Only where letters are different was there a significant difference between values.

In 1999, when male and female NSHD participants were aged 53 y, they consumed 13% of their total daily protein at breakfast (meal 2), 29/28% at lunch (meal 4) and 43/44% at the evening meal (meal 6) (Table 4.6).

4.4.4 Meal Muscle Protein Synthesis Score

Chapter 2 (Section 2.4) provides an overview of the evidence for the impact of quantity (and quality) of ingested protein per meal on muscle protein synthesis. A rationale for the derivation of the score, the use of a 20 g marker and an explanation of its implementation and calculation is given.

Table 4.7 Number and percentage of NSHD participants, who reported dietary data in all years, who consumed as much as 20 g of protein at eight eating occasions across the day

	Males (*n*=568)			Females (*n*=695)		
	1982	1989	1999	1982	1989	1999
1	*n*=1 (0.2)	0	*n*=1 (0.2)	0	0	0
2 Breakfast	*n*=36 (6.3%)	*n*=57 (10%)	*n*=53 (9.3%)	*n*=5 (0.7%)	*n*=12 (1.7%)	*n*=8 (1.2%)
3	8 (1.4)	15 (2.6)	5 (0.9)	0	0	0
4 Lunch	*n*=390 (68.7%)	*n*=417 (73.4%)	*n*=385 (67.8%)	*n*=287 (41.3%)	*n*=325 (46.8%)	*n*=325 (46.8%)
5	6 (1.1)	6 (1.1)	10 (1.8)	0	1 (0.1)	7 (1)
6 Evening meal	*n*=511 (90%)	*n*=512 (90.1%)	*n*=524 (92.3%)	*n*=551 (79.3%)	*n*=599 (86.2%)	*n*=617 (88.8%)
7	6 (1.1)	23 (4)	11 (1.9)	2 (0.3)	6 (0.9)	4 (0.6)
Extras	0	2 (0.4)	0	0	0	1 (0.1)

In 1999, when NSHD participants were aged 53 y, ~9% of males consumed as much as 20 g of protein at breakfast (meal 2), the percentage of females who consumed ≥ 20 g of protein at breakfast was 1.2%. At lunch (meal 4) ~68% of males consumed ≥ 20 g of protein, by comparison the equivalent percentage of females consuming this amount of protein was 47%.

At the evening meal, across all 3 measurement years, ≥ 90% of males consumed as much as 20 g of protein. In females, the percentage consuming at least this amount of protein ranged from 79.3 – 88.8% across 3 measurement years (Table 4.7).

4.4.5 Daily MPSS

The frequency with which ≥ 20 g of protein was consumed, at any of eight eating occasions (scored 1), was summed to provide daily muscle protein synthesis scores in each measurement year. Gender differences in group membership were tested using crosstabs/the Chi-square test of association (Pearson Chi-Square) (2-sided)(adjustment for multiple testing was not possible (increased chance of a type 1 error)).

Table 4.8 Total daily muscle protein synthesis scores, 1982 – 1999, among NSHD participants who provided dietary data in all years

	Males (*n*=568)			Females (*n*=695)		
Muscle Protein Synthesis Score	1982	1989	1999	1982[1]	1989[2]	1999[3]
0	28 (4.9)	15 (2.6)	8 (1.4)	93 (13.4)	59 (8.5)	43 (6.2)
1	160 (28.2)	138 (24.3)	176 (31)	364 (52.4)	340 (48.9)	349 (50.2)
2	343 (60.4)	357 (62.9)	340 (59.9)	233 (33.5)	285 (41)	296 (42.6)
3	36 (6.3)	53 (9.3)	43 (7.6)	5 (0.7)	11(1.6)	7 (1)
4	1 (0.2)	4 (0.7)	1 (0.2)			
5		1 (0.2)				

[1]1982 Chi-Square test for estimated misreporting: $\chi 2$ (4) = 148.5, p<0.001; [2]1989 Chi-Square test for estimated misreporting: $\chi 2$ (5) = 140.8, p<0.001; [3]1999 Chi-Square test for estimated misreporting: $\chi 2$ (4) = 99.2, p<0.001.

Across 3 measurement periods, the greatest proportion of males consistently consumed ≥ 20 g of protein on 2 occasions across the day. In 1982 this was 60.4% increasing slightly to 62.9% in 1989. In 1999 the proportion of males consuming ≥ 20 g of protein on two occasions fell to < 60%. Concurrently, those consuming ≥ 20 g on 1 occasion in the day increased from 24.3% in 1989 to 31% in 1999. In 1982 when males were aged 36 y, ~5% never consumed as much as 20 g of protein at any of eight eating occasions across the day. When they were 53 y, this proportion had fallen to 1.4% of males. In 1982, 52.4% of females aged 36 y consumed ≥ 20 g of protein on 1 occasion in the day whereas 13.4% never consumed as much 20 g at any of eight eating occasions (Table 4.8).

In 1989, when females were aged 43 y, the proportion never consuming as much as 20 g fell to 8.5% and 41% consumed ≥ 20 g on 2 occasions across the day. In 1999 (when aged 53 y) the proportion never consuming as much as 20 g at any eating occasion across the day had fallen to 6.2% and the proportion consuming at least 20 g on 2 occasions, had increased to 42.6% (Table 4.8).

4.4.6 Derivation of adulthood MPSS

An adulthood muscle protein synthesis score was calculated as described in Chapter 2, i.e. daily scores for 1982 – 1999 were summed for individuals who had provided dietary data in all years (see Figure 4.1). These scores reflected the frequency with which ≥ 20 g protein had been consumed across the day throughout adulthood (36 – 53 y).

Figure 4.1 Derivation of an adulthood muscle protein synthesis score in NSHD participants who provided dietary data in all 3 years

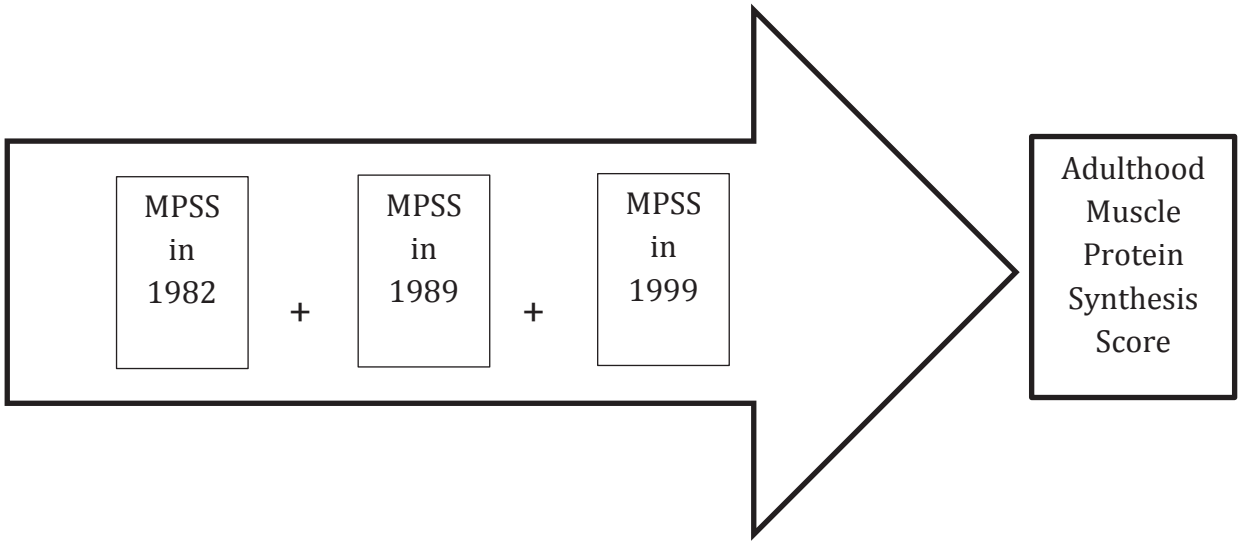


Table 4.9 The number and percentage of NSHD participants who provided dietary data in all years by adulthood muscle protein synthesis score

Adulthood muscle protein synthesis score	Males (*n*=568)	Females (*n*=695)
0		2 (0.3)
1		15 (2.2)
2	14 (2.5)	72 (10.4)
3	41 (7.2)	172 (24.7)
4	98 (17.3)	191 (27.5)
5	160 (28.2)	157 (22.6)
6	180 (31.7)	78 (11.2)
7	51 (9)	6 (0.9)
8	18 (3.2)	2 (0.3)
9	5 (0.9)	
10	1 (0.2)	

The adulthood muscle protein synthesis score ranged from 2 to 10 in males and 0 to 8 in female NSHD participants who had provided dietary data in all years. The identification of low protein consumers using the adulthood muscle protein synthesis score is described in Chapter 2, i.e. those in the lowest gender-specific quartile of score. This is shaded for males and females in Table 4.9 above.

Among males, 180 (31.7%) had an adulthood muscle protein synthesis score of 6, this equates to consumption of ≥ 20 g of protein on two occasions across the day in each measurement year; a pattern of protein consumption seen in 78 (11.2%) of females.

4.5 Discussion

This chapter examined diurnal patterns of protein and energy consumption in NSHD participants, who provided dietary data via a 5 d food diary at all 3 measurement periods (1982, 1989 and 1999) when they were aged 36, 43 and 53 years, respectively. Diurnal eating occasions (meal slots) were labelled as described in (Table 4.1). The labelling of meals in this manner may have imposed a particular structure of diurnal consumption onto NSHD participants and introduced an element of subjectivity into the data collection.

Protein intakes at all main meals (breakfast, lunch and the evening meal) and in all years, was higher in males compared with females. Male intakes were consistently 2 – 3 g higher at breakfast, 5 – 6 g higher at lunch and 6 – 7 g higher at the evening meal.

The research available on diurnal patterns of consumption, i.e. that of (Tieland *et al.*, 2012a) and (Ruiz Valenzuela RE, 2013) concerns older (≥ 65 y) subjects and as such is not directly comparable to the present cohort. Where comparisons are made with the (Tieland *et al.*, 2012a) study these are with the younger community-dwelling group (65 – 74 y) and not those aged 75 – 97 y. In the (Tieland *et al.*, 2012a) study, protein intakes were not split by gender as in (Ruiz Valenzuela RE, 2013) which made comparisons with NSHD female intakes difficult. Difficulties also arose in relation to meal time nomenclature; lunch may often comprise the main protein-containing meal, especially in older cohorts whereas dinner may be a smaller meal, arguably more comparable to lunch among the NSHD cohort.

In the NSHD cohort, protein intakes at breakfast were 8 – 11 g which was in accordance with the 10 g intakes observed by (Tieland *et al.*, 2012a) among community-dwelling Dutch subjects, but much less than the 15 – 19 g intakes observed among Caucasian Mexican adults by (Ruiz Valenzuela RE, 2013); this may be explained by differences in habitual diet and the frequent consumption of protein from animal sources among this cohort.

Lunch time protein intakes among the NSHD cohort were 25 – 26 g in males and 19 – 20 g in females. Average lunch time protein intakes among the Dutch community-dwelling subjects (Tieland *et al.*, 2012a) were 27 ± 15 g; however, 70% of these subjects consumed a 'bread containing meal' which provided 19 ± 9 g. Where a hot meal was consumed, protein intakes averaged 39 ± 16 g. Protein provided by a bread-containing meal was consistent with intakes reported by female NSHD participants, but among males, protein consumption was more consistent with overall mean lunchtime intakes, which included those who consumed a hot meal. Among the Mexican cohort, the midday meal (lunch) was typically the main meal of the day and was arguably more comparable with the evening meal consumed by the NSHD cohort. Protein intakes at dinner in this cohort (comparable to lunch intakes in the NSHD) were 14 and 20 g among females and males, respectively. Comparing these intakes to lunchtime intakes among the NSHD cohort, females were consuming 5 – 6 g more and males 5 – 6 g more.

At the evening meal protein intakes among the NSHD cohort were 35 – 37 g in males and 28 – 30 g in females. While increases in absolute protein intakes were insignificant (with the exception of 1982 – 1989 in females only), these meal intakes reflected a general decline in protein consumption, as a percentage of total daily protein consumption. In 1982 males and females consumed 45 – 46% of daily protein at the evening meal, this declined significantly in 1989 and remained unchanged in 1999.

Among the community-dwelling Dutch cohort (Tieland *et al.*, 2012a) average protein intake at the evening meal was ~32 g while among the Mexican cohort, protein intake at the main meal was 27 and 33 g in females and males, respectively. Among male NSHD participants, protein intakes at the evening meal were 2 – 5 g higher; whereas among NSHD females, protein intakes were 1 – 3 g higher than those seen in the Mexican cohort.

Drawing on observations provided by NHANES III data, (Volpi *et al.*, 2013) observed that older Americans typically consume ~50% of daily protein at dinner. By comparison, the trend amongst NSHD participants was towards a lower percentage of total daily protein at the evening meal. As this cohort ages, this trend (accompanied by increasing protein intakes at lunch and maintenance of adequate daily intakes) may attenuate the age-related effects of anabolic resistance.

In terms of the (Paddon-Jones D, 2009) 25 – 30 g per meal recommendation, NSHD males (at 53 y) met the recommendation at lunch and at dinner, whereas females met the recommendation only at the evening meal. However, it must be stated that these recommendations are primarily aimed at older subjects who exhibit protein anabolic resistance and higher splanchnic extraction of amino acids (altered protein metabolism) and may also have higher protein needs due to inadequate protein consumption, chronic and acute (inflammatory) diseases and greater inactivity/immobility (Bauer *et al.*, 2013).

CHAPTER 5

Physical Activity and Physical Capability

5.1 Introduction

5.1.1 Physical activity

This aims of this chapter are to report adulthood habitual leisure time physical activity as measured in 1982 (at 36 y), 1989 (at 43 y) and 1999 (at 53 y) and various measures of physical capability and anthropometry (including body composition) at different measurement points. Secular trends and important relationships are statistically investigated.

The particular value of longitudinal cohort studies are that they provide data on physical activity for the same individuals at several points in the lifecourse and changes in physical activity behaviour across adulthood may be investigated. The individual physical activity trajectory may be important – regardless of the level of physical activity in early adulthood, those who reduce their physical activity over time may fare differently from those who maintain physical activity across adulthood. Cross-sectional studies have the limitation of temporality – it is impossible to determine the time order of events and to impute causation, and randomised control studies (RCTs) may specifically investigate a particular types of activity which may not represent habitual physical activity in the general population.

Physical inactivity worldwide causes 6% of the burden of disease from CHD, 10% of breast and colon cancer and 9% of premature mortality (Lee *et al.*, 2012). Physical inactivity is one of the major modifiable risk factors associated with myocardial infarction (Anand *et al.*, 2008) and stroke (O'Donnell *et al.*, 2010).

The association between physical activity during the lifecourse and bone mineral content (BMC) in later life was evaluated in a systematic review (Bielemann *et al.*, 2013). Pooled analyses were not possible due to the heterogeneity of the studies, mainly in the different instruments used to measure physical activity, but positive associations between physical activity and bone mass were found (more in males than in females).

In a systematic review (Fogelholm, 2010) risk for all-cause and cardiovascular mortality was lower in individuals with good aerobic fitness notwithstanding a high BMI, compared with individuals with poor fitness and normal BMI. However, a high BMI was associated with a greater risk of type 2 diabetes (and the prevalence of cardiovascular and diabetes risk factors) notwithstanding higher physical activity compared with a normal BMI and low physical activity. These finding were consistent with a systematic review by Blair and Brodney (Blair SN, 1999) who concluded *inter alia* that physical activity attenuated the health risks associated with overweight and obesity; active obese individuals had lower morbidity and mortality than their sedentary, normal weight counterparts and inactivity and lower cardiorespiratory fitness were as important as adiposity in predicting mortality.

A major determinant of cardiovascular fitness is habitual physical activity (a genetic component explains 25 – 40% of the variability in fitness) (Wei *et al.*, 1999) and low cardiovascular fitness adds to overweight and obesity in adversely influencing mortality. The relative risk associated with low cardiovascular fitness was found to be comparable to those for diabetes, hypercholesterolemia, hypertension and smoking (Wei *et al.*, 1999).

In the Hertfordshire Cohort Study, 275 women (mean age 68.2 y) and 229 men (mean age 67.9 y) completed a 69 item physical activity questionnaire on the basis of which women were classified as either 'keep fit' or 'indoors' types and men, 'keep fit', 'indoors' or 'less active'. Cluster analysis revealed that females classified as 'keep fit' had significantly better hand grip strength and performances at the 3 m walk and chair rise test compared with those classified as 'indoors'. Between male physical activity clusters, there were no significant differences in muscle strength or physical performance. In describing gender differences, women had significant higher median total energy expenditure (TEE) than men – with walking & home activity driving the difference. Median-estimated monthly TEE was 665.3 MET.h/month in women and 482.7 MET.h/month in men. The difference was shown not to be explained by the over-reporting of physical activity in women (Martin *et al.*, 2008).

Predictors of midlife participation in sports and recreational activity were investigated in the NSHD. Those who took part in sports in at 36 y were a 'selected group' compared with the less active; they had fewer childhood health problems, were assessed as socially outgoing in adolescence, were above average at school games, well-educated with secondary-educated mothers. The observation that those who were active at work engaged in less leisure activity was consistent with evidence from other studies which also suggested that those who frequently engaged in sport/recreational activities were better educated and had non manual occupations (Kuh, 1992).

In a systematic review and meta-analysis, older adults with chronic musculoskeletal pain were found to be less active than asymptomatic controls. The authors concluded that physical activity was integral for healthy aging and should be regarded as a central non-pharmacological strategy in the management of chronic pain (Stubbs B, 2013).

In a systematic review by (Sun *et al.*, 2013) into global levels of physical activity in older people (≥ 60 y) 53 studies were included, 49 cross sectional and 4 longitudinal. Physical activity included that undertaken as leisure time (most often measured), occupational, household and transportation. Physical activity volume was calculated differently across studies. Most studies reported that 20 – 60% of their sample met the guideline of 150 minutes/week (in 10 minute bouts). Only 6 studies used accelerometers (objective data) while 48 measured self-reported physical activity (subjective data). Two studies compared the subjective and objective data: in an American study (Tucker *et al.*, 2011) (using NHANES data) the proportion classified as 'sufficiently active' when measured by accelerometry was 7.25% and 17.24% (using 2 different guidelines); however this increased to 54.2% when measured subjectively (by questionnaire). In a Swedish study (Hurtig-Wennlöf *et al.*, 2010) the equivalent proportions were 87% and 72.2% respectively. This latter (contradictory) finding was believed to result from a lower cut-off point for moderate PA compared with other studies and the inclusion of exercise bouts of < 10 minutes duration. Gender differences in self-reported physical activity (reported by 22 studies) ranged from 0.8 – 21.4% but when physical activity was measured by accelerometry gender differences were 0.2 – 1.5%. Two studies measured physical activity objectively and 18 subjectively across different age groups, and reported that the older old were more sedentary than the younger old. When divided into narrower bands (compared with dichotomising the data) physical activity decreased progressively with age in males and females. The authors observed that when investigating trends over time it was crucial that there was comparability in the methods; differences in instruments, definitions and physical activity domains posed a significant challenge. The authors concluded that more evidence of physical activity amongst older physical performance (using validated measurement instruments) was required to inform public health strategies.

In a prospective cohort study of 416 175 individuals, followed-up after 8 y, participants were categorised, based on self-administered physical activity questionnaire, into 5 categories, inactive, low, medium, high or very high activity. Every 15 minutes of exercise (beyond the minimum amount of 15 minutes/d) was associated with a 4% reduction in all-cause mortality and a 1% reduction in all-cancer mortality (Wen *et al.*, 2011). Benefits of daily physical activity were applicable males and females in all age groups.

5.1.2 Physical capability

Age and gender differences in physical capability levels were examined using harmonised (cross-sectional) data from eight UK cohort studies including NSHD (Cooper R, 2011); physical capability was objectively measured HALCyon cohorts by hand grip, chair rise, walking speed and timed up and go. Higher levels were recorded by younger participants and males (hand grip strength, chair rise). Gender differences in hand grip strength (likely to be explained by differences in body composition) were found to diminish with age.

Objective measures of physical capability – hand grip strength, walking speed, chair rise time and standing balance time – were found to be suggestive of subsequent health (Cooper *et al.*, 2011a) and predictive of all-cause mortality in older populations in quantitative systematic reviews and meta-analyses (Cooper *et al.*, 2010).

Diet and its relationship with grip strength were examined in the Hertfordshire Cohort Study where muscle function (as measured by grip strength) was found to be positively influenced by a single dietary factor, namely fatty fish consumption. In this population, at this age, every one additional weekly portion of fatty fish was associated with a 0.43 kg increase in hand grip strength (in males) and a 0.48 kg increase in women, independent of their height, age and birth weight (Robinson *et al.*, 2008).

5.2 Leisure time physical activities in 1982

In the NSHD, data on the nature, frequency and duration of leisure time physical activity were collected by questionnaire at ages 36 y (1982), 43 y (1989) and 53 y (1999) as described in Chapter 2. In 1982 NSHD participants were asked about their leisure time activity e.g. walking, cycling, gardening, DIY and a range of sporting/ recreational activities, and classified as either inactive, less active or most active as described in Chapter 2.

Table 5.1 NSHD cohort members' participation in cycling and walking in 1982

	Inactive	Less active	Most active
Males *n*(%)	406 (24.8)	758 (46.2)	475 (29.0)
Females *n*(%)	321 (19.3)	737 (44.0)	602 (36.3)

When NSHD cohort members were 36 y their participation in cycling and walking was recorded and these data were available for 3299 individuals; 1639 males and 1660 females. 22% (*n*=727) of all respondents reported no participation in this activity and were classified as inactive. 45.3% of all respondents were classified as less active and 32.6% most active, i.e. reporting cycling and walking 5 or more times a month. Walking and cycling were reported more frequently by females than males at 36 y (Table 5.1).

Table 5.2 NSHD cohort members' participation in DIY and heavy gardening in 1982

	Inactive	Less active	Most active
Males *n*(%)	572 (34.8)	605 (36.8)	468 (28.4)
Females *n*(%)	948 (57)	516 (31)	200 (12)

Participation in Do It Yourself (DIY) activities (household maintenance/ repair and modification) and heavy gardening was recorded at age 36 y and such data were available for 3309 cohort members; 1645 males and 1664 females. 1520 individuals (45.9%) reported no participation in these activities in the previous month; 1121 individuals (33.9%) were classified as less active and 668 individuals (20.2%) as most active. This activity was reported more frequently by males than females at 36 y (Table 5.2).

Table 5.3 NSHD cohort members' participation in sport and recreational activities in 1982

	Inactive	Less active	Most active
Males *n*(%)	514 (31.2)	435 (26.4)	697 (42.3)
Females *n*(%)	705 (42.4)	402 (24.2)	556 (33.4)

Participation in a range of 27 sport and recreational activities was recorded at 36 y and this data was available for 3309 cohort members, 1646 males and 1663 females. Most frequently reported activities (reported by at least one in 10 men in the previous month) were swimming (23.3%), exercises at home (16%), golf (11.6%), jogging (11.1%), squash (10.9%), dancing (10%) and football (9.8%). Most frequently reported (by at least one in 10 women in the previous month): swimming (24.7%), exercises at home (18.2%), dancing (15.4) and movement to music (9.5%)(Kuh, 1992). Because they reported no participation in any of the listed leisure time sport/ recreational activities in the preceding month, 36.8% of all responders were classified as inactive; 25.3% of individuals were classified as less active and 37.9% as most active. The latter reported participation in these activities 5 or more times in the previous month. Sport and recreational activity was reported more frequently by males than females at 36 y (Table 5.3).

5.2.1 Physical activity at 36 y

As described in Chapter 2, a summary value for leisure time physical activity at 36 y was created and used to classify NSHD respondents as either inactive, moderately active or most active, across three investigated activities. These summary statistics are presented in Table 5.4

Table 5.4 1982 leisure time physical activity in NSHD participants at 36 y

	Inactive	Moderately active	Most active
Males *n*(%)	77 (4.7)	1123 (68.6)	438 (26.7)
Females *n*(%)	114 (6.9)	1223 (73.7)	322 (19.4)

In 1982 when cohort members were 36 y data on three leisure time physical activities were available for 3297 individuals, 1638 males and 1659 females. Of the total cohort in 1982, 5.8% were classified as inactive, 71.2% as moderately active and 23.1% as most active. In this group of individuals more females were inactive and less were most active at 36 y than among males. Females had reported more walking and cycling but less participation in DIY / heavy gardening and sports/ recreational activities than males at this age.

5.3 Physical activity at 43 y

Leisure time physical activity data at 43 y was available for 3262 individuals; 1635 males and 1627 females

Table 5.5 1989 leisure time activity in NSHD participants at 43 y

	Inactive	Moderately active	Most active
Males *n*(%)	795 (48.6)	386 (23.6)	454 (27.8)
Females *n*(%)	904 (55.6)	367 (22.6)	356 (21.9)

At age 43 y 52.1% of NSHD participants were classified as inactive, 23.1% as moderately active and 24.8% as most active with the latter participating in sports, vigorous leisure activities or exercise five or more times a month.

5.4 Physical activity at 53 y

In 1999 at 53 y, leisure time physical activity data was available for 2986 NSHD participants; 1466 males and 1520 females

Table 5.6 1999 leisure time activity in NSHD participants at 53 y

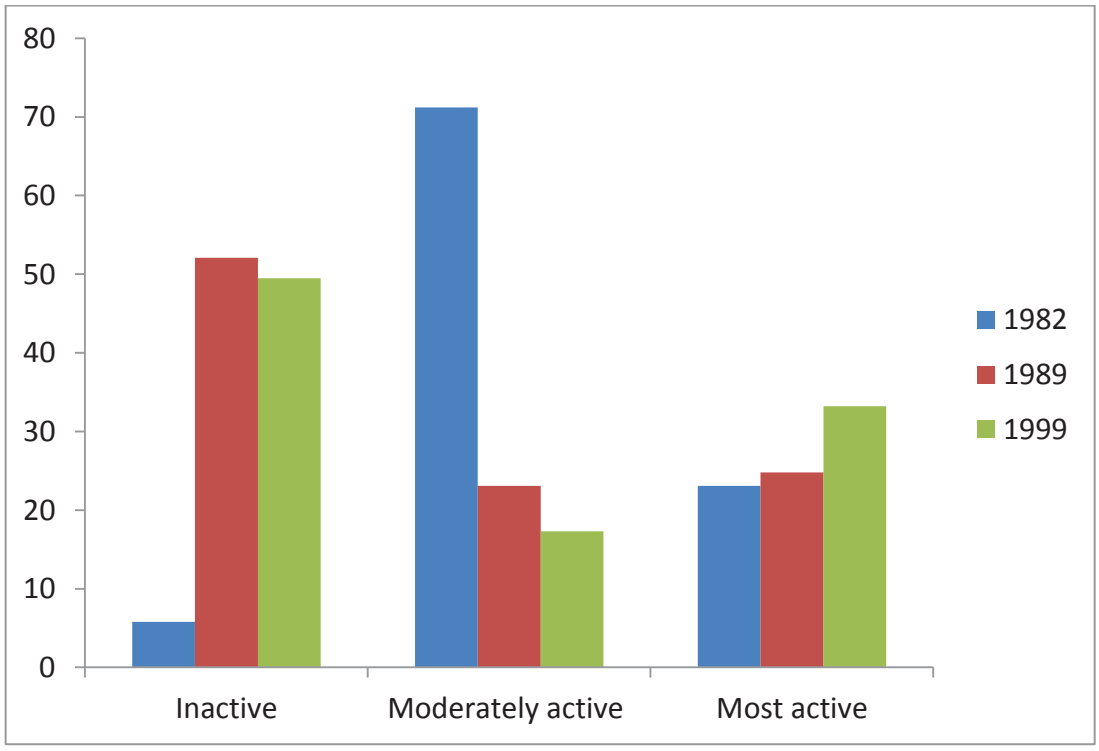
	Inactive	Moderately active	Most active
Males *n*(%)	705 (48.1)	273 (18.6)	488 (33.3)
Females *n*(%)	772 (50.8)	245 (16.1)	503 (33.1)

Of the NSHD participants who provided physical activity data in 1999 49.5% were classified as inactive, 17.3% as moderately active and 33.2% as most active.

5.5 Secular trends in physical activity

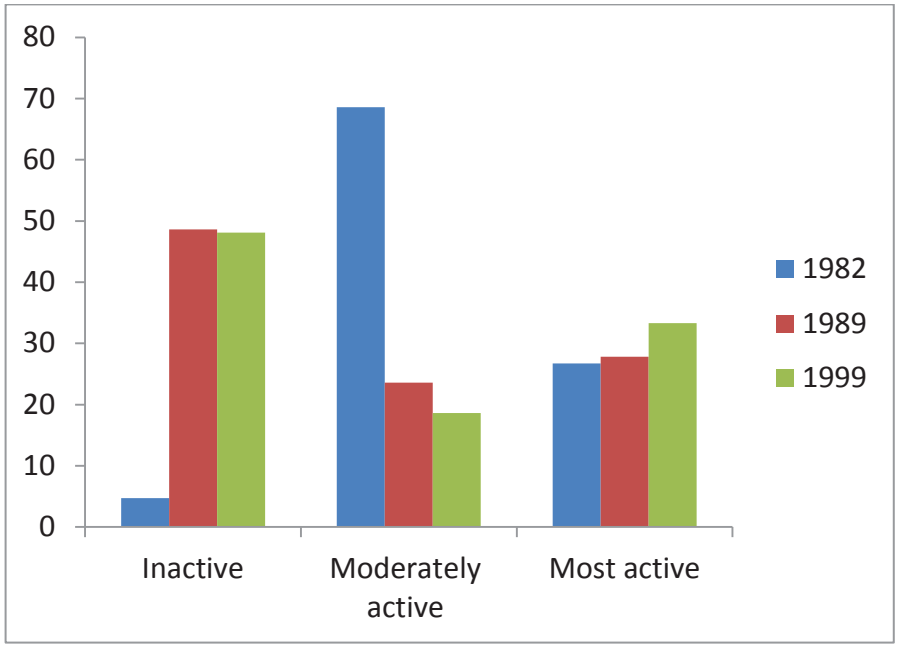
Physical activity data were available in 1982 for 3297 NSHD participants (49.7% were male), in 1989 for 3262 (50% were male) and in 1999 for 2986 (49% were male).

Figure 5.1 The proportion of NSHD participants classified as inactive, moderately active and most active in 1982 (aged 36 y), 1989 (aged 43 y) and 1999 (aged 53 y)



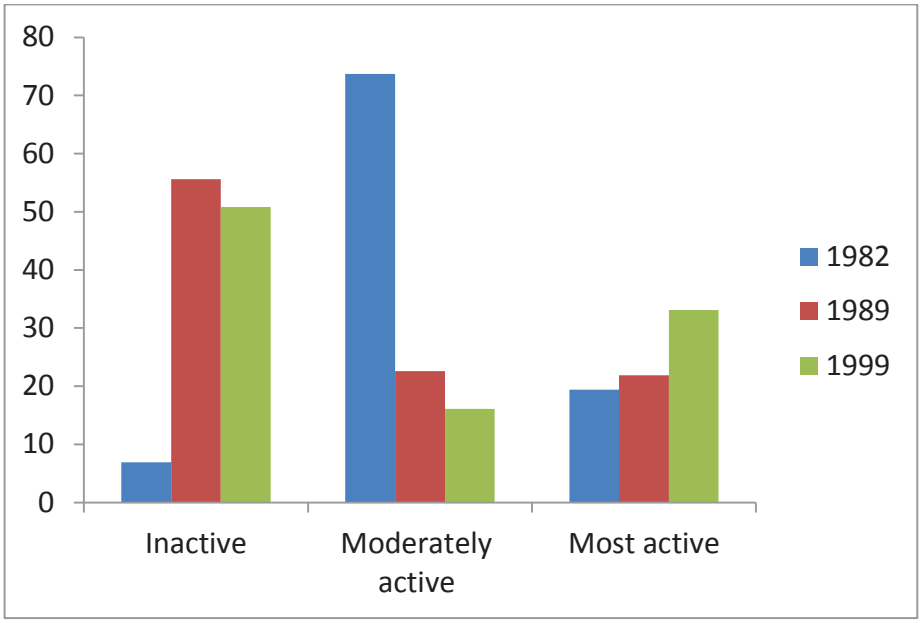
In 1982 only 5.8% of all responders were classified as inactive, this increased dramatically to 52.1% in 1989 and falling slightly in 1999 to 49.5%. Conversely, those classified as moderately active fell from 71.2% of individuals in 1982 to 17.3% in 1999. In comparison, the proportion of individuals classified as most active increased steadily from 23.1% in 1982, to 24.8% in 1989 and 33.2% in 1999 (Figure 5.1).

Figure 5.2 Percentages of male NSHD participants classified as inactive, moderately active and most active in 3 measurement points, 1982 - 1999



The proportion of males classified as inactive increased very markedly between 1982 and 1989 – up from just under 5% to 48.6% in 1989 and then remained stables in 1999 (48.1%). In contrast, those classified as most active increased steadily over this period from 26.7% at age 36 y, to 27.8% at age 43 y and 33.3% at 53 y (Figure 5.2).

Figure 5.3 Percentages of female NSHD participants classified as inactive, moderately active and most active in 3 measurement points, 1982 - 1999



The temporal pattern of change in the proportion of women classified as inactive was similar to that in men (see Figure 5.2) although the proportion of females classified as inactive dropped by nearly 5% in 1999 to 50.8% (Figure 5.3). In all measurement years, the proportion of inactive females was higher than inactive males. As with males, the proportion of most active females increased steadily over the whole measurement period to reach 33.1% in 1999 which is very similar to the proportion of most active males in 1999 (33.3%).

5.6 The adulthood physical activity score

A composite physical activity score (PA score), which reflected habitual, leisure-time physical activity over the measurement periods 1982 – 1999, was determined by the method described in Chapter 2. As described, this score was used to categorise individuals as inactive at all 3 ages, more active, active or most active at all 3 ages. The present analysis considers those NSHD participants who provided physical activity data in all 3 measurement years and such, repeated estimates of adulthood physical activity were available for 2589 individuals, 1252 males and 1337 females.

Table 5.7 Adulthood physical activity in NSHD participants (*n*=2589)

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males *n*(%)	30 (2.4)	529 (42.3)	448 (35.8)	245 (19.6)
Females *n*(%)	54 (4.0)	628 (47.0)	453 (33.9)	202 (15.1)

Pearson Chi-square analysis (without adjustment for multiple testing) revealed that there were significant gender differences in physical activity group membership (χ^2 = 16.7 (3) p=0.001). Averaged across all 3 measurement periods, it is apparent that a higher proportion of males reported being active or most active than did females (Table 5.7).

5.6.1 Physical activity and BMI

Physical activity is an important determinant of energy balance and influences the risk of obesity. The other major determinant is, of course, dietary energy intake. Conversely, those with higher BMI tend to undertake less leisure time physical activity. In addition, obesity is an important modulator of both cardiovascular and musculoskeletal health so, via the effects on BMI, physical activity may influence physical capability in later life.

This analysis focussed on 1023 NSHD participants (459 males and 564 females) who provided dietary data at all three time points, BMI data at all four time points and adulthood physical activity data:

Table 5.8 Adulthood physical activity in NSHD participants who provided BMI (kg/m^2) data at all four time points (*n*=1021)

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males *n*(%)	8 (1.7)	166 (36.2)	192 (41.8)	93 (20.3)
Females *n*(%)	15 (2.7)	230 (40.9)	223 (39.7)	94 (16.7)

In this subset of individuals, approximately 80% of participants were either 'more active' or 'active' at all 3 measurement periods. Just under 2% of males and nearly 3% of females were 'inactive' at all 3 ages, whereas 20.3 and 16.7% of males and females respectively were 'most active' at all ages (Table 5.8). Pearson Chi-square analysis (without adjustment for multiple testing) revealed there was no significant gender difference in physical activity group membership ($\chi^2 = 4.45$ (3) p=0.217).

5.6.1.1 Physical activity and 4 year mean BMI

For both genders, 4 y mean BMI (mean BMI across 4 measurement points in adulthood i.e. 1928, 1989, 1999 and 2006 – 10) was highest for those participants who were inactive at all 3 time-points during adulthood and the overall mean BMI was very similar for males and females, i.e. 27.3 and 27.5 kg/m^2 respectively. In contrast, those who were most active at all ages had the lowest 4 y mean BMI for both genders (Table 5.9).

Table 5.9 Four year mean BMI (kg/m^2) in NSHD participants by adulthood physical activity group averaged across all 3 measurement points

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males (*n)*	27.3 (8)	25.9 (166)	26.2 (192)	25.2 (93)
Females (*n*)	27.5 (15)	25.6 (230)	24.9 (223)	24.3 (94)

Linear regression analysis was used to investigate the relationship between adulthood physical activity score and 4 year mean BMI (kg/m^2). For this purpose, a simple physical activity scoring system was derived which pooled physical activity measurements across 3 measurement time-points to produce a composite physical activity score which ranged from 0 – 6 (see Chapter 2 (Section 2.7.4) for details). Individual physical activity score was used as the independent variable in a linear regression analysis with 4 y mean BMI as the dependent variable. This analysis was undertaken separately for males and females. In females (n=562) adulthood physical activity score was a good predictor of 4 y mean BMI; a one unit increase in the physical activity score was associated with a 0.45 kg/m^2 fall in BMI (p<0.001). In males (n=459) adulthood physical activity score was not a good predictor of 4 y mean BMI (p=0.219).

5.6.1.2 Physical activity and mean BMI at 60 – 64 y

As above, for both genders, mean BMI at age 60 – 64 y was highest for those participants who were inactive at all 3 time-points during adulthood and mean BMI was again very similar for males and females, i.e. 29.4 and 29.8 kg/m^2 respectively. Those who were most active at all ages had the lowest mean BMI at 60 – 64 y (Table 5.10).

Table 5.10 Mean BMI (kg/m^2) at age 60 – 64 y in NSHD participants by adulthood physical activity group averaged across all 3 measurement points

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males (*n)*	29.4 (8)	27.4 (166)	27.8 (192)	26.4 (93)
Females (*n*)	29.8 (15)	27.8 (230)	26.9 (223)	26.1 (94)

Linear regression analysis was used to investigate the relationship between adulthood physical activity score (the independent variable) and mean BMI (kg/m^2) at age 60 – 64 y (the dependent variable), separately for males and females. In females (n=562) a one unit increase in the physical activity score was associated with a 0.53 kg/m^2 reduction in BMI at 60 – 64 y (p<0.001). In males (n=459) the relationship between adulthood physical activity score and BMI at 60 – 64 was not statistically significant (p=0.163).

5.6.2 Adulthood physical activity and abdominal circumference

The distribution of stored body fat has an important effect on health outcomes, in particular, abdominal fat storage is associated with poorer health outcomes especially for metabolic disease including cardiovascular disease. Abdominal (or waist) circumference is a readily-measured surrogate for abdominal adiposity and was used as such in the present analysis.

Table 5.11 Mean abdominal circumference at 60 – 64 y by adulthood physical activity in NSHD participants

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males (*n)*	100.2 (8)	99.9 (166)	100.7 (191)	95.7 (92)
Females (*n*)	98.7 (15)	91.8 (230)	90.4 (223)	86.7 (94)

For females, there was a progressive reduction in abdominal circumference at age 60 – 64 y with increasing physical activity across adulthood with the physically inactive women having, on average, 12 cm greater waist circumference than the most active women (Table 5.11). In contrast, there was very little difference in mean abdominal circumference for men in the inactive, more active and active groups (means ranged from 99.9 – 100.7 cm) whereas those in the most active group had a mean waist circumference which was 4 – 5 cm smaller.

Using the same scoring system described above, linear regression analysis was used to investigate the relationship between adulthood physical activity score and abdominal circumference (cm) at 60 – 64 y. Adulthood physical activity score was a good predictor of abdominal circumference for both genders. In females (n=562) a one unit increase in adulthood physical activity score was associated with a 1.5 cm reduction in abdominal circumference at 60 – 64 y (p<0.001) whereas in males (n=457) it was associated with a 0.95 cm reduction (p=0.004).

5.6.3 Adulthood physical activity and body weight at age 60 – 64 y

Body weight data were available in 2006 – 10 for 2220 individuals, 1062 males and 1158 females.

Table 5.12 Mean body weight (kg) at age 60 – 64 y in NSHD participants by adulthood physical activity group averaged across all 3 measurement points

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males (*n)*	85.5 (17)	84.2 (341)	86.4 (348)	82.6 (185)
Females (*n*)	80 (37)	74.9 (447)	72. 7 (361)	70.1 (166)

As with abdominal circumference there was a progressive decline in body weight at age 60 – 64 y with increasing physical activity across adulthood in females but little difference in mean weight for men in the less active groups; only in the most active group did males have a mean body weight which was approximately 3 kg less (Table 5.12).

Linear regression analysis was used to investigate the relationship between adulthood habitual physical activity and body weight at 60 – 64 y. In females (n=1011) a 1 unit increase in the adulthood physical activity score was associated with a 1.5 kg decrease in body weight at 60 – 64 y (p<0.001). In males (n=891) this relationship was not statistically significant (B = -0.162) (p=0.562).

5.6.4 Adulthood physical activity and body composition

BMI and abdominal circumference are relatively crude measures of adiposity and e.g. cannot distinguish between lean and adipose tissue. The availability of data from DEXA scans (Hologic Inc., Bedford, MA) when NSHD participants were aged 60 – 64 y permits a more detailed investigation of body composition including consideration of the distributions of lean and fatty tissue. In the subset of NSHD participants for whom dietary data in all 3 measurement years and BMI data in four measurement years were available, DEXA-derived body composition data were available as follows: appendicular lean and fat mass (kg) for 768 individuals and whole body fat mass and lean mass (kg) for 739 individuals.

Table 5.13 Mean body composition measures (kg) in NSHD participants at 60 – 64 y by category of adulthood physical activity

	Inactive at all 3 ages	More active	Active	Most active at all 3 ages
Males				
Appendicular lean mass (*n*)	26.4 (4)	24.3 (116)	24.7 (147)	24.6 (79)
Whole body lean mass (*n*)	54.6 (4)	53.1 (114)	53.7 (141)	52.8 (74)
Appendicular fat mass (*n*)	11.3 (4)	9.9 (116)	10.3 (147)	9.5 (79)
Whole body fat mass (*n*)	27.2 (4)	23.5 (114)	24.3 (141)	21.5 (74)
Females				
Appendicular lean mass (*n*)	15.4 (8)	15.9 (162)	16.3 (173)	16.1 (78)
Whole body lean mass (*n*)	36 (8)	36.7 (156)	37.4 (168)	36.8 (74)
Appendicular fat mass (*n*)	16.1 (8)	14.2 (162)	14.0 (173)	13.5 (78)
Whole body fat mass (*n*)	32.6 (8)	28.4 (156)	27.8 (168)	25.9 (74)

Among men, appendicular lean mass (kg) at age 60 – 64 y appeared to be unaffected by physical activity level across adulthood (range 24.3 – 26.4 kg) whereas appendicular lean mass (kg) tended to increase as activity increased for women (Table 5.13). In both genders, both appendicular fat mass (kg) and whole body fat mass (kg) declined progressively with increasing activity across adulthood. The difference in mean whole body fat mass between inactive and most active groups was 5.7 and 6.7 kg for males and females respectively.

Using the scoring system for adulthood physical activity level as described in Chapter 2, linear regression analysis was used to investigate whether physical activity predicted body composition at 60 – 64 y.

Table 5.14 Outcomes of linear regression analyses of relationships between adulthood PA score and measures of body composition (kg) at age 60 – 64 y

Body composition measurement	Number of participants	B	*p*-value
Males			
Mean appendicular lean mass	348	0.090	0.438
Mean whole body lean mass	335	0.054	0.829
Mean appendicular fat mass	348	-0.054	0.593
Mean whole body fat mass	335	-0.304	0.242
Mean body fat percentage	335	-0.329	0.070
Females			
Mean appendicular lean mass	429	0.009	0.901
Mean whole body lean mass	414	-0.046	0.778
Mean appendicular fat mass	429	-0.314	**0.015***
Mean whole body fat mass	414	-0.869	**0.001***
Mean body fat percentage	414	-0.723	**<0.001***

In females, the adulthood physical activity score predicted appendicular and whole body fat mass and body fat percentage at 60 – 64 y. A one unit increase in the physical activity score was associated with a 0.3 kg reduction in appendicular fat mass (p=0.015), a 0.9 kg reduction in whole body fat mass (p=0.001) and a 0.7% reduction in body fat percentage (p<0.001) (Table 5.14). There were no significant (p>0.05) relationships between adulthood physical score and body composition at 60 – 64 y in males.

5.6.5 Adulthood physical activity and physical capability at age 60 – 64 y

Linear regression was used to test for relationships between adulthood leisure time physical activity (as measured by the lifetime physical activity score) and physical capability at 60 – 64 y in a subset of NSHD participants who provided dietary data in all 3 years of measurement.

Table 5.15 Outcomes of linear regression analyses of relationships between adulthood PA score and measures of physical capability at age 60 – 64 y

Physical capability measurement	Number of participants	B	*p*-value
Males			
Chair rise time (s) (*n*)	433	-0.853	**<0.001**
Timed up and go (s) (*n*)	421	-0.100	0.093
Hand grip strength (kg) (*n*)	425	0.829	**0.027**
Females			
Chair rise time (s) (*n*)	537	-1.002	**<0.001**
Timed up and go (s) (*n*)	532	-0.258	**<0.001**
Hand grip strength (kg) (*n*)	527	0.673	**0.001**

Adulthood leisure time physical activity predicted performance in all three physical capability tests administered at 60 – 64 y in females; a 1 unit increase in the score (which ranged from 0 – 6) was associated with a 1 second decrease in chair rise time, a 0.3 s decrease in timed up and go and a 0.7 kg increase in hand grip strength (Table 5.15).

In males, the relationship between physical activity score and physical activity were qualitatively similar to those observed in females. Adulthood physical activity was associated significantly with chair rise time and hand grip strength at 60 – 64 y where a 1 unit increase in PA score was associated with a 0.8 s decrease in chair rise time (a better performance) (p<0.001) and a 0.8 kg increase in hand grip strength (p=0.03). For timed up and go a one unit increase in PA score was associated with better performance, but this effect was not statistically significant (p=0.093).

5.7 Physical capability at age 53 and 60 – 64 y

NSHD cohort members provided various physical capability, anthropometric and metabolic measures during adulthood. Where these values were provided at 2 measurement points, they are shown below. For analysis of variance (Paired-Samples T Test and GLM Repeated Measures (with Bonferroni adjustment)) values were compared when provided by the same individuals (Tables 5.16 and 5.17). For regression analyses, values were merged into the dietary dataset which comprised a smaller subset of NSHD participants who had provided dietary data at all 3 measurement points.

Table 5.16 Chair rise time and hand grip strength in 1999 (at 53 y) and in 2006 – 10 (at 60 – 64 y) in NSHD participants, by gender

Physical capability measurement	Mean value in **1999** at 53 y[11]	Mean value in **2006 – 10** at 60 – 64 y[12]	*p*-value
Males			
Chair rise time (s) (*n*)	21.5 (1357)	24.4 (988)	<0.001 (893)
Hand grip (kg) (*n*)	47.6 (1406)	45.9 (1005)	<0.001 (908)
Females			
Chair rise time (s) (*n*)	22.9 (1400)	25.7 (1074)	<0.001 (992)
Hand grip (kg) (*n*)	27.8 (1444)	26.8 (1064)	<0.001 (988)

For both genders, chair rise time increased (a poorer performance) and hand grip strength declined significantly between 1999, when they were aged 53 y, and 2006 – 10, when they were 60 – 64 y. The increases in chair rise time were much greater for women than for men (24.7 and 11.3% change for women and men respectively) whereas women showed a smaller loss of hand grip strength (2.7 and 3.6% change for women and men respectively) over this approximately 9 year period in mid-adulthood.

[11] Dataset [munro-1.sav]; these individuals may not have provided dietary data
[12] Dataset [munro_nov12.sav]; these individuals may not have provided dietary data

5.8 Abdominal circumference at age 43 and 60 – 64 y

Table 5.17 Mean abdominal circumference (cm) in 1989 (at 43 y) and 2006 – 10 (at 60 – 64 y) in NSHD participants, by gender

Anthropometric measurement	Mean value in **1989**[13] at 43 y	Mean value in 2006 – 10 at 60 – 64 y	*p*-value
Males			
Abdominal circumference (*n*)	91.9 (1609)	100.9 (1061)	<0.001 (987)
Females			
Abdominal circumference (*n*)	77.9 (1613)	92.4 (1156)	<0.001 (1096)

For both genders, abdominal circumference increased significantly between 1989 when they were 43 y and 2006 – 10 when they were 60 – 64 y (Table 5.17). Increases in abdominal circumference were much greater for females than for males (18.6% (14.5 cm) and 9.8% (9 cm) change for women and men respectively) over this approximately 20 y period.

[13] Dataset [munro-1.sav]; these individuals may not have provided dietary data

5.9 Discussion

5.9.1 Physical activity

This chapter examined adulthood habitual leisure time physical activity as measured in 1982, 1989 and 1999 at age 36 y, 43 y and 53 y respectively. Also examined were various measures of physical capability, anthropometry (including body composition) and metabolic biomarkers in NSHD participants between the ages of 43 y and 60 – 64 y. Secular trends and relationships were investigated statistically.

Habitual physical activity is a major determinant of cardiovascular fitness (Wei *et al.*, 1999) and low cardiovascular fitness in combination with overweight/ obesity operate synergistically to influence mortality adversely. Individuals who are more active have lower rates *inter alia* of all-cause mortality, CHD, hypertension, type 2 diabetes and metabolic syndrome and are more likely to maintain a healthy weight and body composition (WHO, 2011).

In the Hertfordshire Cohort Study (Martin *et al.*, 2008), higher levels of self-reported customary physical activity (over the previous 12 months) were associated with better muscle (hand grip) strength and physical performance (3 m walk and chair rise time) in female participants only (mean age 68.2 y). In contrast in NSHD participants, higher adulthood physical activity scores (derived from self-reported leisure activity at ages 36, 43 and 53 y) were positively associated with all 3 tests of physical capability in females and with chair rise time and hand grip strength in males. Methodological differences may explain this finding, including the use of longitudinal data in the present analysis and the fact that NSHD participants were slightly younger when physical capability tests were administered.

In the Age Gene/Environment Susceptibility (AGES) – Reykjavik Study (Chang *et al.*, 2013) regular leisure time physical activity in midlife was associated with better lower extremity function in later life (at 76 y) in men and women. Lower extremity function in that study was determined by performance at gait speed, timed up and go and knee extension. In the present study adulthood physical activity was also associated with a better timed up and go performance at age 60 – 64 y in females, but the association was not significant in males.

Gender differences in health (and mortality) were investigated by (Oksuzyan A, 2013) and explained in terms of 1. biological endowment – the protective effect of oestrogen in women, the greater susceptibility of males to infection and genetic factors; 2. Lifestyle behaviours – risk taking behaviours (e.g. smoking, alcohol consumption) more commonly engaged in by men; and 3. Social roles and health behaviours – the reluctance of some men to report and seek help for illness and infection.

To stay healthy, UK (NHS) physical activity guidelines for adults (19 – 64 y) recommend at least 150 minutes a week of moderately-intensive aerobic activity (fast walking or cycling) combined with muscle-strengthening activities, working all major muscle groups on 2 or more days a week (NHS Choices). This equates to an average of 30 minutes of physical activity per day, 5 d/week. These recommendations are echoed in the WHO 2011 Global Recommendations on Physical Activity for Health for adults aged 18 – 64 y. Only those categorised as most active in terms of the adulthood physical activity score in the present study would have been meeting these recommendations.

A limitation of the present study is that occupational physical activity was not taken into account and lack of data on this potentially important component of daily physical activity may obscure and confound some of the findings reported in the Chapter. For example, there is evidence that individuals with physically demanding occupations are less likely to engage in leisure time physical activity (Kuh, 1992). If this was applied in the NSHD cohort, such individuals would be categorised as inactive which would not be a true reflection of their overall level of habitual physical activity.

Domestic physical activity was also not measured or taken into account in the present study. In the Hertfordshire Cohort Study (Martin *et al.*, 2008) walking and home activity drove the gender difference in higher females median total energy expenditure compared to that of males, i.e. 665.3 vs. 482.7 MET.h/month. When gender differences in subjectively (questionnaire) and objectively (accelerometry) reported physical activity were investigated by (Sun *et al.*, 2013) the difference fell from 0.8 – 21.4% to 0.2 – 1.5%.

Questionnaires on habitual participation in structured leisure time sports and recreational activity, especially where travel to/ from work and occupational activity are excluded do not provide a true reflection of overall physical activity. The advent of new accelerometry technology will provide accurate, objective data on habitual physical activity which may assist in the clarification of relationships between physical activity and long term health.

5.9.2 Physical capability

Trajectories of hand grip strength after the age of 45 y were examined using cross sectional and longitudinal data in 8,342 Danes (46 – 102 y) (Frederiksen *et al.*, 2006). Grip strength was found to decline throughout life and could be described by the formulae 24.38 + 0.38 * height (cm) – 0.59 * age (y) in males and 11.63 + 0.21 * height (cm) = 0.31 * age in females. Using these formulae, hand grip strength in the NSHD cohort at ages 53 and 62 years were predicted and compared with the observed values for hand grip strength (Table 5.18) below

Table 5.18 Mean hand grip strength observed in NSHD participants in 1999 and 2006 – 10 compared with hand grip strength predicted by the (Frederiksen *et al.*, 2006) formulae

	At 53 y		At 62 y	
	Males	Females	Males	Females
Hand grip strength predicted by Frederiksen *et al.*, (*n*)	59.5 (1403)	29.2 (1437)	54.3 (1002)	26.4 (1063)
Hand grip strength in NSHD participants (*n*)	47.6 (1406)	27.8 (1444)	45.9 (1005)	26.8 (1064)

When the (Frederiksen *et al.*, 2006) formulae were applied to NSHD participants at ages 53 y and 60 – 64 y (using median age 62 y), observed hand grip strength was less than predicted in males at both ages. In female NSHD participants, observed hand grip strength was less than predicted age 53 y but slightly more than predicted at 60 – 64 y (Table 5.18). The Danish study population comprised participants of 3 nationwide studies, the Study of Middle-Aged Twins, the Longitudinal Study of Aging Danish Twins and the Danish 1905 Cohort Study. The authors noted that the Danish population may not be completely comparable to other similar populations and the Smedley dynamometer in the Danish cohort was not used in the NSHD.

5.9.3 Abdominal circumference

In the current study, the relationship between adulthood physical activity and abdominal circumference was strongest in females – a 1 unit increase in the PA Score was associated with a 1.5 cm reduction in abdominal circumference at 60 – 64 y. Waist circumference (WC) was highest in females classified as inactive at all 3 ages (98.7 cm) and mean waist circumference in all females at age 60 – 64 y was 92.4 cm. In terms of WHO Guidelines (Waist Circumference and Waist-Hip Ratio, Report of a WHO Expert Consultation) 2011, this places them at a very high disease risk relative to normal weight/ waist circumference. These postmenopausal women may have experienced a redistribution of fat to the abdominal area (menopause transition) and this is associated with increased risk of cardiovascular disease (Toth *et al.*, 2000).

In the Canada Heart Health Surveys (1986 – 1992) the use of waist circumference in overweight and obese women assisted in the identification of those at higher CVD risk (Ardern CI, 2003). Waist circumference was reported to be more predictive of coronary heart disease risk (CHD) in overweight (BMI 25 – 37 kg/m^2) premenopausal women (aged 20 – 45 y) by (Lofgren *et al.*, 2004) who also reported that waist circumference reflected levels of physical activity – a finding consistent with that in the present study where increasing adulthood physical activity was associated with a marked progressive reduction in abdominal circumference at age 60 – 64 y in females. The pattern was much less clear in males. Adulthood physical activity was also significantly, and positively, associated with reductions in whole body and appendicular fat mass and body fat percentage in NSHD females, whereas these relationships were insignificant in males.

The use of waist circumference and BMI was found to be superior to their use as separate indices in predicting risk of cardiometabolic disorder and CVD in over 46,000 Chinese participants (Hou X, 2013). This finding is consistent with that proposed by (Janssen *et al.*, 2004) and (Janssen *et al.*, 2002a). In the former study (of ~15 000 adult participants of the third National Health and Nutrition Examination Survey) waist circumference and not BMI, explained the obesity-related risk of the clustering of hypertension, diabetes, dyslipidaemia and the metabolic syndrome characteristic of abdominal obesity.

In the present study the increase in chair rise time between 1999 (at 53 y) and 2006 – 10 (at median age 62 y) was much greater in females than in males. This gender-dependent slowing in ability to move from a seated to a standing position may be exacerbated by the greater increase in central adiposity, and therefore in mass to be raised, in females. Between the ages of 43 y and 62 y abdominal circumference increased by a mean of 14.5 cm in males compared with 9 cm in males.

CHAPTER 6

Predictors of Physical Capability at 60 – 64 Years

6.1 Introduction

Midlife grip strength (Kuh *et al.*, 2006b) and physical performance (Kuh *et al.*, 2006a) have developmental origins. Birth weight (in 1946) was positively associated with adult grip strength in 2775 NSHD participants at age 53 y after adjustment for adulthood and childhood height and weight (Kuh *et al.*, 2002), and in 2983 participants of the Hertfordshire Cohort Study (born 1931 – 1939) birth weight and height were positively related to grip strength, in males at age 65.7 y and females at age 66.6 y (Robinson *et al.*, 2008). In 4304 participants of the Northern Finland Birth Cohort 1966, birth weight was positively associated with muscle (hand grip) strength and aerobic fitness at age 31 y, independently of adult body mass (p<0.001); whereas greater infant weight gain (between 0 – 1 y) was associated with poorer aerobic fitness (p=0.002)(Ridgway CL, 2009). In a systematic review and meta-analysis of 19 studies, 17 showed a positive association between birth weight and muscle strength. The meta-analysis of 13 studies (Dodds *et al.*, 2012) demonstrated that every additional 1 kg of birth weight was associated with a 0.86 kg increase in hand grip strength, after adjustment for gender and current age and height.

Research in young (age 19 y) (Jensen *et al.*, 2007) and older men (mean age 72.5 y) (Patel *et al.*, 2012) have suggested that an adverse intrauterine environment may negatively influence (or programme) skeletal muscle morphology, contributing to the development of type 2 diabetes and sarcopenia in later life.

Postnatal factors were examined in 2850 participants of the 1946 British birth cohort in whom grip strength was measured at age 53 y; these included birth weight, height and weight "velocities" e.g. rate of weight change between 0 – 7 y, 7 – 15 y and 15 – 53 y, motor milestones (first standing/walking, timing of puberty) and childhood cognitive ability (at ages 8, 11 and 15 y) (Kuh *et al.*, 2006b). After adjustment for potential confounders of midlife grip strength – lifetime social class, current physical activity and health status, birth weight was associated with grip strength at 53 y (p=0.009). Also, in males pubertal (7 – 15 y) weight gain was positively associated with grip strength at 53 y (p<0.001) whereas in females pubertal height gain was most beneficial (p<0.001). Effects of the same postnatal factors were examined in relation to chair rise time (n=2757) and standing balance (n=2784) performance among NSHD participants when aged 53 y (Kuh *et al.*, 2006a). Weight gain < 7 y was beneficial for balance and chair rising in males, hypothesised to reflect muscle growth whereas weight gain in early life (15 – 26 y) was detrimental to performance. In females, pubertal and adult weight gain were detrimental to performance in females – weight gain in adulthood representing gains in fat and not lean mass.

Physical capability at 53 y was poorer among individuals living in disadvantaged socioeconomic conditions, with greater body weight, poorer health status and inactive lifestyles (Kuh *et al.*, 2005). In 2956 NSHD participants, two measures of childhood socioeconomic position (SEP) (mother's educational attainment and father's occupational class) and adulthood socioeconomic position (head of household's occupational) were positively associated with chair rise time but not grip strength at age 53 y (Strand *et al.*, 2011). In a systematic review and meta-analysis of 19 studies, a lower childhood SEP was associated with reductions in grip strength and gait speed and poorer chair rise and standing balance time in adulthood (Birnie *et al.*, 2011a).

After adjustments for age, adulthood SEP and body size, only the association with gait speed (–0.02 m/s) (p=0.015) and chair rises time (+ 3%) (p=0.02) remained significant. SEP in adulthood was a better predictor of physical capability than childhood SEP. The authors hypothesised that growth & early life nutrition influenced the peak level of physical capability attained in early adulthood thereby affecting levels in later life.

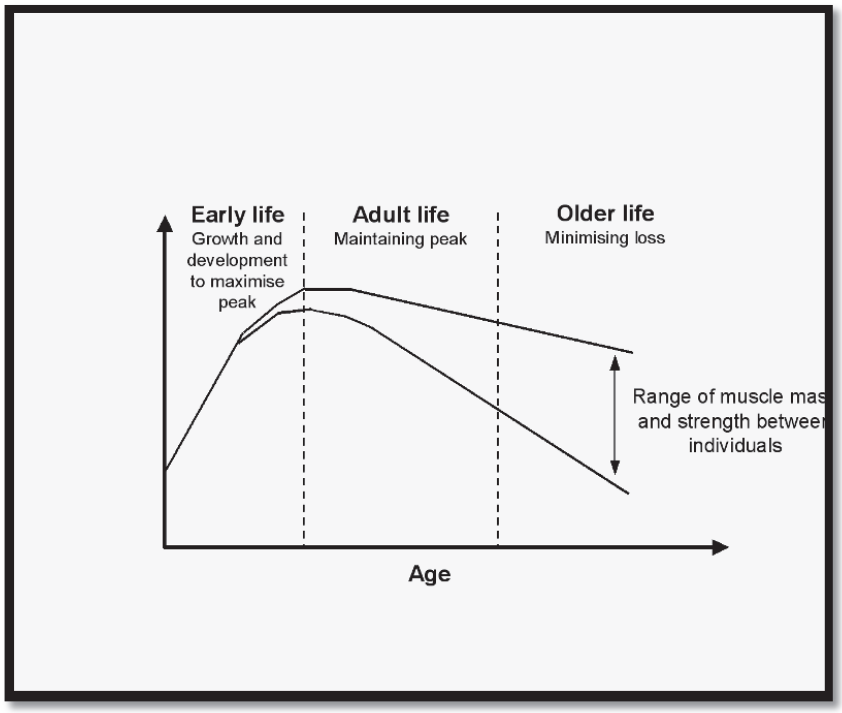
Nutrition, motor development, physical activity and fitness in early life are socioeconomically graded and track into adulthood. In the NSHD, lifecourse area deprivation, individual socioeconomic position and physical capability at 53 y were examined (Murray *et al.*, 2013b). Poorer standing-balance and chair rise time were most strongly associated with current deprivation, but deprivation in midlife was not related to grip strength. Higher area deprivation was associated with poorer dietary habits, less physical activity and higher rates of smoking.

Socio-economic disadvantage over a lifetime (from childhood to adulthood) was significantly associated with gait speed (at 63 – 86 y) in the Boyd Orr and Caerphilly prospective cohorts (Birnie *et al.*, 2011b). At timed up and go, increased educational attainment and duration (per extra year at school) were associated with a 2 – 4% faster gait speed. Lower adulthood SEP, smoking, a greater BMI and history of stroke and angina were associated with slower gait speed. Adjusting for health behaviours (smoking, alcohol and exercise) and diseases in adulthood attenuated the associations, but significance remained (p<0.001). Participants who moved from a low childhood SEP to a high adulthood SEP had a 3% slower gait speed whereas movement in the opposite direction was associated with 5% slower gait; those with low childhood and adulthood SEP had 10% slower gait speed.

Habitual levels of physical activity across adulthood were not associated with grip strength in females (at age 53 y) and in males only physical activity at 53 y was associated with grip strength at 53 y (Cooper *et al.*, 2011b). The joint associations of leisure-time physical activity and BMI on physical and mental capability at age 49.5 y were examined by (Lindholm *et al.*, 2013). After adjustments for age and gender, overweight and physical inactivity jointly contributed to poor physical functioning although weight tended to dominate the association; those who were inactive and overweight were most strongly associated with poor physical functioning.

In 15 longitudinal studies included in a systematic review (Vincent *et al.*, 2010) all except one study reported relationships between adiposity and declining mobility. Chair rise ability was found to be compromised with obesity with obese women at an increased risk for mobility impairment than men. BMI and waist circumference were seen to be emerging as the more consistent predictors of the onset or worsening of mobility disability.

Figure 6.1 A lifecourse model of sarcopenia (Sayer *et al.*, 2008b)



Adult muscle mass and strength is significantly associated with birth weight and childhood/early adulthood growth, and these factors contribute to the peak attained in early adult life (Figure 6.1). However, it is also explained in terms of factors that operate across adulthood (e.g. diet and physical activity) which impact on the rate at which muscle mass and strength is lost (Sayer *et al.*, 2008b).

In 1569 males (mean 65.7 y) and 1414 females (mean age 66.6 y) of the Hertfordshire Cohort Study, relationships between birth weight, diet in the preceding 3 months and grip strength were investigated (Robinson *et al.*, 2008) Using Principal Components Analysis (PCA) a "prudent" diet was characterised and a prudent diet score attributed to each individual. Grip strength was positively related to the prudent diet score, higher scores were associated with higher grip strength. In males and females, the most important food in terms of its association with grip strength was fatty fish, each weekly portion was associated with an additional 0.43 kg and 0.48 kg hand grip strength in males and females, respectively. After selected nutrient intakes were energy-adjusted there were positive associations only with selenium and carotene in males. In females, all selected nutrients (with the exception of vitamin E) were related to grip strength (protein, vitamin C, carotene, selenium and vitamin D) and remained so after intakes were energy-adjusted.

In the transition from independence to disability in older adults (Inzitari *et al.*, 2011) muscle impairment appeared a relevant step in the pathway that linked poor nutrition with functional decline. As muscle quality shows an even greater deterioration than muscle mass, oxidative stress and inflammatory markers may mediate the relationship between nutrition and function in older people. No study has so far has assessed the impact of diet on physical performance decline in older adults, outcomes are often intermediate (e.g. changes in nutritional or anthropometric parameters) and not strong clinical events, e.g. disability.

Among 10,308 participants of the UK Whitehall II Study (Stafford M, 1998) cigarette smoking, lower levels of physical activity and a higher BMI (kg/m^2) were associated with poor physical functioning in males; whereas in females, lower levels of physical activity, an unhealthy or average diet (compared with a healthy diet) and a higher BMI (kg/m^2) were associated with poor physical functioning. All associations were independent of current disease and physical functioning was assessed at 5 year follow up (median age 49 y) by the short-form 36 health survey, which questioned 10 items related to sports and activities of daily living.

The aims of this chapter are to present and explain the results of hierarchical linear regression to predict the determinants of objectively-measured physical capability at 60 – 64 while specifically testing for the effects of adulthood protein intakes (daily and diurnal).

6.1.1 Overview of methodology

In the subset of NSHD participants who provided dietary data in all years, hierarchical linear regression analysis was used to determine the order (or hierarchy) of predictors of performance at three objectively measured physical capability tests; hand grip strength, chair rise and timed up and go at age 60 – 64 y. The variables tested were selected as they were believed *a priori* to be associated with physical capability in later life. Models were split by gender because of the significant gender differences in physical capability at 60 – 64 y: males performed significantly better at hand grip strength (p<0.001), chair rise time (p=0.021) and timed up and go (p=0.015) compared with females (see Table 6.1). The gender difference was most marked for hand grip strength where, on average, grip strength was 73% greater among males than females whereas chair rise time was only 4.5% faster in men.

Table 6.1 Gender differences in physical capability test performance at 60 – 64 y in NSHD participants who provided dietary data in all years

Physical capability test	Mean (n)		p-value
	Male	Female	
Hand grip strength (kg)	46.6 (426)	27 (528)	<0.001
Chair rise time (s)	24.15 (434)	25.3 (538)	0.021
Timed up and go (s)	8.8 (422)	9.2 (533)	0.015

In this subset of individuals, protein intake data were available at age 36, 43 and 53 y and were expressed as g/d, relative to body mass (g/kg/d) and adjusted for daily energy intake, i.e. protein intake as a percentage of total daily energy. Three year means and quintiles of consumption were calculated and three new variables were derived to facilitate comparison of those individuals in the lowest quintile (quintile 1) of mean daily protein consumption with those in the higher quintiles of protein consumption (as described in Chapter 2).

The potential impact of quantity of protein eaten at any meal (or snack) across the day was captured by a novel muscle protein synthesis score (MPSS) which scored consumption of ≥ 20 g protein at any of eight possible eating occasions across the day (see Chapter, Section 2.4 for details). An adulthood MPSS was calculated in those who had provided dietary data in all 3 years and a new variable derived to compare those in quartile 1 of MPSS with those in the higher quartiles of MPSS.

6.1.2 Data transformations and adjustments

DEXA-derived whole body lean mass (kg) and appendicular lean mass (kg) at 60 – 64 y were divided by (adjusted for) height (m) (at 60 – 64)^2.

Gender-specific chair rise time (CRT) and timed up and go (TUG) values were not normally distributed (skewed). They were logarithmically transformed and the e-base logarithm multiplied by 100. Coefficients in these models are therefore interpreted as percentages. Hand grip strength (HGS) values were normally distributed and thus not transformed.

Table 6.2 Assessing the normality of gender-specific physical capability distributions using skewness and excess kurtosis among NSHD participants who provided dietary data in all years (*n*=1263)

Physical capability variable	Skewness (SE)(*n*)		Excess kurtosis (SE)(*n*)	
	M	F	M	F
Hand grip strength	.14 (.118) *n*=426	.30 (.106) *n*=528	.27 (.236) *n*=426	-.08 (.212) *n*=528
Chair rise time	1.2 (.117) *n*=434	2.7 (.105) *n*=538	3.0 (.234) *n*=434	15.4 (.210) *n*=538
Transformed CRT	.058 (.117) *n*=434	.167 (.105) *n*=538	.628 (.234) *n*=434	3.1 (.210) *n*=538
Timed up and go	.95 (.119) *n*=422	3.6 (.106) *n*=533	2.4 (.237) *n*=422	24.5 (.211) *n*=533
Transformed TUG	.16 (.119) *n*=422	.83 (.106) *n*=533	.24 (.237) *n*=422	4.8 (.211) *n*=533

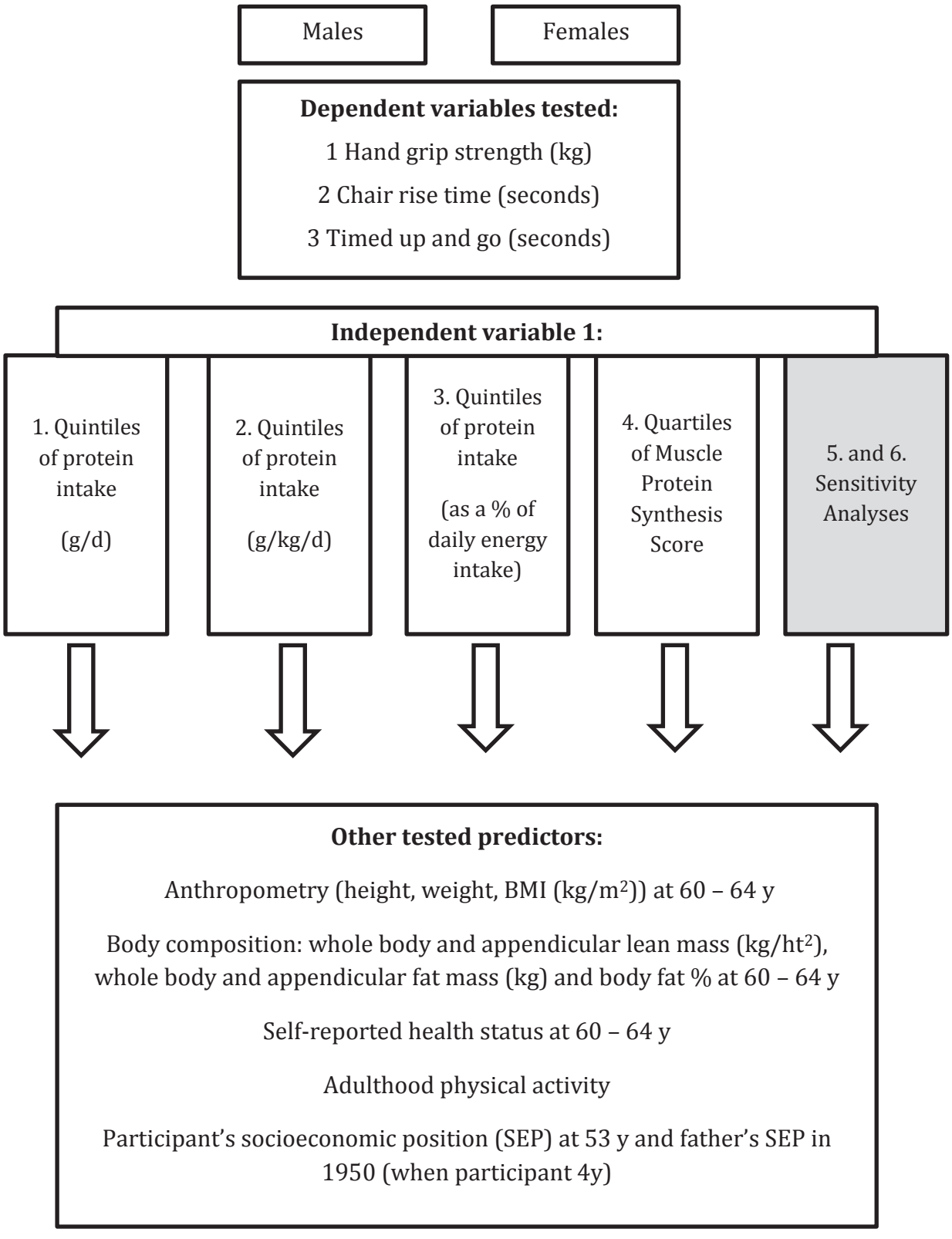
After e-base logarithmic transformation, all values of skewness were < 2 and all values for kurtosis proper (calculated by adding 3 to the value provided by SPSS (above)) were < 7 (Table 6.2).

6.1.3 Sensitivity analyses

Two sensitivity analyses were undertaken to determine the effect of predicted under- and over-reporting on outcomes of regression analyses. For this purpose, new quintiles of 3 y mean protein consumption (expressed as (g/d) and as (g/kg/d)) were calculated after excluding all individuals ever predicted to have under- or over-reported their energy intake. From this, two new variables were derived to compare individuals in quintile 1 with those in the higher quintiles of protein consumption.

In this chapter, the process undertaken to select the order of predictors is described in detail for models 1.1 – 1.6 (sections 6.2.1 – 6.2.6) only. In subsequent models (2 – 6) outcomes of regression analysis only are described since the process of selection of predictors followed exactly the same approach as that described in the first set of models. The structure of this chapter and analytical strategy adopted is shown in Figure 6.1.

Figure 6.2. Analytical strategy and structure of Chapter 6



6.2 Derivation of predictors of physical capability in analysis of impact of quintiles of protein consumption (g/d) using hierarchical linear regression

In the first set of models (6.2.1 – 6.2.6), the protein variable selected as independent variable 1 was quintile 1 versus the other quintiles of 3 y mean protein consumption (g/d). The outcome (dependent) variable first examined was hand grip strength (kg) as measured at 60 – 64 y.

6.2.1 Predictors of hand grip strength in NSHD males

Table 6.3 Determining the hierarchy of predictors of hand grip strength in NSHD males.

Selection of independent variable 2

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06		.450
BMI (kg/m^2)	aBMI_06		.509
Body fat percentage	aFat_Perc	.023	**.006**
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN	.017	**.017**
Whole body fat mass (kg)	aFM_kg		.605
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	.024	**.020**
Adulthood physical activity	_MoreActive _MostActive		.107
Height (m)	ahtn09	**.089***	**<.001**
Appendicular fat mass (kg)	AppFAT_kg		.406
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN	.034	**.001**
Weight (kg)	aWeight2006	.014	**.013**
Participant's SEP at 53 y	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.123
Father's SEP (in 1950 when participant 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.087

The sequential addition of each new predictor gave rise to a R Square (R^2) Change statistic (see Table 6.3 above) which quantified the amount of change in the dependent variable (hand grip strength) that could be explained by the model by the addition of the new predictor. The significance of this R^2 change was calculated using an *F*-ratio ($F = (N - k - 1)R^2 / k(1 - R^2)$) where N is the number of participants and k the number of predictors in the model. In Table 6.3 the significance of this change is shown as a *p*-value. New variables (predictors) that resulted in a significant *F* change ($p<0.05$) were considered first. Of these, only one variable gave rise to a significant ($p<0.001$) *F* change *viz.* height (m). Since height resulted in the greatest R^2 Change (8.9%), this predictor was selected as the second independent variable (after the protein variable).

Table 6.4 Determining the hierarchy of predictors of hand grip strength in NSHD males.

Selection of independent variable 3

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06		.075
BMI (kg/m^2)	aBMI_06		.989
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN	**.032***	**.001**
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN	.020	**.007**
Body fat %	aFat_Perc	.018	**.012**
Whole body fat mass (kg)	aFM_kg		.204
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.154
Self-reported health status at 60 – 64 y	_Good _Fair _Poor		.051
Adulthood PA	_MoreActive _MostActive		.128
Appendicular fat mass (kg)	AppFAT_kg		.057
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.298
Weight (kg)	aWeight2006		.958

After the protein variable and height had been included in the regression model, three variables produced significant (p<0.05) F changes. Since adjusted appendicular lean mass (kg/ht^2) gave rise to the greatest R^2 change (3.2%), this was selected as the third variable (Table 6.4).

Table 6.5 Determining the hierarchy of predictors of hand grip strength in NSHD males.

Selection of independent variable 4

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	p-value
Abdominal circumference (cm)	AbCirc_06	**.054***	**<.001**
BMI (kg/m^2)	aBMI_06	.039	**<.001**
Body fat %	aFat_Perc	.032	**.001**
Whole body fat mass (kg)	aFM_kg	.034	**<.001**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.160
Self-reported health status at 60 – 64 y	_Good _Fair _Poor		.056
Adulthood PA	_MoreActive _MostActive		.241
Appendicular fat mass (kg)	AppFAT_kg	.036	**<.001**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.062
Weight (kg)	aWeight2006	.037	**<.001**

After the protein variable, height and adjusted appendicular lean mass had been included in the regression model, five variables produced significant (p<0.001) F changes; since abdominal circumference gave rise to the greatest R^2 change (5.4%) this was selected as the fourth and final independent variable (Table 6.5). After abdominal circumference had been included in the model, no other tested variable produced a significant (p<0.05) F change.

When there is a strong correlation between two or more predictors in a regression model, this is known as multicollinearity. The Variance Inflation Factor (VIF) indicates when a predictor has a strong linear relationship with another predictor. Field (Field, 2011) suggested that a value of 10 was cause for concern. Variance Inflation Factors were continually monitored for signs of multicollinearity.

Table 6.6 Outcomes of hierarchical linear regression analysis to predict hand grip strength (kg) in NSHD males (*n*=337) at 60 – 64 y (**Model 1.1**)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d)	.807	.027	.589	1.012
Height (m)	53.373	.308	**<.001**	1.028
Adjusted appendicular lean mass (kg/ht^2) at 60 – 64 y	4.620	.376	**<.001**	1.695
Abdominal circumference (cm) at 60 – 64 y	-0.313	-0.305	**<.001**	1.714

In NSHD males, height and adjusted appendicular lean mass (kg/ht^2) were positively associated with hand grip strength at 60 – 64 y; each additional 1 kg in appendicular lean mass was associated with an additional 4.6 kg of hand grip strength (p<0.001). In contrast, abdominal circumference (cm) was negatively associated with grip strength; each additional 1 cm in circumference was associated with 0.3 kg less hand grip strength (p<0.001). Quintiles of protein consumption (g/d) across adulthood were not significantly associated with hand grip strength in males (Table 6.6). This model (with four predictors) explained 16.4% of the variability in hand grip strength at 60 – 64 y in NSHD males who provided dietary data in all years of measurement.

6.2.2 Predictors of hand grip strength in NSHD females

Table 6.7 Determining the hierarchy of predictors of hand grip strength in NSHD females. Selection of independent variable 2

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06		.996
BMI (kg/m^2)	aBMI_06		.758
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN	.017	**.008**
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN		.065
Body fat %	aFat_Perc	.012	**.025**
Whole body fat mass (kg)	aFM_kg		.761
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.479
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	.025	**.005**
Adulthood PA	_MoreActive _MostActive	.024	.002
Height (m)	ahtn09	**.065***	**<.001**
Appendicular fat mass (kg)	AppFAT_kg		.909
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.072
Weight (kg)	aWeight2006	.013	**.008**

In females, only height gave rise to a significant (p<0.001) F change and as height gave rise to the greatest R^2 change (6.5%), it was selected as the second independent variable (after the protein variable) (Table 6.7).

Table 6.8 Determining the hierarchy of predictors of hand grip strength in NSHD females. Selection of independent variable 3

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06		.546
BMI (kg/m^2)	aBMI_06		.321
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN	.018	**.005**
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN	.011	**.028**
Body fat %	aFat_Perc		.075
Whole body fat mass (kg)	aFM_kg		.489
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.535
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	**.026***	**.002**
Adulthood PA	_MoreActive _MostActive	.017	**.008**
Appendicular fat mass (kg)	AppFAT_kg		.446
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.106
Weight (kg)	aWeight2006		.295

After the protein variable and height had been included in the model, four variables produced significant (p<0.05) F changes; adjusted appendicular and whole body lean mass (kg/ht^2), self-reported health status at 60 – 64 y and adulthood physical activity (Table 6.8). As self-reported health status gave rise to the greatest R^2 change (2.6%) it was selected as the third independent variable.

Table 6.9 Determining the hierarchy of predictors of hand grip strength in NSHD females Selection of independent variable 4

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06		.876
BMI (kg/m2)	aBMI_06		.163
Appendicular lean mass (kg) *adjusted for height²*	Adj_AppLEAN	**.016***	**.008**
Whole body lean mass (kg) *adjusted for height²*	Adj_LEAN	.011	**.031**
Body fat %	aFat_Perc		.136
Whole body fat mass (kg)	aFM_kg		.710
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.516
Adulthood PA	_MoreActive _MostActive	.012	**.036**
Appendicular fat mass (kg)	AppFAT_kg		.652
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.114
Weight (kg)	aWeight2006		.143

After the protein variable, height and self-reported health status at 60 – 64 y had been included in the model, three tested variables produced significant *F* changes ($p<0.05$); adjusted appendicular and whole body lean mass (kg/ht²) and adulthood physical activity (Table 6.9). Since adjusted appendicular lean mass gave rise to the greatest R^2 change (1.6%) it was selected as the fourth independent variable.

Table 6.10 Determining the hierarchy of predictors of hand grip strength in NSHD females Selection of independent variable 5

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.031*	**<.001**
BMI (kg/m^2)	aBMI_06	.014	**.012**
Body fat %	aFat_Perc	.015	**.010**
Whole body fat mass (kg)	aFM_kg	.016	**.008**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.429
Adulthood PA	_MoreActive _MostActive		.153
Appendicular fat mass (kg)	AppFAT_kg	.015	**.010**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.489
Weight (kg)	aWeight2006	.013	**.015**

After the protein variable, height, self-reported health status at 60 – 64 y and adjusted appendicular lean mass (kg/ht^2) had been included in the model, only abdominal circumference (cm) resulted in a significant change in the *F* ratio ($p < 0.001$); with an R^2 change of 3.1% abdominal circumference was selected as the fifth and final independent variable (Table 6.10).

Table 6.11 Outcomes of hierarchical linear regression analysis to predict hand grip strength (kg) in NSHD females at 60 – 64 y (**Model 1.2**)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d)	-0.817	-.041	.373	1.016
Height (m)	29.745	.240	**<.001**	1.028
Self-reported health status at 60 – 64 y _Good	-0.531	-.035	.461	1.073
_Fair	-5.630	-.206	**<.001**	1.061
_Poor	-10.377	-.101	**.029**	1.016
Adjusted appendicular lean mass (kg/ht^2)	2.570	.300	**<.001**	1.985
Abdominal circumference (cm)	-0.157	-.251	**<.001**	2.032

This model (with 5 predictors) explained 16.1% of the variability in hand grip strength in NSHD females (n=405) at 60 – 64 y. A fair health status at 60 – 64 y compared with a self-reported health status of excellent/ very good (the reference category) was associated with 5.6 kg less hand grip strength (p<0.001), while a poor health status was associated with 10.4 kg less hand grip strength (p=0.029). Each additional 1 kg of appendicular lean mass was associated with an additional 2.6 kg of hand grip strength whereas each additional 1 cm of abdominal circumference was associated with 0.2 kg less hand grip strength (p<0.001) (Table 6.11).

Females in quintile 1 of mean protein consumption (g/d) across adulthood had approximately 1 kg less hand grip strength compared to those in the higher quintiles of consumption but this difference was not statistically significance (p>0.05).

6.2.3 Predictors of chair rise time in males

The second outcome (dependent) variable examined was performance at the chair rise test (s) at 60 – 64 y. The protein variable selected as the first independent variable was quintile 1 versus all higher quintiles of 3 y mean protein consumption (g/d).

Table 6.12 Determining the hierarchy of predictors of chair rise time in NSHD males.
Selection of independent variable 2

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.059	**<.001**
BMI (kg/m^2)	aBMI_06	.027	**.001**
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		.341
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN	.015	**.028**
Body fat %	aFat_Perc	.014	**.036**
Whole body fat mass (kg)	aFM_kg	.021	**.009**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled	.024	**.046**
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	.106*	**<.001**
Adulthood PA	_MoreActive _MostActive	.042	**<.001**
Height (m)	ahtn09		.703
Appendicular fat mass (kg)	AppFAT_kg	.029	**.002**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled	.029	**.012**
Weight (kg)	aWeight2006	.027	**.001**

In the prediction of chair rise time in males at 60 – 64 y, three variables resulted in a R^2 change which, when tested by *F* ratio, were significant (p<0.001); abdominal circumference (cm), self-reported health status and adulthood physical activity (Table 6.12). Since health status resulted in the greatest R^2 change (10.6%) it was selected as the second independent variable.

Table 6.13 Determining the hierarchy of predictors of chair rise time in NSHD males.

Selection of independent variable 3

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.034*	**<.001**
BMI (kg/m^2)	aBMI_06	.014	**.012**
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		.452
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN		.069
Body fat %	aFat_Perc		.199
Whole body fat mass (kg)	aFM_kg		.062
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.110
Adulthood PA	_MoreActive _MostActive	.024	**.004**
Height (m)	ahtn09		.657
Appendicular fat mass (kg)	AppFAT_kg	.013	**.032**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.057
Weight (kg)	aWeight2006	.015	**.008**

After the protein variable and self-reported health status had been included in the model, only abdominal circumference (cm) produced a significant (p<0.001) R^2 change (3.4%) and was selected as the third independent variable in the model (Table 6.13).

Table 6.14 Determining the hierarchy of predictors of chair rise time in NSHD males – selection of variable 4

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
BMI (kg/m^2)	aBMI_06	.009	**.036**
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		.076
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN		.613
Body fat %	aFat_Perc		.261
Whole body fat mass (kg)	aFM_kg		.127
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.113
Adulthood PA	_MoreActive _MostActive	**.019***	**.010**
Height (m)	ahtn09		.781
Appendicular fat mass (kg)	AppFAT_kg		.517
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.076
Weight (kg)	aWeight2006	.009	**.042**

After abdominal circumference had been included in the model, BMI (kg/m^2), adulthood physical activity and body weight (kg) produced significant *F* changes ($p<0.05$) (Table 6.14). As adulthood PA produced the greatest R^2 change (1.9%), it was selected as the fourth and final independent variable. After physical activity had been included in the model, no other tested variable produced a significant ($p<0.05$) *F* change.

Table 6.15 Outcomes of hierarchical linear regression analysis to predict chair rise time in NSHD males at 60 – 64 y (**Model 1.3**)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d)	-1.511	-.023	.620	1.023
_Good	4.719	.083	.078	1.075
_Fair	22.417	.239	**<.001**	1.090
_Poor	34.208	.159	**.001**	1.019
Abdominal circumference (cm)	.422	.177	**<.001**	1.071
_MoreActive	-5.503	-.106	**.039**	1.255
_MostActive	-9.440	-.149	**.004**	1.262

This model (with 4 predictors) explained 15.9% of the variability in chair rise time in males at 60 – 64 y. Compared with a self-reported health status of excellent/ very good, fair health was associated with a 4.7% poorer performance at chair rise (p<0.001) while poor health was associated with a 34% poorer performance (p=0.001). Increasing abdominal circumference (cm) was also associated with a poorer performance (p<0.001). Adulthood physical activity was positively associated with chair rise time – being 'more active' compared with sedentary was associated with a 5.5% improvement (p=0.039) while being 'most active' was associated with a 9.4% improvement in chair rise performance (p=0.004) (Table 6.15). Quintiles of 3 y mean protein consumption (g/ d) were not significantly predictive of chair rise time in males at 60 – 64 y.

6.2.4 Predictors of chair rise time in females

Table 6.16 Determining the hierarchy of predictors of chair rise time in NSHD females.

Selection of independent variable 2

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.050	**<.001**
BMI (kg/m^2)	aBMI_06	.023	**<.001**
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		.052
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN		.051
Body fat %	aFat_Perc	.012	**.028**
Whole body fat mass (kg)	aFM_kg	.018	**.008**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.110
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	**.099***	**<.001**
Adulthood PA	_MoreActive _MostActive	.032	**<.001**
Height (m)	ahtn09		.053
Appendicular fat mass (kg)	AppFAT_kg	.017	**.008**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.417
Weight (kg)	aWeight2006	.034	**<.001**

In the prediction of chair rise time in females at 60 – 64 y, abdominal circumference (cm), BMI (kg/m^2), self-reported health status and adulthood physical activity resulted in significant R^2 changes (p<0.001) (Table 6.16). Self-reported health status resulted in the greatest R^2 change (9.9%) and was selected as the second independent variable (after the protein variable).

Table 6.17 Determining the hierarchy of predictors of chair rise time in NSHD females.

Selection of independent variable 3

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.036*	**<.001**
BMI (kg/m^2)	aBMI_06		**.002**
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		**.015**
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN		**.048**
Body fat %	aFat_Perc		**.039**
Whole body fat mass (kg)	aFM_kg		**.011**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.184
Adulthood PA	_MoreActive _MostActive		**.010**
Height (m)	ahtn09		**.046**
Appendicular fat mass (kg)	AppFAT_kg		**.004**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.671
Weight (kg)	aWeight2006	.027	**<.001**

After the protein variable and self-reported health status at 60 – 64 y had been included in the model, only abdominal circumference (cm) and body weight (kg) at 60 – 64 y produced significant (p<0.001) R^2 changes. The former was included as the third and final independent variable with an R^2 change of 3.6% (Table 6.17). After abdominal circumference was included in the regression model, no other tested variable produced a significant (p<0.05) F change.

Table 6.18 Outcomes of hierarchical linear regression analysis to predict chair rise time in NSHD females at 60 – 64 y (**Model 1.4**)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d)	-4.165	-.055	.184	1.007
_Good	12.224	.204	**<.001**	1.069
_Fair	19.249	.178	**<.001**	1.057
_Poor	54.300	.166	**<.001**	1.021
Abdominal circumference (cm)	0.465	.192	**<.001**	1.031

In females, this model (with 3 predictors) explained 13.6% of the variability in chair rise time at 60 – 64 y. Declining self-reported health status was significantly associated with a poorer performance in a progressive manner. Compared with individuals who reported being in excellent/very good health, those who reported that their health status was good, fair or poor took significantly longer to complete the chair rise test by 12.2%, 19.2% and 54.3% respectively ($p<0.001$). Abdominal circumference (cm) was also negatively associated with chair rise time performance ($p<0.001$) (Table 6.18). Quintiles of 3 y mean protein consumption (g/d) were not significantly associated with chair rise performance in females at 60 – 64 y.

6.2.5 Predictors of timed up and go in males

The third outcome (dependent) variable examined was performance at timed up and go at 60 – 64 y. The protein variable selected as the first independent variable was quintile 1 versus the other quintiles of 3 y mean protein consumption (g/d).

Table 6.19 Determining the hierarchy of predictors of timed up and go in NSHD males. Selection of independent variable 2

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06		.622
BMI (kg/m^2)	aBMI_06		.555
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		.343
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN		.828
Body fat %	aFat_Perc		.173
Whole body fat mass (kg)	aFM_kg		.277
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.157
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	**.065***	**<.001**
Adulthood physical activity	_MoreActive _MostActive		.177
Height (m)	ahtn09		.129
Appendicular fat mass (kg)	AppFAT_kg		.092
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled	.033	**.008**
Weight (kg)	aWeight2006		.889

In the prediction of timed up and go performance in NSHD males only self-reported health status at 60 – 64 y produced a significant (p<0.001) F change (Table 6.19). After health status had been included in the model, no other tested variable produced a significant (p<0.05) F change.

Table 6.20 Outcome of hierarchical linear regression analysis to predict timed up and go in NSHD males at 60 – 64 y (**Model 1.5**)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d)	-2.977	-.056	.251	1.009
_Good	5.649	.124	**.012**	1.051
_Fair	16.367	.220	**<.001**	1.048
_Poor	24.154	.128	**.009**	1.012

In NSHD males (*n*=407) a health status of good was associated with a 5.6% poorer performance (*p*=0.012) when compared to those who had reported a health status of excellent/very good (the reference category). Fair health was associated with a 16.3% (*p*<0.001) and poor health a 24.2% poorer timed up and go performance (*p*=0.009) (Table 6.20). This model (with two predictors) explained only 6.7% of the variability in timed up and go in NSHD males at 60 – 64 y.

6.2.6 Predictors of timed up and go in females

Table 6.21 Determining the hierarchy of predictors of timed up and go in NSHD females.

Selection of independent variable 2

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.054	**<.001**
BMI (kg/m2)	aBMI_06	.039	**<.001**
Appendicular lean mass (kg) *adjusted for height²*	Adj_AppLEAN		.442
Whole body lean mass (kg) *adjusted for height²*	Adj_LEAN		.256
Body fat %	aFat_Perc	.040	**<.001**
Whole body fat mass (kg)	aFM_kg	.032	**<.001**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		**.018**
Self-reported health status at 60 – 64 y	_Good _Fair _Poor	**.105***	**<.001**
Adulthood physical activity	_MoreActive _MostActive		**.001**
Height (m)	ahtn09		.374
Appendicular fat mass (kg)	AppFAT_kg		**.001**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		**.016**
Weight (kg)	aWeight2006	.032	**<.001**

In the prediction of timed up and go performance in females, six variables produced a significant (p<0.001) F change; abdominal circumference (cm), BMI (kg/m^2), body fat percentage, whole body fat mass (kg), self-reported health status and body weight (kg) (Table 6.21). Self-reported health status resulted in the greatest R^2 change (10.5%) and was selected as the second independent variable.

Table 6.22 Determining the hierarchy of predictors of timed up and go in NSHD females.

Selection of independent variable 3

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
Abdominal circumference (cm)	AbCirc_06	.032*	**<.001**
BMI (kg/m2)	aBMI_06	.024	**<.001**
Appendicular lean mass (kg) *adjusted for height²*	Adj_AppLEAN		.681
Whole body lean mass (kg) *adjusted for height²*	Adj_LEAN		.582
Body fat %	aFat_Perc	.028	**<.001**
Whole body fat mass (kg)	aFM_kg		**.004**
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		**.042**
Adulthood physical activity	_MoreActive _MostActive		**.008**
Height (m)	ahtn09		.445
Appendicular fat mass (kg)	AppFAT_kg		**.006**
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.110
Weight (kg)	aWeight2006		**.001**

After the protein variable and self-reported health status at 60 – 64 y were included in the regression model, three variables produced a significant (p<0.001) F change; abdominal circumference (cm) at 60 – 64 y, BMI (kg/m^2) at 60 – 64 y and body fat percentage at 60 – 64 y (Table 6.22). Of these three, abdominal circumference produced the greatest R^2 change (3.2%) and was selected as the third independent variable.

Table 6.23 Determining the hierarchy of predictors of timed up and go in NSHD females.

Selection of independent variable 4

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
BMI (kg/m^2)	aBMI_06		.876
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN	.016	**.005**
Whole body lean mass (kg) *adjusted for height^2*	Adj_LEAN	**.018***	**.004**
Body fat %	aFat_Perc		.079
Whole body fat mass (kg)	aFM_kg		.942
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.062
Adulthood physical activity	_MoreActive _MostActive		**.033**
Height (m)	ahtn09		.262
Appendicular fat mass (kg)	AppFAT_kg		.613
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.167
Weight (kg)	aWeight2006		.504

In selecting the fourth independent variable, there was very little to differentiate between adjusted whole body lean mass (kg/ht^2) and appendicular lean mass (kg/ht^2) in females (Table 6.23). Adjusted whole body lean mass was selected as the fourth independent variable as it resulted in the greatest R^2 change (p=0.004).

Table 6.24 Determining the hierarchy of predictors of timed up and go in NSHD females.

Selection of independent variable 5

Variable Name	Variable code	Model Summary Change Statistics	
		R^2 Change	*p*-value
BMI (kg/m^2)	aBMI_06		.695
Appendicular lean mass (kg) *adjusted for height^2*	Adj_AppLEAN		.628
Body fat %	aFat_Perc		.310
Whole body fat mass (kg)	aFM_kg		.700
Father's SEP (at 4 y)	FSC_IIINM FSC_IIIM FSC_Partly FSC_Unskilled		.292
Adulthood physical activity	_MoreActive _MostActive	**.018***	**.015**
Height (m)	ahtn09		.242
Appendicular fat mass (kg)	AppFAT_kg		.382
SEP (at 53 y)	SEP_IIINM SEP_IIIM SEP_Partly SEP_Unskilled		.131
Weight (kg)	aWeight2006		.529

After the protein variable (quintiles of protein intake (g/d)), self-reported health status, abdominal circumference (cm) and adjusted whole body lean mass (kg/ht^2) at 60 – 64 y had been included in the regression model, only adulthood physical activity produced a significant (p<0.05) F change (Table 6.24). Once this variable was included in the regression model, no other tested variable produced a significant F change.

Table 6.25 Outcomes of hierarchical linear regression analysis to predict timed up and go in NSHD females at 60 – 64 y (**Model 1.6**)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d)	6.177	.107	**.021**	1.024
_Good	3.699	.084	.077	1.079
_Fair	22.090	.277	**<.001**	1.069
_Poor	24.702	.103	**.027**	1.042
Abdominal circumference (cm)	.526	.295	**<.001**	2.328
Adjusted whole body lean mass (kg/ht^2)	-2.227	-.190	**.006**	2.228
_MoreActive	-5.881	-.139	**.006**	1.217
_MostActive	-5.297	-.099	.051	1.235

This model (with 5 predictors) explained 19.2% of the inter-individual variation in timed up and go performance in NSHD females (n=397) at 60 – 64 y. For this measure of physical capability, quintiles of protein consumption (g/d) were predictive of performance (p=0.021). On average, those in quintile 1 of mean protein consumption (g/d) across adulthood took 6.2% longer to complete the task (Table 6.25).

A declining self-reported health status was associated with a poorer performance, females who declared themselves to be in poor health took 24.7% longer to complete the task compared with females in excellent/very good health (p=0.027). Increasing abdominal circumference (cm) was negatively associated with performance (p<0.001) while increasing appendicular lean mass (kg/ht^2) and adulthood physical activity were positively (p=0.006) associated with timed up and go performance at 60 – 64 y.

6.3 Summary of all model outcomes

All regression models tested the same three dependent variables, i.e. hand grip strength, chair rise time and timed up and go at 60 – 64 y. The difference between the models was the first independent variable, the protein variable, which compared those in quintile 1 (or quartile 1 for the muscle protein synthesis score) of mean protein consumption across adulthood with those in higher quintiles (or quartiles) of consumption. Protein intake across adulthood i.e. quantified in 1982, 1989 and 1999 was expressed in several ways, detailed in Table 6.26

Table 6.26 Models and first independent variable (the protein variable)

Models	First independent variable and how it was determined
1.1 – 1.6	Quintile 1 of 3 y mean protein consumption compared with higher quintiles of consumption (g/d)
2.1 – 2.6	Quintile 1 of 3 y mean protein consumption compared with higher quintiles of consumption (g/kg/d)
3.1 – 3.6	Quintile 1 of 3 y mean protein consumption compared with higher quintiles of consumption, daily protein as a percentage of total energy (%TE)
4.1 – 4.6	Quartile 1 of muscle protein synthesis score compared with higher quartiles of MPSS
5.1 – 5.6	Sensitivity analysis 1: Quintile 1 of 3 y mean protein consumption compared with higher quintiles of consumption (g/d) excluding predicted misreporters
6.1 – 6.6	Sensitivity analysis 2: Quintile 1 of 3 y mean protein consumption compared with higher quintiles of consumption (g/kg/d) excluding predicted misreporters

There was considerable homogeneity in the outcomes of the regression analyses for all 6 modes of expression of protein intake for any one of the 3 dependent variables (hand grip strength, chair rise time and timed up and go). To facilitate comparisons between effects of measures of protein intake, the results for each of the 3 measures of physical capability are presented separately in Tables 6.27 – 6.32, by gender. Only significant coefficients are reported. All outcomes of hierarchical linear regression analyses (all models) are in the appendices to this Chapter (Tables 6.33 – 6.62).

6.3.1 Predictors of hand grip strength in males – all models

Table 6.27 Predictors of hand grip strength (kg) at 60 – 64 y in NSHD males who provided dietary data in all years. Outcomes of hierarchical linear regression analyses investigating different measures of protein intake

Model and first independent variable	**Hierarchy of Predictors**	**B**	***p*-value**	**Model R^2**
1.1 Quintiles of protein intake (g/d)	Height (m)	53.4	<.001	16.4%
	Adjusted appendicular lean mass (kg/ht^2)	4.6	<.001	
	Abdominal circumference (cm)	-0.3	<.001	
2.1 Quintiles of protein intake (g/kg/d)	Height (m)	53.2	<.001	16.7%
	Adjusted appendicular lean mass (kg/ht^2)	4.6	<.001	
	Abdominal circumference (cm)	-0.3	<.001	
3.1 Quintiles of protein intake (as a proportion of daily energy intake)	Height (m)	53.0	<.001	16.4%
	Adjusted appendicular lean mass (kg/ht^2)	4.6	<.001	
	Abdominal circumference (cm)	-0.3	<.001	
4.1 Quartiles of diurnal protein intake (MPSS)	Height (m)	53.2	<.001	16.4%
	Adjusted appendicular lean mass (kg/ht^2)	4.6	<.001	
	Abdominal circumference (cm)	-0.3	<.001	
5.1 Quintiles of protein intake (g/d) excluding predicted misreporters	Height (m)	66.5	<.001	24%
	Adjusted whole body lean mass (kg/ht^2)	2.8	<.001	
	Abdominal circumference (cm)	-0.3	<.001	
6.1 Quintiles of protein intake (g/kg/d) excluding predicted misreporters	Height (m)	64.0	<.001	23.2%
	Adjusted whole body lean mass (kg/ht^2)	2.7	<.001	
	Abdominal circumference (cm)	-0.3	.003	

Regardless of how protein intake was expressed, there was no evidence that protein consumption across adulthood was a significant predictor of hand grip strength in males at age 60 – 64 y (Table 6.27). Height was consistently the most predictive of hand grip strength in all regression models. Also common to all models was abdominal circumference – each additional 1 cm was consistently associated with 0.3 kg less hand grip strength at 60 – 64 y.

In models 1.1 – 4.1 (excluding the sensitivity analyses) adjusted appendicular lean mass (kg/ht^2) was consistently predictive of hand grip strength at 60 – 64 y; each additional 1 kg was associated with an additional 4.6 kg (p=0.001). These first four models explained 16.4 – 16.7% of the variability in hand grip strength in NSHD males at 60 – 64 y.

The sensitivity analyses (models 5.1 and 6.1) were conducted in 174 men who provided apparently reliable dietary data in all years of measurement and who were predicted never to have under- or over-reported their energy intakes. In this subset of males, adjusted whole body lean mass (kg) and not appendicular lean mass (kg) was predictive of hand grip strength (after height); each additional 1 kg was associated with an additional 2.7/2.8 kg hand grip strength (p<0.001). Increases in adjusted whole body lean mass, predictive of performance in the sensitivity analyses, were associated with a smaller increase in hand grip strength (2.7/2.8 kg) than those associated with increases in adjusted appendicular lean mass (4.6 kg) in models 1.1 – 4.1. Models conducted as sensitivity analyses explained more of the variability (23.2/24%) in hand grip strength than any of the other analyses (which included all males who provided dietary data in all measurement years).

6.3.2 Predictors of hand grip strength in females – all models

Table 6.28 Predictors of hand grip strength (kg) at 60 – 64 y in NSHD females who provided dietary data in all years. Outcomes of hierarchical linear regression analyses investigating different measures of protein intake

Model and first independent variable	**Hierarchy of predictors**	**B**	***p*-value**	**Model R^2**
1.2 Quintiles of protein intake (g/d)	Height (m)	29.7	<.001	16.1%
	Fair health status	-5.6	<.001	
	Poor health status	-10.4	.029	
	Adjusted appendicular lean mass (kg/ht^2)	2.6	<.001	
	Abdominal circumference (cm)	-0.2	<.001	
2.2 Quintiles of protein intake (g/kg/d)	Height (m)	30.0	<.001	15.9%
	Fair health status	-5.6	<.001	
	Poor health status	-10.6	.026	
	Adjusted appendicular lean mass (kg/ht^2)	2.6	<.001	
	Abdominal circumference (cm)	-0.2	<.001	
3.2 Quintiles of protein intake (as a proportion of daily energy)	Height (m)	30.2	<.001	16.2%
	Fair health status	-5.5	<.001	
	Poor health status	-11.0	.021	
	Adjusted appendicular lean mass (kg/ht^2)	2.6	<.001	
	Abdominal circumference (cm)	-0.2	<.001	
4.2 Quartiles of diurnal protein intake (MPSS)	Height (m)	31.0	<.001	16.5%
	Fair health status	-5.6	<.001	
	Poor health status	-11.3	.017	
	Adjusted appendicular lean mass (kg/ht^2)	2.6	<.001	
	Abdominal circumference (cm)	-0.2	<.001	
5.2 Quintiles of protein intake (g/d) excluding predicted misreporters	Height (m)	38.0	<.001	17.2%
	Adjusted appendicular lean mass (kg/ht^2)	3.7	<.001	
	Abdominal circumference (cm)	-0.2	<.001	
6.2 Quintiles of protein intake (g/kg/d) excluding predicted misreporters	Height (m)	37.0	<.001	17.5%
	Adjusted appendicular lean mass (kg/ht^2)	3.5	<.001	
	Abdominal circumference (cm)	-0.2	<.001	

As with males, height was consistently and significantly the most predictive of hand grip strength in females (Table 6.28). Abdominal circumference was consistently negatively associated with performance at 60 – 64 y, each additional 1 cm of circumference was associated with 0.2 kg less hand grip strength (p<.001).

After height (in models 1.2 – 4.2 only, excluding the sensitivity analyses) a 'fair' self-reported health status (compared with a health status of excellent/very good) was consistently associated with 5.5/5.6 kg less hand grip strength (p<0.001) and 'poor' health with 10.4 – 11.3 kg less hand grip strength (p<0.05).

Adjusted appendicular lean mass (kg/ht^2) was common to all regression models in NSHD females. In models 1.2 – 4.2 each additional 1 kg/ht^2 was associated with 2.6 kg greater hand grip strength (p<.001) whereas in the sensitivity analyses (models 5.2 and 6.2) each additional 1 kg/ht^2 of appendicular lean mass was associated with 3.7 kg and 3.5 kg greater hand grip strength, respectively (p<.001).

The sensitivity analyses (models 5.2 and 6.2) were each conducted in 209 females who provided apparently reliable dietary data in all years of measurement and who were predicted never to have under- or over-reported their energy intakes. In these models self-reported health status at 60 – 64 y was not predictive of hand grip strength.

6.3.3 Predictors of chair rise time in males – all models

Table 6.29 Predictors of chair rise time at 60 – 64 y in NSHD males who provided dietary data in all years. Outcomes of hierarchical linear regression analyses investigating different measures of protein intake

Model and first independent variable	**Hierarchy of Predictors**	**B (%)**	***p*-value**	**Model R^2**
1.3 Quintiles of protein intake (g/d)	Fair health status	22.4	<.001	15.9%
	Poor health status	34.2	.001	
	Abdominal circumference (cm)	0.4	<.001	
	Adulthood PA_MoreActive	-5.5	.039	
	Adulthood PA_MostActive	-9.4	.004	
2.3 Quintiles of protein intake (g/kg/d)	Quintiles of protein intake (g/kg/d)	-6.6	.033	16.7%
	Fair health status	22.2	<.001	
	Poor health status	35.5	<.001	
	Abdominal circumference (cm)	0.5	<.001	
	Adulthood PA_MoreActive	-5.3	.043	
	Adulthood PA_MostActive	-9.7	.003	
3.3 Quintiles of protein intake (as a proportion of daily energy)	Fair health status	22.4	<.001	15.8%
	Poor health status	34.0	.001	
	Abdominal circumference (cm)	0.4	<.001	
	Adulthood PA_MoreActive	-5.5	.039	
	Adulthood PA_MostActive	-9.5	.004	
4.3 Quartiles of diurnal protein intake (MPSS)	Fair health status	22.3	<.001	15.8%
	Poor health status	34.0	.001	
	Abdominal circumference (cm)	0.4	<.001	
	Adulthood PA_MoreActive	-5.6	.036	
	Adulthood PA_MostActive	-9.4	.004	
5.3 Quintiles of protein intake (g/d) excluding predicted misreporters	Fair health status	17.1	.016	11.6%
	Adulthood PA_MoreActive	-9.7	.013	
	Abdominal circumference (cm)	0.4	.039	
6.3 Quintiles of protein intake (g/kg/d) excluding predicted misreporters	Quintiles of protein intake (g/kg/d)	9.8	.019	8.0%
	Fair health status	21.0	.004	
	Poor health status	37.0	.038	

Quintiles of protein intake when expressed relative to body mass (model 2.3) were significantly predictive of chair rise performance in males at 60 – 64 y; however, quintile 1 of mean protein consumption (g/kg/d) was associated with a *better* (6.6% faster) chair rise time (p=0.033). After excluding predicted energy misreporters (model 6.3) the direction of the association changed from being negative to positive i.e. quintile 1 of protein consumption (g/kg/d) was associated with a 9.8% *poorer* performance at this test (p=0.019) (Table 6.29).

In the first four regression models (1.3 – 4.3) a 'fair' health status (compared with excellent/very good) was consistently associated with a 22.2 – 22.4% poorer performance at chair rising (p<0.001) and 'poor' health with a 34.0 – 35.5% poorer performance (p=0.001). In the first sensitivity analysis (model 5.3) only a 'fair' health status was predictive of a poorer (17.1%) performance. In the second sensitivity analysis, in which protein was expressed relative to body mass (g/kg/d) and predicted energy misreporters were excluded, a fair and a poor self-reported health status were both predictive of a poorer chair rise performance – poor health cf. excellent/very good was associated with a 37% poorer performance (p=0.038) in males.

With the exception of model 6.3, increasing abdominal circumference was consistently associated with a poorer chair rise performance, each additional 1 cm of circumference was associated with an additional 0.4/0.5% chair rise time (p<0.001).

In models 1.3 – 4.3 (excluding the sensitivity analyses) adulthood physical activity was positively associated with chair rising; compared to those who were 'sedentary' throughout adulthood, being 'more active' was consistently associated with a 5.3 – 5.6% better performance, whereas being 'most active' was associated with a 9.4 – 9.7% better performance (p<0.05). In the first sensitivity analysis (model 5.3), conducted in 215 males who provided apparently reliable dietary data the effect of being 'more active' compared with being sedentary, was associated with a 9.7% better chair rise time (p=0.013). In the second sensitivity analysis (model 6.3) conducted in 216 males who provided apparently reliable data, only quintiles of protein consumption (g/kg/d) and self-reported health status were predictive of chair rise time in males.

6.3.4 Predictors of chair rise time in females – all models

Table 6.30 Predictors of chair rise time at 60 – 64 y in NSHD females who provided dietary data in all years. Outcomes of hierarchical linear regression analyses investigating different measures of protein intake

Model and first independent variable	**Hierarchy of Predictors**	**B (%)**	***p*-value**	**Model R^2**
1.4 Protein quintiles (g/d)	Good health status	12.2	<.001	13.6%
	Fair health status	19.2	<.001	
	Poor health status	54.3	<.001	
	Abdominal circumference (cm)	0.5	<.001	
2.4 Protein quintiles (g/kg/d)	Good health status	11.0	<.001	15.1%
	Fair health status	19.0	<.001	
	Poor health status	52.0	<.001	
	Abdominal circumference (cm)	0.4	<.001	
	Height (m)	46.8	.021	
	Adulthood PA_MostActive	-8.7	.012	
3.4 Protein quintiles (as a proportion of daily energy)	Good health status	11.0	<.001	15.2%
	Fair health status	19.0	<.001	
	Poor health status	51.0	<.001	
	Abdominal circumference (cm)	0.4	<.001	
	Height (m)	46.0	.022	
	Adulthood PA_MostActive	-8.8	.011	
3.4 Quartiles of MPSS	Good health status	11.0	<.001	15.1%
	Fair health status	19.0	<.001	
	Poor health status	52.2	<.001	
	Abdominal circumference (cm)	0.4	<.001	
	Height (m)	46.0	.023	
	Adulthood PA_MostActive	-8.7	.012	
5.4 Protein quintiles (g/d) excluding predicted misreporters	Abdominal circumference (cm)	0.7	<.001	11.5%
	Fair health status	21.6	.004	
6.4 Protein quintiles (g/kg/d) excluding predicted misreporters	Abdominal circumference (cm)	0.7	<.001	11.2%
	Fair health status	22.1	.004	

In regression models 1.4 – 3.4 (excluding the sensitivity analyses) self-reported health status at 60 – 64 y was most predictive of chair rise time in females at 60 – 64 y and a declining health was consistently associated with a poorer performance (Table 6.30).

Compared to those declaring themselves in excellent/very good health, females declaring their health to be 'good', 'fair' or 'poor' took significantly longer to complete the task; i.e. 11 – 12%, 19% and 51 – 54.3% longer (p<0.001), respectively.

Increasing abdominal circumference (cm) was consistently negatively associated with chair rise performance, each additional 1 cm of circumference was associated with a 0.4/0.5% poorer performance (p<0.001). These first four regression models explained 13.6 – 15.1% of the variability in chair rise time in NSHD females at 60 – 64 y.

The sensitivity analyses (models 5.4 and 6.4) were each conducted in 258 females who provided apparently reliable dietary data in all years of measurement. In these models, increasing abdominal circumference (each additional 1 cm) was most predictive of chair rise time; each additional 1 cm of circumference was associated with a 0.7% poorer performance (compared with 0.4/0.5% in the first four models, unadjusted for predicted misreporters) (p<0.001). Only a 'fair' health status, compared with excellent/very good, was predictive of performance and was associated with a 21.6/22.1% poorer performance (p=.004) in females. There was no significant difference in chair rise time between those who reported excellent/very good health and those who reported 'good' or 'poor' health. When predicted misreporters were excluded from the analyses, regression models explained less (11.2 – 11.5%) of the variability in chair rise time in females at 60 – 64 y.

6.3.5 Predictors of timed up and go in males – all models

Table 6.31 Predictors of timed up and go performance (s) at 60 – 64 y in NSHD males who provided dietary data in all years. Outcomes of hierarchical linear regression analyses investigating different measures of protein intake

Model and first independent variable	**Hierarchy of Predictors**	**B (%)**	***p*-value**	**Model R^2**
1.5 Quintiles of protein intake (g/d)	Good health status	5.6	.012	6.7%
	Fair health status	16.4	<.001	
	Poor health status	24.2	.009	
2.5 Quintiles of protein (g/kg/d)	Good health status	5.8	.010	6.8%
	Fair health status	16.4	<.001	
	Poor health status	24.3	.008	
3.5 Quintiles of protein intake (as a proportion of daily energy)	Good health status	5.7	.011	6.4%
	Fair health status	16.1	<.001	
	Poor health status	23.5	.011	
4.5 Quartiles of diurnal protein intake (MPSS)	Good health status	5.8	.010	6.5%
	Fair health status	16.8	<.001	
	Poor health status	24.2	.009	
5.5 Quintiles of protein intake (g/d) excluding predicted misreporters	SEP IV (at 53 y) (partly skilled)	14.4	.010	5%
6.5 Quintiles of protein intake (g/kg/d) excluding predicted misreporters	Quintiles of protein intake (g/kg/d)	7.4	.035	6.5%
	Father's SEP V (when participant 4 y) (unskilled)	13.4	.040	

In the first four regression analyses (models 1.5 – 4.5) only self-reported health status was predictive of timed up and go performance in males at 60 – 64 y (Table 6.31). Compared to those in excellent/very good health, males declaring their health to be good took ~6% longer to complete the task. A fair health status was associated with a 16 – 16.8% poorer performance (p<0.001) and poor health, a 24% poorer performance. These four models explained 6.4 – 6.8% of the variability in timed up and go in males at 60 – 64 y.

In the first of the sensitivity analyses (model 5.5) conducted in 213 males who provided apparently reliable dietary data in all years of measurement, only socioeconomic position at 53 y was predictive of performance at time up and go at 60 – 64 y. Compared with SEP I/II (professional/intermediate), SEP IV (partly unskilled) was associated with a 14.4% poorer performance (p=0.010). In the second sensitivity analysis, also conducted in (n=213) males, quintiles of protein consumption when expressed relative to body mass (g/kg/d) were significantly associated with timed up and go performance at 60 – 64 y. Compared with those reporting higher intakes of protein, quintile 1 was associated with a 7.4% poorer performance (p=0.035). Also predictive of performance was father's SEP (when participant was 4 y). Compared with father's SEP I/II (professional/intermediate), father's SEP V (unskilled) was associated with a 13.4% poorer performance (p=0.040) in male participants at 60 – 64 y.

6.3.6 Predictors of timed up and go in females – all models

Table 6.32 Predictors of timed up and go performance (s) at 60 – 64 y in NSHD females who provided dietary data in all years. Outcomes of hierarchical linear regression analyses investigating different measures of protein intake

Model and first independent variable	**Hierarchy of Predictors**	**B (%)**	***p*-value**	**Model R^2**
1.6 Quintiles of protein intake (g/d)	Quintiles of protein intake (g/d)	6.2	.021	19.2%
	Fair health status	22.0	<.001	
	Poor health status	25.0	.027	
	Abdominal circumference (cm)	0.5	<.001	
	Whole body lean mass (kg/ht^2)	-2.2	.006	
	Adulthood PA_MoreActive	-6.0	.006	
2.6 Quintiles of protein intake (g/kg/d)	Good health status	4.6	.030	16.7%
	Fair health status	23.3	<.001	
	Poor health status	28.0	.013	
	Body fat percentage (%)	0.6	.002	
	Adulthood PA_MoreActive	-5.8	.008	
	Adulthood PA_MostActive	-5.7	.038	
3.6 Quintiles of protein intake (as a proportion of daily energy)	Fair health status	22.2	<.001	18.7%
	Poor health status	26.0	.023	
	Abdominal circumference (cm)	0.5	<.001	
	Whole body lean mass (kg/ht^2)	-2.2	.007	
	Adulthood PA_MoreActive	-6.0	.006	
	Adulthood PA_MostActive	-5.9	.031	
4.6 Quartiles of diurnal protein intake (MPSS)	Fair health status	22.0	<.001	18.1%
	Poor health status	27.0	.016	
	Abdominal circumference (cm)	0.5	<.001	
	Whole body lean mass (kg/ht^2)	-2.3	.005	
	Adulthood PA_MoreActive	-6.2	.004	
	Adulthood PA_MostActive	-5.6	.038	
5.6 Quintiles of protein intake (g/d) excluding predicted misreporters	Fair health status	16.5	.012	15.4%
	Body fat percentage (%)	0.9	.001	
	SEP V at 53 y (Unskilled)	28.0	.002	
6.6 Quintiles of protein intake (g/kg/d) excluding predicted misreporters	Fair health status	16.5	.011	16.1%
	Body fat percentage (%)	1.0	<.001	
	SEP V at 53 y (Unskilled)	29.0	.002	

There was heterogeneity in the outcomes of regression analyses to predict timed up and go at 60 – 64 y in females (Table 6.32).

In model 1.6, quintile 1 of absolute mean protein consumption (g/d) was associated with a 6.2% poorer performance compared with those in higher quintiles of protein consumption (p=0.021). However, after the exclusion of predicted misreporters (model 5.6) this association disappeared.

In all regression models, self-reported health status at 60 – 64 y was most predictive of timed up and go performance in females. In models 1.6 – 4.6, compared with a self-reported health status of excellent/very good, a fair health status was associated with a 22.0 – 23% poorer performance (p<0.001). In the sensitivity analyses (models 5.6 and 6.6) excluding predicted misreporters, a fair health status was associated with a 16.5% poorer performance (p<0.05) at timed up and go.

In models 1.6 – 4.6 adulthood physical activity was predictive of TUG performance. Compared with being sedentary throughout adulthood, being 'more active' or 'most active' was associated with a 6% better performance.

Only in models 2.6, where protein intake was expressed relative to body mass (g/kg/d) and in the sensitivity analyses (models 5.6 and 6.6) was body fat percentage (and not abdominal circumference) predictive of timed up and go performance in females. Each additional 1% of body fat was associated with a 0.6 – 1.0% poorer performance. Abdominal circumference (and not body fat percentage) was predictive of performance in models 1.6, 3.6 and 4.6; each additional 1 cm was associated with a 0.5% poorer performance (p<0.001). When abdominal circumference was predictive of TUG performance, whole body lean mass (kg/ht^2) was also predictive of performance; each additional 1 kg associated with a 2% better performance.

The sensitivity analyses (models 5.6 and 6.6) were each conducted in 201 females who provided apparently reliable dietary data in all years of measurement and who were predicted never to have under- or over-reported their energy intakes. In these subsets of females, socioeconomic position at 53 y was also predictive of TUG performance. Compared with SEP I/II (professional/intermediate) SEP V (unskilled) was associated with a 28 – 29% poorer performance at 60 – 64 y (p=0.002).

6.4 Discussion

6.4.1 Overview of hypothesis and analytical approach

This project aimed to test the hypothesis that relatively low protein intake across adulthood would predict poorer physical capability in middle age (60 – 64 y). For this purpose, the analysis was restricted to those NSHD participants who provided dietary data in all years of measurement (1982, 1989 and 1999) and intakes across all measurement years were aggregated to provide summary measures which were the best available evidence for adult protein intakes. Protein intake was expressed in four different ways i.e. as absolute amounts eaten (g/d), as quantities per day scaled to body mass (g/kg/d) and as a percentage of total energy intake (PPTE). Diurnal protein intakes ≥ 20 g were expressed as a muscle protein synthesis score (MPSS). In addition, 3 measures of physical capability were examined *viz*. hand grip strength, chair rise time and timed up and go. All analyses were undertaken using hierarchical linear regression analysis which, in addition to considering measures of protein intake, considered anthropometric measures, adulthood leisure-time physical activity, measures of self-reported health and socioeconomic status as potential predictors of physical capability. Finally, to assess the possible impact of dietary misreporting, the analyses were repeated (for protein intake in g/d and as g/kg/d) restricted to the subset of NSHD participants who appeared to report 'valid' energy intakes on all 3 measurement occasions (please see Chapter 2 for details of assessment of misreporting). These were referred to as sensitivity analyses in this chapter. Given the significant differences between men and women in all 3 measures of physical capability, all analyses were undertaken for males and females separately.

The analyses provided little support for the hypothesis that relatively low protein intake across adulthood would predict poorer physical capability at 60 – 64 y. This was true regardless of how protein intake was expressed and which physical capability measure was considered. Only in males, after excluding those predicted to have misreported their EI, were quintiles of protein consumption, expressed relative to body mass (g/kg/d) associated with poorer outcomes at chair rise time and timed up and go.

Longitudinal changes in DEXA-determined skeletal muscle mass after age 60 y were examined by (Gallagher *et al.*, 2000). Mean age at baseline and follow up was 73 y and 78 y in males (*n*=24) and 70 y and 75 y in females (*n*=54). In males, loss of total appendicular skeletal muscle was 0.8 kg (0.7 kg in leg and 0.2 kg in arm skeletal muscle) and the annual rate of change was – 0.2 ± 0.5 kg/ y. There were also significant losses in fat free body mass and increases in fat mass. In females, loss of total appendicular skeletal muscle mass was 0.4 kg (0.3 kg in legs and 0.1 kg in arms) and the annual rate of change was – 0.1 ± 0.4 kg/ y, half the rate of change seen in males. There were insignificant increases in fat free body mass and decreases in fat mass in females. The authors concluded *inter alia* that musculoskeletal relationships in males and females could be expected to develop very differently, with important implications for mobility and physical function in later life (Gallagher *et al.*, 2000).

Among the eight UK cohort studies which comprise the HALCyon programme (including the NSHD at 53 y), and using cross sectional data, a higher BMI (kg/m^2) was associated with a poorer performance at the chair rise test and a better performance at hand grip strength (in males) (Hardy *et al.*, 2013). In the present study (at 60 – 64 y) BMI was never predictive of performance at any of the objectively measured physical capability tests. However, increasing abdominal circumference (cm) was predictive of a poorer performance at chair rise in males and females. In females the association was significant in all models, even after the exclusion of predicted misreporters whereas in males the association was lost after protein intake was adjusted for body weight and the model adjusted for misreported energy intakes. Increasing abdominal circumference and body fat percentage were also predictive of a poorer performance at timed up and go in females. High body fatness but not low fat free mass (assessed by bioelectrical impedance) was predictive of self-reported, mobility-related disability (walking and stair climbing) in older (≥ 65 y) men and women (Visser *et al.*, 1998b). DEXA-determined total body and lower extremity muscle mass were not associated with self-reported physical disability among 753 participants of the Framingham Heart Study (72 – 95 y). However, there was a strong positive association between body fat percentage and disability.

Compared to those in the lowest tertile of body fat percentage, the odds ratio for disability among those in the highest tertile was 2.69 in females and 3.08 in males (Visser *et al.*, 1998a). Self-reported disability was assessed by nine physical function questions i.e. stooping, crouching and kneeling, standing, walking, arm reach above shoulder height, handling a small/lifting a large object, getting in and out of a car and putting on socks/ stockings. In addition, hand grip strength (by dynamometer) and fat distribution (waist circumference and the waist-hip ratio) were determined and physical activity and self-reported health status included as potential confounders. Grip strength was positively correlated with whole body skeletal muscle mass in males (r = .50) and females (r = .46) an observation consistent with the current study.

Physical (and mobility-related) disability were positively associated with percent body fat but not with the distribution of body fat in (Visser *et al.*, 1998a), a finding not observed in the current study. Body fat percentage was significantly associated with a poorer performance at timed up and go, but only in females. By comparison, in sensitivity analyses (using reliable dietary data), abdominal circumference, a measure of central adiposity, was predictive of poorer hand grip strength and chair rise time in females. In males, abdominal circumference was predictive of poorer hand grip strength and chair rise time (where protein was expressed in absolute intakes).

Gender differences in the anthropometric predictors of physical performance in older adults were examined by (Fragala *et al.*, 2012) as males have more absolute and relative lean mass and less fat mass than females. In 470 older men and women (mean age 73 y) body composition was determined by DEXA, leg strength/power by a leg press and mobility performance/functional strength by gait speed and chair rise. After accounting for age, BMI (kg/m^2) was associated with poorer chair rise (0.4) (p<0.001) in females but not in males (p=0.146). In the present study BMI was not predictive of chair rise performance and there was considerable overlap in gender models. Factors common to both were a 'fair' self-reported health status (associated with a 17 – 22% poorer performance in males and a 19 – 22% poorer performance in females) and abdominal circumference (associated with a 0.4 – 0.5% poorer performance in males and a 0.4 – 0.7% poorer performance in females). Increasing adulthood physical activity predicted a better performance at chair rise time, but only in males.

In a systematic review by (Vincent *et al.*, 2010) it was reported that maintaining mobility was more challenging for women than men. In longitudinal studies that examined chair rise time, weight gain and increased BMI contributed to the decline in body transfer ability and multiple comorbidities increased the susceptibility to mobility loss. In a systematic review by (Shin *et al.*, 2011) studies suggested that adiposity was a stronger determinant of physical performance than muscle mass in older community dwelling adults. However, the positive relationship between muscle mass and physical performance was clearly shown when functionality was assessed by hand grip strength. These observations were consistent with the findings in the current study.

In the English Longitudinal Study of Ageing (age at baseline 72.3 y in males and 73.2 y in females), higher BMI was associated with impaired physical function at 5 y follow up but not mortality. Physical function was assessed by activities of daily living and the Short Physical Performance Battery (Lang *et al.*, 2008). As those who were overweight/obese were more likely to become disabled but not more likely to die, this suggested long periods of living with a disability.

In 2876 participants of the Health, Aging and Body Composition Study the joint effects of adiposity (BMI, body fat percentage, waist circumference) and physical activity on incident mobility limitation in older adults were examined (Koster *et al.*, 2008). BMI (kg/m^2) was categorised into 3 groups (<25, 25 – 29.9 and ≥30) and total body fat into sex-specific quartiles, high(est) (>31.3% (males) and >43.7% (females)), and low(est) (<24.7% (males) and <35.8% (females). A high waist circumference was ≥ 102 cm (in males) and 88 cm (in females). Physical activity was divided into quartiles, high physical activity (> 106.5 kcal/ kg per week) and low physical activity (< 38.4 kcal.kg per week). Second and third quartiles were combined for medium. Incident mobility limitation was defined as self-reported difficulty walking ¼ mile or climbing 10 steps at 2 consecutive assessments. Self-rated health status was classified as in the present study i.e. excellent – poor, and depressed mood assessed by a MMMSE score. Cox proportional hazard regression models were stratified by gender and race.

In (white) males, the highest combined risk of mobility limitation (HR = 2.52, 95% CI = 1.48 – 4.28) was in those with a high BMI and medium levels of physical activity. In (white) females, it was in those with a high total body fat percentage and low physical activity (HR = 3.53, 95% CI = 1.91 – 6.52) (Koster *et al.*, 2008). This study highlights how gender differences in body composition differentially impact on mobility limitation at follow up. In the present study, body fat percentage was only predictive of physical capability in females; in models adjusted and unadjusted for predicted misreporters, each additional 1% was associated with 0.1 s at timed up and go ($p<0.05$).

6.5 Appendices

6.5.1 Predictors of hand grip strength in NSHD males at 60 – 64 y (Models 2.1 – 6.1)

Table 6.33 Model 2.1 Predicting HGS in NSHD males at 60 – 64 y using quintiles of protein intake (g/kg/d)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d)	-1.610	-.057	.277	1.104
Height (m)	53.247	.307	**<.001**	1.022
Adjusted appendicular lean mass (kg/ht^2)	4.619	.376	**<.001**	1.688
Abdominal circumference (cm)	-.295	-.288	**<.001**	1.796

Table 6.34 Model 3.1 Predicting HGS in NSHD males at 60 – 64 y using quintiles of protein intake (as a percentage of total daily energy)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (%TE)	.320	.012	.818	1.017
Height (m)	52.907	.306	**<.001**	1.022
Adjusted appendicular lean mass (kg/ht^2)	4.6	.374	**<.001**	1.692
Abdominal circumference (cm)	-.311	-.303	**<.001**	1.717

Table 6.35 Model 4.1 Predicting HGS in NSHD males at 60 – 64 y using quartiles of diurnal protein intake (MPSS)

	B	**Beta**	***p*-value**	**VIF**
Quartiles of diurnal protein intake (MPSS)	.228	.009	.866	1.022
Height (m)	53.195	.307	**<.001**	1.043
Adjusted appendicular lean mass (kg/ht^2)	4.595	.374	**<.001**	1.689
Abdominal circumference (cm)	-.312	-.304	**<.001**	1.716

Table 6.36 Model 5.1 Predicting HGS in NSHD males at 60 – 64 y using quintiles of protein intake (g/d) excluding predicted misreporters (sensitivity analysis 1)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d) excluding predicted misreporters (SA 1)	2.552	.095	.175	1.075
Height (m)	66.517	.397	**<.001**	1.100
Adjusted whole body lean mass (kg/ht^2)	2.833	.489	**<.001**	2.026
Abdominal circumference (cm)	-.334	-.298	**.003**	2.092

Table 6.37 Model 6.1 Predicting HGS in NSHD males at 60 – 64 y using quintiles of protein intake (g/kg/d) excluding predicted misreporters (sensitivity analysis 2)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d) excluding predicted misreporters (SA 2)	.133	.005	.945	1.143
Height (m)	64.015	.382	**<.001**	1.088
Adjusted whole body lean mass (kg/ht^2)	2.729	.471	**<.001**	2.002
Abdominal circumference (cm)	-.338	-.301	**.003**	2.156

6.5.2 Predictors of chair rise time in NSHD males at 60 – 64 y (Models 2.3 – 6.3)

Table 6.38 Model 2.3 Predicting CRT in NSHD males at 60 – 64 y using quintiles of protein intake (g/kg/d)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d)	-6.643	-.103	**.033**	1.142
Self-reported health status _Good	4.472	.079	.093	1.074
_Fair	22.191	.236	**<.001**	1.085
_Poor	35.450	.164	**<.001**	1.022
Abdominal circumference (cm)	.501	.211	**<.001**	1.193
Adulthood PA _MoreActive	-5.345	-.102	**.043**	1.252
_MostActive	-9.662	-.152	**.003**	1.263

Table 6.39 Model 3.3 Predicting CRT in NSHD males at 60 – 64 y using quintiles of protein intake (as a percentage of total daily energy)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (%TE)	-1.321	-.021	.654	1.028
Self-reported health status _Good	4.818	.085	.071	1.070
_Fair	22.429	.239	**<.001**	1.092
_Poor	33.996	.158	**.001**	1.017
Abdominal circumference (cm)	.416	.175	**<.001**	1.083
Adulthood PA _MoreActive	-5.500	-.105	**.039**	1.257
_MostActive	-9.486	-.149	**.004**	1.264

Table 6.40 Model 4.3 Predicting CRT in NSHD males at 60 – 64 y using quartiles of diurnal protein intake (MPSS)

	B (%)	**Beta**	***p*-value**	**VIF**
Quartiles of diurnal protein intake (MPSS)	-.038	-.001	.989	1.048
Self-reported health status _Good	4.810	.085	.072	1.070
_Fair	22.274	.237	**<.001**	1.125
_Poor	34.005	.158	**.001**	1.024
Abdominal circumference (cm)	.421	.177	**<.001**	1.071
Adulthood PA _MoreActive	-5.593	-.107	**.036**	1.249
_MostActive	-9.413	-.148	**.004**	1.264

Table 6.41 Model 5.3 Predicting CRT in NSHD males at 60 – 64 y using quintiles of protein intake (g/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 1)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d) excluding predicted misreporters (SA 1)	-6.752	-.103	.128	1.058
Self-reported health status _Good	3.395	.059	.381	1.053
_Fair	17.071	.163	**.016**	1.055
_Poor	34.435	.128	.053	1.013
Adulthood PA _MoreActive	-9.740	-.186	**.013**	1.271
_MostActive	-4.268	-.069	.350	1.280
Abdominal circumference (cm)	.370	.140	**.039**	1.065

Table 6.42 Model 6.3 Predicting CRT in NSHD males at 60 – 64 y using quintiles of protein intake (g/kg/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 2)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d) excluding predicted misreporters (SA 2)	9.770	.156	**.019**	1.005
Self-reported health status _Good	4.672	.081	.228	1.034
_Fair	20.567	.196	**.004**	1.029
_Poor	37.321	.139	**.038**	1.007

6.5.3 Predictors of timed up and go in NSHD males at 60 – 64 y (Models 2.5 – 6.5)

Table 6.43 Model 2.5 Predicting TUG in NSHD males at 60 – 64 y using quintiles of protein intake (g/kg/d)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d)	-3.6	-.069	.154	1.005
Self-reported health status _Good	5.774	.127	**.010**	1.050
_Fair	16.410	.221	**<.001**	1.047
_Poor	24.286	.128	**.008**	1.011

Table 6.44 Model 3.5 Predicting TUG in NSHD males at 60 – 64 y using quintiles of protein intake (as a percentage of total daily energy)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (%TE)	1.261	.025	.608	1.001
Self-reported health status _Good	5.747	.127	**.011**	1.050
_Fair	16.102	.217	**<.001**	1.046
_Poor	23.536	.124	**.011**	1.008

Table 6.45 Model 4.5 Predicting TUG in NSHD males at 60 – 64 y using quartiles of diurnal protein intake (MPSS)

	B (%)	**Beta**	***p*-value**	**VIF**
Quartiles of diurnal protein intake (MPSS)	-1.856	-.039	.428	1.050
Self-reported health status _Good	5.807	.128	**.010**	1.051
_Fair	16.759	.225	**<.001**	1.089
_Poor	24.228	.128	**.009**	1.017

Table 6.46 Model 5.5 Predicting TUG in NSHD males at 60 – 64 y using quintiles of protein intake (g/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 1)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d) excluding predicted misreporters (SA 1)	1.312	.026	.701	1.013
Socioeconomic status SEP_IIINM	2.884	.043	.533	1.052
SEP_IIIM	6.896	.130	.060	1.061
SEP_Partly	14.404	.176	**.010**	1.040
SEP_Unskilled	-10.452	-.075	.263	1.013

Table 6.47 Model 6.5 Predicting TUG in NSHD males at 60 – 64 y using quintiles of protein intake (g/kg/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 2)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d) excluding predicted misreporters (SA 2)	7.368	.146	**.035**	1.017
Father's SEP FSC_IIINM	-5.749	-.115	.160	1.420
FSC_IIIM	-.940	-.020	.809	1.451
FSC_Partly	3.584	.065	.412	1.347
FSC_Unskilled	13.431	.151	**.040**	1.143

6.5.4 Predictors of hand grip strength in NSHD females at 60 – 64 y (Models 2.2 – 6.2)

Table 6.48 Model 2.2. Predicting HGS in NSHD females at 60 – 64 y using quintiles of protein intake (g/kg/d)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d)	-.120	-.006	.900	1.161
Height (m)	30.298	.254	**<.001**	1.029
Self-reported health status _Good	-.529	-.035	.464	1.073
_Fair	-5.610	-.205	**<.001**	1.062
_Poor	-10.597	-.104	**.026**	1.017
Adjusted appendicular lean mass (kg/ht^2)	2.586	.302	**<.001**	2.027
Abdominal circumference (cm)	-.158	-.253	**<.001**	2.070

Table 6.49 Model 3.2. Predicting HGS in NSHD females at 60 – 64 y using quintiles of protein intake (as a percentage of total daily energy)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (%TE)	.903	.050	.280	1.016
Height (m)	30.185	.244	**<.001**	1.019
Self-reported health status _Good	-.583	-.039	.420	1.078
_Fair	-5.536	-.202	**<.001**	1.063
_Poor	-10.934	-.107	**.021**	1.016
Adjusted appendicular lean mass (kg/ht^2)	2.582	.302	**<.001**	1.985
Abdominal circumference (cm)	-.157	-.251	**<.001**	2.030

Table 6.50 Model 4.2 Predicting HGS in NSHD females at 60 – 64 y using quartiles of diurnal protein intake (MPSS)

	B	**Beta**	***p*-value**	**VIF**
Quartiles of diurnal protein intake (MPSS)	1.099	.074	.112	1.019
Height (m)	30.865	.249	**<.001**	1.024
Self-reported health status _Good	-.519	-.034	.471	1.073
_Fair	-5.577	-.204	**<.001**	1.061
_Poor	-11.345	-.111	**.017**	1.022
Adjusted appendicular lean mass (kg/ht^2)	2.574	.301	**<.001**	1.984
Abdominal circumference (cm)	-.155	-.249	**<.001**	2.032

Table 6.51 Model 5.2 Predicting HGS in NSHD females at 60 – 64 y using quintiles of protein intake (g/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 1)

	B	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d) excluding predicted misreporters (SA 1)	.745	.039	.542	1.016
Height (m)	37.852	.296	**<.001**	1.009
Adjusted appendicular lean mass (kg/ht^2)	3.707	.388	**<.001**	1.654
Abdominal circumference (cm)	-.209	-.301	**<.001**	1.649

Table 6.52 Model 6.2 Predicting HGS in NSHD females at 60 – 64 y using quintiles of protein intake (g/kg/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 2)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d) excluding predicted misreporters (SA 2)	1.329	.075	.270	1.137
Height (m)	36.634	.287	**<.001**	1.011
Adjusted appendicular lean mass (kg/ht^2)	3.548	.372	**<.001**	1.687
Abdominal circumference (cm)	-.220	-.317	**<.001**	1.689

6.5.5 Predictors of chair rise time in NSHD females at 60 – 64 y (Models 2.4 – 6.4)

Table 6.53 Model 2.4 Predicting CRT in NSHD females at 60 – 64 y using quintiles of protein intake (g/kg/d)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d)	.545	.007	.871	1.166
Self-reported health status _Good	11.088	.185	**<.001**	1.103
_Fair	18.727	.173	**<.001**	1.066
_Poor	51.832	.159	**<.001**	1.019
Abdominal circumference (cm)	.409	.169	**<.001**	1.211
Height (m)	46.782	.096	**.021**	1.036
Adulthood PA _MoreActive	-3.879	-.067	.140	1.221
_MostActive	-8.709	-.114	**.012**	1.222

Table 6.54 Model 3.4 Predicting CRT in NSHD females at 60 – 64 y using quintiles of protein intake (as a percentage of total daily energy)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (%TE)	2.100	.030	.465	1.016
Self-reported health status _Good	10.974	.183	**<.001**	1.107
_Fair	18.775	.173	**<.001**	1.066
_Poor	51.223	.157	**<.001**	1.022
Abdominal circumference (cm)	.418	.173	**<.001**	1.061
Height (m)	46.438	.096	**.022**	1.035
Adulthood PA _MoreActive	-3.808	-.065	.147	1.222
_MostActive	-8.778	-.115	**.011**	1.221

Table 6.55 Model 4.4 Predicting CRT in NSHD females at 60 – 64 y using quartiles of diurnal protein intake (MPSS)

	B (%)	**Beta**	***p*-value**	**VIF**
Quartiles of diurnal protein intake (MPSS)	-.636	-.011	.795	1.028
Self-reported health status _Good	11.077	.185	**<.001**	1.103
_Fair	18.706	.173	**<.001**	1.066
_Poor	52.205	.160	**<.001**	1.023
Abdominal circumference (cm)	.412	.171	**<.001**	1.067
Height (m)	46.339	.095	**.023**	1.046
Adulthood PA _MoreActive	-3.889	-.067	.139	1.219
_MostActive	-8.709	-.114	**.012**	1.221

Table 6.56 Model 5.4 Predicting CRT in NSHD females at 60 – 64 y using quintiles of protein intake (g/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 1)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d) excluding predicted misreporters (SA 1)	4.842	.069	.253	1.024
Abdominal circumference (cm)	.675	.257	**<.001**	1.062
Self-reported health status _Good	4.559	.077	.208	1.059
_Fair	21.637	.175	**.004**	1.052
_Poor	6.700	.021	.728	1.032

Table 6.57 Model 6.4 Predicting CRT in NSHD females at 60 – 64 y using quintiles of protein intake (g/kg/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 2)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d) excluding predicted misreporters (SA 2)	-3.100	-.045	.465	1.071
Abdominal circumference (cm)	.682	.259	**<.001**	1.112
Self-reported health status _Good	4.948	.084	.171	1.054
_Fair	22.087	.178	**.004**	1.051
_Poor	7.585	.024	.694	1.030

6.5.6 Predictors of timed up and go at 60 – 64 y in NSHD females (Models 2.6 – 6.6)

Table 6.58 Model 2.6 Predicting TUG in NSHD females at 60 – 64 y using quintiles of protein intake (g/kg/d)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d)	1.240	.022	.645	1.087
Self-reported health status _Good	4.568	.104	**.030**	1.061
_Fair	23.346	.293	**<.001**	1.050
_Poor	28.059	.117	**.013**	1.037
Body fat%	.568	.156	**.002**	1.113
Adulthood PA _MoreActive	-5.792	-.137	**.008**	1.220
_MostActive	-5.710	-.107	**.038**	1.243

Table 6.59 Model 3.6 Predicting TUG in NSHD females at 60 – 64 y using quintiles of protein intake (as a percentage of total daily energy)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (%TE)	4.177	.081	.083	1.039
Self-reported health status _Good	3.286	.075	.118	1.085
_Fair	22.196	.278	**<.001**	1.071
_Poor	25.535	.107	**.023**	1.040
Abdominal circumference (cm)	.536	.301	**<.001**	2.325
Adjusted whole body lean mass (kg/ht^2)	-2.190	-.187	**.007**	2.233
Adulthood PA _MoreActive	-5.934	-.140	**.006**	1.218
_MostActive	-5.872	-.110	**.031**	1.234

Table 6.60 Model 4.6 Predicting TUG in NSHD females at 60 – 64 y using quartiles of diurnal protein intake (MPSS)

	B (%)	**Beta**	***p*-value**	**VIF**
Quartiles of diurnal protein intake (MPSS)	.533	.012	.791	1.023
Self-reported health status _Good	3.579	.081	.089	1.079
_Fair	21.895	.275	**<.001**	1.068
_Poor	27.185	.113	**.016**	1.044
Abdominal circumference (cm)	.537	.302	**<.001**	2.328
Adjusted whole body lean mass (kg/ht^2)	-2.250	-.192	**.005**	2.229
Adulthood PA _MoreActive	-6.194	-.147	**.004**	1.212
_MostActive	-5.648	-.106	**.038**	1.232

Table 6.61 Model 5.6 Predicting TUG in NSHD females at 60 – 64 y using quintiles of protein intake (g/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 1)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/d) excluding predicted misreporters (SA 1)	.762	.014	.837	1.045
Self-reported health status _Good	3.513	.080	.240	1.049
_Fair	16.521	.174	**.012**	1.053
_Poor	10.736	.037	.592	1.049
Body fat %	.914	.236	**.001**	1.036
Socioeconomic status SEP_IIINM	-3.439	-.080	.267	1.163
SEP_IIIM	-3.795	-.045	.515	1.084
SEP_Partly	1.000	.013	.848	1.108
SEP_Unskilled	28.051	.212	**.002**	1.042

Table 6.62 Model 6.6 Predicting TUG in NSHD females at 60 – 64 y using quintiles of protein intake (g/kg/d) excluding those ever predicted to have misreported their EI (sensitivity analysis 2)

	B (%)	**Beta**	***p*-value**	**VIF**
Quintiles of protein intake (g/kg/d) excluding predicted misreporters (SA 2)	-4.328	-.086	.216	1.093
Self-reported health status _Good	3.608	.082	.226	1.050
_Fair	**16.515**	.174	**.011**	1.047
_Poor	9.920	.034	.615	1.029
Body fat %	**.999**	.258	**<.001**	1.108
SEP at 53 y SEP IIINM	-3.591	-.083	.244	1.162
SEP IIIM	-3.218	-.038	.580	1.091
SEP Partly	1.217	.016	.813	1.094
SEP Unskilled	**28.932**	.218	**.002**	1.047

CHAPTER 7

General Discussion and Future work

7.1 Overview

This project aimed to test the hypotheses that low protein consumption throughout adulthood would impair physical capability in later life and that diurnal patterns of protein consumption throughout adulthood would influence physical capability in later life. Energy consumption was considered in order that protein density of daily and mealtime energy (protein as a % of total energy) could be determined. Daily energy intakes were also used to identify predicted under- and over-reporters and selected analyses were repeated without these individuals to determine the influence of misreporting on relationships between dietary protein and physical capability. A previous study has shown that associations between diet and physical capability outcomes can be distorted by measurement error and that by excluding individuals who appear to have misreported their intakes, associations may be strengthened (Beasley *et al.*, 2010).

There is evidence that diurnal patterns of protein consumption influence short term protein retention and body composition (Arnal *et al.*, 1999; Bouillanne *et al.*, 2013) and that protein intakes are associated with long term change in body composition (Houston *et al.*, 2008; Meng *et al.*, 2009; Scott *et al.*, 2010). In addition, low nutrient intakes (including protein) are associated with incident disability in older women (Bartali *et al.*, 2006b), frailty (Bartali *et al.*, 2006a; Beasley *et al.*, 2010) and muscle strength (Bartali *et al.*, 2012) although the direction of causality, if any, in such studies is often problematical.

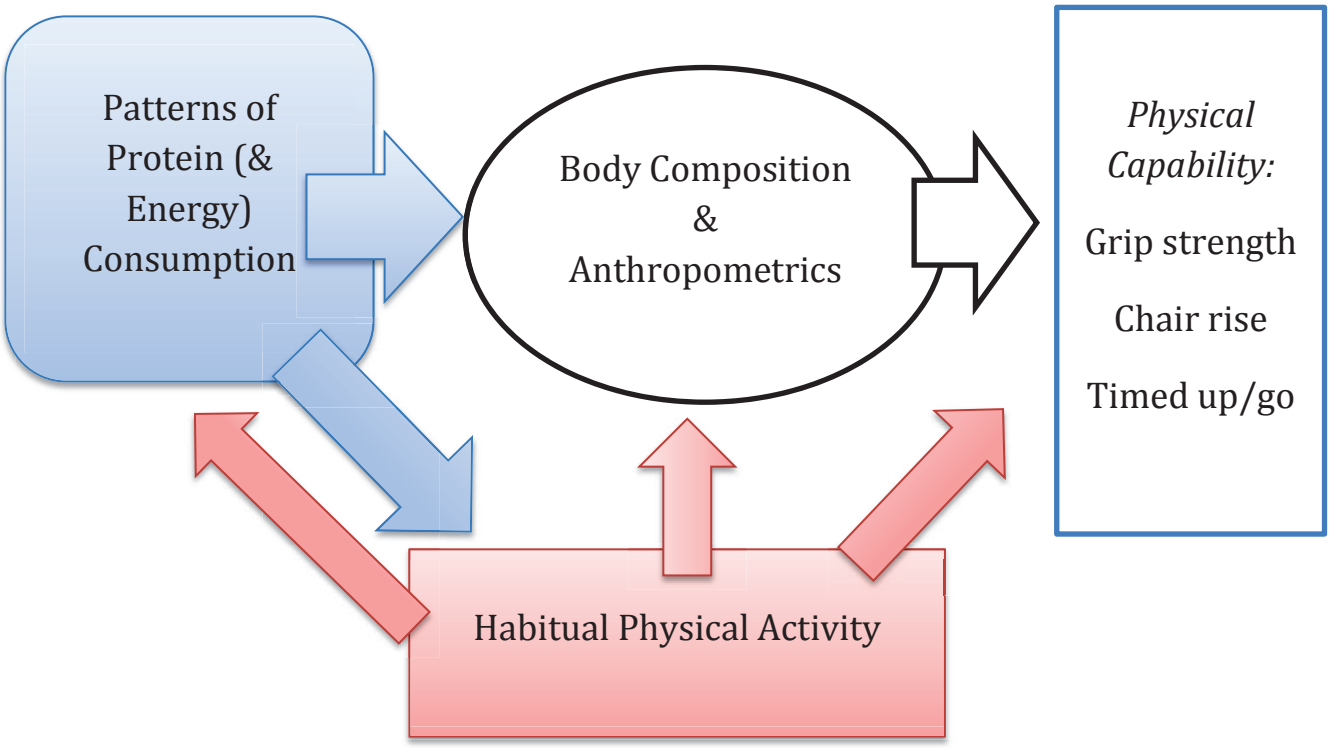
Resistance exercise, when combined with adequate dietary protein, preserves muscle in middle aged individuals (Morris and Jacques, 2013) compared with general aerobic activities with adequate protein intakes which did not offset age-related loss of skeletal muscle mass (Starling *et al.*, 1999). In addition, there is experimental evidence demonstrating that, in both older and younger adults, protein supplementation can enhance the effect of exercise training on measures of physical capability (Cermak *et al.*, 2012).

There is a paucity of research on effects of diurnal patterns of protein consumption (Tieland *et al.*, 2012a), only one in relation to changes in body composition (Ruiz Valenzuela RE, 2013) and none in relation to objectively determined measures of physical capability. Using longitudinal data from the MRC National Survey of Health and Development, a large British cohort of community living adults, this study aimed to address this research gap.

7.2 Analytical model and research strategy

The analytical model and research strategy for this project are depicted in Figure 7.1. *A priori*, daily energy and protein intakes throughout adulthood were expected to influence anthropometry, body composition and levels of habitual physical activity. Adulthood patterns of protein consumption (whether daily (protein intakes expressed in various ways) or diurnal) were hypothesised to be particularly associated with measures of body composition in later life, via their association with muscle protein synthesis, protein degradation and net protein balance.

Figure 7.1 Analytical model and research strategy



Anthropometry and body composition (including measures of lean and fat mass) were hypothesised to influence performance at objectively measured tests of strength and physical capability. Absolute amounts of lean mass were expected to be predictive of grip strength whereas higher adiposity was expected to be associated with poorer measures of physical capability (here tested by chair rise time and timed up and go). Since habitual physical activity (PA) is a major determinant of diet, energy balance, anthropometry and body composition, it was hypothesised that habitual PA would impact on performance in all tests of physical capability and, as such, should be quantified and included in statistical modelling.

7.3 Project aims

This project aimed:

1. To characterise and to quantify patterns of protein consumption (both mean daily intake and diurnal patterns of intake) in a cohort of individuals providing dietary data by 5 d food diary in 1982, 1989 and 1999 when aged 36 y, 43 y and 53 y, respectively.

2. To determine and to characterise physical capability at age 60 – 64 y using a range of techniques including hand grip strength, timed up and go and chair rise time.

3. To determine and to characterise other variables identified *a priori* as potentially mediating (or confounding) the relationship between protein consumption and physical capability. These variables included body composition and anthropometrics, habitual physical activity, socioeconomic status, health status and other related (meta)data.

4. To apply a range of statistical techniques, including hierarchical linear regression, to this dataset to determine which variables, including patterns of protein consumption during adulthood, predict physical performance at age 60 – 64 y.

7.4 Interpretation of findings

The extent to which body composition and anthropometry were predictive of physical capability at 60 – 64 y was the subject of the main hierarchical linear regression analyses. As models were split by gender they are discussed separately below, although there was extensive commonality in the outcomes of these analyses.

7.4.1 Females

In females, in models unadjusted for predicted misreporters, **hand grip strength** at age 60 – 64 y was predicted by height, self-reported health status, appendicular lean mass (kg/ht^2) and abdominal circumference. In the sensitivity analyses (using reliable dietary data) grip strength was predicted only by height, appendicular lean mass (kg/ht^2) and abdominal circumference. Appendicular (and whole body) lean mass was predicted by absolute intakes of protein (and energy-adjusted protein intakes) throughout adulthood. These findings suggest that diet, and in particular protein consumption, is operating *via* body composition in the maintenance of muscle mass and strength into later life. Conversely, the low levels of physical activity observed in females throughout adulthood may have resulted in increased abdominal circumference which affected muscle strength adversely.

In female models, adjusted for predicted misreporters, **chair rise time** was predicted by self-reported health status and abdominal circumference at 60 – 64 y only, whereas **timed up and go** was predicted by health status, body fat percentage and socioeconomic position (at 53 y). As adulthood energy intakes were not predictive of body fat percentage in females, it may be surmised that higher levels of body fat percentage (and abdominal circumference) resulted from increasing sedentarism. In simple linear regression, adulthood physical activity was significantly and positively associated with reductions in whole body and appendicular fat mass and body fat percentage in females (but not in males).

A fair health status compared to excellent/very good health (the reference category) was consistently associated with a poorer performance at chair rise and timed up and go. There was insufficient differentiation between the reference category and 'good' health and it may be inferred that when health status is subjectively interpreted as 'fair', this is the point at which health status begins to impact on this aspect of physical capability. A self-reported health status of 'poor' was also associated with an increase in time to complete the task (a poorer performance) but this did not reach statistical significance.

As central adiposity, quantified as abdominal circumference, is strongly associated with age-related chronic disease (CHD, diabetes, CVD and cancer) and all-cause mortality (Taylor *et al.*, 2010; Donini *et al.*, 2012; Staiano *et al.*, 2012), it may be hypothesised that increasing abdominal circumference is a major contributory factor in the self-reported decline in health status. In NSHD females, between the ages of 43 y and 60 – 64 y, abdominal circumference increased on average by 14.5 cm whereas in males, the increase was 9 cm.

Low levels of adulthood habitual physical activity may affect indices of physical capability by increasing adiposity. Greater adiposity would be expected to affect negatively the movements required in these tests (Bohannon RW, 2005; Vincent *et al.*, 2010; Shin *et al.*, 2011). For example, in chair rising (also an element of timed up and go) higher body weight would carry a penalty as the mass to be lifted is greater (Hardy R, 2010).

Other prerequisites of a good performance at these tests are lower limb strength, good balance, agility and coordination (Hardy R, 2010; Schoene *et al.*, 2013), all likely affected by higher levels of central adiposity. It may also be argued that higher levels of central and whole body adiposity may impair muscle function via their operation on muscle quality (Goodpaster *et al.*, 1997; Goodpaster *et al.*, 2001).

7.4.2 Males

In males, in models unadjusted and adjusted for predicted misreporters, **hand grip strength** at 60 – 64 y was predicted by height, measures of lean mass and abdominal circumference. In the sensitivity analyses (using reliable dietary data), grip strength was predicted by height, whole body lean mass (kg/ht^2) and abdominal circumference. Whole body lean mass was significantly and positively associated with absolute intakes of protein and energy-adjusted protein intakes throughout adulthood in males. Physical activity in males also predicted hand grip strength and this, together with protein intake, may explain these results. Abdominal circumference was also predicted by physical activity, and where this was low throughout adulthood, such inactivity would have resulted in an increasing waist circumference, influencing negatively, muscle strength at 60 – 64 y. In NSHD males, physical inactivity (a lower adulthood physical activity score) was associated with a 0.95 cm increase in abdominal circumference (p=0.004).

Exceptionally, a low protein intake relative to body mass (g/kg/d) (unadjusted for predicted misreporters) was significantly predictive of a better performance at **chair rise time**. However, after adjusting for predicted misreporters, this association became significantly negative i.e. a low protein intake (quintile 1) predicted a poorer performance at chair rise time. It may be hypothesised that as this was not a measure of absolute protein intake (g/d) but a measure of protein adequacy relative to body mass, it would ultimately be a reflection of muscle mass in older subjects. As ageing is accompanied by alterations in protein metabolism, including higher splanchnic extraction of amino acids and protein anabolic resistance (Bauer *et al.*, 2013), low protein intakes relative to body mass might manifest as reduced muscle quantity and quality with concomitant effects on whole body metabolism and strength. In this cohort, at these ages (≤ 55 y) the age-related changes in protein metabolism may not have occurred and the possible adverse effect of a low protein intake relative to body mass, may not yet have manifested. It this arguments holds, then the effect of low protein intakes relative to body mass would be observed in tests of muscle strength in adults older than those investigated in the present study.

Chair rise time in male regression models adjusted and unadjusted for predicted misreporters (and excluding the effect of protein) was predicted by health status, abdominal circumference and habitual physical activity. As already discussed for females, abdominal circumference and health status are causally interrelated. As was hypothesised *a priori*, habitual physical activity positively influenced physical capability. This result was consistent with those of (Cooper *et al.*, 2011b) who reported, in the same cohort, that leisure-time physical activity at 36 and 43 y was positively associated with chair rise performance at age 53 y after adjusting for covariates (but not hand grip strength). In simple linear regression analysis, adulthood PA was significantly, and positively associated with chair rise time and hand grip strength in males at 60 – 64 y (but not with timed up and go). These findings suggest that the cumulative benefits of physical activity across adulthood on physical capability continue to operate into older ages (60 – 64 y). The fact that the effects of physical activity were observed only in males may be due to the fact that among female NSHD participants, reported levels of habitual physical activity were relatively low. Across all 3 measurement periods, a higher proportion of males reported being active or most active than did females and gender differences in physical activity group membership were significant.

In male regression models unadjusted for predicted misreporters, **timed up and go** was predicted by self-reported, current health status only. This may be because health status impacted on all the requirements of this test i.e. agility, balance, gait and the transferring and turning subtasks (Herman T, 2011) to the exclusion of all other potential predictors. In the sensitivity analyses, predictors of timed up and go were completely different from models in which predicted misreporters were not excluded; i.e. low protein intake and a lower socioeconomic position (at 4 y and 53 y) predicted a poorer performance.

7.5 Strengths and limitations of current study

7.5.1 Protein intakes relative to current recommendations

It is noted that protein intakes among NSHD participants who provided dietary data in all years were high relative to current recommendations (the Reference Nutrient Intake for protein of 0.83 g/kg/d (Rand *et al.*, 2003)). Among those who provided dietary data in all years, the percentage of participants who met this protein intake recommendation in 1982, 1989 and 1999 was 76.1, 81.2 and 80.2% respectively. In this sub-cohort of NSHD participants (*n*=1263) only 71 individuals (24 males and 47 females) reported a protein intake < 0.83g/kg/d in every measurement years.

It is further noted that the diurnal protein score (here referred to as the Muscle Protein Synthesis Score (MPSS)) was set at consumption of ≥ 20 g of protein at any eating occasion across the day. The use of a higher or lower threshold of protein intake may have impacted upon the results of the analyses.

7.5.2 Hierarchical linear regression

The use of hierarchical linear regression has potential limitations, specifically that the strict stepwise procedure may allow the data 'to drive the theory.' Variables may no longer contribute to the regression model because of the other variables in the model, even if they did contribute at an earlier point in time. Hierarchical linear regression analysis may be viewed as a strict, procedural method, in which direction and control of the analyses may be abdicated or 'given over' to the methodology. In mitigation, considerable care was given to the choice of all variables tested in the analyses.

7.5.3 Contemporary dietary variables

Dietary variables collected at 60 – 64 y (at the time physical capability was assessed) were not available and thus not used in regression analyses. Their inclusion may have had two broad effects; a reduction in sample size (when selecting participants who reported dietary data on all occasions) and in terms of the outcomes of regression analyses.

7.5.4 Participant subgroups

For the purposes of the analyses, only NSHD participants who provided dietary data in all years were used (*n*=1263). It should be noted that these individuals represent a special, self-selected group. The anthropometric characteristics, traits and lifestyle behaviours of participants of longitudinal cohort studies who consistently report dietary intake and anthropometric data in all measurement years may differ considerably from those who do not report in all years. Conclusions drawn from this group may not be generally applicable to all NSHD participants and not capable of extrapolation to the general UK population. In addition, sensitivity analyses were undertaken to determine the influence of misreporting on relationships between dietary protein and physical capability, in participants predicted never to have misreported their energy intake at all 3 measurement occasions. In these analyses, gender-specific sample sizes ranged from *n*=284 to *n*=318. In some instances, outcomes of hierarchical linear regression analyses in these subgroups were substantially different from outcomes of analyses which included all participants. Outcomes in these subgroups may reflect the greater reliability/validity of dietary intakes, alternatively they may reflect the underlying characteristics of the smaller subgroup under investigation.

7.5.5 Measurement of habitual physical activity

In the present study, habitual physical activity was self-reported by questionnaire and was restricted to assessment of leisure time physical activity only. As such, this was subject to two significant limitations i.e. physical activity in non-leisure time activities was not measured and quantification of physical activity by questionnaire may lack the required objectivity and precision. Although much of the inter-individual variation in energy expenditure among UK adults appears to relate to non-occupational activities, occupation-related physical activity may be more important for some especially those in manual occupations and those which require the individuals to spend much of the day standing and walking. There are strong arguments for using tools such as pedometers and accelerometers when assessing physical activity (Corder K, 2007) and such, objective measures are now widely used in epidemiological studies.

Where the physical activity assessment is restricted to self-report, recent recommendations on improving the accuracy of such approaches may be of benefit (Ainsworth BE, 2012). The present study found that leisure time physical activity was much higher in males than in females. However, this did not take into account occupational activity, getting to or from work or household duties/childcare responsibilities. The physical activity questionnaires investigated specific, structured activities (gardening, cycling, sports and recreation). Arguably household duties/childcare responsibilities may have precluded female participation in more structured activities, particularly in 1982/89 and the physical activity associated with household duties/childcare responsibilities would not have been reflected in the physical activity score. In the Hertfordshire cohort study (Martin *et al.*, 2008) walking and home activity drove the considerable difference in median total energy expenditure, and in (Sun *et al.*, 2013) gender differences fell to 0.2 – 1.5% when assessed objectively by accelerometry. It is notable that participation in sports/recreational activities did increase in females in 1999. As a consequence, associations between physical activity and diet, anthropometry/body composition and physical capability in females (and also in some males) may have been weakened or obscured by the lack of information on total physical activity.

7.5.6 Measurement of habitual dietary intake

Predicted under-reporting was extensive and the use of such data may obscure/confound associations between diet/dietary components and outcome measures of muscle strength and physical capability. After adjusting for predicted misreporting (in the sensitivity analyses) associations were observed to strengthen and alter. The identification of predicted misreporting was by the application of appropriate formulae to total daily energy intakes. However, dietary misreporting is macronutrient specific and evidence indicates that protein may be better reported than total energy intake. By excluding predicted energy misreporters, in sensitivity analyses, it is possible that individuals who correctly reported their protein intake were excluded.

In (Beasley *et al.*, 2010) protein and energy intakes were statistically corrected for measurement error using biomarkers (biomarker-calibrated intakes). A 20% increase in uncalibrated protein intake was associated with a 12% lower risk of frailty compared with a 32% lower risk associated with a 20% increase in calibrated intakes.

7.6 Future work

Dietary protein intakes in NSHD participants were investigated when cohort members were aged between 36 y – 53 y and physical capability outcomes assessed at 60 – 64 y. The overall lack of effect of total daily and diurnal protein intakes on outcomes of physical capability in this cohort may be explained by the fact that the cohort members were too young for the hypothesised effect to be seen. Indeed, most of the research indicating an effect of diurnal protein ingestion on physical capability and body composition has been in much older, frail, hospitalised, institutionalised or at risk subjects. Also in this cohort (Mulla *et al.*, 2013) found modest positive associations between energy intakes at 36 and 43 y and hand grip strength at 53 y and some indication of a relationship between protein intake, grip strength and standing balance time. The use of hierarchical linear regression analyses which included measures of DEXA-derived lean mass, self-reported health status and anthropometrics at 60 – 64 y, may explain, in part, the lack of any such association between protein intakes and grip strength at 60 – 64 y. Future work should examine dietary protein intakes (daily and diurnal patterns) in relation to physical capability at older ages, ideally by continuing to track this cohort or by the investigation of these effects in a much older cohort e.g. the Newcastle 85+ Study.

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