Gastroparesis symptoms in Parkinson's disease

Correlation with motor and non-motor symptoms and exploration of a novel drug to improve gastric emptying

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Abstract 1

Gastroparesis symptoms in Parkinson's disease

Background: Parkinson's disease (PD) can result in impaired motility throughout the gastrointestinal tract. Delayed gastric emptying or gastroparesis is estimated to affect over 70% of all people with PD. However, the prevalence of gastroparesis symptoms in people with PD is unknown. This study sought to define the prevalence and associated features of gastroparesis symptoms in people with PD.

Methods: More than 1,000 people with PD completed the Gastroparesis Cardinal Symptom Index (GCSI), a validated structured questionnaire to assess the presence and severity of gastroparesis symptoms. Two groups of patients were recruited to this UK wide study: those with recently diagnosed PD (within the last three years) and those with young onset disease (diagnosed before the age of 50).

Results: More than half of the 1,003 participants reported at least one gastroparesis symptom in the two weeks prior to completing the GCSI questionnaire. In those with recently diagnosed PD (n = 691) only 4.1% had a GCSI total score consistent with significant gastroparesis. In the young onset group 12.5% of participants had symptoms in keeping with gastroparesis. Features associated with a greater gastroparesis symptom burden across both groups included more advanced PD features, more non-motor symptoms, autonomic symptoms, anxiety and depression.

Conclusions: Although delayed gastric emptying is estimated to affect over 70% of all people with PD, in this large study we found that significant gastroparesis symptoms were only reported by 4-12% of patients. This suggests that although gastroparesis is a common abnormality in PD it may be largely asymptomatic.

Abstract 2

Exploration of a novel treatment for delayed gastric emptying in Parkinson's disease

Background: Delayed gastric emptying can impair Levodopa absorption and contribute to motor fluctuations in people with PD. Prokinetic medications may improve gastric emptying and consequently Levodopa absorption and motor function. However, there are few licenced prokinetics suitable for use in PD. This is an interim report of an on-going Phase II, double blind, placebo controlled study to assess the effect of a novel motilin agonist (Camicinal) on Levodopa absorption in people with PD, delayed gastric emptying and motor fluctuations.

Methods: 15 patients with PD were enrolled in this study. All had bilateral disease (Hoehn & Yahr stage II-IV) and troublesome motor fluctuations. There were 9 men and 6 women with a mean age of 64.40 (± 10.32) years. Participants were randomised to Camicinal 50mg (n=10) or placebo (n=5) once daily for 7-9 days. The treatment and placebo group were matched for clinical and demographic features. Clinical and pharmacokinetic parameters were measured serially at baseline, on the first day of treatment and the final dosing day. Statistical analyses were performed using non-parametric tests.

Results: Camicinal was as well tolerated as placebo. By the end of the dosing period there was a non-significant trend towards increased Levodopa absorption (Cmax) in the Camicinal treated group but not the placebo group. Gastric emptying rates and gastroparesis symptoms did not change significantly in either group. There was a significant improvement in motor function (reduced MDS UPDRS Part III score) in the treatment group by the end of the dosing period compared with baseline ($P < 0.05$). The percentage of the waking day spent in the 'off' state fell in the Camicinal group and rose in the placebo group.

Conclusions: These interim results suggest that Camicinal has potential as a novel therapy to enhance Levodopa absorption and motor function in people with PD. Therefore on-going study and evaluation of this agent is justified.

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I am grateful to all of my colleagues and friends who have supported me during my research and the writing of this thesis. I would particularly like to thank my supervisors Professor David Burn and Dr Anton Emmanuel who throughout have offered me support and guidance and have generously shared their experience and time.

I would like to thank Professor Donald Grosset who has led the PRoBaND study and co-ordinated the compilation of data for analysis in this thesis. I would also like to thank Dr Katherine Grosset and Dr Naveed Malik who have been central members of the PRoBaND team. Parkinson's UK funded the PRoBaND (Tracking Parkinson's) study and my contribution to the project over the last two years.

I have worked closely with GlaxoSmithKline's Academic Discovery Performance Unit and would particularly like to thank Rachel Gibson, George Dukes, Matthew Barton, Bhopinder Sarai, Lakshmi Vasist Johnson and Tal Otiker. My work on this project was funded jointly by GSK and the Michael J Fox Foundation and I am grateful to both for their financial support.

The Parkinson's disease specialist nurses at the Royal Victoria Infirmary, Newcastle upon Tyne were invaluable in assisting my identification of possible study participants. The North East DeNDRoN team were also helpful in identifying participants for both of my research projects.

I would like to thank all of the nurses at the Clinical Ageing Research Unit and especially Joanne Brown who was a great partner to work alongside during long visit days. Many colleagues at CARU have helped me over the last two years but I would particularly like to thank Dr Alison Yarnall, Dr Jo Jefferis, Dr Sue Lord and Dr Gordon Duncan for their advice, support and friendship.

Finally I would like to thank all of the participants and their families who contributed to my research. Without their interest, effort and time none of this would have been possible.

Statement of work undertaken

No portion of the work in this thesis has been submitted in support of an application for another degree or qualification at this or any other university or other institute of learning.

During my two year research degree I divided my time between two research projects and the work presented in this thesis represents my contribution to both studies.

Parkinson's Repository of Biosamples and Networked Datasets (PRoBaND) study

Professor Donald Grosset is the chief investigator for the PRoBaND study and was responsible for devising the project, securing funding from Parkinson's UK and obtaining ethical approval. The study was co-ordinated by the PRoBaND clinical consortium, consisting of a number of co-investigators from around the UK. The inclusion of a gastroparesis symptom questionnaire in the PRoBaND study protocol was suggested by Professor David Burn and I and was facilitated by Donald Grosset.

In Newcastle, Professor David Burn was principal investigator for the PRoBaND study and I was the lead study doctor for two years. I was responsible for the recruitment of study participants and from February 2012 to November 2013 I recruited a total of 141 participants with either recent or young onset Parkinson's disease. I undertook all baseline assessments for these participants which included obtaining informed consent, clinical evaluation, history taking, performance of the MDS UPDRS and MoCA and administration of patient questionnaires. Research nurses at the Clinical Ageing Research Unit (CARU) assisted in obtaining and processing blood samples and measurement of vital signs.

The data in this thesis is primarily taken from baseline visits however study participants were invited to attend for follow-up visits every six months. During my two year period working on the PRoBaND study I undertook a total of 145 follow-up visits with the assistance of the CARU research nurses and two research doctors (Dr John Boucher and Dr David Nesbitt).

This thesis includes data from Newcastle participants as well as those recruited to the other 56 UK sites. All study data was anonymously compiled in Glasgow and was

Quality Control checked by Donald and Katherine Grosset. The gastroparesis data was prepared specifically for use in this thesis and publications arising from it. I have performed all analysis of this data independently and the writing of this thesis is all my own work.

Exploration of a novel treatment for delayed gastric emptying in Parkinson's disease

This study was a collaborative project between GlaxoSmithKline (study sponsors) and Newcastle University. The study was conceived and designed by GSK's Academic Discovery Performance Unit. The original protocol was written by Matthew Barton, the GSK study team, Professor David Burn and myself. Additionally I had direct involvement in all amendments to the protocol and presented the study to the local Research and Ethics Committee alongside Professor Burn.

In the first phase of the project (May 2012 – November 2012) I consented and screened a total of nine participants. Following a major protocol amendment, a second phase of recruitment began (May 2013 – October 2013) during which I consented and evaluated a further 14 participants. In total I performed 22 screening visits, 24 dosing visits and 12 follow-up visits. At these study visits, I obtained clinical and demographic details and performed all clinical assessments. Serial blood sampling for pharmacokinetic analysis was performed by myself and the study nurses.

Breath samples to measure gastric emptying rates were analysed by Advanced Breath Diagnostics, Nashville, TN, USA. Pharmacokinetic bioanalyses were performed in GSK laboratories and parameters (Levodopa AUC) estimated from a model developed by the study's clinical pharmacologist (Lakshmi Vasist Johnson). The statistical analysis plan for this thesis was discussed with the study statistician (Tal Otiker) however I undertook all analysis and write up independently at Newcastle University.

The GSK study team have reviewed this thesis but have not influenced the analysis, results or conclusions that I have drawn. I can confirm that the study write up presented in this thesis is all my own work.

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Abbreviations

BP = blood pressure

CBD = corticobasal degeneration

CCK = cholecystokinin

CNS = central nervous system

DBS = deep brain stimulation

DLB = dementia with Lewy bodies

DMNV = dorsal motor nucleus of the vagus nerve

EDS = excessive daytime somnolence

ENS = enteric nervous system

FOG = freezing of gait

GBA = glucocerebrosidase

GCSI = Gastroparesis Cardinal Symptom Index

GCSI-DD = Gastroparesis Cardinal Symptom Index Daily Diary

GES = gastric electrical stimulation

GE t1/2 = gastric emptying half time

GHSR = growth hormone secretagogue receptor

GI = gastrointestinal

GLP-1 = glucagon-like peptide-1

GLP-2 = glucagon-like peptide-2

GSK = GlaxoSmithKline

H&Y = Hoehn & Yahr stage

H.pylori = Helicobacter pylori

ID = indeterminate motor phenotype

iRBD = idiopathic REM sleep behaviour disorder

LADS = Leeds anxiety and depression scale

LRRK2 = leucine-rich repeat kinase 2

MDS UPDRS = Movement Disorder Society Unified Parkinson's Disease Rating Scale

mH&Y = modified Hoehn & Yahr stage

MMC = migrating motor complex

MoCA = Montreal cognitive assessment

MSA = multiple system atrophy

NMS = non-motor symptoms

NMSS = non-motor symptom scale

PD = Parkinson's disease

PDD = Parkinson's disease dementia

PD-MCI = Parkinson's disease mild cognitive impairment

PDQ8 = Parkinson's disease quality of life 8 part scale

PIGD = postural instability and gait difficulty

PRoBaND = Parkinson's Repository of Biomarkers and Networked Datasets

PSP = progressive supranuclear palsy

PYY = peptide YY

SCOPA-AUT = autonomic symptom questionnaire

Chapter 1 Introduction

Parkinson's disease (PD) is the second commonest neurodegenerative disorder in the UK after Alzheimer's disease (Tanner and Aston, 2000). Diagnosis is based upon the identification of cardinal motor symptoms however non-motor symptoms are evident in all stages of the disease.

Gastrointestinal (GI) dysfunction is one of the commonest examples of non-motor impairment associated with PD. Pathological and functional abnormalities have been described at all levels of the GI tract. Most GI abnormalities associated with PD are attributable to impaired motility in the tract (Cloud and Greene, 2011). At the level of the stomach, impaired motility may cause delayed gastric emptying or gastroparesis. Gastroparesis has been demonstrated in all stages of PD. However, the clinical presentation of delayed gastric emptying in people with PD is poorly understood.

This introductory chapter will review the current literature relating to gastroparesis in PD. An abridged version of this introduction has been published as a review article in Movement Disorders (Marrinan *et al.*, 2014).

The broad themes of this thesis are the exploration of gastroparesis symptoms in people with PD and the evaluation of a novel treatment for delayed gastric emptying in PD.

1.1 Parkinson's disease

1.1.1 Epidemiology

PD is the second commonest neurodegenerative condition after Alzheimer's disease and the commonest movement disorder after essential tremor (Tanner and Aston, 2000). Varied prevalence rates are reported, which is likely to reflect differences in study methodologies and diagnostic stipulations. In Europe the prevalence of PD is estimated to be between 100 and 200 cases per 100,000 of the population (von Campenhausen *et al.*, 2005; Alves *et al.*, 2008). Incidence rates, which may be more accurate than estimates of prevalence (Alves *et al.*, 2008), range from 8.6 to 19.0 per 100,000 of the population (Twelves *et al.*, 2003).

PD affects all ethnicities although some geographical variation in incidence has been described, with higher rates of the disease reported in the developed rather than the developing world. However, this may simply reflect greater diagnostic accuracy and a longer life expectancy in countries with more developed health care systems (Alves *et al.*, 2008).

The greatest single risk factor for the development of PD is advancing age. The average age of onset is 60 years old (Lees *et al.*, 2009). Approximately 1% of those aged over 60 years old are affected and in those aged over 80 years, the prevalence is estimated to be 4% (de Rijk *et al.*, 1995; Fahn, 2003; Nussbaum and Ellis, 2003). PD is a progressive, incurable neurodegenerative disease. The average life expectancy from the point of diagnosis is approximately 12 years (Hely *et al.*, 2008).

1.1.2 Aetiology

The aetiology of PD is unknown and is likely to be multifactorial. In addition to advancing age, other risk factors identified include male gender, genetic predispositions and environmental exposures. Although advancing age is the greatest risk factor for the development of PD, approximately 4% of all cases develop clinical signs of the disease prior to the age of 50 years old (Van Den Eeden *et al.*, 2003). Genetic abnormalities may account for some of these young onset cases.

The risk of developing PD is generally reported to be greater for men than women, particularly so with advancing age (Taylor *et al.*, 2007). A meta-analysis which adjusted for age, reported the ratio of male to female incident PD cases was 1.46 : 1 (Taylor *et al.*, 2007). The reasons for this gender imbalance are unclear. It has been postulated that female hormones may be neuroprotective or that men have greater risk of occupational exposure to neurotoxins (Alves *et al.*, 2008).

The extent to which genetic abnormalities contribute to the risk of developing PD is complex and an evolving area of understanding. Individuals who have a first degree relative with PD have approximately a 3 to 4 fold increased risk of developing the disease (Autere *et al.*, 2000; Kurz *et al.*, 2003). Monogenetic causes of PD account for fewer than 5% of cases (Alves *et al.*, 2008). However, the study of cases of familial PD has led to the identification of pathogenic mutations in several genes including: alpha-synuclein, LRRK2, Parkin, DJ-1 and PINK-1. A detailed description of these mutations and their functional consequences is beyond the scope of this thesis, but there is good evidence for both autosomal dominant (Gasser *et al.*, 1998; Leroy *et al.*, 1998; Paisan-Ruiz *et al.*, 2004; Zimprich *et al.*, 2004) and autosomal recessive patterns of inheritance (Kitada *et al.*, 1998; Valente *et al.*, 2001; van Duijn *et al.*, 2001). The relevance of genetics to non-familial, idiopathic PD is less clear. The LRRK2 mutation is the commonest identifiable cause of dominantly inherited PD (Gasser *et al.*, 2011) and causes a parkinsonian phenotype very similar to idiopathic PD (Aasly *et al.*, 2005; Haugarvoll *et al.*, 2008). However, some LRRK2 mutations may be better characterised as contributory rather than causal risk factors (Gasser *et al.*, 2011). In a study from China, the G2385R mutation of the LRRK2 gene was found in 9% of PD patients but was also identified in 3% of healthy controls (Farrer *et al.*, 2007).

Several environmental factors have been identified as potential risk factors for the development of PD. Cumulative exposure to pesticides (Priyadarshi *et al.*, 2000; Lai *et al.*, 2002) and chemical solvents (Guehl *et al.*, 1999) may increase the life-time risk of PD. Repeated head injury has historically been cited as a risk factor for the development of PD but recent studies have called into question this aetiological association (Fang *et al.*, 2012). Conversely, some environmental exposures may actually reduce the risk of developing PD, namely cigarette smoking and high caffeine

intake (Ross *et al.*, 2000; Hernan *et al.*, 2002). The role of nicotine and caffeine as potential neuroprotective targets is still being explored.

In summary, the exact aetiology of PD is still uncertain. However, it appears likely that there is not one single cause but perhaps a complex interplay of multiple risk factors including genetic and environmental factors.

1.1.3 Pathology and pathogenesis

The pathological hallmark of PD is abnormal accumulation of alpha-synuclein in the basal ganglia. Alpha-synuclein is a neural protein which for unknown reasons mis-folds and aggregates in inclusion bodies. In nerve cells, these inclusion bodies are called Lewy bodies (LBs) and in neural processes, the spindle-like aggregations are called Lewy neurites (LNs). Some neurons appear particularly vulnerable to abnormal accumulation of Lewy pathology, namely long-axoned neurons with little or no myelination (Braak and Del Tredici, 2004). There is evidence that alpha-synuclein can spread between adjacent neurons (Danzer *et al.*, 2007; Danzer *et al.*, 2009; Desplats *et al.*, 2009; Angot *et al.*, 2012) in a manner likened to some prion-mediated diseases (Olanow and Prusiner, 2009; Angot *et al.*, 2010).

The spread of alpha-synuclein through the brain occurs in a predictable and progressive manner. The first region of the brain affected by Lewy pathology is the brainstem. Thereafter alpha-synuclein spreads in an ascending caudo-rostral direction, affecting the basal ganglia and ultimately cortical regions of the brain. Heiko Braak and colleagues have undertaken extensive post-mortem studies to characterise the topography of alpha-synuclein spread in PD. This work led to the development of a pathological staging system for PD (Braak *et al.*, 2003) (Table 1-1). The proposed six step pathological staging system can be correlated with many of the clinical features of PD. The first three stages, in which alpha-synuclein ascends through the brainstem and lower portions of the basal ganglia, correspond with the ‘pre-motor’ phase of the disease. In the later three stages, as alpha-synuclein extends through the basal ganglia and higher cortical areas, motor signs appear and progress.

Table 1-1 Pathological staging system for PD - adapted from (Braak *et al.*, 2004)

Pathological stage	Site of Lewy pathology	Disease phase
1	Dorsal motor nucleus of the vagus nerve	
2	Gain setting nuclei	Pre-motor
3	Substantia nigra & amygdala	
4	Mesocortex & thalamus	
5	Neocortex	Motor
6	Neocortex & premotor areas	

Although the pattern of alpha-synuclein spread through the brain is specific to PD, Lewy pathology is also evident in other neurodegenerative diseases including Alzheimer's disease and Dementia with Lewy bodies (DLB). Although alpha-synuclein deposition is a prerequisite for the pathological diagnosis of PD, increasingly there is evidence to suggest that other abnormal proteins including β-amyloid and Tau may also contribute to the pathogenesis of PD (Jellinger, 2003). Evidence of mitochondrial dysfunction in PD is also emerging (Schapira and Gegg, 2011; Schapira and Jenner, 2011) and an area of increasing research interest.

1.1.4 Diagnosis

Current approaches to the diagnosis of idiopathic PD are imperfect. There are no reliable serological or cerebrospinal fluid biomarkers in early disease. Despite many advances in structural and functional brain imaging, these techniques are still only supportive to the clinical diagnosis. PD can only be diagnosed with certainty after post-mortem examination of the brain. Therefore despite advances in our understanding of the pathogenesis of PD, the diagnosis still hinges upon the recognition of cardinal clinical features.

Although PD is diagnosed clinically, studies correlating ante-mortem features and post-mortem pathological findings, have sought to refine and standardise the diagnostic process. The most widely accepted clinical diagnostic criteria are those produced by the UK Parkinson's Disease Society Brain Bank (Hughes *et al.*, 1992). These criteria were devised after the examination of brain specimens and clinical records from 100 people diagnosed with PD by consultant neurologists. From this work, a three step approach to the diagnosis of idiopathic PD has been advocated (Table 1-2). The first

step requires identification of the motor features of Parkinsonism. Secondly, features which may suggest an alternative cause of Parkinsonism should be excluded. Thirdly, additional supportive features should be sought by the clinician although such supportive features largely rely upon the passage of time and hence are of less relevance when a patient first presents.

Table 1-2 UK brain bank criteria for the clinical diagnosis of idiopathic Parkinson's disease (Hughes *et al.*, 1992)

Step 1. Diagnosis of a Parkinsonian Syndrome Bradykinesia and at least one of the following: • Muscular rigidity • Rest tremor (4-6Hz) • Postural instability
Step 2. Exclusion criteria for Parkinson's disease • History of repeated strokes with stepwise progression of parkinsonian features • History of repeated head injury • History of definite encephalitis • Oculogyric crises • Neuroleptic treatment at onset of symptoms • More than one affected relative • Sustained remission • Strictly unilateral after three years • Supranuclear gaze palsy • Cerebellar signs • Early severe autonomic involvement • Early severe dementia • Babinskis sign • Presence of cerebral tumour or communication hydrocephalus on imaging • Negative response to large doses of levodopa in absence of malabsorption • MPTP exposure
Step 3. Supportive prospective positive criteria for Parkinson's disease Three or more required for diagnosis of definite Parkinson's disease in combination with step 1: • Unilateral onset • Rest tremor present • Progressive disorder • Persistent asymmetry affecting side of onset most • Excellent response to levodopa • Severe levodopa-induced chorea • Levodopa response for 5 years or more • Clinical course of 10 years or more

Application of the UK Parkinson's Disease Society Brain Bank criteria in routine clinical practice is associated with approximately a 90% diagnostic accuracy rate, when compared with post-mortem pathological diagnosis (Hughes *et al.*, 2001). Common misdiagnoses include other parkinsonian disorders such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and vascular parkinsonism. Tremor disorders such as essential tremor and dystonic tremor may also be erroneously diagnosed as PD (Meara *et al.*, 1999; Schneider *et al.*, 2007; Newman *et al.*, 2009).

Table 1-3 Differential diagnosis for parkinsonian disorders – adapted from (Alves *et al.*, 2008)

Type of parkinsonism	Subtype / Cause
Parkinson's disease	• Idiopathic • Familial
Symptomatic parkinsonism	• Drug induced • Vascular disease • Toxic • Post-traumatic • Post-infectious • Neoplasm • Normal pressure hydrocephalus

Parkinsonism secondary to other neurodegenerative diseases	• Atypical parkinsonism
	  ○ Multiple system atrophy (MSA)
	  ○ Progressive supranuclear palsy (PSP)
	  ○ Corticobasal degeneration (CBD)
	• Dementia with Lewy bodies (DLB)
	• Alzheimer's disease

1.1.5 Motor features

As far back as 1817, when James Parkinson described the 'shaking palsy' (Parkinson, 1817), the motor features of PD have been well characterised. Bradykinesia, tremor, rigidity and postural instability are the hallmarks of Parkinsonism and underpin the clinical diagnosis (Hughes *et al.*, 1992). Despite the apparent simplicity of these four cardinal motor features, there is considerable heterogeneity in how these features present and progress.

Resting tremor is the commonest presenting symptom in PD (Hughes *et al.*, 1993) but is not universal; approximately one quarter of patients are never affected by tremor (Jankovic *et al.*, 1990; Schrag *et al.*, 2002). Postural instability which encompasses

abnormalities of gait, posture and balance, is more typical of advanced PD but can also be a presenting feature (Alves *et al.*, 2008). The classification of patients according to their predominant motor features can overlook much of the phenotypic heterogeneity. However, the distinction between tremor dominant (TD) and postural instability and gait difficulty (PIGD) subtypes has some prognostic relevance. Tremor dominant patients tend to have slower rates of motor progression (Jankovic *et al.*, 1990) and a lower risk of developing dementia (Burn *et al.*, 2003; Alves *et al.*, 2006) compared with the PIGD subtype. Therefore the TD subtype is sometimes felt to represent a more benign disease phenotype. However, such classifications are broad and dynamic, as with advancing disease severity, the proportion of patients with PIGD predominant features increases (Alves *et al.*, 2008).

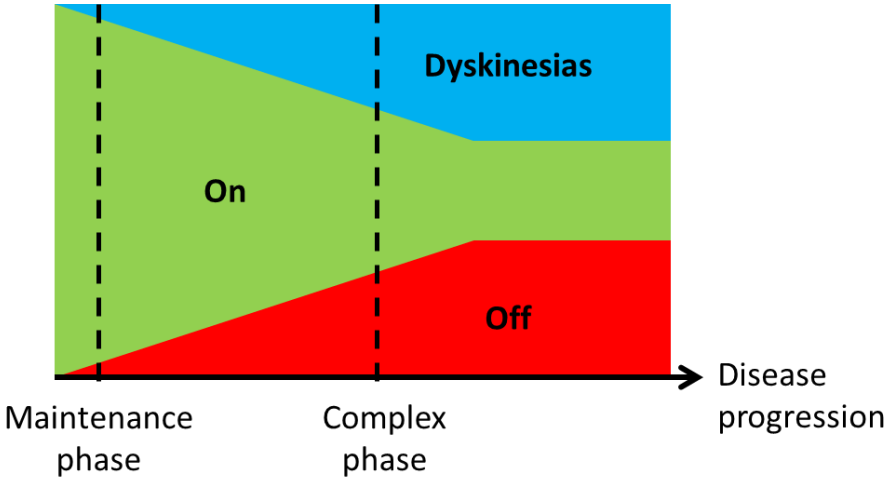
The motor features of PD usually progress along a predictable trajectory with respect to the distribution of symptoms and the rate of change. Motor signs usually present unilaterally in the upper limbs (Uitti *et al.*, 2005) before progressing to the ipsilateral lower limb after an interval of 1-3 years (Poewe and Wenning, 1998). Thereafter, the contralateral side usually develops motor signs 3-8 years after diagnosis (Poewe and Wenning, 1998). Despite the progressive nature of these abnormalities, some degree of asymmetry usually persists with the first affected side typically persisting as the most affected side (Hughes *et al.*, 2001).

In the later stages of PD, a variety of motor complications may occur; some are inherent to the disease process and others are complications of long-term treatment. In the maintenance phase of the disease, patients typically have a predictable and smooth response to medications. However, the development of response fluctuations and dyskinesias, herald the onset of the 'complex' disease phase. After 4-6 years, 40% of patients will have developed motor complications (Ahlskog and Muenter, 2001) and by 17 years they are near universal (Hely *et al.*, 2005).

Patients with response fluctuations experience unpredictable responses to their medications often culminating in less 'on' time and more 'off' time. Additionally patients with fluctuations may experience 'wearing off' and 'delayed on' phenomena which can have a major impact upon their activities of daily living and quality of life.

Associated with response fluctuations, patients may also experience dyskinesias which are involuntary choreiform movements associated with long-term levodopa therapy.

Figure 1-1 Diagram representing the development of motor complications in advancing PD - adapted from (Worth, 2013)



The greater an individuals cumulative exposure to Levodopa, the greater their risk of motor complications. Other pharmacokinetic and pharmacodynamic factors have been identified as risk factors for the development of motor complications (Table 1-4). The importance of delayed gastric emptying as a contributory factor to the development of motor fluctuations will be covered in detail in section 1.5.

Table 1-4 Factors contributing to the development of motor complications – adapted from (Worth, 2013)

Pharmacokinetic factors	• Delayed gastric emptying (gastroparesis) • Dietary amino acids competing for absorption • Short Levodopa half-life (approx. 90 minutes)
Pharmacodynamic factors	○ Loss of presynaptic 'buffering' as the nigrostriatal pathway degenerates ○ Plastic changes in the postsynaptic receptor population ○ Changes in striatal gene transcription

An array of postural, balance and gait abnormalities may also complicate the more advanced stages of the disease. In the early phase of PD, falls are an atypical feature however they increase in frequency as the disease progresses , with up to 70% of all patients falling in any one year (Hely *et al.*, 2005). Aside from disease duration and severity, other risk factors for falls include female gender, older age, autonomic dysfunction and symmetrical symptoms at diagnosis (Williams *et al.*, 2006). Freezing of gait (FOG), where patients have difficulty in initiating or maintaining walking (Stolze *et al.*, 2004), is a relatively poorly understood phenomenon which again becomes more frequent and severe as the disease process progresses (Alves *et al.*, 2008).

1.1.6 Non-motor symptoms

A great many non-motor symptoms (NMS) are recognised as part of the broad clinical phenotype of idiopathic PD(Table 1-5). Large out-patient based studies have demonstrated that NMS are extremely common in all stages of the disease. In 2007, a large, international, multi-centre observational study assessed the prevalence of NMS in patients attending routine outpatient appointments (Martinez-Martin *et al.*, 2007). A total of 545 patients with PD completed a 30 item questionnaire: the NMSQuest. Nearly all patients (98.4%) reported at least one NMS; the commonest symptoms were nocturia (61.90%), urinary urgency (55.81%) and constipation (52.48%). Older age, longer disease duration and more advanced disease stage were all significantly associated with a greater NMS burden. The PRIAMO study (Barone *et al.*, 2009) also demonstrated a high prevalence of NMS in people with PD. More than 1,000 patients attending clinic completed a structured NMS questionnaire, with 98.6% of patients reporting at least one NMS and the mean number of symptoms per patient was approximately 8.

Table 1-5 Non-motor symptoms of Parkinson's disease - adapted from (Chaudhuri and Schapira, 2009)

Neuropsychiatric • Cognitive impairment: mild cognitive impairment, dementia • Depression • Anxiety • Apathy, anhedonia • Hallucinations, illusions, delusions • Psychosis
Sleep disorders • REM sleep behaviour disorder • Restless legs • Excessive daytime somnolence • Insomnia • Vivid dreaming • Sleep-disordered breathing
Autonomic symptoms • Urinary urgency, frequency • Nocturia • Excessive sweating • Orthostatic hypotension • Erectile dysfunction
Gastrointestinal symptoms • Sialorrhoea • Ageusia • Dysphagia • Gastroparesis • Constipation • Defecatory difficulty
Sensory symptoms • Pain • Paraesthesia • Olfactory disturbance
Other symptoms • Fatigue • Diplopia • Blurred vision • Weight loss or gain

Although the number of NMS increases with advancing disease duration (Chaudhuri *et al.*, 2011), NMS are also evident in very early PD (Khoo *et al.*, 2013) and may be present at diagnosis (O'Sullivan *et al.*, 2008). Some autonomic features including constipation, urinary dysfunction, erectile dysfunction and orthostatic hypotension have been shown to pre-date the diagnosis of PD by anything from 5 to 20 years (Postuma *et al.*, 2013). These pre-motor features are being examined in the on-going Parkinson's at risk (PARS) study (Stern and Siderowf, 2010) which aims to identify groups of people at risk of PD who could then be studied for biomarkers and considered for neuroprotective interventions.

Despite the prevalence of NMS in PD, in more than half of cases, symptoms go unreported by patients or unrecognised by physicians (Shulman *et al.*, 2002; Chaudhuri *et al.*, 2010; Gallagher *et al.*, 2010). Chaudhuri et al (Chaudhuri *et al.*, 2010) explored some of the reasons underlying this and identified the following common explanations:

- *Patients were unaware that their symptoms related to PD*
- *Patients reported feeling embarrassed at discussing their symptoms*
- *Patients reported that the consultation focused upon their motor symptoms*

Recognition and management of NMS is important, as a greater NMS burden has been shown to correlate with worse quality of life scores and in some cases this effect was greater for NMS than for motor symptoms (Qin *et al.*, 2009; Gallagher *et al.*, 2010; Martinez-Martin *et al.*, 2011; Hinnell *et al.*, 2012; Sjödahl Hammarlund *et al.*, 2012).

The pathophysiology of NMS is still speculative. However, it has been suggested that many NMS could be attributable to early abnormalities in the lower brainstem nuclei (Chaudhuri *et al.*, 2011). Neurochemically, there is evidence that both dopaminergic and non-dopaminergic dysfunction is contributory to many of the recognised NMS (Chaudhuri and Schapira, 2009).

As PD progresses, the NMS burden increases (Chaudhuri *et al.*, 2011) and certain categories of impairment become more prominent. Cognitive impairment is an important non-motor complication and is not merely a feature of late disease. The point-prevalence of Parkinson's disease dementia (PDD) is estimated to be 25-30%

(Aarsland *et al.*, 2005) and ultimately approximately 80% of all patients will develop dementia (Hely *et al.*, 2008). Identification of those individuals most at risk of future dementia is important and it is now evident that mild cognitive impairment (PD-MCI) may be present at diagnosis (Foltynie *et al.*, 2004; Khoo *et al.*, 2013).

In summary, the non-motor features of PD are diverse and common. They occur in all stages of the disease and may commence in the pre-motor period of the disease. However, despite their detrimental impact upon quality of life, non-motor symptoms are often under-recognised in clinical practice.

1.2 Gastrointestinal dysfunction in Parkinson's disease

This thesis focuses upon gastric dysfunction in PD however there are many other types of gastrointestinal (GI) abnormalities associated with this condition. As will be discussed, GI dysfunction in PD is anatomically extensive, common and evident in all stages of the disease.

1.2.1 Clinical features of gastrointestinal dysfunction in Parkinson's disease

Symptoms resulting from GI dysfunction are among the commonest examples of NMS associated with PD. Almost the entire length of the GI tract is vulnerable to dysfunction (Table 1-6).

Table 1-6 Sites and types of gastrointestinal dysfunction associated with Parkinson's disease

Level of GI tract	Site	Type of dysfunction
Upper GI tract	Mouth	• Sialorrhoea • Dental deterioration
	Pharynx	• Oropharyngeal dysphagia
	Oesophagus	• Oesophageal dysphagia • Gastro-oesophageal reflux
	Stomach	• Delayed gastric emptying (gastroparesis) • Weight loss
Lower GI tract	Small intestine	○ Dilatation
	Large intestine	○ Dysmotility ○ Constipation ○ Volvulus ○ Megacolon ○ Perforation
	Rectum	○ Defecatory difficulties

1.2.2 Epidemiology of gastrointestinal dysfunction in Parkinson's disease

In large international outpatient, questionnaire-based studies (Martinez-Martin *et al.*, 2007; Barone *et al.*, 2009; Rodriguez-Violante *et al.*, 2010) approximately 30% of PD patients report at least one GI symptom at any point in time. Although GI symptoms are common in other chronic conditions and in the general population, they are significantly more prevalent in people with PD (Edwards *et al.*, 1991).

GI dysfunction is evident in all stages of disease including very early disease. A recent study (Khoo *et al.*, 2013) which assessed NMS in 159 newly diagnosed patients (median disease duration 4.4 months) and 99 age and sex-matched healthy controls, reported significantly higher rates of troublesome GI symptoms in those with early PD compared with controls. Even in later stages of the disease, the prevalence of GI symptoms in people with PD is greater than that reported by controls (Cersosimo *et al.*, 2013).

A wide array of GI symptoms are described in the context of PD but sialorrhoea, dysphagia, gastroparesis, weight loss and constipation are amongst the most common. Sialorrhoea; a sensation of excessive saliva is estimated to affect up to 70% of patients (Edwards *et al.*, 1991). Dysphagia is reported to occur in anything from 30% to 80% of people with PD (Pfeiffer, 2011). This wide range in prevalence is likely to be a consequence of different definitions of dysphagia; although symptomatic swallowing difficulties are common in PD, even greater numbers have evidence of mechanical swallowing difficulties when assessed by video fluoroscopy or barium swallow. Gastroparesis will be described in detail in later sections of this chapter but estimates suggest that 70 to 100% of all patients with PD may have delayed gastric emptying (Heetun and Quigley, 2012). Approximately half of all people with PD are estimated to have significant weight loss attributable to the disease (Abbott *et al.*, 1992). Constipation is arguably the most widely recognised GI symptom associated with PD, estimated to affect up to 89% of patients (Pfeiffer, 2011) with prevalence rates rising as the disease advances (Edwards *et al.*, 1991; Sakakibara *et al.*, 2001).

1.2.3 Aetiopathogenesis of gastrointestinal dysfunction in Parkinson's disease

Almost all examples of GI dysfunction in PD can be attributed to underlying impaired motility in the GI tract (Cloud and Greene, 2011). Sialorrhoea is a perception of excessive saliva but in fact salivary production in people with PD is normal or even reduced; rather it is a reduced frequency of swallow that accounts for this symptom (Bagheri *et al.*, 1999). Swallowing is a complex process and several stages may be vulnerable to impaired function in PD (Pfeiffer, 2003). The aetiopathogenesis of weight loss is still speculative and likely multifactorial. Proposed underlying mechanisms include: reduced energy intake, increased energy expenditure,

dyskinesias, hyposmia, dysphagia and depression (Pfeiffer, 2003; Bachmann and Trenkwalder, 2006). Constipation is one symptom clearly attributable to slowed colonic motility. Retrospective and prospective studies have demonstrated that constipation can be a premotor feature of PD (Abbott *et al.*, 2001; Abbott *et al.*, 2007; Savica *et al.*, 2009). This association offers an interesting perspective on the aetiology of PD. Constipation in the premotor period of PD may be a very early symptom or a risk factor. It is postulated that an ingested pathogen may trigger PD and if this is the case then slower GI transit could increase the exposure time to such a pathogen (Pfeiffer, 2003).

1.2.4 Pathophysiology of gastrointestinal dysfunction in Parkinson's disease

The aetiology of GI dysfunction in PD is unknown but is likely attributable to pathological changes in both the dorsal motor nucleus of the vagus nerve (DMNV) and the enteric nervous system (ENS) (Cersosimo and Benarroch, 2012). The GI tract is under both intrinsic and extrinsic neural control. Intrinsic innervation is via the ENS and extrinsic innervation involves preganglionic sympathetic and parasympathetic fibres of the vagus and sacral nerve plexuses (Cersosimo and Benarroch, 2008).

The ENS is an extensive neural network which runs the entire length of the GI tract and is sometimes referred to as the 'second brain' (Langley, 1903) as it contains nearly 100 million neurons and can function independently of the central nervous system (CNS) (Derkinderen *et al.*, 2011). The ENS consists of two ganglionated nerve plexuses; the myenteric (or Auerbach's) plexus and the submucosal (or Meissner's) plexus. The myenteric plexus primarily controls smooth muscle activity in the GI tract and the submucosal plexus regulates mucosal secretions and blood supply (Derkinderen *et al.*, 2011). These two plexuses are embedded within the walls of the GI tract: the myenteric plexus sits between the longitudinal and circular muscle layers and the submucosal plexus is located between the submucosa and circular muscle layer (Figure 1-2). Although the ENS can function autonomously, the vagus nerve synapses with the myenteric plexus in the stomach, creating a direct link between the DMNV in the medulla and the gastric ENS. This two-way interaction between the ENS and the CNS has been termed the 'brain-gut axis.'

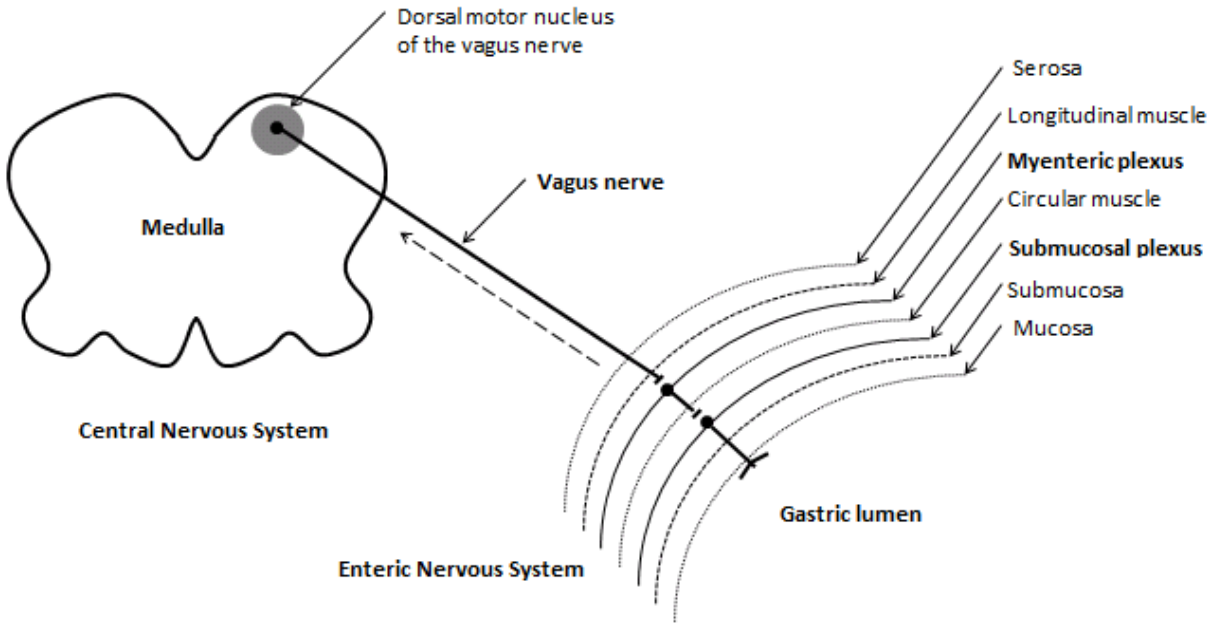


Figure 1-2 Interaction of the enteric nervous system and the vagus nerve in the stomach

One of the first cerebral sites infiltrated by Lewy pathology is the DMNV in the brainstem (Braak *et al.*, 2003). We now know that this earliest pathological feature is mirrored by alpha-synuclein aggregation beginning in the gastric ENS (Braak *et al.*, 2006). The synchronous appearance of alpha-synuclein in the DMNV and the gastric ENS and the fact that these two anatomically disparate sites are linked by the vagus nerve, underpins the so called 'dual hit hypothesis' of PD (Hawkes *et al.*, 2007; Hawkes *et al.*, 2009). It is hypothesised that PD is caused by a putative pathogen which may be carried in nasal secretions. When swallowed this pathogen could then have direct access to the ENS in the stomach with subsequent trans-synaptic spread via the vagus nerve to the DMNV. It is suggested that there is simultaneous spread to the olfactory bulb from nasal secretions which may accounting for the olfactory deficits sometimes seen in early PD (Hawkes *et al.*, 2007).

If there is an infective trigger underlying the onset of PD, identification of it remains elusive. However, another infective agent which has been considered as potentially relevant to the pathogenesis of PD is the gastric bacterium *Helicobacter pylori* (*H.pylori*). *H.pylori* infection has been reported to be a risk factor for PD (Bjarnason *et al.*, 2005; Nielsen *et al.*, 2012) and yet the prevalence of *H.pylori* infection in those with PD is not significantly higher than in the general population (Rees *et al.*, 2011; Fasano *et al.*, 2013). It may be that *H.pylori* infection is not a cause of PD but a determinant of its progression. There are reports of improved Levodopa absorption and better motor control in *H.pylori* infected patients with motor fluctuations who receive eradication therapy (Pierantozzi *et al.*, 2006; Lee *et al.*, 2008; Fasano *et al.*, 2013). However, interestingly the prevalence of *H.pylori* infection in people with fluctuations is reported to be just over 36% (Pierantozzi *et al.*, 2006) which is not significantly higher than in those without fluctuations or than the general population at large (Rees *et al.*, 2011).

As the distal bowel is relatively readily accessible to biopsy via endoscopy, several recent studies have sought to characterise the deposition of alpha-synuclein in the bowel. Lewy pathology is evident in the colonic ENS of patients with established PD (Lebouvier *et al.*, 2010) and interestingly is also present in people with early untreated PD (Shannon *et al.*, 2011). Furthermore these pathological abnormalities develop in the pre-motor period. A recent study (Shannon *et al.*, 2012) retrospectively assessed

the colonic biopsies of three patients with PD who had undergone colonoscopy several years prior to their diagnosis of PD. In all three cases, an abnormal pattern of alpha-synuclein deposition was evident in the colon prompting suggestions that alpha-synuclein in the colon could be exploited as a potential pre-motor biomarker (Olanow, 2012).

Studies of GI function and pathology in animal models of PD have also helped to develop understanding of the likely pathogenesis of GI dysfunction in this condition. Rotenone is a cytotoxic agent which if administered to rodents results in reduced striatal dopamine and serotonin, thereby replicating parkinsonism (Heikkila *et al.*, 1985). Rotenone-treated rats develop alpha-synuclein aggregates in the myenteric plexus (Drolet *et al.*, 2009a), along with delayed gastric emptying and reduced stool frequency (Greene *et al.*, 2009); changes which precede the appearance of alpha-synuclein in the brain. This reinforces the suggestion that PD may begin in the GI tract rather than the brain. Study of transgenic mice that over-express alpha-synuclein has also yielded valuable insights into the role of the gut in PD. Such transgenic mice have been reported to have impaired colonic motility (Wang *et al.*, 2008; Kuo *et al.*, 2010) and delayed gastric emptying (Noorian *et al.*, 2012). Additionally transgenic mice that over-express human alpha-synuclein develop alpha-synuclein aggregates in the ENS before their appearance in the brain (Kuo *et al.*, 2010). One particularly interesting study reported that in addition to finding alpha-synuclein in the gastric myenteric plexus of transgenic mice, they also identified alpha-synuclein in cholinergic efferent vagal nerve processes (Noorian *et al.*, 2012). A sub-study then reported that when transgenic mice underwent vagotomy, alpha-synuclein did not accumulate in the gastric ENS (Noorian *et al.*, 2012). This demonstrates the importance of the vagus nerve as the neural bridge in the 'brain-gut axis.'

Animal studies focusing on the appearance and spread of alpha-synuclein from the GI tract have also provided new insights into the pathogenesis of PD and the role of the stomach in this process. Mice chronically exposed to rotenone have been shown to develop alpha-synuclein inclusions within the myenteric plexus (Drolet *et al.*, 2009b). Additionally direct intra-gastric rotenone administration in rodents not only led to alpha-synuclein accumulation in the ENS but also remotely in the DMNV (Pan-Montojo *et al.*, 2010). However, when this experiment was repeated after lesioning the

sympathetic and parasympathetic nerves, the spread of alpha-synuclein from the ENS to the brain was halted (Pan-Montojo *et al.*, 2012). Whilst animal models of PD have inherent limitations, these studies are strongly supportive of the hypothesis that the abnormal aggregation of alpha-synuclein may originate in the stomach.

The vagus nerve and the ENS work in synchrony to regulate normal GI motility. Abnormal accumulation of alpha-synuclein in both of these sites is an early feature of idiopathic PD. It is therefore reasonable to hypothesise that these pathological changes may explain many if not all of the GI motility disturbances associated with PD (Cersosimo and Benarroch, 2012). However, there is an apparent discrepancy between pathology, functional impairment and symptoms in the GI tracts of people with PD. Whilst abnormal accumulation of alpha-synuclein in the GI tract is thought to account for impaired motility the relationship between alpha-synuclein burden and GI symptoms is not linear. A recent study (Cersosimo *et al.*, 2013) looked at the prevalence of upper and lower GI symptoms in PD patients and controls. They also attempted to address the chronology of these symptoms in relation to the onset of motor dysfunction. Although upper GI symptoms preceded motor dysfunction in more than half of PD participants questioned, the prevalence was not significantly different to the control group. The authors suggest that in the upper GI tract there is a mismatch between functional impairment and symptoms.

Gastric motility and gastroparesis

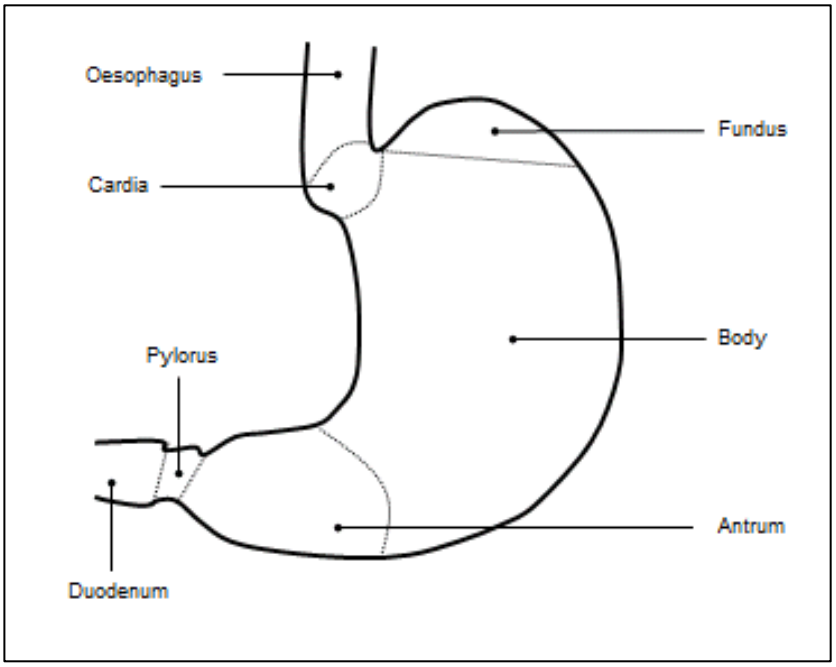
In the context of PD, the primary example of gastric dysfunction is delayed gastric emptying, also called gastroparesis. This section describes patterns of gastric motility and factors which regulate the process of gastric emptying under normal conditions, before considering gastroparesis.

1.3 Gastric motility

1.3.1 Gastric structure and function

The stomach plays an early but vital role in the complex process of digestion. The stomach acts as a large receptive chamber for ingested substances and manages the mechanical and chemical breakdown of particles, in readiness for the on-going digestion. Although the stomach has only one chamber, within it there are distinct anatomical areas with different properties and functions (Figure 1-3).

Figure 1-3 Anatomical structure of the human stomach



The stomach is functionally divided into three areas: the proximal stomach, distal stomach and pylorus (Yamada *et al.*, 2009). The proximal stomach consists of the cardia, fundus and proximal part of the stomach body. The distal stomach includes the distal part of the stomach body and the antrum. The pylorus is an anatomically small region just proximal to the duodenum but it has a major role in regulating gastric emptying.

The stomach is also an important hormonal organ; it secretes many chemicals and peptides and also responds to hormones produced elsewhere in the GI tract and other

parts of the body. There are a great many known GI hormones and there are likely to be other GI peptides that have yet to be identified. Several hormones are involved in the regulation of gastric emptying, with secretion occurring in the fed and fasted states.

1.3.2 Gastric motility in the fed state

After a meal, the first role of the stomach is to act as a receptive chamber which adapts in size to receive the ingested volume whilst maintaining a stable intra-gastric pressure: the so-called gastric accommodation reflex (Cannon WB, 1911). Gastric accommodation is mediated by a vagovagal reflex pathway (Kindt and Tack, 2006). Impaired relaxation of the proximal stomach may contribute to the symptoms of functional dyspepsia and various agents including; nitrates, sildenafil, paroxetine, cisapride & clonidine have been explored as potential therapies to improve proximal stomach relaxation (Kindt and Tack, 2006). In diabetic patients, an association between impaired gastric accommodation and impaired gastric emptying has been suggested (Samsom *et al.*, 1998) and therefore it is possible that agents targeted at augmenting proximal stomach relaxation, may also have a role in the management of gastroparesis.

After ingestion of a meal, the stomach exhibits a repetitive pattern of contractile motor waves which begin proximally and travel distally, breaking-up and mixing particles (Hellstrom *et al.*, 2006). Strong contractions against the closed pylorus help to grind and break down large food particles.

The rate of emptying for solids and liquids varies considerably. Liquids with no nutritional value leave the stomach almost immediately and at a rate proportional to their volume (Hellstrom *et al.*, 2006). However, gastric emptying of solids follows a biphasic pattern: in the first 'lag phase' particles are broken down to approximately 1-2mm diameter so that in the second 'linear emptying phase' they can pass through the pylorus easily (Hellstrom *et al.*, 2006).

Gastric emptying rate is at least partially responsive to the caloric content of a meal, such that a feedback system operates between the duodenum and stomach so that nutrients are delivered to the small bowel at a steady rate (Hunt and Stubbs, 1975;

Brener *et al.*, 1983; Collins *et al.*, 1984). This feedback system is primarily regulated by the hormone cholecystokinin (CCK) (Hellstrom *et al.*, 2006). Other peptides which contribute to this regulatory process are glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), both of which are secreted by intestinal L-cells and act via afferent vagal pathways to exert an inhibitory effect upon gastric emptying (Hellstrom *et al.*, 2006).

These mechanical and hormonal changes take place over a four hour period after ingestion of a meal (Quigley *et al.*, 1997). Thereafter, the stomach remains active but a 'fasting state' pattern of motility is ensues.

1.3.3 Gastric motility in the fasted state

Gastric motility in the fasted state follows a three phase cyclical pattern called the migrating motor complex (MMC). These stereotypic motor complexes help to clear the stomach of digestive remnants and are vital for the maintenance of normal gastric emptying. Phase I of the MMC is a quiescent period, in phase II there are irregular but escalating gastric contractions and in phase III regular intense contractions spread from the gastric body to the pylorus, clearing the stomach of any large (>2mm) digestive remnants (Hellstrom *et al.*, 2006; Yamada *et al.*, 2009). This three phase cycle repeats approximately every 90 minutes during inter-digestive periods. Once again, hormonal regulation is important in this process. Table 1-7 summarises some of the hormones known to be active in the fed and fasted states. More attention will be given to the peptides motilin and ghrelin in the following sections, as both act to stimulate the MMC and both have the potential to be utilised as treatments for delayed gastric emptying.

Table 1-7 Gastrointestinal hormones secreted in the fed and fasted state adapted from (Sanger and Lee, 2008; Khoo *et al.*, 2010)

Hormones secreted in response to a meal	Hormones secreted in the fasting state
• Gastrin • Cholecystokinin (CCK) • Leptin • Enterostatin • Peptide YY (PYY) • Apolipoprotein A-IV • Glucagon-like peptide-1 (GLP-1) • Glucagon-like peptide-2 (GLP-2) • Glucose dependent insulinotropic polypeptide (GIP) • Pancreatic polypeptide • Oxyntomodulin • Amylin	○ Motilin ○ Ghrelin ○ Somatostatin ○ Xenin ○ Orexin A and B

1.3.4 Motilin

Motilin is an endogenous peptide which was first described in the early 1970s (Brown *et al.*, 1971; Brown *et al.*, 1972; Schubert and Brown, 1974). Motilin and its receptors are found throughout the GI tract but are mostly concentrated in the duodenum (De Smet *et al.*, 2009). Motilin receptors have also been found in lower numbers elsewhere in the body, including in brain, thyroid and bone marrow (De Smet *et al.*, 2009). Soon after the identification of motilin, it was demonstrated that this peptide helps trigger phase III of the MMC and hence can enhance gastric emptying (Christofides *et al.*, 1979; Christofides *et al.*, 1981). However, it appears likely that the prokinetic effect of motilin and its agonists is mediated by changes in cholinergic and vagal activity (Sanger, 2014). Low doses of motilin have been reported to result in increased activity in vagal afferent fibres of the stomach (Sanger *et al.*, 2013) and motilin agonists may modify vagal activity in the upper GI tract (Mathis and Malbert, 1998; Suzuki *et al.*, 1998).

Macrolide antibiotics (including erythromycin, azithromycin and clarithromycin) are motilin agonists and have been shown to enhance gastric emptying rates (Janssens *et al.*, 1990; Urbain *et al.*, 1990; Arts *et al.*, 2005; Abell *et al.*, 2006; Larson *et al.*, 2010; Moshiree *et al.*, 2010). Two non-antimicrobial motilin agonists (ABT-229 and Mitemcinal) have been studied in diabetic and idiopathic gastroparesis. However, in a

randomised, double blind trial comparing ABT-229 with placebo in patients with type 1 diabetes and GI symptoms, the motilin agonist did not significantly improve symptoms compared with placebo (Talley *et al.*, 2001). Two randomised controlled trials looking at Mitemcinal, reported improved GI symptoms (McCallum and Cynshi, 2007b) and significantly increased gastric emptying rates in in people with diabetic and idiopathic gastroparesis (McCallum and Cynshi, 2007a). The success of motilin agonists in the treatment of gastroparesis have to date been modest but there is evidence to suggest that more selective, small molecule motilin agonists, given at low doses may be effective prokinetic agents (Sanger, 2014). Chapter 3 of this thesis considers a novel motilin agonist (Camicinal) as a treatment for delayed gastric emptying in people with PD and motor fluctuations.

1.3.5 Ghrelin

Ghrelin is an endogenous 28 amino acid peptide, first described in 1999 (Kojima *et al.*, 1999). It is produced in the stomach but the growth hormone secretagogue receptor (GHSR) at which it acts has been identified at numerous sites in the GI tract, the ENS and also parts of the CNS. In the GI tract ghrelin exerts prokinetic effects and centrally it enhances appetite (Ejskjaer *et al.*, 2009). Ghrelin levels peak before eating and fall rapidly as ingestion starts. The hormone is regarded as a key orexigenic hormone. Although ghrelin is a gastric derived hormone, its secretion appears to be at least in part dependent upon vagal activity as following vagotomy in rodents, the expected pattern of ghrelin secretion is not seen (Williams *et al.*, 2003). This may occur because GHSRs are located distally in the dorsal vagal complex (Unger *et al.*, 2011a).

Ghrelin has a structural homology with the gastric-derived peptide motilin (Tomasetto *et al.*, 2000) and both enhance upper GI motility. Several studies have evaluated ghrelin and ghrelin agonists as therapeutic options for diabetic gastroparesis (Murray *et al.*, 2005; Ejskjaer *et al.*, 2009; Ejskjaer *et al.*, 2013b; Shin *et al.*, 2013). In 2009 favourable results for infusions of a ghrelin receptor agonist (TZP-101) were reported in people with diabetic gastroparesis (Ejskjaer *et al.*, 2009). Significant improvements in both gastric emptying rates and gastroparesis symptoms were described. More recently, the same group (Ejskjaer *et al.*, 2013b) published results of a randomised, placebo-controlled, double-blind study of an oral ghrelin receptor agonist (TZP-102).

Gastric emptying parameters did not differ significantly between groups but gastroparesis symptoms improved in the group receiving active treatment. A further study (Shin *et al.*, 2013) looked at a different oral ghrelin agonist (RM-131) and reported significant increases in gastric emptying rates compared with placebo. More recent studies have failed to replicate these positive findings (Ejskjaer *et al.*, 2013a; McCallum *et al.*, 2013) however there remains potential for ghrelin to be a useful therapy for gastroparesis (Camilleri and Acosta, 2013).

The importance of ghrelin as a peptide operating along the 'brain-gut axis' is emphasised by the finding of ghrelin receptors in dopaminergic neurons within the substantia nigra (Guan *et al.*, 1997; Zigman *et al.*, 2006; Andrews *et al.*, 2009). Furthermore, from work with MPTP animal models of PD, there is evidence to suggest that ghrelin could have a neuroprotective role in augmenting dopamine cell loss (Williams *et al.*, 2003; Jiang *et al.*, 2008; Andrews *et al.*, 2009; Moon *et al.*, 2011).

1.3.6 Neural control of gastric emptying

Both intrinsic and extrinsic nerve pathways are involved in the regulation of gastric emptying. Intrinsic innervation comes from the ENS, which has already been extensively described (section 1.2.4). Extrinsic innervation of the stomach comes via the vagus and splanchnic nerves, both of which relay efferent and afferent fibres to and from the stomach. The vagus nerve supplies three types of efferent fibres to the stomach; preganglionic parasympathetic excitatory cholinergic fibres, preganglionic inhibitory fibres and sympathetic fibres (Liedberg *et al.*, 1973; Lundberg *et al.*, 1976; Larson and Hixenbaugh, 1983). The splanchnic nerve also provides sympathetic innervation to the stomach, and has both excitatory and inhibitory roles, but the excitatory component dominates (Yamada *et al.*, 2009).

The number of afferent vagal and splanchnic fibres supplied to the stomach vastly outnumbers the efferent component (Yamada *et al.*, 2009). Vagal afferents travel to the nucleus of the tractus solitarius which, in turn, sends projections to the DMNV (Gwyn *et al.*, 1979; Sawchenko, 1983). The importance of an intact vagus nerve for the normal regulation of gastric emptying has been demonstrated through human and animal studies after vagotomy. Post-vagotomy gastric emptying for liquids increases

but gastric emptying of solids is delayed (Wilbur and Kelly, 1973; MacGregor *et al.*, 1977; Lavigne *et al.*, 1979).

In summary gastric emptying is a complex process influenced by neural and hormonal factors. Neural regulation requires intact vagal and enteric nervous systems but both of these are vulnerable to dysfunction early in the pathogenesis of PD.

1.3.7 Measuring gastric motility - gastric emptying studies

There are several methods by which gastric emptying can be measured (Table 1-8). Scintigraphy is sometimes referred to as the gold standard however this technique requires the use of a gamma camera at regular intervals over a four hour period. This is impractical or simply impossible in some non-specialist settings and may prove inappropriate for certain patient groups, for example elderly or frail individuals. It may be a particularly difficult test in people with advanced PD and dyskinesias, who may struggle to lie still during the repeated scans, which could influence the results (Goetze *et al.*, 2005). Breath testing can be performed more readily in non-specialist centres as it requires no imaging or specialist equipment on site. Breath testing results are comparable to those obtained via scintigraphy (Ghoos *et al.*, 1993; Braden *et al.*, 1995) and the technique has good reproducibility (Choi *et al.*, 1998; Arts *et al.*, 2005). Breath testing typically involves ingestion of a meal labelled with Carbon^{13} (C^{13}) derived from octanoic acid (Perri *et al.*, 2005) or spirulina, a naturally occurring cyanobacterium. The spirulina breath test gives results comparable with those obtained by scintigraphy (Lee *et al.*, 2000).

All validated methods for measuring gastric emptying require the subject to consume a test meal. This may be solid, liquid or a mixed consistency. Increasingly it appears that solid test meals are more sensitive for detecting delayed gastric emptying and also appear to correlate better with reported symptom severity (Sachdeva et al., 2011).

It is important to highlight that whichever technique is used to measure gastric emptying, there is no universally accepted definition of what constitutes impaired or delayed gastric emptying. For each technique, normality must be defined through study of healthy controls who then serve as a reference point for defining abnormal according to that particular method.

Table 1-8 Techniques to measure gastric emptying

Testing method	Description	Advantages	Disadvantages
Scintigraphy	• Solid or liquid test meal labelled with a radioisotope (usually Tc-99m). • Gamma camera used to scan the stomach at regular intervals for up to 4 hours.	• Often felt to be the gold-standard for testing. • Reliable and reproducible.	• Specialist equipment and staff required. • Patient movement can influence results.
Breath testing	○ Solid or liquid test meal labelled with a non-radioactive isotope (^{13}Carbon). ○ Once ingested, it is absorbed in the duodenum, metabolised in the liver, oxidised to $^{13}CO_2$ and exhaled. ○ Breath samples collected at regularly over 4 hours.	○ Non-invasive. ○ No imaging or specialist equipment required at the site of testing. ○ Results are comparable with scintigraphy. ○ Reliable and reproducible.	○ Reproducibility may be slightly inferior to scintigraphy.
Other imaging modalities	▪ MRI and ultrasound have been piloted but are as yet not widely accepted tests.	▪ Non-invasive.	▪ Experienced radiologists required to interpret images. ▪ Reliability and reproducibility unknown.

1.3.8 The effect of age and gender on gastric motility

Before discussing the impact of pathological processes on gastric motility, it is important to consider the effect of age and gender upon gastric emptying in healthy individuals.

One study has reported advancing age to be associated with slower gastric emptying (Brogna *et al.*, 1999). However, gastric emptying in this study was measured via ultrasonography which at present is not a validated technique. Other studies using scintigraphy reported no significant effect of age upon gastric emptying (Moore *et al.*, 1983; Madsen and Graff, 2004).

Evidence regarding the effects of gender upon gastric motility is also equivocal. Some studies have reported slower gastric emptying rates in women compared with men (Madsen and Graff, 2004).

1.4 Gastroparesis

1.4.1 Definition

Gastroparesis is defined as a chronic, symptomatic disorder of delayed gastric emptying without an underlying obstructive cause (Parkman *et al.*, 2004). The term is sometimes attributed to patients with suggestive symptoms and risk factors for gastroparesis. In order to definitely diagnose gastroparesis, gastric emptying studies must be performed (Quigley, 2000). However, these tests are not widely available in clinical practice and can be difficult to perform in some patients Therefore, the symptoms of gastroparesis are often used as a marker for at risk groups or individuals.

1.4.2 Epidemiology and aetiology

Relatively few studies have addressed the epidemiology of gastroparesis in the general population. A large community study in the USA (Jung *et al.*, 2009) reported an age-adjusted incidence of 2.4 per 100,000 person-years for men (95% CI, 1.2-3.8) and 9.8

for women (95% CI, 7.5-12.1). From this same study, the reported prevalence of gastroparesis for men was 9.6 per 100,000 (95% CI, 1.8-17.4) and for women 37.8 per 100,000 (95% CI, 23.3-52.4). Other studies have also shown gastroparesis to be more common in women (Soykan *et al.*, 1998; Dudekula *et al.*, 2011), which may reflect the tendency for slower gastric emptying rates even in healthy women (Graff *et al.*, 2001). Table 1-9 summarises some of the recognised causes of gastroparesis. The commonest aetiologies are idiopathic (36-61%) and diabetic (10-13%), with PD estimated to account for 7.5% of cases (Soykan *et al.*, 1998; Dudekula *et al.*, 2011).

Table 1-9 Causes of gastroparesis - Adapted from (Quigley, 2000)

Endocrine / Metabolic disturbances	• Diabetes mellitus • Hypothyroidism • Uraemia • Amyloidosis • Pregnancy
Neurological disease	○ Parkinson's disease ○ Muscular dystrophy ○ Spinal cord disease ○ Brain stem tumours ○ Peripheral neuropathy
Connective tissue disorders	• Scleroderma • Systemic lupus erythematosus
Gastrointestinal disorders	○ Gastro-oesophageal reflux ○ Peptic ulcers ○ Viral gastritis ○ Chronic intestinal pseudo-obstruction
Medications	• Anticholinergic agents • Opiates • Dopaminergic drugs • Chemotherapeutic agents
Other	○ Idiopathic ○ Post- surgery ○ Following radiation therapy ○ Chronic liver disease ○ Anorexia nervosa

1.4.3 Clinical features

Gastroparesis can result in a variety of upper GI symptoms including; nausea, vomiting, retching, early satiety, bloating, loss of appetite and epigastric pain. A retrospective study (Parkman *et al.*, 2011) considered the presenting symptoms in 243 patients with idiopathic gastroparesis. The three commonest presenting symptoms were nausea

(34%), abdominal pain (23%) and vomiting (19%). Another recent study (Rey *et al.*, 2012) reported that in 104 patients with established gastroparesis, the commonest persistent upper GI symptoms were nausea and vomiting (92.3%), early satiety (84.6%), regurgitation (72.1%), loss of appetite (70.2%) and bloating (65.4%).

One of the difficulties in assessing patients with possible gastroparesis is that many of the associated symptoms are shared by other disorders of the upper GI tract, namely functional dyspepsia (Parkman *et al.*, 2004). One study (Sarnelli *et al.*, 2003) which measured gastric emptying rates in people with apparent functional dyspepsia, reported that over one third actually had evidence of delayed gastric emptying. Hence there is a degree of overlap between these two diagnoses.

1.4.4 Quantified assessment of gastroparesis symptoms

In clinical practice and research settings, it is important to have a consistent method for assessing and quantifying the symptoms associated with gastroparesis. The patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM) (Rentz *et al.*, 2004) was designed as a self-reporting tool to be used by patients with a variety of upper GI disorders. From this general tool, a more specific instrument was constructed specifically for gastroparesis symptoms. The gastroparesis cardinal symptom index (GCSI) (Revicki *et al.*, 2003) is a nine item questionnaire which sub-divides into three symptom clusters (Appendix B). Patients complete three questions regarding nausea and vomiting, four questions regarding fullness and two questions about bloating. They are instructed to recall their typical symptoms over the preceding two-weeks. Each symptom is graded in severity on an incremental six-point scale (0 = none, through to 5 = very severe). The GCSI was validated in 169 patients with gastroparesis and correlated well with patients' overall perceptions of symptom severity and with physicians' assessments (Revicki *et al.*, 2003).

A global measure of gastroparesis symptom severity can be calculated from the nine-item questionnaire. The 'GCSI total score' is a composite score that is derived from the mean of the three subsections. The GCSI total score ranges from 0 to 5 with higher scores representing a greater symptom burden. Revicki et al (Revicki *et al.*, 2003) reported 2.56 to be the mean GCSI total score for the 169 patients in their validation study hence this score is often taken to represent significant gastroparesis symptoms.

A GCSI total score of ≥1.90 has also been proposed as a marker of significant gastroparesis symptoms (Kofod-Andersen and Tarnow, 2012).

A daily diary version of the GCSI has also been devised (the GCSI-DD) (Revicki *et al.*, 2009) which includes two additional questions regarding upper abdominal discomfort and pain (Appendix B). The GCSI-DD has been validated for reliability and responsiveness to changing symptoms (Revicki *et al.*, 2012) and is recommended for use in a clinical trial setting.

The GCSI was designed as a tool to assess symptoms in people with known gastroparesis but in the context of diabetes, it has been used as a screening tool with which to identify possible gastroparesis (Kofod-Andersen and Tarnow, 2012). Although the GCSI was not intended as a screening tool, it is the most comprehensive method for assessing gastroparesis symptoms and hence is utilised in this way in clinical and research settings.

1.4.5 Relationship between gastric emptying delay and symptoms

The relationship between the severity of gastric emptying delay and symptoms is only partially understood. One reason may be that studies have often used different methods to measure gastric emptying and to assess symptoms. However, certain upper GI symptoms recurrently emerge as correlating with delayed gastric emptying, namely, nausea and vomiting, post-prandial fullness, early satiety and inability to finish a normal sized meal (Stanghellini *et al.*, 1996; Sarnelli *et al.*, 2003; Stanghellini *et al.*, 2003; Talley *et al.*, 2006; Cassilly *et al.*, 2008; Grad *et al.*, 2012). A recent study (Olausson *et al.*, 2013) assessed gastric emptying and symptoms in 115 patients with insulin treated diabetes and either GI symptoms, a clinical suspicion of gastroparesis, or poor glycaemic control. They used the GCSI to assess gastroparesis symptoms and reported that the subsections of this tool which significantly correlated with severity of gastric emptying delay were the nausea and vomiting items ($r = 0.30$; $P < 0.001$) and the fullness questions ($r = 0.34$, $P < 0.0001$). Such paired evaluations of gastric emptying and gastroparesis symptoms has not previously been performed in the context of PD but will be addressed in this thesis.

1.4.6 Non-interventional management of gastroparesis

The mainstay of management for gastroparesis is pharmacological therapy although the agents currently available remain somewhat suboptimal.

Dopamine receptor antagonists such as metoclopramide and domperidone have been shown to improve gastric emptying rates and gastroparesis symptoms (Abell *et al.*, 2006) however both agents have their limitations. Metoclopramide is a systemically acting dopamine receptor antagonist and can cross the blood brain barrier. This means that with long-term use it can cause extrapyramidal side effects. Domperidone is a peripherally acting dopamine antagonist and as such can be used in the context of PD, although it is not currently approved for use in the United States.

Cisapride is a 5-HT4 receptor agonist with a potent prokinetic effect. It was used extensively in the 1990s to manage gastroparesis however it was later shown to be associated with an increased risk of arrhythmia and sudden death and was therefore withdrawn from the market in 2000 (Smalley *et al.*, 2000; Quigley, 2011).

As previously described, macrolide antibiotics such as erythromycin, azithromycin and clarithromycin are motilin agonists and as such can be used in the treatment of delayed gastric emptying (Arts *et al.*, 2005; Abell *et al.*, 2006; Larson *et al.*, 2010; Moshiree *et al.*, 2010). Motilin agonists without antimicrobial activity have been explored in only a few studies to date with mixed results reported (Talley *et al.*, 2001; McCallum and Cynshi, 2007a; McCallum and Cynshi, 2007b). Ghrelin agonists are not currently licenced for the treatment of gastroparesis, but initial studies suggest that they may have potential as a novel therapeutic option (Murray *et al.*, 2005; Ejskjaer *et al.*, 2009; Ejskjaer *et al.*, 2013b; Shin *et al.*, 2013).

The fact that gastric emptying can be measured in a variety of ways means that meta-analysis to compare the effects of different prokinetics on gastric motility is extremely difficult. However, a systematic review published in 1999 (Sturm *et al.*, 1999) suggested that in terms of joint improvements in gastric emptying and symptoms, the motilin agonist erythromycin was probably superior to dopamine receptor antagonists.

1.4.7 Interventional management of gastroparesis

Interventional management strategies for gastroparesis are reserved for those rare cases that are refractory to all available medical therapies. Implantation of a gastric electrical stimulator (GES) or gastric pacemaker has been reported to improve symptoms and to a lesser degree gastric emptying rate, although complication rates are relatively high (Abell *et al.*, 2003; Lin *et al.*, 2005). Intra-pyloric injection of botulinum toxin (botox) has also been tried in several studies but a recent systematic review suggests that there is currently insufficient evidence to recommend this as a routine treatment for gastroparesis (Bai *et al.*, 2010).

Gastroparesis in Parkinson's disease

Gastroparesis is the commonest example of gastric dysfunction in PD. The features and clinical associations of gastroparesis in the context of PD will be discussed in this section including its wider implications for the absorption of Levodopa and PD motor control more generally.

1.5 Gastroparesis in Parkinson's disease

1.5.1 Epidemiology

The prevalence of delayed gastric emptying in PD is unknown. The main studies to have considered gastric emptying and gastroparesis in PD are summarised in Table 1-10. The sample size in these studies is relatively small; the largest included 80 patients (Goetze *et al.*, 2006). This reflects the complexity and time-consuming nature of gastric emptying studies, particularly in people with PD where motor dysfunction may prove a challenge to some testing methods.

The mixed methodologies of previous studies, prevents a meta-analysis. However a recent systematic review of the literature concluded that gastroparesis is likely to affect 70-100% of all people with PD (Heetun and Quigley, 2012). This suggests that gastroparesis is a common problem, although it is arguably under-recognised.

Table 1-10 Summary of existing studies evaluating gastroparesis in Parkinson's disease

Author (reference)	Key aim of the study	Subject demographics	Gastric emptying study method	Key results	Limitations / comments
Djaldetti (Djaldetti *et al.*, 1996)	Comparison of GE rates in PD patients (with and without motor fluctuations) and healthy controls.	PD without motor fluctuations(n=15): ○ Age: 64.8±9.9y. ○ DD: 4.0±3.3y. PD *with* motor fluctuations(n=15): ○ Age: 62.0±9.0y. ○ DD: 8.8±4.6y. Healthy controls (n=22): ○ Age: 45.0±3.5y.	• Scintigraphy. • Solid test meal (99m-technetium colloid). • Fasted prior to test.	○ Delayed GE defined as the upper limit of the controls. ○ All 30 PD patients had delayed GE. ○ GE delay was significantly greater in those with motor fluctuations than in those without.	▪ Controls not age matched. ▪ GE rates for PD patients more heterogeneous than controls. ▪ Scintigraphy results are affected by patient movement. ▪ Not all non-fluctuators were on levodopa. ▪ Timing of last levodopa dose not stated.
Hardoff (Hardoff *et al.*, 2001)	Comparison of GE rates in PD patients (mild and moderate disease stage) and age-matched healthy controls.	Mild PD (H&Y:1-2) (n=29): ○ Age: 61.5±5.9y. Moderate PD (H&Y:2.5-3) (n=22): ○ Age: 65.1±5.6y. Healthy controls (n=22): ○ Age: 61.9±6.1y.	• Scintigraphy. • Solid test meal (99m-technetium colloid). • Fasted prior to test. • PD patients assessed in 'on' state.	○ Delayed GE defined by the upper limit of the controls. ○ 48.3% of those with mild PD & 36.4% of those with moderate PD had delayed GE. ○ Patients with motor fluctuations had quicker GE rates than those without.	▪ Heterogeneous GE results amongst all PD patients. ▪ Assessments undertaken in the 'on' state, unlike most other studies which may explain why fluctuators had better GE rates.

Goetze (Goetze *et al.*, 2005)	Comparison on GE rates in PD patients (of different disease stages) and age-matched healthy controls.	PD patients (n=36): ○ Age: 63.6±11.2y. Healthy controls (n=22): ○ Age: 63.6±6.8y.	• ^{13}C-octanoic acid breath test. • Solid test meal. • Breath samples obtained for 4 hours after ingestion of meal. • Fasted overnight.	○ GE delayed in 97% of PD patients. ○ Disease duration did not correlate with degree of GE delay but more severe PD was associated with worse GE delay.	● GE rates in PD patients very heterogeneous, which may reflect small subject numbers.
Thomaides (Thomaides *et al.*, 2005)	Assessed GE rates in PD, MSA and healthy controls.	PD patients (n=12): ○ Age: 57.9±10.1y. MSA patients (n=12): ○ Age: 59.1±9.8y. Healthy controls (n=12): ○ Age: 58.4±9.2	• Scintigraphy. • Semi-solid test meal labelled with 99m-technetium colloid. • All patients were off anti-parkinsonian medications for 4 days before test.	○ MSA & PD patients had significantly slower rates of GE than controls.	▪ A semi-solid test meal was used, which differs from the majority of studies. ▪ Direct comparison with other studies is therefore limited.
Goetze (Goetze *et al.*, 2006)	Comparison of solid and liquid GE rates in patients with PD and healthy controls.	PD patients given liquid test meal (n=40): ○ Age: 65±8y. ○ DD: 5.8±4.0y. PD patients given solid test meal (n=40): ○ Age: 63±11y. ○ DD: 6.2±4.8y. Healthy controls given liquid and solid test meals (n=40): ○ Age: 61y.	• Fasted and off medications for at least 12 hours. • Oral breath test with ^{13}C-acetate labelled liquid or ^{13}C-octanoic acid labelled solid meal.	○ No significant difference in liquid GE between PD and controls. ○ GE of solid test meal was significantly slower in PD than controls. ○ Slower GE of solids, associated with advancing PD, tremor & rigidity.	▪ No clear definition of delayed GE offered by authors.

Krygowska-Wajs (Krygowska-Wajs *et al.*, 2009)	Comparison of GE rates in familial PD, sporadic PD and healthy controls.	Familial PD defined as 2 or more affected individuals within 3 generations, without a recognisable genetic aetiology. Familial PD (n=10): ○ Age: 59±8.2y. Sporadic PD (n=35): ○ Age: 60.5±9.9y. Healthy controls (n=15): ○ Age:59.5±9.7y.	• Scintigraphy following a solid test meal labelled with 99m-technetium.	○ GE was slower than controls in 70% of those with familial PD but only 55% of those with sporadic PD.	▪ Scintigraphy images were only obtained for 92 minutes after the test meal was ingested. ▪ The timing of the last dose of levodopa was not specified.
Tanaka (Tanaka *et al.*, 2009)	Comparison on GE rates in PD patients (with and without motor fluctuations) and healthy controls.	PD patients with motor fluctuations (n=20). PD patients without motor fluctuations (n=20). ○ median age of all 40 patients: 67.0y. Healthy controls (n=20): ○ median age: 69.0y.	• Overnight fast. • No levodopa for 24 hours prior to testing. • Oral breath test following a ^{13}C-sodium acetate labelled liquid test meal.	○ GE was significantly delayed in PD patients. ○ No significant difference between fluctuators and non-fluctuators.	▪ The liquid test meal may be less sensitive than solid meal in detecting GE delay in PD.
Tanaka (Tanaka *et al.*, 2011)	Comparison of GE rates in early PD patients (untreated), patients with advanced (treated) PD and healthy controls.	Early, untreated PD (n=20): median age: 70.5y. Advanced, treated PD (n=40): median age: 67y. Healthy controls (n=20): median age: 69y.	• Overnight fast. • No levodopa for 24 hours prior to testing. • Oral breath test following a ^{13}C sodium acetate labelled liquid test meal.	○ Delayed GE was evident in early untreated and advanced cases of PD.	▪ Liquid test meal may be less sensitive than solid meals.

Key: *DD = disease duration, GE = gastric emptying,* age and disease duration given as mean ± standard deviation unless otherwise specified

1.5.2 Aetiology

As previously described (section 1.2.4) impaired GI motility in PD is likely attributable to abnormal pathology in the ENS and the DMNV. The same pathophysiology is implicated in PD associated gastroparesis.

1.5.3 The relationship between gastric emptying delay and gastroparesis symptoms in Parkinson's disease

Exploration of the association between symptom severity and gastric emptying delay is important for two reasons. Firstly it is relevant to our understanding of the links between pathology, physiology and symptoms. Secondly, as gastric emptying studies are time consuming and may be impractical for some patients (e.g. those with frailty or advanced motor symptoms), the exploration of symptoms which are predictive of gastric emptying delay could help to identify patients at risk of gastroparesis without the need for prolonged testing.

Based upon the current evidence, it is difficult to draw any definitive conclusions regarding associations between gastric emptying delay and GI symptoms in PD. Two studies have reported no correlation (Hardoff *et al.*, 2001; Goetze *et al.*, 2005) whilst one suggested that upper GI symptoms were commoner in those patients with delayed gastric emptying (Goetze *et al.*, 2006).

Although not a clinical feature of gastroparesis, constipation has been reported to be more common in patients with delayed gastric emptying (Unger *et al.*, 2011b). This is an interesting association which has also been demonstrated in rodent models (Greene *et al.*, 2009). It is likely that this represents a continuum of impaired motility extending through the upper and lower GI tracts.

Little work has been done to assess if particular parkinsonian features are associated with delayed gastric emptying. To date one study (Goetze *et al.*, 2006) has reported that rigidity and tremor are independent predictors of delayed gastric emptying ($r = 0.68$, $P < 0.001$). This is an area worthy of further exploration and has practical relevance in the identification of patients at risk of gastroparesis.

1.5.4 Disease duration and gastric emptying in Parkinson's disease

One might hypothesise that as disease duration advances and Lewy pathology becomes more extensive, gastric emptying speed may consequently deteriorate. However, almost all of the research to date has found no significant association between disease duration and gastric emptying delay (Djaldetti *et al.*, 1996; Hardoff *et al.*, 2001; Goetze *et al.*, 2005; Thomaides *et al.*, 2005; Goetze *et al.*, 2006; Tanaka *et al.*, 2011; Unger *et al.*, 2011b).

One study (Tanaka *et al.*, 2011) which elegantly demonstrated the apparent lack of association between disease duration and gastroparesis, assessed gastric emptying in three groups: 20 patients with early untreated PD (median age 70.5 years, median disease duration 0.9 years), 40 patients with established treated PD (median age 67.0 years, median disease duration 6.0 years, median daily Levodopa intake 400mg) and 20 healthy controls (median age 69.0 years). The gastric emptying half time (GE $t_{1/2}$) of people with early untreated PD was significantly slower than controls (median 2.04 vs. 1.44 hours, $P < 0.001$) but there was no significant difference between GE $t_{1/2}$ in those with early PD and those with advanced disease ($P = 0.77$).

Another group (Unger *et al.*, 2011b) assessed gastric emptying in people with idiopathic REM sleep behaviour disorder (iRBD), a recognised pre-motor feature of PD. Gastric emptying was measured in 13 people with iRBD, 21 drug-naïve PD patients, 18 people with established treated PD and 20 healthy controls. Once again, significantly slower gastric emptying rates were evident in PD patients compared to controls ($P < 0.001$) but there was no significant difference between early and advanced PD and iRBD subjects were not significantly different to controls.

1.5.5 Disease severity and gastric emptying in Parkinson's disease

As PD is a progressive degenerative disease, disease duration and severity are inherently linked. Although disease duration does not appear to be a predictor of delayed gastric emptying, there is evidence to suggest that disease severity does influence gastric emptying.

A 2005 study by Goetze et al (Goetze *et al.*, 2005) compared gastric emptying in 36 patients with PD and 22 age-matched controls. When the PD cohort were stratified by

disease severity (according to UPDRS part III score and Hoehn & Yahr stage), those with more severe PD had significantly slower gastric emptying. A later, larger study by the same group (Goetze *et al.*, 2006) reported significantly longer GE $t_{1/2}$ in subjects with higher UPDRS part III scores (≥45) compared to those with lower scores (<45) (181 ± 49 mins vs. 156 ± 30 mins, respectively, $P < 0.05$). When compared according to Hoehn & Yahr stage, the same trend was evident (H&Y 3-5: GE $t_{1/2}$ 191 ± 44 mins vs. H&Y 1-2.5: GE $t_{1/2}$ 149 ± 28 mins, $P < 0.01$).

One study (Hardoff *et al.*, 2001) has reported that delayed gastric emptying is more prevalent in mild rather than severe PD. However, this study assessed patients' gastric emptying in the 'on' state, with no medication wash-out period whereas the majority of studies have assessed gastric emptying after a 12 hour medication-free period.

Overall, the available evidence would seem to suggest that more advanced disease severity is associated with slower gastric emptying.

1.5.6 The effect of Levodopa on gastric emptying

The relationship between Levodopa ingestion and gastric emptying is interdependent. Levodopa affects gastric emptying but additionally gastric emptying rates affect Levodopa absorption. Both aspects of this bidirectional relationship will be considered.

Levodopa is the oldest but still one of the commonest treatments for PD. For several decades it has been suggested that Levodopa delays gastric emptying. In 1980, it was reported that healthy young men (mean age 42 years) given oral Levodopa (single dose of 1,000mg) had delayed gastric emptying which was reversible with co-administration of metoclopramide (Berkowitz and McCallum, 1980). A subsequent study (Robertson *et al.*, 1990) assessed gastric emptying after ingestion of a more typical therapeutic dose of oral Levodopa (125mg) and also reported delayed gastric emptying although the effect was not exacerbated by repeated doses (Waller *et al.*, 1991). In healthy older men (mean age 73 years) Levodopa administration has also been shown to result in slower gastric emptying (Robertson *et al.*, 1992).

Although Levodopa may contribute to delayed gastric emptying in PD it is certainly not the sole cause as gastroparesis has been described in early untreated PD (Tanaka *et al.*, 2011; Unger *et al.*, 2011b).

1.5.7 The effect of delayed gastric emptying on Levodopa absorption

Levodopa is a large amino acid which is de-carboxylated into dopamine and O-methylated into 3-O-methyldopa (Nyholm and Lennernas, 2008). Absorption of Levodopa is confined to the small intestine (Gundert-Remy *et al.*, 1983; Kim *et al.*, 2007) and importantly there is no gastric absorption (Rivera-Calimlim *et al.*, 1971); hence gastric emptying has been described as the rate limiting step in the absorption of Levodopa (Nyholm and Lennernas, 2008).

Although the pharmacokinetic properties of Levodopa would suggest that gastric emptying and levodopa absorption are closely interrelated, surprisingly few studies have undertaken simultaneous assessment of these two parameters in the context of PD. Using an animal model, the co-administration of Levodopa with an anticholinergic to replicate conditions of delayed gastric emptying, resulted in reduced Levodopa absorption. In humans, similar findings have been described in two studies to date. Muller et al (Muller *et al.*, 2006) measured plasma Levodopa levels and gastric emptying in 11 patients with PD (mean age 58 ± 10 years). They reported that patients with slower gastric emptying had lower plasma Levodopa levels. A more recent, larger study (Doi *et al.*, 2012) undertook similar assessments in 31 people with PD (mean age 68.1 ± 7.8 years, mean disease duration 4.2 ± 3.8 years, mean UPDRS part III score 18.37 ± 8.60). Patients were retrospectively stratified into those with an early (1 hour) peak in serum Levodopa levels (58% of subjects) and those with a late (2 hour) peak in Levodopa (42%). Patients with slow gastric emptying were significantly more likely to have a later peak in serum Levodopa ($P < 0.05$).

Additionally, there is indirect evidence to support the importance of gastric emptying for Levodopa absorption, from studies of prokinetics in PD. Cisapride, a serotonin 5-HT_4 receptor agonist, is a prokinetic agent which is no longer licenced but was previously studied in the context of PD. An open-label study (Neira *et al.*, 1995) of cisapride in 20 people with PD and motor fluctuations, reported that co-administration of cisapride resulted in a 37% increase in peak plasma Levodopa levels and a 13%

increase in mean plasma Levodopa levels. A recent randomised, cross-over design study (Nishikawa *et al.*, 2012) compared plasma Levodopa levels in 18 people with PD, with and without co-administration of domperidone. All measures of Levodopa absorption were significantly increased by the co-administration of domperidone.

Further supportive evidence of the importance of gastric emptying upon Levodopa absorption comes from studies of direct duodenal delivery of Levodopa. Comparison of continuous intra-duodenal Levodopa infusion versus optimal oral dosing has demonstrated improved levels of absorption (Nyholm *et al.*, 2003; Nyholm *et al.*, 2005) and importantly improved motor function as a consequence (Puente *et al.*, 2010).

In summary, there is strong evidence to suggest that delayed gastric emptying in PD can result in impaired Levodopa absorption, which may contribute to response fluctuations in the complex phase of the disease.

1.5.8 Gastric emptying and motor fluctuations in advanced Parkinson's disease

Several studies have assessed gastric emptying in patients with and without motor fluctuations. Djaldetti (Djaldetti *et al.*, 1996) evaluated 15 patients with motor fluctuations (mean age 62 ± 9 years, mean disease duration 8.8 ± 4.6 years), 15 patients without fluctuations (mean age 65 ± 10 years, mean disease duration 4.0 ± 3.3 years) and 22 healthy controls (mean age 45 ± 3.5 years). All fluctuating patients were on combination therapy consisting of Levodopa plus at least one other dopaminergic agent, whilst eight of the non-fluctuators were taking regular Levodopa and seven were receiving Selegiline only. Gastric emptying was significantly slower in those with fluctuations compared to those without (77.4 ± 15.5% test meal retention at 1 hour vs. 64.0 ± 14.3%, respectively, $P < 0.05$).

Conversely a later study (Hardoff *et al.*, 2001) comparing gastric emptying rates in fluctuators and non-fluctuators found slower emptying rates in the non-fluctuating participants. However, this study assessed gastric emptying in the 'on' state where most similar studies have assessed gastric emptying in the practically defined 'off' state after a medication free overnight period.

A recent study (Tanaka *et al.*, 2009) compared fluctuators and non-fluctuators on comparable doses of Levodopa (425mg and 400 mg daily respectively, P = 0.55). This group reported no significant difference in gastric emptying rates between fluctuators and non-fluctuators.

In summary, there is physiological and pharmacological evidence to suggest that gastroparesis may contribute to response fluctuations in PD. However, the evidence base is still small and further research is required to clarify this relationship. This thesis will describe the relationship between gastric emptying and Levodopa absorption in people with PD and motor fluctuations.

1.5.9 Management of delayed gastric emptying in Parkinson's disease

The evidence base for the management of delayed gastric emptying in the context of PD is relatively limited compared with the extensive number of studies looking at this issue in people with diabetes.

As previously described, cisapride was shown to improve levodopa absorption and motor control in patients with PD and fluctuations (Djaldetti *et al.*, 1995; Neira *et al.*, 1995). Mosapride, another 5-HT_4 receptor agonist, was also reported to improve gastroparesis in PD (Asai *et al.*, 2005).

Systemic dopamine antagonists such as metoclopramide may be highly effective in the management of delayed gastric emptying associated with diabetic or idiopathic gastroparesis, but are contraindicated in people with PD as they can cause extra-pyramidal side effects.

Currently the only licenced therapy suitable for long-term use in the context of PD is domperidone. Domperidone has been reported to improve Levodopa absorption (Nishikawa *et al.*, 2012), gastric emptying and GI symptoms (Soykan *et al.*, 1997) in people with PD. However, this medication is not licenced in all countries.

Injection of botulinum toxin into the gastric pylorus of two people with PD was positively reported in a case series (Gil *et al.*, 2011) but has yet to be evaluated on a larger scale.

A recent pilot study evaluated the selective histamine H2-receptor antagonist Nizatidine as a therapy for delayed gastric emptying in PD (Doi *et al.*, 2013). This open label study involved 20 subjects with PD and gastroparesis symptoms. GI symptoms and gastric emptying rate were evaluated before and after three months of treatment with Nizatidine. At the conclusion of the study there was a significant improvement in gastric emptying and GI symptoms in those with baseline gastroparesis however the effects upon Levodopa absorption and motor function were not reported.

There is a clear need for more effective treatments for delayed gastric emptying in people with PD. The implications of such therapies would be far reaching as they hold the potential to improve not only GI symptoms but also the absorption of Levodopa and hence motor fluctuations. The number of currently available agents is very limited and hence the need to identify novel agents is great.

1.6 Conclusions

Parkinson's disease results in abnormal pathology and symptoms that extend the entire length of the GI tract. Gastroparesis has been demonstrated in early and advanced PD and is estimated to affect 70-100% of all patients. However, the prevalence of gastroparesis symptoms in people with PD is unknown.

Delayed gastric emptying can impair the absorption of Levodopa and contribute to motor fluctuations in the later stages of the disease. Although improved gastric emptying has been shown to enhance Levodopa absorption, there are currently very few licenced prokinetics suitable for use in PD. Exploration of alternative agents to enhance gastric emptying and Levodopa absorption is therefore indicated.

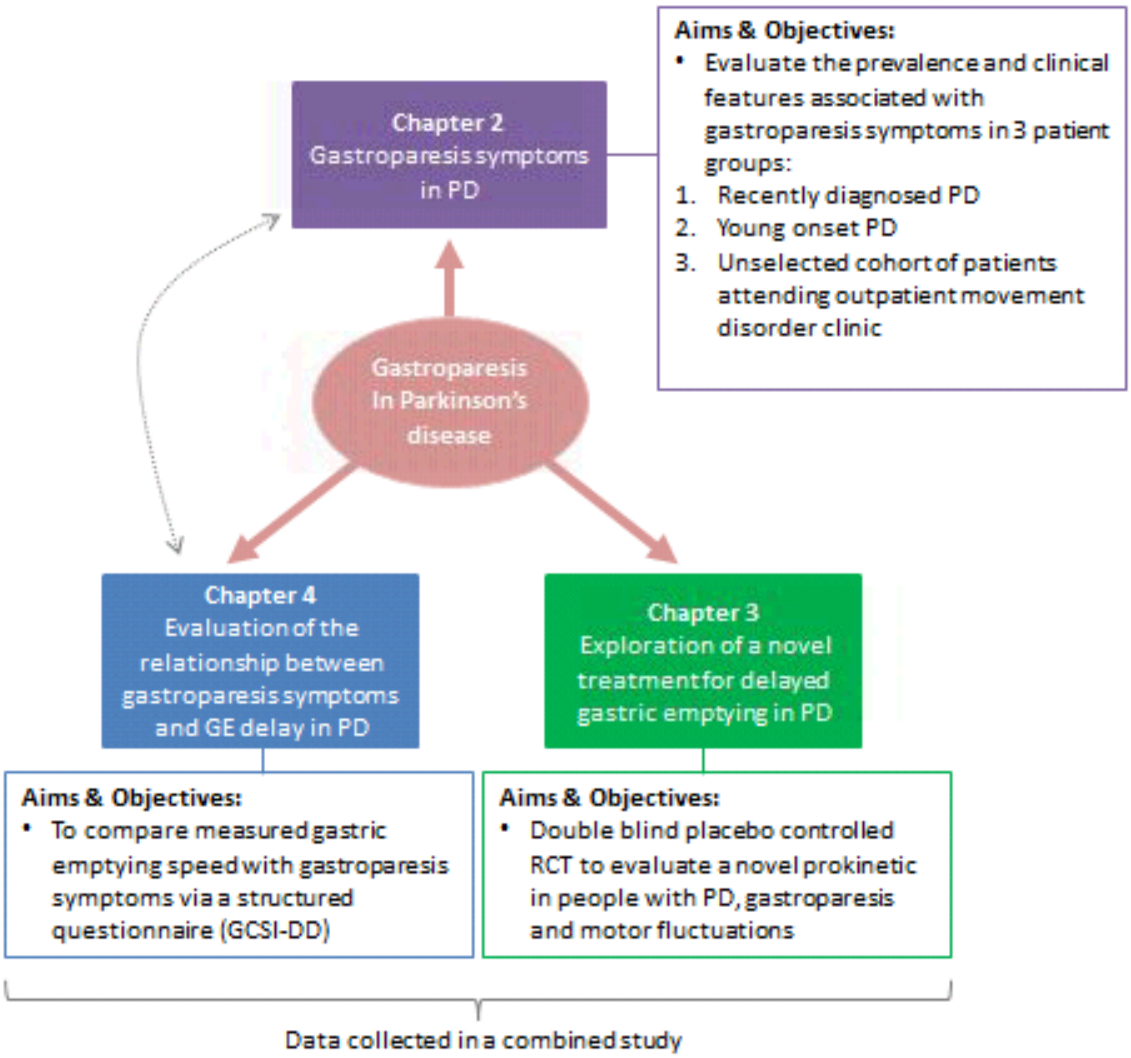
1.7 Overview of thesis structure and content

The underlying theme and focus of this thesis is gastroparesis in PD. A critical review of the literature led to the identification of the following unanswered research questions:

- *What is the prevalence of gastroparesis symptoms in people with PD and what clinical features are associated?*
- *Can a novel motilin agonist improve Levodopa absorption in people with PD and delayed gastric emptying?*
- *Are gastroparesis symptoms predictive of gastric emptying delay in people with PD?*

These research questions are addressed in the subsequent three chapters as outlined in Figure 1-4.

Figure 1-4 Overview of thesis structure and content



Chapter 2 Gastroparesis symptoms in Parkinson's disease

2.1 Background

Delayed gastric emptying has been demonstrated in all stages of PD with an overall prevalence estimated at 70-100% (Heetun and Quigley, 2012). However, the prevalence of gastroparesis symptoms associated with delayed gastric emptying is unknown in people with PD.

2.2 Aims and objectives

The aim of this study was to evaluate the occurrence of gastroparesis symptoms in people with PD. Three patient groups were considered: recently diagnosed PD, young onset PD and unselected PD patients attending for routine movement disorder clinic follow-up. The prevalence of significant gastroparesis symptoms in each group was described. Correlations between gastroparesis symptoms, motor and non-motor features of the disease were examined. The broad intention was to better characterise the symptoms of gastroparesis in people with PD.

2.2.1 Hypotheses

A priori hypotheses of this study were as follows:

1. Gastroparesis symptoms will be more prevalent and severe in female PD patients compared to males.
2. Age at the time of assessment will not be a significant determinant of gastroparesis symptoms in PD.
3. Longer PD disease duration will not be associated with a greater gastroparesis symptom burden.
4. Gastroparesis symptoms will be more severe in those participants with more advanced PD.
5. Participants with the PIGD motor subtype will have a greater gastroparesis symptom burden than those with the TD phenotype.

6. Gastroparesis symptoms form part of a wider spectrum of NMS. PD participants with more NMS will be more likely to report gastroparesis symptoms.
7. Participants with autonomic symptoms or evidence of autonomic dysfunction will have a greater gastroparesis symptom burden.
8. Gastroparesis symptoms will be more marked in participants with constipation.
9. Participants with more severe gastroparesis symptoms will have a greater measureable deficit in olfactory function.

2.3 Data collection

The assessment of gastroparesis symptoms in recently diagnosed and young onset cases of PD was performed as a sub-study of a multi-centre research project. The Parkinson's Repository of Biosamples and Networked Datasets (PRoBaND) study is an on-going prospective observational study which will be described in the following section.

A smaller data set was obtained as part of a service evaluation study to assess gastroparesis symptoms in consecutive PD patients attending a movement disorder clinic at the Newcastle upon Tyne Hospitals NHS Foundation Trust.

2.4 Parkinson's Repository of Biosamples and Networked Datasets (PRoBaND)

The PRoBaND study (also known as Tracking Parkinson's) is an on-going prospective, observational, multicentre study funded by Parkinson's UK and co-ordinated by NHS Greater Glasgow and Clyde and The University of Glasgow. Ethical approval for this study was obtained from the West of Scotland Research Ethics Committee 1 in January 2012. Patient recruitment began in January 2012 and the study is expected to run for a total of five years. A brief overview of the aims and structure of the PRoBaND study are provided here as context to the gastroparesis sub-study.

2.4.1 Hypothesis and aims for the PRoBaND study

The primary hypothesis of the PRoBaND study was that genetic and biomarker variability underpins the phenotypic heterogeneity of PD. Variation in the following four clinical domains was assessed prospectively and serially:

1. Motor features.
2. Cognitive status.
3. Therapy responsiveness.
4. Non-motor features.

Alongside the collection of clinical and demographic data, genetic and biomarker analysis was performed to identify markers which may explain observed clinical variations.

2.4.2 PRoBaND study population

Three subject groups were eligible for inclusion in the study: two cohorts of people with PD and selected first degree relatives of participants in these two patient groups (Table 2-1).

Table 2-1 Overview of participant groups in the PRoBaND study

Study participants	Description	Target number of subjects
Recent onset PD patients	PD diagnosed within the preceding three years	2,000
Young onset PD patients	PD diagnosed before the age of 50 years	240
First degree relatives	Siblings of the above two patient groups	840
		Total 3,080

This thesis will consider baseline data from a subset of participants in the recent onset PD group and all participants in the young onset PD group. No results from the first degree relatives are considered in this thesis.

2.4.3 Eligibility criteria for the PRoBaND study

Patient participants in this study were diagnosed as having idiopathic PD according to the UK Brain Bank Criteria (Hughes *et al.*, 1992) and had this diagnosis established by a specialist, either within the preceding three years or prior to the age of 50 years old. All participants were aged 18 to 90 years old (inclusive) and were willing and able to provide written informed consent. Participants were allowed to already be taking anti-parkinsonian medications prior to entry into the study. The main exclusion criteria for the PRoBaND study included: severe co-morbid illness that would limit participation, features suggestive of another cause of degenerative parkinsonism (e.g. progressive supranuclear palsy, multiple system atrophy), drug-induced parkinsonism or symmetrical lower body parkinsonism suggestive of underlying vascular aetiology. Neuroimaging was not a pre-requisite of enrolment however patients who previously had negative or normal functional imaging of the presynaptic dopamine system were excluded.

2.4.4 Identification of participants for the PRoBaND study

Over 50 sites (hospitals, universities and research centres) around the United Kingdom were involved in the identification, consent and assessment of study participants. The PRoBaND study was co-ordinated and led by a research team in Glasgow. Newcastle acted as the second leading site with a research doctor (SM) undertaking all recruitment and baseline assessments of participants.

2.4.5 PRoBaND study procedures

Patients in the recently diagnosed cohort were assessed every six months over a three year period (seven study visits in total). Patients in the young onset group were assessed on two occasions approximately six months apart. Assessments relevant to this thesis are detailed in section 2.5.

Genetic testing as part of the PRoBaND study included testing for the LRRK2 and GBA mutations in all participants. Those patients with young onset PD were additionally tested for abnormalities in Parkin (PARK 2) & PINK-1 (PARK 6). The results of these genetic tests are not considered in this thesis.

2.4.6 PRoBaND study timeline

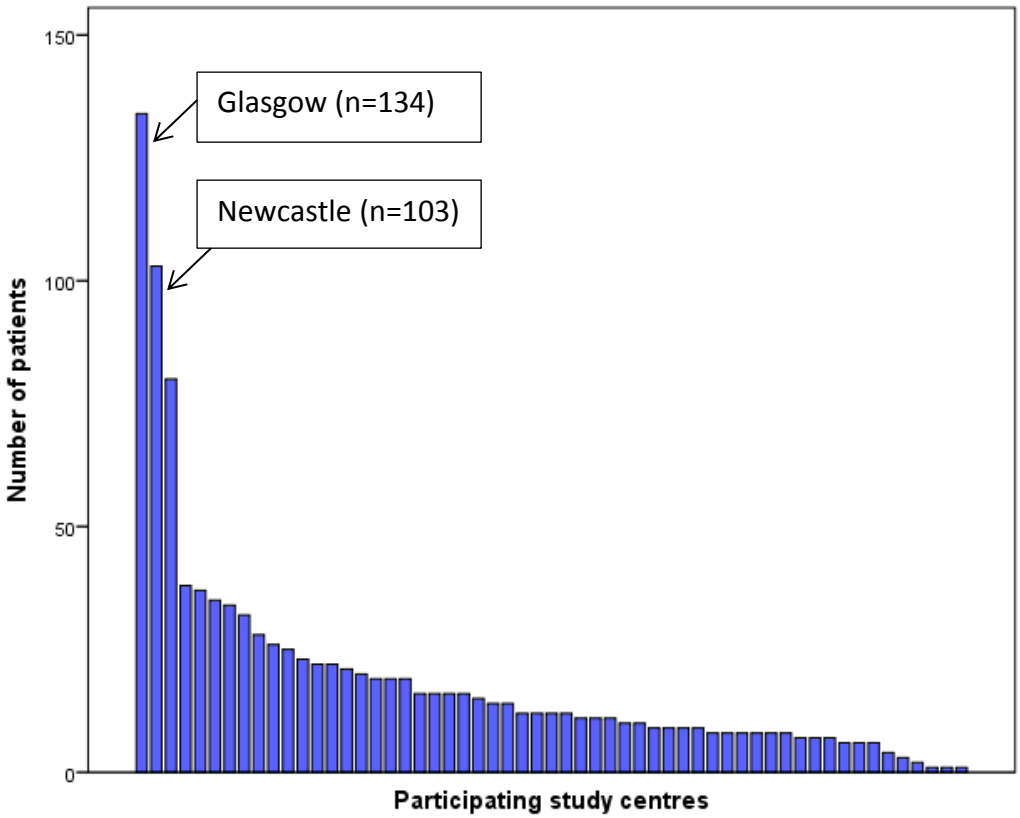
Recruitment of subjects to the PRoBaND study began in January 2012 and the first patient assessment was performed on 2 February 2012. The national recruitment target of 240 patients for the young onset PD cohort was surpassed (n = 337) in December 2012. Recruitment to the recently diagnosed PD cohort was on-going at the time of writing this thesis. Current recruitment rates suggest that patient enrolment will conclude in early 2014 with the study completing in 2017.

The data presented in this thesis represent an interim analysis of the first PRoBaND participants recruited to the study between January 2012 and July 2013.

2.5 General study methodology

Between 2 February 2012 and 22 July 2013, 1,084 people with PD (recently diagnosed or young onset) were recruited to the PRoBaND study. All patients provided written informed consent and met the study eligibility criteria previously summarised (section 2.4.3). A total of 57 sites around the UK contributed patients to the study. The lead study site (Glasgow) contributed 134 patients (12.4% of participants) and Newcastle contributed 103 (9.5%) (Figure 2-1).

Figure 2-1 Number of patients recruited to the PRoBaND study per site (from February 2012 to July 2013)



2.5.1 Baseline clinical assessment

At baseline, demographic and clinical data were obtained from each participant by trained study staff. Details of individuals' past medical history and family history were obtained via patient self-completed questionnaires. All current medications (for the treatment of PD and all other concomitant medications) were recorded by study staff. Height and weight were measured and used to calculate participants' body mass index (BMI = weight in Kg / height in metres^2). Postural blood pressure measurements were recorded manually or with an automated sphygmomanometer following three minutes in a supine position and again after two minutes of standing. Orthostatic hypotension was defined as a lying to standing systolic deficit of ≥20mmHg or a diastolic deficit of ≥10mmHg ('Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. The Consensus Committee of the American Autonomic Society and the American Academy of Neurology,' 1996).

Clinical assessment was performed by experienced raters and included use of the revised Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS UPDRS) (Goetz *et al.*, 2008). The MDS UPDRS includes clinician rated sections and patient or carer completed questions. The MDS UPDRS comprises four sections; Part I: Non-motor experiences of daily living (score range 0-52), Part II: Motor experiences of daily living (score range 0-52), Part III: Motor examination (score range 0-132), Part IV: Motor complications (score range 0-24). Scores for each part of the MDS UPDRS as well as the total score (potential range 0-260) were reported as measures of disease severity, with higher scores reflecting more severe disease parameters. Scores from the baseline MDS UPDRS assessment were used to categorise patients according to their motor phenotype: tremor dominant (TD), postural instability and gait difficulty (PIGD) or indeterminate (ID). Calculation of the mean tremor score and mean postural instability and gait score for each subject, allowed classification into motor subtypes where a ratio of ≥ 1.15 was deemed TD and ≤ 0.90 was PIGD with intervening ratio values classified as ID (Stebbins *et al.*, 2013). Disease stage was reported according to the modified Hoehn and Yahr (mH&Y) staging system (Hoehn and Yahr, 1967; Jankovic *et al.*, 1990).

At baseline patients completed a series of validated questionnaires to provide a wider context to their PD symptoms. The presence and severity of non-motor symptoms was

evaluated with the 30-item Non-Motor Symptom Scale (NMSS) (Chaudhuri *et al.*, 2007). This was used to calculate a global score as well as scores for nine individual NMS domains (Cardiovascular & falls, Sleep, Mood & cognition, Perceptual problems & hallucinations, Attention & memory, Gastrointestinal, Urinary, Sexual function and Miscellaneous). Autonomic symptoms associated with PD were assessed using the SCOPA-AUT tool (Visser *et al.*, 2004). This questionnaire was used to calculate a global score reflective of the total autonomic symptom burden and domain sub-scores (Gastrointestinal, Urinary, Cardiovascular, Thermoregulatory, Pupillomotor, Sexual). Symptoms of anxiety and depression were assessed with the Leeds scales for the self-assessment of Anxiety and Depression (LADS) (Snaith *et al.*, 1976) with scores ≥ 7 for the anxiety or depression questions respectively taken to represent significant anxiety or depression. Quality of life (QoL) was evaluated using the PDQ8 (Jenkinson *et al.*, 1997) which is a brief 8-item tool specifically designed to assess PD-related QoL parameters. All responses were combined to give a global severity score; the PDQ8 summary index (range 0-100%) (Katsarou *et al.*, 2004).

Alongside the baseline assessments described, one piece of follow-up data is included in this thesis. At the six-month follow-up visit, olfactory testing was performed and where available (for the recently diagnosed cases), this was evaluated alongside baseline gastroparesis data. It was hypothesised that olfactory impairment and gastroparesis symptoms would correlate as according to the 'Dual hit hypothesis' of PD the olfactory bulb and DMNV are the earliest sites of abnormal pathology in the pathogenesis of PD (Hawkes *et al.*, 2007). For the PRoBaND study olfactory testing was performed in a standardised, quantitative manner using a British version of the University of Pennsylvania Smell Identification Test (UPSIT), produced by Sensonics Inc, New Jersey, USA. This 40-item validated test of olfactory function (Doty *et al.*, 1984) has been used reliably in established PD (Hawkes *et al.*, 1997) as well as early untreated disease (Doty *et al.*, 1992). For those participants aged ≥60, an UPSIT score of ≤23/40 was deemed abnormal and for those aged <60, a higher threshold (≤28/40) was set as a marker of significant hyposmia (Silveira-Moriyama *et al.*, 2009).

2.5.2 Assessment of gastroparesis symptoms

The presence and severity of gastroparesis symptoms was assessed in all patients at baseline using the Gastroparesis Cardinal Symptom Index (GCSI) (Revicki *et al.*, 2003). The GCSI is a structured questionnaire in which patients self-report the severity of 9 symptoms typically associated with gastroparesis (Table 2-2). Participants were asked to report the symptoms they experienced in the preceding two week period.

Table 2-2 Components of the Gastroparesis Cardinal Symptom Index

Symptom cluster	Question / symptom
Nausea & vomiting	1 Nausea
	2 Retching
	3 Vomiting
Fullness & early satiety	4 Stomach fullness
	5 Early satiety
	6 Post-prandial fullness
	7 Loss of appetite
Bloating	8 Bloating
	9 Stomach visibly larger

The severity of each of the 9 symptoms was graded on an ordinal numeric scale as follows: None = 0, Very mild = 1, Mild =2, Moderate = 3, Severe = 4 and Very severe = 5. Each symptom was subsequently re-coded as either 'present' (score ≥1) or 'absent' (score = 0). This was used to describe the prevalence but not severity of each of the 9 gastroparesis symptoms. Similarly, answers to all 9 questions were summated to give a cumulative score (0 – 45). Again, participants were re-coded as either 'gastroparesis symptoms present' (scores ≥1) or 'no gastroparesis symptoms' (score 0).

Gastroparesis symptom severity was described with the GCSI total score. The GCSI validation study (Revicki *et al.*, 2003) described a calculation for the GCSI total score combining all 9 questions of the GCSI to give a global measure of gastroparesis symptom severity (Equation 1).

$$\frac{\left[\frac{(Q1+Q2+Q3)}{3}+\frac{(Q4+Q5+Q6+Q7)}{4}+\frac{(Q8+Q9)}{2}\right]}{3}$$

Equation 1 Formula for calculating the GCSI total score

Higher GCSI total scores reflected a greater burden of gastroparesis symptoms. The GCSI total score could range from 0 to 5. A large community based study of gastroparesis symptoms in people with type 1 diabetes suggested that a GCSI total score of ≥ 1.90 is abnormal (Kofod-Andersen and Tarnow, 2012). A validation study in 169 people with known gastroparesis (Revicki *et al.*, 2003) suggested that scores of >2.56 are consistent with severe symptoms of delayed gastric emptying.

GCSI responses from those participants with young onset PD were dichotomised into those diagnosed with PD before the age of 40 and those diagnosed aged ≥ 40 and <50 years old. It was speculated that those with very young onset PD would be more likely to have a monogenetic cause and hence comparison of GCSI total scores between young and very young onset cases may hint towards a genetic influence upon rates of gastroparesis in PD.

2.5.3 Assessment of other gastrointestinal symptoms

The bowel function of all participants was evaluated at baseline alongside gastroparesis symptoms. Participants were asked to report their typical daily bowel motion frequency during the preceding week (less than once daily, once daily, twice daily or more than twice daily). Furthermore, Part I of the MDS UPDRS includes a question on the severity of constipation difficulties on a scale from 0 to 4, where 0 is normal (no problems) and 4 is severe (requiring physical help from others to empty their bowels). Additionally, participants were asked if they had used laxatives in the last week, if they had undertaken any exercise in the last week.

2.6 Statistical analysis

Statistical analyses were performed using SPSS 19 (SPSS, Chicago, IL). Normality of the data was assessed visually with histograms and objectively with the Kolmogorov-Smirnov test.

Normally distributed data are presented as mean and standard deviation (SD). Means between groups were compared with un-paired Student t-tests. Relationships between normative data series were assessed with Pearson's correlation.

Non-normally distributed data are presented as median and inter quartile range (IQR). Non-parametric tests (Mann-Whitney or Kruskal-Wallis test) were used for non-normally distributed data. Relationships within non-normative data were assessed with Spearman's rank correlation.

Where multiple comparisons of the data were undertaken, post hoc pairwise comparisons were undertaken with Bonferroni's correction and significance reported according to the revised P value.

Logistic regression was used to evaluate binary outcome measures. Linear regression was used to model the effect of continuous predictor variables on the GCSI total score. Multiple regression was used to model the influence of mixed predictor variables on the GCSI total score.

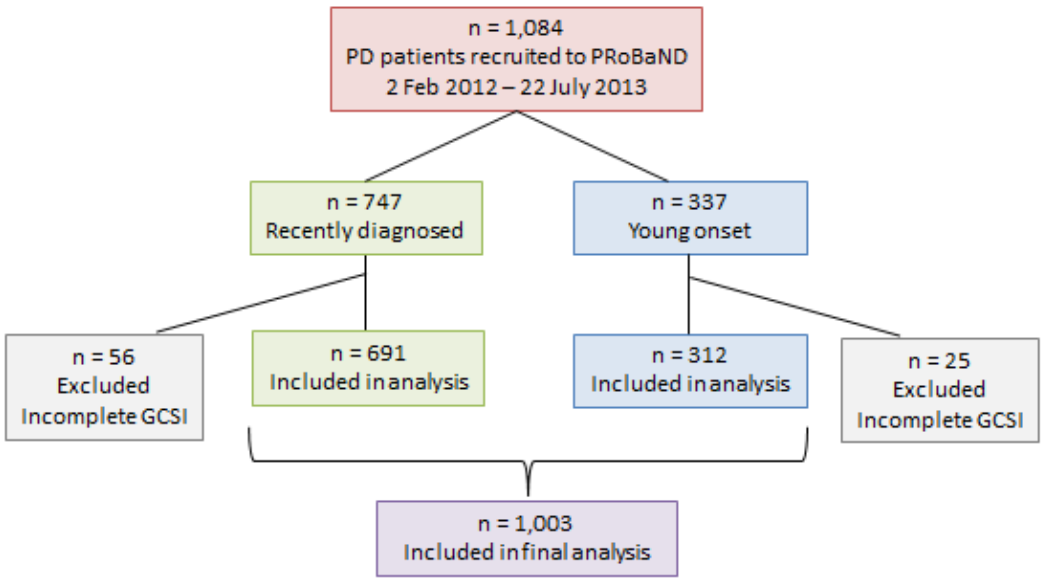
Frequencies of categorical variables between groups were compared with the Chi-Square test, with Odds Ratios (OR) with 95% confidence intervals (95% CI) reported for significant differences.

All P values are presented a two-tailed unless otherwise stated; where one-tailed tests were used because a directional effect was hypothesised a P value of < 0.05 was deemed significant.

2.7 Selection of cases for analysis

Of the first 1,084 patients recruited to the PRoBaND study, 747 were recently diagnosed cases (clinical diagnosis of PD within three years) and 337 were young onset cases (diagnosed before the age of 50). A small number of participants (n = 81) did not complete the full GCSI questionnaire. Results of the GCSI were taken as the primary outcome measure for this study of gastroparesis symptoms in PD. Therefore those who did not complete the GCSI were excluded from subsequent analyses. There were no significant differences with respect to age, gender, cognition (MoCA score) or motor disease severity (MDS UPDRS Part III) in the 1003 participants who fully completed the GCSI compared with the 81 subjects who did not. Full details of these statistical comparisons are provided later in the appropriate results sections (Table 2-3 and Table 2-17).

Figure 2-2 Overview of cases selected for analysis by type



2.8 Results – Gastroparesis symptoms in people with recently diagnosed Parkinson's disease

2.8.1 Baseline characteristics of study participants – recently diagnosed Parkinson's Disease

747 patients diagnosed with PD within the preceding three years were recruited to the PRoBaND study between 2 February 2012 and 22 July 2013. Of these, 691 completed the GCSI questionnaire in its entirety and 56 had either partially or wholly incomplete questionnaires. There were no significant differences in age, gender, cognition (MoCA score) or motor severity (MDS UPDRS Part III) between these two groups (Table 2-3). All subsequent analyses will focus solely on the 691 participants with recently diagnosed PD who at baseline fully completed the GCSI.

Table 2-3 Comparison of included vs excluded recently diagnosed PD cases

Characteristic	All recently diagnosed cases n = 747		P value
	Included n = 691	Excluded n = 56	
Age (years)	68.09 (7.76)	68.75 (10.37)	0.642[a]
Male gender (%)	466 (67.3%)	39 (69.6%)	0.724[b]
MoCA score	25.00 (4.00)	25.00 (3.00)	0.403[c]
MDS UPDRS Part III	19.00 (15.00)	19.5 (19)	0.085[c]

Data presented as: mean age (SD), number (%) male, median MoCA & MDS UPDRS Part III scores (IQR). Statistical tests: [a]Unpaired t-test, [b]Chi-Square, [c]Mann Whitney test.

Baseline characteristics of the 691 recently diagnosed PD participants are summarised in Table 2-4. Participants were staged according to the modified Hoehn and Yahr (mH&Y) staging system. 85% of participants were mH&Y stage 2 or less with nearly half of all participants only showing unilateral signs only (49.8% mH&Y stage 0, 1 or 1.5). When classified according to motor phenotype, 46.7% of participants were of the tremor dominant (TD) subtype, 40.8% postural instability and gait disorder (PIGD) subtype and 12.4% were indeterminate.

Table 2-4 Baseline characteristics of participants with a recent diagnosis of PD

Characteristic		Descriptors
Age		68.1 (7.8, 49.4 - 89.0)
Male gender		465 (67.3%)
Age at diagnosis		66.7 (7.8, 48.9 - 87.3)
Disease duration (yrs)*		1.3 (1.7, 0 - 3.6)
Caucasian ethnic origin		677 (98.0%)
mH&Y stage	0	10 (1.5%)
	1	210 (32.1%)
	1.5	106 (16.2%)
	2	231 (35.3%)
	2.5	64 (9.8%)
	3	31 (4.7%)
	4	0 (0%)
	5	2 (0.3%)
MDS UPDRS*	Part I	9 (7, 0 - 34)
	Part II	9 (9, 0 - 48)
	Part III	19 (15, 0 - 66)
	Part IV	0 (0.0, 0 - 12)
	Total	38 (24.0, 6 - 141)
Anxiety		165 (24.3%)
Depression		150 (22.2%)

Data presented as mean (SD, range), except non-normally distributed variables indicated by * which are reported as median (IQR, range). Categorical variables are presented as frequency (%).

Patients self-reported their past medical history as summarised in Table 2-5. The most commonly reported comorbidities were hypertension and hypercholesterolaemia (33.0% and 32.2% of participants, respectively).

Table 2-5 Comorbidities reported by study participants with a recent diagnosis of PD

Comorbid condition	Positive history reported
Hypertension	33.0%
Hypercholesterolaemia	32.2%
Asthma	10.4%
Angina	8.6%
Diabetes (Type 1 or 2)	8.5%
Stroke or TIA	4.8%
Myocardial infarction	4.2%
Heart failure	3.1%
Rheumatoid arthritis	2.5%
Breast cancer	2.2%
Prostate cancer	2.0%
Bronchitis	2.0%
Emphysema	1.2%
Bowel cancer	1.2%
Lung cancer	0.1%

A majority of participants (90.4%) was taking some form of antiparkinsonian therapy at baseline assessment (Table 2-6). The most commonly prescribed medication was Levodopa-based therapy (64.2%) followed by dopamine agonists (42.2%). 48% of participants were taking monotherapy whilst 41.6% were taking two or more antiparkinsonian drugs.

Table 2-6 Medications of recently diagnosed study participants at baseline assessment

Medication type	Number of patients	Percentage
Levodopa	641	64.2%
Dopamine agonist	421	42.2%
MAOBI	296	29.7%
Amantadine	74	7.4%
COMT Inhibitor	48	4.8%
Anticholinergic	22	2.2%
None	96	9.6%

2.8.2 Presence of gastroparesis symptoms – recently diagnosed PD

More than half of all patients (62.5%) reported at least one symptom associated with gastroparesis (Figure 2-3). The most commonly reported gastroparesis symptom was stomach fullness, present in 36.5% of participants with early PD. Post-prandial fullness (32.7%), bloating (32.1%) and the stomach being visibly larger or distended (30%) were the next most frequently reported symptoms Table 2-7.

Figure 2-3 Number of gastroparesis symptoms positively reported on the GCSI questionnaire per patient with a recent diagnosis of PD

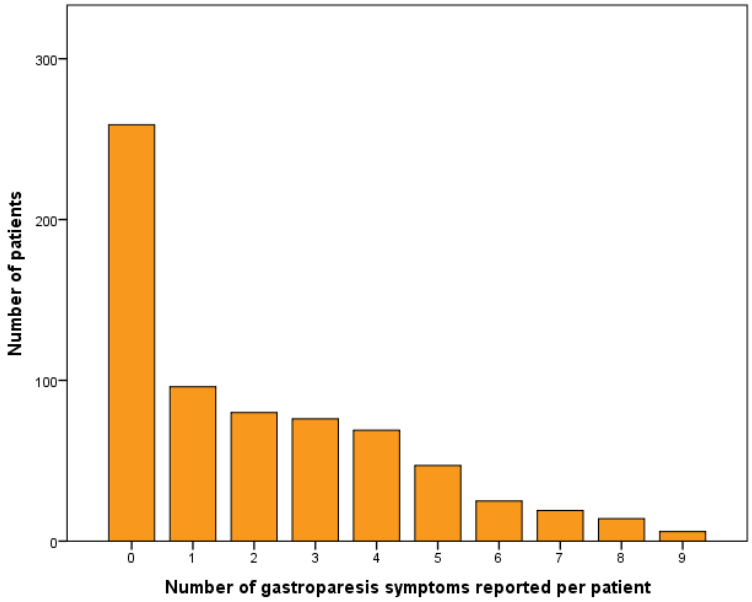


Table 2-7 Prevalence of positive gastroparesis symptoms reported in the GCSI questionnaire by participants with recently diagnosed PD

Gastroparesis symptom	Patients reporting symptoms in the preceding 2 weeks
Stomach fullness	36.5%
Post-prandial fullness	32.7%
Bloating	32.1%
Stomach larger / distended	30.0%
Loss of appetite	23.0%
Early satiety	22.1%
Nausea	20%
Retching	9.6%
Vomiting	3.0%

An analysis was undertaken to compare those participants who reported at least one gastroparesis symptom (n = 432, 62.5%) with those who reported no symptoms (n = 259, 37.5%) (Table 2-8). Gastroparesis symptoms were significantly more likely to be reported by women compared to men (Chi-square test, P = 0.001, OR 1.77, 95% CI 1.26 – 2.50). Patients classified as PIGD motor subtype were more likely to report gastroparesis symptoms than those of a TD phenotype (Chi-square test, P < 0.001, OR 0.510, 95% CI 0.36 – 0.73). Those patients who reported gastroparesis symptoms had significantly more severe PD (higher MDS UPDRS Part III score) (Mean 21.9 vs 19.9, t-test, P = 0.034). Patients who reported less than one bowel motion per day were also significantly more likely to have experienced gastroparesis symptoms than those who opened their bowel at least once daily (Chi-square test, P = 0.022, OR 1.58, 95% CI 0.36 – 0.73). Accordingly, those patients who reported more severe constipation difficulties in the MDS UPDRS (question 1.11; none vs slight - severe) were significantly more likely to also experience gastroparesis symptoms (Chi-square test, P < 0.001, OR 2.25, 95% CI 1.64 – 3.08). After Bonferroni correction for multiple comparisons; gender, motor phenotype and constipation difficulties (MDS UPDRS question 1.1) all remained significant variables at the new level of significance (P = 0.01).

Table 2-8 Comparison of demographic and clinical differences between those recently diagnosed participants with and without gastroparesis symptoms

Variable	Gastroparesis symptoms reported n = 432	No gastroparesis symptoms reported n = 259	P value
Female gender	161 (23.3%)	65 (9.4%)	**0.001**[a]
Age*	68.1 (7.9)	68.0 (7.6)	0.837[b]
Age at diagnosis*	66.7 (7.9)	66.6 (7.5)	0.911[b]
Disease duration*	1.4 (0.9)	1.4 (0.9)	0.436[b]
PIGD phenotype	182 (29.0%)	74 (11.8%)	**<0.001**[a]
MDS UPDRS III*	21.9 (11.8)	19.9 (10.4)	**0.034**[b]
Bowel motion <1/d	103 (14.9%)	43 (6.2%)	**0.022**[a]
Constipation problems	265 (38.5%)	108 (15.7%)	**<0.001**[a]
Orthostatic hypotension	71 (10.6%)	34 (5.1%)	0.218[a]
mH&Y stage ≥ 2	211 (32.3%)	117 (17.9%)	0.234[a]
Weight:			0.281[a]
Underweight (BMI: <18.5)	7 (1.0%)	2 (0.3%)	
Normal (BMI: ≥18.5, <25)	129 (19.2%)	88 (13.1%)	
Overweight (BMI: ≥25, <30)	181 (26.9%)	113 (16.8%)	
Obese (BMI: ≥30)	104 (15.5%)	49 (7.3%)	

Data are presented as frequency (% of all cases) for categorical variables, continuous variables (indicated by *) are reported as mean (SD). [a]Chi-square test, [b]Unpaired t-test.

Stepwise logistic regression was used to determine the predictors of gastroparesis symptoms being present or absent in patients with recently diagnosed PD. Female gender, constipation problems and a greater Non-motor symptom burden predicted the presence of gastroparesis symptoms accounting for over 17% of the variance. The model had a high specificity (83.0%) but a low sensitivity (38.1%) (Nagelkere R square 0.177)(Table 2-9).

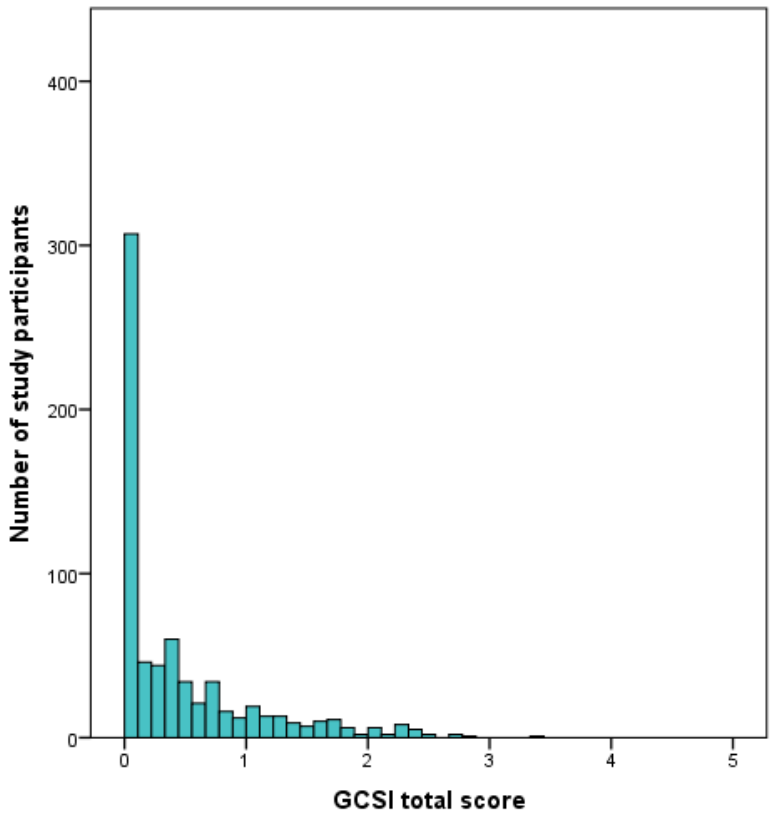
Table 2-9 Logistic regression model for predictors of gastroparesis symptoms in early PD

Variable	β	S.E.	P value	Exp (β)
Female gender	0.530	0.224	0.018	0.589
Constipation problems	-0.605	0.214	0.005	0.546
UPDRS part I score	0.133	0.025	0.000	1.142
Constant	0.023	0.321	0.942	1.024

2.8.3 Severity of gastroparesis symptoms – recently diagnosed PD

The GCSI total score was calculated as a global marker of gastroparesis symptom severity. For the 691 participants with a recent diagnosis of PD, the GCSI total scores were non-normally distributed (Kolmogorov-Smirnov P<0.001), with a median score of 0.17 (0 - 3.42) and a mean of 0.45 (0.61) (Figure 2-4).

Figure 2-4 Baseline GCSI total scores for 691 PRoBaND participants with a recent diagnosis of PD



As diabetes mellitus is a well-recognised cause of delayed gastric emptying, it was necessary to establish if the presence of diabetes as a co-morbidity was a significant factor influencing GCSI scores. A total of 58 participants (8.5%) reported having diabetes but their median GCSI total score (0.25) was not significantly different to that of participants without diabetes (0.17) (Mann-Whitney, U = 19,424, z = 0.89, P = 0.376, r = 0.001).

28 participants (4.1%) had a GCSI total score of ≥ 1.90 which is regarded as abnormally high and consistent with gastroparesis (Kofod-Andersen and Tarnow, 2012). Only four

participants (0.6%) had a GCSI total score of > 2.56 consistent with severe gastroparesis (Revicki *et al.*, 2003).

Higher GCSI total scores were associated with female gender, PIGD motor phenotype, a lack of recent exercise, symptoms of anxiety and depression, constipation and recent laxative use (Table 2-10). Participants age, age at the time of diagnosis and disease duration were not significantly associated with GCSI total scores (Spearman's rho, P = 0.563, 0.466 and 0.262, respectively).

Table 2-10 Comparison of GCSI total scores according to patient characteristics

Patient characteristic	Median GCSI total score of participants with characteristic	Median GCSI total score of participants without characteristic	P value
Female gender	0.28	0.17	**0.001**
PIGD phenotype	0.33	0.08	**<0.001**
OH	0.25	0.17	0.33
No exercise in past week	0.25	0.17	**0.009**
Laxativesused in last week	0.50	0.11	**<0.001**
Anxiety (LAD ≥7)	0.53	0.08	**<0.001**
Depression (LADS≥7)	0.67	0.10	**<0.001**

Data presented: Mann-Whitney test. OH: orthostatic hypotension (postural drop in systolic blood pressure ≥ 20mmHg or diastolic blood pressure drop ≥ 10mmHg).

The influence of anti-parkinsonian medications on gastroparesis symptoms was evaluated with non-parametric tests. Taking of Levodopa medication and anticholinergics did not significantly influence GCSI total score (Mann-Whitney test, P = 0.844 and 0.227, respectively). The use of dopamine agonist medications was however associated with a higher GCSI total score (Mann-Whitney test, P < 0.001).

The GCSI total score was significantly influenced by modified H&Y disease stage (Kruskall-Wallis, P = 0.038) with a trend towards higher GCSI total scores with advancing disease stage (Table 2-11).

Table 2-11 GCSI total scores according to disease stage

mH&Y stage	Number of participants	Median GCSI total score
0	10	0.08
1	210	0.11
1.5	106	0.17
2	231	0.17
2.5	64	0.28
3	31	0.42
5	2	1.56

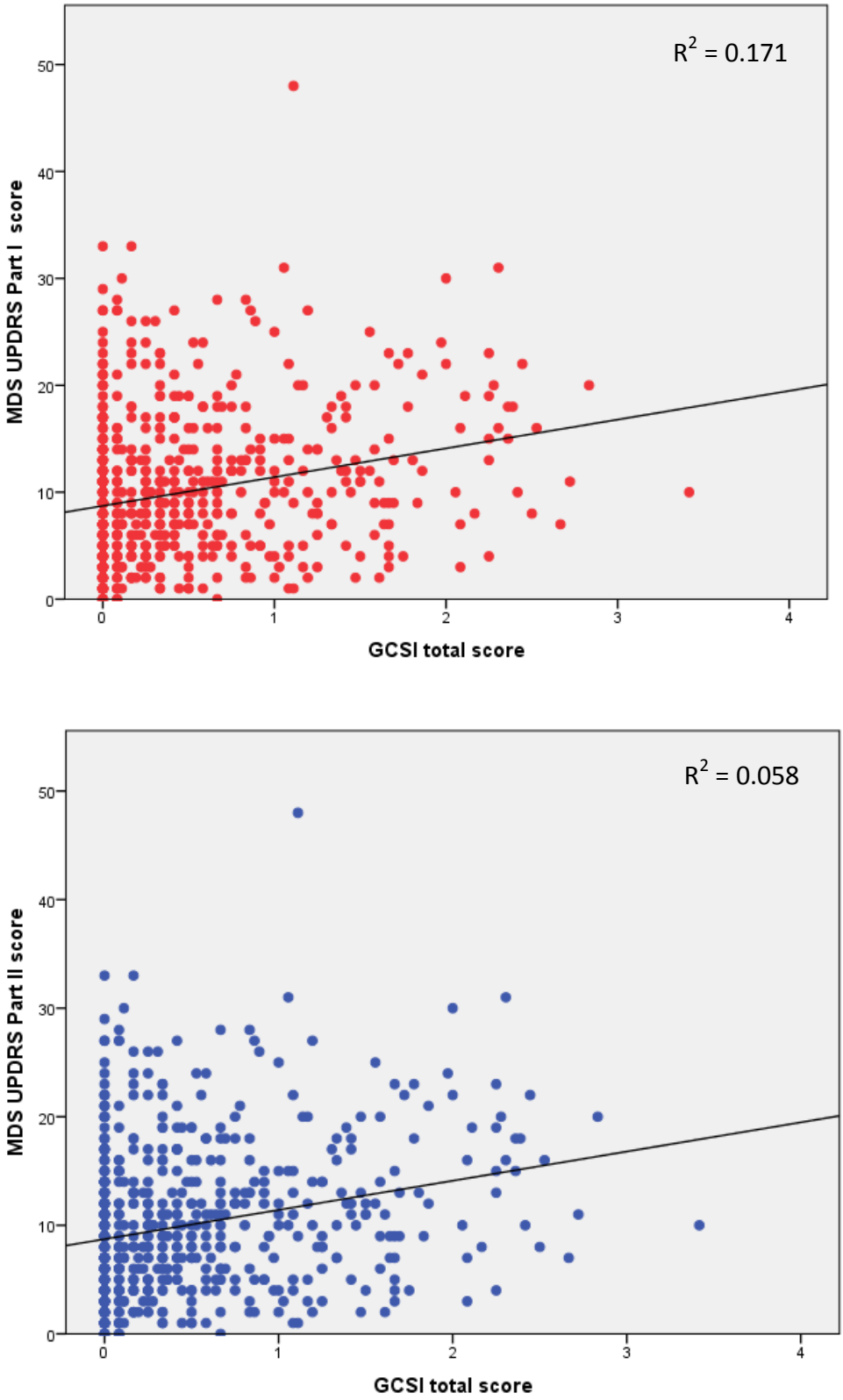
Advancing disease severity significantly correlated with more severe gastroparesis symptoms. Higher scores in each sub-section of the MDS UPDRS were associated with higher GCSI total scores (Table 2-12 and Figure 2-5). Using a stepwise linear regression model, MDS UPDRS Part I scores had the greatest influence on GCSI total scores (R^2 = 0.171)

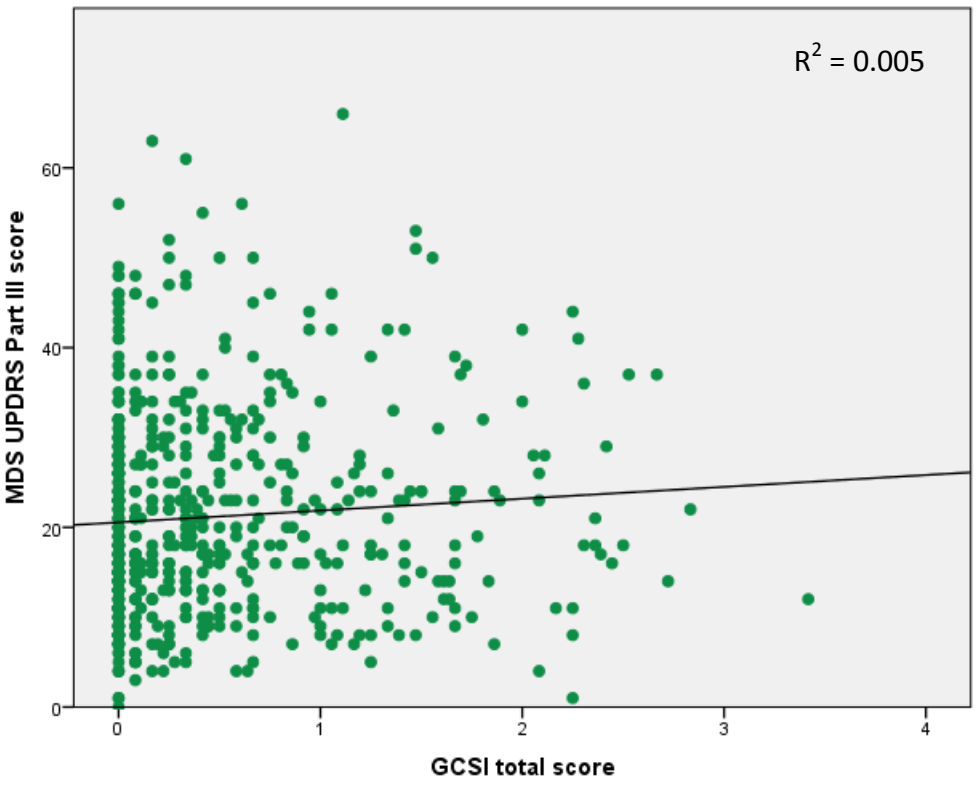
Table 2-12 Correlation between MDS UPDRS scores and the GCSI total score

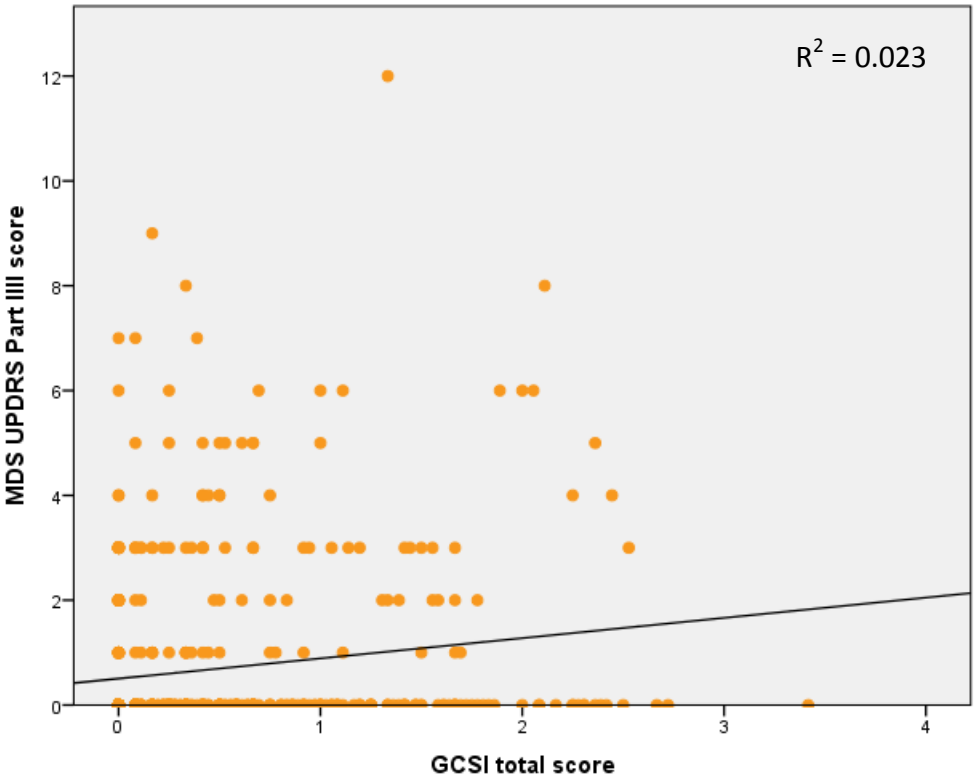
MDS UPDRS sub-section	Descriptor of the sub-section	Correlation coefficient	P value
Part I	Non-motor experiences of daily living	0.395	<0.001
Part II	Motor experiences of daily living	0.258	<0.001
Part III	Motor examination	0.093	0.010
Part IV	Motor complications	0.136	<0.001
Total score		0.256	<0.001

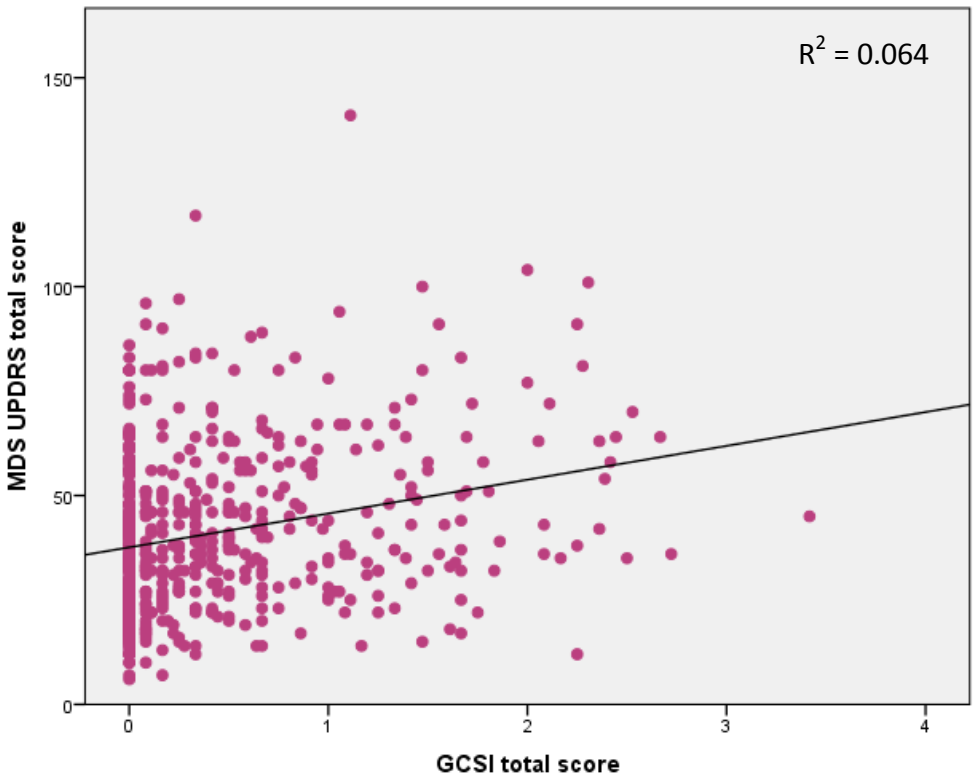
Data are presented as Spearman's rho, 1-tailed significance. After Bonferroni's correction for multiple comparisons, all variables remained significant ($P < 0.0125$).

Figure 2-5 Scatter plots demonstrating the relationship between GCSI total scores and sub-sections of the MDS UPDRS (with R^2 values for linear regression model)




R^2 = 0.005
MDS UPDRS Part III score
0
20
40
60
0
1
2
3
4
GCSI total score


R^2 = 0.023
MDS UPDRS Part IIII score
0
2
4
6
8
10
12
0
1
2
3
4
GCSI total score



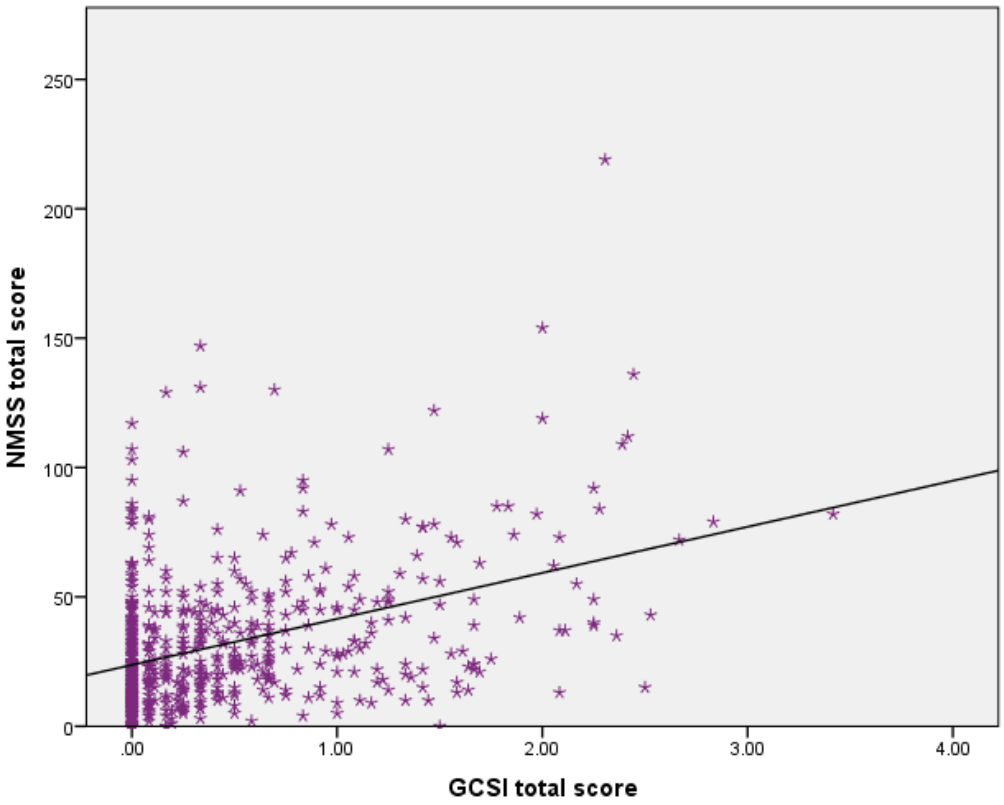
There was a positive correlation between non-motor aspects of daily living (MDS UPDRS Part I scores) and greater gastroparesis symptom severity. This relationship was explored further using the 30-item Non-Motor Symptom Scale (NMSS). This questionnaire has nine symptom domains, each of which was evaluated for an association with the GCSI total score (Table 2-13). Higher symptom scores in all nine NMSS domains significantly correlated with higher GCSI total scores. Using a stepwise linear regression model, the NMSS total scores accounted for 16.3% of the variance in GCSI total scores (Figure 2-6).

Table 2-13 Association between the domains of the NMSS (non-motor symptoms questionnaire) and GCSI total scores in participants with recently diagnosed PD

NMSS domain number	Domain type	Correlation coefficient	P value
1	Cardiovascular & falls	0.247	<0.001
2	Sleep & fatigue	0.322	<0.001
3	Mood & cognition	0.274	<0.001
4	Perceptual & hallucinations	0.187	<0.001
5	Attention & memory	0.200	<0.001
6	Gastrointestinal	0.277	<0.001
7	Urinary	0.229	<0.001
8	Sexual	0.117	0.001
9	Miscellaneous	0.280	<0.001
Total	All domains combined	0.365	<0.001

Data are presented as Spearman's rho, 1-tailed significance.

Figure 2-6 Scatter plot to show the relationship between the NMSS total score and the GCSI total score



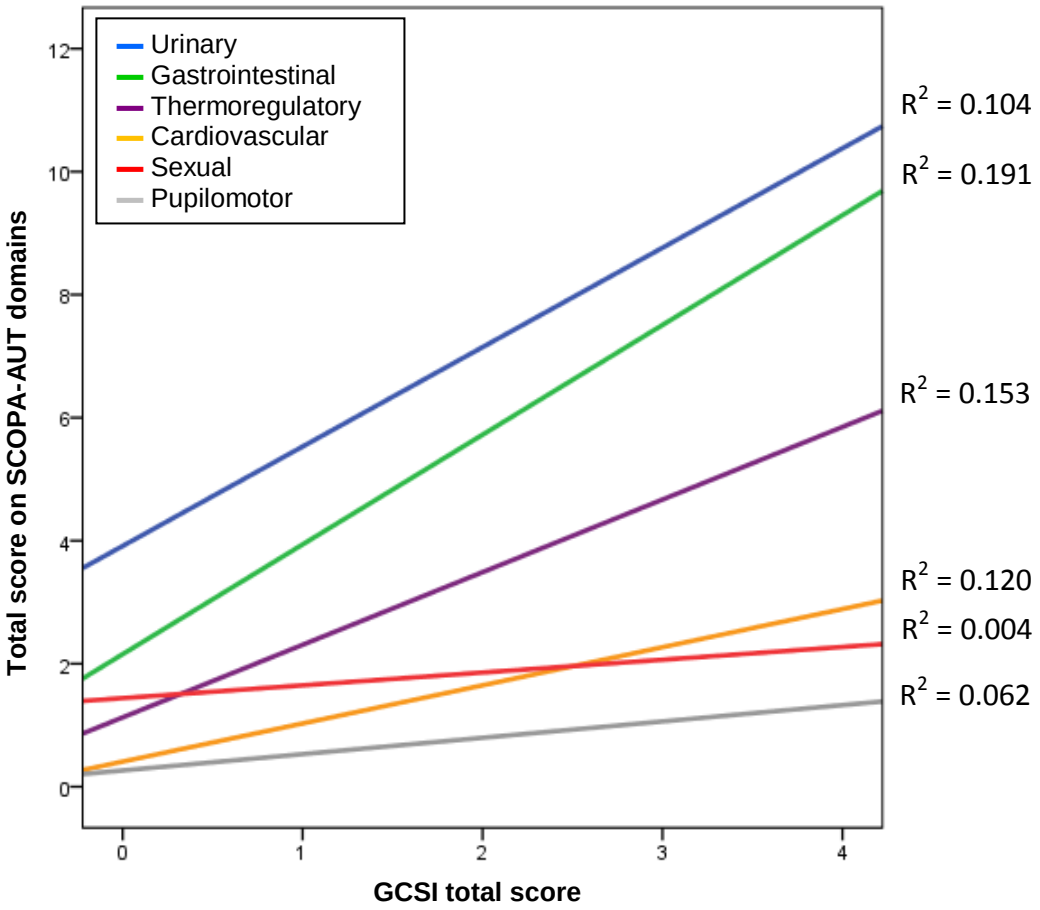
Correlation between autonomic dysfunction and gastroparesis symptoms was further explored through analysis of the SCOPA-AUT questionnaire. All six items of this autonomic symptom questionnaire positively correlated with higher GCSI total scores (Table 2-14), the strongest relationship was seen with the gastrointestinal and urinary domain scores (Figure 2-7).

Table 2-14 Association between domains of the SCOPA-AUT (autonomic symptoms questionnaire) and GCSI total score in recently diagnosed PD

SCOPA-AUT domain	Correlation coefficient	P value
Gastrointestinal	0.409	<0.001
Urinary	0.282	<0.001
Cardiovascular	0.280	<0.001
Thermoregulatory	0.303	<0.001
Pupillomotor	0.232	<0.001
Sexual	0.071	0.031
Total	0.426	<0.001

Data are presented as Spearman's rho, 1-tailed significance.

Figure 2-7 Association between domain scores of the SCOPA-AUT and GCSI total scores in participants with recently diagnosed PD



Best fit lines are presented with corresponding linear regression models (R^2 values) for each component of the SCOPA-AUT and the relationship with GCSI total score.

The PDQ8 summary index was used as a measure of PD-related quality of life with higher scores (0-100%) reflecting a greater disease burden (worse QoL). The median summary index score for participants was 15.6% (21.8, 0-75%). Increasing gastroparesis symptom severity correlated with worse QoL (Spearman's rho, correlation coefficient = 0.314, $P < 0.001$).

Variables identified as being significantly associated with the GCSI total score were entered into a stepwise multiple regression model (summarised in Table 2-15). Autonomic symptoms, gender and MDS UPDRS Parts I, II and IV scores all contributed significantly to the prediction of GCSI total scores. When these variables were combined in a stepwise multiple regression model, they accounted for 31.3% of the variability in GCSI total scores.

Table 2-15 Multiple regression model for predicting the GCSI total score in recently diagnosed PD participants

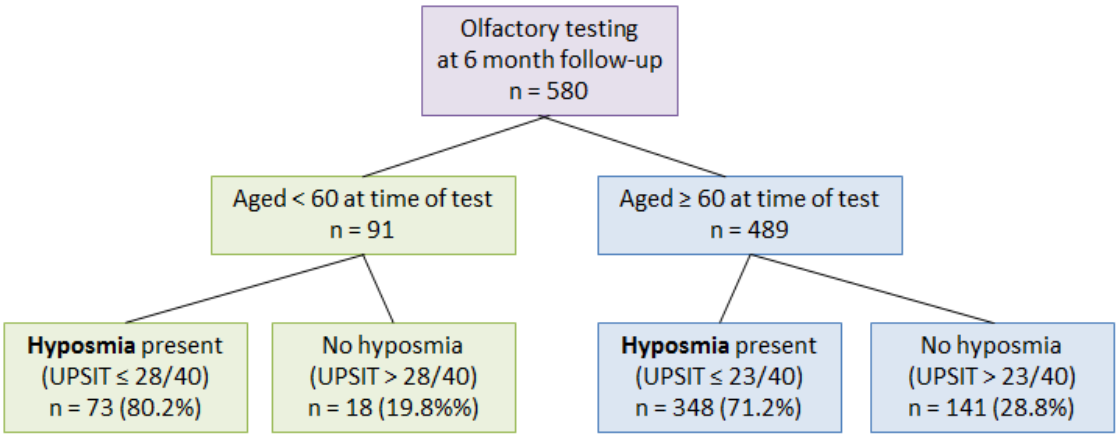
Variables	B	S.E. B	β
Constant	-0.393	0.091	
SCOPA-AUT total score	0.036	0.005	0.388*
MDS UPDRS Part I score	0.030	0.007	0.260*
Male gender	0.189	0.055	0.144**
MDS UPDRS Part II score	-0.014	0.005	-0.159**
MDS UPDRS Part IV score	0.038	0.017	0.096**

R^2 = 0.313 for the model, *P<0.001, **P<0.005

2.9 Olfactory function and association with gastroparesis symptoms in recently diagnosed Parkinson's disease

Of the 691 recently diagnosed cases included in the main analysis, 580 participants had fully completed the olfactory function test at the time of this analysis. The mean UPSIT score (out of a possible total of 40) was 20.27 (SD 6.93) with a median of 20 (IQR 11). Hyposmia defined according to age was evident in the majority of study participants (Figure 2-8).

Figure 2-8 Summary of olfactory testing results from 580 participants with recently diagnosed Parkinson's disease



Amongst those patients aged under 60 years old, 65.7% of those with hyposmia reported at least one gastroparesis symptom with the equivalent figure for those without hyposmia being 64.7%, but there was no statistically significant difference in

the absolute rates of gastroparesis symptoms between those with and without hyposmia (Chi square test, P = 0.576). Similarly the proportion of patients reporting at least one gastroparesis symptom did not differ significantly between those with and without hyposmia in the older age group (≥ 60 years) (Chi square test, P = 0.513). The median GCSI total scores did not differ significantly between those participants with and without hyposmia (Table 2-16).

Table 2-16 GCSI total scores in participants with and without hyposmia.

	GCSI total scores		P value
	Hyposmia	**No hyposmia**	
Age < 60 years	0.22 (0.92)	0.08 (1.29)	0.923
Age ≥ 60 years	0.17 (0.58)	0.25 (0.67)	0.479

Data are presented as median (inter quartile range). Mann-Whitney test to compare medians between those with and without hyposmia according to age.

Comparison of GCSI total scores and UPSIT scores across the whole group (n = 580) did not show a significant relationship between these two variables, disproving the *a priori* hypothesis that higher gastroparesis scores would be associated with worse olfactory function.

2.10 Results – Gastroparesis symptoms in young onset PD cases

2.10.1 Baseline characteristics of study participants – young onset PD

337 patients diagnosed with PD prior to the age of 50 were recruited to the PRoBaND study between 2 February 2012 and 22 July 2013. Of these, 312 completed the GCSI questionnaire in its entirety and 25 had either partially or wholly incomplete questionnaires. There were no significant differences in age, gender, cognition (MoCA score) or motor severity (MDS UPDRS Part III) between these two groups (Table 2-17). All subsequent analyses will focus on the 312 participants with young onset PD who at baseline fully completed the GCSI.

Table 2-17 Comparison of included vs excluded young onset PD cases

Characteristic	All young onset cases n = 337		P value
	Included n = 312	Excluded n = 25	
Age (years)	51.87 (7.55)	50.49 (11.21)	0.578[a]
Male gender (%)	200 (64.7%)	15 (68.2%)	0.821[b]
MoCA score	26.00 (4.00)	25.00 (8.00)	0.123[c]
MDS UPDRS III score	23.00 (20.00)	20.00 (19.00)	0.663[c]

Data presented as: mean age (SD), number (%) male, median MoCA & MDS UPDRS III scores (IQR). Statistical tests: [a]Unpaired t-test, [b]Chi-Square, [c]Mann Whitney test.

Baseline characteristics of the 312 participants with young onset PD are summarised in Table 2-18. The mean age of participants was 51.9 years old with a median age at diagnosis of 44.8 years. 67 participants (21.5%) were diagnosed prior to the age of 40 with the remainder (245, 78.5%) diagnosed aged over 40 but under 50. Disease duration was non-normally distributed across the 312 participants with a median number of years since diagnosis of 6.7 years and a range from just one month through to 35.4 years. More than half of young onset cases had bilateral signs (61% mH&Y ≥2) in keeping with the longer disease duration of this cohort relative to the previously described recently diagnosed cases. Accordingly, average MDS UPDRS section scores were also higher than seen in the recently diagnosed participants. The commonest motor phenotype was the PIGD subtype (59.1%), with 29.5% classed as TD and 11.4% indeterminate.

Table 2-18 Baseline characteristics of participants with young onset PD

Characteristic		Descriptors
Age		51.9 (7.57, 28.23-75.07)
Male gender		202 (64.7%)
Age at diagnosis*		44.8 (7.63, 20.12-52.30)
Disease duration (yrs)*		6.7 (9.05, 0.05-35.46)
Caucasian ethnic origin		296 (94.9%)
mH&Y stage	0	4 (1.4%)
	1	65 (22.6%)
	1.5	43 (14.9%)
	2	94 (32.6%)
	2.5	42 (14.6%)
	3	26 (9.0%)
	4	13 (4.5%)
	5	1 (0.3%)
MDS UPDRS*	Part I	12 (10, 1-38)
	Part II	15 (13, 1-41)
	Part III	22 (21, 0-74)
	Part IV	4 (8, 0-20)
	Total	56 (42, 10-138)
Anxiety		147 (48.2%)
Depression		128 (42.0%)

Data presented as mean (SD, range), except non-normally distributed variables indicated by * which are reported as median (IQR, range). Categorical variables are presented as frequency (%).

Significant symptoms of anxiety and depression (as reported in the LADS questionnaire) were common (48% and 42% respectively), other comorbidities are summarised in Table 2-19.

Table 2-19 Comorbidities reported by study participants with young onset PD

Co-morbid condition	Positive history reported
Hypertension	10.6%
Hypercholesterolaemia	10%
Asthma	7.1%
Angina	3.2%
Stroke / TIA	2.3%
Diabetes (Type 1 or 2)	1.9%
Heart failure	1.9%
Rheumatoid arthritis	1.6%
Bronchitis	1.6%
Myocardial infarction	1.3%
Prostate cancer	1%
Breast cancer	1%
Emphysema	0.3%
Lung cancer	0.3%
Bowel cancer	0.3%

A majority of participants (95.5%) were taking at least one antiparkinsonian medication. The most commonly prescribed medications were Levodopa (74.9%) and dopamine agonists (62.7%). 45% were prescribed a Monoamine-oxidase B inhibitor, 21.2% amantadine and 4.8% anticholinergics.

2.10.2 Presence of gastroparesis symptoms – young onset PD

Of all 312 young onset PD cases analysed, 242 (77.6%) reported at least one symptom associated with gastroparesis (Figure 2-9). The most commonly reported symptoms were bloating and stomach fullness (Table 2-20).

Figure 2-9 Bar chart of the number of gastroparesis symptoms positively reported on the GCSI questionnaire per patient with young onset PD

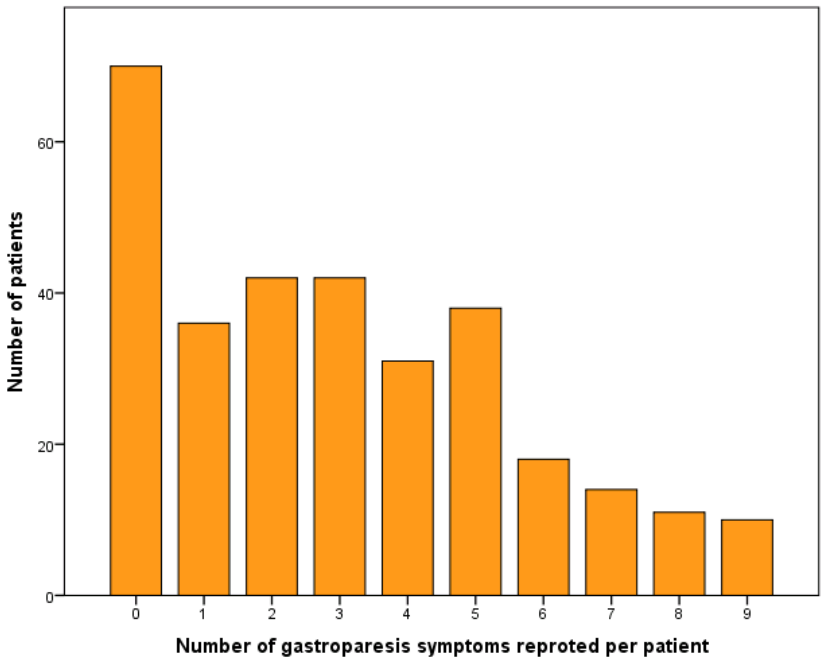


Table 2-20 Prevalence of positive gastroparesis symptoms reported in the GCSI questionnaire by participants with young onset PD

Gastroparesis symptom	Patients reporting positive symptoms in the preceding 2 weeks
Bloating	48.4%
Stomach fullness	46.2%
Stomach larger / distended	43.6%
Postprandial fullness	42.0%
Nausea	35.9%
Early satiety	32.4%
Loss of appetite	30.4%
Retching	16.7%
Vomiting	7.1%

An analysis was undertaken to compare the clinical characteristics of those participants who reported at least one gastroparesis symptom (n = 242) with those who reported none (n = 70). There were no significant gender differences between these two groups (Chi square test, P = 0.847). Patients with and without gastroparesis symptoms did not differ significantly with regard to motor subtype (Chi square test

P=0.742) or disease stage (mH&Y <2 vs ≥ 2, Chi square test P =0.232). Bowel frequency (<1 motion/day vs ≥1 motion/day) did not differ significantly between those with and without gastroparesis symptoms (Chi square test, P = 0.058). However, gastroparesis symptoms were significantly more likely in those participants who reported constipation problems on the MDS UPDRS Part I (Chi square test, P = 0.010). This association remained significant after Bonferroni's correction for multiple comparisons (P < 0.0125). There was a nearly two-fold increased risk of gastroparesis symptoms in those who experienced constipation problems (OR 1.97, 95% CI 1.15 – 3.37). The effects of age, disease duration and MDS UPDRS scores upon the reporting of gastroparesis symptoms are summarised in Table 2-21. The mean MDS UPDRS Part I score was significantly higher in those with gastroparesis symptoms that those with no symptoms but other components of the MDS UPDS did not differ significantly between the two groups.

Table 2-21 Comparison of demographic and clinical differences between those young onset participants with and without gastroparesis symptoms

Variable	Gastroparesis symptoms reported	No gastroparesis symptoms reported	P value
Age	51.5 (7.6)	53.1 (7.3)	0.124
Age at diagnosis	43.9 (5.5)	43.7 (4.7)	0.802
Disease duration	7.7 (6.6)	9.4 (6.7)	0.051
MDS UPDRS Part I	14.2 (6.9)	9.8 (6.3)	**<0.001**
MDS UPDRS Part II	16,9 (9.3)	13.5 (9.5)	0.009
MDS UPDRS Part III	25.7 (14.7)	23.5 (15.7)	0.302
MDS UPDRS Part IV	4.8 (4.8)	4.6 (4.8)	0.816
Total MDS UPDRS	61.2 (28.5)	51.1 (29.6)	0.020

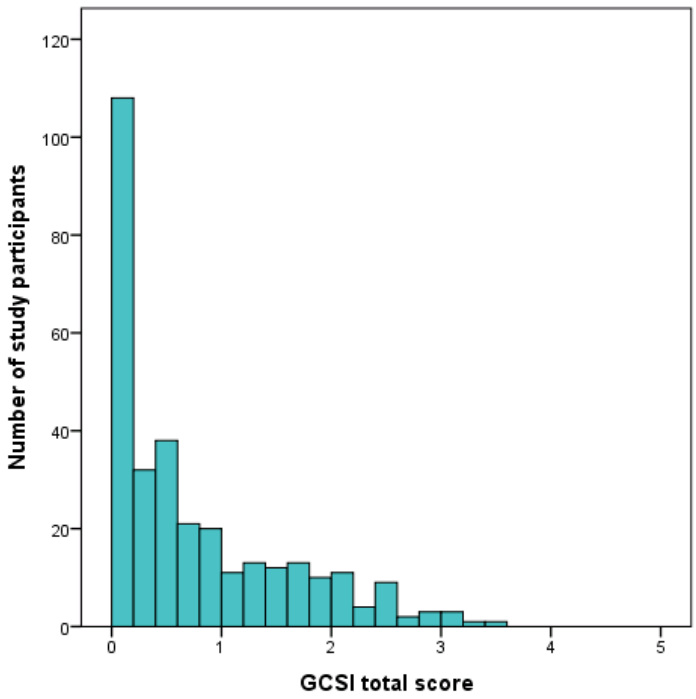
Data presented as mean (standard deviation) with un-paired t-tests used to compare means between the two groups.

Stepwise logistic regression modelling was used to assess the predictive value of constipation problems and the MDS UPDRS Part I score in predicting the GCSI total score. However, constipation problems did not add significantly to the value of the MDS UPDRS Part I score alone in predicting the GCSI total score.

2.10.3 Severity of gastroparesis symptoms – young onset PD

The GCSI total scores of the 312 participants were non-normally distributed (Kolmogorov-Smirnov test P < 0.001) with a median score of 0.43 (IQR 1.16), a mean of 0.76 (SD 0.82) and a reported range from 0 to 3.58 (Figure 2-10). 39 participants (12.5%) had GCSI total scores of ≥ 1.90, consistent with gastroparesis and 11 (3.5%) had GCSI total score of > 2.56 consistent with severe gastroparesis.

Figure 2-10 Baseline GCSI total scores for 321 PRoBaND participants with young onset PD



Diabetes mellitus, a recognised cause of gastroparesis, was present in 6 (1.9%) participants. However, diabetes did not influence the severity of gastroparesis symptoms; the median GCSI total score of those with diabetes (0.93 ± 2.15) was not significantly different to those without diabetes (0.44 ± 1.25) (Mann-Whitney test, P = 0.285).

GCSI total scores of participants did not differ significantly by gender or motor subtype. However, GCSI total scores were significantly higher in those participants with anxiety and depression and also those who had reported recent laxative use (Table 2-22).

Table 2-22 Comparison of GCSI total scores according to patient characteristics

Patient characteristic	Median GCSI total score of participants with characteristic	Median GCSI total score of participants without characteristic	P value
Female gender	0.44 (1.50)	0.42 (1.14)	0.225
PIGD phenotype	0.44 (1.28)	0.42 (1.10)	0.581
Orthostatic hypotension	0.67 (1.44)	0.42 (1.25)	0.247
No exercise in last week	0.50 (1.17)	0.33 (1.29)	0.987
Laxative use in last week	0.83 (1.60)	0.42 (0.94)	**0.026**
Anxiety (LADS ≥ 7)	0.71 (1.40)	0.28 (0.83)	**<0.001**
Depression (LADS ≥ 7)	0.79 (1.49)	0.28 (0.83)	**<0.001**

Data presented as group medians with the Mann-Whitney test for comparisons between groups.

The influence of medication type upon gastroparesis symptom severity was evaluated, but for all categories (Levodopa, Dopamine Agonist, Monoamine-oxidase B inhibitor, Amantadine, COMT Inhibitor, Anticholinergic) GCSI total scores did not differ significantly between those taking or not taking the medication (Mann-Whitney, $P > 0.05$ for all comparisons). The total number of anti-parkinsonian therapies taken by participants was evaluated but did not significantly influence the GCSI total score (Kruskall-Wallis, $P = 0.215$).

The age of participants and their age at diagnosis did not correlate with the GCSI total score (Spearman's rho, $P = 0.936$ and 0.406, respectively). Disease duration was also not significantly associated with gastroparesis symptom severity (Spearman's rho, $P = 0.508$). Disease stage, according to the mH&Y staging system was not a significant determinant of GCSI total score in participants (Kruskall-Wallis, $P = 0.072$). However, disease severity and gastroparesis symptom severity were positively associated. Higher scores in each subsection of the MDS UPDRS, as well as the total score correlated significantly with increasing GCSI total scores (Table 2-23), with the strongest correlation seen with Non-motor activities of daily living (MDS UPDRS Part I). In a linear regression model, MDS UPDRS Part I scores accounted for 18% of the variance in GCSI total scores ($R^2 = 0.180$).

Table 2-23 Correlation between MDS UPDRS scores and the GCSI total score

MDS UPDRS sub-section	Descriptor of the sub-section	Correlation coefficient	P value
Part I	Non-motor experiences of daily living	0.425	<0.001
Part II	Motor experiences of daily living	0.294	<0.001
Part III	Motor examination	0.105	0.046
Part IV	Motor complications	0.115	0.029
Total score		0.268	<0.001

Data are presented as Spearman's rho, 1-tailed significance.

The relationship between non-motor features of PD and the severity of gastroparesis symptoms in participants with young onset PD was further explored with analysis of the NMSS questionnaire. All 9 domains of the NMSS as well as the total score positively correlated with the GCSI total score (Table 2-24).

Table 2-24 Association between the domains of the NMSS (non-motor symptoms questionnaire) and GCSI total scores in participants with young onset PD

NMSS domain number	Domain type	Correlation coefficient	P value
1	Cardiovascular & falls	0.353	<0.001
2	Sleep & fatigue	0.358	<0.001
3	Mood & cognition	0.357	<0.001
4	Perceptual & hallucinations	0.225	<0.001
5	Attention & memory	0.281	<0.001
6	Gastrointestinal	0.267	<0.001
7	Urinary	0.221	<0.001
8	Sexual	0.209	<0.001
9	Miscellaneous	0.304	<0.001
Total	All domains combined	0.427	<0.001

Data are presented as Spearman's rho, 1-tailed significance.

The association between autonomic features of PD and gastroparesis symptoms was examined by comparison of the SCOPA-AUT and the GCSI total score. All domains of the SCOPA-AUT positively correlated with GCSI total scores (Table 2-25). Using a linear regression model to evaluate which autonomic domains were predictive of the GCSI total score, the strongest relationships were seen with the gastrointestinal and

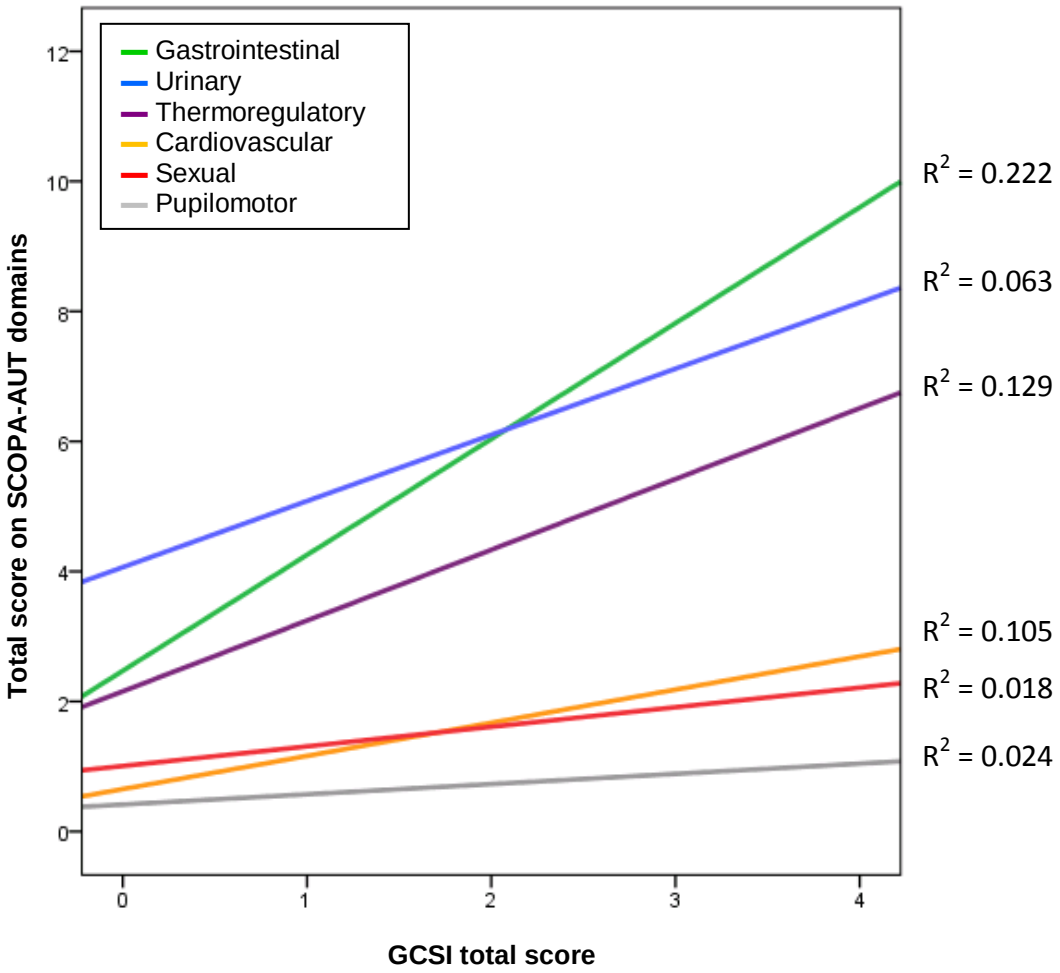
thermoregulatory domains, which respectively accounted for 22% and 12.9% of the variance in GCSI total scores (Figure 2-11).

Table 2-25 Association between domains of the SCOPA-AUT (autonomic symptoms questionnaire) and GCSI total score in young onset PD

SCOPA-AUT domain	Correlation coefficient	P value
Gastrointestinal	0.413	<0.001
Urinary	0.279	<0.001
Cardiovascular	0.352	<0.001
Thermoregulatory	0.368	<0.001
Pupilomotor	0.207	<0.001
Sexual	0.159	0.031
Total	0.460	<0.001

Data are presented as Spearman's rho, 1-tailed significance.

Figure 2-11 Association between domain scores of the SCOPA-AUT and GCSI total scores in participants with young onset PD



Best fit lines are presented with corresponding linear regression models for each component of the SCOPA-AUT.

All variables identified as being associated with higher GCSI total scores were entered into a stepwise multiple regression model. Autonomic symptoms, depression and the MDS UPDRS Part I & III score were all included in the model as significant predictor variables (Table 2-26). Overall the model accounted for 30.8% of the observed variance in GCSI total scores.

Table 2-26 Multiple regression model for predictors of GCSI total score in young onset PD patients

Variables	B	S.E. B	β
Constant	0.074	0.111	
SCOPA-AUT total score	0.033	0.008	0.339*
Depression	0.291	0.114	0.174**
MDS UPDRS Part III score	-0.008	0.004	-0.148**
MDS UPDRS Part I score	0.023	0.011	0.194**

R^2 = 0.308 for the model, *P<0.001, **P<0.005

2.10.4 Sub-analysis of young onset PD participants: Comparison of cases diagnosed before 40 versus diagnosed aged 40 to 50 years.

Participants diagnosed before the age of 50 were dichotomised into those who were diagnosed prior to the age of 40 (n = 67, 21.5%) and those diagnosed aged 40 to 50 years old (n = 245, 78.5%). There was no significant difference in the reporting of gastroparesis symptoms between these two groups (Chi Square, P = 0.502). The GCSI total scores were non-normally distributed for both groups. The median GCSI total score of the <40 group (0.42 ± 0.83) was not significantly different to that of the ≥ 40 group (0.44 ± 1.25) (Mann-Whitney test, P = 0.790). The reporting of constipation in both groups was also comparable (18 out of 67 in the < 40 group and 47 out of 243 in the ≥ 40 group, Chi Square test, P = 0.180). Furthermore the proportions reporting constipation problems on the MDS UPDRS Part I, was not significantly different between the two groups (Chi Square test, P = 0.226).

2.11 Gastroparesis symptoms in an unselected cohort of people with Parkinson's disease attending the Newcastle movement disorder clinic

2.11.1 Background

The previous two sections of this thesis considered the frequency and clinical features associated with gastroparesis symptoms in two large PD groups; those with a recent diagnosis and those with young onset PD. This section presents data concerning gastroparesis symptoms in consecutive patients with PD attending the movement disorder clinic at the Newcastle upon Tyne Hospitals NHS Foundation Trust. The aim of the study was to determine the frequency of gastroparesis symptoms in a non-research, out-patients setting. This service evaluation was approved by the hospitals Research and Development (R&D) department, the Directorate lead for Neurosciences and the lead consultant for this clinic. Permission to use the GCSI in this setting was obtained from the copyright controllers: MAPI Research Trust, France.

2.11.2 Methods

Consecutive patients attending the movement disorder clinic from 12th December 2012 to 3rd April 2013 were asked if they would be willing to complete an gastroparesis symptom questionnaire. Patients were not required to provide written informed consent but were clearly told that they were free to decline the offer to complete this questionnaire. Patients were informed that this questionnaire would be analysed anonymously alongside the following information: gender, age, disease duration, disease stage and current medications. The GCSI was used to report gastroparesis symptom prevalence and severity as previously described in this chapter.

2.11.3 Statistical analysis

Statistical analyses were performed using SPSS 19.0 (IBM, SPSS, Chicago, IL, USA) with appropriate parametric and non-parametric tests performed as previously described (section 2.6).

2.11.4 Results

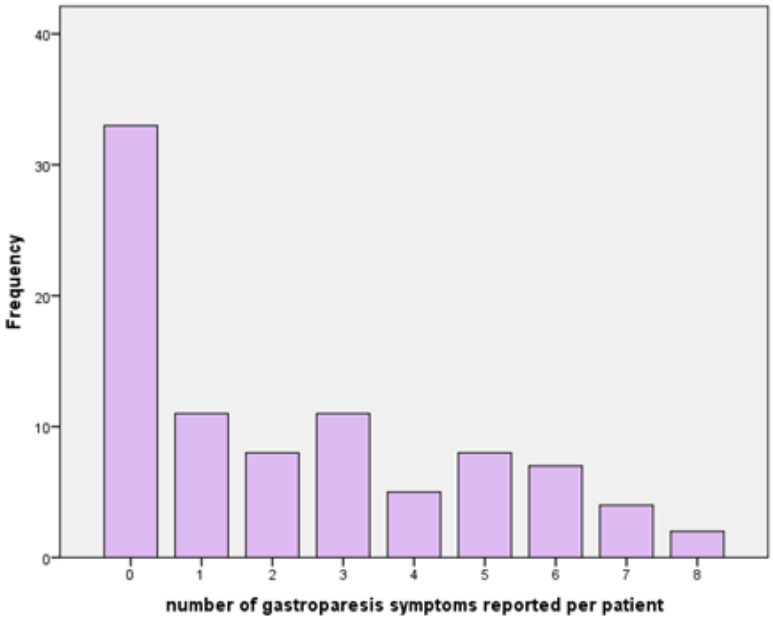
90 patients, from 13 clinics in a four month period completed the GCSI. 62 patients (68.9%) were male. Age was normally distributed by visual assessment of histograms and formal testing (Kolmogorov-Smirnov test $P > 0.05$). The mean age of patients was 66.6 years (SD 9.6). Analysis of patients revealed one outlier with a disease duration of 31 years. It is possible that this case was erroneously diagnosed as idiopathic PD and in fact represented a non-neurodegenerative tremor disorder (e.g.: essential tremor). Excluding this outlier from analysis, disease duration for the remaining 89 patients was non-normally distributed and positively skewed, with a median disease duration of 6.0 years (IQR 7.5).

62.9% of patients reported at least one gastroparesis symptom during the preceding two week period. The most commonly reported gastroparesis symptom was feeling excessively full after meals, which was reported by 34.8% of all patients (Table 2-27).

Table 2-27 Frequency and percentage of gastroparesis symptoms reported by 89 PD patients attending the movement disorder clinic

GCSI question / symptom	Positive symptoms reported
Q1 Nausea	27 (30.3%)
Q2 Retching	11 (12.4%)
Q3 Vomiting	7 (7.9%)
Q4 Stomach fullness	28 (31.5%)
Q5 Not able to finish a normal sized meal	28 (31.5%)
Q6 Feeling excessively full after meals	31 (34.8%)
Q7 Loss of appetite	23 (25.8%)
Q8 Bloating	28 (31.5%)
Q9 Stomach visibly larger	23 (25.8%)

Figure 2-12 Number of gastroparesis symptoms reported by each patient



Patients were dichotomised according to their GCSI total score but no statistically significant differences were observed between those with high (≥1.90) versus low (<1.90) scores (Table 2-28).

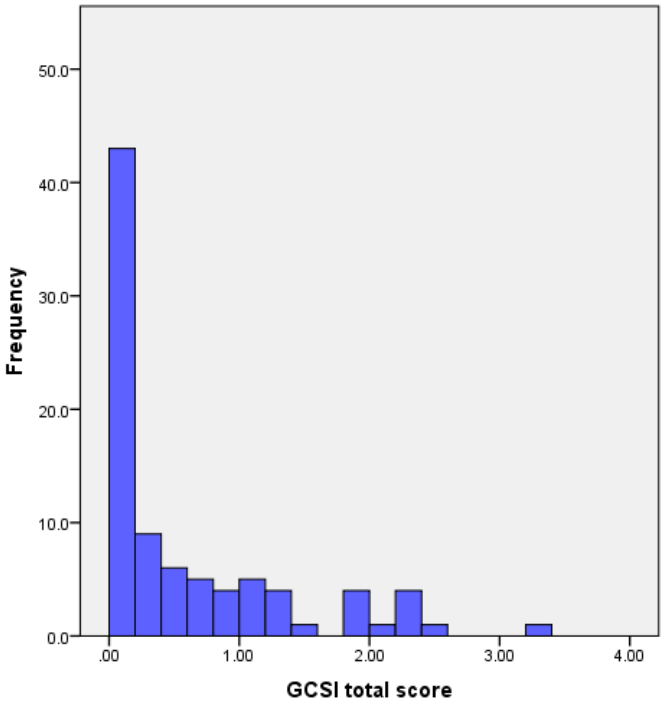
Table 2-28 Comparison of clinic patients with high and low GCSI total scores.

Variable	High GCSI total score n = 9 (10.1%)	Low GCSI total score n = 80 (89.9%)	P value
Age yrs	62.4 (10.4)	67.2 (9.5)	0.177[a]
Male gender (%)	5 (55.6)	56 (70)	0.376[b]
Disease duration yrs	4.0 (7.0)	6.0 (8.0)	0.870[c]
mH&Y stage: 0	0 (0)	0 (0)	0.414[b]
1	4 (44.4)	13 (16.3)	
1.5	0 (0)	1 (1.3)	
2	3 (33.3)	37 (46.3)	
2.5	0 (0)	9 (11.3)	
3	2 (22.2)	18 (22.5)	
4	0 (0)	2 (2.5)	
5	0 (0)	0 (0)	
Disease stage:			0.056[b]
Unilateral (mH&Y<2)	4 (44.4)	14 (17.5)	
Bilateral (mH&Y≥2)	5 (55.6)	66 (82.5)	
Bowel frequency:			0.706[b]
<1/d	4 (44.4)	30 (38)	
≥1/d	5 (55.6)	49 (62)	

Statistical analysis: [a]independent t-test, [b]Chi Square test, [c]Mann-Whitney test.

The GCSI total scores for the 89 patients considered in this analysis were non-normally distributed (Figure 2-13). The mean GCSI total score was 0.58 (SD 0.76) and the median was 0.24 (IQR 0.9).

Figure 2-13 GCSI total scores for 89 patients attending the Movement Disorder Clinic



The GCSI total score was not significantly affected by patient gender (Mann Whitney test P = 0.137), age (Spearman's correlation P = 0.535) or disease duration (Spearman's correlation P = 0.734). GCSI total score was not significantly different by modified H&Y stage (Kruskall Wallis, P = 0.412) or bowel frequency (Mann Whitney, P = 0.650). The use of levodopa containing medications, dopamine agonists and anticholinergics each had no significant effect upon GCSI total score (Mann Whitney, all P>0.05).

2.12 Discussion

This study was a large scale evaluation of gastroparesis symptoms in people with PD. We have been able to characterise the prevalence of gastroparesis symptoms in early disease, young onset cases and unselected consecutive outpatient attenders. The data obtained from the PRoBaND study is unsurpassed in scale, with gastroparesis symptom data available for over 1,000 patients with PD. This data was obtained from more than 50 centres around the United Kingdom, minimising geographical or centre bias.

2.12.1 Gastroparesis symptoms in recently diagnosed PD

This study involved over 600 patients with early PD, which is the largest study to date to assess gastroparesis symptoms in this patient group. Demographically our participants were typical of early disease and comparable with other studies (Khoo *et al.*, 2013). More than half of all recently diagnosed participants reported at least one symptom associated with gastroparesis. Previous cross-sectional studies have reported that approximately 30% of PD patients experience GI symptoms at any point in time (Martinez-Martin *et al.*, 2007; Rodriguez-Violante *et al.*, 2010). However, these studies focused upon lower GI symptoms with a relative scarcity of questions relating to upper GI dysfunction and none specific to gastroparesis. Our study is the first to evaluate the prevalence of upper GI symptoms in early PD in such a large cohort (691 participants). Our findings suggest that in early PD, symptoms of upper GI dysfunction are common and may be as frequent as lower GI symptoms. Although more than half of patients (62.5%) reported at least one upper GI symptom, only a small proportion of participants (4.1%) had abnormally high GCSI total scores consistent with clinically significant gastroparesis.

Most of our *a priori* hypotheses proved to be correct in participants with a recent diagnosis of PD. Large epidemiological studies have reported that gastroparesis (of all aetiologies) is approximately four-times more common in women compared to men (Jung *et al.*, 2009). Therefore, it was hypothesised that the same gender imbalance would be evident in the context of PD. Indeed upper GI symptoms were nearly twice as likely to be reported by women compared with men and GCSI total scores were significantly higher in female participants.

Advancing age appears not to be a major predictor of delayed gastric emptying (Madsen and Graff, 2004) and accordingly, as hypothesised we demonstrated no relationship between age and severity of gastroparesis symptoms.

Evidence regarding the effect of disease duration on gastric emptying is conflicting (Hardoff *et al.*, 2001; Goetze *et al.*, 2005; Goetze *et al.*, 2006; Tanaka *et al.*, 2011; Unger *et al.*, 2011b). Our *a priori* hypothesis that disease duration would not significantly influence gastroparesis symptoms was correct. Although disease duration and disease severity are inherently linked in this neurodegenerative condition, there is evidence to suggest that gastric emptying is slower in more advanced disease stages (Goetze *et al.*, 2005; Goetze *et al.*, 2006). Accordingly we found that gastroparesis symptoms were more marked with advancing disease severity.

In early PD, the PIGD phenotype has been associated with a greater NMS burden than the TD phenotype (Khoo *et al.*, 2013). Therefore, we hypothesised that gastroparesis symptoms would be more severe in those with the PIGD phenotype and this was indeed borne out in our results. We hypothesised that those patients with more severe NMS (as assessed by the NMSS questionnaire and MDS UPDRS Part I) would be more likely to experience gastroparesis symptoms. Our data support this relationship and suggest that gastroparesis symptoms are an important part of the NMS spectrum in early disease. This is a striking finding as gastroparesis symptoms are not included in the current NMS questionnaires used commonly in clinical and research settings.

The aetiology of delayed gastric emptying in PD is unproven but abnormalities in the ENS and the DMNV are strongly implicated. We hypothesised that patients with evidence of autonomic dysfunction would be more likely to experience gastroparesis symptoms. The presence of orthostatic hypotension, an example of autonomic impairment associated with PD (Allcock *et al.*, 2006) was not significantly associated with gastroparesis symptoms. However, the reporting of autonomic symptoms in the SCOPA-AUT questionnaire did strongly correlate with the presence of gastroparesis symptoms. In people with diabetes, gastroparesis has been shown to be associated with fatigue (Cherian *et al.*, 2012) and autonomic and cardiac neuropathies (Campbell *et al.*, 1977; Bian *et al.*, 2011). It has been suggested that in diabetes, gastroparesis is part of a wider progressive autonomic neuropathy process (Mayaudon *et al.*, 1999).

We would speculate based upon our findings that this could also be the case in PD, although further study and evaluation is needed to explore this apparent relationship.

Impaired motility is evident throughout the GI tract in PD (Pfeiffer, 2003). The suggestion that impaired upper and lower GI motility in PD are consequences of the same pathophysiology (Cloud and Greene, 2011) underpinned our *a priori* hypothesis that constipation and gastroparesis symptoms would correlate. This relationship has been demonstrated previously in a small number of patients with PD (Unger *et al.*, 2011b) but ours is the largest study to date to show an association between constipation and gastroparesis in PD. However, given the continuous nature of the GI tract it is important to also consider the possibility that constipation results in 'back-pressure' and consequently causes some upper GI symptomatology.

The relationship between gastroparesis symptoms and mood was not an *a priori* hypothesis. However, we found that both anxiety and depression were associated with higher GCSI total scores, a relationship also described in the context of people with diabetes (de Kort *et al.*, 2012). From our data, it is not possible to further explore the directional nature of this relationship. Participants with anxiety or depression may be more likely to report gastrointestinal symptoms or gastroparesis symptoms could be a cause of abnormal mood.

We demonstrated that worse QoL scores were seen in those participants with more gastroparesis symptoms. The extent to which gastroparesis impacts upon QoL cannot be fully explored by our current data but is an interesting association which we feel is worthy of further evaluation in future research.

We hypothesised that gastroparesis symptoms and olfactory dysfunction would be associated in early PD as the olfactory bulb and gastric ENS are early sites of alpha-synuclein deposition in PD (Hawkes *et al.*, 2007). We were unable to demonstrate a relationship between hyposmia and gastroparesis symptoms in our study. However, as discussed it may be that gastric dysfunction in early PD is largely asymptomatic. To further explore this relationship, future work could look at paired comparisons of olfactory function and gastric emptying speed rather than gastroparesis symptoms.

2.12.2 Gastroparesis symptoms in young onset PD

The PRoBaND study evaluated young onset PD patients, likely to be at greater risk of an underlying genetic cause of Parkinson's. Genotype was not considered in this thesis but it was assumed that a higher frequency of monogenetic cases would be more likely in this young onset cohort.

The median disease duration of participants in this group was 6.7 years, allowing a broader evaluation of the effect of disease duration upon gastroparesis symptoms. However, our *a priori* hypothesis that disease duration would not affect gastroparesis symptoms still pertained.

We dichotomised participants into those diagnosed before the age of 40 and those diagnosed aged 40 to 50 years old. The younger the age of onset, the more likely a monogenetic aetiology and hence it was postulated that if genetic Parkinsonism affected the reporting of gastroparesis symptoms, then comparing groups in this way may reveal different symptom burdens. However, we did not identify any significant differences in gastroparesis symptomatology between these two groups.

Gastroparesis symptoms were more prevalent in the young onset than the recently diagnosed cohort of this study (77.6% vs 62.5%) and severe symptoms suggestive of gastroparesis were more common in the young onset (12.5%) than in the recently diagnosed cases (4.1%). As advanced disease severity but not age or age of onset were shown to impact upon gastroparesis symptoms, it is likely that the greater gastroparesis burden in this young onset group is a reflection of their more advanced disease stage. Factors associated with more severe gastroparesis symptoms in young onset PD included; more severe non-motor features (MDS UPDRS Part I & NMSS), higher MDS UPDRS Part II scores, autonomic symptoms (SCOPA-AUT), laxative use, anxiety and depression. These correlations were largely in line with our *a priori* hypotheses.

2.12.3 Gastroparesis symptoms in PD patients attending routine clinic follow-up

This smaller case series (89 patients) was studied to evaluate the prevalence of gastroparesis symptoms in a cross-sectional sample of clinic attenders. A total of 9 patients (10.1%) had abnormally high GCSI total scores consistent with gastroparesis.

However, when potential influencing variables (gender, disease severity, constipation, medications) were considered, none significantly correlated with gastroparesis symptom severity. It is most likely that this reflects a lack of statistical power owing to the small sample size, rather than a true discrepancy with the previously proven hypotheses.

2.12.4 Strengths and limitations of the study

The main strength of the recently diagnosed and young onset data, obtained as part of the PRoBaND study is the size of the data set; over 1,000 patients. Gastroparesis symptoms in PD have never previously been evaluated on such a large scale. Another important strength of the study is the breath of clinical data collected for comparison with the GCSI. Again, such comparisons have not been undertaken in the previous literature relating to gastroparesis in PD.

One limitation of the study is that it was not possible to measure the gastric emptying speed of participants in the PRoBaND study. Gastric emptying studies are prolonged and impractical outside of a specialist research or tertiary clinic setting. Although the GCSI is not a diagnostic tool, it has been validated as a good indicator of patients' symptoms (Revicki *et al.*, 2003) and in the context of diabetes higher GCSI symptom scores have been shown to correlate with slower gastric emptying (Olausson *et al.*, 2013).

The scale of our study meant that it was not possible to obtain a detailed gastrointestinal past medical history from all participants although we were able to consider diabetes as a potential confounder. The study design did not allow for a comparator control group at this stage. However, the within group analyses did allow for full testing of all of our *a priori* hypotheses. As the PRoBaND study progresses some first degree relatives of enrolled PD participants will be recruited for evaluation and the GCSI results from these non-PD participants could serve as a control group.

The evaluation of gastroparesis symptoms in PD patients attending the movement disorder clinic was limited by its small scale. Hence, there was a major limitation with regard to statistical power in this study. However, the evaluation of gastroparesis symptoms in unselected PD outpatients is still a valid project and something which we hope to integrate into routine clinical practice.

2.12.5 Summary and future directions

The major finding of this study is that in all stages of PD, including early disease, upper GI symptoms are common. The estimated rates of symptomatic gastroparesis in PD are relatively low (4.1 – 12.5%) which is in contrast to the conclusions of a systematic review which suggested that 70-100% of all patients with PD are likely to have delayed gastric emptying (Heetun and Quigley, 2012). The apparent disparity between measured gastric emptying delay and associated symptoms of gastroparesis is interesting but is not unique to patients with PD. Similar studies in people with diabetes have reported the prevalence of significant gastroparesis symptoms to be approximately 5-12% (Haans and Masclee, 2007; Kofod-Andersen and Tarnow, 2012). Research into gastroparesis in diabetes is more established than in people with PD and in diabetic populations a relatively poor relationship between gastric emptying and symptoms has been described (Samsom *et al.*, 2009). Therefore we have concluded that whilst delayed gastric emptying is common in PD, much of this dysfunction appears to be asymptomatic. However, this remains an important finding as even asymptomatic impairment of gastric motility could have major implications for the absorption and action of Levodopa medications.

The PRoBaND study is on-going at the time of writing this thesis. Participants in the recent onset group will be evaluated prospectively and will complete the GCSI on three occasions in total (baseline, 18 month and 36 month visits). This will allow prospective evaluation of changes in gastroparesis symptoms over time which has not previously been undertaken in a PD population.

Chapter 3 Exploration of a novel treatment for delayed gastric emptying in Parkinson's disease

3.1 Background

This chapter describes the interim findings of a phase II study to investigate the effects of a novel treatment for gastroparesis in patients with PD, motor fluctuations and delayed gastric emptying.

Delayed gastric emptying can impair the absorption of Levodopa (Muller *et al.*, 2006; Doi *et al.*, 2012) and there is evidence to suggest that this may contribute to motor response fluctuations associated with long-term Levodopa therapy (Djaldetti *et al.*, 1996). Furthermore, the use of prokinetic agents in patients with fluctuations has been reported to improve Levodopa absorption and motor function (Neira *et al.*, 1995; Nishikawa *et al.*, 2012). However, currently there are limited options for the treatment of delayed gastric emptying in people with PD.

Camicinal (GSK962040) is a novel motilin agonist which stimulates phase III of the MMC in the stomach thereby enhancing gastric emptying. In initial studies of Camicinal in healthy volunteers and people with diabetes it was found to be an effective and safe prokinetic agent. This project was a phase II, double-blind, placebo controlled study to assess the safety and efficacy of Camicinal in people with PD. The results presented are part of an interim analysis of the first 15 participants.

The study began as a single site project in Newcastle in May 2012. However, in November 2012 the study was paused due to issues with the study design. This facilitated a review of the protocol and a major amendment which was implemented from May 2013 until October 2013. This chapter will describe the methods pertaining to the original protocol. Section 3.3.9 explains the changes made during the second phase of this project and the rationale behind redesigning the study.

3.2 Aims & objectives

The aim of this study was to perform an interim analysis to evaluate the safety and efficacy of Camicinal in people with Parkinson's disease with motor fluctuations and delayed gastric emptying. The efficacy of Camicinal was evaluated through its effect upon the following parameters:

- Levodopa pharmacokinetics
- Gastric emptying
- Gastroparesis symptoms
- PD motor function
- PD non-motor symptoms
- Bowel function

3.2.1 Hypotheses

The *a priori* hypotheses of this study were that, compared with placebo Camicinal would:

- Accelerate gastric emptying.
- Improve Levodopa absorption.
- Improve gastroparesis symptoms.
- Reduce 'off' time and increase 'on' time
- Improve MDS UPDRS scores (reflecting improved PD motor and non-motor function).
- Improve constipation problems.

3.3 Methods

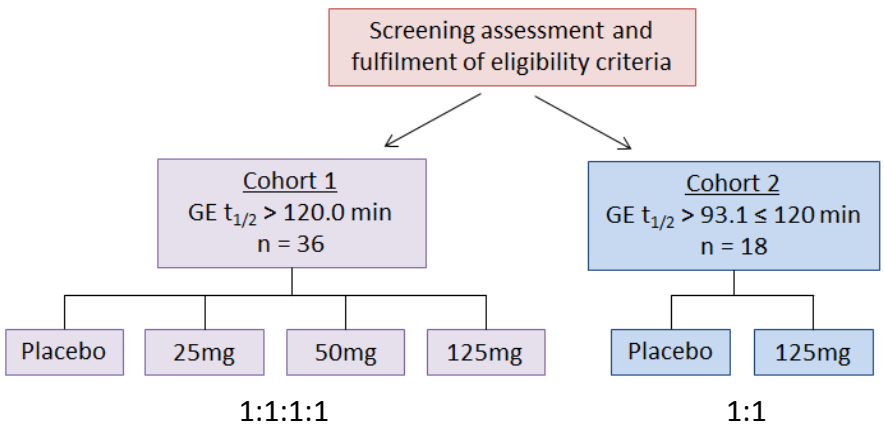
This study was conceived by the Worldwide Development Division of GlaxoSmithKline (GSK). Newcastle University contributed to the protocol design and subsequent amendments. It was a single site study, led by Newcastle University and sponsored by the pharmaceutical company GlaxoSmithKline (GSK).

3.3.1 Study design and overview

This was a randomised, double-blind, placebo-controlled, parallel group, dose ranging study to assess the effect of repeated doses of Camicinal (GSK962040) on Levodopa absorption in patients with PD, motor fluctuations and delayed gastric emptying.

Eligible study participants were assigned to one of two treatment groups according to the severity of their gastric emptying delay at baseline. Cohort 1 comprised subjects with severely delayed gastric emptying half times (GE $t_{1/2}$ > 120 minutes). Cohort 2 comprised those with moderately delayed gastric emptying (GE $t_{1/2}$ > 93.1 ≤ 120 minutes). Patients in Cohort 1 were randomised to placebo or Camicinal 25mg, 50mg or 125mg in a 1:1:1:1 ratio. Patients in Cohort 2 were randomised to placebo or Camicinal 125mg in a 1:1 ratio. The intention was to recruit 36 participants to Cohort 1 and 18 to Cohort 2 (Figure 3-1).

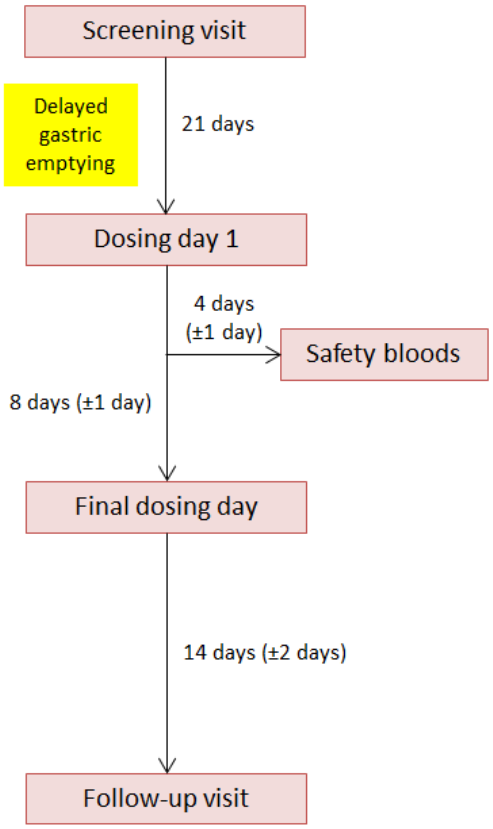
Figure 3-1 Flow diagram summarising participant randomisation and Cohort structure



Randomisation of study participants was pre-determined by the study statistician using a validated randomisation software package (RandAll). The randomisation was stratified such that the treatment to placebo ratio within each cohort was maintained.

Eligible study participants were required to attend a total of five study visits over approximately a six week period (Figure 3-2). The primary aim of the screening visit was to measure gastric emptying speed alongside evaluation of baseline Levodopa absorption, motor function and non-motor features. If following the screening visit, delayed gastric emptying was demonstrated and other eligibility criteria were met, the first dosing day took place within 21 days of the screening date. The total dosing period was 8 days (± 1 day). For the remainder of this report, the first day of dosing will be referred to as 'day 1' and the final day (7-9 days later) will be referred to as 'day 8.' The screening visit served as a 'baseline' assessment for the study and will be referred to as such in the results section. On the fourth day of dosing (± 1 day) blood samples were taken for safety and monitoring purposes, given the investigational nature of this phase II study. A fifth and final follow-up visit was performed 14 days (± 2 days) after the final dosing day.

Figure 3-2 Summary of study visit schedule



The protocol design included plans for an interim analysis once 15 subjects had completed the study. This was intended to assess futility and allow re-estimation of the overall sample size, if necessary. This interim analysis is the basis of this thesis report. Follow-up data was not presented in this thesis however it was analysed by the study team and sponsors, with no major problems identified.

3.3.2 Patient identification and selection

The study protocol was approved by the NHS Local Research Ethics Committee (NRES Committee North East - Newcastle & North Tyneside 1). Thereafter, approvals were obtained from Medicines and Healthcare products Regulatory Agency (MHRA) and the Trust Research and Development (R&D) department. All potential participants were patients attending the movement disorder service in Newcastle upon Tyne Hospitals NHS Foundation trust.

Between June 2012 and November 2012 a total of 36 patients were approached regarding the study and provided with patient information sheets. Of these, 9 consented to participate. All patients provided written informed consent prior to participation in the study.

3.3.3 Eligibility criteria for the study

Inclusion criteria:

1. Diagnosis of idiopathic Parkinson's disease (according to modified Hoehn & Yahr criteria Stages II-IV) and with suboptimal motor control on Levodopa or Levodopa combination therapy (i.e. wearing off, peak dose dyskinesias, delayed on or no-on effects).
2. Subjects receiving a stable regimen of Levodopa for at least four weeks prior to screening.
3. Patient has gastroparesis at screening:
    - A patient is eligible for Cohort 1 if the gastric half-time of emptying >120.0 min as determined by ^{13}C-oral breath test.
    - A patient is eligible for Cohort 2 if the gastric half-time of emptying >ULN (93.1 min) and ≤120.0 min as determined by ^{13}C-oral breath test.

4. Between 40 and 80 years of age, inclusive.
5. Patient has never had a gastrectomy, nor major gastric surgical procedure or any evidence of bowel obstruction or strictures within the previous 12 months.
6. Dosage of any concomitant medications has been stable for at least 4 weeks.
7. A female subject is eligible to participate if she is of:
    a. Non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea (in questionable cases a blood sample will be used to confirm menopausal status).
    b. Child-bearing potential and is abstinent or agrees to use an appropriate contraceptive method prior to the study and for at least five days after the last dose.
8. ALT < 2xULN; alkaline phosphatase and bilirubin ≤ 1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
9. ECG shows single or average QTc, QTcB or QTcF< 450 msec; or QTc < 480 msec in subjects with Bundle Branch Block.

Exclusion criteria:

1. Late stage advanced subjects with incapacitating peak dose or biphasic dyskinesia on a stable Levodopa regime.
2. Presence, or history within the previous 3 months, of significant and/or uncontrolled psychiatric, neurological (other than Parkinson's disease), gastro-intestinal, haematological, endocrine, neurological (other than Parkinson's disease),cardiovascular disease, active malignancy (other than basal cell cancer) or other condition that would in the opinion of the investigator or medical monitor make the subject unsuitable for inclusion in this clinical study.
3. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening.
4. Patient has a gastric pacemaker.
5. Patient is on chronic enteral or parenteral feeding.
6. Patient has evidence of severe cardiovascular autonomic neuropathy (e.g. history of recurrent syncope in the last 6 months).

7. Estimated or measured glomerular filtration rate ≤ 30 mL/min.
8. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones).
9. Pregnant females as determined by positive serum or urine hCG test at screening or prior to dosing.
10. Lactating females.
11. Unable to refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice from 7 days prior to the first dose of study medication until follow-up.
12. Use of medications potentially influencing upper gastrointestinal motility or appetite within one week of the study (e.g., prokinetic drugs, macrolide antibiotics (erythromycin), GLP-1 mimetics).
13. Unable to refrain from use of prohibited medications (listed in Appendix C) within the restricted timeframe relative to the first dose of study medication.
14. The patient has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer).
15. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.
16. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56-day time-period.

3.3.4 Study visits and general procedures for participants

All consented and eligible study participants attended for five visits as previously outlined (Figure 3-2). For three of these visits (screening / baseline, day 1 and day 8), participants were required to attend the research unit in the morning, having fasted overnight and omitted all morning medications. The overnight fast was necessary in order to accurately evaluate gastric emptying speed and the omission of medications prior to arrival ensured that baseline assessments were performed in a clinically defined 'off' state. At each visit, patients took their usual Levodopa containing

medications at a specified time: referred to as time 0. Thereafter, serial assessments were undertaken to measure: Levodopa absorption, gastric emptying and motor function. The timings of all assessments were in minutes, relative to the time of the levodopa dose (at time 0) such that a timed assessment 10 minutes prior to Levodopa dosing would be at time '-10 minutes'.

At the screening visit, after obtaining written informed consent, a full medical history was obtained and details of all current medications were recorded. A detailed, systematic physical examination was performed alongside measurement of postural blood pressure (after lying for at least five minutes and standing for three minutes). Participants' height and weight were also recorded. A 12 lead electrocardiogram (ECG) was performed prior to Levodopa ingestion and again at time 0. Baseline urinalysis and blood sampling (including for Hepatitis B and C testing) was performed prior to Levodopa dosing on the screening day. In female participants with reproductive capacity (i.e. pre-menopausal with an intact uterus) pregnancy testing (urinary HCG) was performed.

One of the main aims of the screening visit was the evaluation of gastric emptying speed. Gastric emptying was measured with breath testing after a ^{13}C-labelled meal which was ingested 10 minutes prior to Levodopa dosing. Details of the gastric emptying breath test are summarised in section 3.3.5.

After Levodopa dosing, motor function was evaluated serially over the next four hours. The MDS UPDRS Part III was repeated on three occasions (120, 180 and 240 minutes post-Levodopa). Motor function was also evaluated with finger tap testing. This required the patient to alternately tap two counters mounted 30 cm apart (Figure 3-3), as many times as possible in 60 seconds. Participants were asked to perform this with the same hand: the more affected side or dominant hand in the case of symmetrical disease). This test was repeated twice at 8 specified time points (-90, 0, 30, 60, 90, 120, 180 and 240 minutes). Mean scores for each time point were analysed.

Figure 3-3 Picture of the finger tap assessment board

Throughout the assessment period, participants were asked to remain in bed in a semi-supine position (45 degree angle). They were also asked not to consume any food other than the test meal and were asked only to drink 180 ml of tap water. Additionally, where possible only Levodopa based medications were taken at time 0 and other parkinsonian medications (dopamine agonists, monoamine oxidase B-inhibitors etc.) and second doses of Levodopa were withheld until the end of the four hour assessment period. This restriction was in place to facilitate paired motor and pharmacokinetic assessments as patients transitioned from the 'off' state, to 'on' and then 'off' again towards the end of the visit. At the end of the four hour testing period, participants were allowed to eat and drink normally and were encouraged to take any usual medications which had been omitted that morning. Table 3-1 summarises the procedures performed during the screening / baseline assessment.

The sequence of assessments and procedures performed on days 1 and 8 were largely similar to those described for screening. The main difference from screening was that the study medication was given 90 minutes prior to ingestion of Levodopa and additionally four plasma samples for pharmacokinetic testing were obtained prior to time 0. Table 3-2 summarises the sequence of assessments and procedures performed on dosing days.

Table 3-1 Screening / baseline visit: assessments and procedures

	Time (min)														
	Pre-Dose	-90 min	-10 min	0 min	15 min	30 min	45 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	Post Testing
Complete physical examination	✓														
12-lead ECG	✓			✓											
Blood pressure, height & weight	✓			✓											
Medication Review	✓														
Urinalysis & blood tests	✓														
^{13}C breath test	✓	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	
PK blood sample	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Levodopa dose				✓											
Test Meal			✓												
Full MDS-UPDRS evaluation	✓														
MDS UPDRS Part III										✓		✓		✓	
Finger taps	✓			✓		✓		✓	✓	✓		✓		✓	
End of Fasting period															✓

PK = pharmacokinetic

Table 3-2 Dosing days 1 and 8: assessments and procedures

	Time (min)																	
	Pre-Dose	-90 min	-75 min	-60 min	-30 min	-10 min	0 min	15 min	30 min	45 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	Post Testing
Brief physical examination	✓																	
12-lead ECG	✓						✓											
Blood pressure, height & weight	✓						✓											
Urinalysis & blood tests	✓																	
AE assessment	✓																	
Medication Review	✓																	
^{13}C breath test	✓	✓							✓	✓	✓	✓	✓	✓	✓	✓	✓	
PK blood sample	✓		✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Levodopa dose							✓											
Study Treatment Dosing		✓																
Test Meal						✓												
Full MDS-UPDRS evaluation	✓																	
MDS UPDRS Part III													✓		✓		✓	
Finger taps	✓						✓		✓		✓	✓	✓		✓		✓	
End of Fasting period																		✓

AE = adverse events

PK = pharmacokinetic

3.3.5 Measurement of gastric emptying speed

Gastric emptying was measured using a ^{13}C oral breath test following a labelled test meal. The test meal was a standardised breakfast consisting of scrambled egg labelled with ^{13}C-*Spirulina plantensis,* six Saltine Crackers and 180ml of tap water at room temperature. The meal was consumed by all participants within 10 minutes and none reported significant dysphagia.

Spirulina plantensis is a non-radioactive, algae-based substance which can be taken as a health food supplement. Breath testing kits and sample analysis were provided by GSK and Advanced Breath Diagnostics, Nashville, TN, USA.

The ^{13}C-*Spirulina* breath test has previously been validated through comparison with scintigraphy (Lee *et al.*, 2000). After ingestion ^{13}C is absorbed in the intestine, oxidised in the liver and excreted in the breath (Perri *et al.*, 2005). Serial measurement of ^{13}C in expired breath is therefore a surrogate marker of gastric emptying speed.

Standardised test kits were provided by ABDiagnostics (Figure 3-4). Two baseline breath samples were obtained in the fasting state, prior to ingestion of the test meal. Following consumption of the ^{13}C-*Spirulina plantensis* labelled test meal, serial breath sampling was performed at designated time intervals up to four hours after consumption of the meal (30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes post-meal). This collection sequence was followed at screening and dosing visits. Contained breath samples were stable stored at room temperature for up to four weeks. However, transport of samples to the ABD Diagnostics laboratories in Nashville was typically arranged within 48 hours of sampling.

Figure 3-4 Spirulina gastric emptying testing kit (courtesy of ABDiagnostics)

Serial measurement of the ^{13}C content of expired breath allowed the calculation of the gastric emptying half time (GE $t_{1/2}$). Higher GE $t_{1/2}$ scores represented slower gastric emptying. Patients in cohort 1 had a GE $t_{1/2}$ of > 120 minutes and patients in cohort 2 had a GE $t_{1/2}$ of >93.1 and ≤ 120 minutes.

3.3.6 Clinical assessments

Throughout the study period standardised assessments were used to assess: motor function, non-motor symptoms and gastroparesis symptoms as outlined below.

Motor and non-motor symptoms

The MDS-UPDRS (Goetz *et al.*, 2008) is a validated tool for the assessment of the motor and non-motor features of PD. The MDS UPDRS was administered by trained and experienced raters. The full MDS UPDRS was performed at screening / baseline, day 1, day 8 and follow-up.

Additionally patients were asked to complete a daily diary, recording their motor symptoms when at home. This diary asked patients, for each 30 minute period of the day and night, to report their motor state as one of the following:

- 'Off': time when the medication has worn off and is no longer providing benefit with regard to mobility, slowness and stiffness.

- 'On' without dyskinesia: time when medication is providing benefit with regard to mobility, slowness and stiffness.
- 'On' with non-troublesome dyskinesias: dyskinesia does not interfere with function or cause meaningful discomfort.
- 'On' with troublesome dyskinesias: interferes with function and causes meaningful discomfort.
- Asleep

Gastroparesis symptoms

Gastroparesis symptoms were assessed using the Gastroparesis Cardinal Symptom Index Daily Diary (GCSI-DD) (Revicki *et al.*, 2009) (Appendix B). This daily equivalent of the nine item self-reported GCSI (which was described extensively in the previous chapter), has been validated as a reliable tool for assessing gastroparesis symptoms and importantly it has been shown to be responsive to change and hence suitable for use in an interventional research study setting (Revicki *et al.*, 2012).

Patients were asked to complete the GCSI-DD after their evening meal, every night for one week following the screening visit, every evening in the dosing week and every evening in the two week follow-up period. Clear verbal and written instructions were given regarding how to complete the GCSI-DD and the completed diaries were reviewed with participants at each subsequent study visit.

3.3.7 Study drug

The following details regarding the study drug have been provided by the manufacturer and study sponsor GlaxoSmithKline.

Camicinal (GSK962040) is a novel motilin agonist believed to enhance gastric emptying through a stimulatory effect on phase III of the gastric MMC. Single and repeated oral dosing for periods of up to 14 days in healthy volunteers and patients with type 1 diabetes have been safe and as well-tolerated as placebo. Pharmacokinetic parameters from these studies have been reported to be dose proportional up to a maximum studied dose of 150mg daily. Improvement in gastric emptying speed has

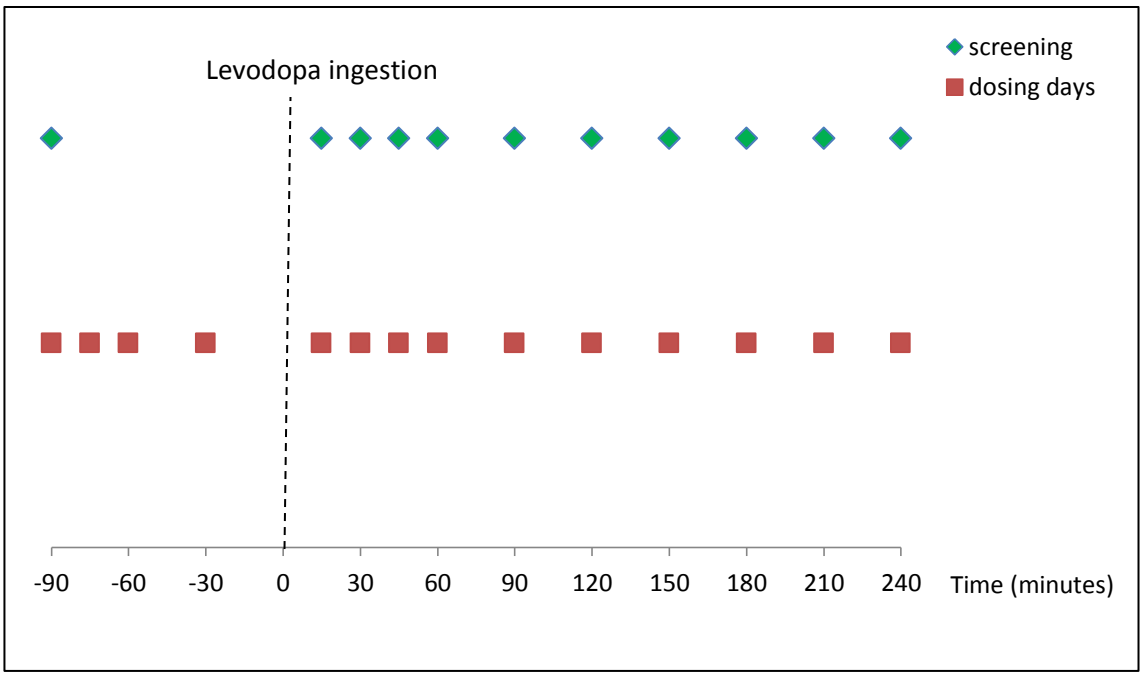
also been described as dose proportional in these initial studies, with mean reduction in GE $t_{1/2}$ of 30-64% compared with placebo.

The study medication is primarily metabolised by the liver, hence the need to exclude patients with underlying significant hepatic impairment at baseline. Liver function tests were performed at all study visits. Renal elimination is estimated to account for approximately 30% of the metabolism of Camicinal. Patients with an estimated glomerular filtration rate (eGFR) of less than 30ml/min were excluded from participation in the study.

3.3.8 Pharmacokinetic analysis

Plasma samples for pharmacokinetic analysis were obtained serially at screening, and on dosing days (Figure 3-5). At baseline (screening) one sample was taken prior to Levodopa ingestion (-90 minutes) and at 10 time points post-dosing (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes). On dosing days 1 and 8 the same sequence of post-Levodopa sampling was followed however in the pre-dose period four plasma samples were obtained (-90, -75, -60 and -30 minutes).

Figure 3-5 Time points for plasma pharmacokinetic sampling



Two pharmacokinetic measures of Levodopa absorption were evaluated in this study. The maximum plasma levodopa concentration (Cmax) measured in the post-dosing period at each assessment visit was reported. A second measure of Levodopa absorption; the area under the curve (AUC) was also reported across four time points (in hours) post Levodopa ingestion: $AUC_{(0\text{-}0.5)}$, $AUC_{(0\text{-}1)}$, $AUC_{(0\text{-}2)}$ and $AUC_{(0\text{-}4)}$.

3.3.9 Study progression, evaluation and re-design

Recruitment to the study began in May 2012. By November 2012 a total of 36 patients had been approached and provided with written information regarding the study. A total of 9 patients consented to participate and attended for screening. The remaining 27 were not enrolled in the study for the following reasons:

- Prescribed a prohibited concomitant medication (n = 8)
- Comorbid illness limiting participation in the study (n = 7)
- No significant motor fluctuations at the time of evaluation (n = 3)
- Patient not interested in participation (n = 9)

One of the 9 participants withdrew from the study mid-way through the screening visit as the patient had a delayed 'on' during the visit and felt unable to continue with the assessments. Of the remaining 8, two had $GEt_{1/2} > 93.1$ minutes and therefore delayed gastric emptying as defined by the study protocol. One of these two participants was randomised to active treatment (125mg Camicinal / GSK962040) and one received placebo. These recruitment rates were lower than had been anticipated. This prompted a review of the study design by the team at Newcastle University and GSK.

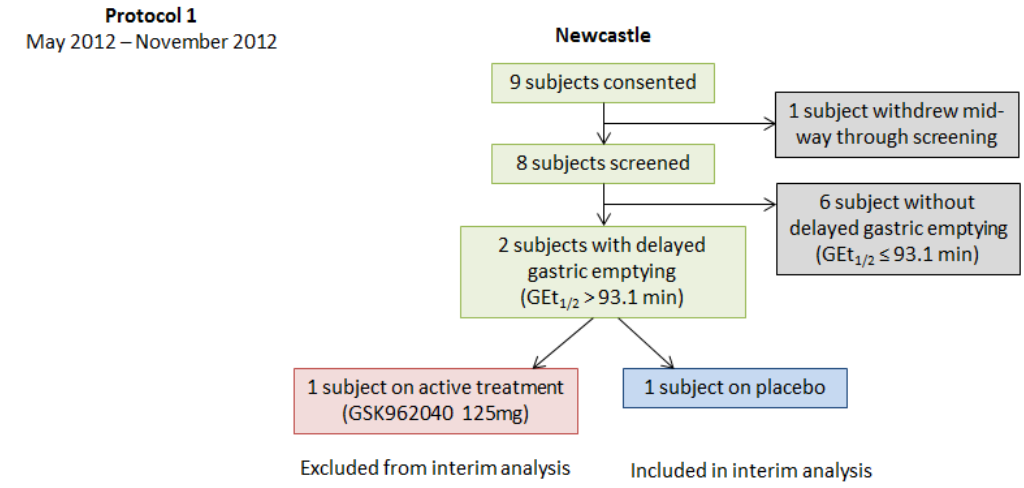
Additionally, in November 2012, GSK reviewed interim results from a study of Camicinal in patients with diabetes and delayed gastric emptying. This analysis showed that whilst the 125mg dose of Camicinal was well tolerated, the exposure to the drug was more variable than anticipated. Pharmacokinetic analysis of some individuals revealed higher exposure levels at the 125mg dose than expected. Therefore, the sponsors decided to pause the study and amend the protocol such that, as a precautionary measure, the Camicinal 125mg dose was removed from the PD study.

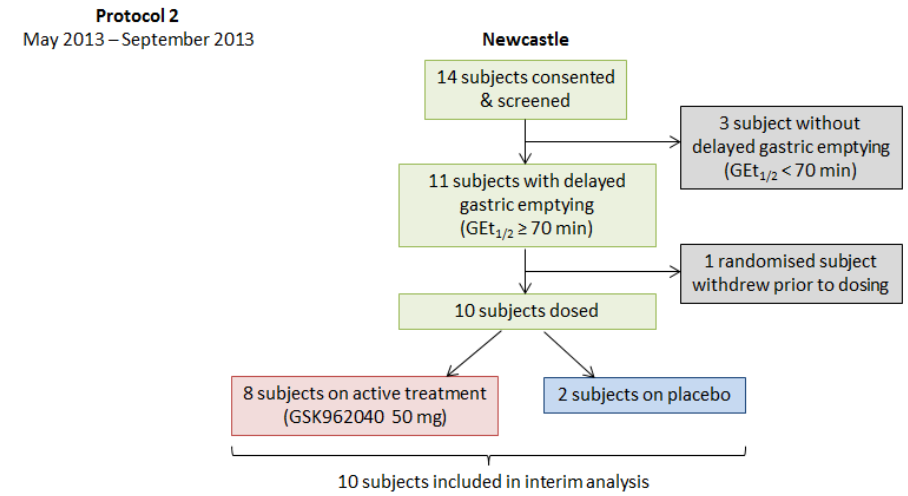
These two events led to a major protocol amendment which was approved by REC and the MHRA in April of 2013. The methods and study procedures were largely unchanged however the following adjustments were implemented in the revised protocol:

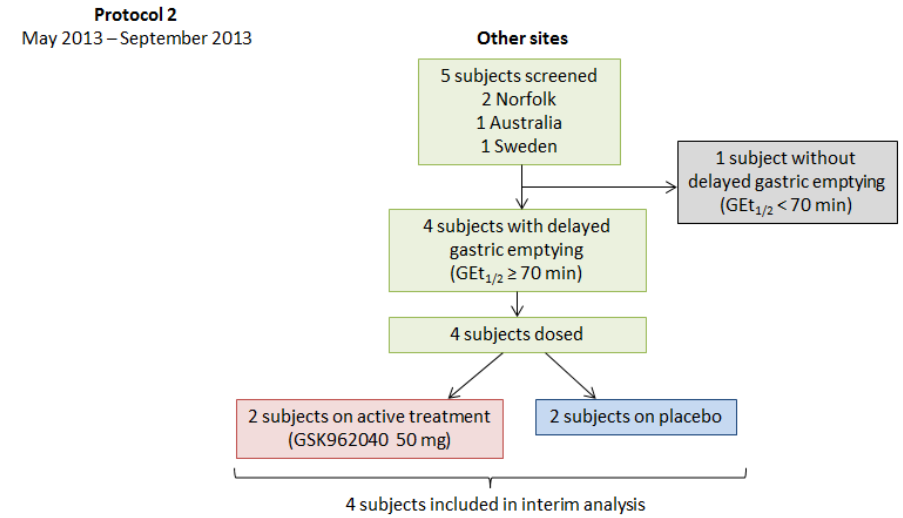
1. Gastric emptying criteria for entry into the study were changed from > 93.1 minutes to $GEt_{1/2} \geq 70$ minutes.
2. The dose-ranging aspect of the study design was removed and instead a single dose of Camicinal (50mg) was compared with placebo (randomised in a 2:1 ratio).
3. The two cohort design was removed. However, participants were dichotomised according to the severity of gastric emptying delay ('Moderate': $GEt_{1/2} \geq 70$ but ≤ 100 min or 'Severe': $GEt_{1/2} > 100$ min). Randomisation of participants to the treatment and placebo groups was stratified such that an even distribution of 'Moderate' and 'Severe' cases was assigned to each of the study arms.
4. The study was extended from a single centre to involve additional UK and international sites (Norwich, Cambridge, Australia and Sweden).
5. Within in the North East of England, additional participant identification centres (PIC sites) were approved to identify potential participants from other regional movement disorder clinics.

Recruitment to the study according to this revised protocol began in May 2013. Figure 3-6 summarises study activity (under both protocols) from May 2012 until September 2013.

Figure 3-6 Summary of study activity according to protocol version and study site







Between May 2013 until September 2013, 56 patients from Newcastle and the North East were approached and provided with details of the study. 14 patients consented and attended for screening and a further two agreed to participation but had not completed the study at the time of this analysis. A total of 40 patients were ineligible or uninterested in the study for the following reasons:

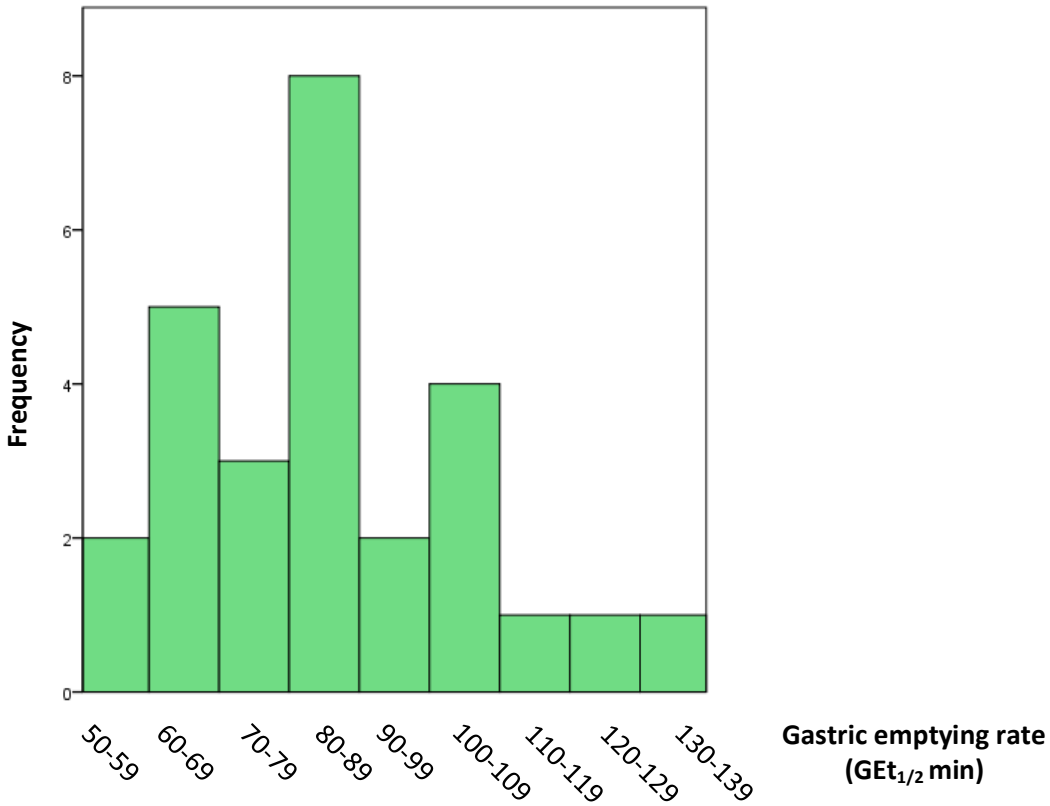
- Prescribed a prohibited concomitant medication (n = 4)
- Comorbid illness limiting participation in the study (n = 4)
- No significant motor fluctuations (n = 3)
- Participating in another on-going trial (n = 1)
- Patient not interested in participation (n = 15)
- Unable to contact in person / did not respond to invitation (n = 13)

Of the 14 subjects screened in Newcastle, 11 had delayed gastric emptying ($GEt_{1/2} \geq 70$ min) and three did not. One of the 11 eligible patients withdrew after randomisation but prior to dosing. This was due to an acute episode of ill-health (urinary sepsis and urinary retention) which was unrelated to the patient's PD or any of the study procedures. In Newcastle, eight participants were randomised to Camicinal 50mg once daily and two were randomised to placebo once daily. Dosing was double blinded and the blind was maintained throughout the study.

In the additional study centres, over the same time period five subjects were consented and screened. Four had delayed gastric emptying ($GEt_{1/2} \geq 70$ min) and one did not. Two of these subjects received Camicinal 50mg once daily and two received placebo once daily.

A summary of the baseline gastric emptying rates for all screened participants can be found in Appendix C. The range of gastric emptying rates measured at screening is represented graphically in Figure 3-7.

Figure 3-7 Gastric emptying rates for all consented participants



The interim analysis, which forms the basis of this thesis report, considered the first 15 subjects to complete the study. Newcastle contributed 11 of these 15 cases. Data from the Newcastle subject (recruited under protocol 1) who received 125mg Camicinal is not analysed in this thesis. The subject experienced no adverse events. However, the dose this subject received is not directly comparable with the 50mg dose taken by the other 10 subjects. The participant who received placebo under protocol version 1 was included in the interim analysis as their study schedule was comparable with that of subjects who received placebo according to protocol 2.

3.4 Statistical analysis

Statistical analyses were performed using SPSS 19 (SPSS, Chicago, IL). Descriptive analyses were used to report trends in the data between groups and within groups. Graphical representations of group means and standard deviations are presented to support the description of trends.

Given the small sample size non-parametric tests were used to compare the study groups. Medians were reported with the appropriate interquartile range. Comparison of medians between the treatment and placebo groups was undertaken with the Mann-Whitney test. Paired comparisons within groups over time were performed with the Wilcoxon signed rank test. Comparison of proportions between groups was undertaken with Chi square testing.

For each statistical analysis, a P value of < 0.05 was deemed significant. Due to the small sample size and exploratory nature of this study, no statistical adjustments for multiple testing were applied. Additionally, non-significant trends in the data were described as these were considered pertinent to the wider on-going study.

3.5 Results

3.5.1 Baseline characteristics of study participants

The mean age of the 15 participants was 64.40 (± 10.32) years (range 45 – 80). All 15 participants were of Caucasian origin. There were 9 male participants in the study (60%). Comparison of the treatment and placebo groups showed no significant differences in baseline demographics (Table 3-3).

Table 3-3 Comparison of the treatment and placebo groups according to baseline demographic features

Clinical features	Placebo group (n = 5)	Treatment group (n = 10)	P value
Age (years)	68.60 (8.08)	62.3 (11.05)	0.281[a]
Gastric emptying ($GEt_{1/2}$ minutes)	96.60 (19.58)	94.00 (15.46)	0.782[a]
Number of male participants (%)	2 (40%)	7 (70%)	0.329[b]
BMI	27.10 (5.83)	25.55 (2.58)	0.594[a]
Orthostatic hypotension present	0 (0%)	2 (20%)	0.524[b]

Data presented as mean (standard deviations). Statistical comparisons: [a]Independent t-test, [b]Chi square test.

Past medical history of important co-morbidities was recorded for each participant. None of the 15 subjects had a current history of angina but one had a past history of angina. No subjects reported any history of myocardial infarction or stroke. One participant had treated hypertension. Three participants had treated hyperlipidaemia. One subject had diabetes, a recognised cause of gastroparesis however their degree of gastric emptying delay ($GEt_{1/2}$ 101 minutes) was not particularly longer than that of the remainder of the screened population (Figure 3-7).

3.5.2 Effect of Camicinal on Levodopa pharmacokinetics

The Levodopa pharmacokinetic end-points evaluated in this study were the area under the curve (AUC) and the maximum plasma concentration (Cmax).

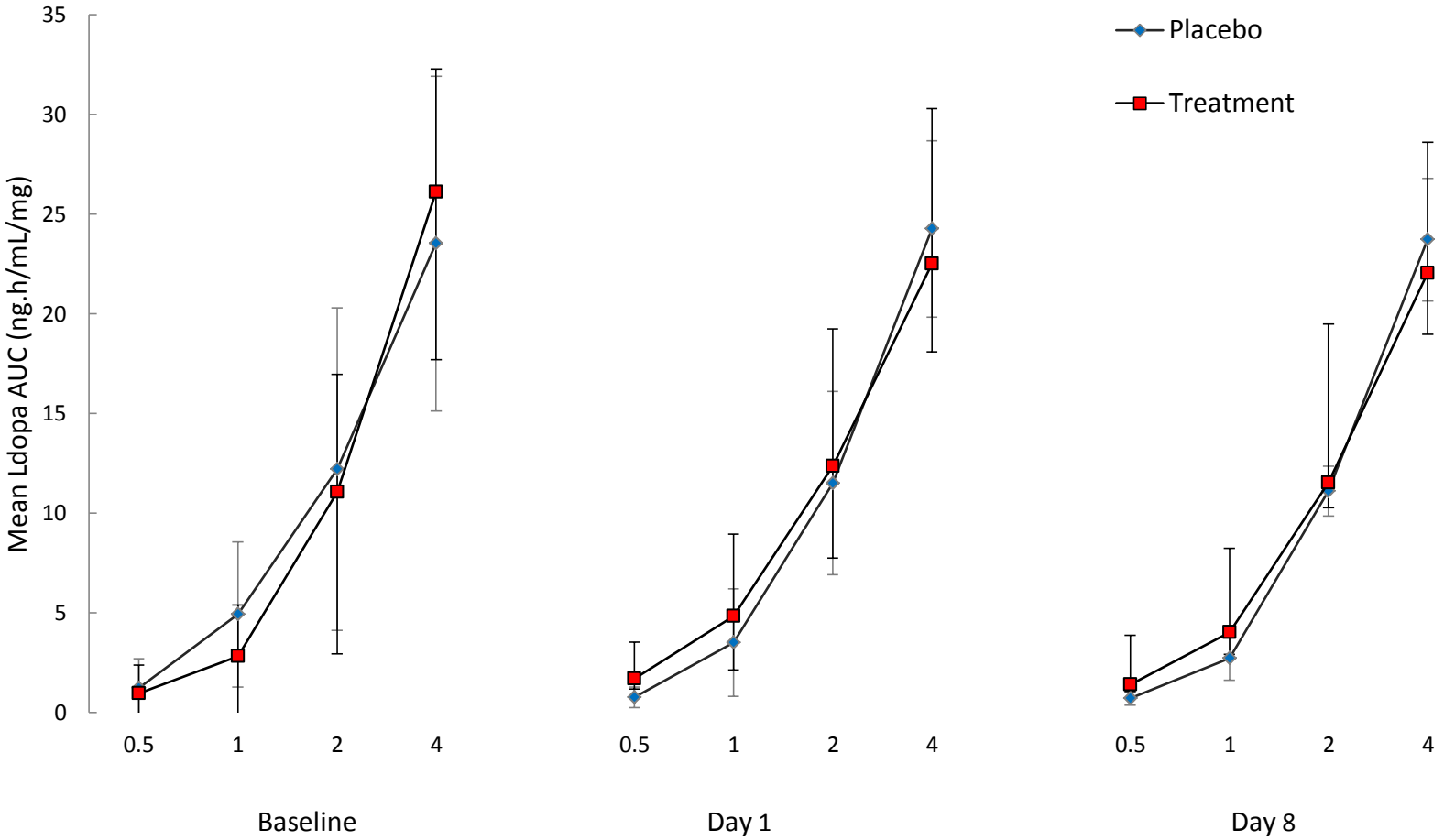
Plasma Levodopa AUC for 0-0.5 hours, 0-1 hour, 0-2 hours and 0-4 hours were calculated for each participant at baseline, day 1 and day 8. As all subjects had different Levodopa equivalent doses, the AUC values were dose-normalised. For each participant, the changes in AUC over time were plotted and visually inspected to assess for trends in the data (Appendix C). Given the small sample size, inspection on a case by case basis was an important step prior to analysis of whole group trends.

It was noted that for six of the 10 participants in the treatment group (subject nos: 10, 11, 14, 15, 19, 101), there was a trend towards increased AUC in the first 2 hours on days 1 and 8, relative to baseline. This suggested quicker absorption of Levodopa during the dosing period in these subjects. In the remaining four participants in the treatment group (subject nos: 401, 16, 20, 22), AUC on the dosing days was either unchanged or decreased relative to baseline.

In the placebo group, four of the five subjects (subject nos: 2, 13, 18, 202) had relatively unchanged or decreased AUC levels in the dosing period compared to baseline. Only one subject's AUC increased on placebo compared to baseline (subject no: 201).

Comparison of mean AUC levels over time suggested no significant differences between the treatment and placebo groups at any of the three visits (Figure 3-8).

Figure 3-8 Mean Levodopa AUC levels over time by study group and visit day



Comparison of the placebo and treatment arms for change in AUC over time showed no significant differences between the groups at any time point on either day 1 or day 8 relative to baseline (Table 3-4).

Table 3-4 Inter-group comparisons of change in Levodopa AUC from baseline to dosing days 1 and 8

Dose normalised	**Change in L-dopa AUC from baseline to day 1**		
Levodopa AUC (ng.h/ml/mg)	Placebo arm n = 5	Treatment arm n = 10	**P value**
AUC: time 0-0.5 hr	0.20 (2.06)	0.39 (1.76)	0.327
AUC: time 0-1 hr	-1.95 (6.21)	1.49 (5.57)	0.086
AUC: time 0-2 hr	-0.49 (7.21)	2.66 (8.71)	0.462
AUC: time 0-4 hr	0.28 (8.24)	-0.14 (11.26)	0.391
Dose normalised	**Change in L-dopa AUC from baseline to day 8**		
Levodopa AUC (ng.h/ml/mg)	**Placebo arm n = 5**	**Treatment arm n = 10**	**P value**
AUC: time 0-0.5 hr	-0.43 (2.31)	0.04 (1.65)	0.327
AUC: time 0-1 hr	-1.76 (8.82)	0.70 (6.41)	0.142
AUC: time 0-2 hr	-1.00 (15.16)	1.10 (12.20)	0.713
AUC: time 0-4 hr	-1.51 (15.44)	-2.99 (16.85)	0.391

Data are presented as median (interquartile range). Comparison of medians with the Mann-Whitney test.

Cmax was calculated as a second measure of Levodopa absorption. Changes in Cmax over the three assessment days were examined visually for each individual in the treatment group (Figure 3-9) and placebo group (Figure 3-10). The change in Cmax from baseline to day 8 was compared across the groups. In the placebo group the Cmax fell from baseline to day 8 (median change -2.93 ± 6.90 ng/mL/mg). In the treatment group there was a modest increase in Cmax (median change 0.36 ± 7.74 ng/mL/mg). However, the trend towards an increased Cmax in the treatment group relative to placebo was not statistically significant (Mann-Whitney, P = 0.462).

Figure 3-9 Changes in Cmax over time for each participant in the **treatment** group

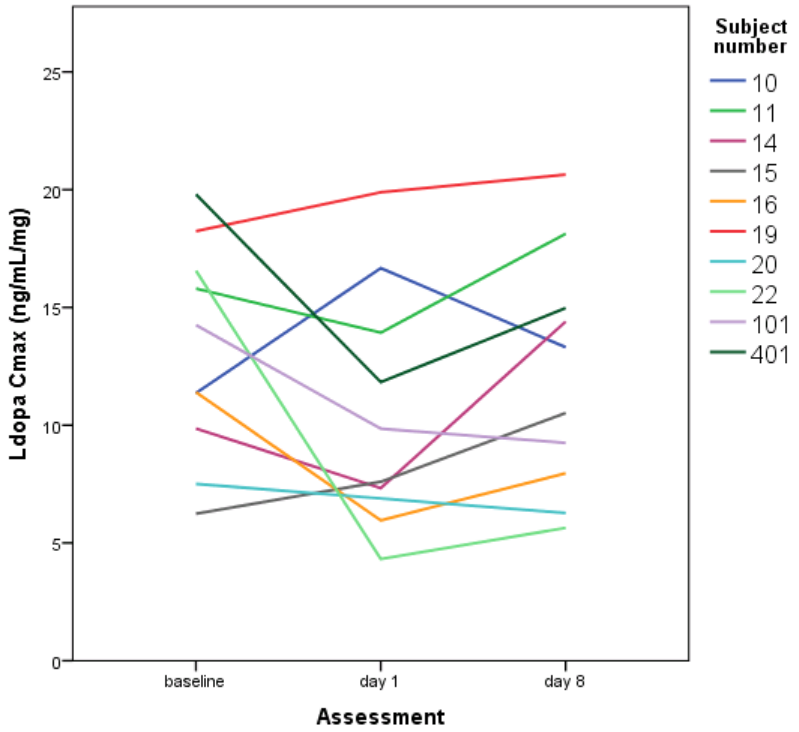
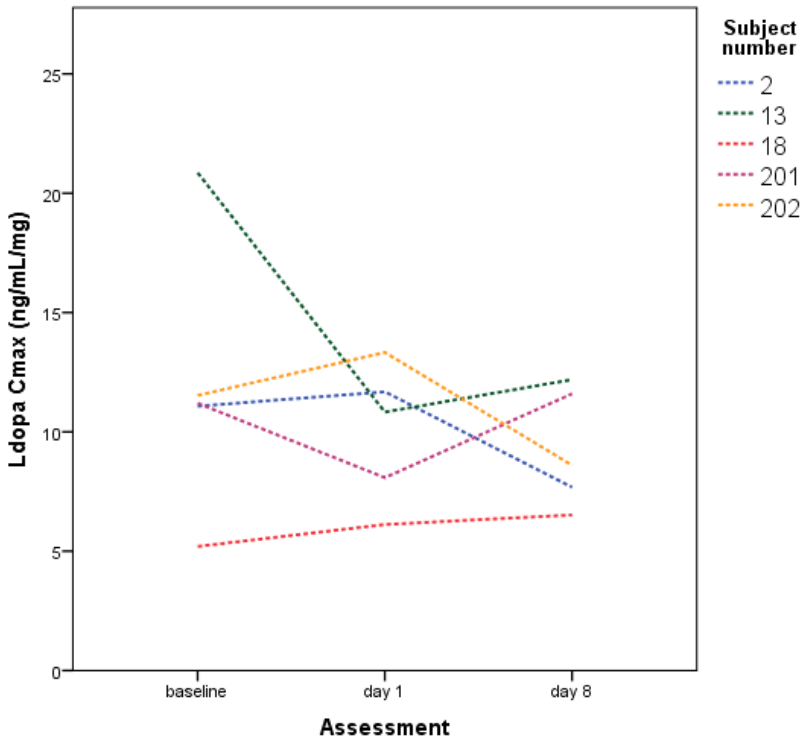


Figure 3-10 Changes in Cmax over time for each participant in the **placebo** group



3.5.3 Effects of Camicinal on gastric emptying

Gastric emptying half time ($GEt_{1/2}$) was calculated for each subject at each visit. Higher scores represented more severe gastric emptying delay. The change in $GEt_{1/2}$ for each participant was plotted according to their study group. Visual inspection of plots for participants on active treatment (Figure 3-11) suggested relatively static $GEt_{1/2}$ levels throughout the study. In some cases there was a modest reduction in $GEt_{1/2}$ over the dosing period which was in keeping with our *a priori* hypothesis that Camicinal would enhance gastric emptying. However, one subject (subject no: 101) had a marked increase in $GEt_{1/2}$ on day 8, representing slower gastric emptying than baseline. This was an unexpected finding which could not be clearly accounted for. This anomaly was strikingly different to the trend seen in the remainder of the group. Therefore, this outlier case was excluded from subsequent whole group analyses. Visual inspection of the $GEt_{1/2}$ levels for the placebo group (Figure 3-12) showed some variation in gastric emptying over time, perhaps reflecting the fact that even in healthy subjects, there is inherent intra-individual heterogeneity in gastric emptying speed.

Figure 3-11 Gastric emptying (GE) half times according to assessment visit for participants in the **treatment** group

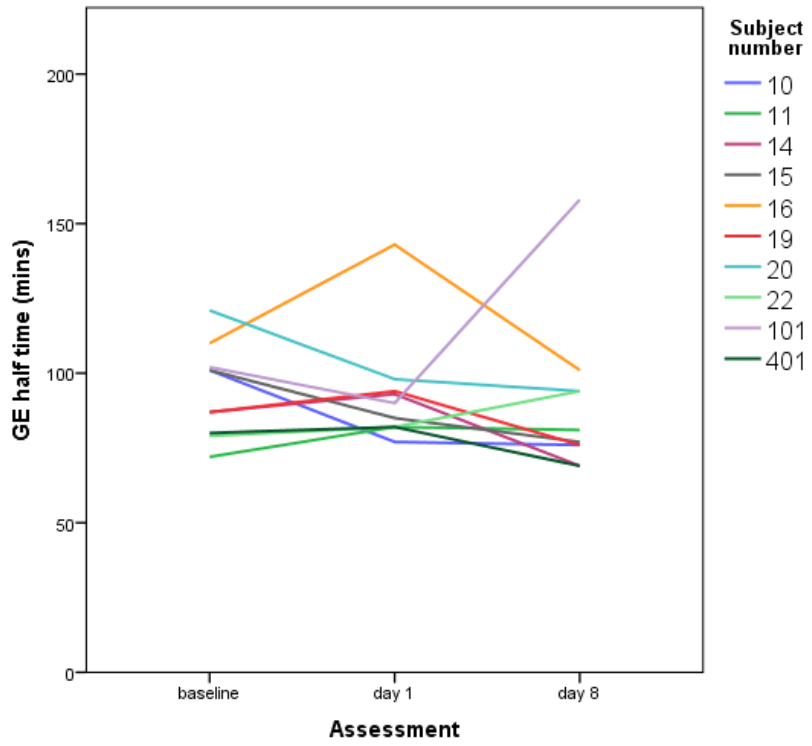
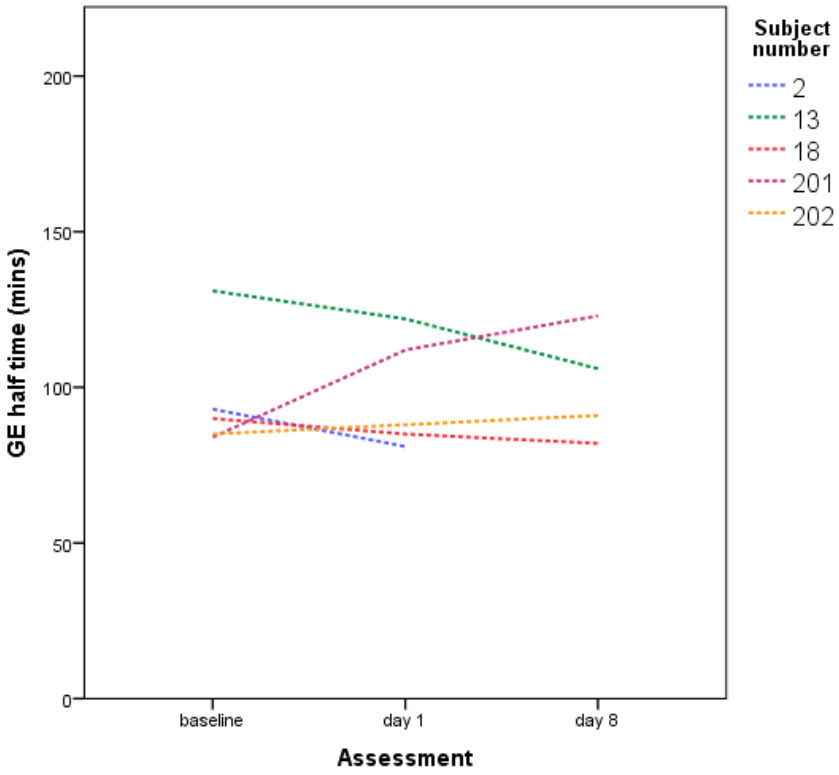
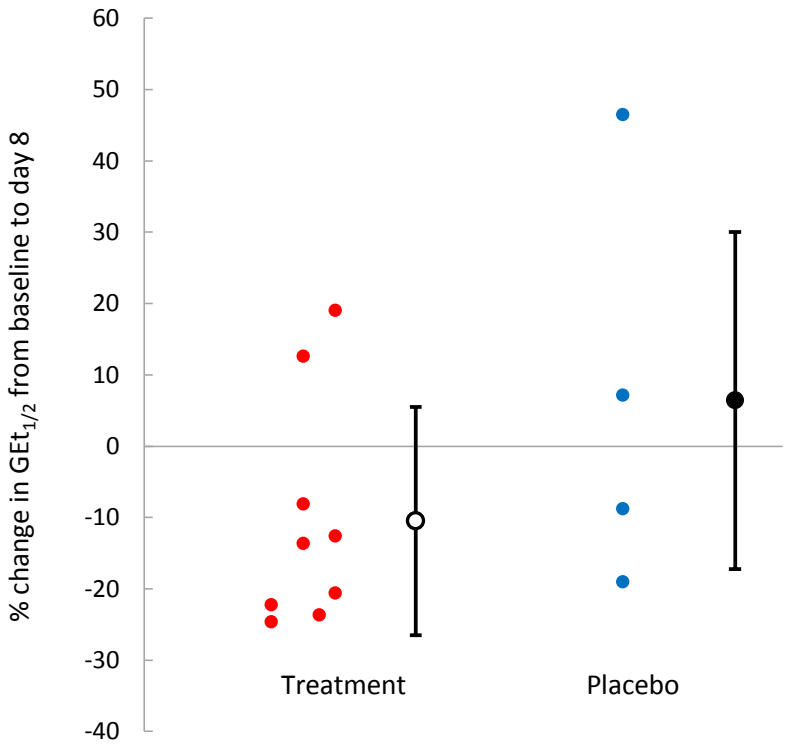


Figure 3-12 Gastric emptying (GE) half times according to assessment visit for participants in the **placebo** group



The relative change in $GEt_{1/2}$ (as a percentage change from baseline to day 8) was analysed for each participant. There was a trend towards a greater reduction (quicker gastric emptying) in the treated participants compared with the placebo group (Figure 3-13).

Figure 3-13 Comparison of treatment and placebo groups according to percentage change in gastric emptying from baseline to day 8

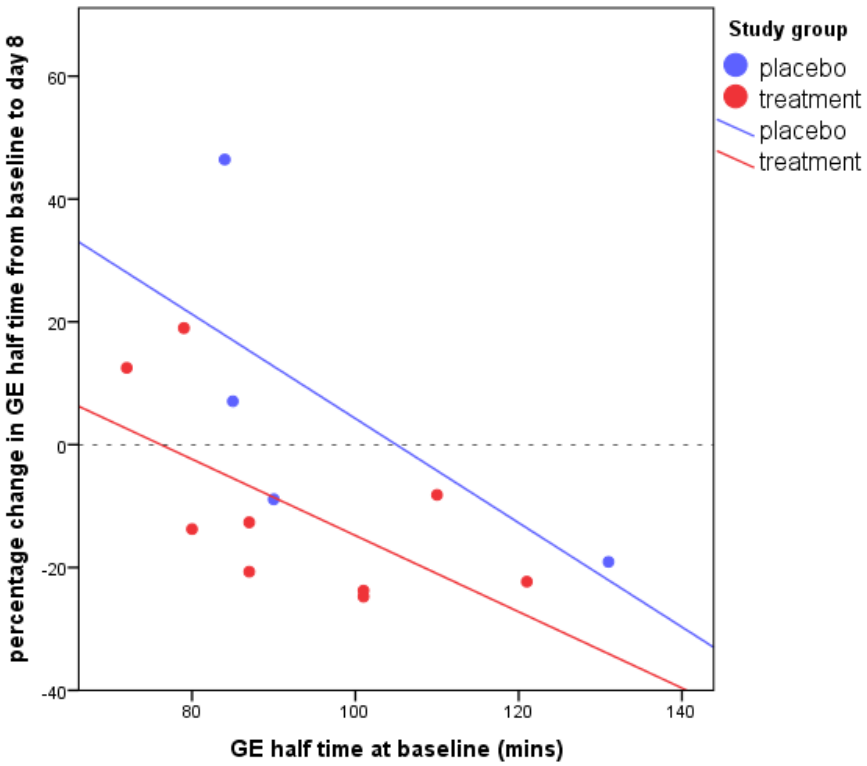


Individual points represent the relative change in $GEt_{1/2}$ for each subject according to treatment group. Error bars represent group means and standard deviations. Subject 101 excluded.

In the treatment group, the $GEt_{1/2}$ fell by an average of 13.75% (median) from baseline to day 8, representing approximately a 14% improvement in gastric emptying in the treatment group during the dosing period. There was also an improvement in gastric emptying in the placebo group over the same period (median $GEt_{1/2}$ change of -0.92%). Although gastric emptying speed improved more in the treatment than the placebo group, the difference was not statistically significant (Mann-Whitney, P = 0.217).

It was postulated that participants with the slowest gastric emptying at baseline would show the greatest response to treatment with Camicinal. Therefore the percentage change in $GEt_{1/2}$ from baseline to day 8 was compared with absolute $GEt_{1/2}$ levels at baseline (Figure 3-14). Although there was a trend towards a greater reduction in $GEt_{1/2}$ in those participants of the treatment group with worse gastroparesis at baseline, the same trend was also evident in the placebo group.

Figure 3-14 Graph representing the effect of baseline gastroparesis severity upon change in gastric emptying half time in response to Camicinal or placebo



Data points represent individual study participants. Lines of best fit represent trends (linear regression) for each group.

It was postulated that Camicinal would improve Levodopa absorption through enhanced gastric emptying. Therefore, the relationship between dose adjusted plasma Levodopa AUC levels and gastric emptying rates were examined for serial paired measurements in the Camicinal treated group. The expectation was that longer $GEt_{1/2}$ times would correlate with lower AUC levels and this was indeed the case (Pearson Correlation coefficient -0.334, 1-tailed significance P = 0.036).

3.5.4 Effects of Camicinal on gastroparesis symptoms

The effect of Camicinal on the symptoms of gastroparesis was assessed via patients' responses on the GCSI daily diary. For each of the 15 study participants, mean gastroparesis symptom scores at baseline (screening week) were compared with mean scores for the final two days of the dosing period. Each symptom was scored on a standardised scale (from 0 to 5) with higher scores representing more severe gastroparesis symptoms. If the average score fell by at least one point, this was deemed 'Improved' if the average score rose by at least one point it was deemed 'Deteriorated' whilst intermediate scores were deemed 'No change.'

For each participant, the relative change in symptoms is summarised in Figure 3-15. In most participants, symptoms were unchanged from baseline. Two of the 10 participants on active treatment had improvement in at least two symptoms (subject nos: 10 and 101). In the placebo group, none of the five participants had improvement in two or more symptoms.

Figure 3-15 Representation of relative change in mean GCSI-DD symptom scores from baseline to end of dosing period

Subject number	GCSI-DD question number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Participants on active **treatment**														
401														
10														
11														
14														
15														
16														
19														
20														
22														
101														
Participants on **placebo**														
2														
13														
18														
201														
202														

GCSI-DD questions by number:

1: Episodes of vomiting
2: Episodes of retching
3: Nausea
4: Excessively full after meals
5: Bloating
6: Early satiety
7: Retching
8: Vomiting
9: Stomach or belly visibly larger
10: Stomach fullness
11: Loss of appetite
12: Upper abdominal pain
13: Upper abdominal discomfort
14: Overall severity of gastroparesis

Key:
- Improved symptom
- No change
- Deteriorated symptom

For each participant, symptom scores were combined to give mean severity scores for the three gastroparesis symptom clusters: 1) nausea & vomiting, 2) fullness, 3) bloating. Additionally, a global severity score (the GCSI total score) was calculated for each participant. Non-parametric tests were performed to compare changes in these scores over time (Table 3-5). In the treatment group there was a trend towards improved fullness and bloating in the dosing period relative to baseline however the differences were not statistically significant.

Table 3-5 Change in symptom scores from baseline to dosing by treatment group

Symptom cluster	Screening week	Dosing week	P value
	Treatment group (n = 10)		
Nausea & vomiting	0.00 (0.24)	0.00 (0.06)	0.144
Fullness	0.22 (0.52)	0.04 (0.64)	0.263
Bloating	0.43 (1.37)	0.00 (0.98)	0.173
GCSI total score	0.45 (0.69)	0.70 (0.52)	0.128
	Placebo group (n = 5)		
Nausea & vomiting	0.00 (1.08)	0.10 (1.19)	0.180
Fullness	2.21 (3.02)	1.96 (2.67)	0.273
Bloating	1.86 (2.75)	2.00 (2.69)	0.655
GCSI total score	1.63 (2.14)	1.54 (2.06)	0.273

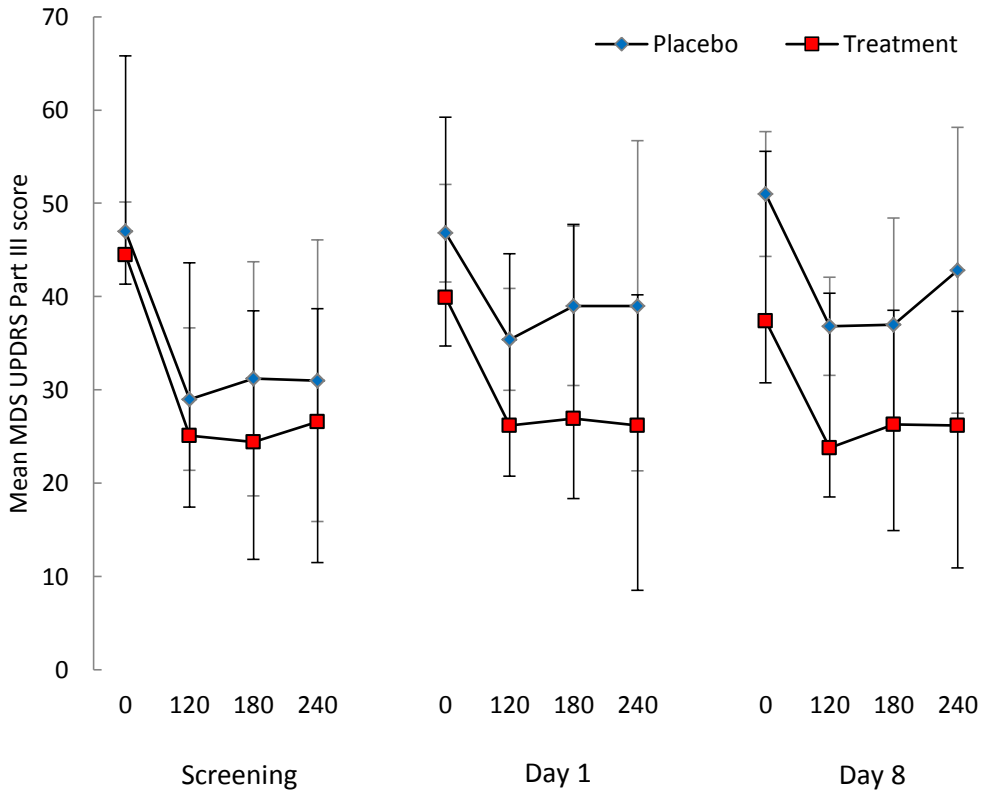
Data presented as group medians (interquartile range) with intra-group comparisons via the Wilcoxon signed rank test.

3.5.5 Effects of Camicinal on motor function

MDS UPDRS Part III

The MDS UPDRS Part III (motor examination) was undertaken on each assessment day just prior to dosing with Levodopa (time 0) and then at three further time points; 120, 180 and 240 minutes later. Mean MDS UPDRS Part III scores for the treatment and placebo groups at each time point were compared graphically (Figure 3-16).

Figure 3-16 MDS UPDRS Part III scores over time for the treatment and placebo groups

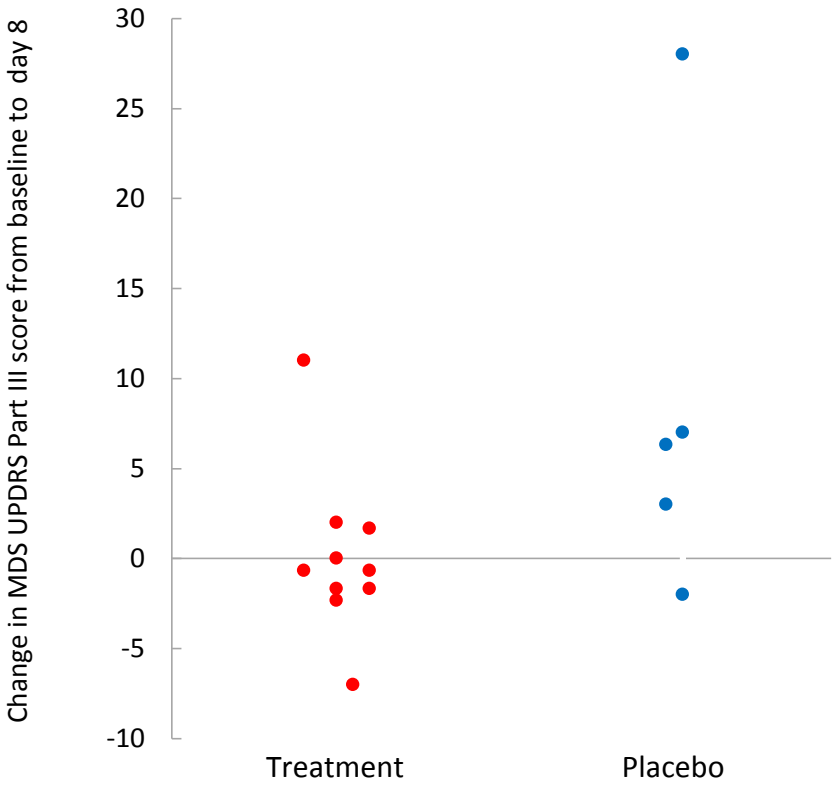


Data points represent group means and standard deviations at each time point.

For each participant, the mean MDS UPDRS Part III score over the post-dosing period (times: 120, 180 and 240 minutes) for each visit was calculated. The change in average score from baseline to day 8 was plotted for each participant according to study group (Figure 3-17). There was a trend towards decreased MDS UPDRS Part III scores, representing improved motor function (median change from baseline to day 8 was -0.67 ± 3.58) whilst in the placebo group there was a trend towards deteriorating scores

over the dosing period (median change 6.33 ± 17.00) however the difference between the two groups was not statistically significant (Mann-Whitney, P = 0.086).

Figure 3-17 Change in MDS UPDRS Part III scores from baseline to day 8

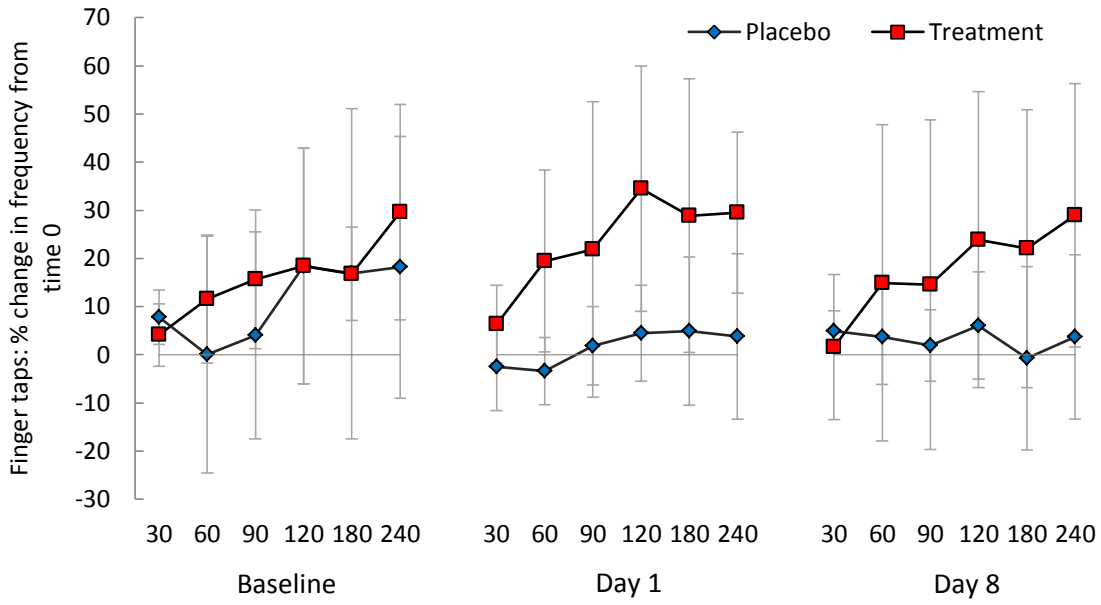


The average change in MDS UPDRS Part III score within each group over the course of the study was analysed. For those participants given placebo, the group median score at baseline (28.33) was not significantly different to that at day 8 (42.67) (Wilcoxon signed rank test, P = 0.08). However, the median scores for the treatment group were significantly lower on day 8 than at baseline (26.17 vs 28.00, Wilcoxon signed rank test, P = 0.007).

Finger taps

Mean finger tap frequency at each time point was used as a surrogate marker of bradykinesia severity. Absolute scores varied considerably across the 15 participants, therefore the data was analysed as percentage change relative to the score at time 0 (Figure 3-18).

Figure 3-18 Percentage change in finger tap frequency relative to time 0 at each study visit



Data points represent group means with standard deviations.

Comparison of average finger tap frequencies for the placebo and treatment groups at the baseline visit, showed no significant inter-group difference (medians 70.5 and 80.07 respectively, Mann-Whitney test, P = 0.462). The difference between the two groups had increased by day 8 with a higher average finger tap frequency in the treatment group but again, relative to placebo, the difference was not statistically significant (medians 95.45 and 67.79 respectively, Mann-Whitney test, P = 0.221).

Intra-group comparisons showed a non-significant trend towards greater finger tap frequency on day 8 in the treatment group relative to the baseline visits (Table 3-6).

Table 3-6 Comparison of finger tap frequency from baseline to day 8

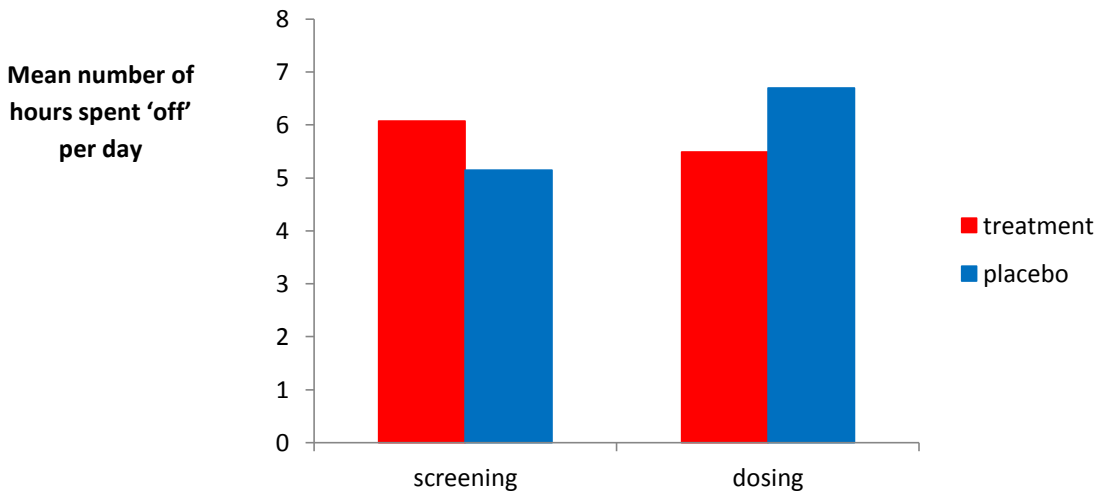
Study group:	Baseline	Day 8	P value
Placebo	70.50 (17.25)	67.79 (20.78)	0.686
Treatment	80.07 (46.44)	95.45 (27.99)	0.173

Data are presented as median (interquartile range) with paired comparisons using the Wilcoxon signed rank test.

Motor symptom diaries

Participants reported their motor state in daily diaries completed throughout the dosing period and for 48 hours after screening. The mean number of hours spent in the 'off' state during the screening and dosing periods was compared (Figure 3-19) with a trend towards a reduction in 'off' time in the treatment group during the dosing period.

Figure 3-19 Bar chart comparing hours spent in the 'off' state during the screening and dosing periods



The treatment and placebo groups were compared with regard to percentage change in 'off' time from baseline to day 8. In the placebo group; from baseline to day 8, 'off' time increased (median increase 9.77% relative to baseline) whilst in the treatment group 'off' time reduced (median decrease from baseline to day 8: 12.61%). However, this difference was not statistically significant (Mann-Whitney test, P = 0.270).

Intra-group comparisons of average time spent 'off' in the screening and dosing periods revealed no significant difference for the placebo group (Wilcoxon signed rank test, P = 0.345) or treatment group (Wilcoxon signed rant test, P = 0.445).

Patient diaries subdivided periods spent in the 'on' state into 'on' without dyskinesias, 'on' with non-troublesome dyskinesias, and 'on' with troublesome dyskinesias. Although there was a trend towards more time with non-troublesome dyskinesias in the treatment group, there was no significant difference when compared with the placebo group (Table 3-7).

Table 3-7 Percentage change in motor state during the dosing period relative to baseline

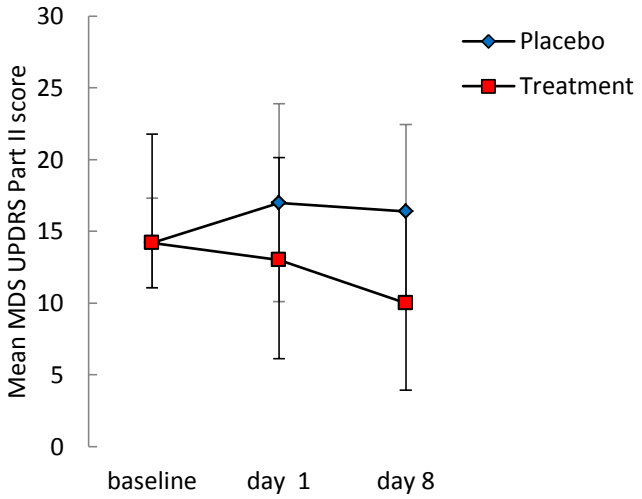
Motor state	**Placebo group (n = 5)**	**Treatment group (n = 10)**	**P value**
'on' without dyskinesias	-3.67 (31.68)	-8.28 (30.53)	1.000
'on' with non-troublesome dyskinesias	0.00 (9.67)	7.91 (14.12)	0.061
'on' with troublesome dyskinesias	0.00 (3.49)	0.00 (0.04)	0.307

Data are presented as medians (interquartile range) with comparisons between groups using the Mann-Whitney test.

MDS UPDRS Part II

The MDS UPDRS Part II was completed at each visit to reflect experiences of motor activities of daily living during the previous week. At baseline, mean MDS UPDRS Part II scores for the treatment and placebo groups were the similar but by day 8 there was a trend towards lower scores in the treatment arm (Figure 3-20). .

Figure 3-20 Comparison of mean MDS UPDRS Part II scores according to study visit



Data points represent group means (with standard deviation error bars).

Comparison of Part II scores between the treatment and placebo groups revealed no significant differences either at baseline or on day 8 (Table 3-8). The change in Part II score from baseline to day 8 was not significant for the placebo group. However, in the treatment group, there was a significant reduction in Part II scores by day 8 relative to baseline, reflecting a significant improvement in motor experiences of daily living.

Table 3-8 Comparison of MDS UPDRS Part II scores by study group and visit day

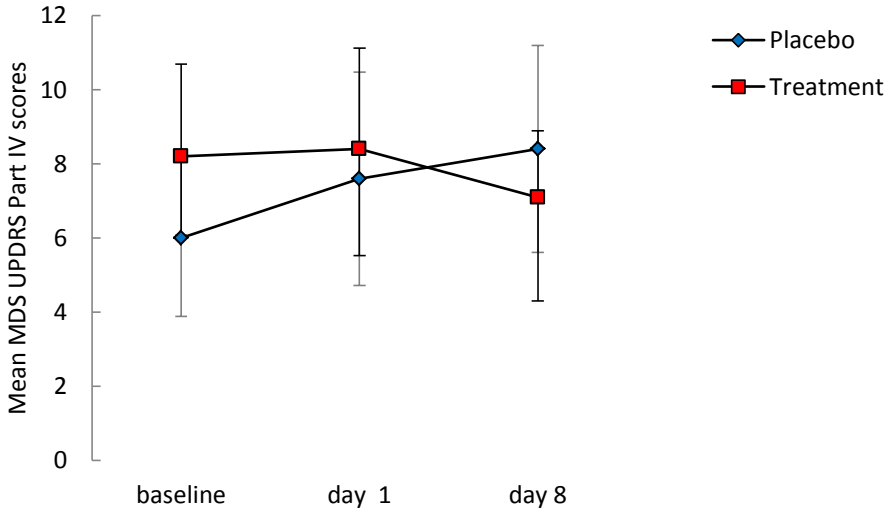
	Placebo group (n = 5)	**Treatment group (n = 10)**	**P value**
Baseline	13.0 (6.0)	12.0 (12.0)	0.805[a]
Day 8	13.0 (12.0)	9.5 (8.0)	0.085[a]
P value	0.357[b]	**0.021[b]**	

Data are presented as median (interquartile range). Inter-group comparisons: [a]Mann-Whitney test. Intra-group comparisons: [b]Wilcoxon signed rank test.

MDS UPDRS Part IV

The occurrence of motor complications was recorded in Part IV of the MDS UPDRS which was completed at the start of each visit. Comparison of mean MDS UPDRS Part IV scores across the three assessment visits are shown graphically in Figure 3-21.

Figure 3-21 Mean MDS UPDRS Part IV scores by assessment visits



Data points represent group means (with standard deviation error bars).

Inter and intra-group differences in MD UPDRS Part IV scores are summarised in Table 3-9. Comparison of Part IV scores for the placebo versus treatment group at baseline and day 8 revealed no significant inter-group differences. Between baseline and day 8 there was a non-significant trend towards reduced part IV scores in the treatment group and increased scores in the placebo group.

Table 3-9 Comparison of MDS UPDRS Part IV scores by study group and visit day

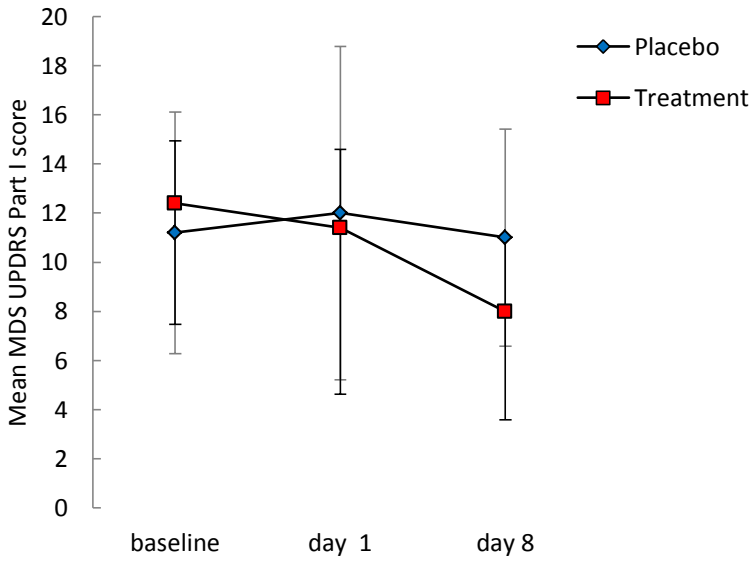
	Placebo group (n = 5)	**Treatment group (n = 10)**	**P value**
Baseline	6.0 (4.0)	9.5 (4.0)	0.099[a]
Day 8	9.0 (6.0)	7.0 (3.0)	0.440[a]
P value	0.102[b]	0.181[b]	

Data are presented as median (interquartile range). Inter-group comparisons: [a]Mann-Whitney test. Intra-group comparisons: [b]Wilcoxon signed rank test.

Effects of Camicinal on non-motor function

A global assessment of the severity of non-motor symptoms of PD was undertaken through analysis of total MDS UPDRS Part I scores. Mean MDS UPDRS Part I scores in the treatment and placebo groups are represented graphically in Figure 3-22.

Figure 3-22 Mean MDS UPDRS Part I scores across assessment visits by group



At baseline and day 8, median MDS UPDRS Part I scores were not significantly different between the treatment and placebo groups however within the treatment group there was a significant reduction in Part I score from baseline to day 8 which was not evident in the placebo group (Table 3-10).

Table 3-10 Comparison of MDS UPDRS Part I scores by study group and visit day

	Placebo group (n = 5)	**Treatment group (n = 10)**	**P value**
Baseline	9.0 (9.0)	12.5 (3.0)	0.324[a]
Day 8	11.0 (8.0)	8.5 (5.0)	0.157[a]
P value	1.00[b]	**0.005**[b]	

Data are presented as median (interquartile range). Inter-group comparisons: [a]Mann-Whitney test. Intra-group comparisons: [b]Wilcoxon signed rank test.

At baseline, all participants apart from one reported some constipation difficulties (a score of one or more on the MDS UPDRS question 1.11). Comparing baseline to day 8, in the treatment group two participants reported worse constipation, two improved and six were unchanged. In the placebo group, one of the five deteriorated, one improved and three reported no change in their constipation symptoms. On this limited evaluation of bowel function, significant differences were not evident between the treatment and placebo groups with regard to change in bowel function during the study.

3.5.6 Safety data and adverse events

No study terminating events occurred during the study. Two serious adverse events (SAEs) were recorded during the study, one in the active treatment group and one in the placebo group. The SAE in the patient receiving Camicinal was an episode of pruritus during the dosing period, which required hospital admission but was related to an underlying co-morbidity and not felt to be related to the investigational product (IP). The other SAE occurred in a participant receiving placebo. This subject experienced an 'extreme off' period with altered sensation in their legs. Clinicians deemed that both participants were suitable to continue with the study and both subjects chose to continue in the study.

There were a further 37 non-serious adverse events (AEs) reported by 12 participants (one of whom also reported one of the SAEs). In the Camicinal treated group, 8 subjects reported 14 AEs whilst in the placebo group 4 subjects reported 23 AEs. Table 3-11 summarises the types of AE reported by each group. By the end of participation in the study, of the 37 AEs reported, all had resolved apart from one; leg cramps which were felt to be unrelated to the medicinal product.

Of the 14 AEs reported in the treatment group, blinded study clinicians reported that 6 were potentially related to the IP. These 6 AEs were: fatigue, lower abdominal ache, increased dyskinesias, increased bowel motion frequency, upper abdominal pain and headache. All were graded as mild in severity apart from the lower abdominal ache which was of moderate severity. All six had resolved by the end of the study.

Serial ECGs and bloods were performed on all subjects on all visit days. A detailed summary of these individual results is not provided here. However, review of the results revealed no significant abnormalities requiring withdrawal of any participants on safety grounds.

It was concluded that Camicinal was as well-tolerated as placebo in this interim analysis of 15 subjects with PD and delayed gastric emptying. Furthermore there were no safety concerns limiting the continuation of the study in this patient group.

Table 3-11 Summary of adverse events reported during the study by treatment group

Category / system affected	**Number of AEs reported**	
	Treatment group	**Placebo group**
Gastrointestinal disorders	4	5
General disorders and administration site conditions	1	1
Infections and infestations	1	3
Injury poisoning and procedural complications	2	
Nervous system disorders	6	5
Ear and labyrinth disorders		1
Eye disorders		2
Musculoskeletal and connective tissue disorders		2
Psychiatric disorders		1
Renal and urinary disorders		1
Respiratory, thoracic and mediastinal disorders		1
Vascular disorders		1
Total	14	23

3.6 Discussion

In this study we examined the effect of Camicinal 50mg or placebo in people with PD, delayed gastric emptying and motor fluctuations. The effect of Camicinal upon Levodopa absorption, gastric emptying, motor and non-motor function was evaluated.

3.6.1 Summary of the study findings

We had hypothesised that Camicinal would enhance Levodopa pharmacokinetics. There were no significant differences in Cmax or AUC over the course of the study. However we have described a non-significant trend towards a slight improvement in Cmax for the Camicinal treated participants. We had hypothesised that absorption would be enhanced as a result of faster gastric emptying. However, the gastric emptying rates of participants in both groups were heterogeneous and no significant improvement was demonstrated with Camicinal. It has previously been shown that delayed gastric emptying can delay Levodopa absorption (Doi *et al.*, 2012). Domperidone has been reported to improve Levodopa absorption through its prokinetic effect (Shindler *et al.*, 1984; Nishikawa *et al.*, 2012). At this stage we are unable to demonstrate such an effect with Camicinal. However, the results presented in this thesis are interim findings only and the sample size at this stage was not sufficiently powered to prove or disprove the study hypotheses. The non-significant trend towards improved Levodopa absorption in some Camicinal treated participants has shown that the study is not futile and therefore the study will continue.

We hypothesised that Camicinal would improve gastroparesis symptoms. However, the GCSI-DD from baseline to the end of the dosing period did not differ significantly between the Camicinal and placebo groups. One explanation for this could be the relatively low baseline gastroparesis symptom scores in this study which rendered little room for improvement. It is also possible that these relatively static symptom scores reflect an underlying disparity between the severity of gastric emptying delay and the symptoms experienced. Gastroparesis studies in people with diabetes have also demonstrated a mismatch between symptoms and severity of gastric emptying delay (Samsom *et al.*, 2009). A recent meta-analysis of studies in which prokinetics were evaluated for changes in gastric emptying and symptoms of gastroparesis reported no

relationship between changing symptoms and gastric emptying. We highlighted this apparent disparity between pathophysiology and symptoms in Chapter 2 and will explore it more directly in the next chapter where were will look at clinical predictors of gastric emptying speed in people with PD.

When considering changes in gastroparesis symptoms over time, it is also important to consider the influence of psychosocial factors. In this study we did not take account of patients' mood. However, in our previous study of gastroparesis symptom prevalence (Chapter 2) we demonstrated a relationship between the severity of gastroparesis symptoms and levels of anxiety and depression. It remains unclear whether gastroparesis results in mood disturbance or whether an underlying mood disorder may make an individual more prone to report gastrointestinal symptoms. In a non-PD population, it has been reported that psychological factors have a greater influence upon gastroparesis symptoms than the severity of the gastric emptying delay (Hasler *et al.*, 2010).

Our results suggest a greater improvement in motor and non-motor symptoms in the Camicinal group compared with placebo. Studies where domperidone was co-administered with Levodopa, also reported non-significant improvements in motor function (Soykan *et al.*, 1997; Nishikawa *et al.*, 2012). However, in our study we saw only modest changes in Levodopa absorption which calls into question the mechanism by which the treatment group participants had improved function. This relationship may become clearer with further study and greater patient numbers.

We had hypothesised that Camicinal would enhance colonic transit time and hence improve constipation problems. We did not see a significant effect of Camicinal upon bowel function. However, our only measure of bowel function in this interim analysis was patients reporting of constipation difficulties on Part I of the MDS UPDRS. This is a subjective interpretation of symptom severity and may therefore be influenced by psychosocial factors as we previously alluded to. A more objective measure would be the recording of daily bowel motion frequency and consistency and this data will be considered in the final study results.

As this was an experimental medicine study, it was essential to consider the safety of Camicinal. Although most study participants reported at least one adverse event, the

rates were similar amongst the treatment and placebo groups. Analysis of the AEs and SAEs reported in both arms of the study suggested that there were no major safety concerns with the use of Camicinal 50mg once daily for 7 to 9 days and furthermore Camicinal was as well tolerated as placebo in people with PD.

3.6.2 Strengths and limitations of the study

This was a 'proof of principle' experimental medicine study of a novel treatment for delayed gastric emptying in PD. Initial recruitment to the study was difficult and hence a period of review and re-design was needed. The process of revision and amendment described in section 3.3.9 was successful in leading to improved study recruitment however it led to a pause in the study and hence at the time of writing this thesis, interim and not completed study results are available to report. This interim analysis of 15 participants was not sufficiently powered to definitively report the efficacy of Camicinal. Therefore we have primarily reported trends in the data, which although not statistically robust, do suggest that the on-going study of Camicinal is appropriate and relevant.

The dosing period in this study was only 7-9 days in total. This relatively short treatment period may have been too limited to detect change in some of the outcome measures for example changes in gastroparesis symptoms. It is plausible to suggest that more striking treatment effects would be seen after longer dosing periods. Therefore, in any future studies of Camicinal in people with PD, it might be appropriate to extend the dosing period to several weeks or months.

One of the major strengths of this study is the breadth and depth of clinical assessments undertaken. We undertook parallel assessments of: Levodopa pharmacokinetics, gastric emptying, gastroparesis symptoms and PD motor function serially in all patients across multiple visits. From these detailed evaluations we have been able to report a trend towards impaired Levodopa absorption in those patients with slower gastric emptying.

3.6.3 Study progression and future directions

The interim results from this study have suggested that continued study of Camicinal as a novel treatment for delayed gastric emptying in PD is justified. The study is on-going at the time of writing this thesis and it is due to conclude once 45 participants have completed the study. Current projections suggest that the study will be complete in spring 2014. Pending the results of the final study, future avenues of research would include the evaluation of different doses of Camicinal and longer dosing periods. Additionally the scope for this novel therapy could be widened to include use in the earlier stages of PD. If Camicinal is demonstrated to enhance Levodopa absorption, it could potentially be used in all disease stages to enhance the efficacy of existing doses thereby helping to keep total Levodopa doses as low as possible.

From the baseline data obtained for this study we were also able to perform a sub-study looking at predictors of delayed gastric emptying in PD. This will be reported in the next chapter.

Chapter 4 Examination of the relationship between gastroparesis symptoms and gastric emptying speed in people with Parkinson's disease

4.1 Background

The previous two chapters explored the prevalence of gastroparesis symptoms in people with PD and a potential novel treatment for delayed gastric emptying in PD. This chapter builds upon these two studies, to evaluate the relationship between gastroparesis symptoms and gastric emptying delay in PD.

Previous studies have suggested that 70-100% of people with PD have abnormally slow gastric emptying (Heetun and Quigley, 2012) and yet our study of over 1,000 patients with PD suggested that the prevalence of significant gastroparesis symptoms is only 4 - 12%. This suggests a disparity between pathophysiological abnormalities of gastric motility and manifest symptoms of gastroparesis. This was therefore explored through the simultaneous evaluation of gastric emptying and gastroparesis symptoms.

Existing methods for measuring gastric emptying are time consuming and not widely available out with research and specialist centres. Therefore there is a need to identify clinical predictors of delayed gastric emptying in PD. The ability to identify those patients most at risk of delayed gastric emptying would help clinicians to identify those individuals who might benefit from a prokinetic agent without the necessity for intrusive or poorly available gastric emptying studies.

Current evidence regarding the association between symptom severity and gastric emptying delay in PD is equivocal (Hardoff *et al.*, 2001; Goetze *et al.*, 2005; Goetze *et al.*, 2006). In a non-PD population the severity of symptoms can be a useful indicator of the severity of underlying gastroparesis (Stanghellini *et al.*, 1996; Sarnelli *et al.*, 2003; Stanghellini *et al.*, 2003; Talley *et al.*, 2006; Cassilly *et al.*, 2008; Grad *et al.*, 2012). A recent study (Olausson *et al.*, 2013) in over 100 people with diabetes used the GCSI in people at risk of gastroparesis and correlated questionnaire scores with gastric emptying rates (measured using scintigraphy). The authors reported that the

nausea / vomiting and fullness subsections of the GCSI correlated significantly with the severity of gastric emptying delay.

The apparent disparity between gastric pathology and symptoms of gastroparesis in early PD was highlighted by a recent study (Cersosimo *et al.*, 2013). However, ours is the first in PD to perform paired gastric emptying studies and structured gastroparesis symptom evaluations in people with PD.

4.2 Aims and objectives

The aim of this study was to assess the association between gastroparesis symptoms and gastric emptying speed in people with PD. This was an exploratory analysis to assess the potential of the GCSI-DD to be used as a screening tool with which to identify PD patients most at risk of gastroparesis. Additionally, demographic and disease characteristics were considered alongside the GCSI-DD as factors potentially impacting upon the risk of gastric emptying delay in PD.

4.3 Methods

This was a sub-study of the previously described randomised controlled trial comparing Camicinal with placebo in people with PD, delayed gastric emptying and motor fluctuations. The methods were described in detail in section 3.3 and will not be repeated here.

All consented patient who attended for screening were asked to complete the GCSI-DD for one week after their screening visit. Questionnaire responses were compiled along with all screened subjects' gastric emptying rates ($GEt_{1/2}$). All participants consented to have their screening data used in this sub-study, even if they failed screening. Of the 28 screened subjects included in this sub-study, 19 were eligible for participation in the randomised controlled trial and nine were ineligible due to a gastric emptying rate faster than permitted by the protocol.

Patients completed a daily diary equivalent of the GCSI; hence reported scores were mean values for the whole reported week. The GCSI total score was calculated as previously described (section 2.5.2) and mean scores for each of the three symptom

clusters are reported (nausea / vomiting, fullness, bloating). Four additional composite scores were calculated (Table 4-1) based upon the previous work of Revicki (Revicki *et al.*, 2012) which suggested that these selective composite scores are most strongly associated with the severity of underlying gastric emptying delay.

Table 4-1 Composite scores to evaluate gastroparesis symptom severity from the GCSI-DD (Revicki *et al.*, 2012)

Composite score	GCSI-DD question number	Gastroparesis symptom
1 – mean of items:	Q3	Nausea
	Q5	Bloating
	Q10	Stomach fullness
	Q4	Excessively full after meals
2 – mean of items:	Q3	Nausea
	Q6	Early satiety
	Q5	Bloating
	Q12	Upper abdominal pain
3 – mean of items:	Q3	Nausea
	Q5	Bloating
	Q4	Excessively full after meals
4 – mean of items:	Q3	Nausea
	Q5	Bloating
	Q6	Early satiety

The influence of medications upon gastric emptying speed was evaluated by calculation of the Levodopa Equivalent Daily Dose (Tomlinson *et al.*, 2010).

4.4 Statistical analysis

Statistical analyses were performed using SPSS 19 (SPSS, Chicago, IL). Normality of the data was assessed visually with histograms and objectively with the Kolmogorov-Smirnov test.

Normally distributed data are reported as mean (with standard deviation) whilst for non-normally distributed data median values (with interquartile range) are reported. Correlations within parametric data sets were performed with Pearson's correlation with 2-tailed significance applied unless there was an *a priori* directional hypothesis in which case the 1-tailed significance level was used (as stated in the text). Correlations within non-normally distributed data sets, was performed with Spearman's rank correlation where again 1 or 2-tailed significance levels were used depending upon the relationship being evaluated. One-way analysis of variance (ANOVA) was used to compare means across groups. Linear and multiple regression models were used in some cases to evaluate predictors of gastric emptying speed.

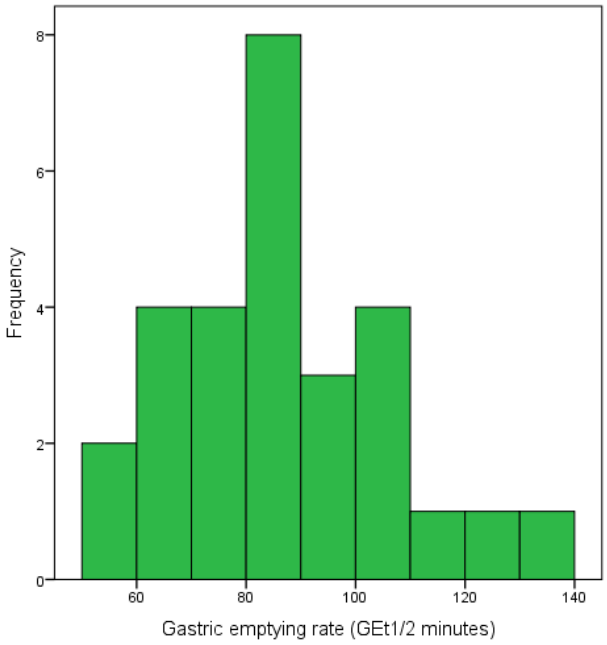
For all statistical analysis a P value of < 0.05 was deemed significant. However, given the small sample size in this interim, exploratory analysis, non-significant trends in the data are also reported and adjustments for multiple comparisons were not applied.

4.5 Results

4.5.1 Demographic factors and their relationship to gastric emptying rate

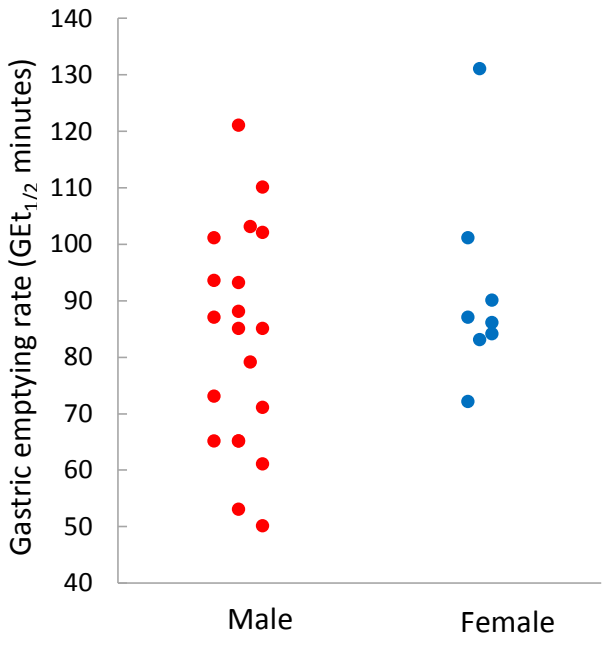
From May 2012 until 12th November 2013, 28 participants completed a screening assessment and underwent gastric emptying measurement. These participants were evaluated across three sites (Newcastle: 24 subjects, Norwich: 3, Australia: 1, Appendix C). Gastric emptying rates ($GEt_{1/2}$) for these 28 participants were normally distributed (Figure 4-1, Kolmogorov-Smirnov, P = 0.200).

Figure 4-1 Histogram showing all measured gastric emptying rates for the 28 screened study participants



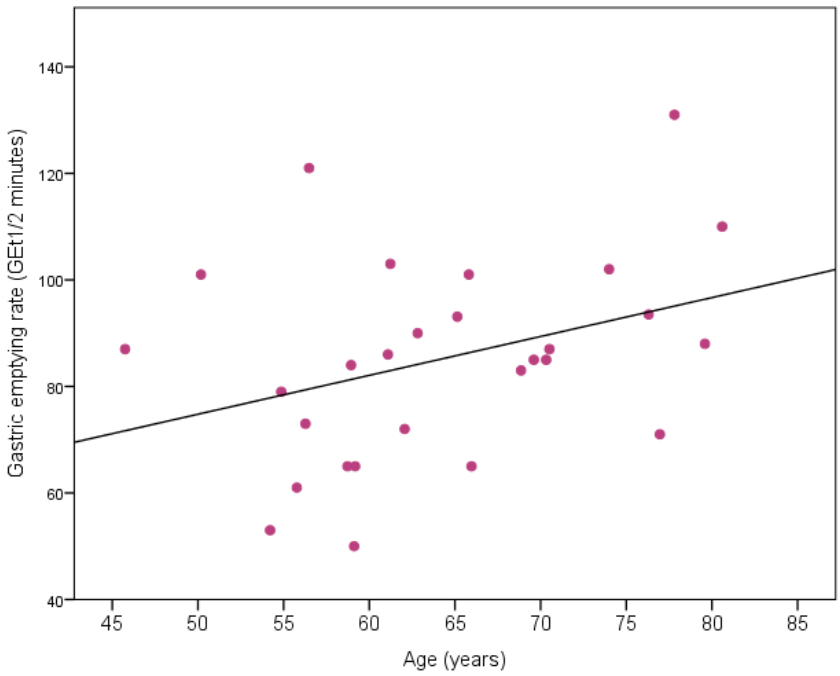
The mean $GEt_{1/2}$ of the group was 85.16 minutes (± 19.17). The quickest $GEt_{1/2}$ recorded was 50 minutes and the slowest was 131 minutes. Eight women were screened and in there was a trend towards slower gastric emptying rates in these participants compared to male subjects (Figure 4-2). However, the difference was not statistically significant (median $GEt_{1/2}$ 85 and 86.5 minutes respectively, Mann-Whitney test, P = 0.415).

Figure 4-2 Scatter plot of gastric emptying speed by gender



The age of participants was normally distributed (Kolmogorov-Smirnov, P = 0.200) with a mean age of 64.22 years (± 9.19). Advancing age and slower gastric emptying rates were significantly correlated (Pearson Correlation coefficient 0.350, 1-tailed significance P = 0.034), with age accounting for 12.3% of the variability in gastric emptying rates (Linear Regression, R^2 = 12.3) (Figure 4-3).

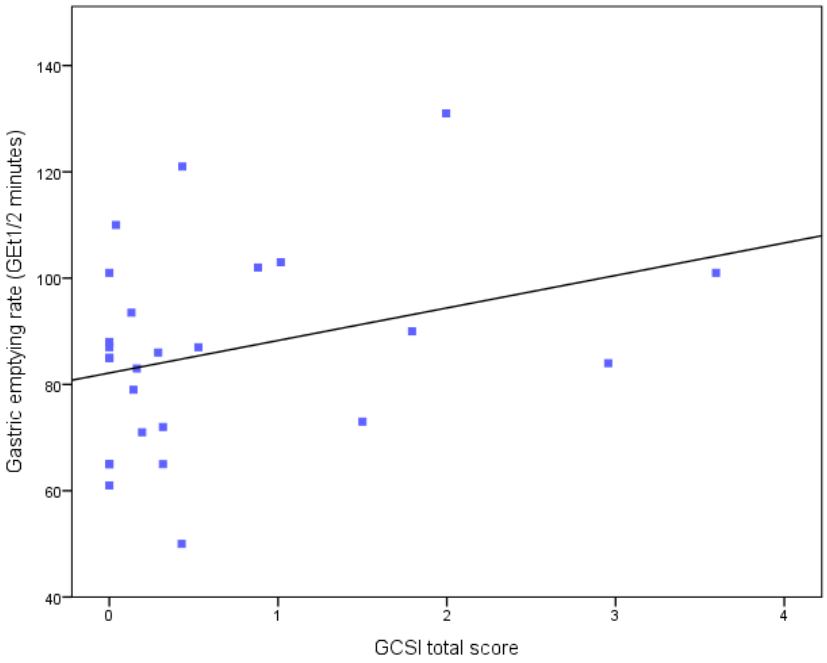
Figure 4-3 Scatter plot comparing age with gastric emptying speed (with line of best fit)



4.5.2 Gastroparesis symptoms and their relationship to gastric emptying rate

The GCSI total scores of the 28 screened participants were non-normally distributed (Kolmogorov-Smirnov, P < 0.001) with a median score of 0.24 (minimum: 0, maximum: 3.6). There was a trend which just failed to reach significance towards higher GCSI total scores in those participants with slower gastric emptying rates (Pearson's correlation coefficient 0.314, 1-tailed significance P = 0.059) with gastric emptying speed accounting for 9.9% of the variance in GCSI total scores (Figure 4-4).

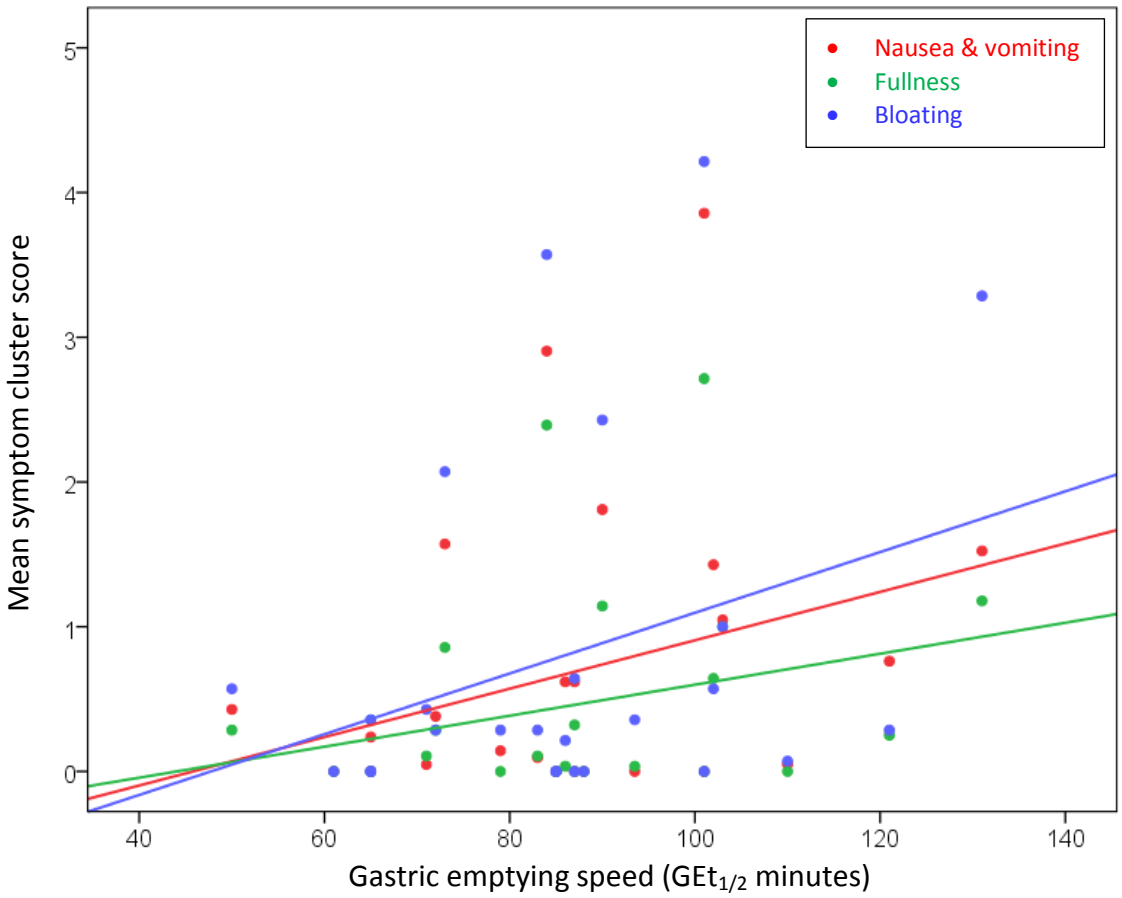
Figure 4-4 Scatter plot comparing change in GCSI total score with gastric emptying speed (with line of best fit)



The correlation between gastric emptying speed and each individual symptom component of the GCSI-DD was explored. Only one symptom of the 11 evaluated was significantly associated. The severity of an individual's abdominal distension (question 9) was positively correlated with the severity of their gastric emptying delay (Spearman correlation coefficient: 0.335, 1-tailed significance P = 0.047).

The relationship between gastroparesis symptom clusters and gastric emptying speed was also evaluated. Mean scores for the three nausea items positively correlated with $GEt_{1/2}$ rates (more severe symptoms associated with slower gastric emptying) although the association did not reach statistical significance (Spearman's correlation coefficient 0.304, 1-tailed significance P = 0.066, linear regression model, R^2 = 0.100). There were also non-significant trends towards slower gastric emptying in those subjects with higher mean fullness and bloating scores (Correlation 0.243, P = 0.116 and correlation 0.244, P = 0.115, respectively) (Figure 4-5).

Figure 4-5 Scatter plots of mean scores on the GCSI-DD by symptom clusters compared with gastric emptying speed (with lines of best fit)



The relationship between gastric emptying speed and four composite symptom scores was examined. There was a non-significant trend towards slower gastric emptying in those with more severe symptom scores. The correlation between $GEt_{1/2}$ and composite score 1was the strongest (Table 4-2).

Table 4-2 Relationship between composite scores derived from the GCSI-DD and gastric emptying speed

Composite	Constituent symptoms	Correlation coefficient	P value
1	Nausea Bloating Stomach fullness Excessively full after meals	0.323	0.054
2	Nausea Early satiety Bloating Upper abdominal pain	0.297	0.070
3	Nausea Bloating Excessively full after meals	0.304	0.066
4	Nausea Bloating Early satiety	0.293	0.073

Spearman's correlation with 1-tailed significance test.

4.5.3 Disease characteristics and their relationship to gastric emptying rate

The disease duration of participants was non-normally distributed (Kolmogorov-Smirnov, P = 0.010) with a median PD duration of 9.5 years. Disease duration and gastric emptying speed were not significantly associated with each other (Pearson Correlation coefficient -0.045, 1-tailed significance P = 0.821). This is especially noteworthy in light of the association between age and gastric emptying rate.

Using participants' baseline MDS UPDRS Part III scores, they were classified into one of three motor phenotypes: tremor dominant, PIGD or indeterminate. Comparison of mean gastric emptying rates between these groups showed no significant differences (one-way ANOVA, P = 0.184).

The influence of disease severity upon gastric emptying was evaluated through analysis of sub-section and total MDS UPDRS scores. The MDS UPDRS total scores of the 28 screened participants were normally distributed (Kolmogorov-Smirnov test, P =

0.142) with a mean score of 78.93 (± 18.89). The average scores for each sub-section of the MDS UPDRS were compared with trends in gastric emptying speed (Table 4-3). There was a non-significant trend towards slower gastric emptying in those participants with higher Part I, III, IV and total MDS UPDRS scores.

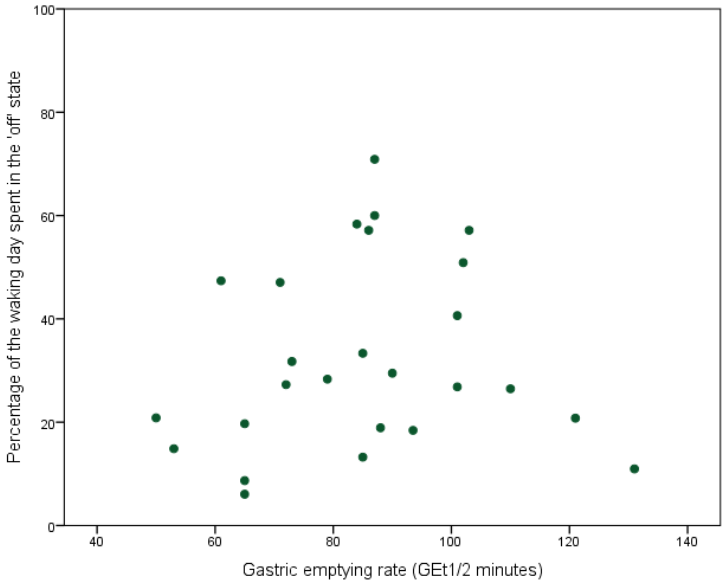
Table 4-3 Correlation between MDS UPDRS sections and gastric emptying speed

MDS UPDRS section	Mean score	Correlation coefficient	P value
Part I	11.50 (± 6.00)*	0.358[b]	0.061
Part II	15.79 (± 7.50)	-0.074[a]	0.708
Part III	43.54 (± 13.11)	0.147[a]	0.456
Part IV	7.00 (± 4.00)*	0.156[b]	0.429
Total	78.93 (± 18.89)	0.134[a]	0.497

Data presented as mean (± standard deviation) except * for medians (± interquartile range). [a]Pearson's correlation. [b]Spearman's correlation. All P values represent 2-tailed tests.

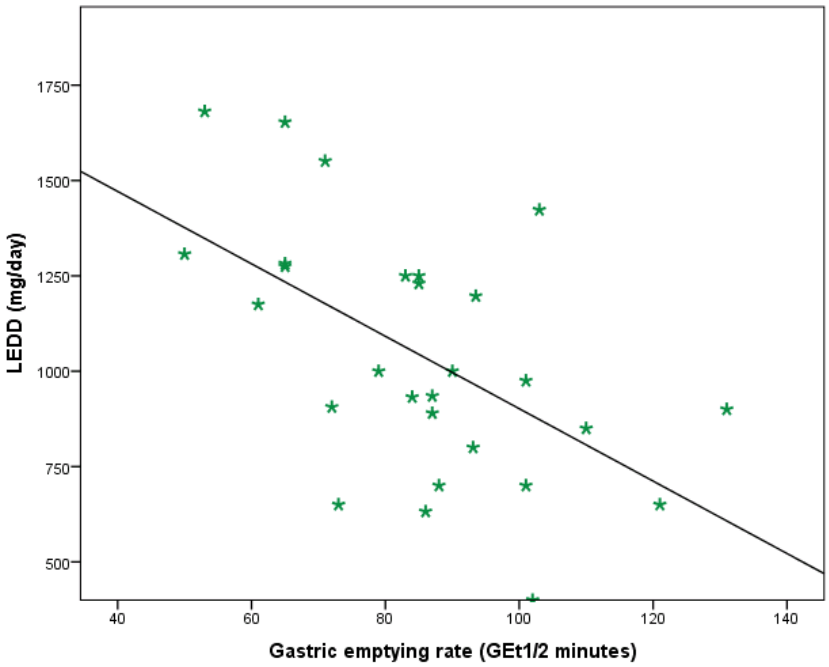
Participants reported their motor state in a detailed diary for the 48 hours after screening. 26 participants completed this diary in its entirety and for these subjects the mean percentage of the waking day spent in the 'off' state was 32.51 ± 18.23 There was a trend towards slower gastric emptying in participants with more 'off' time but the relationship did not reach the threshold of statistical significance (Spearman's correlation 0.106, 2-tailed significance P = 0.303).

Figure 4-6 Scatter plot of the relationship between gastric emptying rate and percentage of the waking day spent in the 'off' state



The Levodopa equivalent daily doses of the 28 participants were normally distributed with a mean of 1,042.64mg/day (± 324.43, minimum 400, and maximum 1,681mg/day). There was a strong negative correlation between LEDD and $GEt_{1/2}$, with lower daily doses associated with slower rates of gastric emptying (Pearson Correlation coefficient -0.561, 2-tailed significance value P = 0.002). Using a linear regression model, participants' LEDD accounted for 31.5% of the observed variation in gastric emptying speed.

Figure 4-7 Scatter plot (with line of best fit) showing the relationship between Levodopa equivalent daily dose (LEDD) and gastric emptying speed



Constipation difficulties were reported by participants in Part I of the MDS UPDRS (question 1.11). Comparing the average gastric emptying rate of those participants (n = 7) with no constipation problems (median 72.00 ± 28.1 minutes) and those with 'Mild' to 'Severe' constipation problems (median 87.00 ± 25.5 minutes) there were no significant differences between the two groups (Mann-Whitney test, P = 0.137). Similarly, when those participants with orthostatic hypotension (n = 3) were compared to those without, no difference in gastric emptying rates was apparent (medians 87 and 85 minutes, respectively).

4.5.4 Predictors of delayed gastric emptying in Parkinson's disease

From the above analyses, the following variables were identified as potential predictors of delayed gastric emptying in people with PD: advancing age, higher GCSI total score, lower LEDD and higher MDS UPDRS part I scores. When these variables were entered into a multiple regression model, they were shown to account for 43.8% of the variance in gastric emptying rate (Table 4-4).

Table 4-4 Multiple regression model for predictors of gastric emptying rate in people with PD

Variables	B	S.E. B	β
Constant	55.715	28.541	
Age	0.801	0.350	0.399*
GCSI total score	6.935	3.496	0.356
LEDD	-0.025	0.010	-0.414*
MDS UPDRS Part I	0.011	0.545	0.003

R^2 = 0.438 for the model, *P<0.005

A further model including only the patient age and LEDD showed that 39.8% of the variance in gastric emptying speed was accounted for by this model.

4.6 Discussion

This study explored the relationship between gastroparesis symptoms and gastric emptying speed in people with PD and was a sub-study of the previously described phase II pharmacokinetic study.

4.6.1 Summary of the study findings

In our series of 28 patients with paired gastric emptying measures and gastroparesis symptom diaries, we found a non-significant trend towards slower gastric emptying in those with higher GCSI total scores. An American study (Cassilly *et al.*, 2008) of over 200 patients referred for gastric emptying studies, reported that certain gastroparesis symptom scores (nausea, early satiety and post-prandial fullness) were better predictors of gastric emptying than global gastroparesis severity scores. However, in our participants, when mean scores for each of 11 typical gastroparesis symptoms

were compared with gastric emptying speed, the only significant correlation was for stomach distension (P = 0.047). None of the symptom clusters significantly correlated with gastric emptying speed but there was a trend towards slower emptying in those with more symptoms of nausea and vomiting. Of the composite scores evaluated, composite 1 (comprising: nausea, bloating, stomach fullness, excessive fullness after meals) was most closely correlated with gastric emptying speed which was in keeping with the findings of Revicki's validation study (Revicki *et al.*, 2012).

Ours is the first study in people with PD to compare gastric emptying and gastroparesis symptom severity. The relationship was not particularly close which is in keeping with recent studies in people with diabetes where a relatively poor association between changing gastric symptoms and gastric emptying has been demonstrated (Sanger, 2014).

The most striking feature of this exploratory data was our demonstration of a significant negative relationship between LEDD and gastric emptying. This may seem a somewhat unexpected finding as in healthy individuals Levodopa has been shown to slow gastric emptying (Berkowitz and McCallum, 1980; Robertson *et al.*, 1990; Robertson *et al.*, 1992). However, there is evidence from two studies (Murata *et al.*, 1996; Hardoff *et al.*, 2001) to suggest that in the context of PD patients on long-term Levodopa with motor fluctuations, Levodopa may paradoxically pseudo-normalise gastric emptying. Hardoff et al (Hardoff *et al.*, 2001) postulate that when first introduced, Levodopa therapy does indeed delay gastric emptying, as has been demonstrated in healthy volunteers but suggested that long-term exposure of the basal ganglia to Levodopa may result in changes to the dopaminergic system of the DMNV, resulting in hypersensitivity and enhanced vagal activity in the 'on' state resulting in enhanced gastric emptying (Hardoff *et al.*, 2001). This is of course speculative but may go some way towards explaining the negative relationship we demonstrated between Levodopa dose and gastric emptying.

We also evaluated demographic and disease specific measures as predictors of gastric emptying speed in people with established PD. There was a non-significant trend towards slower gastric emptying in female participants. With additional participant numbers we would anticipate that this gender difference may become more marked,

as previous studies have reported that women are at greater risk of gastroparesis (Stanghellini *et al.*, 2003) and in our earlier prevalence study (Chapter 2) we also showed this association in early PD. We demonstrated a significant relationship between advancing age and slower gastric emptying (p = 0.034). Some studies have reported no effect of age upon gastric emptying (Madsen and Graff, 2004) whilst others have reported slower gastric emptying in older individuals (Moore *et al.*, 1983; Brogna *et al.*, 1999). The fact that we found slower gastric emptying in older individuals may in fact be a surrogate marker of advancing disease severity, as PD is a neurodegenerative disease of older people. Indeed in our data series there was a slight trend towards more severe disease (higher MDS UPDRS total score) in older participants (Pearson Correlation 0.213, 2 tailed significance P = 0.227). Accordingly, we saw a trend towards slower gastric emptying in those participants with higher MDS UPDRS Part III scores.

4.6.2 Strengths and limitations

The main limitation of this sub-study was the relatively small number of participants. The original intention was to present results from all screened patients in the completed study. However, due to the need to re-design the main study at the end of 2012, there was a delay in recruitment which meant that only an interim analysis could be performed. However, given the exploratory nature of this evaluation which has never previously been undertaken in people with PD, we feel that even these preliminary findings and trends in the data are of academic and clinical relevance. The data we have obtained certainly forms the basis of a potential power calculation for future studies. One further limitation of this study was that it did not include a control group. Comparison with healthy volunteers with respect to gastric emptying speed and gastroparesis symptoms would be of interest. However, we were specifically interested in variations within PD patients and the relevance of their PD characteristics to gastric emptying rates. Future study including comparisons with healthy age matched controls would, nevertheless be of value.

A major strength is that the study represents the first parallel assessment of gastroparesis symptoms and gastric emptying rates in patients with PD. As has been reported in people with diabetes, the relationship between the degree of gastric

emptying delay and the severity of gastroparesis symptoms is complex and not direct (Samsom *et al.*, 2009). Our results suggest that whilst the GCSI-DD alone may have a low predictive value, when used in conjunction with the identification of higher risk demographic and disease characteristics, it may be a useful tool for identifying people with PD at risk of gastroparesis, especially in individuals with prominent 'off' periods and low Levodopa equivalent daily doses.

4.6.3 Study progression and future directions

In order to further evaluate the relationship between gastric emptying and gastroparesis symptoms, this sub-study will continue in tandem with the main investigational study. When the study concludes, paired gastric emptying and GCSI results will be available for 60-70 people, allowing more meaningful statistical analysis to be undertaken. Ultimately we would hope to identify symptom and clinical parameters predictive of delayed gastric emptying in PD, such that patients could be stratified for their risk of gastroparesis and then managed or investigated as appropriate.

Chapter 5 Conclusions and future studies

Parkinson's disease is increasingly thought of as a multi-system disorder, with an array of non-motor symptoms present at all stages of the disease. Gastroparesis is an example of gastric dysfunction which can occur in early and advanced PD. Gastroparesis is important not only because of the symptomatic implications but also because of its potential detrimental effect upon Levodopa absorption and action. The broad aims of our studies were: 1) to characterise the prevalence and associations of gastroparesis symptoms in people with PD, 2) to explore a novel treatment for delayed gastric emptying in PD.

5.1 Gastroparesis symptoms in Parkinson's disease

5.1.1 Summary of main findings

As part of the PRoBaND study, gastroparesis symptoms in over 1,000 people with PD were evaluated alongside detailed clinical evaluations. This represents the largest study of gastroparesis symptoms in PD to date. In early PD we demonstrated that upper GI symptoms were reported by more than half of all patients suggesting that upper GI symptoms are as prevalent as lower GI symptoms in early disease. However, the prevalence of significant gastroparesis symptoms was low at just over 4%. This was lower than might have been anticipated given that the overall prevalence of delayed gastric emptying in PD is estimated to exceed 70% (Heetun and Quigley, 2012). The prevalence of probable gastroparesis in those patients who had young onset disease was higher at 12.5%. However, it is likely that this reflects more advanced disease stage in these participants rather than a true increased gastroparesis risk in those participants diagnosed prior to the age of 50.

Based upon small studies of gastric motility in PD, we had identified several clinical variables which we hypothesised would be associated with a greater risk of gastroparesis symptoms. In early PD, female gender, PIGD phenotype, advanced disease severity, a greater number of NMS, the presence of autonomic symptoms, anxiety, depression and constipation were all significantly correlated with a greater gastroparesis symptom burden. These were generally in accordance with our *a priori*

hypotheses. The early, parallel appearance of alpha-synuclein in the DMNV and the olfactory centre led us to hypothesise that in early PD, hyposmia and gastroparesis symptoms would be associated however we were unable to demonstrate any such relationship in our study.

5.1.2 Conclusions

Upper GI symptoms are common in PD although rates of symptomatic gastroparesis are relatively low. In those patients who do report gastroparesis symptoms, these abnormalities form part of a wider spectrum of non-motor and autonomic symptoms. It is possible that some of these cases may ultimately be diagnosed with an alternative atypical parkinsonian disorder (e.g. multiple system atrophy). We are unable to discount this possibility in some cases; however on-going prospective evaluation of recently diagnosed participants will allow diagnostic re-evaluation as the study progresses.

The discrepancy seen between high rates of gastric emptying delay and relatively low rates of gastroparesis symptoms in PD suggests that this gastric motility abnormality may be asymptomatic in many cases. The strength of the relationship between motility and symptoms has also been called into question in the context of people with diabetes (Samsom *et al.*, 2009). Furthermore, a dissociation between alpha-synuclein burden and functional abnormalities of the upper GI tract has been reported in PD (Cersosimo *et al.*, 2013). Therefore it appears that the progression from pathological abnormality to functional impairment and then symptoms is indirect and heterogeneous in people with PD.

5.1.3 Future directions

The PRoBaND study, from which our gastroparesis symptom study was derived, is on-going. Participants with a recent diagnosis of PD will be asked to complete the GCSI again at 18 months and 36 months after baseline. This will allow longitudinal evaluation of gastroparesis symptoms. Such a prospective evaluation of changes in gastroparesis symptoms has not been undertaken before in PD. The results of this study have suggested a disparity between functional and symptomatic abnormalities of the stomach in PD. A sub-study of our evaluation of a novel treatment for delayed

gastric emptying has begun to assess this relationship between gastric motility and symptoms but this remains an area worthy of investigation. Matched assessments of 1) gastric ENS pathology, 2) gastric emptying, 3) gastroparesis symptoms in all stages of PD would be the optimal way to explore the relationship between pathology, physiology and symptoms.

There is extensive research on-going to identify biomarkers of early PD, with particular interest in non-motor features of the disease, as many of these pre-date the onset of motor dysfunction. A retrospective study reported that gastroparesis symptoms precede motor symptoms in PD although the prevalence of gastroparesis symptoms was not significantly different to that of the control group (Cersosimo *et al.*, 2013). Given the apparent discrepancy between symptoms and dysfunction of the stomach in early PD, measurement of gastric emptying may serve as a more reliable early biomarker. To date only one study (Unger *et al.*, 2011b) has tried to address this by measuring gastric emptying in healthy controls, early PD and people with idiopathic REM sleep behaviour disorder (iRBD), a recognised premotor feature of PD. Relative to the healthy controls, gastric emptying rates in the early untreated PD patients were abnormally slow but no significant difference was evident between controls and iRBD cases. However, the median disease duration for the iRBD subjects was 48 months and it is recognised that the interval from onset of iRBD to diagnosis with PD may be up to 15 years in many cases (Postuma, 2014). Therefore, further research to establish when in the chronology of pre-motor PD, gastric motility becomes impaired. Thereafter, in conjunction with other non-motor features such as RBD, constipation and hyposmia, gastroparesis may prove a useful biomarker for idiopathic PD.

5.2 Exploration of a novel treatment for delayed gastric emptying in Parkinson's disease

5.2.1 Summary of main findings

We have presented interim findings from an on-going phase II study to assess the efficacy of Camicinal as a novel treatment for delayed gastric emptying in PD. We recruited 15 participants with PD who experienced motor fluctuations on oral Levodopa and had proved delayed gastric emptying. In those participants treated with Camicinal, by the end of the 7-9 day dosing period there was a trend towards improved Levodopa absorption (increased Cmax) relative to baseline, although the improvement was not statistically significant. It was hypothesised that Levodopa absorption would improve as a consequence of faster gastric emptying. However, the interim analysis of the first 15 participants did not reveal a significant difference in gastric emptying between the two study groups. Gastroparesis symptoms from baseline to the end of the dosing period did not differ significantly in either group although baseline gastroparesis severity scores were relatively low in most cases. We noted some significant improvements in motor function, non-motor function and reduced off time in the Camicinal treated participants. Given the modest improvements in Levodopa absorption seen, at this interim analysis phase, it is not possible to conclude that Camicinal has improved PD function through enhanced gastric emptying and Levodopa absorption. Camicinal was as well tolerated as placebo with no safety issues evident in this study.

A sub-study was undertaken looking at all screened study participants with the aim of trying to assess the relationship between gastroparesis symptoms and gastric emptying in people with PD. This sub-study also explored other potential clinical and demographic predictors of delayed gastric emptying. This was the first such study to perform paired physiological and symptom evaluations alongside detailed PD assessments. A total of 28 screened participants were considered in the sub-study which demonstrated a non-significant trend towards higher GCSI total score in those participants with slower gastric emptying. The only individual gastroparesis symptom which positively and significantly correlated with gastric emptying speed was 'Stomach

distension.' Participants' ages and Levodopa equivalent daily dose (LEDD) were the only other variables which significantly correlated with gastric emptying speed. Advancing age was associated with significantly slower gastric emptying, which has been demonstrated in studies with healthy older people (Moore *et al.*, 1983; Brogna *et al.*, 1999). Contrary to expectation we saw a negative correlation between LEDD and gastric emptying. Levodopa is typically thought to result in slowed gastric motility (Berkowitz and McCallum, 1980; Robertson *et al.*, 1990; Robertson *et al.*, 1992) however the reverse was evident in our study results. This inverse relationship has previously been described in the literature on two occasions (Murata *et al.*, 1996; Hardoff *et al.*, 2001), where it was postulated that in the short term Levodopa delays gastric emptying but with long-term use, plastic changes in the DMNV result in a pseudo-normalisation of gastric motility in the presence of Levodopa (Hardoff *et al.*, 2001). The average disease duration of the 28 participants in this sub-study was just under a decade. To further explore the suggestion that chronic Levodopa use pseudo-normalises gastric emptying, it would be of interest to study gastric motility in people who have taken Levodopa for different durations of time; additionally paired measures before and after the introduction of dopaminergic therapies would be valuable.

5.2.2 Conclusions

This was an experimental medicine study. The intention of the interim analysis presented in this thesis was not to definitively report upon the efficacy of Camicinal, but rather to evaluate the potential of this novel agent, its safety and also suitability for the study to continue. The conclusion of the interim review was that the investigation should continue to explore the potential of Camicinal as a treatment for delayed gastric emptying in PD.

The sub-study has suggested that gastroparesis symptoms alone are not predictive of gastric emptying speed. However, when used in conjunction with clinical and demographic parameters, gastroparesis symptoms evaluated using the GCSI-DD, can be helpful in identifying those patients with PD most at risk of delayed gastric emptying.

5.2.3 Future directions

Following the interim analysis, the study statisticians at GSK concluded that a sample size of 45 should be sufficient to evaluate the efficacy of Camicinal in enhancing Levodopa absorption. The study is on-going at the time of writing this thesis and based upon current recruitment rates it is forecast that the study will conclude in the spring of 2014. Pending the final results of this mechanism of action study, future directions for this project could include the study of Camicinal over longer and titrated dosing periods, to evaluate its longer term effects upon pharmacokinetic and clinical parameters. If Camicinal is shown to be effective at enhancing Levodopa absorption and motor function, it could also be evaluated in earlier stages of PD as an adjunct therapy to help keep total Levodopa doses as low as possible.

The sub-study to evaluate clinical predictors of delayed gastric emptying is also on-going and will run in parallel to the main study until its conclusion. It is envisaged that from this work a list of risk factors will be identified to help clinicians identify those patients with PD at greatest risk of delayed gastric emptying such that prokinetic agents could be most appropriately prescribed.

5.3 Summary

Severe gastroparesis symptoms are rare in PD despite gastric motility problems being common in all stages of the disease. There may not be a direct relationship between gastroparesis symptoms in the presence of impaired gastric emptying. However, our work has helped to identify symptoms and clinical factors which may identify those patients most at risk of delayed gastric emptying. Even if relatively asymptomatic, it is important for clinicians to be able to identify such patients so that they can be considered for appropriate therapies to improve gastric motility and potentially enhance Levodopa absorption and action.

Appendix A - Publications, awards and presentations arising from this thesis

Review article in *Movement Disorders* (Marrinan *et al.*, 2014)

REVIEW

Delayed Gastric Emptying in Parkinson's disease

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ABSTRACT: Gastrointestinal symptoms are evident in all stages of Parkinson's disease (PD). Most of the gastrointestinal abnormalities associated with PD are attributable to impaired motility. At the level of the stomach, this results in delayed gastric emptying. The etiology of delayed gastric emptying in PD is probably multifactorial but is at least partly related to Lewy pathology in the enteric nervous system and discrete brainstem nuclei. Delayed gastric emptying occurs in both early and advanced PD but is underdetected in routine clinical practice. Recognition of delayed gastric emptying is important because it can cause an array of upper gastrointestinal symptoms, but additionally it has important implications for the absorption and action of levodopa. Delayed gastric emptying contributes significantly to response fluctuations seen in people on long-term L-dopa therapy. Neurohormonal aspects of the brain-gut axis are pertinent to discussions regarding the pathophysiology of delayed gastric emptying in PD and are also hypothesized to contribute to the pathogenesis of PD itself. Ghrelin is a gastric-derived hormone with potential as a therapeutic agent for delayed gastric emptying and also as a novel neuroprotective agent in PD. Recent findings relating to ghrelin in the context of PD and gastric emptying are considered. This article highlights the pathological abnormalities that may account for delayed gastric emptying in PD. It also considers the wider relevance of abnormal gastric pathology to our current understanding of the etiology of PD.

Key Words: Parkinson's disease; gastroparesis; gastrointestinal motility; gastrointestinal hormones; ghrelin

Gastrointestinal (GI) dysfunction is common in all stages of PD,[1] with approximately 30% of patients reporting GI symptoms.[2,3] Structural and functional abnormalities of the GI tract are evident in PD and almost the entire length of the GI tract is vulnerable to dysfunction[4] (Table 1).

Impaired motility underlies most of the GI symptoms associated with PD.[5] Constipation, an example of impaired colonic motility, is the most recognized example of GI dysfunction in PD. Constipation is reported by close to 90% of all patients,[4] with the rates rising as the disease progresses.[6,7] Constipation is also a well described pre-motor feature.[8–10]

Pathological and functional abnormalities of the stomach have to date received less attention than colonic abnormalities but it can be argued that they are just as important from a patient and physician perspective. The stomach is one of the earliest sites of alpha-synuclein deposition in PD,[11] suggesting that the stomach is integral to the pathogenesis of PD.

This review focuses upon delayed gastric emptying in PD, a common but under recognized problem. Delayed gastric emptying can cause problematic upper GI symptoms and can negatively impact upon the absorption of levodopa in PD, contributing to response fluctuations.

The pathophysiology of delayed gastric emptying in PD is unproven but is likely multifactorial. As is described in this review, alpha-synuclein aggregation

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TABLE 1. GI symptoms associated with Parkinson's disease (adapted from Pfeiffer[1])

Level of the GI tract	Site of dysfunction	Problem
Upper GI tract	Mouth	Sialorrhea
		Dental deterioration
	Pharynx	Oropharyngeal dysphagia
	Esophagus	Esophageal dysphagia
	Stomach	Delayed gastric emptying
		Gastroesophageal reflux
Lower GI tract	Small intestine	Dilatation
	Large intestine	Constipation
		Dysmotility
		Volvulus
		Megacolon
		Perforation
	Rectum	Defecatory difficulties

GI, gastrointestinal.

TABLE 2. Gastrointestinal hormones secreted in the fed and fasted state (adapted from Khoo et al.[22] and Sanger and Lee[13])

Hormones secreted in response to a meal	Hormones secreted in the fasting state
Gastrin	Motilin
Cholecystokinin (CCK)	Ghrelin
Leptin	Somatostatin
Enterostatin	Xenin
Peptide YY (PYY)	Orexin A and B
Apolipoprotein A-IV	
Glucagon-like peptide-1 (GLP1)	
Glucagon-like peptide-2 (GLP2)	
Glucose-dependent insulinotropic polypeptide (GIP)	
Pancreatic polypeptide	
Oxyntomodulin	
Amylin	

and abnormalities in the dorsal motor nucleus of the vagus nerve (DMNV) and the enteric nervous system (ENS) are implicated.[12] Interplay between these 2 areas, the so-called brain-gut axis, involves complex neural and hormonal processes. Examples of hormones operating along this axis in the regulation of appetite and GI motility include: ghrelin, motilin, orexin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY).[13] In this review we focus on ghrelin as an exemplar hormone with both GI and central nervous system (CNS) actions. Exploiting ghrelin's dual neural and GI actions, it has been tested as a therapy for delayed gastric emptying[14,15] and, most excitingly, ghrelin also shows promise as a neuroprotective agent in PD.[16,17]

Search Strategy

This review focuses on gastric dysfunction in PD but is not an exhaustive appraisal of the literature. It focuses on seminal studies or those with novel findings, with a particular emphasis on histopathological and clinical correlates.

Articles were identified through a Medline search of English language articles from 1946 to the first week of May 2013 using the following MeSH terms: Parkinson disease, gastroparesis, gastrointestinal motility, nonmotor, non-motor, gastrointestinal hormones, ghrelin.

Gastric Structure and Function

The stomach plays an early, critical role in the complex process of digestion. Although the stomach has only 1 chamber, it can functionally be considered in 3 regions: the proximal stomach, the distal stomach and the pylorus.[18] The motor activity and contractile patterns of these 3 regions are quite distinct but they operate in unison to maintain normal gastric emptying. The proximal stomach primarily acts as a receptive chamber and is able to adapt its size in response to an ingested meal such that a stable intragastric pressure is maintained,[19] thus allowing continued meal ingestion until satiation. This vagally mediated process[20] is termed the gastric accommodation reflex. The distal stomach is active in the fed and fasted states. Following a meal, strong contractile pulses in the distal stomach break up food particles and in interdigestive periods a cyclical pattern of contractile activity termed the migrating motor complex (MMC) is observed. These stereotypic motor complexes help to clear the stomach of digestive remnants and are vital for the maintenance of normal gastric emptying.[21]

Several GI hormones regulate gastric motility and many also act in the brain to influence appetite.[13] A full description of the roles of each of these hormones is beyond the scope of this article and has been addressed comprehensively in previous reviews.[13,22,23] Table 2 summarizes the hormones secreted in response to a meal and also those which act during periods of fasting.

Control of Gastric Emptying

Neural Control of Gastric Emptying

Gastric emptying is regulated by extrinsic neural influences via vagal and splanchnic pathways and intrinsic innervation via the ENS. The ENS is an extensive neural network that runs the entire length of the GI tract and is sometimes referred to as the "second brain"[24] because it contains nearly 100 million neurons and can function independently of the CNS.[25] The ENS consists of 2 ganglionated nerve plexuses: the submucosal (Meissner's) plexus; and the myenteric (Auerbach's) plexus, which influences

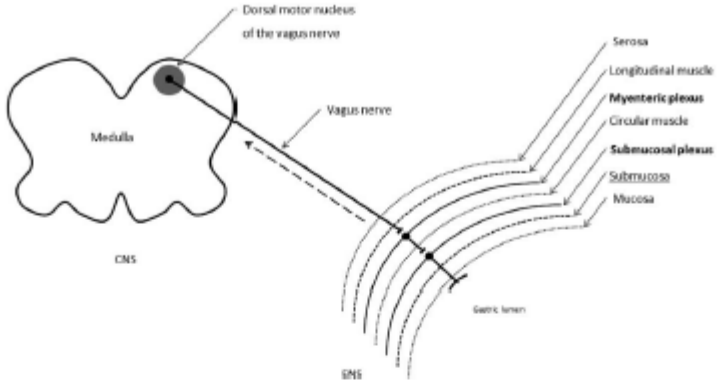

FIG. 1. Interaction of the central nervous system with the enteric nervous system via the vagus nerve

smooth muscle activity in the GI tract.[25] Although the ENS can function autonomously, it closely interacts with the vagal system. In the stomach, the myenteric plexus connects directly with the vagus nerve (see Fig. 1) providing a direct neural link between brain and stomach.

The vagus nerve is integral to the control of gastric emptying and the stomach is richly supplied by vagal efferent and afferent fibers running to and from the smooth muscle layers. Vagal damage is implicated as a cause of delayed gastric emptying as following vagotomy delayed gastric emptying has been demonstrated.[26]

Hormonal Control of Gastric Emptying

Several hormones influence gastric emptying: ghrelin and motilin accelerate emptying while CCK, GLP-1, and PYY delay emptying.[22] The best characterized of these peptides in the context of PD is ghrelin, which we examine in greater depth. To the best of our knowledge, serum levels of the other listed peptides have to date not been extensively evaluated in PD.

The brain-gut axis involves not only neural connections but also hormonal mediators.[13] Specifically ghrelin, CCK, and GLP-1 receptors colocalize with dopaminergic neurons in the basal ganglia. All 3 have been considered as targets for disease-modifying therapy in PD. CCK polymorphisms have been reported to confer an increased risk of hallucinations in PD,[27,28] but therapies targeting CCK have thus far been ineffective.[29] Agonists of the GLP-1 receptor have been reported to have neuroprotective potential based upon studies in animal models of PD.[30–32] More recently a proof of concept study of the GLP-1 agonist exenatide in 45 patients with moderate PD reported improvements in motor function over 12 months of treatment.[33]

Alpha-Synuclein and the Stomach in PD

Phosphorylated alpha-synuclein in Lewy bodies (LBs) and Lewy neurites (LNs) is the pathological hallmark of idiopathic PD. As described, neural control of gastric emptying is influenced by vagal pathways and the ENS and both are infiltrated by alpha-synuclein early in the disease process.

Braak et al.[34] hypothesized that alpha-synuclein spreads through the brain in a predictable ascending manner and proposed a 6-step pathological staging system for PD. In the pre-motor period of the disease, alpha-synuclein is said to ascend through the brainstem with the DMNV universally affected. As the vagus nerve is central to control of gastric emptying, this early pathological change in the DMNV is pertinent to consideration of the pathophysiology of delayed gastric emptying in early PD.[35,36]

Alpha-synuclein also extensively infiltrates the ENS,[37–39] and in particular the gastric myenteric plexus (see Fig. 2). The first appearance of alpha-synuclein in the ENS coincides with its appearance in the DMNV,[11] suggesting that the disease process may begin in the stomach and rapidly ascend to the DMNV via the vagus nerve. A pathogenic trigger to the onset of PD has been proposed with possible simultaneous invasion of the gastric ENS and the olfactory centers.[40,41] Transsynaptic spread of alpha-synuclein to adjacent vulnerable neurons has been described,[42–46] with the process likened to a prion-mediated disorder.[47]

Several recent animal model studies have further enhanced understanding of the role of the stomach in alpha-synuclein accumulation and spread. Mice chronically exposed to rotenone have been shown to develop alpha-synuclein inclusions within the

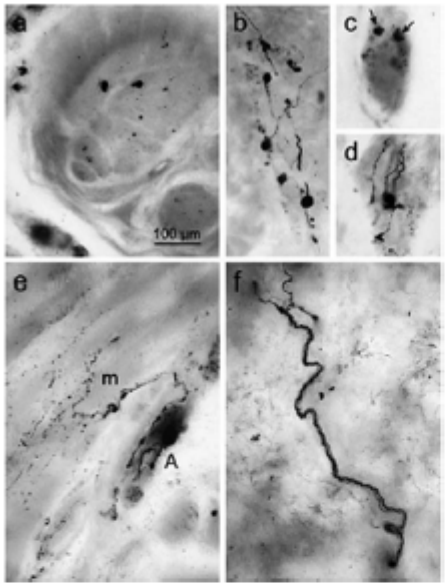

FIG. 2. Aggregated α-synuclein in the gastric wall (Braak et al.[11] Neuroscience Letters 2006;396:67–72; Fig. 1; permission to republish obtained from Elsevier).

TABLE 3. Causes of gastroparesis

Endocrine/metabolic disturbances
Diabetes mellitus
Hypothyroidism
Uremia
Amyloidosis
Pregnancy
Neurological disease
Parkinson's disease
Muscular dystrophy
Spinal cord disease
Brain stem tumors
Peripheral neuropathy
Connective tissue disorders
Scleroderma
Systemic lupus erythematosus
Gastrointestinal disorders
Gastroesophageal reflux
Peptic ulcers
Viral gastritis
Chronic intestinal pseudo-obstruction
Medications
Anticholinergic agents
Opiates
Dopaminergic drugs
Chemotherapeutic agents
Other
Idiopathic
Postsurgery
Following radiation therapy
Chronic liver disease
Anorexia nervosa

myenteric plexus.[48] Additionally, direct intragastric rotenone administration in rodents not only led to alpha-synuclein accumulation in the ENS but also remotely in the DMNV.[49] When this experiment was repeated after lesioning the sympathetic and parasympathetic nerves, the spread of alpha-synuclein from the ENS to the brain was halted.[50] However, such findings have not been consistently replicated. One recent study of rodents chronically exposed to rotenone reported only minor ENS changes and GI alpha-synuclein expression was actually reduced when compared with controls (although, somewhat paradoxically, there was a significant decrease in tyrosine hydroxylase-immunoreactive neurons in the substantia nigra of rotenone-exposed animals).[51]

Although animal models of PD have inherent limitations, and findings with regard to accumulation and spread of alpha-synuclein are not always consistent, there is an increasing body of evidence to suggest that the stomach may be critical in the pathogenesis of PD.

Delayed Gastric Emptying in PD

When delayed gastric emptying is chronic and symptomatic it is termed gastroparesis. The typical symptoms of gastroparesis include nausea, vomiting, retching, early satiety, bloating loss of appetite, and abdominal discomfort.[52]

There are many recognized causes of gastroparesis (see Table 3), with PD estimated to account for 7.5% of all cases.[53,54] In the general population, the prevalence of gastroparesis is estimated to be 9.6 per 100,000 men and 37.8 per 100,000 women.[55]

There are several recognized, validated ways to measure gastric emptying.[52] However, results obtained through different testing methods are not always comparable, preventing meta-analysis. However, a systematic review has estimated the prevalence of delayed gastric emptying in PD to be 70% to 100%,[56] although much of this may be asymptomatic.[57]

Pathophysiology of Delayed Gastric Emptying in PD

The pathophysiology of delayed gastric emptying in PD is unproven but there are several plausible explanations, suggesting that the etiology may be multifactorial. The roles of the vagal system and the ENS in control of gastric emptying have been outlined and as both are early sites of alpha-synuclein deposition, it is postulated that resultant neural damage and dysfunction contributes to impaired gastric motility. Indirect evidence supporting the hypothesis that abnormal

alpha-synuclein accumulation underlies impaired gastric motility in PD comes from studies demonstrating delayed gastric emptying in multiple system atrophy, another synucleinopathy.[58,59] To the best of our knowledge, no evaluations of gastric emptying in the context of dementia with Lewy bodies have been published. Such wider evaluations of gastric motility in synucleinopathies other than PD would be clinically relevant and may also advance understanding of the pathophysiology of gastroparesis.

Dopaminergic medications may also contribute to delayed gastric emptying. In young and old healthy volunteers, single and multiple doses of L-dopa have been reported to delay gastric emptying.[60–63] While dopaminergic medications may contribute, they are certainly not the sole cause of delayed gastric emptying, because this abnormality has been demonstrated in early untreated cases of PD, as will be described in the next section.[35,36]

A recent study that reported improved gastric emptying after subthalamic nucleus–deep brain stimulation (STN-DBS)[64] adds a further dimension to current understanding of the pathophysiology of gastroparesis in PD. STN-DBS has previously been reported to improve some non-motor symptoms,[65–68] although the mechanism remains unclear. While the STN and autonomic centers of the brain are interconnected[69] and STN-DBS has been shown to activate autonomic areas,[70–72] the exact mechanism by which STN-DBS influences gastric emptying remains speculative. The authors postulate that the mechanism could in fact be indirect, such that improved motor function, reduced medication doses, and weight gain postsurgery may all contribute to improved gastric motility.[64]

Delayed Gastric Emptying in Early PD

Two studies have assessed gastric emptying in early untreated PD. Tanaka et al.[35] measured gastric emptying rates in 20 treatment-naïve early PD patients (median age 70.5 years, Hoehn & Yahr [H&Y] stage 1–2, median disease duration 0.9 years), 40 patients with advanced treated PD (median age 67.0 years, H&Y stage 3–4, median disease duration 6 years), and 20 healthy controls (median age 69 years). Gastric emptying, measured using the ^{13}C-acetate breath test, was significantly slower in both PD groups compared with controls ($P < 0.001$) but there was no significant difference between early and advanced disease. Another study[36] reported comparable rates of gastric emptying delay in early and advanced PD but no significant abnormality in subjects with idiopathic rapid eye movement (REM) sleep behavior disorder (iRBD), a pre-motor phenotype of PD. This suggests that while delayed gastric emptying may be an early feature of PD, screening for gastroparesis as part of the Parkinson's at risk syndrome (PARS) may not be helpful.

Delayed Gastric Emptying in Advanced PD

Longer PD duration alone does not appear to be associated with worse gastroparesis.[35,36,73–77] However, advancing motor severity has been reported to correlate with worse gastric emptying delay.[74,75]

L-Dopa is only absorbed once it reaches the level of the proximal duodenum. Hence gastric emptying speed has been described as the rate-limiting step in the absorption of L-dopa.[78] Gastric emptying rates and plasma L-dopa levels are closely related,[79,80] underpinning the suggestion that gastroparesis contributes to response fluctuations. In a 1996 study[73] comparing gastric emptying rates in PD patients with and without fluctuations and in healthy controls, gastric retention rates were significantly higher in those with fluctuations compared with non-fluctuators ($77.4 \pm 15.5\%$ vs $64.0 \pm 14.3\%$, $P < 0.05$). Not all studies have replicated this finding[76] but indirect evidence in support of gastric emptying contributing to response fluctuations comes from the study of prokinetics, which have been reported to improve L-dopa absorption and motor function.[81,82]

In non-Parkinsonian individuals, gastric colonization with *Helicobacter pylori* (*H. pylori*) is not a cause of delayed gastric emptying. However, there is evidence to suggest that such colonization may also contribute to motor fluctuations.[83] Following *H. pylori* eradication, L-dopa absorption and motor fluctuations can be improved.[83,84]

Ghrelin

Ghrelin is an endogenous 28–amino acid peptide, first described in 1999.[85] It is produced in the stomach but the growth hormone secretagogue receptors (GHSR), at which it acts, have been identified in numerous sites within the GI tract, the ENS, and also the CNS. In the GI tract ghrelin exerts prokinetic effects and centrally it enhances appetite.[14] Ghrelin levels peak before eating and fall rapidly as ingestion starts. The hormone is regarded as a key orexigenic hormone. Although ghrelin is a gastric-derived hormone, its secretion appears to be at least in part dependent upon vagal activity because following vagotomy in rodents, the expected pattern of ghrelin secretion is not seen.[86] This may be because GHSRs have been identified within the dorsal vagal complex.[87]

Ghrelin and Gastric Emptying

Several studies have considered ghrelin and ghrelin agonists as therapy for diabetic gastroparesis.[14,15,88,89] Although research into ghrelin agonists is still in an early stage, and has not been undertaken in the context of PD, such agents are promising potential treatments for gastroparesis.

A recurrent feature of the gastroparesis literature has been the poor correlation between gastric emptying measurement and subjective symptoms: it is possible that PD patients offer a novel metric for studying gastric function in terms of dopamine pharmacokinetics as well as motor symptom response.

To date only 1 study has simultaneously measured ghrelin levels and gastric emptying in the context of PD. Arai et al.[64] measured ghrelin levels and gastric emptying in 16 patients with PD before and after STN-DBS. There was no significant association between ghrelin levels and gastric emptying speed in these patients either before or after surgery or *on* or *off* L-dopa medications. A recently published study[90] reported that in rodents given L-dopa, ghrelin coadministration prevented L-dopa–induced delayed gastric emptying.

Ghrelin and PD

Aside from the relevance of ghrelin to gastric emptying in PD, this peptide also holds promise in 3 interesting areas: (1) as a contributory factor in PD-associated weight loss; (2) as a biomarker for early PD; and (3) as a neuroprotective agent.

Approximately half of all PD patients have unintentional weight loss,[91] a feature associated with reduced life expectancy.[92] The orexigenic action of ghrelin has spurred interest in it as a potential mediator of weight loss in PD. In PD patients, ghrelin levels are lower in those patients with lower weight.[93] While one would expect ghrelin levels to rise in a malnourished state, the reported lower levels in underweight PD patients may reflect some abnormality of this homeostatic process, which could contribute to spiraling problem of weight loss in PD. Weight gain following DBS surgery is well recognised,[94–101] but the pathophysiology underlying this remains subject of debate. Ghrelin levels have been measured following STN DBS in 4 studies to date: 3 reported no significant change in ghrelin levels post-DBS[64,102,103] and 1 reported a positive association between weight gain and ghrelin levels.[104]

Abnormal postprandial ghrelin response patterns may be a useful biomarker in early PD. Unger et al.[87] measured fasting and postprandial ghrelin levels in 19 drug naïve PD patients, 20 treated PD patients, 11 subjects with iRBD, and 20 healthy controls. After the initial normal postprandial decline, ghrelin levels rose significantly slower in the PD and iRBD patients compared with controls ($P = 0.002$ and $P = 0.037$, respectively). Whether or not such abnormal ghrelin secretion patterns are secondary to underlying abnormalities of the vagal system or a primary feature of early disease remains unclear. The intertwined relationship between ghrelin and the vagus nerve as demonstrated through animal models[86] suggests that synergy between these neural and hormonal elements of the brain-gut axis may be key to understanding the pathogenesis of PD.

The study of ghrelin as a potential neuroprotective agent in PD is at an early stage but the peptide holds promise as a novel disease-modifying target. Rodent studies using a 1-methyl-4-phenly-1,2,5,6 tetrahydropyridine (MPTP) model for PD have reported reduced dopaminergic cell damage after administration of exogenous ghrelin.[105,106] Ghrelin reduces dopaminergic damage through decreased microglial and caspase activation and it is postulated that 5′adenosine monophosphate activated protein kinase (AMPK) increases mitophagy, thereby enhancing overall mitochondrial function.[17] Ghrelin also modulates mood, anxiety, learning, and memory,[16] and hence may be a useful therapy in a variety of disease settings.

Treatment of Delayed Gastric Emptying in PD

Cisapride was an effective prokinetic and useful adjunct therapy in patients with motor fluctuations.[81,82] However, cisapride was withdrawn from the market in the late 1990s due to reports of cardiotoxicity.[107] Prucalopride is a highly selective 5-HT4 receptor agonist that accelerates small and large intestine transit.[108] It may have a role as a gastric prokinetic, but this has yet to be studied.

Dopamine receptor agonists such as metoclopramide are the mainstay of treatment for gastroparesis in the general population[109] but due to the potential for extrapyramidal side effects, metoclopramide is not suitable for long-term use in PD. Domperidone does not cross the blood brain barrier and has been shown to enhance gastric emptying and L-dopa bioavailability in PD,[110] but is not licensed in every country.

Macrolide antibiotics are motilin agonists that stimulate phase III of the MMC and hence enhance gastric emptying. Macrolides are effective in gastroparesis,[109,111–113] but the antimicrobial properties limit their long-term use. Non-antimicrobial motilin agonists have been studied in diabetic and idiopathic gastroparesis with mixed results,[114–116] but to date such agents have not been assessed in people with PD.

Ghrelin has a structural homology with the gastric-derived peptide motilin. Both enhance upper gut transit. In 2009,[14] favorable results for infusions of a ghrelin receptor agonist (TZP-101) were reported in people with diabetic gastroparesis. More recently, the same group[88] published results of a randomized, placebo-controlled, double-blind study of an oral ghrelin receptor agonist (TZP-102). Gastric emptying parameters did not differ significantly between groups but gastroparesis symptoms improved in the group receiving active treatment. A further study[15] of another oral ghrelin agonist (RM-131)

reported significant increases in gastric emptying rates compared with placebo.

There is currently insufficient evidence to support the routine use of intrapyloric botulinum toxin injections for gastroparesis,[117] although a case report of its use in 2 patients with PD was favorable in the short term.[118] Implantation of gastric electrical stimulators has had some favorable reports,[119,120] although complication rates are not insignificant and long-term outcome data is lacking.

In summary, current therapeutic options for gastroparesis in the context of PD are somewhat limited. The judicious use of domperidone or macrolide antibiotics may be helpful in some cases. Other novel agents, including motilin and ghrelin agonists, have potential but are currently still at an investigational stage of development. Further practical management steps include review of concomitant medications and avoidance where possible of agents known to delay gastric emptying (eg, anticholinergics and opiates). Finally, dietary adjustments such as reducing meal volume while increasing meal frequency ("little and often") has been advocated as a useful approach in gastroparesis generally.[109]

Conclusions and Future Horizons

Pathological and functional abnormalities of the stomach have been described in all stages of PD. Delayed gastric emptying is a seemingly common consequence of these abnormalities and is a problem with wide ranging implications for many patients with PD. The study of gastric pathology and function in PD holds promise of both enhancing knowledge of the etiopathogenesis of the disease as well as offering potential opportunities for treatment.

There is compelling evidence to suggest that the stomach is integral to the pathophysiology of PD although there remain many unknowns. Whether the early appearance of alpha-synuclein in the stomach is indicative of the disease originating there is still speculative. However, we feel that further exploration of pathological, physiological and hormonal variations in the stomach associated with PD, are likely to provide valuable insights into the condition as a whole.

The search for effective prokinetic agents in the context of PD is ongoing and it may be that exploitation of endogenous peptides such as ghrelin and motilin is valuable in this respect. Exploration of the neuroprotective potential of GI hormones such as ghrelin is at an early stage but is an exciting development in the search for disease modifying therapies. Further examination of the complex neurohormonal brain-gut axis may reveal additional novel therapeutic targets. ●

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Publications and posters

- **Marrinan S**, Emmanuel AV, Burn DJ. Delayed gastric emptying in Parkinson's disease. *Movement Disorders*. 2013 Oct 21. doi: 10.1002/mds.25708. [Epub ahead of print].
- Duncan GW, Yarnall AJ, **Marrinan S**, Burn DJ. New horizons in the pathogenesis, assessment and management of movement disorders. *Age Ageing* 2013; 42 (1): 2-10.
- **S Marrinan**, N Bajaj, R Barker, Y Ben-Shlomo, A Emmanuel, T Foltynie, D Grosset, H Morris, N Williams, N Wood, D Burn. Gastroparesis symptoms in early Parkinson's disease. Poster presented at the Spring BGS, November 2013, Harrogate.
- **Sarah Marrinan**, Anton Emmanuel, Donald Grosset, David Burn. Prevalence of gastroparesis symptoms in patients with early Parkinson's disease. Poster presented at the 17th International Congress of Parkinson's Disease and Movement Disorders, June 2013, Sydney, Australia.
- **Sarah Marrinan**, George Dukes, Rachel Gibson, Anton Emmanuel, Donald Grosset, David Burn. Gastroparesis in Parkinson's disease. Parkinson's UK Research Conference, November 2012, York.

Awards

- North East Postgraduate Research Conference 2013 – second place for best oral presentation. **S Marrinan**, N Bajaj, R Barker, Y Ben-Shlomo, A Emmanuel, T Foltynie, D Grosset, H Morris, N Williams, N Wood, D Burn. Gastroparesis symptoms in early Parkinson's disease.

Presentations

- Newcastle University Ageing Symposium: 'Parkinson's disease and the stomach' – Institute for Ageing and Health – 06/09/2013.
- Parkinson's UK South of Tyne branch meeting: 'Parkinson's disease and the stomach' – Stockton – 20/07/2013.

- MDS International Congress: ‘Prevalence of gastroparesis symptoms in patients with early Parkinson’s disease’ – Sydney - 18/06/2013.
- Regional BGS meeting: ‘Prevalence of gastroparesis symptoms in patients with early Parkinson’s disease’ – Newcastle University - 14/03/2013.
- Parkinson’s UK Research Conference, Patient group meeting: ‘Gastroparesis in Parkinson’s disease’ – 04/11/2012.
- Parkinson’s Interest Group Meeting: *‘Gastroparesis in Parkinson’s disease’* – Gateshead - 18/10/2012.
- Demands Journal club meeting: ‘Gastroparesis in Parkinson’s disease’ – Institute for Ageing and Health - 07/01/2013.

Appendix B - Gastroparesis questionnaires

The Gastroparesis Cardinal Symptom Index (GCSI)

GCSI

This questionnaire asks you about the severity of symptoms you may have related to your gastrointestinal problem. There are no right or wrong answers. Please answer each question as accurately as possible.

For each symptom, please *circle the number* that best describes how *severe* the symptom has been during the past 2 weeks. If you have not experienced this symptom, circle 0. If the symptom has been very mild, circle 1. If the symptom has been mild, circle 2. If it has been moderate, circle 3. If it has been severe, circle 4. If it has been very severe, circle 5. Please be sure to answer every question.

Please rate the severity of the following symptoms during the past 2 weeks.

	None	Very Mild	Mild	Moderate	Severe	Very Severe
1. nausea (feeling sick to your stomach as if you were going to vomit or throw up)	0	1	2	3	4	5
2. retching (heaving as if to vomit, but nothing comes up)	0	1	2	3	4	5
3. vomiting	0	1	2	3	4	5
4. stomach fullness	0	1	2	3	4	5
5. not able to finish a normal-sized meal	0	1	2	3	4	5
6. feeling excessively full after meals	0	1	2	3	4	5
7. loss of appetite	0	1	2	3	4	5
8. bloating (feeling like you need to loosen your clothes)	0	1	2	3	4	5
9. stomach or belly visibly larger	0	1	2	3	4	5

GCSI - United States/English - Mapi Research Institute.
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Gastroparesis Cardinal Symptom Index – Daily Diary (GCSI-DD)

GASTROPARESIS CARDINAL SYMPTOM INDEX - DAILY DIARY (GCSI-DD)

Instructions: *These questions ask about symptoms you may have each day. Please complete the daily diary after your evening meal every day.*

Primary Symptoms

1. During the past 24 hours, how many episodes of vomiting did you have? ☐☐

2. During the past 24 hours, how many episodes of retching (heaving as if to vomit, but nothing comes up) have you had? ☐☐

For each symptom listed below, please mark with an X the box that best describes the worst severity of each symptom during the past 24 hours. Please be sure to answer each question.

	None	Very Mild	Mild	Moderate	Severe	Very Severe
3. Nausea (feeling sick to your stomach as if you were going to vomit or throw up)	☐	☐	☐	☐	☐	☐
4. Feeling excessively full after meals	☐	☐	☐	☐	☐	☐
5. Bloating (feeling like you need to loosen your clothes)	☐	☐	☐	☐	☐	☐
6. Not able to finish a normal-sized meal (for a healthy person)	☐	☐	☐	☐	☐	☐

Secondary Symptoms

For each symptom listed below, please mark with an X the box that best describes the worst severity of each symptom during the past 24 hours. Please be sure to answer each question.

	None	Very Mild	Mild	Moderate	Severe	Very Severe
7. Retching (heaving as if to vomit, but nothing comes up)	☐	☐	☐	☐	☐	☐
8. Vomiting	☐	☐	☐	☐	☐	☐
9. Stomach or belly visibly larger	☐	☐	☐	☐	☐	☐
10. Stomach fullness	☐	☐	☐	☐	☐	☐
11. Loss of appetite	☐	☐	☐	☐	☐	☐

US:ENG (United States/English)

GASTROPARESIS CARDINAL SYMPTOM INDEX - DAILY DIARY (GCSI-DD) (Continued)

	None	Very Mild	Mild	Moderate	Severe	Very Severe
12. Upper abdominal pain (above the navel)	☐	☐	☐	☐	☐	☐
13. Upper abdominal discomfort (above the navel)	☐	☐	☐	☐	☐	☐

	No Symptoms	Very Mild	Mild	Moderate	Severe	Very Severe
14. What was the overall severity of your gastroparesis symptoms today (during the last 24 hours)	☐	☐	☐	☐	☐	☐

15. How many times have you had a bowel movement during the past 24 hours? _____

16. Rate the overall consistency of your bowel movement over the past 24 hours from the following choices: (1) very hard, (2) hard, (3) formed, (4) loose, (5) watery? _____

US:ENG (United States/English)

Appendix C – Study of Camicinal in people with PD and delayed gastric emptying

Summary of prohibited concomitant medications

Use of medications that potentially influence upper gastrointestinal motility or appetite or medication that may influence gastric emptying rate:

- Prokinetic drugs
- Macrolide antibiotics (erythromycin, azithromycin)
- GLP-1 mimetics
- Anticholinergics known to affect gastric emptying (oxybutynin)
- Opiates

Strong inhibitors of P-glycoprotein (quinidine, cyclosporine)

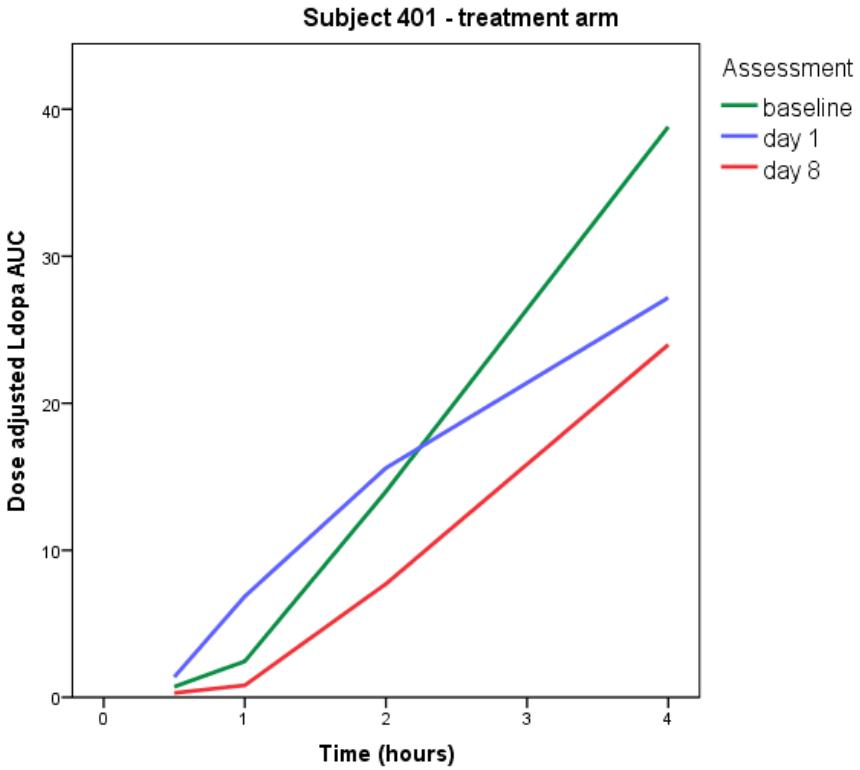
Strong CYP3A4 inducers or inhibitors are prohibited:

- Cardiac (verapamil, amiodarone)
- Antibiotics (clarithromycin, erythromycin, troleandomycin)
- Anti-fungals (oral azoles)
- Antidepressants (fluvoxamine, nefazadone)
- Antiepileptics (carbamazepine, phenytoin, phenobarbital)
- Antiretrovirals (ritonavir, delaviridine, indanavir, nelfinavir, saquinavir)

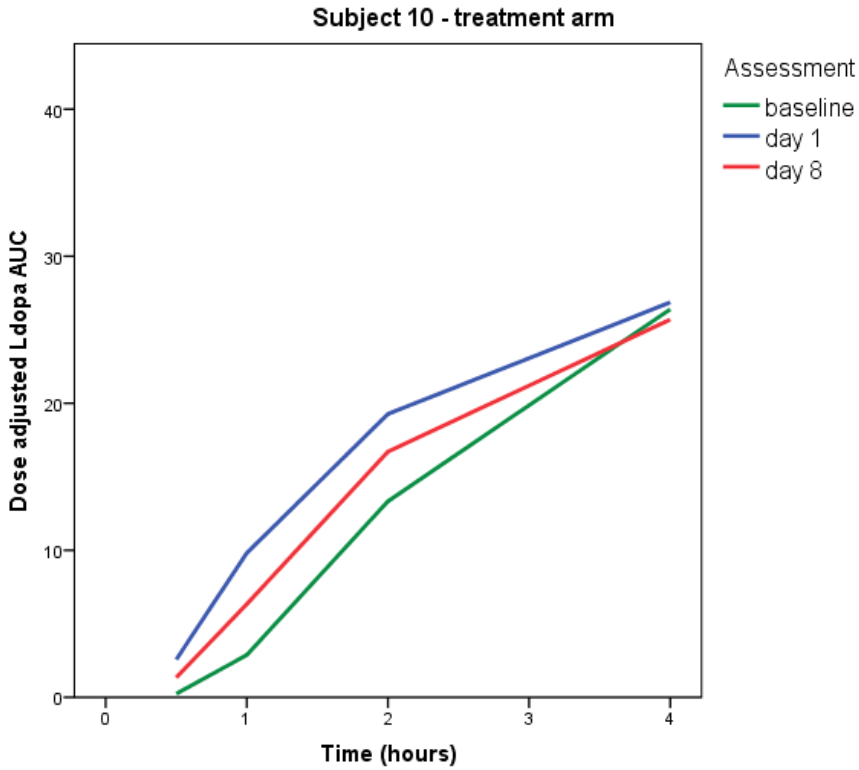
Gastric emptying rates for all screened subjects by study centre

Subject number	Study site	Screening date	$GEt_{1/2}$ (min)	Outcome
Protocol 1 (May – November 2012)				
000001	Newcastle	29 June 2012	65	Ineligible
000002	Newcastle	10 Aug 2012	**93**	**Eligible**
000003	Newcastle	13 Sept 2012	65	Ineligible
000004	Newcastle	11 Oct 2012	86	Ineligible
000005	Newcastle	12 Oct 2012	50	Ineligible
000006	Newcastle	19 Oct 2012	**103**	**Eligible**
000007	Newcastle	1 Nov 2012	85	Ineligible
000008	Newcastle	9 Nov 2012	83	Ineligible
000009	Newcastle	16 Nov 2012	incomplete	Withdrew
Protocol 2 (May – September 2013)				
000010	Newcastle	22 May 2013	**101**	**Eligible**
000011	Newcastle	31 May 2013	**72**	**Eligible**
000012	Newcastle	11 June 2013	61	Ineligible
000013	Newcastle	13 June 2013	**131**	**Eligible**
000014	Newcastle	25 June 2013	**87**	**Eligible**
000015	Newcastle	5 July 2013	**101**	**Eligible**
000016	Newcastle	9 July 2013	**110**	**Eligible**
000017	Newcastle	1 Aug 2013	53	Ineligible
000018	Newcastle	15 Aug 2013	**90**	**Eligible**
000019	Newcastle	22 Aug 2013	**87**	**Eligible**
000020	Newcastle	23 Aug 2013	**121**	**Eligible**
000021	Newcastle	10 Sept 2013	**71**	**Eligible** (Withdrew)
000022	Newcastle	17 Sept 2013	**79**	**Eligible**
000023	Newcastle	3 Oct 2013	65	Ineligible
000101	*Australia*	*14 Aug 2013*	***102***	***Eligible***
000201	*Norwich*	*16 July 2013*	***84***	***Eligible***
000202	*Norwich*	*24 Sept 2013*	***85***	***Eligible***
000400	*Sweden*	*24 Sept 2013*	*69*	*Ineligible*
000401	*Sweden*	*24 Sept 2013*	***80***	***Eligible***

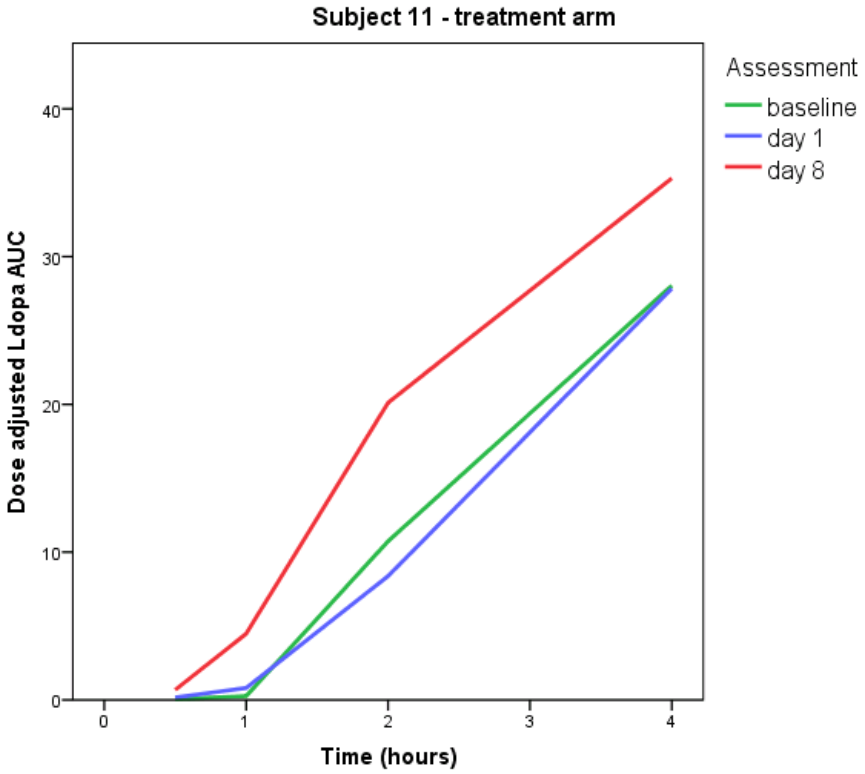
Dose adjusted Levodopa AUC profiles over time for each subject with parallel gastric emptying measures



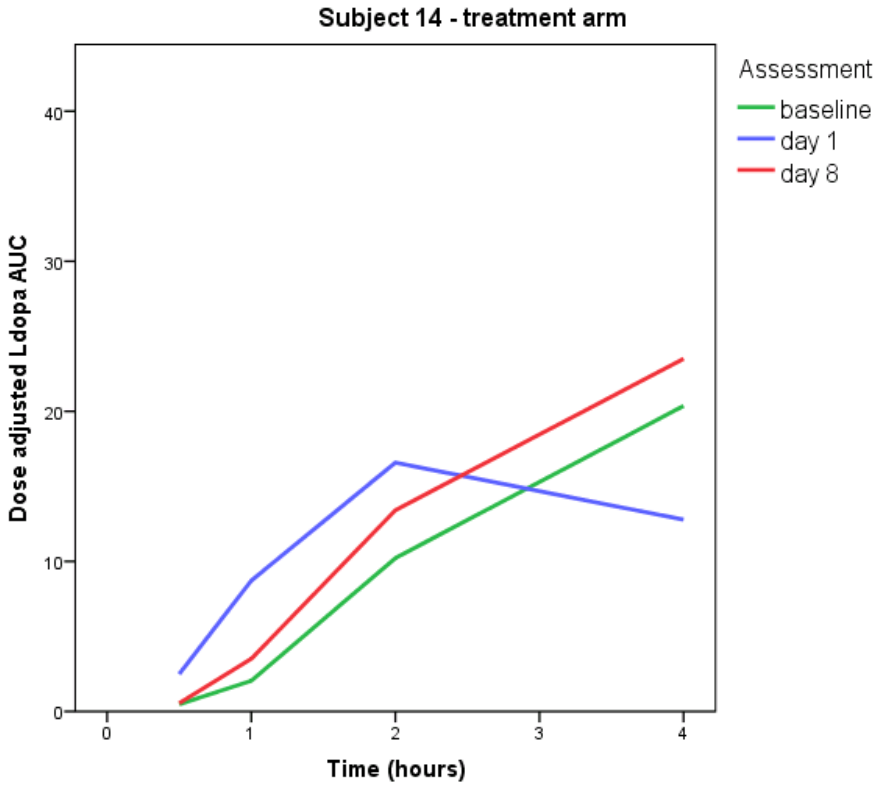
Assessment	$GEt_{1/2}$ (minutes)
Baseline	101
Day 1	77
Day 8	67



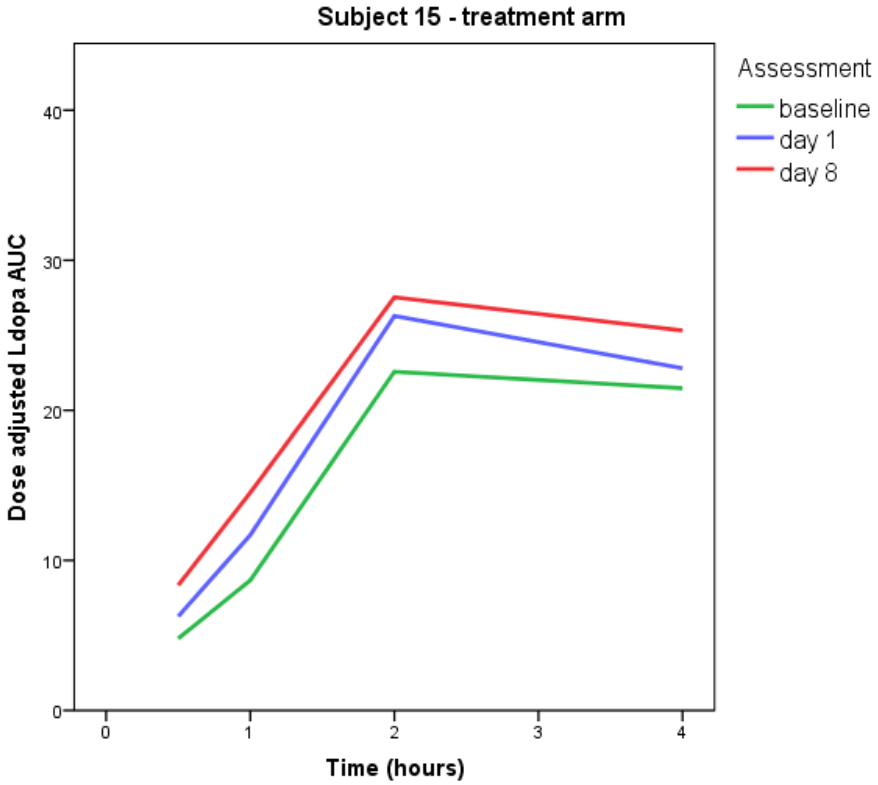
Assessment	$GEt_{1/2}$ (minutes)
Baseline	80
Day 1	82
Day 8	69



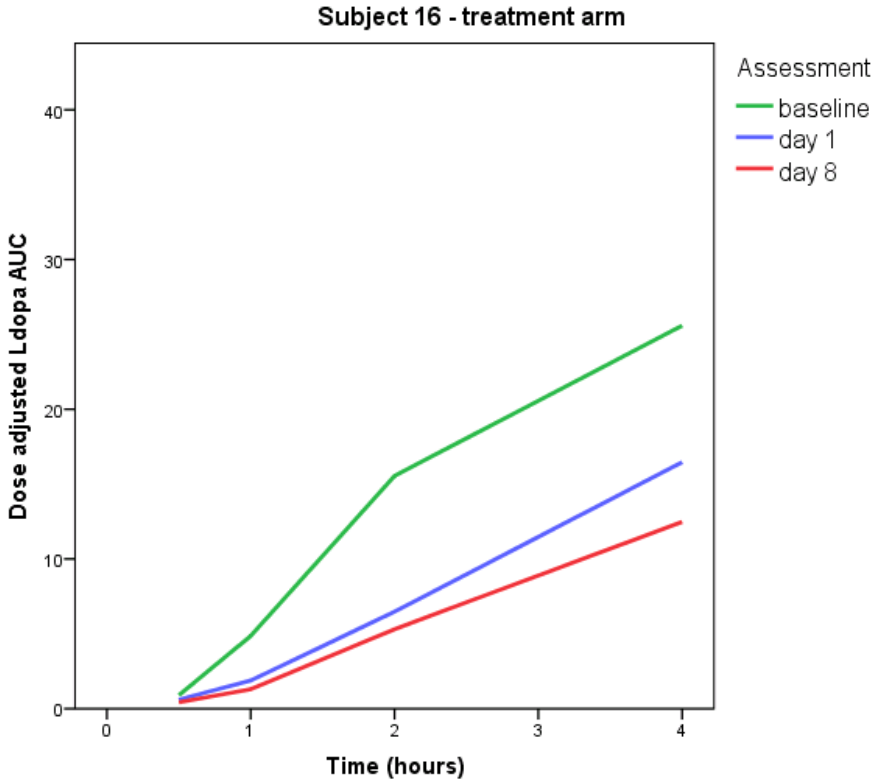
Assessment	$GEt_{1/2}$ (minutes)
Baseline	72
Day 1	82
Day 8	81



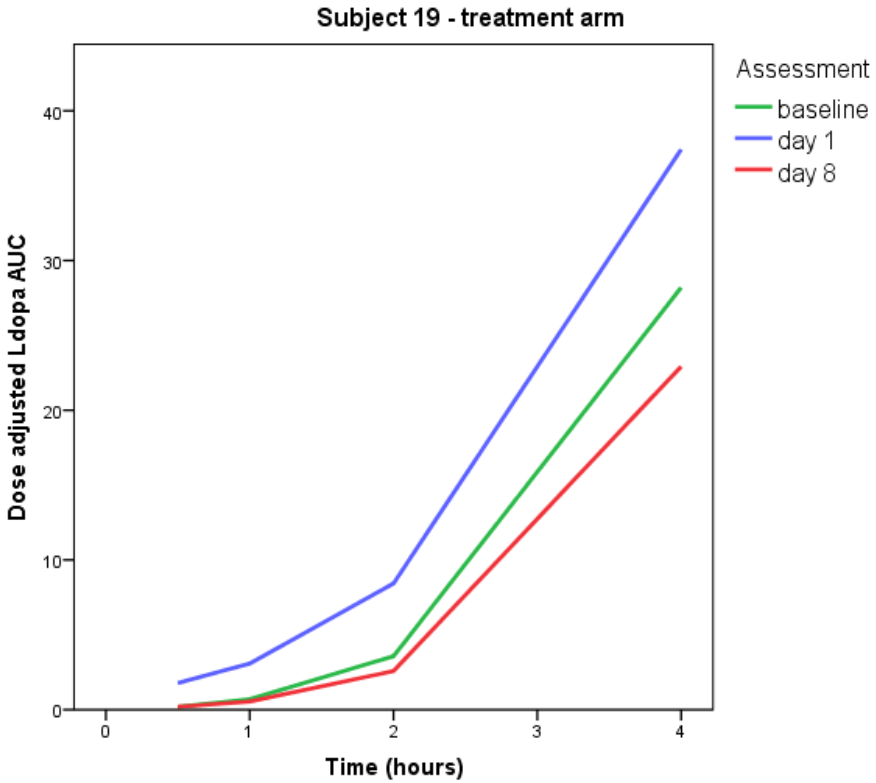
Assessment	$GEt_{1/2}$ (minutes)
Baseline	87
Day 1	93
Day 8	69



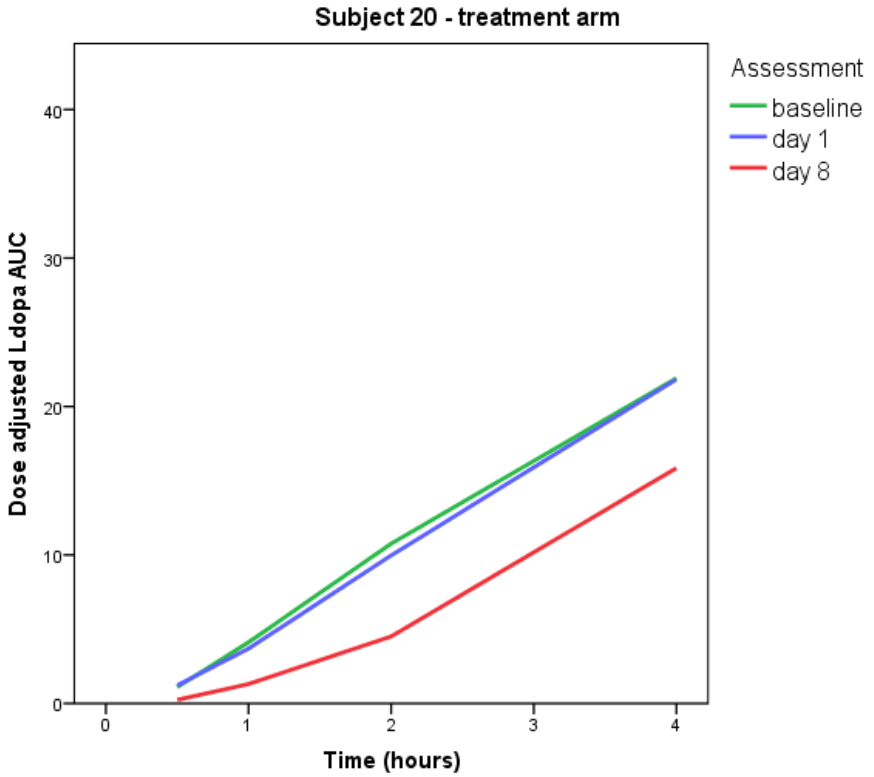
Assessment	$GEt_{1/2}$ (minutes)
Baseline	101
Day 1	85
Day 8	77



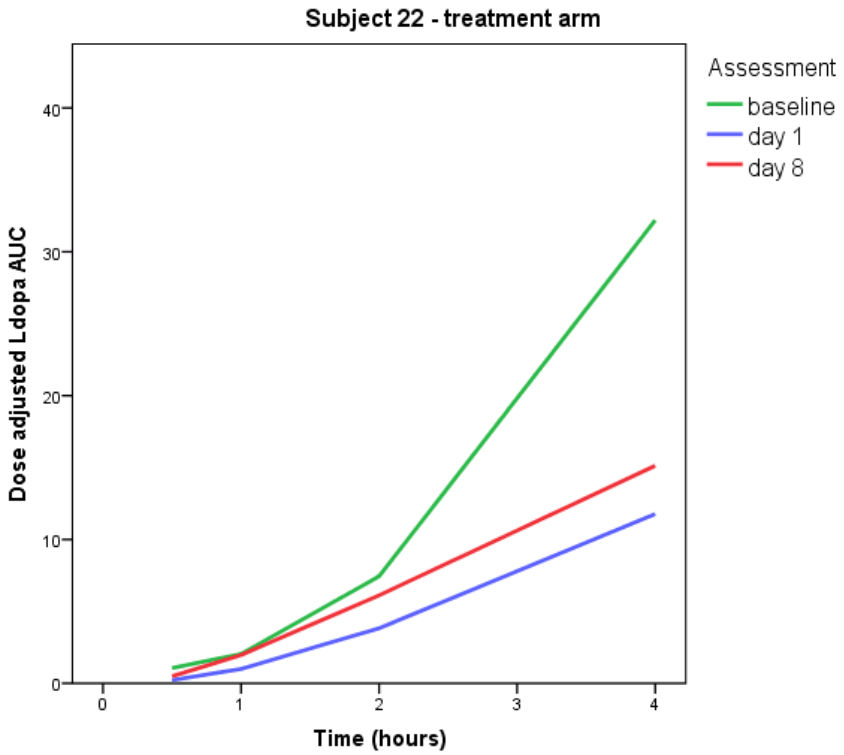
Assessment	$GEt_{1/2}$ (minutes)
Baseline	110
Day 1	143
Day 8	101



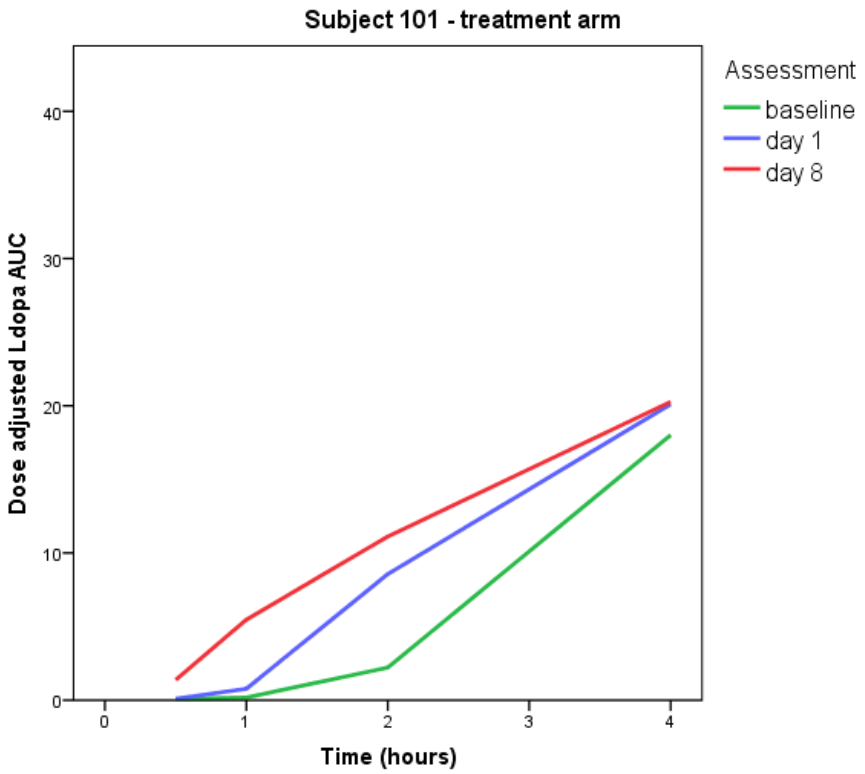
Assessment	$GEt_{1/2}$ (minutes)
Baseline	87
Day 1	94
Day 8	76



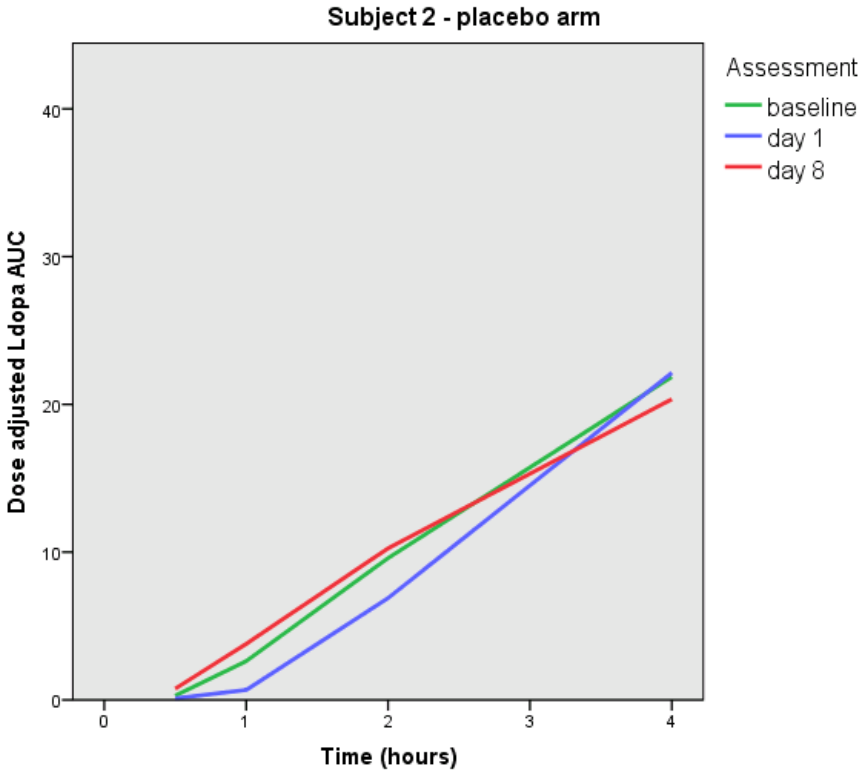
Assessment	$GEt_{1/2}$ (minutes)
Baseline	121
Day 1	98
Day 8	94



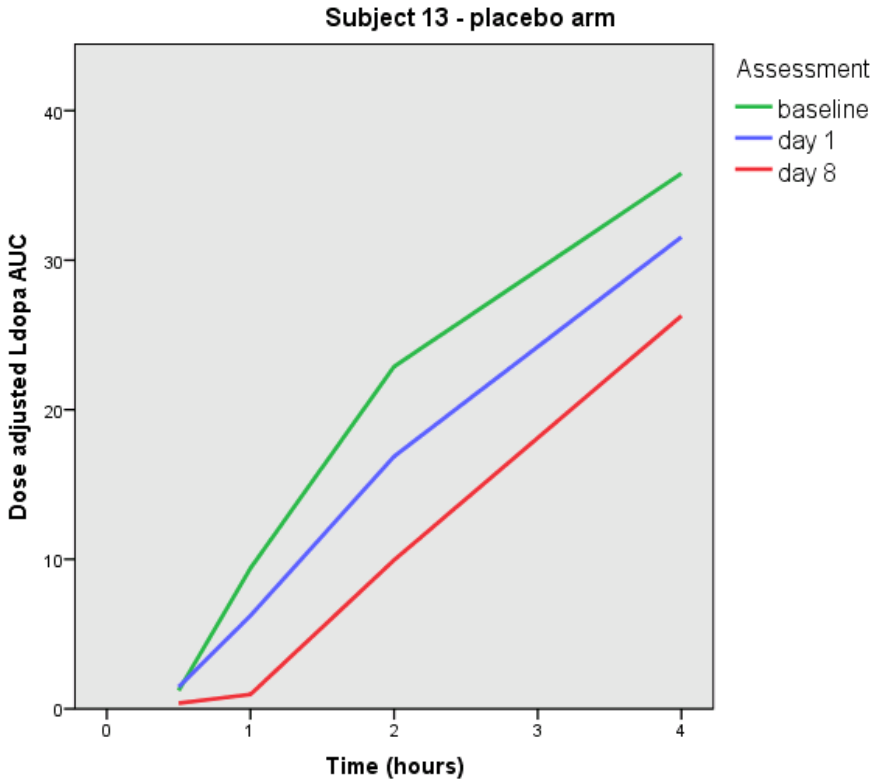
Assessment	$GEt_{1/2}$ (minutes)
Baseline	79
Day 1	82
Day 8	94



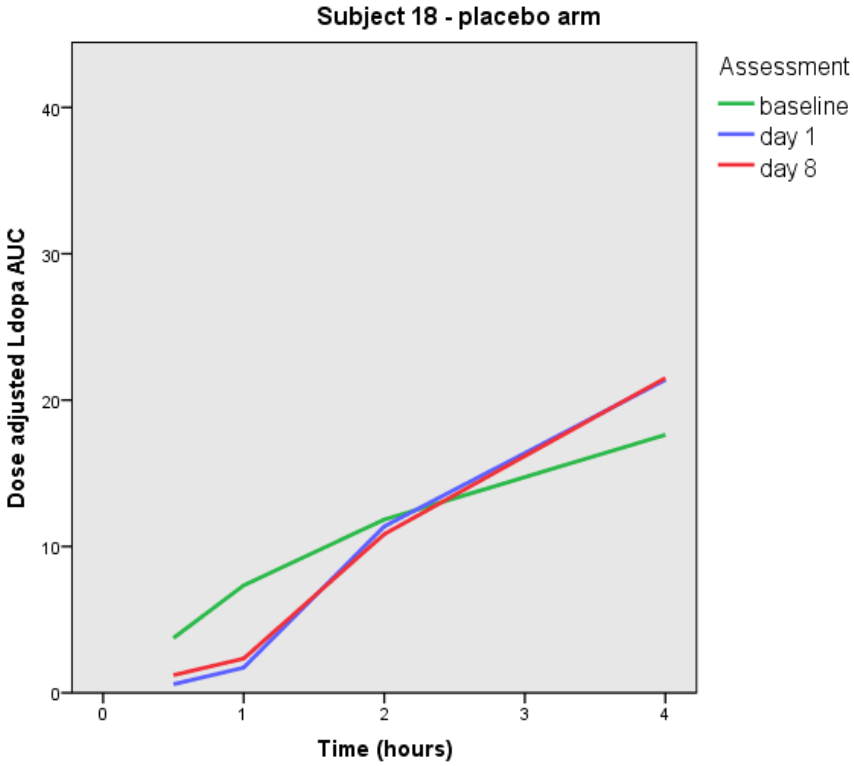
Assessment	$GEt_{1/2}$ (minutes)
Baseline	102
Day 1	90
Day 8	158



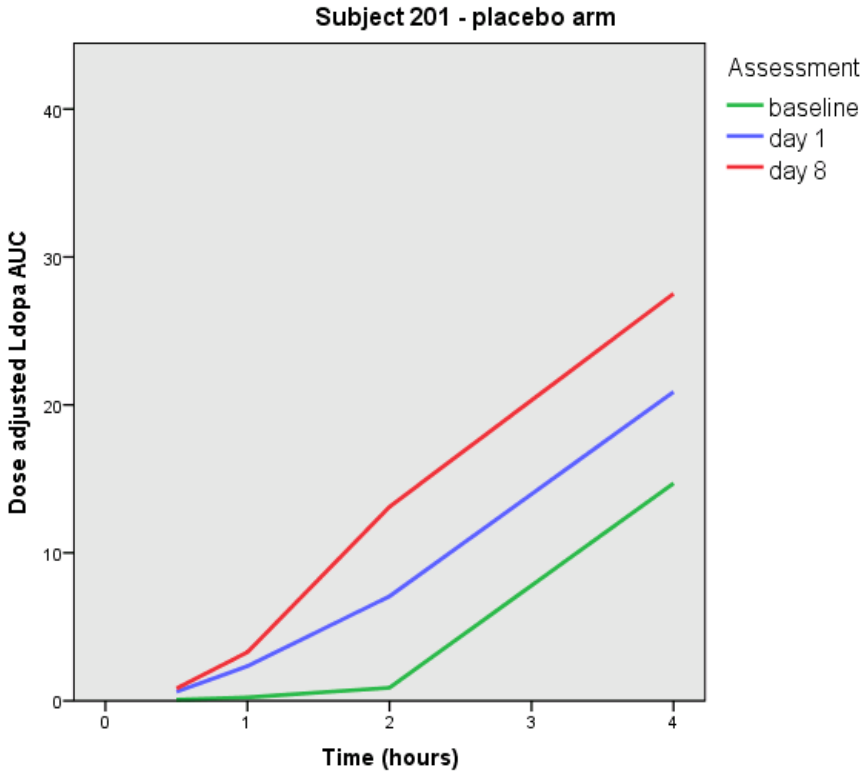
Assessment	$GEt_{1/2}$ (minutes)
Baseline	93
Day 1	81
Day 8	missing



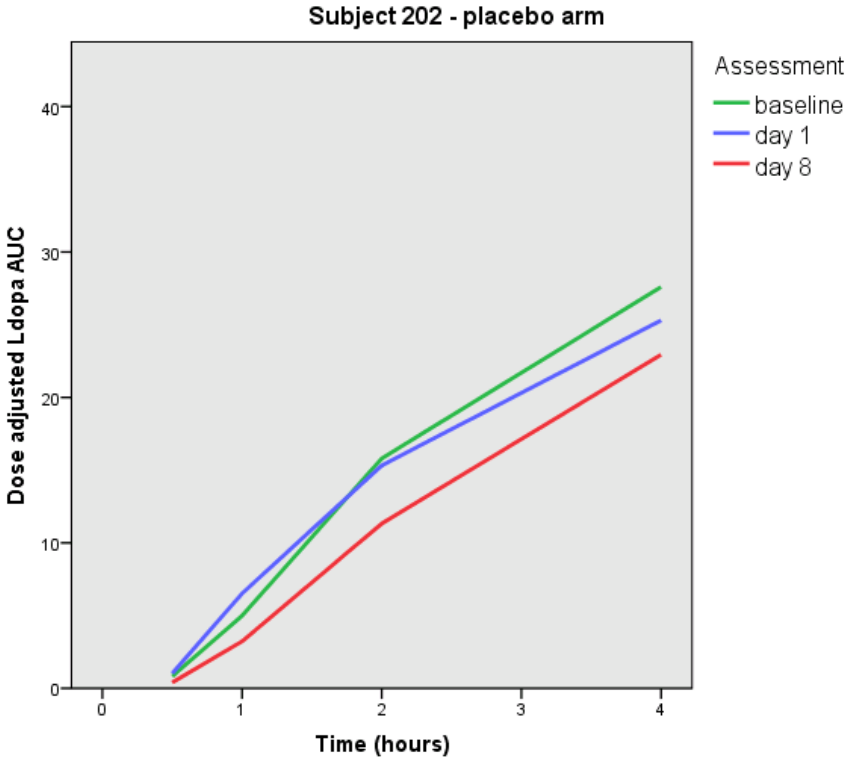
Assessment	$GEt_{1/2}$ (minutes)
Baseline	131
Day 1	122
Day 8	106



Assessment	$GEt_{1/2}$ (minutes)
Baseline	90
Day 1	85
Day 8	82



Assessment	$GEt_{1/2}$ (minutes)
Baseline	84
Day 1	112
Day 8	123



Assessment	$GEt_{1/2}$ (minutes)
Baseline	85
Day 1	88
Day 8	91

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