

NEWCASTLE UNIVERSITY

Hormones of energy homeostasis; linking weight loss and cognition in Parkinson's disease?



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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease in man and is complicated by dementia in up to 83% of people with PD. The risk of dementia is increased in people with PD who lose weight. Stable body weight relies on maintaining a balance of energy intake and energy output; a process coordinated by a number of circulating hormones of energy homeostasis. There is evidence that some of these; ghrelin, insulin and leptin may have pro-cognitive and neuroprotective effects in animal models of PD. Ghrelin secretion may be disordered in PD as levels are lower than in healthy controls. It is not known whether hormones of energy homeostasis are disrupted in people with PD and cognitive impairment (PD-CI). We conducted a cross-sectional quasi-experimental pilot study of 16 people with PD-CI, 19 people with PD and normal cognition and 20 healthy controls. Fasting and post-prandial acyl-ghrelin, total ghrelin, growth hormone, insulin-like growth factor-1, insulin and leptin levels were measured. Fasting values and area under the curve for each value was analysed using ANOVA and post-hoc testing using the least-significant difference test. Post-hoc testing demonstrated lower acyl-ghrelin in the PD-CI group ($p=0.02$). Moreover, there was a correlation between acyl-ghrelin and cognition across the cohort ($p=0.01$) and acyl ghrelin significantly predicted cognition in multiple linear regression, accounting for 15% of the variance. Insulin and leptin were not different between groups and did not predict cognition. Hunger and fullness were measured using visual analogue scales. Energy intake at lunch was also recorded. Groups were compared using ANOVA. There were no significant differences in hunger, fullness or energy intake between groups. The data show reduced acyl-ghrelin in PD-CI. Acyl-ghrelin is a potential biomarker for cognitive decline in PD and further longitudinal studies should be carried out to further investigate this.

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Dedication

For my husband Simon who has been so good to me.

Statement of work undertaken

This study was designed by my supervisors Prof. David Burn and Dr. Mario Siervo, our collaborator Prof. Jeff Davies and myself. Target sample size was calculated by Dr Shirley Coleman. Subsequent statistical analysis was carried out by myself and Dr Mario Siervo. The day to day running of the study was conducted by me with the support of my supervisors and the research nurses at the Clinical Ageing Research Unit (CARU), especially Helen Pilkington. I was responsible for obtaining and complying with ethical approval, recruitment (including capacity assessment and discussion with consultees), clinical assessments (including administering questionnaires) at screening and test visits, processing samples on site and data entry. The research nurses at CARU measured height, weight, fat mass and blood pressure at the screening visit. During the test visit they performed blood draws and visual analogue scales, provided water and meals according to the protocol, set up the bite counter and counted bites manually during meals. They also weighed food before and after the *ad libitum* meal. Helen Pilkington carried out data cleaning for the study. Biochemical analysis was carried out by Dr Jeff Davies at Swansea University.

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List of abbreviations

Abbreviation	Meaning
ABO	Beta amyloid oligomers
Ach	Acetylcholine
AchE	Acetylcholinesterase
ACTH	Adrenocorticotrophic hormone
AD	Alzheimer's disease
ADLs	Activities of daily living
AG	Acyl-ghrelin
AgRP	Agouti related peptide
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
AMPK	Adenosine 5' monophosphate-activated protein kinase
APP	Amyloid precursor protein
ARC	Arcuate nucleus of the hypothalamus
AUC	Area under the curve
Aβ	Beta-amyloid
αMSH	A-melanocortin stimulating hormone
BBB	Blood brain barrier
BMI	Body mass index
BrdU	bromodeoxyuridine
CAMCOG	Cambridge cognitive examination
CARU	Clinical Ageing Research Unit
CCK	Cholecystokinin
CI	Confidence interval
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
DaT	Dopamine transporter
DBS	Deep brain stimulation
DLB	Dementia with Lewy bodies
DM	Diabetes mellitus
DMNV	Dorsal motor nucleus of the vagus
DPP4i	Dipeptidyl peptidase 4 inhibitor
fMRI	Functional magnetic resonance imaging
GDS	Geriatric depression scale
GH	Growth hormone
GHRH	Growth hormone stimulating hormone
GHSR1a	Growth hormone secretagogue receptor 1a (Ghrelin receptor)
GOAT	Ghrelin O acyltransferase
GSK3	Glycogen synthase 3
HOMA-IR	Homeostasis assessment method of measuring insulin resistance
HVLT	Hopkins verbal learning test
ICV	Intra-cerebroventricular
IDE	Insulin degrading enzyme
IGFBP	IGF binding protein
IGF-1	Insulin-like growth factor 1
i.p.	intraperitoneal
IR	Insulin receptor
iRBD	Idiopathic REM-sleep behaviour disorder
IV	intravenous
LED	Levodopa equivalent dose
LEPR	Leptin receptor (synonym of OB-R)
LHA	Lateral hypothalamic area
LnAG	Log transformed acyl-ghrelin
LnTG	Log transformed total ghrelin
LPS	Lipopolysaccharide
LSD	Least significant difference test
LTP	Long term potentiation
MAOIB	Monoamine oxidase inhibitor
MCI	Mild cognitive impairment
MC3R	Melanocortin 3 receptor
MC4R	Melanocortin 4 receptor
MDS	Movement disorder society
MDS UPDRS	Movement disorder society unified Parkinson's disease rating scale
MMSE	Mini-mental state examination
MoCA	Montreal Cognitive Assessment
MPTP	1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine
MRI	Magnetic resonance imaging

MSG	Monosodium glutamate
mTOR	Mammalian target of rapamycin
NAc	Nucleus accumbens
NO	Nitric oxide
NOS	Nitric oxide synthase
NMS	Non-motor symptoms of Parkinson's disease
NPY	Neuropeptide Y
NTS	Nucleus tractus solitarius
OB-R	Leptin receptor (synonym of LEPR)
PA	Physical activity energy expenditure
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PD-CI	Parkinson's disease cognitive impairment
PD-MCI	Parkinson's disease mild cognitive impairment
PI3K	phosphatidylinositol 3 kinase
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus
PYY	Peptide YY
REE	Resting energy expenditure
ROS	Reactive oxygen species
s.c.	subcutaneous
SD	Standard deviation
SIBO	Small intestinal bacterial overgrowth
SLR	Spontaneous location recognition test
SNP	Single nucleotide polymorphism
SNpc	Substantia nigra pars compacta
TEE	Total energy expenditure
TH	Tyrosine hydroxylase
TMTA	Trail making test A
TMTB	Trail making test B
UAG	Unacylated ghrelin
UCP	Uncoupling proteins
UPDRS	United Parkinson's Disease Rating Scale
VTA	Ventral tegmental area
6-ODHA	6- hydroxydopamine

Chapter 1. Brief background, aims and outline

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (Hirsch and Hunot, 2009). PD is associated with a nearly 6 fold increased risk of dementia compared with healthy ageing (Aarsland et al., 2001). Identifying people with PD who are at greater risk of developing dementia may enable treatment in the pre-clinical phase when disease modifying medications become available. Weight loss is common in PD due to altered energy balance and has been linked with increased risk of dementia. Weight loss and dementia in PD may be linked by dysregulation of pleiotropic hormones of energy homeostasis such as ghrelin, insulin-like growth factor (IGF-1), insulin and leptin which have also been implicated in learning and memory and may be neuroprotective. This thesis aims to explore the relationship between appetite, dysregulation of hormones involved in body weight regulation and cognitive impairment in PD and to determine if hormones of energy homeostasis merit further investigation as potential biomarkers of cognitive decline in PD. This will be achieved through the following objectives;

To explore whether perceived and objective measures of appetite and food intake differ between patients with PD, PD-CI and controls.

To explore whether fasting and post-prandial changes in ghrelin, insulin-like growth factor-1 (IGF-1), insulin, glucagon-like peptide 1 (GLP-1) and leptin concentrations differ between patients with PD, PD-CI and controls.

Chapter 2. Introduction and Literature Review

2.1 Parkinson's disease

2.1.1 *Clinical features of Parkinson's disease*

Parkinson's disease (PD) is a systemic neurodegenerative disorder characterised by bradykinesia, tremor, rigidity and postural instability (Hughes et al., 1992). It was first described by James Parkinson in 1817 in his "Essay on the Shaking Palsy" (Parkinson, 1817) and 200 years later, a cure remains elusive. PD is the second most common neurodegenerative disorder in the UK after Alzheimer's disease (AD) (Ben-Shlomo, 1997) and has an age-adjusted prevalence of 83.9-202.7/100,000 in the UK (Newman et al., 2009, Walker et al., 2010). PD is estimated to cost the UK economy £449 million to £3.3 billion each year (Findley, 2007). Although many treatments provide symptomatic relief, no disease modifying medications are available and the progression of PD is unrelenting (Evans et al., 2011, Hely et al., 2008). Dopamine replacement and augmentation are the mainstay of treatment for the motor symptoms of PD (National Institute for Health and Clinical Excellence, 2006).

PD does not solely affect motor function but rather causes a constellation of other symptoms over a wide range of systems. These are termed non-motor symptoms (NMS) and include constipation, incontinence, orthostatic hypotension, cognitive impairment, apathy, hallucinations and frank dementia amongst others (Barone et al., 2009). Figure 1 outlines common NMS in PD. The PRIAMO study found that 98.6% of patients with Parkinson's disease had NMS (Barone et al., 2009). Moreover, they can be as devastating as they are diverse, causing significant impact on patients' and carers' quality of life (Aarsland et al., 2009b, Martinez-Martin, 2011). Neuropsychiatric NMS such as dementia, apathy, depression and psychosis are especially burdensome, increasing caregiver stress, negatively impacting quality of life and increasing the likelihood of transfer to residential or nursing care (Aarsland et al., 2009b, Barone et al., 2009, Leroi et al., 2012, Schrag et al., 2000, Aarsland et al., 2007). As with motor symptoms there are no curative treatments and therapy is therefore symptomatic.

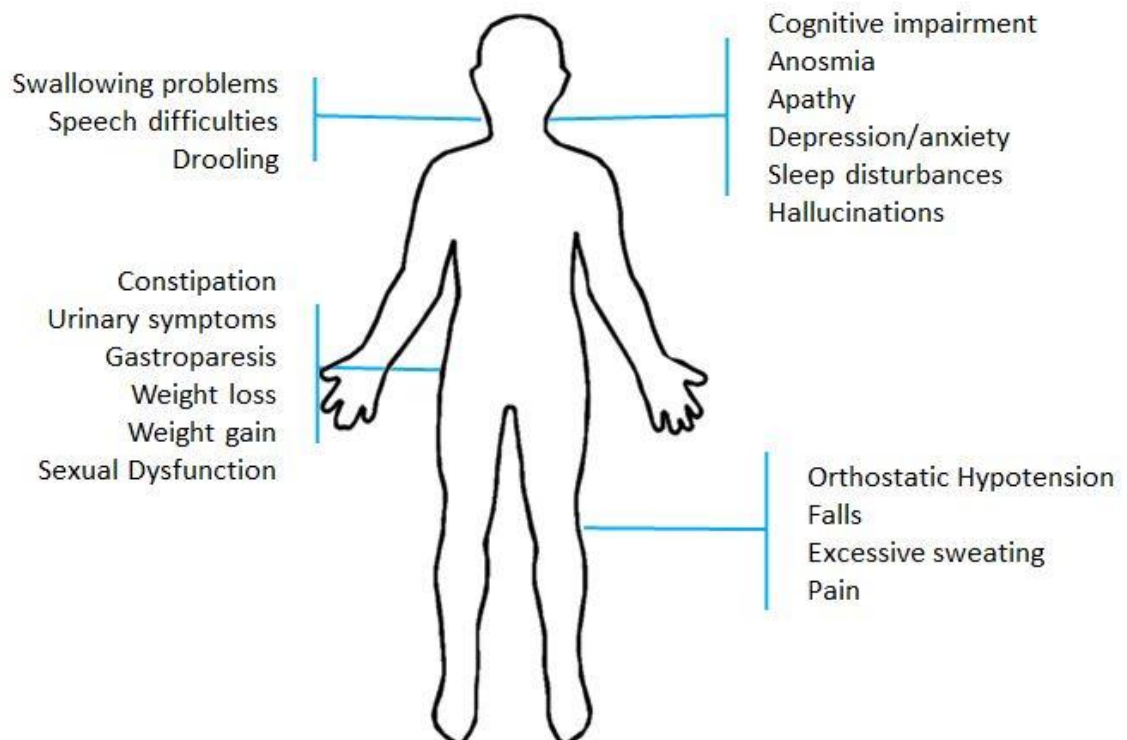


Figure 1. Non-motor features of Parkinson's disease (Todorova et al., 2014, Martinez-Martin, 2011, Chaudhuri et al., 2006, Chaudhuri and Schapira, 2009)

2.1.2 Pathophysiology of Parkinson's disease

The biological basis of PD is a progressive loss of neurones throughout the brain, but particularly dopaminergic cells in the substantia nigra pars compacta (SNpc) of the midbrain. This cell death is associated with the presence of Lewy bodies; these are pathological inclusions in the neuronal cytoplasm made up of misfolded α -synuclein, ubiquitin and neurofilaments (Spillantini et al., 1997). Alpha-synuclein is usually soluble in cytosol but becomes insoluble when misfolded (Goedert, 2001). It then precipitates out in the cell causing Lewy body formation and, ultimately, cell death (Moore et al., 2005). The underlying cause of synuclein misfolding is unknown and the cause of cell death is poorly understood. Mitochondrial dysfunction, leading to increased reactive oxygen species and apoptosis (programmed cell death) has been implicated (Figure 2). The cause of mitochondrial dysfunction is unclear and may be the cause rather than the effect of α -synuclein aggregation (Moore et al., 2005). Cell death may also result from a failure of usual cell maintenance by the ubiquitin proteasome system and autophagy lysozyme pathway. These processes prevent the accumulation of toxic proteins in order to preserve cell function (Pan et al.,

2008). Finally, neuroinflammation has been implicated in the pathogenesis of PD, though again it is unclear whether this is a reaction to the degenerative process or the cause (Hirsch and Hunot, 2009). Recent research has demonstrated that higher pro-inflammatory markers are associated with disease progression and cognitive impairment in PD (Williams-Gray et al., 2016)

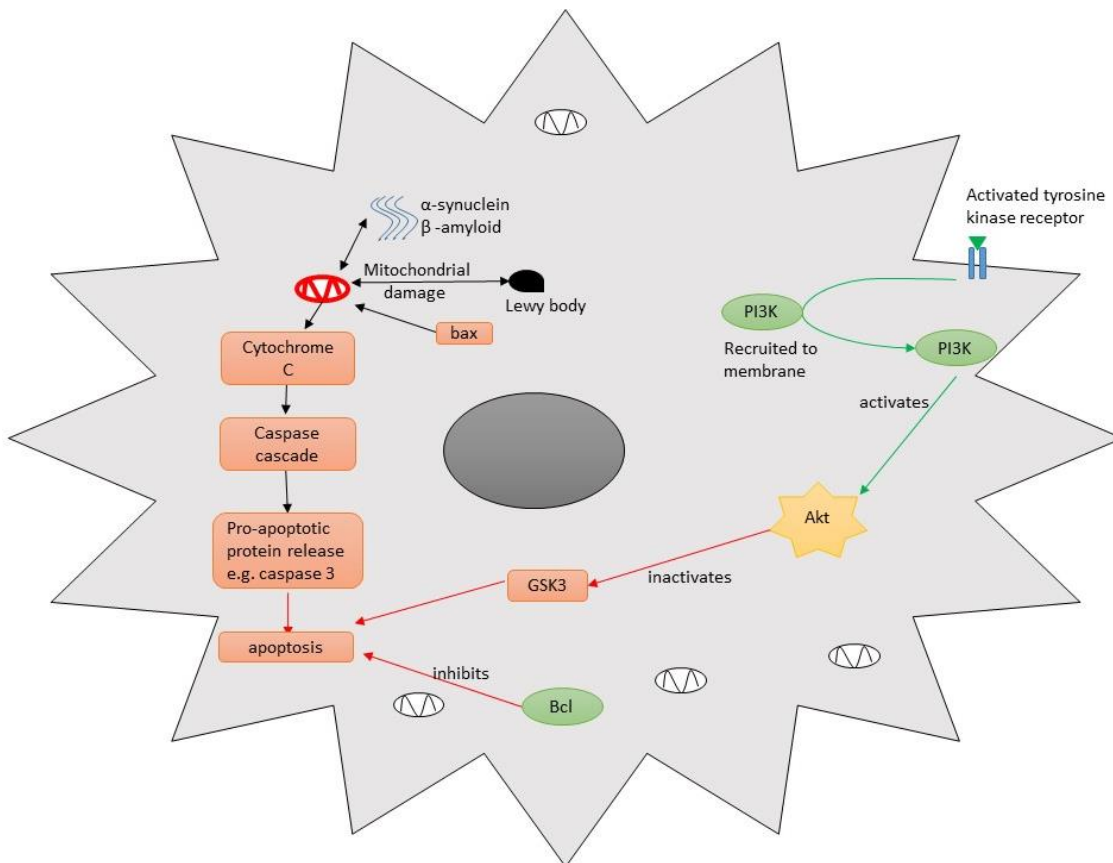


Figure 2. Simplified pathway for neuronal apoptosis. Damaged mitochondria release cytochrome C and initiate apoptotic cascade. Other pro-apoptotic proteins include bax protein. B-cell lymphoma protein 2 (Bcl-2) prevent this cascade and is therefore anti-apoptotic. A cell may be pushed towards apoptosis or survival by the ratio of pro- and anti-apoptotic factors within it (Underwood and Cross, 2009, Brunet et al., 2001).

If the mechanism by which cells are damaged is unclear, so is the underlying trigger for the disease. Some theories implicate toxin exposure, for example with pesticides, other unknown environmental agents (Hubble et al., 1993, Koller et al., 1990), or prion-like disease process with so-called “permissive templating” (Olanow and Prusiner, 2009, de Lau and Breteler, 2006). Braak and colleagues postulate that an unknown environmental agent enters the body through the gut or respiratory tract and spreads to the brain via the enteric nervous system or olfactory bulb in a retrograde fashion. This is based on their

observation that Lewy bodies form sequentially in different areas of the brain. They describe Lewy bodies appearing first in the dorsal motor nucleus of the vagus nerve (DMNV) and olfactory bulb then the brainstem and midbrain (including the SNpc) and eventually the cortex (Braak et al., 2003a, Braak et al., 2003b). This cannot explain the pathological process in patients with the early widespread cortical Lewy bodies seen in patients with early cognitive impairment. Furthermore, pathological stage may not correlate with clinical features and progression from one stage to another is not inevitable (Burke et al., 2008). Despite this the Braak hypothesis does appear to apply to a subgroup of young PD patients (Halliday et al., 2008). This is important as it suggests that neuronal loss precedes the onset of motor features in at least some patients. It has been proposed that there are three stages of PD; pre-clinical, pre-motor and motor (Todorova et al., 2014). Preclinical and premotor disease could represent opportunities for future disease modifying therapies to prevent PD. Prevention will only be possible if patients at risk can be identified, for example, using clinical or biochemical biomarkers.

The pathophysiology of NMS is less well understood but may be due to dopaminergic and non-dopaminergic neuronal loss throughout the brain and body (Todorova et al., 2014). NMS may precede motor symptoms by up to 10 years (Chaudhuri et al., 2006, Braak et al., 2003a, Braak et al., 2003b) and are very common. Their treatment can be difficult and evidence for efficacy of some treatments is lacking (Seppi et al., 2011). Dopaminergic medications used to treat motor symptoms may be ineffective for NMS and can actually exacerbate symptoms such as dementia, psychosis and orthostatic hypotension (Chaudhuri and Schapira, 2009, Chaudhuri et al., 2006). There is a great deal of ongoing research into effective treatments for NMS in PD, with some promising new medicines and new indications for old medicines on the horizon. Despite this, treatment remains symptomatic (Schrag et al., 2015).

2.1.3 Cognitive impairment in Parkinson's disease

Up to 83% of people with PD who survive more than 20 years from diagnosis will develop Parkinson's disease dementia (PDD) (Hely et al., 2008). Advanced age, mild cognitive impairment (MCI), non-tremor dominant phenotype, greater disease severity and weight loss all predispose to PDD (Emre, 2003, Aarsland

and Kurz, 2010, Kim et al., 2012, Burn et al., 2006, Selikhova et al., 2009, Williams-Gray et al., 2007). Clinically, PDD manifests as cognitive impairment within several domains; attention, executive function, visuo-constructive ability and memory (Emre et al., 2007, Docherty and Burn, 2010, Emre, 2003, Aarsland and Kurz, 2010). The Movement Disorder Society (MDS) Task Force diagnostic criteria state that PDD may be diagnosed in patients with a greater than 12 month history of parkinsonism if there is evidence of impairment in more than one of the above cognitive domains. The deficit must be severe enough to impact on activities of daily living and there should be no depression, delirium or other confounding factors (Dubois et al., 2007) (Table 1). PDD has been shown to adversely impact patients' and carers' quality of life (Aarsland et al., 2009b, Barone et al., 2009, Leroi et al., 2012, Schrag et al., 2000, Aarsland et al., 2007) and is associated with increased mortality and dependency (Hely et al., 2008, Hely et al., 2005). As with PD there are no disease modifying therapies for PDD. Treatment, for example with cholinesterase inhibitors, is therefore symptomatic (van Laar et al., 2011, Emre, 2004).

Table 1. Movement disorder society task force level 1 criteria for the diagnosis of Parkinson's disease dementia (Dubois et al., 2007)

MDS task force criteria for the diagnosis of PDD (Level 1)
1. A diagnosis of PD based on Queen's Square (UK) Brain Bank criteria for PD
2. PD developed prior to the onset of dementia
3. Mini mental state examination (MMSE) below 26/30
4. Cognitive deficits severe enough to impact daily living
5. Impairment in at least two of; <ol style="list-style-type: none"> a. Months reversed or seven backwards b. Lexical fluency or clock drawing c. MMSE pentagons d. 3 word recall

PDD is biologically characterised by widespread Lewy body pathology and neuronal loss throughout the brain (Aarsland et al., 2005). It is felt to be a pathological continuum with dementia with Lewy bodies (DLB) but differs clinically from DLB in that the onset of cognitive impairment occurs more than one year after the motor deficit develops (McKeith et al., 1996). The pattern of neuronal loss in PDD varies according to the patient's symptoms. Hippocampal atrophy is seen in people with PDD who have hallucinations and difficulties with verbal learning, for example, whereas those with impaired visual memory have more orbitofrontal neuronal loss (Kehagia et al., 2010). Degeneration of dopaminergic (ventral tegmental area), cholinergic (nucleus basalis of Meynert),

noradrenergic (locus coeruleus) and serotonergic (dorsal raphe nucleus) pathways all occur to varying degrees and, again, may relate to phenotype (Kalaitzakis and Pearce, 2009). Alpha-synuclein is not the only important pathological protein in PDD. Beta-amyloid (A β) plaques in cortical and limbic areas have been shown to correlate with the presence of Lewy body formation. Beta-amyloid plaques are usually associated with AD (Lashley et al., 2008). This observation could be explained by increased fibrillisation of α -synuclein in the presence of A β (Masliah et al., 2001). In other words, A β plaque formation may accelerate neurodegeneration associated with Lewy body formation in PDD. This hypothesis is supported by the Sydney Multicentre Study finding that PDD patients had more Lewy bodies and amyloid plaques in the neocortex than non-demented PD patients with the same disease duration (Halliday et al., 2008). Furthermore, studies by Compta *et al.* and Irwin *et al.* have both demonstrated that a greater burden of A β at reduces the time from the development of motor symptoms to the emergence of dementia in PD (Compta et al., 2011, Irwin et al., 2017).

Almost all of the available research regarding A β deposition has been carried out in humans with AD and in animal models of AD. The burden of A β in AD depends on the rates of A β synthesis and clearance from the brain parenchyma. Amyloid precursor protein (APP) is produced by all cells in the body and is cleaved by γ - and β - secretase to form A β species, some of which (such as A β ₁₋₄₂) are thought to be more toxic than others (A β ₁₋₄₀) (Hardy and Selkoe, 2002). Beta-amyloid clearance from the brain occurs in four main ways; through binding of A β to carrier proteins such as albumin and transthyretin, through degradation by proteases such as insulin degrading enzyme (IDE), endocytosis of A β resulting in breakdown by lysosomes (possibly involving APOE-4), and increased transport across the blood brain barrier (Adamis et al., 2009, Bates et al., 2009, Bu, 2009, Mawuenyega et al., 2010) (Figure 3). The finer details of the molecular processes underlying A β clearance are still not fully understood.

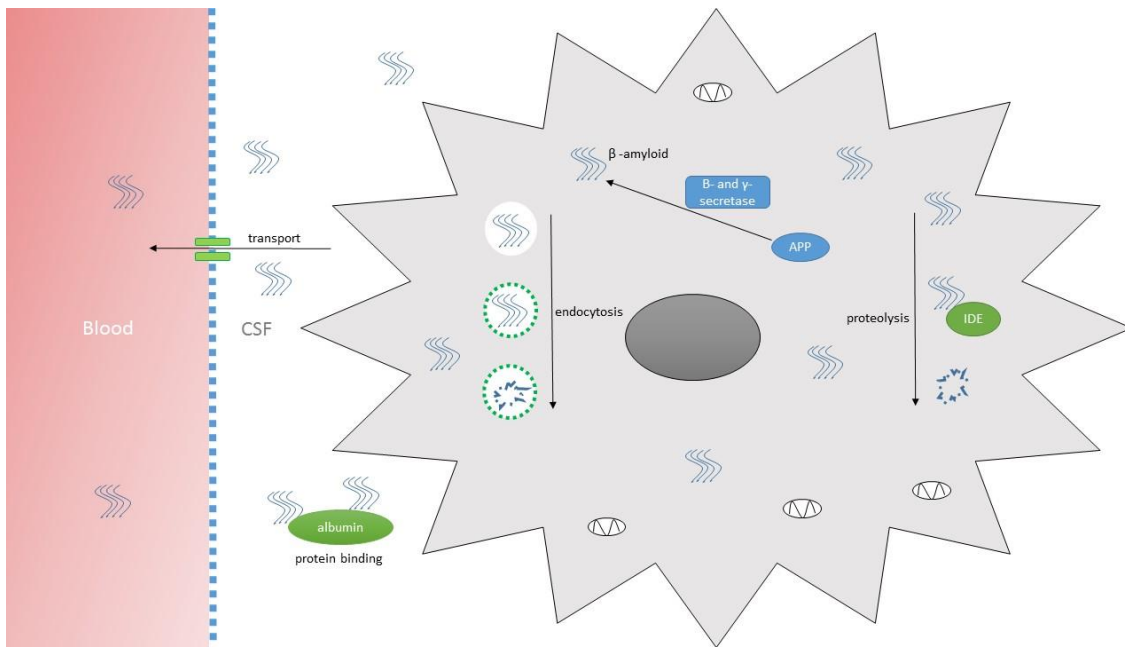


Figure 3. Simplified model of Aβ production and clearance

Mild cognitive impairment (MCI) has been proposed as a pre-dementia state in Parkinson’s disease (PD-MCI) (Litvan et al., 2012). This is defined as “cognitive decline that is not normal for age but with essentially normal functional activities”. PD-MCI comprises a diverse range of cognitive deficits. Diagnosis is made using the MDS task force criteria based on testing of five cognitive domains; attention and working memory, executive function, language, memory and visuospatial function (Litvan et al., 2011) (Figure 3). PD-MCI is even more common than PDD, with studies reporting frequencies of up to 42.5% of PD patients at presentation (Yarnall et al., 2014). Although PD-MCI does not, by definition, impact on activities of daily living it has been shown to negatively impact quality of life for patients and their carers (Leroi et al., 2012). There are no current treatments available for PD-MCI.

Table 2. Movement disorder society task force criteria for the diagnosis of mild cognitive impairment in Parkinson's disease (Litvan et al., 2012)

MDS task force criteria for the diagnosis of PD-MCI (Level 1)

Inclusion Criteria

- Diagnosis of PD as based on UK PD brain Bank Criteria
- Gradual decline, in the context of established PD, in cognitive ability reported by either the patient or informant, or observed by the clinician
- Cognitive deficits on either formal neuropsychological testing or a scale of global cognition
- Cognitive deficits are not sufficient to interfere significantly with functional independence, although subtle difficulties on complex functional tasks may be present

Exclusion criteria

- Diagnosis of PDD based on MDS task force criteria
- Other primary explanations for cognitive impairment (e.g. delirium, stroke, major depression, metabolic abnormalities, adverse effects of medication, or head trauma)
- Other PD-associated comorbid conditions (e.g. motor impairment or severe anxiety, depression, excessive daytime sleepiness, or psychosis) that, in the opinion of the clinician, significantly influence cognitive testing

Specific guidelines for PD-MCI level I categories

- Impairment on a scale of global cognitive abilities validated for use in PD or
 - Impairment on at least 2 tests, when a limited battery of neuropsychological tests is performed (i.e. the battery includes less than 2 tests within each of the 5 cognitive domains assessed)
-

The pathophysiology of PD-MCI has not yet been fully elucidated. PD-MCI is presumed to occur due to the same pathological processes responsible for PDD (Aarsland et al., 2011). Post-mortem examination of the brains of eight people with PD-MCI found a heterogeneous pattern of neurodegeneration associated with both α -synuclein and A β (Adler et al., 2010). As with PDD a number of brain regions have been implicated. Two recent studies have used magnetic resonance imaging (MRI) to assess the brains of patients with PD and normal cognition, PD-MCI and healthy controls. Both studies found cortical thinning in PD-MCI patients compared with PD and controls (Pereira et al., 2014, Danti et al., 2015). The areas involved appeared to be related to the observed deficit but with considerable overlap. Danti *et al.* found reduced temporal thickness associated with reduced verbal fluency (Danti et al., 2015). Pereira *et al.* demonstrated parietal and temporal thinning associated with visuospatial deficits, executive function and attentional deficits associated with cortical thinning in temporal, parietal and frontal cortex and amnesic deficits associated with temporal thinning (Pereira et al., 2014).

Parkinson's disease MCI is associated with an increased risk of PDD but progression from PD-MCI to PDD is not inevitable (Aarsland et al., 2009a, Janvin et al., 2006, Pedersen et al., 2013). Identifying those most at risk of progression will be necessary when disease modifying agents for PDD become available. Early clinical and biochemical predictors of PDD will be needed for

this. Identifying biomarkers may also give clues to pathogenesis thereby paving the way for novel therapeutic agents. The pathological overlap between PDD and AD increases the available pool of scientific research to enable us to achieve this.

2.1.4 Weight loss and cognitive decline in Parkinson's disease

Unintended weight-loss has been proposed as a potential biomarker for cognitive decline in PD. It is linked with poorer cognitive function (Kim et al., 2012, Lorefalt et al., 2004, Uc et al., 2006) and may predict PDD (Kim et al., 2012). To date, no studies have investigated the aetiology of weight loss in PDD though body weight does fluctuate as Parkinson's disease progresses (Bachmann and Trenkwalder, 2006, Beyer et al., 1995, Sharma and Turton, 2012). Weight loss in PD is common. Despite this, weight loss has been shown to increase the risk of cognitive impairment and accelerate the rate of cognitive decline in PD without dementia at baseline (Kim et al., 2012). Moreover, cognitive impairment and neuropsychiatric complications such as hallucinations are risk factors for weight loss in PD (Lorefalt et al., 2004, Uc et al., 2006). Weight loss has also been shown to be important in other dementias, and has been reported to precede cognitive impairment in AD patients (Stewart et al., 2005, Ogunniyi et al., 2011, Johnson et al., 2006). Weight loss in AD continues throughout the disease course and is associated with increased mortality (White et al., 1998). Furthermore, a large multicentre study carried out in eight countries showed that the severity of all cause dementia correlated with the degree of weight loss in 16,538 adults over 65 (Albanese et al., 2013). As expected given that weight loss preceded AD, weight loss was also seen in MCI. Besser *et al.* carried out a longitudinal study of 2268 people with MCI and 1506 people with AD. They demonstrated that symptom progression in amnesic MCI was faster in participants with weight loss and lower baseline body mass index (BMI) than in weight stable participants and those with a higher baseline BMI (Besser et al., 2014). More recently, a number of studies support the hypothesis that weight loss increases the risk of dementia. A 2016 prospective study found that weight loss predicted progression from MCI to all cause dementia and AD in 125 people with MCI followed over 4 years. Moreover, the onset of AD was earlier in patients with weight loss (Cova et al., 2016). A 20 year study of 289 women found that weight loss of just 0.5kg/year

increased the risk of MCI or dementia by 30%(LeBlanc et al., 2016). Finally, these results were very closely replicated by a UK-based retrospective cohort study of nearly 2 million people which demonstrated that those with a BMI <20kg/m² had a 34% increased risk of dementia over 20 years (Qizilbash et al., 2015). Weight loss therefore appears to be associated with increased risk of dementia, earlier dementia onset and greater disease severity, at least in the context of AD. Interestingly, there may be a U-shaped relationship between adiposity and the risk of AD over the life course as mid-life obesity has also been linked to an increased risk of developing the disease (Fitzpatrick et al., 2009, Kivipelto et al., 2005). This has been proposed to reflect deleterious metabolic processes occurring in midlife contributing to disease and associated weight loss over time. The study by Qizilbash *et al* described above did not support this, however, and so a relationship between midlife obesity and dementia remains unclear.

The neural control of energy balance is complex and finely balanced. Myriad feedback signals from the body need to be integrated by several brain areas to ensure that intake is adequate for metabolic needs (Morton et al., 2006, Woods et al., 1998). This neural control includes hormones of energy homeostasis, some of which have been implicated in cognition and neuroprotection. It is possible that neurodegeneration, even at a pre-clinical stage, could disrupt this delicate balance. This thesis will focus on hormones of energy homeostasis as a putative link between cognitive decline and weight loss in Parkinson's disease.

2.2 Parkinson's disease, adiposity and energy homeostasis over the life course

2.2.1 Overview

Meta-analysis has demonstrated that patients with PD have lower BMIs than controls and that lower BMI correlates with severity of disease but not disease duration. They propose that the lack of association between disease duration and weight loss may be due to different rates of progression amongst individuals (van der Marck et al., 2012). This would fit with the proposal by Sharma and Turton that there are two phenotypes of PD with weight change. Phenotype A is characterised by anosmia, higher weight at baseline, weight

loss throughout the disease course, dyskinesia, more severe neurodegeneration and a longer premotor phase. Meanwhile, phenotype B is characterised by lower weight at baseline, stable weight or weight gain throughout the disease course, slower neurodegeneration and a shorter premotor phase (Sauerbier et al., 2016, Sharma and Turton, 2012). As well as more severe motor symptoms weight loss may increase the risk of NMS, including neuropsychiatric symptoms, and has been associated with poorer quality of life (Akbar et al., 2015, Kim et al., 2012, Lorefalt et al., 2004). People with PD are up to 4 times more likely to be malnourished than their age matched counterparts and those with greater disease severity are most likely to be malnourished (Beyer et al., 1995, Sheard et al.). Perhaps unsurprisingly, in view of this, weight loss is associated with increased mortality in men with PD. It is proposed that women are protected from this effect by increased adipose reserve (Walker et al., 2012). It is worth noting that it is *unintended* weight loss that may be deleterious in PD. There is some evidence from animal studies that calorie restriction may be protective in PD (Maswood et al., 2004), through anti-inflammatory, anti-apoptotic or hormonal means (Bayliss and Andrews, 2016, Bayliss et al., 2016b, Fontana, 2009). Weight loss referred to in this thesis may be assumed to be unintended unless otherwise stated. The mechanisms by which weight loss occurs in PD is likely to be multifactorial. Overall in order for weight loss to occur more energy must be expended than consumed resulting in negative energy balance.

There has been much research into the effects of deep brain stimulation (DBS) on weight in Parkinson's disease, and it appears that DBS leads to weight gain (Aiello et al., 2015). The mechanism underpinning this is an area of debate and outwith the scope of this thesis.

2.2.2 Energy balance in Parkinson's disease

Oral intake

It has been proposed that PD patients with weight loss have reduced oral intake due to anosmia, drug side effects, poor swallow and the time taken to complete a meal (Bachmann and Trenkwalder, 2006, Barichella et al., 2009, Beyer et al., 1995). This is intuitive given the high prevalence of GI symptoms and anosmia in PD (Pfeiffer, 2003). Several studies have therefore investigated a possible link between NMS and energy intake in PD. Sharma and Turton assessed olfaction and intake in PD patients with and without weight loss. They found that patients with weight loss were more likely to have more severe anosmia than those with a stable weight. There was a trend towards lower energy intake in anosmic patients though this did not reach statistical significance (Sharma and Turton, 2012). Previously, Lorefalt *et al* assessed 26 PD patients over two consecutive years for problems with eating, swallowing and activities of daily living. Nineteen patients lost weight (0.5-8kg) during follow up. The authors found that patients with weight loss had more dysphagia than controls and patients with stable weights ($p < 0.05$ at baseline and < 0.01 the following year). In keeping with this, the PD patients with weight loss ate less solid food than controls. There was no significant difference in food choices or dysphagia between controls and PD patients with a stable weight. The authors speculate that eating and swallowing difficulties may lead to reduced intake in PD patients with weight loss (Lorefalt et al., 2004). Elsewhere, Barichella *et al.* investigated nutritional risk in the presence of dysphagia, excessive salivation and constipation (dysautonomia symptoms). They found that nutritional risk as measured by the Malnutrition Universal Screening Tool increased in patients with advancing Hoehn and Yahr stage, levodopa dose and the number of dysautonomia symptoms present (Barichella et al., 2013b). Finally, Aden *et al* assessed energy intake, olfaction, swallowing, food cutting ability and salivation in 87 cognitively intact people with incident PD. They found that protein intake, though not total energy intake, was reduced in PD patients with anosmia (Aden et al., 2011). Their cohort was followed up at 3 years and the relationship between these variables and cognition was examined. There was no association between anosmia, energy intake or eating difficulties at baseline and cognitive decline. Energy intake was not re-assessed at follow up (Vikdahl et al., 2015).

Despite this apparent increased nutritional risk, there is as yet no clear evidence that energy intake is reduced in PD patients compared to healthy older adults. In fact, several studies have demonstrated increased energy intake in PD. A small study by Davies *et al.* found that PD patients ate around 400kcal/day more than controls ($p=0.02$) when dietary intake was assessed by a seven day food diary. Participants received training on food portion sizes but portions were not weighed (Davies *et al.*, 1994). The authors concluded that reduced energy intake did not account for weight loss in PD. Their assertion is supported by Logroscino *et al.* who found that energy intake was 350kcal/day more in PD patients than controls ($p=0.001$) when assessed using a semi-quantitative food questionnaire. Importantly, the authors did not analyse data from patients who had lost weight. It is therefore difficult to draw conclusions about the eating habits of weight losing PD patients from this study (Logroscino *et al.*, 1996). This may be important as people with PD who lose weight may be phenotypically different as detailed above (Sauerbier *et al.*, 2016, Sharma and Turton, 2012). In support of this, a further paper published on the same cohort as the Lorefalt paper described above reports increased energy intake in PD patients compared with those with stable weights. Energy intake was significantly increased in weight-losing people with PD when expressed as a function of body weight (kcal/kg/day). It is worth noting that PD patients with weight loss had a significantly lower BMI than the weight stable group, making the validity of a weight corrected measurement questionable (Lorefalt *et al.*, 2004). A subsequent small study of PD patients with and without weight loss by Delikanaki-Skaribas *et al.* revealed very similar results. The authors reported that energy intake was increased in the weight loss group when expressed as a function of body weight. Once again, the weight loss group had significantly lower BMIs than the weight stable groups so that the significance of results is uncertain. (Delikanaki-Skaribas *et al.*, 2009).

There is evidence that both weight loss and increased energy intake may occur early in the disease process, even in the pre-clinical phase. In a large prospective study of more than 170,000 individuals, 468 (267 men and 201 women) developed PD during follow up (14 and 18 years respectively). Weight was self-reported biennially and dietary intake was measured using a food frequency questionnaire. The team found that patients lost weight prior to

diagnosis and that weight loss was significantly more than for controls (p for trend <0.0001). Weight loss started 2-4 years prior to PD diagnosis and energy intake increased during this period. Patients had an average increase of 347 kcal/day compared to controls ($p <0.002$ from 2-4 years prior to diagnosis). However; it is worth noting that confidence levels for energy intake in PD were large and overlapped with those for energy intake in controls at several time points (Chen et al., 2003).

The most compelling evidence that energy intake is increased in PD comes from a large, cross-sectional case controlled study of 600 people with Parkinson's disease and 600 age, physical activity, education and gender matched healthy controls. People with weight loss were not excluded but those requiring prescribed nutritional supplementation were. A trained dietician completed a semi-quantitative food frequency questionnaire with each participant. The authors found that BMI was lower in the PD groups and worsened with increasing disease severity. Despite this, energy intake was higher by around 200 kcal/day in those with PD and continued to increase with disease progression. This was significant both as a function of body weight ($p <0.001$) and in absolute numbers ($p <0.001$). People with cognitive impairment were excluded from this study (Barichella et al., 2017).

Evidence for increased energy intake in PD compared with healthy adults has not been entirely consistent, however. An earlier study by Barichella *et al.* found no significant difference in intake between PD patients and controls (Barichella et al., 2013a). Furthermore, an earlier study by Toth *et al.* investigating energy intake in 16 PD patients and 46 controls found that energy intake was no different between groups. Importantly, this study excluded patients with recent weight loss (Toth et al., 1997), which may have biased their results.

On balance, it does appear likely that energy intake is higher in PD than in controls. It is difficult to reconcile this with increased nutritional risk in the presence of non-motor symptoms. Accurately measuring energy intake is fraught with difficulties. Food diaries are prone to errors due to inaccurate estimation of portion sizes by respondents. None of the above studies used weighed portions. Similarly, food frequency questionnaires are useful to record trends in food consumption in populations but are unable to record intake at an

individual day to day level (Gibson, 1990). The available studies do not adequately account for potential differences between weight stable and weight losing people with PD. It is possible that energy intake is increased in PD but that energy requirements are increased resulting in weight loss overall.

Energy expenditure

It has been proposed that weight losing PD patients have increased energy expenditure due to increased muscle rigidity and the emergence of dyskinesia. Total energy expenditure (TEE) may be separated into resting energy expenditure (REE) and physical activity. Early studies looked at REE alone to determine whether or not energy expenditure is increased in PD. A small study by Levi *et al.* found that REE significantly decreased when patients had a reduction in motor symptoms or “switched on” following PD medication (Levi *et al.*, 1990). Similarly, Markus *et al.* studied REE in 12 PD patients and eight controls. REE was increased in patients with PD compared to controls. Four patients had increased REE in the “off” state but 7 showed no difference (Markus *et al.*, 1992). More recently, Capecchi *et al.* measured REE in 58 PD patients when the patients were fasted and “off” and then again 60 minutes after PD medication. They found an 8% reduction in REE in the “on”-state. Despite this, the participants REE was similar to that expected for gender, age and BMI. The clinical significance of increased REE is therefore uncertain (Capecchi *et al.*, 2013). Conversely, Lorefalt *et al.* found reduced REE per Kg body weight and reduced physical activity in PD patients compared to controls. The reduction in REE was seen in PD patients with and without weight loss. There was no difference in REE between PD patients who lost weight and those whose weight remained stable (Lorefalt *et al.*, 2004). Taking these studies together it is unclear to what extent REE is increased in PD, if at all.

Two well conducted studies have gone on to investigate TEE in PD with a view to obtaining a more complete picture of energy expenditure. Both studies used the doubly labelled water technique to measure TEE. This technique uses water labelled with naturally occurring isotopes of hydrogen and oxygen (H_2 and O_{18}). Energy expenditure can then be calculated using the proportion of labelled hydrogen and oxygen excreted in urine, rather than exhaled as carbon dioxide (Schoeller, 1988). Indirect calorimetry, in which expired CO_2 is measured at rest using a ventilation hood, was used to measure REE (Schoeller, 1988). Toth *et*

al. measured TEE and REE in 16 men with PD and 46 healthy male controls. TEE was found to be reduced in patients with PD compared to controls due to reduced physical activity, proposed to be due to poor mobility and increased dependency (Toth et al., 1997). More recently, Delikanaki-Skaribas *et al.* investigated 10 PD patients with stable weight and 10 PD patients with weight loss. They found that neither TEE nor REE were significantly different between weight loss and weight stable groups (Delikanaki-Skaribas et al., 2009).

There is further evidence in support of the idea that energy expenditure is reduced, rather than increased in PD. Data from a study using accelerometers to measure physical activity in free-living people with PD demonstrated that people with PD are significantly less active than controls, even in early disease (Lord et al., 2013). Moreover, the recent Barichella paper discussed above collected data on daily sleep, hobbies, housework and other daily activities to calculate TEE and physical activity. They also calculated REE based on height, weight and age. They found that physical activity was lower in people with PD and reduced further as disease progressed (Barichella et al., 2017). There is, therefore, relative agreement in the literature that people with PD lose weight in the face of apparent positive energy balance, which is to say increased energy intake and reduced energy expenditure. Let us consider other potential ways in which this excess energy might be “lost” in PD.

Malabsorption

As discussed above, gastrointestinal symptoms such as dysphagia, constipation and abdominal bloating are common in PD. Many of these symptoms have been attributed to disruption in gut motility (Pfeiffer, 2003). This has led some authors to speculate that weight loss in PD may have an enteric cause. Small intestinal bacterial overgrowth (SIBO) has been shown to contribute to occult weight loss in older adults (McEvoy et al., 1983). Intestinal dysmotility is thought to be an important aetiological factor in the development of SIBO (Gasbarrini et al., 2007) so it follows that SIBO should be more common in PD patients. As expected, the prevalence of SIBO is higher in patients with PD than in the healthy population. However; this has not been shown to correlate with weight loss in PD (Fasano et al., 2013, Gabrielli et al., 2011, Tan et al., 2014). An important role for malabsorption in causing weight loss therefore appears unlikely.

Medication

Dopaminergic medications have been implicated in the aetiology of weight loss in people with PD (Kashihara, 2006). These drugs have side effects including nausea and vomiting which may impair appetite. However, even when present, nausea and vomiting do not appear to reduce oral intake (Lorefalt et al., 2004, Palhagen et al., 2005). It was previously proposed that levodopa may contribute to weight loss by enhancing lipolysis (Vardi et al., 1976). This is in keeping with the finding that weight loss in PD tends to be fat loss-based rather than due to sarcopaenia (Lorefalt et al., 2009, Markus et al., 1993). However; direct measurement using microdialysis failed to demonstrate increased lipolysis with levodopa administration (Adams et al., 2008). Prolonged levodopa treatment and higher doses are often accompanied by the emergence of motor fluctuations and dyskinesia in PD. The Barichella study discussed above demonstrated that positive energy balance in PD correlated positively with motor fluctuations, including involuntary dyskinesia (Barichella et al., 2017). Sharma and Turton also found that more severe motor fluctuations and dyskinesia were associated with weight loss in PD (Sharma and Turton, 2012). These data suggest that complications of long term levodopa treatment may increase energy requirements, which is difficult to measure under laboratory conditions.

The principal argument against a significant role for medications directly causing weight loss in PD is that weight loss appears to precede drug therapy and motor symptoms (Chen et al., 2003, Cheshire Jr and Wszolek, 2005). The onset of weight loss prior to motor symptoms and in early disease also makes increased energy expenditure an unlikely sole cause of weight loss.

It seems likely that the cause of weight loss in PD is multifactorial, with increased requirements due to as yet unidentified reasons and inadequate nutrition to maintain homeostasis despite overall increased energy intake. It appears that there is failure of the usual homeostatic mechanisms in PD. As detailed above, weight loss in PD is associated with an increased risk of cognitive decline but the nature of this relationship, whether or not weight loss causes cognitive decline, is unclear (Kim et al., 2012). No studies to date have assessed energy intake in people with PD-CI. Extensive neurodegeneration by α -synuclein and A β may cause both weight loss and cognitive impairment

through damage to limbic and cortical areas of the brain. There are plausible mechanisms by which weight loss in PD may precipitate or accelerate cognitive decline. A number of hormones of energy homeostasis have been proposed to be neuroprotective in PD and in AD. Disruption of these systems may result in loss of neuroprotection and increased neuronal loss. This will be discussed in detail in the chapters below.

2.3 Energy Homeostasis

2.3.1 Overview

Energy homeostasis refers to the active maintenance of total body energy stores at a level appropriate to the environment over time. Our bodies store energy derived from food in muscle, liver and adipose tissue. If more energy is consumed than is expended then more adipose tissue is laid down and body weight increases. Conversely, if energy intake is less than energy spent then body weight decreases. This may be lost from muscle or adipose mass (Woods and D'Alessio).

Energy homeostasis requires signals about body energy stores to be communicated from the peripheral tissues to the central nervous system. These signals must then be integrated and result in appropriate changes in feeding behaviour and energy expenditure. Most humans and several species of animal consume energy in discrete meals. It is thought that satiation, or a feeling of “fullness”, resulting in the limitation of energy consumption at meal times is more important in energy homeostasis than satiety, which is to say meal initiation (Valassi et al., 2008). This makes sense in the social and cultural context of feeding behaviour in humans in which meals are often initiated due to habit, mood and circumstances rather than in response to a lack of circulating nutrients. Regulation of energy homeostasis through satiation (meal termination) rather than satiety (meal initiation) also makes sense in the context of human evolution where scarce food resources meant that food was eaten whenever it was available (Woods and D'Alessio). In order to ensure that homeostasis is maintained, information from the body regarding the amount and type of nutrients ingested at each meal must be conveyed to the central nervous system (CNS) along with information about total background energy stores (Woods and D'Alessio, 2008)

The hypothalamus is the main “hunger centre” of the brain. The arcuate nucleus (ARC) of the hypothalamus contains both appetite suppressant (anorexigenic) and appetite stimulant (orexigenic) neurones. Anorexigenic neurones secrete pro-opiomelanocortin (POMC), which is cleaved into α -melanocyte stimulating hormone (α MSH). Alpha-MSH acts at Melanocortin 3 (MC3R) and Melanocortin 4 receptors (MC4R) to reduce food intake (Cowley et al., 2001, Fan et al., 1997). Orexigenic neurones secrete the neurotransmitters neuropeptide Y (NPY) and agouti-related peptide (AgRP). AgRP antagonises α MSH, whilst NPY acts directly on Y receptors, both resulting in increased food intake. The control of intake depends on the balance between these two sets of neurones, which are stimulated in response to a myriad of peripheral and central factors (Valassi et al., 2008). The downstream effect is likely to be mediated by adenosine 5' monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) (Cota et al., 2006, Minokoshi et al., 2004). The blood brain barrier (BBB) at the ARC is more permeable than in other areas of the brain and consequently may be able to sense peripheral hormones and circulating nutrient levels (Peruzzo et al., 2000). Glucose, free fatty acids and the amino acid leucine have been proposed to inhibit appetite centrally (Woods and D'Alessio, Lam et al., 2005, Obici et al., 2002, Cota et al., 2007). The ARC also receives and integrates input from other areas of the hypothalamus and throughout the CNS resulting in shifts in balance between the stimulation of orexigenic and anorexigenic neurones and hence energy intake (Cone, 2005, Cota et al., 2007, Elmquist et al., 2005, Morton et al., 2006, Small and Bloom, 2004, Woods and D'Alessio, 2008) (Figure 4).

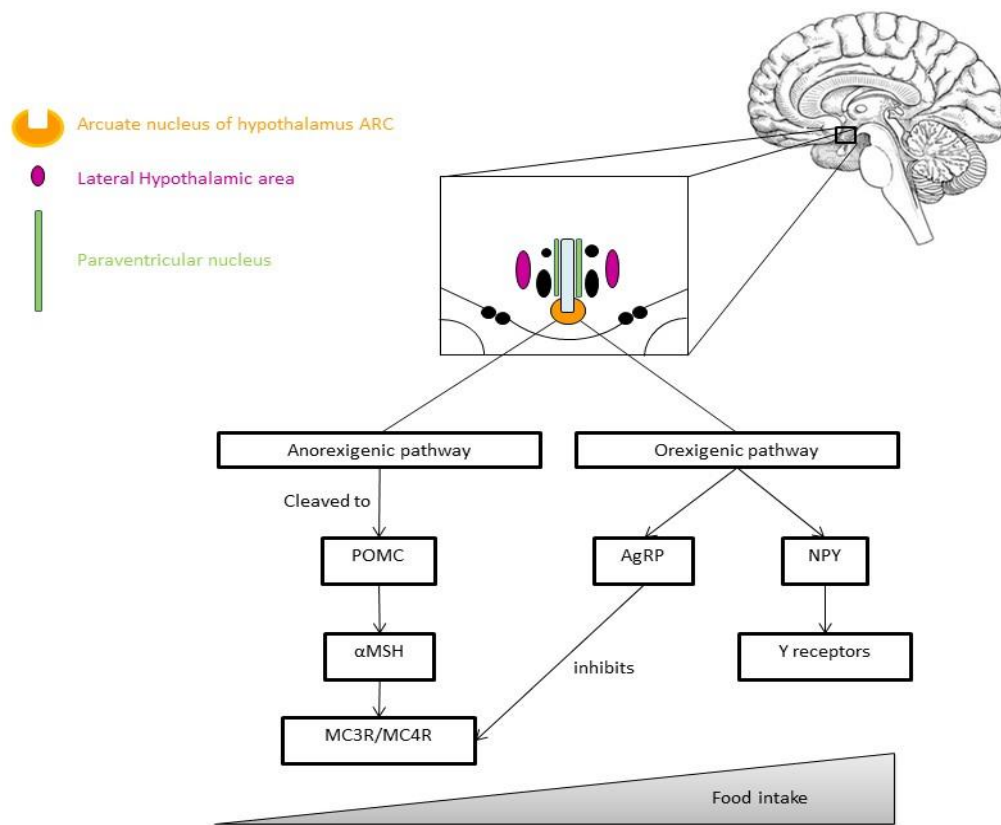


Figure 4. First order neural pathways involved in appetite

Feeding is a complex behaviour requiring the organism to be able to find food, correctly identify it as food and ingest it in appropriate amounts according to their metabolic needs. It is not surprising then that numerous areas of the brain are involved in energy homeostasis (Figure 5). In the brainstem, the nucleus tractus solitarius (NTS) receives sensory input from the tongue regarding the taste and texture of foods and from the vagus nerve under hormonal control and in response to stomach stretching. In the diencephalon, the lateral hypothalamic area (LHA), ventral tegmental area (VTA) and nucleus accumbens (NAc) make up the cholinergic-dopaminergic reward circuit (Morton et al., 2006, Woods, 2013). The NTS and cholinergic-dopaminergic reward circuit work together to assign food reward and communicate with the ARC to regulate intake (Morton et al., 2006). The LHA (part of the cholinergic-dopaminergic reward circuit) receives input from the orbitofrontal cortex, regarding the look, smell, taste and texture of foods (Rolls, 2005). It has a direct neural connection with the ARC and contains orexigenic neurones itself (Broberger et al., 1998). It is thought to be important in initiating food seeking behaviour (Morton et al., 2006). In

contrast, the paraventricular nucleus (PVN) is thought to augment anorexigenic signals by producing corticotropin-releasing hormone and oxytocin, both of which suppress appetite (Woods and D'Alessio, 2008). Peripheral signals regarding adiposity and nutrient status are transmitted to the CNS through the blood brain barrier and via the vagus nerve. There are a number of hormones of energy homeostasis involved in this process (Table 3). This thesis will focus on the actions of ghrelin, insulin, leptin and glucagon-like peptide 1 as these are the compounds most relevant to neurodegenerative disease.

Table 3. Hormones of energy homeostasis (Cholerton et al., 2013, Norton et al., 2014, Rizza et al., 1982, Smith and Thorner, 2012, Woods and D'Alessio, 2008)

Hormone	Receptor	Production	Site of action	Appetite	Other actions
Active Ghrelin	GHSR1a	X/A like cells in Oxyntic glands of stomach Requires intact vagal nerve Likely an element of higher control	Arcuate nucleus of hypothalamus Nucleus Tractus Solitarius Hippocampus	Orexigenic	<u>Stomach</u> Increases motility , gastric emptying and stomach acid secretion <u>Metabolic</u> Increases blood glucose during starvation, reduces insulin release, increases adiposity (reduces utilisation of fat) <u>Central nervous system</u> Increases GHRH,GH, prolactin and ACTH , increases reward <u>Other</u> ↑ cardiac output, ↓ blood pressure, Anti-inflammatory
Insulin	Insulin receptor (IR)	B-cells in the pancreas in response to circulating glucose. Augmented by GLP-1, suppressed by growth hormone and cortisol	Peripheral tissues and throughout the CNS	Anorexigenic	Enables storage of energy, lowers blood glucose Regulates Ach and noradrenaline Decreases ghrelin
Leptin	LEP-R/OB-R	White adipose cells	CNS- especially hypothalamus but including SNpc	Anorexigenic	Augments CCK response May increase insulin sensitivity Resistance occurs Inhibits bone formation May have a role in fertility Suppress AgRP/NPY neurones
Cholecystokinin (CCK)	CCK-A (pancreas and gallbladder) CCK-B (CNS)	I cells duodenum and jejunum	Hypothalamus via the vagus nerve Pancreas Gall bladder	Anorexigenic	Inhibits ghrelin Augments response to leptin Reduces gastric emptying Increases exocrine pancreatic secretions Increases PYY secretion Stimulates gall bladder contraction
Glucagon-like peptide 1 (GLP-1)	GLP-1R	L-cells throughout the GI tract, especially ileum	Hypothalamus and pancreas	Anorexigenic	Augments insulin secretion Reduces glucagon Increases β cell proliferation Reduces gastric emptying Decreases PYY
Peptide YY (PYY)	Y2	L-Cells in GI tract in response to nutrients	Hypothalamus	Anorexigenic	Reduce motility in stomach and GI tract

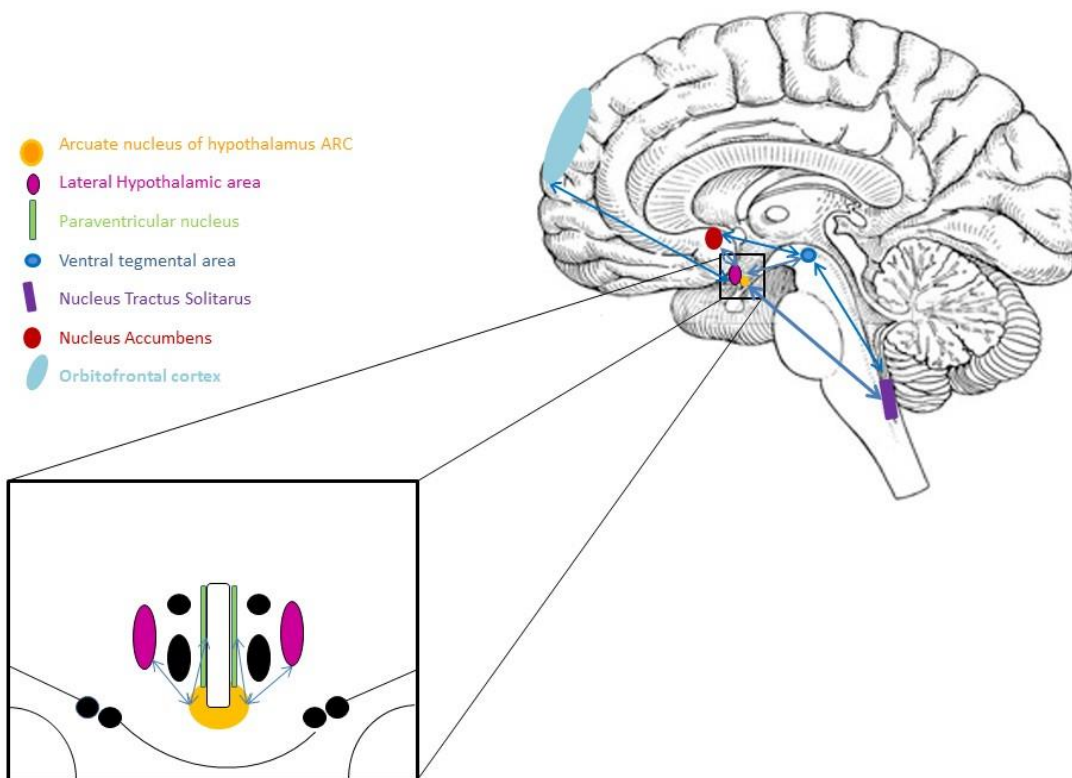


Figure 5. Areas of the brain involved in appetite regulation

2.3.2 Ghrelin

Ghrelin is a 28 amino acid peptide which was discovered by Kojima et al. in 1999 and identified as endogenous ligand for an orphan G-coupled receptor called growth hormone secretagogue receptor (GHSR1a) (Kojima et al., 1999). Ghrelin is a powerful orexigenic hormone produced as des-acyl or unacylated-ghrelin (UAG) in oxyntic X/a-like cells in the stomach in response to fasting (Wren et al., 2001, Date et al., 2000a, Nakazato et al., 2001). Once secreted, ghrelin passes into the blood stream where it is acylated into its active form, acyl-ghrelin (AG) by ghrelin-O-acyltransferase (GOAT) (Yang et al., 2008). Acyl-ghrelin stimulates the DMNV and crosses the blood brain barrier into the brain. Once across the BBB AG binds with growth hormone secretagogue receptor 1 a (GHSR1a) receptors in the hypothalamus and DMNV, resulting in inhibition of anorexigenic POMC neurones and stimulation of orexigenic NPY neurones (Cowley et al., 2003, Horvath et al., 2001, Currie et al., 2005, Nakazato et al., 2001). The net result is increased hunger and food intake (Andrews, 2011, Markaki et al., 2012, Yang et al., 2008, Cummings, 2006). Acyl-ghrelin also acts at the NTS via the vagus nerve where input is integrated with information about food taste and texture. Finally AG acts at the cholinergic-dopaminergic reward circuit to increase reward assigned to palatable foods (Jerlhag et al., 2012a).

Ghrelin has both short term or “phasic” and long term or “tonic” secretion (Figure 6). Phasic ghrelin levels peak before meals and decline thereafter (Davis et al., 2011, Cummings et al., 2001). There is also diurnal variation, with a night-time peak in the early hours of the morning (Nass et al., 2013). The control of ghrelin release is not yet well understood but appears to require an intact vagus nerve (Date et al., 2002, Huda et al., 2010) and to be at least partly under higher cortical control (Seoane et al., 2007, Williams et al., 2003a, Williams et al., 2003b). Suppression after meals occurs when nutrients enter the duodenum after stomach emptying, or after tease feeding (Seoane et al., 2007, Tschöp et al., 2001a). Control appears to be, at least partially, mediated by acetylcholine. Oral pyridostigmine, a cholinergic agonist, was shown to increase ghrelin levels whilst pirenzepine, an anti-cholinergic, decreased ghrelin secretion in 6 healthy humans (Broglio et al., 2004).

Tonic ghrelin and AG levels increase with declining BMI; increasing hunger and promoting food intake in times of negative energy balance as part of a negative feedback loop (Tschöp et al., 2001b, Beberashvili et al., 2017). The tonic level of ghrelin can also be described as the area under the curve of ghrelin over the course of the pre-prandial, post-prandial and recovery phases of secretion. Ghrelin is effective in increasing weight and muscle mass in cachectic patients with severe heart failure and COPD and increases intake in patients with metastatic cancer and anorexia. Exogenous administration of ghrelin results in increased energy intake in humans with heart failure, COPD, end-stage renal failure and cancer (Wynne et al., 2005, Neary et al., 2004, Nagaya et al., 2004, Nagaya et al., 2006, Nagaya and Kangawa, 2006). (Akamizu and Kangawa, 2011, Neary et al., 2004)

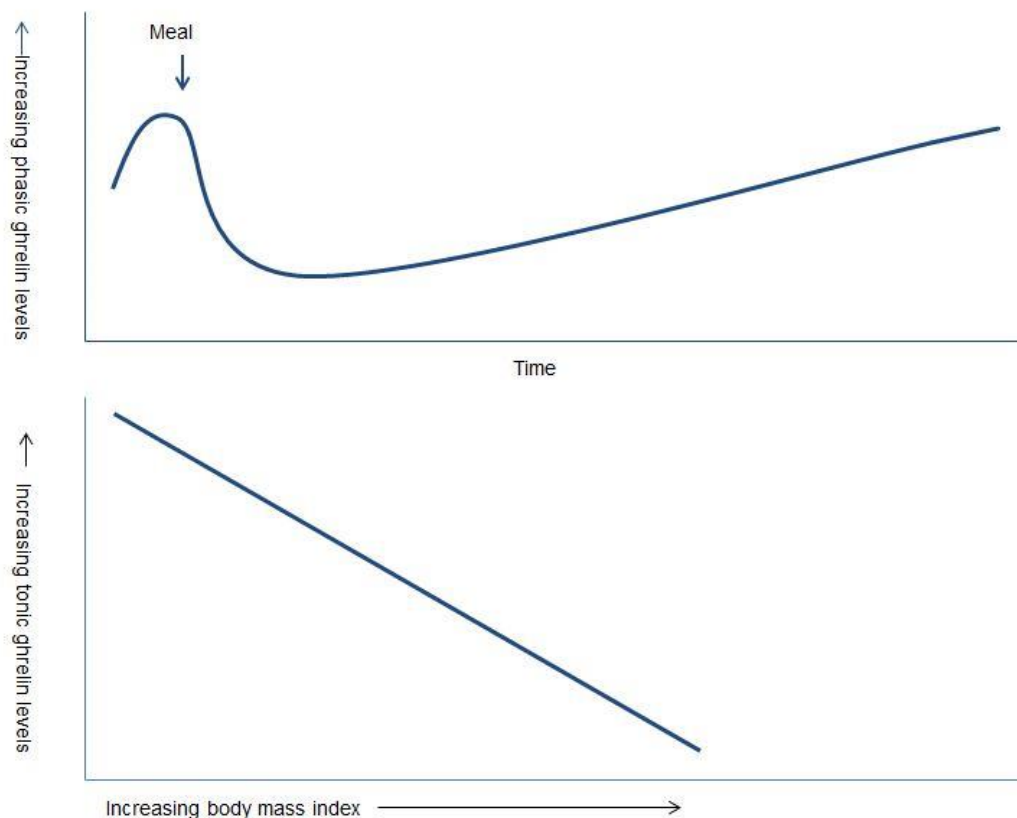


Figure 6. Schematic of ghrelin release

Ghrelin levels have been proposed to decline with age, a phenomenon that has been linked with somatopause (relative growth hormone deficiency in older age) and hypothalamic degeneration (Akamizu et al., 2006, Nass et al., 2013,

Rigamonti et al., 2002). In 2002 Rigamonti *et al.* measured fasting total ghrelin in young people with anorexia nervosa (n= 6, aged 17.5 ± 0.5), obesity (n=7, aged 26.9 ± 2.7) and normal weight (n=12, aged 33.4 ± 1.0) and older people with normal weight (n=7, 79.8 ± 2.1). They found that ghrelin levels were high in young people with anorexia and low in those with obesity relative to young people with normal weight. This was in keeping with the expected pattern of ghrelin according to BMI. They found that older adults with normal weight had lower ghrelin levels despite a similar BMI and that their ghrelin levels were similar to young people with obesity (Rigamonti et al., 2002). This was quickly followed by a 2003 study examining the dynamic total ghrelin response to ingesting ice-cream in 8 well-nourished older adults (aged 77 ± 0.9) 8 undernourished older adults (aged 80.4 ± 2.6) and 8 well-nourished young adults (aged 22 ± 1.3). The authors report that total ghrelin levels were 20% lower in the well-nourished older adults compared with well-nourished younger adults. This effect failed to achieve significance, however ($p=0.2$), making their results inconclusive. The authors felt this may be due to the study being underpowered (Sturm et al., 2003). A Japanese study of 105 older people (aged 73.4 ± 6.3) and 39 younger people (aged 33.5 ± 9) found that fasting AG was lower in older women than younger women but no difference was observed for men (Akamizu et al., 2006). More recently, Nass *et al.* found significantly lower 24 hour AG profile in 6 older adults (aged 65.2 ± 4.9) compared with eight healthy young men (aged 25.8 ± 3.1). Blood tests were taken every 10 minutes over the 24 hour period and a loss of a nighttime peak of ghrelin was seen in the older subjects (Nass et al., 2013).

There is some evidence that the relationship between ageing and ghrelin is not as straightforward as deterioration over time. A recent study by Serra-Prat *et al.* looked at 55 people over 70 and 33 people aged 25-65. The older group were determined to be either sarcopaenic (i.e. had clinical evidence of muscle wasting) or non-sarcopaenic. Of the 55 older participants 23 were sarcopaenic. Ghrelin levels were not significantly different between the young adult group and the non-sarcopaenic older participants. Sarcopaenic participants had lower ghrelin levels than non-sarcopaenic older adults but this effect was lost when analysed by gender, possibly due to small sample size. The authors propose that ghrelin may not decline in healthy ageing but rather may reflect a loss of

homeostasis in unhealthy ageing, perhaps reflecting a pre-frail state (Serra-Prat et al., 2015). This is supported by their previous work showing that lower ghrelin levels at baseline were associated with weight loss and functional decline in elderly people over 2 years (Serra-Prat et al., 2010). Further evidence comes from a small study by Schneider *et al.* who looked at total ghrelin and AG pre-prandially and for 4 hours post-prandially in healthy people and malnourished people requiring dietician input for nutrition. They found that AG levels were higher in undernourished young people (age 26 ± 6 , $n=10$) compared with well-nourished young people (aged 34 ± 8 , $n=10$) but undernourished older people (aged 80 ± 6 , $n=11$) did not have significantly elevated AG compared to healthy older adults (76 ± 9 , $n=9$). There was no significant difference in AG between healthy older adults and healthy younger adults. This study is limited by its small numbers and also because the reason for malnourishment was different between younger and older groups. More young people were suffering from anorexia nervosa and older people were suffering with depression, infection or recent surgery (Schneider et al., 2008). This is important as ghrelin is proposed to be an acute phase reactant (Maruna et al., 2008). Levels do not change in depression (Kluge et al., 2009) but may be elevated in anorexia nervosa (Soriano-Guillén et al., 2004), making interpretation difficult. Although flawed, this study does support the idea that ageing *per se.* may not affect ghrelin secretion but that there may be a failure of homeostasis in ill health in older age. The studies by Rigamonti, Sturm and Akamizu's groups cannot clarify this relationship as their inclusion criteria were broad and measures of frailty and sarcopaenia were not included. It has been suggested that the efficacy of ghrelin may decrease with ageing, due to reduced GHSR1a signaling (Nass et al., 2013, Yin and Zhang, 2015). Further research is needed to determine if decline in ghrelin is part of healthy ageing or a reflection of an increased prevalence of frailty in older age.

Pleiotropic ghrelin

Ghrelin is a pleiotropic hormone, which is to say that it has differing effects in parts of the body. Its receptor, GHSR1a is expressed throughout the body in a number of different tissues (Gnanapavan et al., 2002). Along with its orexigenic effects ghrelin acts as a stimulating hormone for growth hormone (GH) (Arvat et al., 2000). It is thought to have anti-inflammatory properties, to reduce blood

pressure (Wynne et al., 2005) and to improve left ventricular function (Nagaya et al., 2004). Ghrelin has also been implicated in learning and memory and may have a role in neuroprotection. Figure 7 gives an overview of the main actions of ghrelin known to date. We will look at some of these in more detail.

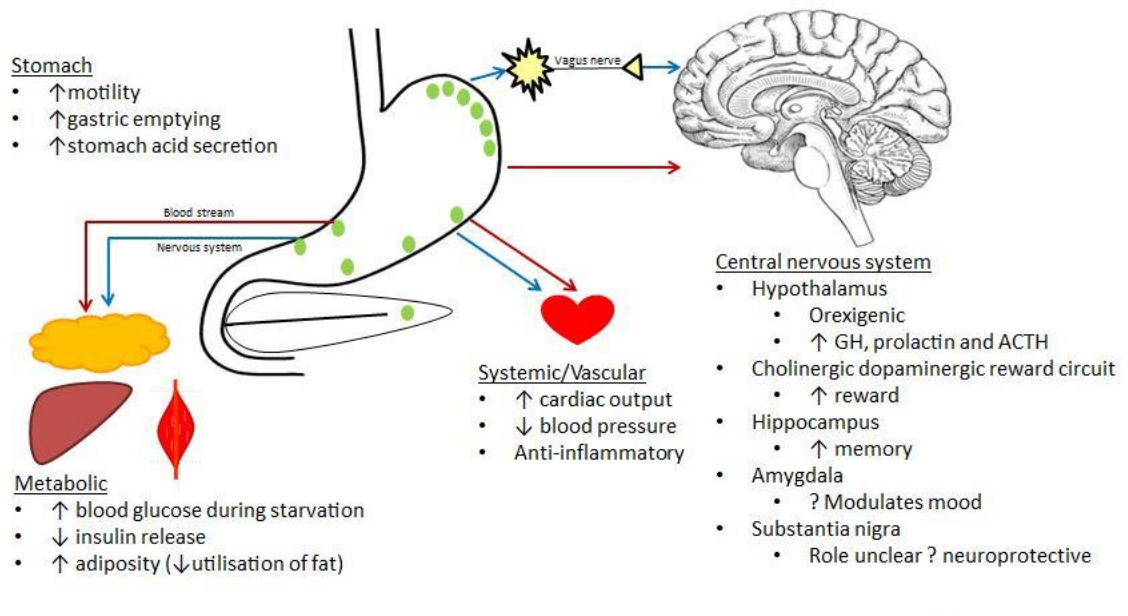


Figure 7. The pleiotropic effects of ghrelin

Ghrelin and the gut

Animal studies have shown ghrelin to increase gut motility, the rate of gastric emptying and stomach acid secretion (Depoortere et al., 2005, Edholm et al., 2004, Fukuda et al., 2004, Levin et al., 2005, Masuda et al., 2000, Trudel et al., 2002). The prokinetic effects of ghrelin have also been demonstrated in humans in two double-blind randomized controlled crossover studies. Levin *et al.* gave intravenous ghrelin to eight healthy adults and six patients with GH deficiency using scintigraphy (radiolabeled foods that can be imaged radiographically). Ghrelin increased gastric motility whether or not GH was administered, suggesting that ghrelin's prokinetic effects are independent of GH (Levin et al., 2006). Tack *et al.* studied nine healthy volunteers and measured their gastric motility using manometry. They administered ghrelin or saline intravenously. They found that gastric motility was increased and that there was sustained stomach contraction after ghrelin infusion (Tack et al., 2006).

Ghrelin has also been investigated as a treatment for gastroparesis. Six people with idiopathic gastroparesis were administered ghrelin or saline in a randomized controlled crossover trial. Gastric emptying was measured using C¹³ breath testing. Gastric emptying was faster following ghrelin treatment than following saline and symptoms of gastroparesis were improved (Tack et al., 2005). Another double blind placebo controlled cross over study looked at ten people with diabetic gastroparesis. They were administered ghrelin or saline IV. Intravenous ghrelin increased gastric emptying as measured using ultrasound. Interestingly this persisted even with vagal neuropathy, suggesting that the effects were independent of the vagus nerve (Murray et al., 2005). The finding that an intact vagal nerve is not required for ghrelin's prokinetic effects is corroborated by another small randomized controlled crossover study in six women with impaired vagal innervation of the stomach; five with autonomic neuropathy due to diabetes and one with gastroparesis following vagotomy. The authors demonstrated that gastric emptying was increased following ghrelin treatment compared with saline infusion (Binn et al., 2006). This is important as it suggests that ghrelin could improve gastric emptying even in the face of neurodegeneration of the vagus nerve. Gastroparesis is a common non motor symptom of PD, for which there are few treatment options at present (Marrinan et al., 2013). Ghrelin treatment could be of potential therapeutic benefit for gastroparesis in PD and human studies examining this are ongoing (Schrag et al., 2015).

Ghrelin and the immune system

There is compelling evidence that ghrelin is an immune modulator. In 2001, Hattori *et al.* found that GHSR1a and ghrelin were present in human T-cells, B cells and neutrophils (Hattori et al., 2001). It has since been demonstrated that GHSR1a is expressed on human T-cells and that expression increases with t-cell activation. *In vitro* ghrelin administration to T-cells inhibits pro-inflammatory cytokines (IL-1 α , IL-1 β TNF α and IL-6). *In vivo*, bacterial lipopolysaccharide (LPS), also known as endotoxin, mimics septic shock. Exogenous ghrelin reduced the inflammatory response to LPS at 4 and 24 hours in mice (Dixit et al., 2004). It has been proposed that ghrelin is important for the maintenance of the thymus; an organ which produces T-cells and is important for immunity. With age, thymic cells are gradually replaced with fatty tissue and the diversity

and number of T-cells produced declines. Dixit *et al.* found that thymic ghrelin expression and GHSR1a expression decline with age in mice, that ghrelin knock-out mice have a greater rate of thymic involution than wild-types and that exogenous ghrelin can reverse this process (Dixit *et al.*, 2007). Similar results are seen in GHSR1a knock out mice (Youm *et al.*, 2009).

Exogenous ghrelin has been shown to reduce inflammation and to improve phenotype in animal models of sepsis (Li *et al.*, 2004), traumatic brain injury (Bansal *et al.*, 2012), myocardial ischaemia (Cao *et al.*, 2013), chronic kidney disease (Deboer *et al.*, 2008), stroke (Cheyuo *et al.*, 2011), inflammatory arthritis (Granado *et al.*, 2005), lung contusion (Guyen *et al.*, 2013), acute lung injury (Imazu *et al.*, 2011, Li *et al.*, 2015a), non-alcoholic fatty liver disease (Li *et al.*, 2013b), multiple sclerosis (Souza-Moreira *et al.*, 2013, Theil *et al.*, 2009) and gut ischaemia (Wu *et al.*, 2008) amongst others. All of these studies found reduced pro-inflammatory cytokine expression in response to treatment with ghrelin.

In addition to direct action on the thymus and T-cells, ghrelin may reduce inflammation via vagal stimulation. Kevin Tracey described the “cholinergic anti-inflammatory pathway” or “inflammatory reflex” in 2002. He proposed that the CNS is able to sense tissue inflammation and rapidly produce an anti-inflammatory response via the autonomic nervous system, specifically sensory vagal afferent and motor vagal efferent neurons. This prevents excessive immune responses, which may be harmful in systemic inflammatory response syndrome or autoimmune disease, for example (Tracey, 2002). This theory was based on previous work demonstrating that acetylcholine (Ach) deactivated macrophages *in vitro* via specific Ach receptors (α -bungarotoxin-sensitive nicotinic receptors) on macrophages. Furthermore, the production of the inflammatory cytokine TNF α was attenuated both at rest and in response to LPS with vagal efferent stimulation (Borovikova *et al.*, 2000). Studies in animal models support the idea that ghrelin exerts its anti-inflammatory action via the cholinergic anti-inflammatory pathway. Wu *et al.* looked at a rat model of gut ischaemia in which the superior mesenteric artery was occluded for 90 minutes. Rats administered ghrelin had reduced inflammatory cytokines and reduced ischaemic injury. This result was lost with both ghrelin receptor antagonism and vagotomy (Wu *et al.*, 2008). Similar results were found by Cheyuo *et al.* in a rat

model of stroke in which the right middle cerebral artery was ligated causing ischaemic brain injury. Half of the rats also underwent vagotomy. Rats were given vehicle or ghrelin intravenously post-operatively. Rats given ghrelin who had an intact vagus nerve had reduced infarct size compared with rats given vehicle or ghrelin and vagotomy. Levels of the inflammatory cytokines TNF- α and IL-6 were similarly reduced (Cheyuo et al., 2011).

There is surprisingly little in the literature regarding exogenous ghrelin in inflammatory conditions in humans. One randomised crossover study examined the ghrelin response to LPS when given to 10 healthy men. They found that ghrelin surged after LPS administration, suggesting that ghrelin is important in immunomodulation in humans (Vila et al., 2007).

Ghrelin and other hormones

Ghrelin was first identified as the ligand for growth hormone secretagogue receptor (GHSR1a). As the name suggests, ghrelin increases GH secretion through binding with this receptor to stimulate GH release at the pituitary (Date et al., 2000b, Kojima et al., 2001, Peino et al., 2000, Wren et al., 2000). It has been shown to have a more potent effect on GH secretion than growth hormone releasing hormone (GHRH) when administered exogenously (Arvat et al., 2000). Furthermore, ghrelin acts synergistically with GHRH to augment the GH release in response to GHRH (Arvat et al., 2001). Interestingly, the GH response to ghrelin is blunted in older adults (Nass et al., 2013). Growth hormone is thought to be important for feelings of vitality. Its secretion is pulsatile and this pattern appears to be lost with ageing. The somatopause has been linked with sarcopaenia and frailty (Lanfranco et al., 2003). It is possible that blunting of the nocturnal ghrelin peak and deteriorating GH response to ghrelin may be important in sarcopaenic patients. Interestingly, decreased nocturnal GH has also been seen in PD (Bellomo et al., 1991)

A downstream effect of GH is to stimulate insulin-like growth factor-1 (IGF-1) production from the liver. Insulin-like growth factor 1 is thought to be important in learning and memory and IGF-1 deficiency has been implicated in neurodegenerative diseases including PD and AD (Giordano et al., 2012). As you might expect, IGF-1 levels decline with age and are part of the

somatopause (Lanfranco et al., 2003). Insulin-like growth factor-1 will be discussed in depth in a later section of this thesis.

In addition to augmenting the somatotrophic axis ghrelin has an important role in glucose metabolism. Ghrelin is required for the maintenance of blood sugar levels in the face of starvation, may promote adiposity independently of food intake and may have an influence on basal metabolic rate. Ghrelin and GHSR1a knockout mice have significantly lower blood glucose under calorie restriction than wild types (Sun et al., 2008). Moreover, GOAT knockout mice become hypoglycaemic and moribund when calorie restricted (Zhao et al., 2010). Exogenous ghrelin impairs glucose tolerance in humans (Broglio et al., 2001, Chuang et al., 2011, Claret et al., 2007, Melville et al., 1990, Smith and Thorner, 2012, Tong et al., 2016, Tong et al., 2010). Ghrelin suppresses insulin in a paracrine fashion through AG binding at GHSR1a receptors in pancreatic islet cells. Growth hormone is known to increase insulin resistance (Rizza et al., 1982). However, the anti-insulin effect of ghrelin is abolished by GHSR1a blockade (Dezaki et al., 2012) and exogenous ghrelin still induces insulin resistance people with GH and ACTH deficiency. Taken together, these data suggest that ghrelin antagonises insulin independently of GH and ACTH (Vestergaard et al., 2008).

Ghrelin in cognition

There is a wealth of evidence from animal studies suggesting a role for ghrelin in learning and memory. The hippocampus is an important structure for laying down new memories (Lynch, 2004). It is situated in the temporal lobes, which are frequently atrophied early in the course of AD (Scheltens et al., 1992). Ghrelin is thought to acts at the hippocampus to improve learning and spatial memory in rodents. The first group to show this was Carlini *et al*, who demonstrated that intra-cerebroventricular (ICV) ghrelin administration increased the time taken for rats to step down onto a platform where they had recently received a shock (increased step-down latency), suggesting improved memory retention (Carlini *et al.*, 2002). The same group later went on to demonstrate that ICV ghrelin improved memory for object recognition in chronically food restricted mice (Carlini et al., 2002, Carlini et al., 2008), whilst intra-hippocampal ghrelin improved long term but not short term memory in rats performing the step-down test (Carlini et al., 2010a). All of these studies used

total ghrelin. Synthetic ghrelin agonists also positively impact memory in rats. Treatment with two different synthetic ghrelin receptor agonists (GSK894490A and CP-464709-18) improved object recognition and spatial memory compared with rats treated with vehicle (Atcha et al., 2009).

The pro-cognitive effects of ghrelin may be mediated through GHSR1a, which is expressed in the hippocampus (Guan et al., 1997). Intra-amygdaloid and intrahippocampal total ghrelin both increase step-down latency in rats but not when co-administered with a GHSR1a blocker (Tóth et al., 2009). Moreover, GHSR1a knockout mice have significantly impaired spatial memory compared to wild types (Davis et al., 2011). Not all studies support the finding that ghrelin improves learning and memory via GHSR1a, however. Albarran-Zeckler *et al.* found that GHSR1a knockout mice had superior performance in the Morris water maze compared to wild types. The authors speculate that the discrepancy between their results and those of Davies *et al.* and Ghersi *et al.* could be due to the different breeding techniques used, resulting in a different phenotype despite the same gene deletion, or to differences in mouse age (Albarran-Zeckler et al., 2012). The balance of evidence suggests, however, that ghrelin signalling via GHSR1a enhances learning and memory at the hippocampus in rodents. This may be through enhanced synaptic plasticity and neurogenesis.

Increased synaptic plasticity, due to enhanced long term potentiation (LTP) and synaptogenesis, has been proposed as a mechanism by which ghrelin may positively impact learning and memory (Carlini et al., 2010b). Long term potentiation is the process by which repeated stimulation of a neurone leads to a lasting (long term) up-regulation (potentiation) of neuronal activity in post synaptic neurones. It has been demonstrated to be important in the laying down of new memories and especially for hippocampal learning (Lynch, 2004). Electrophysiological studies have demonstrated that intra-hippocampal total ghrelin reduces the threshold for LTP and improves spatial memory in rats (Carlini et al., 2010b). An elegant study by Ghersi *et al.* used immunohistochemistry, electrophysiology and behavioural techniques to show that total ghrelin increased LTP and that enhanced LTP improved memory in rats. They did this by labelling NR2B-1R cells, which are important for LTP, and found that these cells are increased with ghrelin administration. The electrophysiological threshold for LTP was reduced in ghrelin treated animals

but this effect was abolished with NR2B-1R antagonism. Finally, step down latency was reduced when ghrelin infusion was preceded by a NR2B-1R antagonist (Gherzi et al., 2015). Total ghrelin has also been shown to increase pre-synaptic glutamate release, which is thought to be the first step in the process of inducing LTP (Izquierdo et al., 2006). Long-term potentiation increases the strength of synaptic connections in response to afferent stimuli. Diano *et al.* showed that total ghrelin administration increases synaptic density in the hippocampus of wild type mice and ghrelin knockout mice (Diano et al., 2006). The exact means by which ghrelin reduces the threshold for LTP in the hippocampus remains to be elucidated but may include ghrelin induced stimulation of receptor expression (AMPA and NMDA receptors) and neurotransmitter release in excitatory synapses (Carlini et al., 2010b, Gherzi et al., 2015, Ribeiro et al., 2014). Despite uncertainty regarding the underlying mechanism, it seems clear that ghrelin enhances LTP and that LTP mediates at least some of the pro-cognitive effects of ghrelin seen in rodents.

Neurogenesis, or the birth of new neurones, occurs in the adult mammalian hippocampus, including in humans (Eriksson et al., 1998). It has been proposed that these new-born neurones may be important in learning and memory (Deng et al., 2010). Animal studies using bromodeoxyuridine (BrdU), which labels newborn neurones by binding to DNA during mitosis, have shown that neurogenesis is enhanced if exogenous ghrelin is administered. Rats treated with total ghrelin and AG have better performance in tests of spatial memory including the spontaneous location recognition task, Y-maze and Morris water maze. These improvements are accompanied by greater numbers of BrdU labelled hippocampal cells, indicating increased neurogenesis compared with animals treated with saline (Kent et al., 2015). A study in ghrelin knock out mice demonstrated that they have fewer progenitor cells in the subgranular zone of the hippocampus than wild types. This corresponded with worse performance on the Y maze task and in novel object recognition. All of the deleterious effects in the ghrelin knockout mice were rescued with exogenous ghrelin administration (Li et al., 2013a). More recently, Cahill *et al.* showed that young GHSR1a knockout rats had fewer dendritic spines and fewer newborn neurones in the hippocampus compared with young wild type rats. The young rats' brains appeared to have aged prematurely, with dendritic spine density and

neurogenesis comparable to middle aged wild type rats. Interestingly, this was not reflected in their cognitive performance as tested by the radial arm maze and Morris water maze. Middle aged rats did worse with the radial arm maze than younger rats, regardless of genotype, and there were no differences in the water maze (Cahill et al., 2014). This is interesting as it has been suggested that ghrelin may improve memory and may enhance neurogenesis but that these effects may be distinct from each other and due to different mechanisms. This assertion is based on work in which mice were administered IP or intrahippocampal total ghrelin, underwent testing of learning and memory, and newborn neurones were labelled *in vivo* using BrdU. Intraperitoneal, but not intrahippocampal, ghrelin increased hippocampal neurogenesis. Conversely intrahippocampal, but not IP, ghrelin improved performance on the Morris water maze (Zhao et al., 2014). Overall however, the evidence from animal models would suggest that ghrelin enhances neurogenesis and that this may have positive cognitive effects.

Most of the animal studies above examined learning and memory acquisition in the context of normal cognitive ageing. Cognitive impairment in PD and in other dementias does not occur in this context, however. Dementias develop due to neurodegeneration of brain structures involved in attention, memory, executive functioning, visuospatial function and language. Accordingly, *in vitro* and *in vivo* studies have been carried out using models of neurodegenerative disease, especially AD. In *in vitro* studies looking at embryonic hypothalamic mouse cell lines treated with A β oligomers (ABO), ABO caused neuronal cell death via apoptosis. This cell death was accompanied by mitochondrial dysfunction and increased reactive oxygen species (ROS). These toxic effects were rescued when cells were pre-treated with total ghrelin. Moreover, GHSR-1a blockade abolished ghrelin's protective effects (Gomes et al., 2014). Similar results were seen for hippocampal neurones, in which total ghrelin improved cell survival, reduced oxidative damage and stabilised mitochondria when cells were treated with ABO (Martins et al., 2013).

In vivo, a number of different models have been used and most show positive effects of ghrelin on the resultant cognitive deficits. SAMP-8 and 5XFAD mice over-express A β and develop early cognitive problems as a result. These mice have improved cognition, fewer amyloid plaques, a reduced A β load and more

neurogenesis when treated with total ghrelin (Moon et al., 2014). In a different model, in which A β is injected into the mouse hippocampi, co-administration of intra-hippocampal total ghrelin attenuated the resultant spatial memory impairment and improved neuronal survival (Moon et al., 2011). Exogenous AG also reduced A β deposition and improved memory performance in rats infused with ICV A β_{25-35} (Kang et al., 2015b). Finally, APP over-expressing APP-SwDI mice had improved Morris water maze performance following treatment with the synthetic ghrelin agonist LY444711 (Dhurandhar et al., 2013).

To date only one animal study has failed to demonstrate positive effect of ghrelin on cognition in the context of neurodegeneration. In 2015 Madhavas *et al* created a rat model of AD using treatment with mono-sodium glutamate (MSG), which causes obesity in rats. They then looked at A β burden, acetylcholinesterase (AChE) levels, known to be high in AD, and performance in the Barnes maze task, a test that requires animals to navigate using learned visual cues. Obese MSG treated rats had a greater A β burden, greater AChE activity and reduced performance on Barnes maze. A GHR1a blocker, [D-Lys3] GHRP-6, reduced A β burden and AChE and improved Barnes maze test performance. Interestingly, [D-Lys3] GHRP-6 also resulted in weight loss and increased endogenous ghrelin levels in MSG treated rats. The metabolic profile of the animals with regard glucose and lipid handling also improved. It may be that improvement in weight and restoration of metabolic profile, including increased endogenous ghrelin levels, could have had a hand in the molecular and behavioural improvements seen with [D-Lys3] GHRP-6 (Madhavadas et al., 2014). The overall body of evidence is strongly in favour of a role for ghrelin in ameliorating cognitive decline in animal models of dementia.

Given the strength of the data for rodent models of AD it may be expected that human studies would show a strong link between ghrelin and learning and memory. There are several studies in support of this. Bellar *et al* carried out an observational study on 28 healthy older adults aged 60-94. Participants had fasting total ghrelin levels taken followed by a light snack before undergoing cognitive testing. Learning and memory was assessed using the Hopkins verbal learning test (HVLT) and global cognition using the mini mental state examination (MMSE). Higher fasting total ghrelin levels were associated with better performance on HVLT (Bellar et al., 2013). The following year a large

observational study looked at single nucleotide polymorphisms (SNPs) of ghrelin and cognition in 280 people (137 men and 143 women). Global cognition was assessed using the MMSE and people were grouped according to MMSE performance, those with a score less than 24 were deemed to have cognitive impairment and those with an MMSE of 24 or above cognitively intact. They found that SNPs in the ghrelin gene were not associated with altered ghrelin levels but that one particular polymorphism called L90G was associated with cognitive impairment, even after adjusting for age, gender, alcohol, depression, glucose impairment, education and ApoE status (Mora et al., 2014). In 2015, Alosco *et al* looked at fasting total ghrelin, leptin and cognition in 84 obese patients aged 20-70 prior to and 12 months following bariatric surgery for the treatment of morbid obesity. Cognition was tested using IntegNeuro computer based cognitive testing which tests attention, executive function, verbal interference, memory and language. The authors found that total ghrelin levels increased after surgery and that these changes predicted improvements in attention and executive function on regression analysis (Alosco et al., 2015). More recently, a study of 356 middle aged women, 247 of whom have chronic HIV infection demonstrated a positive correlation between cognition and AG levels as tested by trail making tests and stroop interference (McFarlane et al., 2017). Finally, a double-blind placebo controlled randomised crossover study examined 21 young men who were administered AG or saline prior to carrying out memory tasks during functional MRI (fMRI). The AG treated group had increased cortical activity on fMRI after ghrelin infusion. The AG treated participants did not demonstrate improved performance in remembering food items that they had been shown on a virtual conveyor belt, however. This was a small study and may have been underpowered to detect differences in memory performance. It could be argued that if there has been a type 2 error here, the degree of improvement may not be clinically significant (Kunath et al., 2016).

Other studies have also shown no, or negative correlations between ghrelin and memory performance in healthy adults. Spitznagel *et al.* studied 35 cognitively intact elderly participants. Participants with higher AG levels had worse cognitive performance than those with lower levels (Spitznagel et al., 2010). In the same year Dresler *et al* administered ghrelin to healthy adults over night but found no improvement in memory consolidation with ghrelin treatment (Dresler

et al., 2010). It remains to be clarified, therefore, whether or not ghrelin is important for learning and memory in adult humans.

The role of ghrelin in patients with neurodegenerative dementia is similarly unclear. Theodoropoulou *et al.* investigated dynamic total ghrelin levels in 10 men and 17 women (17) with AD and age- and sex-matched controls. They found that the area under the curve for total ghrelin was significantly lower in men, but not women, with AD (Theodoropoulou et al., 2012). A study from the Netherlands Brain Bank showed that ghrelin, GHSR1a and GOAT expression were lower in the inferior temporal lobes of AD patients compared with controls. Moreover, GHSR1b, a receptor whose downstream effect is to inhibit ghrelin, was increased in AD patients. Together, these data suggest a disordered ghrelin response in AD (Gahete et al., 2010). Finally, a Japanese study found a small but significant association between a SNP (Leu90GLn) in the gene encoding ghrelin and AD. They found this region to be highly polymorphic, however, and no other SNPs shared this association (Shibata et al., 2011). Conversely, an Iranian study looking at 37 people with AD and 34 controls found that fasting AG levels were higher in AD than in control groups. It is worth noting that the control group was significantly younger than the AD group, which may have confounded results (Akbarzadeh et al., 2013). No studies have examined ghrelin in PDD to date.

Evidence from animal models would suggest that ghrelin is important for memory acquisition, especially spatial memory. Despite this, human studies are not yet able to clarify whether or not ghrelin is important for cognitive function in man. A role for ghrelin in spatial memory is intuitive when we consider the problem in evolutionary terms. Ghrelin levels are high in times of calorie restriction when improved memory for how and where to acquire food may be beneficial. It has been proposed that ghrelin is the “missing link” between calorie restriction and cognitive performance. Like ghrelin, calorie restriction has been shown to increase neurogenesis in rodents (Ferreira-Marques et al., 2016, Li et al., 2013a, Lee et al., 2002). Calorie restriction has also been shown to improve cognition in elderly humans (Witte et al., 2009). Even if ghrelin is not a cognitive enhancer in and of itself, it may have an important role in neuroprotection in neurodegenerative dementias, including PDD.

Ghrelin and neuroprotection

In addition to having a putative role in cognitive enhancement, ghrelin has been shown to be neuroprotective in animal models of stroke, traumatic brain injury and PD. Although, cell death from necrosis does occur in these conditions, apoptosis, or programmed cell death, is a major cause of neuronal loss (Anglade et al., 1997, Cheyuo et al., 2011, Lopez et al., 2012). There is evidence from animal models that ghrelin reduces neuronal apoptosis and improves cell survival in the face of cellular insult. This was first demonstrated in 2006 by Liu *et al* who created an ischaemia/ reperfusion brain injury rat model by ligating both vertebral arteries and then clamping both carotid arteries for 8 minutes a control group underwent sham operation. Rats from each group were treated with i.p. saline or total ghrelin. The animals were sacrificed and their hippocampi examined. Saline treated rats who underwent cerebral ischaemia/reperfusion injury had marked hippocampal apoptosis compared to animals undergoing sham operation. Ghrelin treated rats had little hippocampal apoptosis, whether or not they had ischaemia/reperfusion injury (Liu et al., 2006). This was then replicated *in vitro* when hypothalamic cells were deprived of oxygen and glucose for 30 minutes, 50% of them underwent apoptosis and 50% remained viable. Pre-treatment with total ghrelin shifted this balance so that up to 83.7% of cells were viable after 30 minutes of glucose and oxygen deprivation. The authors demonstrated that ghrelin prevents apoptosis via activation of the ERK pathway, an effect which was abolished with GHSR1a inhibition (Chung et al., 2007). Since then a number of studies have shown that ghrelin increases the Bcl-2-bax ratio, inhibiting caspase 3 activation and thereby reducing apoptosis in neuronal cells in response to a number of different insults (Chung et al., 2011, Chung et al., 2008, Hwang et al., 2009, Lim et al., 2011a, Xu et al., 2009).

When mitochondria are damaged, their membranes depolarise and ROS are produced, augmenting cellular damage and promoting apoptosis. Ghrelin has been shown to reduce apoptosis by preventing both mitochondrial depolarisation (Martins et al., 2013) and the release of pro-apoptotic factors such as cytochrome C (Dong et al., 2009, Lee et al., 2010a, Lee et al., 2010b, Yu et al., 2016). These protective effects may be achieved through uncoupling proteins (UCPs), which reduce mitochondrial respiration by diverting oxidative

phosphorylation away from the electron transport chain. Uncoupling of respiration from mitochondria results in membrane stabilisation, a reduction of ROS production and reduced apoptosis. It has been demonstrated that ghrelin increases UCP2 expression in hippocampal cells *in vitro* (Liu et al., 2009). Lopez *et al* demonstrated increased UCP2 in ghrelin treated rats following traumatic brain injury. This was associated with reduced caspase 3 activation and reduced apoptosis *in vivo* (Lopez et al., 2012).

Other ways in which apoptosis may be prevented is through the removal of toxic proteins and damaged organelles via autophagy and through a reduction of inflammation. Autophagy is important in cellular maintenance and, if disrupted, the cellular environment may become toxic leading to mitochondrial damage and ultimately cell death. Ghrelin has very recently been shown to increase autophagy in rat neurones *in vitro* via the GHSR1a receptor (Ferreira-Marques et al., 2016). Dysregulation of autophagy is thought to be important in neurodegenerative disease, including PD. As discussed above, ghrelin is a powerful anti-inflammatory. This has been proposed as a further mechanism by which ghrelin may be neuroprotective. Neuroinflammation is characterised by microglial activation. Ghrelin treatment has been shown to reduce microglial activation and neuronal apoptosis in rodents treated with neurotoxins such as A β O, kainic acid and 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP), with attendant improvement in neurological performance in each case (Jiang et al., 2008, Lee et al., 2010a, Moon et al., 2011, Moon et al., 2009, Yu et al., 2016). MPTP is a compound known to be toxic to dopaminergic cells and often used to generate animal models of PD (Andrews et al., 2009, Jiang et al., 2008).

There is good evidence that ghrelin is neuroprotective at least in the context of acute insults in rodents. To date, there have been no published trials evaluating ghrelin as a neuroprotective agent in humans.

Ghrelin in Parkinson's disease

There is accumulating evidence that ghrelin may be disrupted in PD and decreased ghrelin levels have even been proposed as a biomarker for the development of PD. In 2011, Unger *et al.* carried out a cross-sectional study of 39 PD patients, 11 with REM-sleep behaviour disorder (iRBD) and 20 healthy controls. Patients with iRBD are at greatly increased risk of PD compared to the

general population and iRBD has therefore been proposed as a pre-motor phase of PD. The authors found that recovery of total ghrelin levels after eating was attenuated in both the PD and iRBD groups compared to controls and that this difference was significant ($p=0.002$ and $p=0.037$ respectively) (Unger et al., 2011). There are limitations to this research. Total ghrelin, rather than AG was used. When PD participants were sub-divided into treated and drug naïve PD there was no difference between drug naïve participants compared with healthy controls, though this may have been due to small sample size. Finally, progression from iRBD to PD is not inevitable. Despite this, it suggests that ghrelin secretion may be disordered in PD patients. There is further evidence to support this hypothesis. A cross-sectional study investigating AG levels in patients with PD with weight loss, without weight loss and controls found a paradoxical correlation between AG levels and BMI in PD patients with weight loss, that is to say that as BMI dropped, ghrelin levels dropped. This is the opposite to the trend seen in healthy people in whom weight loss triggers increased ghrelin production in order to stimulate feeding behaviour and restore body weight. It is worth noting that despite these results, there was no significant difference in AG levels between groups (Fischer et al., 2010). More robust evidence comes from a very recent study of fasting AG and total ghrelin in 291 people with PD and 303 age matched controls. The authors found that both AG and total ghrelin levels were lower in the PD group ($p<0.05$). Interestingly, there was no difference in AG or total ghrelin according to Hoehn and Yahr stage. They went on to study the dynamic AG and total ghrelin responses to glucose ingestion. Participants with PD had an attenuated AG and total ghrelin recovery at 180 minutes (Song et al., 2017). This was a large, well designed study measuring both AG and total ghrelin. Taking all of this research together it seems likely that that ghrelin secretion is abnormal in PD. We know from Braak *et al* that the DMNV is frequently damaged early in the course of PD. Unger *et al* speculate that disruption of the brain-gut axis via the vagus nerve may result in disordered ghrelin secretion in PD (Unger and Oertel, 2014).

Ghrelin is likely to be neuroprotective in PD. Neuroinflammation, mitochondrial instability, disordered autophagy and, ultimately, apoptosis are all implicated in the pathogenesis of PD and reduced by ghrelin. Studies using animal models of

PD support this. Jiang *et al.* pre-treated mice with intra-cerebroventricular total ghrelin prior to administering MPTP and found that ghrelin prevented MPTP induced nigral cell apoptosis (Jiang et al., 2008). Similarly, Andrews *et al.* demonstrated that intraperitoneal ghrelin protected mice from MPTP-mediated dopaminergic cell loss through upregulation of UCP2 and consequent mitochondrial membrane stabilisation. Ghrelin and GHSR1a knockout mice were more susceptible to dopaminergic cell loss from MPTP than wild types (Andrews et al., 2009). In the same year, Dong *et al* showed that total ghrelin stabilised mitochondria and reduced apoptosis in dopaminergic cells *in vitro* and Moon *et al.* found that total ghrelin pre-treatment reduced microglial activation and reduced dopaminergic cell loss in the mouse SNpc *in vivo* (Dong et al., 2009, Moon et al., 2009, Yu et al., 2016). More recently, Yu *et al* treated dopaminergic cells with the mitochondrial poison rotenone, which causes parkinsonism in animal models. The cells showed mitochondrial dysfunction and apoptosis following treatment and these effects were ameliorated by pre-treatment with total ghrelin (Yu et al., 2016). Finally, Bayless *et al* showed that AG, not UAG is responsible for neuroprotection against MPTP as cell loss was reduced with AG, but not UAG, administration to ghrelin knock out mice (Bayliss et al., 2016a). There is, in summary, good evidence for a role for ghrelin in neuroprotection in PD.

If ghrelin levels are low in PD and ghrelin is neuroprotective, it is possible that pathological progression in PD is accelerated by the loss of endogenous neuroprotection by ghrelin. Ghrelin would therefore appear to be an attractive area for research in the development of disease modifying therapy, with potential roles in slowing disease progression and preventing cognitive decline. Ghrelin may also be a candidate for symptomatic treatments in PD. GHSR1a and D1 dopamine receptors co localise throughout the brain, including in the substantia nigra (Jiang et al., 2006). There is evidence that ghrelin increases augments dopamine release in the SNpc, at least in response to MPTP in animal models (Andrews et al., 2009). Ghrelin has also been demonstrated to augment extracellular dopamine release in the dopaminergic-cholinergic reward circuits (NAc and VTA) of rodents (Jerlhag et al., 2007, Jiang et al., 2006, Abizaid et al., 2006). This effect is attenuated by GHSR1a antagonism, suggesting that ghrelin induced dopamine release is mediated by ghrelin acting

at GHSR1a (Jerlhag et al., 2012b). It is therefore plausible that ghrelin could improve motor symptoms. Moreover, ghrelin increases gut motility and may improve symptoms of gastroparesis and constipation, which are common in PD (Barboza et al., 2015). Animal studies have shown that ghrelin reverses levodopa-induced gastroparesis and the ghrelin agonist HM01 is prokinetic and relieves constipation in animal models of PD (Wang et al., 2012, Karasawa et al., 2014). A ghrelin agonist is currently being trialled as a treatment for constipation in people with PD (Schrag et al., 2015).

The effects of dopamine on ghrelin activity are less clear. A study using cultured ghrelin-producing MGN3-1 (mouse ghrelinoma) cells found that the D₁ agonist fenoldopam stimulated ghrelin release but the D₂/D₃ agonist bromocriptine did not (Iwakura et al., 2011). Another study in Sprague-Dawley rats found that high dose antagonism of D₁ (with the experimental drug SCH23390) and D₂ and D₃ receptors (with eticlopride) reduced ghrelin-induced food intake in these animals. The authors also found that D₁ and D₂ stimulation with the agonists SKF38393 (D₁) and quinpirole (D₂) attenuated the orexigenic effects of exogenous ghrelin, an apparently directly opposing finding (Romero-Picó et al., 2013). The role of dopamine in the control of ghrelin release and signalling therefore remains unclear. Weight gain has been reported in patients treated with dopamine agonists, largely attributed to compulsive eating (Nirenberg and Waters, 2006). It is difficult to fully exclude ghrelin as a contributor to this phenomenon. Against this, only D₁ agonism has been shown to increase ghrelin and even then only in vitro studies (Iwakura et al., 2011). The most commonly prescribed dopamine agonists pramipexole and ropinirole have negligible D₁ activity, exerting their effects on D₂ and D₃ receptors instead (Gerlach et al., 2003). Further research is needed to definitively clarify this relationship.

No studies have yet been carried out looking at cognition and ghrelin in PD. Taking together the evidence suggesting that; 1) weight loss predisposes to cognitive decline in PD, 2) weight loss in PD may be associated with abnormally low ghrelin levels, 3) ghrelin may be a cognitive enhancer and 4) ghrelin is likely to be neuroprotective in PD, it is reasonable to hypothesise that lower ghrelin levels may be associated with cognitive decline in PD.

Insulin-like growth factor-1

Insulin-like growth factor 1 (IGF-1) is not directly involved in the maintenance of body energy stores, and may not therefore be described as a hormone of energy homeostasis. It is, however, intimately linked to ghrelin, may have a role in learning and memory and has been proposed to be neuroprotective in neurodegenerative disease so it is important to consider here. The majority of IGF-1 is produced by the liver in response to GH, which is itself stimulated by ghrelin. Ghrelin has been shown to increase insulin-like growth factor when administered to rats (Nagaya et al., 2001b). Interestingly, this has not yet been demonstrated in humans (Nagaya et al., 2001a). Up to 80% of IGF-1 in the circulation and CSF is protein bound to IGF-1 binding proteins (IGFBP) (Paolisso et al., 1997). Insulin-like growth factor-1 is actively transported across the BBB at the choroid plexus (Torres-Aleman, 2000) and locally within the brain, especially during neural development and synaptogenesis (Åberg et al., 2006). IGF-1 binds with its receptor but also insulin and insulin-like growth factor-2 receptors, though it has greatest affinity for its own receptor (Sara and Hall, 1990). IGF-1 receptors are expressed throughout the BBB and brain but especially in the choroid plexus, cortex and striatum (Åberg et al., 2006). As with GH, levels of IGF-1 decline with age (Al-Delaimy et al., 2009, van Dam and Aleman, 2004) and, in keeping with its' relationship to ghrelin, IGF-1 levels tend to decline with increasing BMI (Faupel-Badger et al., 2009). IGF-1 is a mitogenic hormone, which is to say that it promotes tissue maintenance through the birth of new cells. This has been shown to occur in the brain as well as in other tissues (Åberg et al., 2000, Åberg et al., 2006). This was recognised as early as the 1990s and there is therefore a large body of research investigating putative cognitive enhancement and neuroprotective properties of IGF-1.

To my knowledge, the first study to show that IGF-1 may be important in cognition was carried out in 1997 by Paolisso *et al* who demonstrated that free IGF-1 expressed as a high IGF-1:IGFBP ratio correlated with MMSE performance in Italian centenarians (Paolisso et al., 1997). Two further confirmatory studies quickly followed. In 1998 Rollero *et al* demonstrated that serum IGF-1 levels correlated with MMSE in older adults. The following year Aleman *et al.* demonstrated that IGF-1 levels were positively correlated with performance on the digit symbol substitution test and concept shifting tasks in

25 healthy older men, even after adjustment for age. Performance in these tests commonly declines with age, whereas reading ability, general knowledge and vocabulary do not. The authors proposed that declining IGF-1 levels may be responsible for, or at least contribute towards cognitive decline with ageing (Aleman et al., 1999). Since then, these findings have been widely replicated (Table 4) and a meta-analysis in 2009 confirmed that IGF-1 levels positively correlate with cognition in healthy older people (Arwert et al., 2009).

Table 4. Human studies exploring the relationship between IGF-1 and cognition

Study	Population studied	Mean age (years ± SD)	Study design	Outcome
(Aleman et al., 1999)	25 healthy older men	69.1±3.4	Cross-sectional	IGF-1 levels correlated with performance on digit symbol substitution test (p=0.009) and concept shifting task (p=0.005)
(Adamis et al., 2009)	67 people aged over 70 admitted to elderly medical unit (48 women)	82.4 ± 6.3	Observational prospective	IGF-1 negatively correlated with presence and severity of delirium (p<0.05)
(Adamis et al., 2014)	142 people admitted consecutively to elderly care (98 women)	84.8 ± 6.4	Longitudinal observational	IGF-1 negatively correlated with disease severity IGF-1 positively correlated with degree of recovery. Authors report low IGF-1 in reversible cognitive impairment.
(Al-Delaimy et al., 2009)	1535 Healthy older people adults from Rancho Bernardo Study (899 women)	Median age 74	Retrospective cross-sectional	IGF-1 positively correlated with IGFBP-1. MMSE, trail making B and verbal fluency performance in men but not women (p=0.001). IGFBP-1 was inversely correlated with MMSE in men (p=0.02).
(Aleman et al., 2000)	17 healthy men aged 66-76	70.1 ± 3.39	Cross-sectional	IGF-1 positively correlated with concept shifting task (p≤0.01) No correlation with tests that do not decline with age (Benton judgment line of orientation test and Brus reading test).
(Angelini et al.)	75 people with hypertension (48 women)	78	Cross-sectional	IGF-1 correlated with improved global cognition (MMSE p=0.012, CAMCOG 0.027 and FAB 0.004)
(Arai et al.)	49 Japanese male and female centenarians (35 women)	100.4±1.1	Cross-sectional	The cohort was divided along the 50 th percentile for IGF-1. The high IGF-1 group had significantly less dementia than the low IGF-1 group (p=0.043)
(Arwert et al., 2005)	24 elderly people (10 women)	80.8±2.3 low IGF-1 and 78.6 ±3.8 for high IGF-1 (p=0.13)	Cross-sectional	The cohort was split into high and low IGF-1 groups. The high IGF-1 group had improved reaction times on the (p<0.04).
(Bellar et al., 2011)	28 Healthy people older adults (22 women)	70.8±9.3	Cross sectional	Higher levels of IGF-1 correlated with improved TMTB performance (p=0.01) and Ruff's test for attention (p=0.011).
(Dik et al., 2003)	1318 older people from the Longitudinal Aging Study Amsterdam N= 1022 at 3 years (40.7-50.8% men depending on IGF-1 quintile)		Cross-sectional and 3 year follow up	Cognitive performance worst in the lowest IGF-1 quintile at baseline Possible threshold effect after 3 rd quintile. The lowest 3 quintiles had greater cognitive decline over 3 years (RR 2.5 CI 1.37-3.44 in lowest quintile).
(Doi et al., 2014)	3355 Japanese older people from the Obu Study of Health Promotion in the Elderly (53.5% women)	71.4	Cross-sectional	All positively correlated with IGD-1 (p<0.001)

Study	Population studied	Mean age (years \pm SD)	Study design	Outcome
(Euser et al., 2008)	1015 men and women over 85 from the Leiden 85-plus study (68% women)	87.4 \pm 3.1	Longitudinal (5 year follow up)	Number of SNPs in the IGF-1 gene (suggesting disrupted IGF-1 signalling) negatively correlated with cognition in women (OR of cognitive decline 1.28 CI 1.06-1.53 per polymorphism)
(Green et al., 2014)	746 men from the Caerphilly Prospective Study who had blood taken in 1986		Retrospective longitudinal (17 years follow up)	No association between baseline IGF-1 and cognition at follow up.
(Han et al.)	40 alcohol-dependent Korean men	47.98 \pm 6.38	Cross-sectional	IGF-1 was positively correlated with TMT A performance but no other cognitive tests ($p < 0.001$)
(Kalmijn et al., 2000)	186 healthy older men and women from the Rotterdam Study (50% women)	67.4 \pm 5.6 at baseline	Longitudinal (1.9 year follow up)	Reduced rate of cognitive decline with higher IGF-1 levels (OR 0.65 CI 0.44-0.95)
(Landi et al.)	353 older Men and women from the <i>Invecchiamento e Longivita nel Sirente</i> (ILSIRENTE) study (236 women)	85.6 \pm 4.8	Cross-sectional	Lower IGHF-1 levels were associated with cognitive impairment
(Lin et al.)	94 healthy older men and women (55 women)	60.68 \pm 8.42	Cross sectional	IGF-1 positively correlated with performance on AVLT and negatively correlated with mood. Depression and poor cognitive performance were correlated in low, but not high LGF-1 groups.
(Okereke et al., 2005)	590 women from the Nurses' Health Study	63.9-64.6 per IGF-quintile	Longitudinal (10 year follow up)	IGF-1 levels correlated with global cognition combining all test scores ($p = 0.07$)
(Okereke et al., 2006)	460 men from the Physicians' Health Study II (376 had free IGF measured)	57	Longitudinal (20 year follow up)	Higher levels of free IGF-1 correlated with improved global cognition ($p = 0.02$)
(Rollero et al., 1998)	22 older Men and women (7 women)	Median age 77	Cross-sectional	IGF-1 correlated with performance on MMSE
(Paolisso et al., 1997)	30 adults <50, 30 adults aged 75-99, 19 adults >100 Men and women (46%, 56% and 57% women respectively)	44.5 \pm 1.8, 78 \pm 0.7 and 102 \pm 0.8 depending on group	Cross-sectional	IGF and IGFBP-3 declined with age. Centenarians had a greater IGF-1/IGFBP-3 ratio. IGF-1/IGFBP-3 correlated with cognition in centenarians

The picture is less clear in neurodegenerative dementias, with high, low and normal levels of IGF-1 associated with dementia depending on the study (Table 5). This may be due, in part, to different subject selection between studies and may reflect the complex interaction with other hormones and dynamic nature of IGF-1 and insulin signaling across the life course. It has been suggested that IGF-1 action may be disordered in AD due to BBB pathology and disrupted receptor signaling. This is supported by the literature as some of the studies showing higher serum IGF-1 in AD have found that CSF IGF-1 is not elevated (Johansson et al., 2013, Tham et al., 1993). In this model higher IGF in the peripheral circulation may be compensatory (Trejo et al., 2007b). A post-mortem study of the brains of AD sufferers and controls found A β induced IGF-1 resistance and disordered downstream signaling (Talbot et al., 2012). Alternatively, the relationship between cognition and IGF-1 may not be linear in degenerative dementias. In support of this some human studies have demonstrated a threshold effect for cognition and IGF-1 in degenerative dementias, below which lower IGF-1 is associated with greater impairment but above which further increase in IGF-1 does not confer added benefit (Watanabe et al., 2005, Westwood et al., 2014). It therefore remains possible that IGF-1 has positive effects on cognition in neurodegenerative disease. There is certainly compelling evidence from animal studies that brain IGF-1 may be neuroprotective. Numerous studies have shown increased neurogenesis, reduced apoptosis, reduced neuroinflammation and increased cell survival with IGF-1 treatment in animal and *in vitro* models of diabetes-induced cognitive impairment, head injury, stroke and AD (Åberg et al., 2000, Bake et al., 2014, Carro et al., 2006, Carro et al., 2002, De Geyter et al., 2016, De Magalhaes Filho et al., 2016, Doré et al., 1997, Hu et al., 2015, Lichtenwalner et al., 2001, Lupien et al., 2003, Lupien et al., 2005, Saatman et al., 1997, Trejo et al., 2007a, Trejo et al., 2007b). Furthermore, the anti-apoptotic actions of IGF-1 appear to be mediated by PI3K/Akt activation, which is implicated in A β handling and reduced tau hyperphosphorylation (Wen et al., 2008). Finally, there is good evidence that IGF-1 has positive effects on A β clearance from the brain parenchyma through recruitment of amyloid binding proteins such as albumin and transthyretin across the blood brain barrier (Carro et al., 2002). Despite this, therapeutic trials of IGF-1 replacement have been disappointing,

and no clinically meaningful improvement in cognition has yet been demonstrated with exogenous IGF-1 in humans (Sevigny et al., 2008).

In addition to work in AD, there are a number of studies examining IGF-1 in PD. Again, there is discordance in the literature (Table 6) but meta-analysis suggests that IGF-1 levels are increased in early PD compared to controls (Li et al., 2015b). The pattern of change in IGF-1 levels with disease progression is still debated. One study reports greater disease severity (measured by the part 3 of the movement disorder society unified Parkinson's disease rating scale [MDS UPDRS]) in the highest quartile of IGF-1, though similar levels of disease severity are seen in the lowest quartile (Numao et al., 2013). Conversely, another study reported a negative correlation between IGF-1 and disease severity though statistical significance was lost with correction for both age and BMI (Suzuki et al., 2014). Disease duration has also been reported to negatively correlate with IGF-1 levels in PD (Godau et al., 2009). It has been argued that increased IGF-1 in early PD may represent upregulation in the face of neuronal damage (Godau et al., 2009). An alternative interpretation of these data is that elevated IGF-1 levels increase the risk of PD, through as yet unclear means (Godau et al., 2011). This has been suggested due to the observation that reduced IGF-1 signaling may increase longevity, even in humans, and may also increase neuronal tolerance to oxidative stress, implicated in neuronal loss in PD (Holzenberger et al., 2003, Van Heemst et al., 2005). Against this hypothesis, there is evidence of disordered IGF-1 signaling in PD, which would be unexpected if reduced signaling was protective. A post-mortem study of the brains of people with PD, DLB and controls demonstrated impaired IGF-1 signaling in PD and DLB compared to controls, with a greater degree of impairment in DLB. Moreover, data from an epigenetic studies showed that gene regulation for IGF-1/PI3K/AKT signalling was impaired in post-mortem brains of people with PD compared to controls, again suggesting that reduced IGF-1 signalling failed to confer an advantage (Kim et al., 2014). Moreover, there is a wealth of evidence from animal models that IGF-1 may be neuroprotective in PD, through neuroproliferative, anti-inflammatory and anti-apoptotic mechanisms similar to those described above (Arboleda et al., 2007, Guan et al., 2000, Quesada et al., 2008, Wang et al., 2010), along with changes in α -synuclein handling. An *in vitro* study of cultured SH-SY5Y neuroblastoma

cells showed that exogenous IGF-1 was able to prevent α -synuclein aggregation in response to dopamine treatment, through activation of the PI3K/Akt pathway (Kao, 2009).

There has been one study attempting to examine IGF-1 in cognition in PD to date. Pellecchia *et al.* carried out a longitudinal observational study of 65 people with newly diagnosed PD. They found that baseline IGF-1 positively correlated with executive function at baseline and at 2 years. Moreover, lower IGF-1 levels at baseline were associated with steeper cognitive decline (Pellecchia *et al.*, 2013).

Although ongoing research is needed to clarify the role of IGF-1 in neurodegenerative disease, it is plausible that it is neuroprotective in PD and may even ameliorate cognitive impairment in PD. It is possible that this is a further means by which ghrelin may exert neuroprotective effects.

Table 5. Studies exploring the relationship between IGF-1 and dementia

Study	Population studied	Mean age years (\pm SD)	Study design	Outcome
(Alvarez et al., 2005)	141 AD, 56 MCI and 30 controls (total 227)	75.25 \pm 8.59 AD, 72.87 \pm 8.14 MCI and 68.87 \pm 8.77 controls	Case control	IGF-1 lower in AD than controls after age adjustment (p <0.05)
(Duron et al., 2012)	694 older Men and women with and without AD N=224 AD, 257 MCI, 213 controls	78.6 \pm 6.7	Case control	IGF-1 positively correlated with cognition in men across all groups (p <0.01) and higher IGF-1 levels correlated with a reduced risk of AD in men (OR 0.48 CI 0.26-0.88) These relationships not seen in women.
(Garcia et al., 2006)	367 older adults 72 AD, 75 VaD, 14 mixed dementia, 209 controls	77 \pm 6 dementia 69 \pm 5 controls	Case control	IGF-1R gene polymorphisms were more common in women with VaD than other groups.
(Johansson et al., 2013)	80 older men and women N= 32 AD, 13 stable MCI, 15 other dementia and 20 controls.	75 AD, 72 MCI, 74 other dementias, 75 controls	Case control	Serum IGF-1 elevated in AD (p =0.01) and other dementias (p <0.05). Over the whole cohort IGF-1 negatively correlated with CSF amyloid. CSF/serum IGF-1 ratio lower in the dementia group,
(Kimoto et al., 2015)	70 Japanese men and women with AD and 10 with MCI	Median age 78	Cross-sectional	IGF-1 levels correlated with A β 42/A β 40 ratio (p =0.007), which is thought to represent improved A β 42 clearance IGF-1 was correlated with performance on cognitive tests (p <0.001).
(Salehi et al., 2008)	82 men and women N=41 with AD and 41 controls	61-75	Cross-sectional	CSF IGF-1 is higher in AD than in controls
(Talbot et al., 2012)	Post mortem AD and normal controls from the University of Pennsylvania and the Religious Orders Study.		Post-mortem	Severe insulin and IGF-1 resistance in AD brains compared with controls, IGF-1 levels were not different between groups
(Rivera et al., 2005)	Post mortem study of brains from people with AD		Post mortem	IGF-1 and IGF-1 R were reduced in AD brains with increasing disease severity.
(Tham et al., 1993)	20 people; 10 healthy men, 6 men and 4 women with AD	65-83 (controls) 63-74 (AD)	Case-control	Serum IGF-1 was elevated in people with AD compared to controls, though CSF IGF-1 was not.
(Watanabe et al., 2005)	436 Japanese men and women N=106 AD, 103 VaD, 227 controls	79 \pm 7 AD, 77 \pm 8VaD, 76 \pm 10 control	Case control	IGF correlated with MMSE across the whole cohort. No added benefit above 50 th percentile. Low IGF-1 associated with AD (OR 2.1 CI 1.21-3.64) and VaD (OR 3.4 CI 2.1-5.61)
(Watanabe et al., 2004)	60 Japanese men and women over 65, 35 VaD and 25 controls	79 \pm 9 VaD, 76 \pm 6 controls	Case control	IGF-1 correlated with performance on MMSE across all groups. IGF-1 was lower in VaD patients than controls.
(Westwood et al., 2014)	3582 dementia-free older men and women from the Framingham Study	65 \pm 11	Longitudinal (7.9 year follow up)	230 incident cases of AD and 49 with other dementias. Lowest quartile of IGF-1 at baseline was associated with increased risk of AD (HR 1.51 CI 1.14-2.00)
(Van Exel et al., 2014)	406 adults, 206 children of people with AD and 200 people with no family history of AD		Case control	IGF-1 levels higher in the children of AD sufferers than controls.

Table 6. Studies exploring the relationship between IGF-1 and Parkinson's disease

Study	Population studied	Mean age years (\pm SD)	Study design	Outcome
(Bernhard et al., 2016)	37 men and women with PD and 22 healthy controls from the Modelling Epidemiological data to Study Parkinson's disease progression (MODEP) study	64 \pm 7	Longitudinal 4 year follow up	IGF-1 higher in PD than controls at baseline ($p=0.026$). When stratified for disease severity levels only significant in moderate PD ($p=0.004$). No differences in IGF-1 decline over time between groups
(Godau et al., 2009)	12 men and women with established PD 6 with newly diagnosed PD and 12 controls for established PD and controls, 33% for new PD	66 \pm 6 established PD and controls, 67 \pm 9 new PD	Longitudinal 6 month follow up	IGF-1 levels higher in PD patients ($p<0.001$). IGF-1 negatively correlated with disease duration.
(Godau et al., 2011)	15 men and women with untreated PD and 139 healthy controls	69 \pm 9.3 PD and 60.6 \pm 6.6 controls	Cross-sectional	IGF-1 higher in PD than controls ($p=0.004$). Motor disease severity(MDS UPDRS) negatively correlated with IGF-1
(Ma et al., 2015)	216 Chinese men and women 100 with PD, 40 with essential tremor (ET) and 76 controls	67.84 \pm 9.89 PD, 70.85 \pm 11.39 ET and 67.75 \pm 8.55 controls	Cross-sectional	IGF-1 increased in PD compared to ET and controls ($p<0.001$). No correlation between IGF-1 and disease duration or levodopa dose equivalent (LED). IGF-1 correlated with cognition in PD ($p=0.026$). IGF-1 and IGFBP higher in PD (both $p<0.001$)
(Mashayekhi et al., 2010)	76 Iranian men and women people 38 with PD and 38 controls	58-78	Cross-sectional	IGF-1 and IGFBP higher in PD (both $p<0.001$)
(Numao et al., 2013)	172 Japanese Men and women, 79 with PD, 25 with MSA, 16 with PSP and 52 healthy controls	66.9 \pm 1.9 for MSA, 68.1 \pm 1.1 PD, 72.7 \pm 2.0 PSP and 65.6 \pm 1.2controls	Cross-sectional	IGF-1 levels were higher in MSA patients compared to PD and controls ($p<0.001$). Trend towards increased IGF-1 in early PD compared to controls not statistically significant. In PD patient IGF negatively correlated with disease severity but significance was lost when corrected for BMI and age (Suzuki et al.).
(Pellecchia et al., 2013)	65 men and women with newly diagnosed PD	58.5 \pm 8.3	Longitudinal (2 year follow up)	IGF-1 correlated with executive function at baseline and baseline IGF negatively correlated with cognitive decline over 2 years.
(Picillo et al., 2013)	37 men and women with newly diagnosed PD and 60 controls	59.4 \pm 9 PD and 58.8 \pm 7 controls	Longitudinal (2 year follow up)	IGF-1 was higher in PD than in controls ($p=0.01$). The highest IGF-1 quartile has significantly more severe motor symptoms compared to the third and second quartiles, but not the lowest quartile ($p=0.002$. 0.005 and 0.1 respectively).
(Tong et al., 2009)	Brains from 7 people with PD, 8 with DLB and 8 controls		Post-mortem	IGF-1 receptor expression reduced in PD and DLB brains. IGF-1 resistance more pronounced in DLB
(Tuncel et al., 2009)	25 men and women people with PD and 25 controls Mean age	67.9 \pm 9.4 PD and 64.3 \pm 8 controls	Cross-sectional	There was a trend toward higher IGF-1 levels in PD but this did not reach significance.

2.3.3 Insulin

Insulin is a 51 amino acid protein that was identified and isolated by Banting and Best in 1921 (Banting et al., 1922). Their discovery was lifesaving and revolutionised the treatment of type 1 diabetes, where there is profound insulin deficiency. Insulin is produced by pancreatic β -cells in the islet of Langerhans in response to rises in serum glucose. Insulin must be cleaved to release C-peptide before becoming biologically active (Begg and Woods, 2012). It acts via its receptor (IR) to enable glucose uptake into cells for respiration, suppress gluconeogenesis (production of glucose from fats) and glycogenolysis (breakdown of glycogen into glucose) in liver and stimulation of glycogen storage in muscle (Begg and Woods, 2012, Møller et al., 2004). The net result is a reduction in circulating glucose levels. As with ghrelin there is both phasic and tonic variation in insulin levels. Phasic secretion of insulin occurs in response to ingestion of carbohydrates (Holt et al., 1992). Tonic or “basal” insulin secretion is proportional to body fat (Bagdade et al., 1967, Woods et al., 1979).

Insulin is predominantly a metabolic hormone but, like ghrelin, it demonstrates pleiotropy (Kullmann et al., 2016). It was thought historically that insulin did not act within the brain as it cannot diffuse across the blood brain barrier. We now know that insulin crosses the BBB through a saturable transporter system (Banks et al., 1997). There are insulin receptors throughout the brain as well as the peripheral tissues (Hill et al., 1986). Once insulin crosses the BBB it binds with IR throughout the brain and is proposed to reduce food intake, enhance cognition and modulate reward (Lattemann, 2008).

Although insulin is proportional to body fat, type 2 diabetes often occurs in obesity. Obesity and hyperglycaemia in the face of high levels of circulating insulin illustrate the phenomenon of insulin resistance (Reaven, 1988). The exact mechanism of insulin resistance is controversial but may be due to chronically elevated insulin levels leading to inactivation of insulin receptors. This then leads to tissues becoming relatively insensitive to circulating insulin (Shulman, 2000). It is thought that this process occurs both centrally, in brain tissue, and peripherally in tissues such as muscle and solid organs (Anthony et al., 2006, Hallschmid and Schultes, 2009, Reaven, 1988).

Insulin resistance and diminished insulin production are more common as people age due to increased adiposity and sarcopaenia (Møller et al., 2004). It has been suggested that relative insulin deficiency and insulin resistance are an inevitable consequence of biological senescence (Elahi et al., 2002). Neither increased adiposity or sarcopaenia is inevitable in old age, however, and recent studies suggest that type 2 diabetes may be reversible with calorie restriction and its attendant weight loss (Lim et al., 2011b). This would argue against insulin deficiency and resistance as a normal part of ageing.

Insulin and appetite control

There is strong evidence from animal studies that insulin is an appetite suppressant hormone. This may surprise some clinicians as it has long been noted that exogenous insulin therapy in diabetes is associated with weight gain (Russell-Jones and Khan, 2007). It was previously thought that this was due to insulin itself (Rodin, 1985) but it is now felt that weight gain associated with insulin therapy is not due to insulin but due to improved utilisation of ingested glucose and increased carbohydrate intake due to fear of hypoglycaemia (Russell-Jones and Khan, 2007). As previously mentioned, basal insulin levels are proportional to adiposity and insulin is transported across the BBB into the CNS. Insulin is therefore able to provide negative feedback to the brain about total peripheral energy stores as an adiposity signal. Once in the CNS insulin acts via IR at the hypothalamus to suppress appetite in three main ways; 1) increased POMC neurone activity, leading to an increase in anorexigenic α MSH (Benoit et al., 2002), 2) inhibition of orexigenic NPY neurones (Schwartz et al., 1992) and, 3) modulation of responses to other hormones of energy homeostasis such as increased sensitivity to cholecystokinin (CCK) (Riedy et al., 1995) and suppression of ghrelin (Griffen et al., 2006, Ryber et al., 2006).

Central insulin administration has been shown to reduce energy intake in baboons, chicks, pigs, mice and rats (Air et al., 2002a, Air et al., 2002b, Anika et al., 1980, Brown et al., 2006, Chavez et al., 1996, Foster et al., 1991, Honda et al., 2007, Ikeda et al., 1986, McGowan et al., 1992, McGowan et al., 1990, Schwartz et al., 1992, Woods et al., 1979). Moreover, treatment with insulin receptor anti-sense and insulin antibodies have both been shown to induce hyperphagia in animal models (McGowan et al., 1992, Obici et al., 2002). Human studies have also shown insulin to be anorexigenic (Flint et al., 2006,

Kroemer et al., 2013, Pal and Ellis, 2010, Pasiakos et al., 2011, van Golen et al., 2014). Not all findings have been consistent, however, with some studies finding no relationship between insulin levels and appetite (Chapman et al., 1998, Holt et al., 1992, Lavin et al., 1998). To resolve this, a meta-analysis was conducted in 2007 looked at data from 7 test-meal studies in human subjects. One hundred and eighty participants were included in the studies, 136 normal weight and 44 overweight or obese individuals. In all 7 studies insulin levels and measures of hunger were assessed after an overnight fast and participants were offered an *ad libitum* meal where their food choices and caloric intake were recorded. Multivariate analysis showed that insulin level correlated with post-prandial fullness, and had an inverse correlation with energy intake and hunger in normal weight but not obese individuals (Flint et al., 2007). This is supported by subsequent work carried out by Pal and Ellis, who showed that increased insulin responses to ingesting proteins were associated with lower hunger and reduced ad libitum intake at a subsequent meal (Pal and Ellis, 2010). More recently, Kroemer *et al* used functional magnetic fMRI to examine neural responses to food images whilst asking participants to rate their subjective hunger using a visual analogue scale. They found that higher insulin levels in response to an oral glucose tolerance test were associated with reduced subjective hunger and reduced activation of a number of brain areas, (including the temporal and parietal cortices, thalamus, amygdala and hippocampus) which had been activated by food imagery but not by control pictures (Kroemer et al., 2013). Finally, there has been a great deal of interest in the synthetic insulin detemir as this appears to cause less weight gain than other insulins used in the treatment of diabetes. It has been suggested that this is due to changes in its structure making it lipophilic, and therefore able to cross the blood brain barrier, so having a central effect on appetite. A double blind randomised control trial of 32 people with type 1 diabetes found that CNS insulin levels were higher with detemir treatment than with other isophane insulins. Detemir treatment was associated with weight loss of 0.8kg, rather than the weight gain of 0.5kg seen with isophane insulin over the 12 week study period. It is worth noting that this was a drug-company funded study and should, perhaps, be interpreted with caution (van Golen et al., 2014).

Part of the reason that it has taken time for insulin to be recognised as an anorexigenic hormone is due to the confounding effects of hypoglycaemia in the presence of hyperinsulinaemia. Attempts have therefore been made to dissociate the peripheral effects of insulin from its CNS effects. One way that this has been achieved is with intranasal insulin. Intranasal insulin is absorbed into the CSF but does not pass into the peripheral circulation and, therefore, does not cause hypoglycaemia (Kern et al., 1999). In 2004, Hallschmid *et al.* carried out a double blind randomised control trial in which they administered intranasal insulin or placebo to 20 healthy volunteers (12 men and 8 women) over a period of 8 weeks. The men in the treatment group lost on average 1.38kg of adipose tissue compared to the placebo group ($p < 0.05$) but no effect was seen for women. The authors propose that insulin may be a relatively more potent appetite suppressant in men compared to women as men have relatively more insulin-producing visceral fat. Women, by contrast, are proposed to be more responsive to leptin as their fat stores tend to be subcutaneous (Hallschmid et al., 2004). This was corroborated by Benedict *et al.* who found that intranasal insulin reduced *ad libitum* food intake in men but not women following a fast (Benedict et al., 2008).

Insulin and cognition

Insulin receptors are found throughout the brain, not just at the ARC. There are areas of dense expression in the cortex, hippocampus and olfactory bulb (Fernandez and Torres-Alemán, 2012). Over the last 20 years or so it has become increasingly clear that CNS insulin is important in cognition and in neurodegenerative diseases, including PD and AD. Diabetes, especially in mid-life, has been shown to increase the risk of developing AD with an odds ratio of 1.65 according to a recent meta-analysis (Cooper et al., 2015). This may be due at least in part to cerebrovascular disease, analogous to the micro- and macro-vascular sequelae of diabetes seen in the kidneys, peripheral nervous system and retina (Ribe and Lovestone, 2016). There is also, however, good evidence for a more direct link between insulin and cognition. Insulin signalling results in the downstream reduction of glycogen synthase 3 (GSK3) activity, which is pro-apoptotic, tau phosphorylating, reduces LTP and is itself inactivated by LTP (Hooper et al., 2007). In keeping with this, numerous animal studies have shown that hippocampus dependent learning is improved with acute insulin

administration (Biessels et al., 1998, Haj-Ali et al., 2009, Moosavi et al., 2007, Park et al., 2000, Stern et al., 2014). This has been replicated in humans with several studies in people with and without dementia demonstrating improved cognition with acute euglycaemic administration of exogenous insulin IV or intranasally (Benedict et al., 2004, Benedict et al., 2008, Claxton et al., 2015, Craft et al., 2003, Craft et al., 1999, Craft et al., 2012, Craft et al., 1996, Krug et al., 2010, Reger et al., 2008, Watson et al., 2003, Watson et al., 2009). There appears to be a u-shaped relationship between circulating insulin and cognition. Several studies have shown that hyperinsulinaemia, even in people without diabetes, is associated with poorer cognitive performance (Burns et al., 2012, Luchsinger et al., 2001, Schrijvers et al., 2010, Stolk et al., 1997). It is felt that hyperinsulinaemia is associated with down-regulation of IR in the CNS, causing brain insulin resistance (Cholerton et al., 2013). People with AD have been demonstrated to have a lower CSF to plasma insulin ratio compared to age matched controls, despite higher circulating plasma levels (Craft et al., 1998). Brain insulin resistance has been described as the metabolic link between type 2 diabetes and AD (Kullmann et al., 2016). Alzheimer's disease is associated with peripheral insulin resistance *in vivo* and CNS resistance in post-mortem studies. The degree of resistance correlates with cognitive decline (Ekblad et al., 2015, Matsuzaki et al., 2010, Morris et al., 2016, Schrijvers et al., 2010, Talbot et al., 2012).

Insulin sensitisation with rosiglitazone has been shown to reduce A β induced tau hyperphosphorylation *in vitro* (Tokutake et al., 2012). This is likely mediated by an insulin-induced reduction in GSK3 activity resulting in a reduction of tau phosphorylation and prevention of toxic tau protein accumulation (Hooper et al., 2008). Moreover, insulin may shunt APP processing away from toxic A β and towards soluble amyloid species, which are non-pathogenic. This occurs via cleavage of APP in the A β domain due to insulin-induced activation of phosphatidylinositol 3 kinase (PI3K) (Solano et al., 2000). Finally, acute insulin administration appears to increase clearance of A β from the cytosol to the extracellular compartment *in vitro* (Gasparini et al., 2001, Pandini et al., 2012). This provides an intuitive model in which acute insulin administration improves cognition through enhancement of hippocampus dependent learning and reduction of toxic proteins in the CNS, whilst chronic hyperinsulinaemia results

in disrupted insulin signalling, impaired hippocampal learning and the accumulation of AD pathology. This may not provide the whole picture, however. Insulin is broken down in the CNS by IDE. This protein also breaks down A β . In the presence of insulin, IDE will preferentially clear insulin and A β therefore accumulates. Hyperinsulinaemia increases IDE expression but prevents IDE from clearing A β . This is a potential mechanism by which hyperinsulinaemia may contribute to the pathogenesis of AD. The literature around the role of insulin in cognitive impairment is vast and several comprehensive reviews have recently been published (Biessels and Reagan, 2015, Cholerton et al., 2013, Hooper et al., 2008, Kullmann et al., 2016, Messier and Teutenberg, 2005, Ribe and Lovestone, 2016, Stanley et al., 2016, Zhao et al., 2004). As a result, it can now be taken as canon that diabetes and chronic hyperinsulinaemia are associated with the development of AD whilst acute insulin administration appears to ameliorate cognitive deficits, though the mechanisms underlying are yet to be fully elucidated.

Insulin, Parkinson's disease and neuroprotection

It is possible that insulin is protective in PD. Insulin receptors co-localise to dopaminergic neurones in the SNpc (Unger et al., 1991). A post-mortem study of 3 people with PD and 3 controls found that IR and tyrosine hydroxylase (TH), an enzyme important for dopamine synthesis, were reduced in the SNpc of patients with PD compared to controls (Takahashi et al., 1996). Furthermore, streptozotocin treatment resulted in both hypoinsulinaemia and decreased TH expression in SNpc of rats (Figlewicz et al., 1996). Insulin receptor expression in the SNpc may be of functional significance as there is evidence that insulin modulates dopamine release in the basal ganglia and that this relationship may be reciprocal. Morris *et al.* induced insulin resistance in rats by feeding them a high fat diet. Dopamine release was measured in the striatum using micropipettes inserted under stereotactic MRI guidance. Levels of dopamine were significantly reduced in insulin resistant animals. Moreover, there was evidence of a greater degree of neurodegeneration in the SNpc of insulin resistant rats on MRI (Morris et al., 2011b). The same authors found that intracerebral administration of the 6-ODHA resulted in striatal but not peripheral insulin resistance (Morris et al., 2011a) and that more severe dopaminergic cell loss was associated with a greater degree of neuronal insulin resistance (Morris

et al., 2008). Insulin may also be important in dopamine handling at the synaptic cleft, through changes in dopamine transporter (DaT) expression. Dopamine re-uptake at the synaptic cleft may also be modulated by insulin. Dopamine transporters are essential for reuptake of dopamine into the cytosol enabling it to be re-released at a later stage. Chronic intracerebroventricular administration of insulin to rats increases DaT expression in the SNpc compared with those treated with vehicle (Finglewicz et al., 1994). Finally, dysfunctional insulin signalling has been proposed to precede dopaminergic cell death in PD. This is based on a post-mortem study of the brains of 6 people with PD, 5 with AD, 1 with vascular parkinsonism and 2 with amyotrophic lateral sclerosis. Insulin receptor expression was lost in surviving dopaminergic neurones in the SNpc of people with PD but preserved in the other patients (Moroo et al., 1994). Taken together these data suggest that insulin signalling may modulate neuronal dopamine synthesis, release and reuptake in the SNpc, and may be disrupted in PD.

Human studies have largely focused on PD and its relationship with diabetes as opposed to insulin *per se*. Results have been conflicting with studies variously reporting an increased risk of PD with diabetes, no association between PD and diabetes and reduced risk of PD with diabetes (Table 7). A 2013 meta-analysis of 6 studies that met the authors' inclusion criteria found an increased risk of PD in people with diabetes, with a RR of 1.26 (95% CI, 1.03-1.55). This significance was retained when results were corrected to exclude vascular parkinsonism and bias from increased medical monitoring in people with diabetes (RR 1.25, 95% CI 0.93-1.68, $p=0.01$) (Cereda et al., 2011, Cereda et al., 2013). These results suggest that diabetes may confer a small additional risk of developing PD over the life course.

Table 7. Studies examining the relationship between diabetes and Parkinson's disease

Study	Population studied	Mean age (years ± SD)	Study design	Outcome
(Simon et al., 2007)	121046 women and 50833 men from the Nurses' Health Study and Health Professionals Follow up Study	Women 30-55 and Men 40-75 at baseline	Prospective (follow up 12- 22.9 years)	No increase in PD seen in people with diabetes
(Scigliano et al., 2006)	178 PD patients and 533 controls	58.1±11.4 PD 59.8 ±10.2 controls	Case-control	Diabetes less common in PD (OR 0.3 CI 0.13-0.72)
(Savica et al., 2012)	196 PD and 196 matched non PD	Age matched cohort	Case control	No association between PD and diabetes
(Bohnen et al., 2014)	148 PD patients with DM (n=15) and without DM (n=133)	65.96 ±7.4	Cross-sectional	PD patients with DM had lower global cognition (composite measure) than those without (p=0.006)
(Sun et al., 2012)	603416 people with diabetes and 472188 people without diabetes in Taiwan	Adults > 20	Retrospective	Diabetes increased the risk of PD (OR 1.37 (CI 1.32-1.41)) association stronger in women
(Schernhammer et al., 2011)	1931 patients with new onset PD and 9651 healthy controls in Denmark	72.2 ± 10.2	Case control	DM increased the risk of PD (OR 1.36 CI 1.08-1.71) association stronger in women
(D'Amelio et al., 2009)	318 PWP and 318 controls	66.7	Case control	People with PD were less likely to have diabetes preceding diagnosis than controls (OR 0.3 CI 0.1-0.9)
(Hu et al., 2007)	51552 people without PD	25-74 at baseline	Prospective (follow up 18 years)	Type 2 diabetes increased risk of PD (OR 1.85 CI 1.23-3.15)
(Jimenez-Jimenez et al., 2000)	24 PD patients 24 controls	67.6±11.5 PD 64.5±9.5 controls	Case control	Fasted CSF insulin levels no different between groups
(Kotagal et al., 2013)	13 PD with diabetes and 26 PD without diabetes	66.4±5.5	Case control	Patients with PD and DM had more PIGD issues than those no DM (p=0.0005)
(Powers et al., 2005)	352 new PD and 484 controls	Median 69 PD Median 71 controls	Case control	Diabetes reduced the risk of PD in men (OR 0.52 CI 0.28-0.) but not women
(Moroo et al., 1994)	6 PD, 1 vascular PD, 5 AD, 2 ALS and 5 controls		Post-mortem	PD brains had reduced insulin receptor expression in SNpc. This was not seen in vascular parkinsonism
(Morris et al., 2014)	20 AD, 22 PD and 21 healthy controls	71.0 ±5.7 PD 72.4 ±5.7 AD 71.7±6.2 controls	Case control	Insulin resistance increased PD compared to controls (p=0.003) with corresponding reduction in grey matter on voxel based MRI.
(Palacios et al., 2011)	147096 people from the Cancer Prevention Study II Nutrition Cohort	>18 years at baseline	Prospective (follow up 7 years)	No increased risk of PD with diabetes
(Takahashi et al., 1996)	3 people with PD and 3 controls		Post-mortem study	Insulin receptor levels lower in SNpc of people with PD
(Pablo-Fernandez et al., 2017)	Neurological Disorders in Central Spain cohort 79 PD and 4919 controls	Mean 73 years, inter quartile range 68-79	Cross-sectional	There was no association between PD and diabetes except where diabetes disease duration was >10 years (p=0.02)

Many of the mechanisms by which insulin exerts positive effects on cognition have also been proposed to be neuroprotective in PD. Insulin binding to its receptor results in activation of PI3k/Akt pathway, which inactivates pro-apoptotic GSK-3, promotes anti-apoptotic mediators and reduces pro-inflammatory cytokines. As mentioned above the PI3K/Akt pathway is involved in the shunting of APP towards benign amyloid species and away from toxic A β (Bassil et al., 2014, Golpich et al., 2015). The net result is that neuronal cell death is reduced. This model is corroborated by a recent study using intra-nasal insulin in 6-ODHA treated rats. Insulin was commenced 24 hours after 6-ODHA treatment and continued for 2 weeks. The insulin treated animals had less motor impairment and a 74.8% increase in dopaminergic cell survival compared to those treated with vehicle (Pang et al., 2016). Finally, insulin may have an indirect role in α -synuclein handling via upregulation of IDE as IDE binds to α -synuclein and prevents aggregation *in vitro* (Sharma et al., 2015).

Given that insulin may be neuroprotective, and treatments for insulin resistance are freely available, there has been a great deal of interest in anti-diabetic medications as potential treatments for PD. The peroxisome proliferator-activated receptor γ agonists pioglitazone and rosiglitazone are insulin sensitising agents that appear to be neuroprotective in animal models (Dehmer et al., 2004, Laloux et al., 2012, Lee et al., 2012, Quinn et al., 2008, Swanson et al., 2011). Results in human studies have been disappointing, however. A recent multicentre, double-blind randomised control trial of 210 patients with PD was unable to demonstrate any disease modification or symptomatic benefit with pioglitazone (Simuni et al., 2015). Rosiglitazone has been withdrawn from use in the UK due to cardiovascular side effects. Glucagon-like peptide -1 (GLP-1) agonists and dipeptidyl peptase-4 inhibitors (DPP4i) are treatments for diabetes that may be neuroprotective in PD. Their main action is through modulation of GLP-1, another hormone of energy homeostasis, and they will therefore be considered in that section of this thesis.

There are three studies published to date examining insulin in Parkinson's disease dementia. In the context of diabetes, a longitudinal study of 77 PD patients, 12 with diabetes and 65 without, demonstrated that diabetes was associated with a greater rate of cognitive decline over 2.4 years. This equated to a decline in 0.55 points out of 30 on the MoCA in the no diabetes group

compared with 3.29 points in participants with diabetes ($p=0.016$). This represents a clinically meaningful change over time. There were no differences in disease severity or cognition at baseline (Ong et al., 2017). The two studies examining insulin and insulin resistance in PD and PDD have shown opposing results. A well- conducted 2012 observational study of 53 people with PDD and 57 people with PD and normal cognition found a positive association between insulin resistance and dementia. Patients had blood drawn for glucose and insulin after an 8 hour fast and 120 minutes after an oral glucose tolerance test. Insulin resistance was calculated using the homeostasis model assessment (HOMA- IR), calculated by multiplying insulin and glucose levels and dividing by a constant, and an oral glucose tolerance test. The dementia group had more insulin resistance ($p= 0.01$) and higher insulin levels ($p=0.05$) than cognitively intact people with PD. This result persisted after correction for disease duration and motor severity (Bosco et al., 2012). Conversely, a 2017 cross-sectional observational study of 122 people with PD with ($n=75$) and without ($n=47$) dementia found that insulin resistance measured by HOMA-IR was lower in the dementia group than those with normal cognition ($p<0.01$). The reduction in calculated insulin resistance was due to reduced insulin secretion in the face of similar fat mass and BMI. The authors report that this may represent metabolic failure but do not comment on how their findings may contribute to cognitive decline (Schelp et al., 2017). The opposing findings in these studies are difficult to explain. The age, sex, disease duration of participants was similar between the studies but BMI was lower in the Bosco study. Participants with diabetes were not excluded in the Schelp study and rates of diabetes per group were not disclosed. Motor severity was not reported or accounted for in the Schelp study. The role of insulin resistance in PDD therefore remains to be elucidated.

Taking all of the available research into consideration one could propose a model by which people with PDD have chronic hyperinsulinaemia leading to central insulin resistance, impaired insulin signalling and loss of insulin mediated neuroprotection with resultant motor and cognitive decline. In this model hyperinsulinaemia could link weight-loss and dementia if it also leads to appetite suppression and weight loss. Against this, animal studies do not suggest that areas involved in appetite suppression are spared from insulin resistance in the face of hyperinsulinaemia (Obici et al., 2002). Despite this, it is

possible that the relative effects of insulin resistance are different in different brain tissues, or insulin sensitivity is lost due to neurodegeneration in brain areas involved in cognition and motor control, with relative preservation of hypothalamic areas controlling appetite. This is highly speculative.

Nevertheless, insulin cannot be discounted as a possible neurohumoral link between weight loss and cognition

2.3.4 Glucagon-like peptide 1

Glucagon-like peptide 1 is a 30 amino-acid hormone produced in L-cells throughout the gastrointestinal (GI) mucosa with levels increasing distally to the stomach (Athauda and Foltynie, 2016). Secretion occurs shortly after ingestion of nutrients, especially carbohydrates and fats, under autonomic control via the vagal nerve. A second wave of secretion occurs when nutrients reach L-cells in the bowel (Hellstrom and Naslund, 2001). Glucagon-like peptide-1 has a very short half-life in the blood stream as it is rapidly broken down by dipeptidyl peptase-4 (DPP-4) (Alagiakrishnan et al., 2013). Its receptors are found throughout the body, including the gut, lungs, muscle, fat and brain. GLP-1 crosses the blood brain barrier by passive diffusion (Alagiakrishnan et al., 2013). In the brain GLP-1 receptor is expressed in areas involved with appetite such as the NTS and PVN but not in the ARC. There are also GLP-1 receptors in the brain stem, hippocampus and amygdala (Hellstrom and Naslund, 2001, McIntyre et al.). Glucagon-like peptide 1 has been shown to decrease food intake in obese and normal weight humans as well as those with type 2 diabetes (Gutzwiller et al., 1999a, Gutzwiller et al., 1999b, Verdich et al., 2001). It slows gastric emptying and forms part of the “ileal brake” (Verdich et al., 2001); in which GI transit slows when the ileum comes into contact with food, resulting in optimal nutrient absorption (Van Citters and Lin, 1999). It is thought that GLP-1 helps to signal fullness or satiation through its GI effects and through increasing sensitivity to CCK, another anorexigenic hormone discussed below (Dailey and Moran, 2013). It may have satiating effects through stimulation of the PVN, which in turn increases corticotropin-releasing hormone and oxytocin release (Katsurada et al., 2014). Glucagon like peptide 1 may also modulate taste responses, decreasing sensitivity to sweetness and increasing sensitivity to umami (savory flavours) (Martin et al., 2009). Peak levels of secretion tend to fall with higher BMI and advancing age (Nauck et al., 2011)

Finally, GLP-1 induces peripheral insulin secretion from pancreatic β cells, though not during hypoglycaemia. This has led to the development of pharmacologically stable GLP-1 mimetic drugs such as exenatide, liraglutide and lixisenatide and DPP-4 inhibitors (DPP-4i); the “gliptins” such as sitagliptin, linagliptin and vildagliptin for use in the treatment of type 2 diabetes. These drugs have a modest effect on glycosylated haemoglobin without hypoglycaemia and reduced glycaemic variability (Brunton, 2014, Nauck et al., 2002).

Glucagon-like peptide and cognition

The link between type 2 diabetes and dementia has spurred a huge body of research into anti-diabetic agents to treat cognitive impairment. GLP-1, GLP-1 analogues and DPP4is are not exceptions to this and studies in animals have been promising. GLP-1 receptor deficient rodents have impaired hippocampal learning measured by performance in the Morris water maze and novel object recognition. These cognitive deficits can be rescued with GLP-1 receptor gene transfer. Moreover, performance was improved with GLP-1 receptor over-expression (Abbas et al., 2009, Daring et al., 2003). The GLP analogues exenatide, liraglutide, lixisenatide and GLP-1 itself have all been shown to increase long term potentiation in rodents *in vivo*; (Cai et al., 2014, Gault and Hölscher, 2008, Gault et al., 2010, McClean et al., 2010, Porter et al., 2010) and GLP-1 analogues have been shown to induce neurogenesis in rodents (Bertilsson et al., 2008, Hamilton et al., 2011, Hunter and Hölscher, 2012). There is also evidence that they may ameliorate AD pathology. The DPP4i vildagliptin, linagliptin and DPP4 inhibiting ayurvedic plants (*pterocarpus marsupium* and *Eugeia jambolana*) have all been shown to reduce tau phosphorylation in streptozotocin treated rats *in vivo* (Kosaraju et al., 2014, Kosaraju et al., 2013) or in SK-MC cells *in vitro* (Kornelius et al., 2015). This may be via inactivation of GSK3b (Cai et al., 2014), much like the mechanism seen in insulin described above. Moreover, A β levels have been shown to be reduced following long-term vildagliptin treatment in streptozotocin treated mice (Kosaraju et al., 2013), whilst double transgenic A β over-expressing mice had reduced A β load following treatment with sitagliptin. The latter also had reduced APP and reduced neuronal inflammation, with correspondingly improved performance on the open field test and contextual fear conditioning (D’Amico et

al., 2010). These results have been replicated using liraglutide in APP/PS1 over-expressing mice and are further supported by *in vitro* studies showing that exenatide reduces APP in neurone-like rat PC12 cells (McClellan et al., 2011). Finally, there is evidence that GLP-1 analogues may be neuroprotective in *in vitro* models of AD. Exenatide has been shown to prevent axonal degradation in cultured Neurobasal-27 neuronal cells treated with A β and cultured rat hippocampal neurons treated with glutamate (Bomfim et al., 2012). Linagliptin also prevented apoptosis of SK-MC cells in response to treatment with A β (Kornelius et al., 2015). Finally, DPP4is have been shown to up-regulate IDE, and may therefore reduce amyloid burden in AD (Yin et al., 2012).

Despite the large body of promising research in animals, there is very little published research into GLP-1, GLP-1 analogues and DPP4i in cognitive impairment in humans. One retrospective observational study followed 240 people in Naples with diabetes and MCI at baseline and measured cognition at 2 years. Participants were not on any medication for cognition or diabetes at baseline. One hundred and twenty of these went on to be treated with DPP4i and metformin and 120 with sulfonylurea and metformin. The DPP4i treated group had improved cognition, better glycaemic control, reduced glycaemic variability and fewer asymptomatic hypoglycaemic episodes than the sulfonylurea treated group. Cognition correlated with HbA1c across all groups. It is therefore difficult to tease apart any pro-cognitive effects of DPP4is over and above loss of hypoglycaemia and improved glycaemic control in these patients (Rizzo et al., 2014). A randomized controlled trial was started in 2012 looking at treatment of people with AD with liraglutide or placebo for 6 months. No data have yet been published (Egefjord et al., 2012). Another phase 2 randomised control study of people with early AD or MCI treated with exenatide for 18 months completed follow up in November 2016 but again no data have yet been published (NCT01255163). Finally, there is a multicenter randomized control phase 2b trial due to complete in 2019 in which people with AD are randomized to liraglutide or control for 12 months (NCT01843075). There are no studies to date examining whether levels differ between people with normal cognition and dementia. As such it is not possible to determine whether disordered GLP-1 signaling may contribute to cognitive decline in AD or PDD.

Glucagon-like peptide, Parkinson's disease and neuroprotection

GLP-1 has been recognized as a neurotrophic factor (Perry et al., 2002). As such, there has been considerable research into GLP-1 analogues and DPP-4i as potential neuroprotective agents for conditions as wide ranging as peripheral neuropathy, stroke, AD and PD (Darsalia et al., 2013, Holscher, 2012, Perry et al., 2007). There is good evidence from animal studies that GLP-1 analogues and DPP4i may be neuroprotective in PD through anti-inflammatory and anti-apoptotic effects, increased mitochondrial stability and altered α -synuclein handling. Intra-peritoneal exendin-4 has been shown to reduce microgliosis, inflammatory cytokines and dopaminergic neurone loss in mice treated with MPTP (Kim et al., 2009). Similar results were found when systemic saxagliptin, a DPP4i, was administered to a rotenone rat model of PD. The rats had an improved phenotype, with reduced akinesia and improved dopaminergic neuronal survival in the SNpc. This was accompanied by reduced neuronal inflammation, lower levels of inflammatory cytokines and a shift in the ratio of pro-apoptotic to anti-apoptotic factors favouring cell survival (increased bcl2 and reduced bax, caspase3 and cytochrome c) (Nassar et al., 2015). Another GLP-1 receptor agonist derived from Chinese medicine was found to improve motor deficits and prevent neuronal apoptosis in a mouse MPTP model of PD. The authors found that the usual pro-apoptotic response to MPTP was blunted by geniposide treatment, with a reduced active (cleaved) caspase 3: inactive (pro-) caspase 3 ratio resulting in increased cell survival (Chen et al., 2015). It has been proposed that GLP-1 analogues and DPP-4is may reduce α -synuclein deposition through upregulation of IDE (Athauda and Foltynie, 2016). This hypothesis is based on the observations that GLP-1 and DPP-4is both increase IDE in experimental models and that IDE reduces α -synuclein aggregation (Sharma et al., 2015, Yin et al., 2012). GLP-1 analogues and DPP-4is appear to induce neurogenesis in rodent models of PD. Two *in vivo* studies have shown increased BrdU labelling in 6-ODHA treated rats when exendin-4 was administered 7 days after lesioning. These changes were associated with improved motor phenotypes and improved dopaminergic cell survival (Bertilsson et al., 2008, Harkavyi et al., 2013). Finally, it has been suggested that GLP-1 analogues and DPP4is could be neuroprotective through mitochondrial stabilization and mitochondrial biogenesis, as GLP-1 has been shown to increase mitochondrial numbers and survival in animal models of

diabetes and spinal cord injury (Fan et al., 2010, Foltynie and Aviles-Olmos, 2014, Kang et al., 2015a, Li et al., 2015c).

In contrast to many potential neuroprotective agents which show promise in animals and *in vitro* but fail to benefit humans, there is evidence from human studies that GLP-1 analogues and DPP4is may be neuroprotective in people with PD. A small randomized, single-blind open label study of 45 people improved motor and cognitive symptoms as measured by part 3 of the MDS-UPDRS and Mattis dementia rating scale respectively compared to the control group receiving usual care (Aviles-Olmos et al., 2013). Interestingly, these effects persisted 12 months after exenatide was stopped (Aviles-Olmos et al., 2014). A phase 2 randomized control trial into the use of exenatide as a neuroprotective agent in PD has recently been published. Sixty-two patients with moderate PD were randomized to receive 48 weeks of exenatide or placebo. The primary outcome measure was motor symptoms of PD measured using part 3 of the MDS-UPDRS in the “off” state and secondary measures included tolerability and cognitive performance. Participants with diabetes or dementia were excluded. The authors found that exenatide treatment resulted in 2.3 points of motor improvement in the MDS-UPDRS compared with a 1.7 point decline in the placebo group after 48 weeks of treatment. This improvement was sustained, though slightly diminished, following 12 weeks of washout. One point of improvement was retained in the treatment group compared to a 2.3 point decline in the placebo arm. These results were statistically significant but the clinical significance is less clear as changes in “on”-state motor scores, quality of life measures and non-motor symptoms did not differ between groups. Interestingly levodopa equivalent dose (LED) increased proportionally more in the treatment group. The authors adjusted for this in their analysis and felt the difference reflects slightly higher disease severity at baseline. There was no difference in the Mattis Dementia Rating Scale between groups in this cognitively intact cohort. Though further research is needed, this study supports exenatide as an exciting potential disease modifying agent in PD (Athauda et al., 2017).

Despite its potential as a potential neuroprotective agent with respect to both the motor and cognitive symptoms of Parkinson’s disease, GLP-1 may not be a major mechanistic contributor to the link between weight loss and dementia in

PD as neurodegeneration would seem likely to render the brain less, not more, sensitive to the satiating effects of GLP-1 through loss of signaling pathways. Alternatively, a relatively more permeable BBB due to neurodegeneration, as is seen in late AD (Bonda et al., 2014) may result in greater influx of GLP-1 and reduced appetite, though perhaps ameliorating the attendant cognitive and motor deficits. Further research is needed to clarify the physiological role of GLP-1 in dementia and PD.

2.3.5 Leptin

Leptin is a 16Kda protein produced by adipocytes. It was originally identified in 1994 as the missing protein in mutant mice with an obese phenotype (Zhang et al., 1994). Leptin levels are proportional to body fat and show diurnal variation, with a nocturnal peak (Mantzoros et al., 2001). Levels may decrease with prolonged fasting (Chan et al., 2003) and increase with severe overfeeding (Kolaczynski et al., 1996) but are not altered by physiological calorie intake (Korbonits et al., 1997, Ullrich et al., 2015). As with insulin, leptin levels increase with adiposity and provide negative feedback in times of positive energy balance (Friedman and Halaas, 1998, Korbonits et al., 1997). This is possible as leptin crosses the BBB via a saturable transporter (Caro et al., 1996) where it binds with LepR receptors. There are 6 known LepRs, also known as OB-R, which are grouped together according to size as short, long and soluble isoforms (Magalhães et al., 2015). Soluble receptors are detectable in blood and are proportionate to membrane bound receptors (Albala et al., 2016).

With regard to appetite leptin can be considered a direct antagonist to ghrelin, with expression in many of the same neuronal structures and with opposite effects. Leptin acts at the hypothalamus to suppress orexigenic NPY and AgRP neurones and stimulate anorexigenic POMC neurones (Cowley et al., 2001, Erickson et al., 1996, Friedman and Halaas, 1998, Korbonits et al., 1997). Where ghrelin positively stimulates the cholinergic dopaminergic reward system, leptin suppresses it (Leininger, 2009). Humans who are deficient in leptin due to a mutation in the LEP gene lose this negative feedback and become morbidly obese (Friedman and Halaas, 1998). Leptin replacement in this context normalises body weight and improves metabolic profile (Oral et al., 2002). Understandably, therefore, there has been considerable interest in leptin

as an anti-obesity therapy. Unfortunately, exogenous leptin treatment has not proven to be as effective at inducing weight loss in obese humans without leptin deficiency. Leptin treatment may, however, augment weight loss in calorie restricted humans (Heymsfield et al., 1999), and has been shown to reduce hunger in the context of severe calorie restriction (Rosenbaum and Leibel, 2014). Again, like insulin, leptin resistance occurs in obesity such that negative feedback is lost. This has been proposed to be due to down-regulation of the saturable transporter across the BBB and disordered leptin signalling due to the accumulation of inhibitory proteins (Münzberg and Myers, 2005).

Interestingly, leptin has also been demonstrated to modulate insulin sensitivity. Patients with leptin deficiency due to LEP mutations or severe lipodystrophy demonstrate marked insulin resistance, which is corrected by leptin replacement (Licinio et al., 2004, Oral et al., 2002). Conversely, hyperinsulinaemia has been shown to increase leptin in women during glucose clamp testing (Kennedy et al., 1997). Leptin is higher in women than in men at comparable BMIs. This is proposed to be related to differences in the distribution of adipose tissue between men and women (Kennedy et al., 1997). It has been suggested that leptin may be more important for weight regulation in women, whilst insulin is more important in men (Hallschmid et al., 2004).

It may be anticipated that leptin levels may increase with ageing due to relative loss of muscle mass and increased adiposity. This does not appear to be the case, however, with BMI-adjusted leptin levels tending to decline with age, especially in women (Isidori et al., 2000). It has been proposed that a shift towards visceral as opposed to peripheral fat storage and adipocyte dysfunction may account for this (Carter et al., 2013).

Leptin in cognition

Like ghrelin and insulin, leptin demonstrates pleiotropy. Leptin receptors are not confined to the hypothalamus but are found widely throughout the brain; in the VTA where leptin affects the mesolimbic dopaminergic system “reward” responses, the hippocampus where it may have a role in synaptic plasticity and memory formation and the SNpc where its actions are as yet unclear (Harvey, 2007, Zupancic and Mahajan, 2011). Animal studies suggest an important role for leptin in learning and memory. Leptin facilitates long-term potentiation in

rodents *in vitro* and *in vivo* (O'Malley et al., 2007, Oomura et al., 2006, Shanley et al., 2001) and has been shown to improve spatial memory in rats undertaking the Morris water maze (Oomura et al., 2011). Conversely, leptin receptor deficient rodents have reduced spatial memory and impaired long term potentiation compared to wild types (Li et al., 2002).

Leptin may also be important in dementia as it appears to ameliorate the toxic effects of Alzheimer's pathology in rodents. Leptin reduces extracellular A β in mice *in vivo* and in SH- SY5Y human neuroblastoma cells *in vitro* via reduced synthesis by β -secretase and increased clearance by endocytosis (Fewlass et al., 2004, Greco et al., 2009). Moreover, leptin administration reduces tau phosphorylation in mouse neurones, SH-SY5Y cells and human embryonic kidney 293 cells *in vitro* (Greco et al., 2009, Greco et al., 2008, Platt et al., 2016). This reduction of tau phosphorylation appears to be mediated via activation of AMPK and inactivation of GSK-3b (Greco et al., 2009), similar to the downstream actions of insulin binding. These effects may be clinically relevant as Farr *et al.* demonstrated that exogenous leptin improved memory performance in A β -over-expressing SAMP8 mice (Farr et al., 2006, Fewlass et al., 2004).

There have been a number of human studies looking at a possible role for leptin in learning and cognitive impairment. As may be expected from the animal studies described above leptin deficiency is associated with learning difficulties in humans. Learning difficulties in leptin deficient humans, like the attendant obesity and metabolic abnormalities, are ameliorated by exogenous leptin therapy (Paz-Filho et al., 2008). In 2001 Power *et al* hypothesised that inappropriately elevated leptin levels may account for weight loss in dementia, especially AD. They found, however, that leptin levels were low in underweight dementia patients, most likely representing a normal physiological response to weight loss. Interestingly, however, this trend was lower for AD patients than those with vascular dementia despite similar mean BMI in both groups (Power et al., 2001). The literature exploring a putative link between cognition and leptin has been conflicting, with a number of studies finding a positive correlation between leptin and cognition (Table 8) and a comparable number showing no relationship or an inverse correlation (Table 9). There is therefore a lack of consensus regarding the role of leptin in cognition in humans. The differences in

results may be due to methodological differences, especially the inclusion or exclusion of obese participants who are likely to have a degree of leptin resistance (Zeki Al Hazzouri et al., 2012).

Table 8. Studies showing a positive correlation between leptin and cognition

Study	Population studied	Leptin	Follow up (years)	Outcome
(Huang et al., 2007)	HIV positive men N=59 Patients with dementia not excluded Age; 39 ± 7 BMI;27±4.5	CSF	Cross-sectional	Low CSF leptin correlated with impaired learning ($p=-0.31$, $p=0.02$) and memory ($p=-0.39$, $p=0.002$) as tested by the neuropsychological battery.
(Holden et al., 2009)	Health, Ageing and Biology (Health ABC) study N=2871 Dementia free at baseline Age; 70-79 BMI; stratified by leptin tertile low 23.2± 3.1, middle 27.0± 4.0 and high 31.1 ± 4.8	Blood	5	High leptin >1 standard deviation [SD] above the mean associated with reduced risk of cognitive decline (OR 0.66 CI 0.48-0.91) Remained significant after correction for BMI
(Lieb et al., 2009)	Framingham Cohort N= 785 Dementia free at baseline Age; 79± 5 years at baseline BMI 28 ±5	Blood	13-17	High leptin associated with reduced risk of all cause dementia (HR 0.68 CI 0.54-0.87) and Alzheimer's dementia (HR 0.60 CI 0.46-0.79) Causes of non-AD dementia not given Results only significant if BMI <30
(Zeki Al Hazzouri et al., 2012).	Sacramento Area Latino Study on Aging (SALSA) cohort N= 1,480 Dementia free at baseline Age; 60-101 at baseline BMI grouped as <25,25-<30 and ≥30	Blood	10	High leptin and small waist circumference reduced cognitive decline vs. low leptin and small waist ($p=0.01$). Significance lost in participants with larger waist measurements
(Zeki Al Hazzouri et al., 2013).	Study of Osteoporotic Fractures cohort N= 579 Dementia free at baseline Age; mean 82.6 BMI grouped as 25-<30 and ≥30	Blood	4	High leptin associated with reduced risk of dementia/MCI (OR 0.68, CI 0.46-0.99) Leptin not protective in obese women Type of dementia and MCI not recorded
(Beydoun et al., 2012)	National Health and Nutrition Examination Study III cohort N= 1293 aged 20-59 , 1673 aged 60-90 BMI; 26.2 ± 0.2 aged 20-59 and 26.6 ±0.1 aged 60-90	blood	Cross-sectional	Better performance on symbol digits substitution testing in second tertile of leptin in the 20-59 age group. No other positive associations for leptin. No difference in performance between third and first tertiles.

(Littlejohns et al., 2015)	Invecchiare in Chianti (InCHIANTI) study N=809 Dementia free older Italians aged 73.6 ±6.6 at baseline BMI grouped as <25,25-<30 and ≥30	blood	Mean 8	Higher leptin was protective against significant cognitive decline defined as ≥5 point change on MMSE (RR 0.84, 95% CI 0.73-0.97) and the relationship persisted in obese individuals.
(Xing et al., 2015)	Non-obese Chinese women with normal cognition or amnesic MCI N= 42, MCI; 23, controls; 19 Age; MCI; 67.2 ±6.9, controls; 68.1±7.3 BMI 23.5 ±1.5 MCI and 24.7 ±3.1 controls	Fasting blood	Cross-sectional	Leptin levels were lower in the MCI group, even after adjustment for BMI and age (p<0.001). Leptin levels did not correlate with cognitive performance.
(Witte et al., 2016)	German MCI patients and healthy controls N=80 MCI;40, controls 40 age, body fat and sex matched Age 67±7 BMI 26.4 ±3.5 MCI and 26.9 ±2.1 controls	Fasting blood	Cross-sectional	MCI group had lower leptin levels than healthy controls (p<0.001) with associated lower hippocampal volume on MRI
(Baranowska-Bik et al., 2015)	Polish women with and without AD N= 18 severe AD, 40 early AD and 42 age and BMI matched controls BMI 24.4 ±3.6 in AD and 25.9 ±4.0 in controls	Fasting blood	Cross-sectional	Leptin levels were lower in AD than in controls despite no significant difference in BMI (P<0.05) and lower in severe AD than early AD (p<0.05)

Table 9. Studies showing no, or inverse, correlation between leptin and cognition

Study	Population studied	Leptin	Follow up (years)	Outcome
(Gunstad et al., 2008).	Dementia free Americans N=35 Age; 73.69 +/- 6.62 BMI; 28.34 ±4.6	Blood	Cross-sectional study	Negative correlation between leptin levels and executive function as tested by Trail making B test (r=.46, p=.01). Not significant for other measures of cognitive impairment.
(Narita et al., 2009)	Cognitively normal non-obese Japanese people N=34 Age men; 64.8±5.1, 64.3 ±4.4 BMI men 23.2 ±2.7 women 21.9±1.8 Participants with BMI >27 excluded	Blood	Cross-sectional	Higher leptin levels associated with larger hippocampal volumes on Voxel-based morphometric MRI. There was no demonstrable correlation with cognition. This may represent a ceiling effect in this cognitively normal cohort.
(Kamogawa et al., 2010)	Japanese Shimanami Health Promoting Program (J-SHIPP) cohort N=524 Age; mean 67 for normal cognition 72 for MCI BMI mean 23.0 normal cognition and mean 22.7 in MCI	Blood	Cross-sectional	No significant correlation between leptin and cognition when corrected for BMI. Greater central adiposity was associated with a lower risk of MCI.
(Gustafson et al., 2012).	Prospective Population Study of Women cohort. Dementia free at baseline N=1462 Age;38-60 at baseline BMI; 24.1 ±3.8	Blood samples taken in 1968	Median 32	No association between leptin and AD, vascular dementia or mixed vascular and Alzheimer's dementia (OR 1.00 CI 0.98-1.03)
(Warren et al., 2012)	Dallas Heart Study Cohort N=2,731 Dementia free at baseline Age; 30-65 at baseline BMI; 28.0±5.1 to 31.9±7.9 depending on ethnicity	Blood measured in 1998	8	No significant correlation between cognition (tested using MoCA) and leptin. Trend towards lower leptin with greater cognitive impairment
(Labad et al., 2012)	Edinburgh Type 2 Diabetes Study (ET2DS) N=1057 BMI not stated though comment "most were obese"	Fasting blood sample	Cross-sectional	Higher leptin levels associated with poorer cognitive performance on psychometric battery in men. No relationship for women. Most subjects were obese so leptin resistance may account for these results
1.(M Johnston et al., 2014)	Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort	Fasting blood sample	3	1. Leptin levels were lower in MCI than in controls but significance was lost with BM>27 and;

2. (Oania and McEvoy, 2015)	N=352 adults with MCI BMI 27±4.1 normal cognition 26.1 ±4 MCI and 25.6 AD			2. There was no association between baseline leptin levels rate of cognitive decline or development of dementia
(Teunissen et al., 2014)	non-obese people with AD, MCI, VaD or healthy controls n= total; 295 AD; 100, MCI; 99, VaD; 31, controls; 65 Age; AD; 63±6, MCI; 62±7, VaD 67±8, controls; 58 ±7 BMI; AD; 24±3, MCI; 25±3, VaD 27±4, controls; 25 ±2	Non-fasting blood sample	Cross-sectional	Leptin levels were not significantly different between groups. No relationship between leptin and hippocampal atrophy on MRI
(Alosco et al., 2015)	Dementia free patients undergoing bariatric surgery N=84 Age 43.86 ±10.39 BMI 46.88 ± 6.08 at baseline and 30.51±5.39 at 12 months	Fasting blood	1- Baseline and post-operatively	Leptin associated with worse attention/executive function (digit span backwards, trail-making A and B tests) and memory (verbal list learning). Post-operative reduction in leptin predicted improved cognition. Correlation disappeared after correction for BMI.
(Gustafson et al., 2015)	American women without dementia N=354, 247 HIV+ and 107 HIV – Age 38.9±9.1 BMI 29.2 ±7.9	Non-fasting blood sample	Cross-sectional	Leptin inversely correlated with cognition. Most of the participants were overweight or obese but the correlation persisted after correction for BMI, age, education, race and CD4 count (p=0.02)
(Correa-Burrows et al., 2016)	Chilean adolescents Age 16-17 BMI data presented as Z scores but 13.5% obese	Fasting blood sample	Retrospective	Hyperleptinaemia was associated with a lower grade point average at school, even after controlling for obesity (p=0.004)

Several studies demonstrating a correlation between cognitive function and leptin have found that significance is lost when subjects are obese (Lieb et al., 2009, Zeki Al Hazzouri et al., 2012, Zeki Al Hazzouri et al., 2013). This would be in keeping with leptin resistance in obesity resulting in loss of putative positive cognitive effects. Against this are studies carried out in Japan and the Netherlands which excluded obese people and still found no correlation between cognition and leptin following correction for BMI (Kamogawa et al., 2010, Narita et al., 2009, Teunissen et al., 2014). Evidence for circulating levels of leptin directly impacting on cognition in humans in a linear fashion is therefore lacking. It is worth noting that animal studies have suggested that the cognitive response to exogenous leptin may have a U shaped relationship, with benefit seen at low doses but lost at high doses (Oomura et al., 2011). There are no studies to clarify if this is true of physiological leptin levels in humans. The issue is unlikely to be resolved without a good quality meta-analysis, which is likely to be challenging in view of experimental heterogeneity.

Another possibility is that leptin signalling in dementia is disrupted or disordered in such a way that peripheral blood and one-off CSF measurements cannot detect. A 2014 post-mortem study by Bonda *et al* examined the brains of 21 people with AD, 8 with MCI and 13 controls. They found that CSF and intrahippocampal leptin were higher in AD brains compared to controls but Ob-R expression was decreased. This was especially the case for severe AD, while conversely there was a trend towards lower leptin in MCI than in controls. None of these findings correlated with age or BMI. The authors suggest that reduced leptin levels early in disease may accelerate AD pathology but disruption of the BBB in severe AD may lead to leptin influx, downregulation of Ob-R and abnormal leptin signalling (Bonda et al., 2014). These results were corroborated by Maioli *et al.* the following year who found AD patients had reduced Ob-R throughout the brain, including in the hippocampus post-mortem. Meanwhile, the distribution of leptin within the hippocampus differed between controls and AD patients, with controls showing staining for leptin in neurones and AD patients showing staining in reactive astrocytes (Maioli et al., 2015). Finally, it may be that free, unbound, leptin is more relevant to cognitive impairment than total leptin. Soluble Ob-R (sOb-R) binds leptin in peripheral blood and prevents it from binding to membrane bound leptin receptors in the brain. Albala *et al*

looked at 667 older people enrolled in longitudinal studies in Chile with baseline blood test available. 42 of these had developed dementia during follow up. Participants were over 65 years old and dementia free at baseline. They had been followed up for an average of 15 years at the time of the study. Leptin and sOb-R were measured in frozen baseline samples. The authors found that reduced leptin availability due to higher levels of circulating sOb-R was seen in people who went on to develop all cause dementia on follow up. They also found that lower body weight increased the likelihood of developing dementia, in line with the literature around weight loss and dementia (Albala et al., 2016). Given the apparent anti-amyloid and anti-tau properties of leptin *in vitro*, lower leptin levels with reduced BMI and increased risk of dementia with low BMI, leptin remains a candidate for a link between neurodegeneration and weight loss.

Leptin, Parkinson's disease and neuroprotection

Leptin may also be important in PD, perhaps related to LepRb expression in the SNpc. Dopamine stores have been found to be low in leptin-deficient mice (Roseberry et al., 2007) and α -synuclein over-expressing mice are underweight and hypoleptinaemic compared to wild types (Rothman et al., 2013). Leptin is also proposed to be neuroprotective in rodent models of PD. Weng *et al.* injected 6-OHDA unilaterally into the SNpc of mice and found that co-administration of leptin prevented nigral cell death. Furthermore, these mice were rescued from developing a dopamine deficient phenotype. They then went on to look at dopaminergic mouse neurones in culture and found that leptin reduced pro-apoptotic proteins such as caspase-9 and caspase-3 in response to 6-ODHA treatment and that this was associated with improved neuronal survival (Weng et al., 2007). Leptin has also been shown to be neuroprotective in human neuroblastoma cells, (dopaminergic SH-SY5Y cells). Leptin treatment improved cell survival following treatment with 1-methyl-4-pyrinium (MPP⁺) PI3/Akt activation (Lu et al., 2006). Finally, leptin has been shown to act as a mitochondrial stabiliser in SH-SY5Y cells treated with MPP⁺, via activation of mitochondrial UCP-2 (Ho et al., 2010).

In humans, Lorefalt *et al.* carried out a small case control study investigating leptin and weight loss in PD patients. They found that leptin levels were lower in women with PD with weight loss compared to weight stable patients and

controls. This is in keeping with reduced fat mass in the weight loss group (Lorefalt et al., 2009). These findings were corroborated by Aziz *et al* who measured serum leptin every 20 minutes for 24 hours in 8 volunteers with PD who were not on medication and 8 weight matched controls. They found no significant difference between groups and leptin levels correlated with adiposity as expected (Aziz et al., 2011). More interestingly, Fiszer *et al.* demonstrated lower levels of leptin in PD patients with unintentional weight loss than in those with stable weights, despite no significant differences in BMI (Fiszer et al., 2010). Very similar results were obtained by Evidente *et al.* nearly a decade earlier, but the observed trend towards lower leptin in PD patients with weight loss did not reach statistical significance (Evidente et al., 2001). The dominant view in the literature is that leptin levels may be low in PD due to reduced adiposity from another cause (Davis et al., 2014, Folch et al., 2012). That weight losing PD patients have lower leptin than their weight stable counterparts suggests that weight losers respond inadequately to a state of negative energy balance. It has been suggested that weight loss in PD may result in loss of neuroprotection from leptin and exacerbate neurodegeneration and cognitive impairment in PD (Davis et al., 2014, Kim et al., 2012). No studies have yet been published examining a role for leptin in PDD and, to my knowledge, there have been no trials of leptin as a neuroprotective agent to date.

2.3.6 Other hormones of energy homeostasis

There are a number of other hormones of energy homeostasis. These have not yet been demonstrated to have cognitive or neuroprotective effects. The two most important are peptide YY (PYY) and CCK. Peptide YY is produced by the same L-cells in the GI mucosa that produce GLP-1. It acts to reduce motility and slow transit time in both the stomach and the lower GI tract. PYY is secreted in response to the presence of nutrients and bile salts in the lumen of the gastrointestinal tract. It may also be under neural control via the vagus nerve. Levels peak after eating and show a dose response, with higher levels achieved after greater calorie intake (Adrian et al., 1985). Cholecystokinin is produced by the small bowel post-prandially, especially after consumption of peptides and fatty acids. The level of CCK secreted corresponds to the nutrient load consumed. It signals satiety and reduces meal size resulting in reduction of energy intake through binding to its receptor CCK1-R in vagal afferent nerve

fibres. Vagal afferent stimulation of the NTS then promotes satiety and meal termination (Lancha et al., 2012, Morton et al., 2006, Woods and D'Alessio). CCK is a modulator of ghrelin, inhibiting the ghrelin response to fasting when administered to rats (Kobelt et al., 2005). It acts synergistically with other anorexigenic hormones; increasing the anorectic response when co-administered with leptin (Wang et al., 2000) and insulin (Riedy et al., 1995). Finally, CCK also has a number of effects on the GI tract; reduction in gastric emptying, stimulation of gall bladder contraction and increasing exocrine pancreatic secretions (Little et al., 2005). Whilst important for regulation of energy intake, these hormones are unlikely to be linked with neurodegenerative disease and will therefore not be discussed further in this thesis.

2.3.7 Summary

Weight loss and cognition in PD may be mechanistically linked via hormones of energy homeostasis. This may be due to degeneration of ghrelin pathways and the somatotrophic axis resulting in weight loss, accelerated cognitive decline and neurodegeneration; through hyperinsulinaemia causing insulin resistance, cognitive impairment, loss of neuroprotection and appetite suppression; or through loss of pro-cognitive and neuroprotective leptin due to weight loss (Figure 8). Each of these pathways may be disordered and could be potential biomarkers for cognitive decline in PD but evidence is lacking. Further study is therefore needed to help clarify this relationship. This thesis therefore aims to explore whether appetite, food intake and hormonal profiles differ between patients with PD, PD-CI and controls using a cross-sectional pilot study observing these parameters in each group.

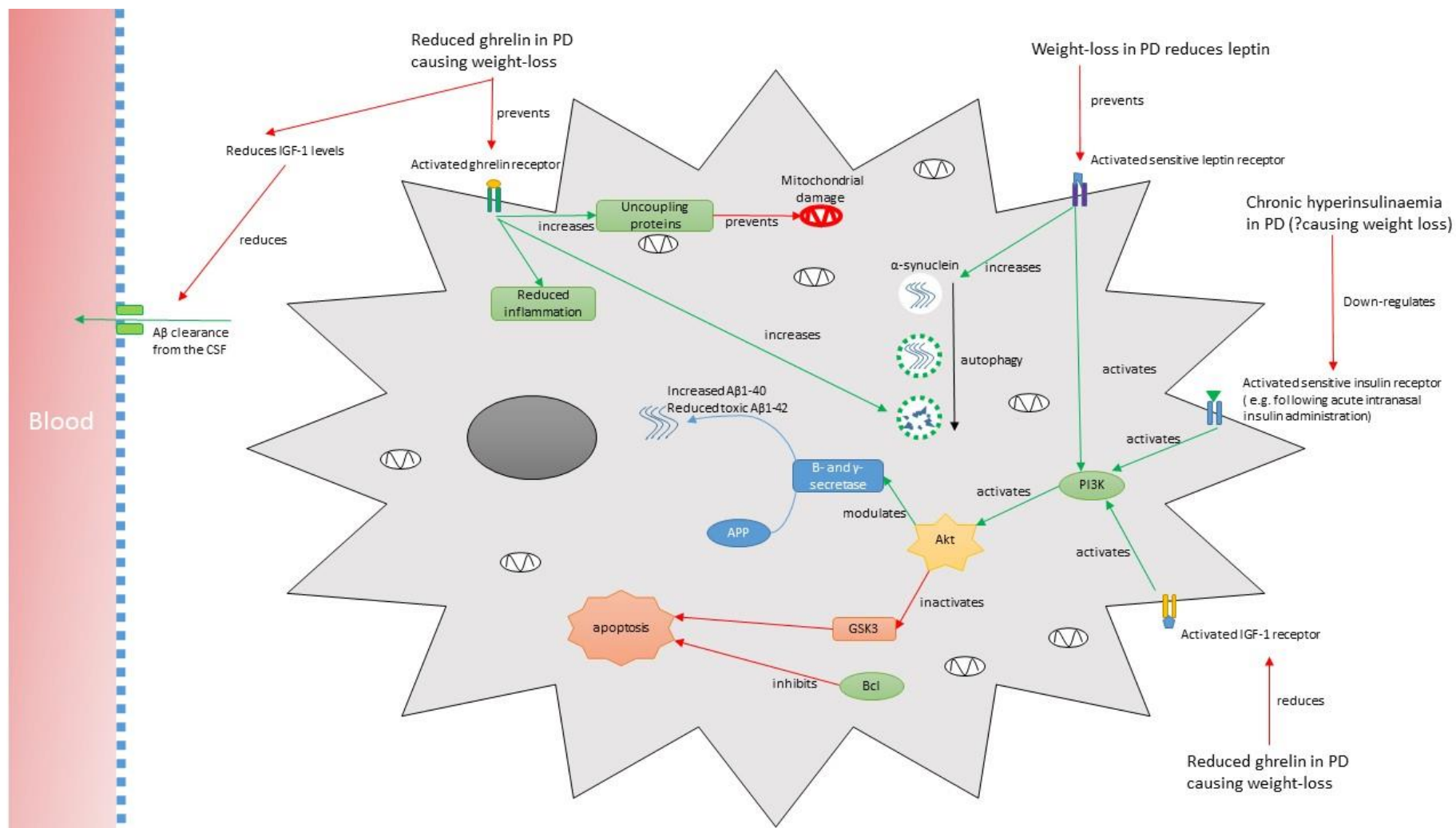


Figure 8. Mechanisms by which weight loss and neurodegeneration may interact in PD and PD-CI

Chapter 3. Methods- Plasma acyl-ghrelin: A biomarker for cognitive decline in Parkinson's disease?

3.1 Ethics and consent

This study received local approval and favourable review by the NHS research ethics service committee North East Newcastle and North Tyneside 1 (reference 14/NE/0002). It was carried out in accordance with the World Medical Assembly Declaration of Helsinki 1964 and subsequent revisions. Tailored written information was provided to all participants and carers according to group. This participant information sheet was given at least 24 hours prior to recruitment. Participants (patients and carers) with capacity to consent to take part signed a consent form prior to screening. In view of the need to include people with cognitive impairment, there was a risk that some participants may have lacked capacity to decide whether or not to take part in the study. All participants in the cognitively impaired group had a formal capacity assessment to determine this. If the participant lacked capacity then a carer, relative or Independent Mental Capacity Advocate was consulted. The consultees were given participant information sheet 24 hours in advance and asked to give a view on whether taking part was in keeping with the potential participant's previous wishes, view and beliefs (adhering to the Mental Capacity Act 2005). If so, consultees were asked to sign a consultation form to confirm this. Consultees were not asked to consent on the potential participant's behalf. Written informed consent was recorded at the first assessment and recorded in the site file. Participants and carers were given copies of the signed documents to keep. See appendices for evidence of ethical approval, participant information sheets, consent forms and consultee forms.

3.2 Study design and aims

This study was designed by Prof. David Burn, Dr. Mario Siervo, Prof. Jeff Davies and myself. The study design was a single-centre, cross-sectional quasi-experimental pilot study. The primary objective was to determine whether hormones of energy homeostasis warrant further investigation as putative biomarkers for cognitive impairment in PD. The secondary objective was to

determine whether perceived and objective measures of appetite and food intake differ between patients with PD, PD-CI and controls. The primary outcome was fasting and dynamic (area under the curve) hormone levels in PD-CI, PD and controls.

3.3 Sample size

The sample size was calculated by Dr. Shirley Coleman (a statistician at Newcastle University) based on the results of the previous study by Unger *et al.* (Unger et al., 2011). That study examined the ratio of ghrelin levels at 60 and 300 minutes and standard deviations were reported at these time points. Based on these values, and taking into account the different study design, Dr Coleman calculated that a sample size of 19 participants per group would be sufficient to detect a difference in AUC and levels over time at a 5% significance level with 80% power. She therefore recommended 20 participants in each group. See appendix D for more details.

3.4 Recruitment

Potential participants with PD and PDD were identified by their clinician within the Newcastle upon Tyne Hospitals Foundation NHS Trust Movement Disorder Service. Healthy controls were spousal or older people who had previously expressed an interest in engaging in research. Potential participants were contacted by telephone, e-mail or post. If they were interested in participating we sent information leaflets outlining the study as detailed above and followed up with a telephone call to further discuss participation. If they were happy to proceed at this point we invited them to attend Clinical Ageing Research Unit (CARU) at Newcastle University for assessment.

3.4.1 Inclusion and exclusion criteria

Subject Population

Adults of both sexes aged 60-85 were recruited into three groups; healthy controls, PD without cognitive impairment (PD) and PD with cognitive impairment (PD-CI). Groups were matched for age and gender. All subjects had a stable weight over the preceding 3 months and no history of diabetes mellitus (DM), gastrointestinal disease, tobacco use or non-selective anticholinergic agents due to potential effects on hormone levels, appetite or cognitive performance (Jo et al., 2002, Broglio et al., 2004). Potential participants were excluded if they were obese or severely underweight. This was in order to reduce confounding factors such as leptin resistance in obesity and abnormal hormone levels in cachexia (Caro et al., 1996). Deep brain stimulation was an exclusion for participants with PD as this may alter central ghrelin levels (Markaki et al., 2012).

Parkinson's disease with normal cognition (PD-NC) group

Participants in this group had an established diagnosis of idiopathic Parkinson's disease made by either a neurologist or a geriatrician with expertise in movement disorders. They had normal cognition as defined by a Montreal Cognitive assessment (MoCA) score of ≥ 26 and the absence of functional impairment resulting from cognitive symptoms.

Parkinson's disease with cognitive impairment (PD-CI)

This group comprised of participants with PDD and with PD-MCI. Participants in both subgroups had diagnoses of PD made by a neurologist or geriatrician with expertise in Parkinson's disease more than 12 months prior to the study. This was to distinguish PDD and PD-MCI from Dementia with Lewy bodies (DLB). Participants with PDD met the Movement Disorder Society Task Force Criteria for PDD, had a MoCA of $\leq 25/30$ and evidence of cognitive deficit impacting on activities of daily living (ADLs). Those with PD-MCI had self or carer reported neuropsychiatric symptoms (including, but not confined to; hallucinations, attention deficit, short term memory loss etc.) and a MoCA of $\leq 25/30$. They had no evidence of impact of cognition on ADLs. This is in keeping with level 1 criteria for the diagnosis of PD-MCI (Litvan et al., 2012). We chose a level 1 cut off for diagnosing PD-MCI in this pilot study due to time constraints and to reduce participant burden. Level 2 criteria requires formal in-depth

neuropsychological testing over 5 cognitive domains, which may be time consuming to deliver. Level 1 criteria for diagnosing PD-MCI is validated but provides a less comprehensive assessment than level 2 criteria and precludes comprehensive subtyping of MCI (Litvan et al., 2012).

Control definition

Healthy controls had no signs or symptoms of movement disorders or dementia. They were spousal or age matched community dwelling adults.

Inclusion and exclusion criteria

Table 10. Inclusion criteria

All participants			
Age 60-85			
Any gender			
English language skills sufficient to allow valid use of cognitive tests (MoCA and MMSE)			
Written consent to participate in the study or, if the participant lacks capacity to consent, written consultation with an appropriate carer in accordance with the Mental Capacity Act 2005.			
PD	PD Cognitive impairment		Controls
	PDD	PD-MCI	
PD diagnosed by a neurologist or a geriatrician	PD diagnosed by a neurologist or a geriatrician	PD diagnosed by a neurologist or a geriatrician	No evidence of Parkinsonism on examination
MoCA \geq 26	Duration of PD > 1 year	Duration of PD > 1 year	MoCA \geq 26
No cognitive symptoms causing functional impairment	Meets MDS task force criteria for PDD	MoCA \leq 25	No evidence of cognitive symptoms causing functional impairment
	MoCA \leq 25	Neuropsychiatric symptoms	
	Functional impairment due to cognition	No functional impairment due to cognition	

Table 11. Exclusion criteria

All Participants		
Age <60 or >85		
Clinically significant depression		
Diabetes Mellitus		
Tobacco use		
BMI <18.5kg/m ²		
BMI \geq 30kg/m ²		
Comorbid gastrointestinal disease		
Concurrent use of non-selective anticholinergic medication		
\geq 3 kg weight change over the preceding 3 months		
Significant comorbidity		
Difficult venous access		
Allergy to ingredients of test meals		
PD	PD cognitive impairment	Controls
Evidence of dementia	Dementia within 12 months of diagnosis of PD	Evidence of Parkinsonism
Deep brain stimulation (DBS)	DBS	Evidence of dementia

Challenges and amendments

Recruitment to this study was challenging due to the older age and comorbidity of participants and carers. As a result, a series of amendments from the research ethics committee and from research and development were required. Reasons for declining to take part in the study are detailed in Figure 9.

In our original protocol we excluded participants who were already taking part in research. Many patients in the movement disorder service who are interested in research, however, were already taking part in low-burden longitudinal observational studies. We therefore extended recruitment to stop excluding participants who are taking part in observational studies.

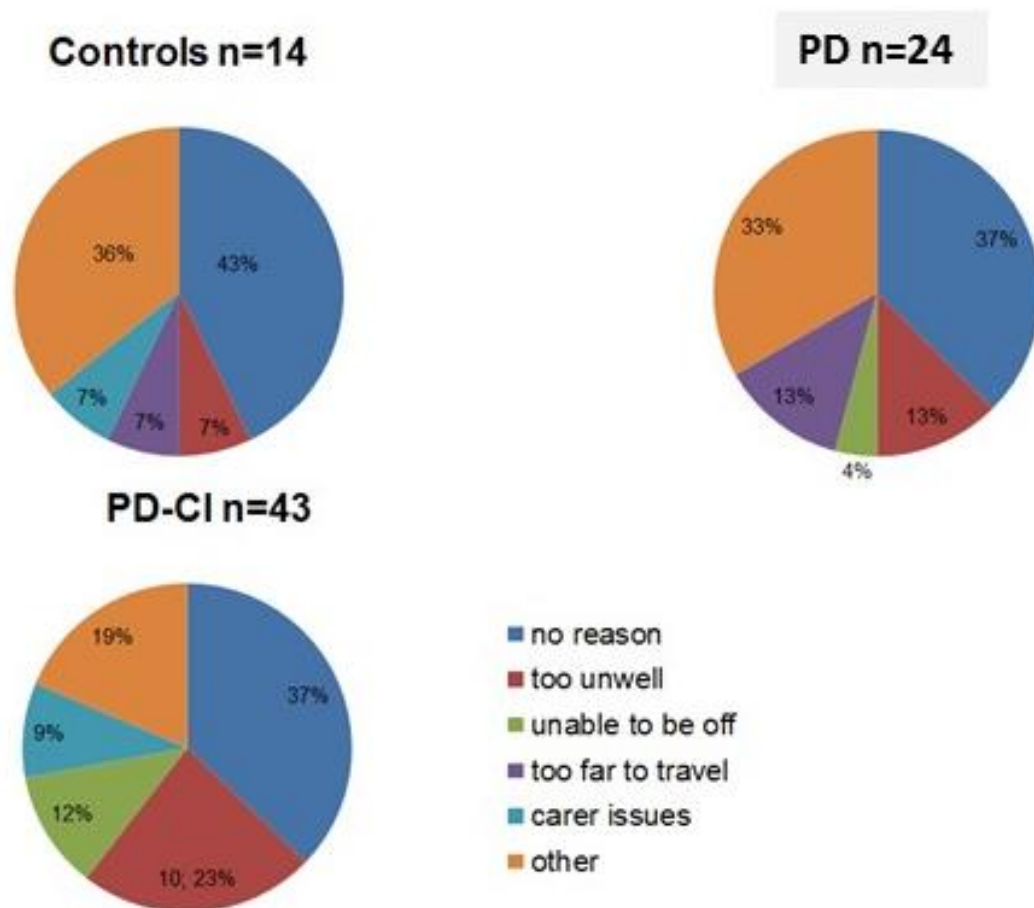


Figure 9. Reasons for declining by group

Similarly, our original protocol specified spousal controls. Healthy spousal controls proved difficult to recruit from clinic as their caring commitments made attending appointments difficult. Several spousal controls were also unwell themselves. We therefore expanded recruitment to include healthy older adults who had previously expressed an interest in taking part in research at CARU and members of the local Parkinson’s UK branch.

There was particular difficulty in recruiting participants to the PD-CI group. This group was more likely to decline to take part than other groups and a greater proportion of participants in this group failed screening (12/30; 40%) compared with PD and (4/24; 16%) and controls HC (6/26; 23%). Figure 10 shows the reasons for screen failure by group.

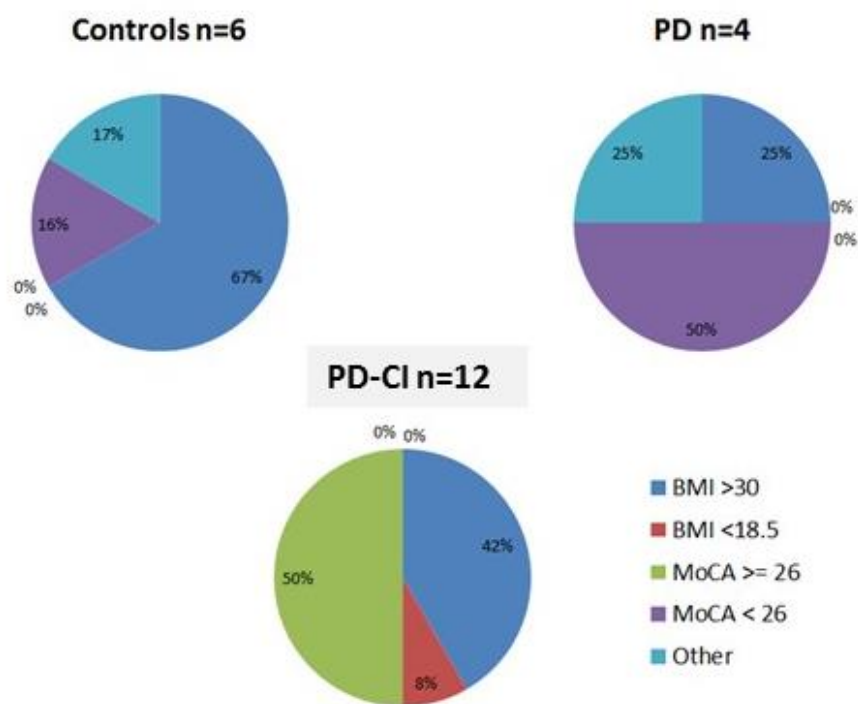


Figure 10. Reason for screen failures by group

We therefore relaxed the inclusion criteria for participants in the cognitively impaired group. The original design of the study was to include participants with clear dementia only in the PD-CI group, with a cut off MoCA score of 21/30 or

below required for inclusion. Following this amendment participants with a diagnosis of PDD and a clear impact of cognitive impairment on activities of daily living can be included despite scoring >21/30 on MoCA. In order to obtain clearly different groups participants in the PDD group were still required to score <26/30 on this test for inclusion. We also added PD-MCI as a subgroup. These participants met the level 1 criteria for MCI according to the MDS task force criteria as detailed above (Litvan et al., 2012). Again, MoCA score was required to be <26/30. Recruitment is summarised in Figure 11.

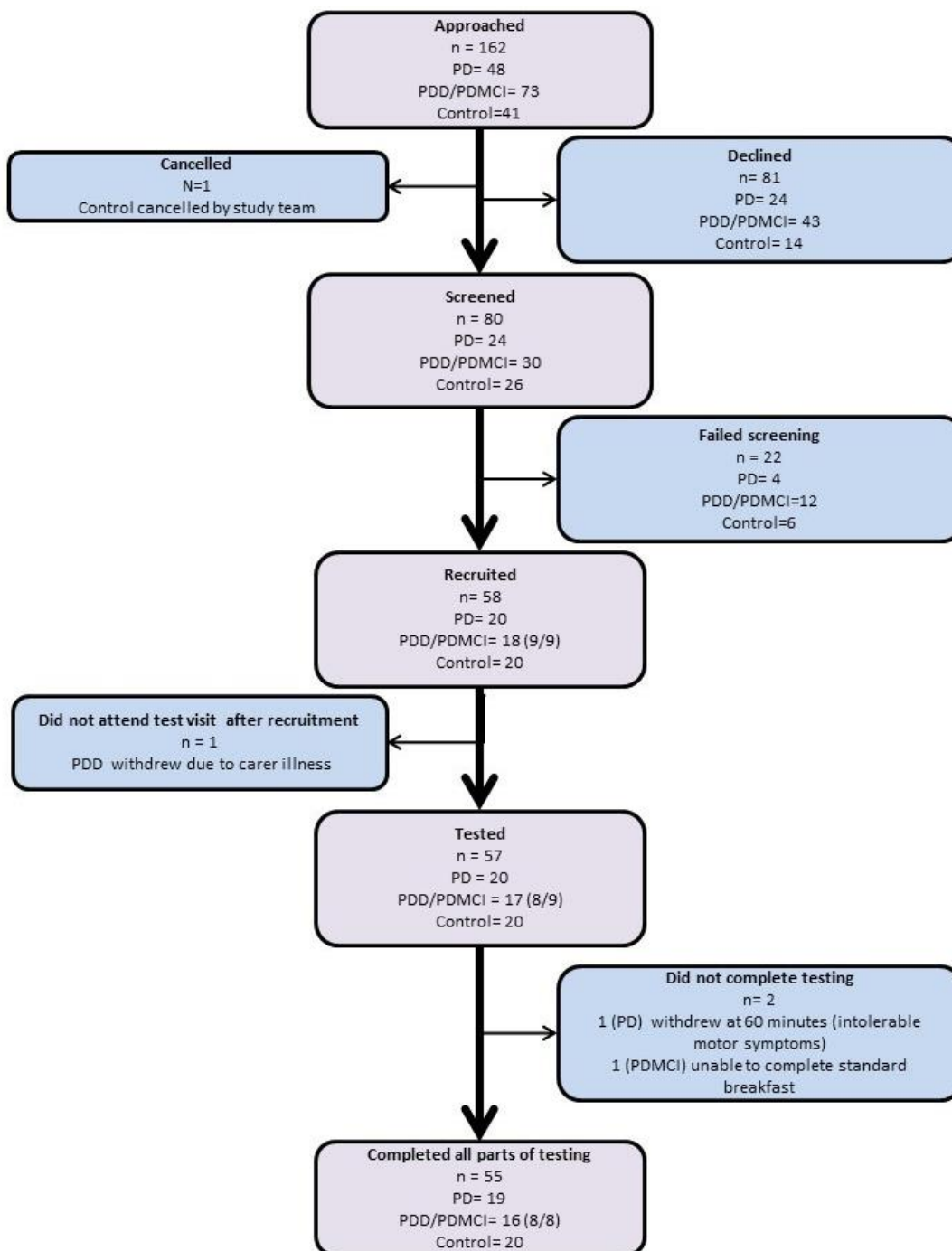


Figure 11. Summary of recruitment

Screening

Once informed consent was obtained participants underwent a screening visit. The main objective of this visit was to make sure that inclusion criteria were met and that no exclusions were present. Participants underwent a review of past medical history, medication review and social history. Impact of cognition on activities of daily living was assessed as part of social history through self-report or a collateral history from a carer. Physical examination including a general examination and examination of the cardiovascular, respiratory, gastrointestinal and neurological systems was carried out. This was in order to exclude undiagnosed exclusion criteria such as Parkinson's disease in control groups.

Participants were weighed and measured and BMI was calculated. Fat mass was assessed using bioimpedance using a set of Tanita scales. Where it was not possible to measure percentage body fat using Tanita (for example, due to pacemaker or inability to stand unaided) fat mass was been calculated using the following formulae (Jebb *et al.*, 2000);

$$\text{Male \% fat} = (1.281 \times \text{BMI}) - 10.13$$

$$\text{Female \% fat} = (1.480 \times \text{BMI}) - 7.00$$

When it was not possible to measure height, previous heights recorded in the medical notes was used.

Motor disease severity was assessed using the movement disorders society unified rating scale (UPDRS) motor section. This assessment is well validated in the PD population (Martinez-Martin *et al.*, 2013). Mood was assessed using the geriatric depression scale (GDS), which is widely used in clinical practice but which has not been validated for use in dementia (Yesavage *et al.*, 1983). This is a limitation of its application. Participants were therefore not excluded on the basis of a high GDS score alone, but only if they had symptoms of uncontrolled clinical depression. Cognition was assessed using the Montreal Cognitive Assessment (MoCA), which is well validated in PD (Dalrymple-Alford *et al.*, 2010). Baseline eating habits were assessed using the three factor eating questionnaire (Stunkard and Messick, 1985). Finally, blood tests were taken to exclude DM and assess general health.

3.5 Testing

Standardised meal

Test visits were carried out no more than 5 weeks after screening. Patients were tested after a 12 hour fast and “off” i.e. having not taken any of their usual PD medication. Other medication was taken as usual. Water was allowed up to 2 hours prior to testing. Patients were re-weighed if more than 7 days had passed since their screening visit. The following standard 300 kcal breakfast was offered;

- One slice of white toast with butter
- One Activia Strawberry yoghurt
- Jam
- Short bread biscuit
- A 200 ml glass of water

Participants were allowed 20 minutes to complete their meal and were required to eat all of it. One hundred and twenty mls of water was given at 60 minutes and 120 minutes for participant comfort and to prevent dehydration.

Assessment of appetite

Visual analogue scales (VAS) were administered to assess fullness and satiety prior to breakfast and then 5,15,30,60,120 and 180 minutes post-prandially (Flint et al., 2000). Usual eating behaviour was assessed using the three-factor eating questionnaire (TFEQ) (Stunkard and Messick, 1985).

Assessment of intake

At 180 minutes participants were given an *ad libitum* meal comprising a pre-measured selection of foods. They were invited to eat as much as they liked and to take their usual PD medications at this time. Presentation of the meal was standardised (see appendix E). The meal comprised of;

- 1 medium banana
- 1 chocolate mousse
- 1 bag ready salted crisps
- Grated cheddar cheese
- Tomato and mozzarella pasta bake
- 1 large glass of water

Each food item was weighed before and after the meal to gain an accurate measure of the food ingested. Energy and macronutrient intake was calculated using the Tesco supermarket's published nutritional data.

A bite counter was also used to measure mouthfuls of food consumed. Bite counters are wrist-worn accelerometers which record each hand movement towards the mouth. They have been used to estimate food consumption in healthy adults as bite rate has been found to correlate with energy intake (Dong et al., 2012, Scisco et al., 2011). No research has evaluated their use in PD patients to date. Bite count and frequency were recorded both manually and using the bite counter during the *ad libitum* meal.

Blood sampling

Blood sampling was carried out via a peripheral venous cannula. Blood was drawn at baseline (fasting) and 5,15,30,60,120 and 180 minutes post-prandially for glucose, lipid profile, leptin, insulin, GH, PYY, GLP-1, total ghrelin, acyl-ghrelin and TNF α . Blood taken in order to test glucose and lipids was drawn into gold topped SST™ BD vacutainers and analysed at the local hospital according to their usual standard operating procedures (SOP). Blood taken in order to analyse leptin, insulin, GH, PYY, GLP-1, total ghrelin, acyl-ghrelin (AG), was drawn into EDTA-containing vacutainer tubes and processed immediately on site before being frozen at -80. Briefly, whole blood treated with EDTA was divided into three 2 millilitre (ml) aliquots for each time point. One aliquot was

added to 80ul of 4-(2-Aminoethyl)-benzenesulfonyl fluoride stock solution (50mg/ml, or 200mM) to give a final concentration of 2mg/ml, or 8mM. This was centrifuged immediately along with one other aliquot at 2,000 x g for 15 minutes at 4 ± 2 °C and 400 microlitre (μ l) of the supernatant pipetted into cryotubes for storage at -80 °C. The final fraction was kept on ice until it was centrifuged at 1,000 x g for 10 mins to produce platelet rich plasma. Again, the supernatant was pipetted into cryotubes for storage at -80 °C. Please see appendix F for the detailed SOP.

Hormonal Analysis

Hormone levels were measured by Prof. Jeff Davis and Ms Amanda Hornsby at Swansea University. AEBSF treated samples were used to quantify leptin, insulin, IL-6, PYY, GLP-1 and AG using the *Milliplex MAP Kit-Human Metabolic Hormone Magnetic Bead Panel 96-well plate* multiplex assay. Total ghrelin was assessed using the *Human Ghrelin (Total) ELISA Kit* (Cat. No. EZGRT-89K, Millipore). Insulin-like growth factor-1 was assessed using *Human IGF-1 DuoSet ELISA kit* (cat. No. DY291, R&D Systems). Finally, GH was assessed using *Human GH DuoSet ELISA kit* (Cat. No. DY1067, R&D Systems).

Completion and follow-up

Each participant completed the study within 6 weeks including follow up contact by telephone. Follow up was carried out 7-10 days after the second visit in order to answer any questions they may have and to gather feedback about their experience.

3.6 Medication

As described above, participants were tested “off” their usual dopaminergic medication. We asked participants not to take their PD medications on the morning of the test visit. In practice, this meant no PD medication from midnight until the *ad libitum* meal; 8-9 hours prior to breakfast and 11-12 hours before lunch. This was in order to reduce potential dopaminergic effects on appetite. We did not ask participants to withhold long acting dopaminergic medications for any longer than their other PD medications, however, as we felt that this would be too burdensome. Controlled release levodopa and levodopa in combination with the catechol-O-methyl transferase (COMT) inhibitor

entacapone fall to less than 10% of peak dose at 6.3 and 7.5 hours respectively (Hsu et al., 2015) but “washout” periods of several days to return to baseline have been reported in the literature (Khor and Hsu, 2007). Other studies have used washout periods of 36 hours for longer acting drugs such as dopamine agonists (Athauda et al., 2017).

Participants were allowed to take other medications, including cholinesterase inhibitors during the study period. We therefore compared cholinesterase inhibitor, antidepressant and long acting PD medication use between groups. We checked each medication that participants were taking for pro-appetitive and anorectic side effects using the British National Formulary (BNF) online (www.bnf.nice.org.uk). Where there was ambiguity (e.g. “weight change”) in the listed side effects, we consulted the manufacturer’s product information. Monoamine oxidase inhibitors (MAOIB) are anorectic drugs. Although they were not taken on the day of testing they were included in the anorectic drug burden as they irreversibly inhibit monoamine oxidase-B (Schapira et al., 2005, LeWitt, 2009). We also calculated participants’ usual levodopa equivalent dose (LED) in the PD and PD-CI groups using the well-validated tool by Tomlinson *et al* (Tomlinson et al., 2010).

3.7 Statistical analysis

Statistical analysis was carried out with Dr. Mario Siervo using IBM SPSS statistics software version 22. Graphs were produced using StatSoft Statistica version 13. Age, disease duration, BMI, fat mass and medications were not normally distributed. The demographic characteristics; age, BMI and fat mass were therefore compared between groups using Kruskal Wallis testing. Mann-Whitney U tests were used to compare disease duration and total UPDRS score between PD and PDD groups. Non-PD medications were compared using the Kruskal Wallis test whilst PD medications and LED were compared using the Mann-Whitney U test. Gender was compared using the chi-squared test. All other variables that were not normally distributed, such as hormone levels scores were log-transformed for analysis. MoCA was reversed and then log transformed (Ln [31-MoCA score]) in order to increase normality. Assumptions for analysis of variance (ANOVA) and linear regression were checked using SPSS.

Repeated measures linear regression was used to compare analytes between groups over time. Disease duration was included as a covariate in this model. Area under the curve was also calculated for each analyte and for visual analogue scales using the trapezoidal method. The AUC for each analyte was compared between groups using ANOVA. Post-hoc comparisons were carried out using the least significant difference method. Analysis was then repeated including potentially confounding covariates with analysis of covariance (ANCOVA). Where PD duration was included as a covariate disease severity measured by motor UPDRS was not included in order to avoid over correction for very similar information. We had an accurate record of date of diagnosis for each participant in the PD and PD-CI groups. We chose to include disease duration rather than UPDRS in our analysis as disease duration is not prone to day-to-day fluctuation or medication effects.

This study was novel in that appetite, energy intake and many of the hormones tested had never been examined in the context of cognitive impairment in PD previously. Analysis was therefore exploratory rather than hypothesis-driven. To this end we tested a large number of potential associations between variables using Pearson's correlation the results of which are presented in Appendix I.

Variables which correlated with reversed log transformed MoCA were entered into a linear regression model to determine predictors of cognition across the whole cohort. This process was repeated for energy intake, with the additional inclusion of AG in the analysis to ensure that appetite suppressant factors and stimulant factors were balanced in the model.

Retrospective analysis of insulin resistance was undertaken using the Homeostasis Assessment Method which is calculated as follows (Wallace et al., 2004, Matthews et al., 1985);

HOMA-IR= (fasting plasma insulin microU/l x fasting plasma glucose mmol/l)

22.5

Units were converted as proposed guidelines from the World Health Organisation (Burns et al., 2010).

Throughout the statistical analysis process, where hormone levels were not detectable they were treated as missing values. Missing data was not interpolated or extrapolated but area under the curve was recalculated taking missing data into account by adjusting the length of time between data points. Area under the curve was not calculated where more than 3 values were missing per participant.

Chapter 4. Do appetite and food intake differ between people with PD, PD-CI and controls?

Weight loss is a risk factor for cognitive impairment in PD (Kim et al., 2012, Lorefalt et al., 2004, Uc et al., 2006), though the mechanisms underlying this are not well understood. It is not yet known whether energy intake is higher in people with PD-CI as is seen in people with PD and normal cognition. Energy intake could equally be reduced due to higher burden of non-motor symptoms, difficulty with self-feeding or hormonal consequences of neurodegeneration (Aarsland et al., 2001, Bachmann and Trenkwalder, 2006, Barichella et al., 2009, Beyer et al., 1995). This study examined 55 weight stable older adults with a healthy BMI in three groups 1) healthy controls (n=20), 2) PD without cognitive impairment (n=19) and 3) PD-CI (n= 16, 8 PD MCI and 8 PDD). All participants in the PD and PD-CI groups were tested “off” their usual PD medication to avoid possible confounding effects of levodopa. The aim of this chapter is to determine whether perceived and objective measures of appetite and food intake differ between patients with PD, PD-CI and controls.

4.1 Results

4.1.1 Demographics

There were no significant differences between age, gender, BMI, fat mass or the number of comorbidities other than PD between groups. MoCA was significantly lower in the PD-CI group compared to other groups ($p < 0.001$). The duration of PD and the severity of motor symptoms as measured by the MDS UPDRS part 3 were greater in the PD-CI group than the PD group ($p = 0.02$ and $p = 0.007$ respectively) Because PD duration and motor UPDRS reflect similar information, PD duration alone was used for correction in this analysis in order to prevent over-correction of data. No participant had clinical depression but GDS scores were higher in the PD-CI group (Kruskal-Wallis $p < 0.001$, pairwise post-hoc test with Bonferroni correction $p < 0.001$ significance between control and PD-CI, $p = 0.000$ between PD and PD-CI and $p = 0.56$ between controls and PD group)). GDS has therefore been included in the ANCOVA comparing area under the curve (AUC). A summary of demographic data is presented in Table 12.

Table 12. Summary of demographic details

Variable (mean ±SD)	Controls	PD	PD-CI	P value
Age in years	74.00 ±6.28	72.53±5.46	74.31±6.01	P=0.648‡
Gender % female	45	47.4	43.8	P=0.976‡‡
Fat Mass %	28.78 ± 7.25	28.48±8.46	25.39±7.66	P=0.268‡
BMI kg/m ²	25.75±2.04	25.18±2.83	24.03±3.31	P=0.242‡
Number of comorbidities	2.85±1.93	2.74±1.56	2.50±1.79	P=0.874‡
MoCA score	28.35±1.14	27.58±1.50	17.25±5.43	P<0.001‡
Motor UPDRS score	NA	31.95±13.22	49.19±19.16	P=0.007‡‡‡
Duration of PD in months	NA	69.53±69.10	107.25±59.48	P=0.022‡‡‡
GDS score	1.25±0.91	2.00±1.41	5.81±2.51	P=0.000‡

‡ Kuskal-Wallis test ‡‡ Pearson Chi-squared test ‡‡‡ Mann-Whitney U test

There was no significant difference in age ($p=0.34$, Mann Whitney U test) or percentage female ($p=0.104$ Pearson Chi-squared test) between those who passed or failed screening. Our cohort had an average of 2.96 comorbidities other than PD overall, with no significant difference between those who passed or failed screening ($p=0.72$)

4.1.2 Medication

Table 13. Medication use by group

Medication	Controls (n=20)	PD (n=19)	PD-CI (n=16)	P value
Standard preparation levodopa	N/A	16 (84.2%)	16 (100%)	P=0.101‡‡‡
Controlled release levodopa	N/A	3 (15.8%)	9 (56.2%)	P=0.013‡‡‡
Dopamine agonists	N/A	7 (36.8%)	0 (0%)	P=0.007‡‡‡
MAOIB	N/A	11 (57.9%)	2 (12.5%)	P=0.006‡‡‡
COMT inhibitors	N/A	4 (21.0%)	7 (43.8%)	P=0.156‡‡‡
LED	N/A	557.8±377.8	737.5±493.3	P=0.259‡‡‡
Cholinesterase inhibitors	0 (0%)	0 (0%)	7 (43.8%)	P<0.001‡
Selective anticholinergics	1 (5%)	3 (15.8%)	3 (18.8%)	P=0.422‡
• Solifenacin	1	3	1	
• Trospium	0	0	2	
Antidepressants	0 (0%)	0 (0%)	7 (43.8%)	P<0.001‡
• Mirtazapine	0	1	3	
• Citalopram	0	0	1	
• Venlafaxine	0	0	2	
• Fluoxetine	0	0	1	
Pro-appetitive medicines	0 (0%)	0 (0%)	3* (18.8%)	P=0.022‡
• Mirtazapine	0	0	3	
• Pregabalin	0	0	2	
Anorectic medications	0 (0%)	11 (57.9%)	6 (37.5%)	P<0.000‡
• MAOIB	N/A	11	2	
• Citalopram	0	0	1	
• Venlafaxine	0	0	2	
• Fluoxetine	0	0	1	
• Clozapine	0	0	1	
• Amitriptyline	0	1	0	

‡ Kuskal-Wallis test ‡‡ Pearson Chi-squared test ‡‡‡ Mann-Whitney U test

* 2 PD-CI participants on both mirtazapine and pregabalin

** 1 PD-CI participant on both clozapine and venlafaxine

There was no significant difference in usual LED or COMT inhibitor use between the PD and PD-CI groups ($p=0.259$ and $p=0.156$, respectively). Dopamine agonist and MAOIB use were significantly greater in the PD group compared to the PD-CI group ($p=0.007$ and $p=0.006$ respectively), whilst controlled release levodopa use was more common in the PD-CI group ($p=0.013$). No participants in the control or PD groups were taking antidepressants or cholinesterase inhibitors. Pro-appetitive medication use was more common in the PD-CI group ($p=0.022$). Anorectic medication use was more common in the PD group ($p<0.001$), almost entirely due to MAOIB use. There was no significant difference in selective anticholinergic use between groups.

4.1.3 Missing data

There was no missing demographic data. One participant in the PD-CI group did not have the frequency of PD medication recorded due to oversight. Type and dose of medication were recorded. There was little missing data regarding appetite and energy intake. All participants had visual analogue scales for hunger and fullness completed, though 4 participants did not have data recorded at 5 minutes due to time pressure. All participants had energy intake at the *ad libitum meal* recorded. Three participants did not have an accurate record of the duration of their *ad libitum meal*. Missing data for appetite and intake are summarized in table 14.

Table 14. Number of participants with data missing by variable

	Control	PD	PD-CI	Comments
VAS Hunger	0	1	3	Time point 5 missing in all cases due to time pressure all other data complete
VAS fullness	0	1	3	Time point 5 missing in all cases due to time pressure all other data complete
Duration of <i>ad libitum meal</i>	1	1	1	Time meal finished not documented in source data

4.1.4 Appetite

Subjective feelings of hunger and fullness were measured using 100mm visual analogue scales (VAS). Participants with cognitive impairment were supported by their carers in completing the VAS. Participants from all groups showed similar patterns of hunger over the course of the morning, with a reduction in hunger after breakfast which recovered to baseline at 3 hours. Repeated

measures ANCOVA showed that change over time was not significantly different between groups when corrected for PD duration ($p=0.41$) (Figure 12).

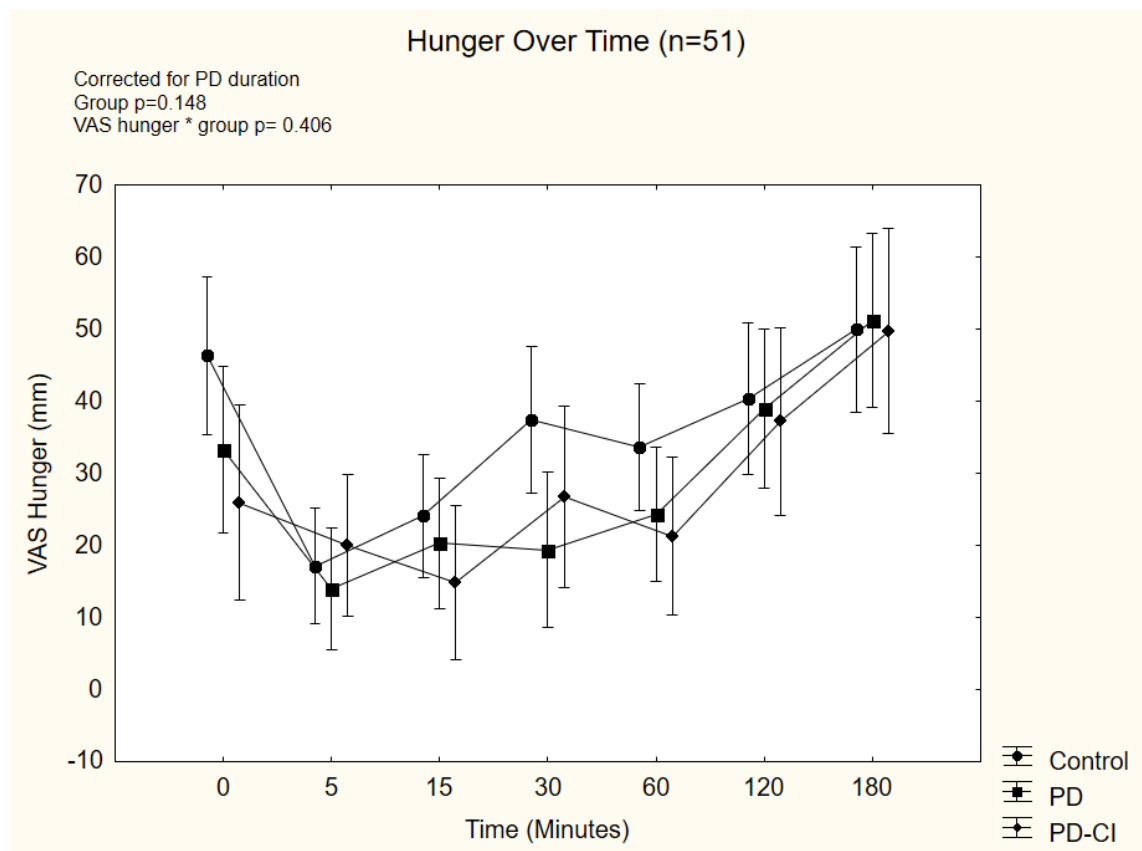
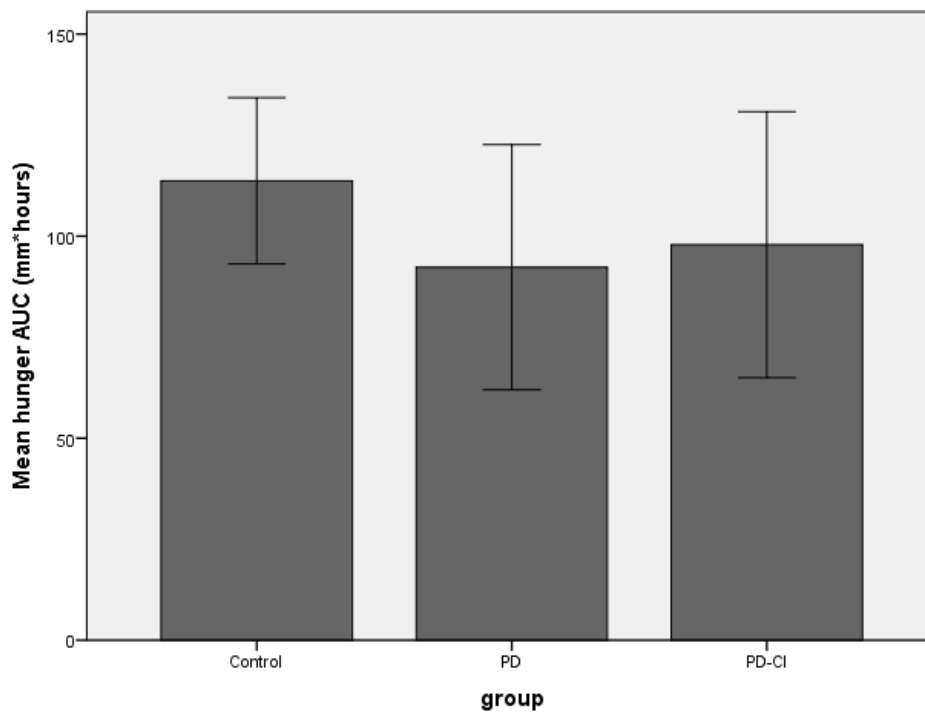


Figure 12. Hunger over time by group

Area under the curve for hunger was compared between groups using ANOVA. There was no significant difference between groups ($p=0.48$), even after controlling for PD duration and depression in the analysis ($p=0.105$) (Figure 13).



Error Bars: 95% CI

Figure 13. Mean hunger area under the curve

Results for fullness followed a similar pattern, with all groups showing an increase in fullness after breakfast which declined over 3 hours. Repeated measures ANCOVA showed that change over time was not significantly different between groups when corrected for PD duration ($p=0.15$) (Figure14).

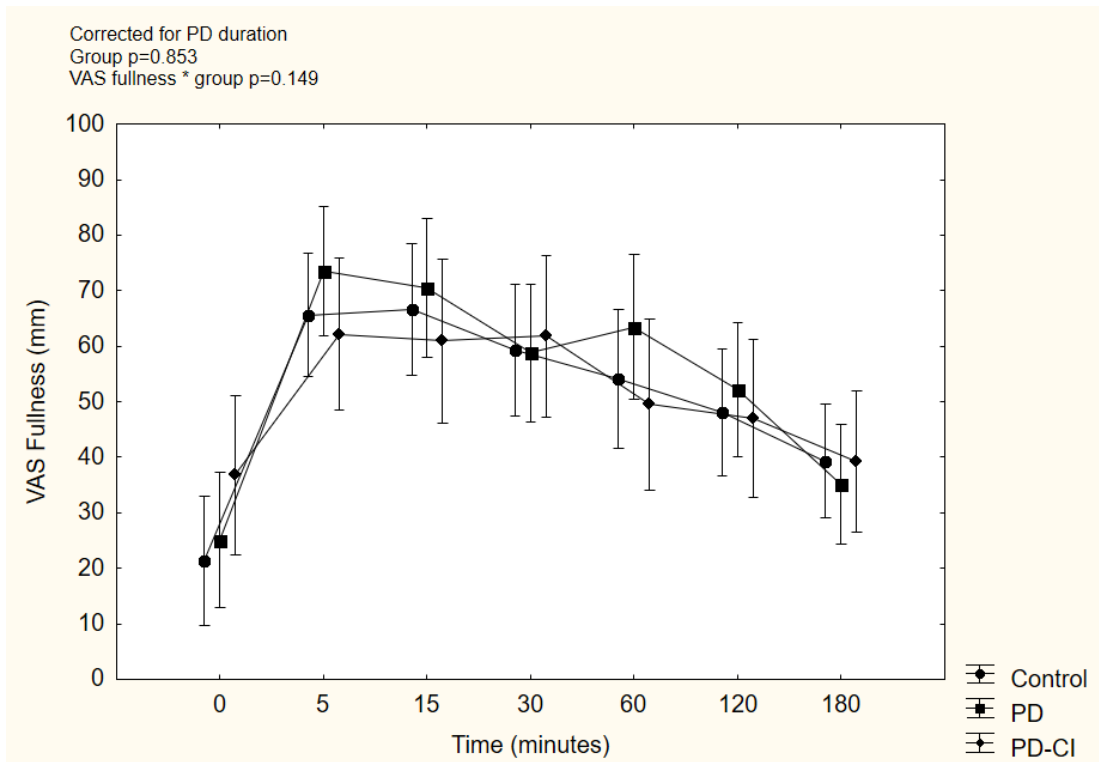


Figure 14. Fullness over time by group

Area under the curve was once again not significantly different between groups ($p=0.58$) even after correction for depression and PD duration ($p=0.44$) (Figure 15).

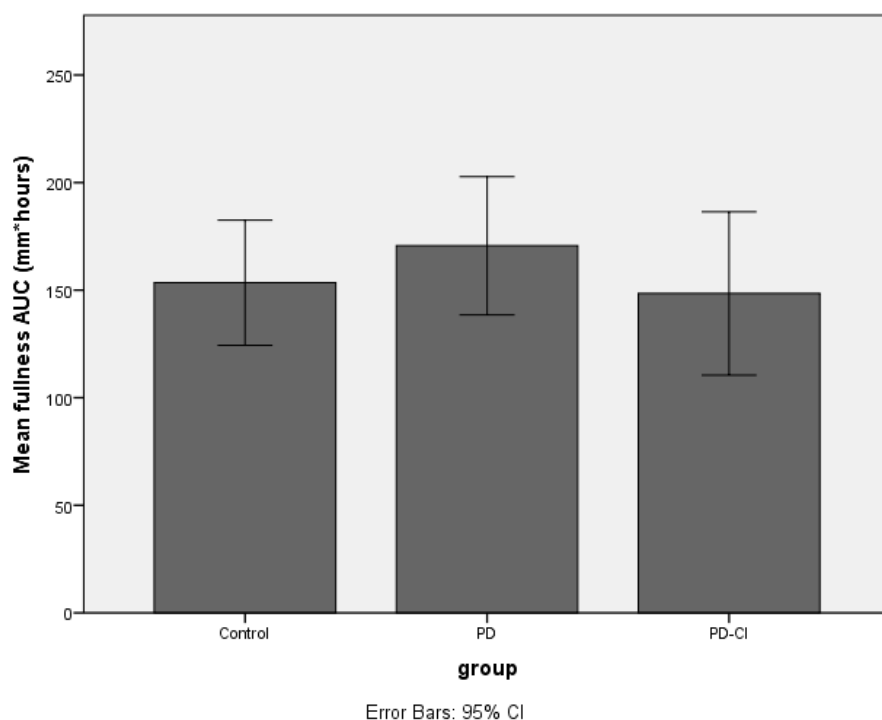


Figure 15. Mean fullness area under the curve

Usual eating behaviour with regard to restraint, disinhibition and hunger were recorded at the screening visit using the Three Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985). Restraint was significantly different across the groups (Kruskall-Wallis test $p=0.03$). Pairwise comparison with Bonferroni correction confirmed the PD-CI group had significantly lower restraint than the control group ($p=0.02$). There was no difference in hunger or disinhibition between groups

4.1.5 Energy intake

Energy intake was calculated by weighing food before and after the meal and calculating the total energy consumed in kilocalories (kcal) based on the manufacturers published information. Energy intake did not differ significantly between groups ($p=0.29$) (Figure 16).

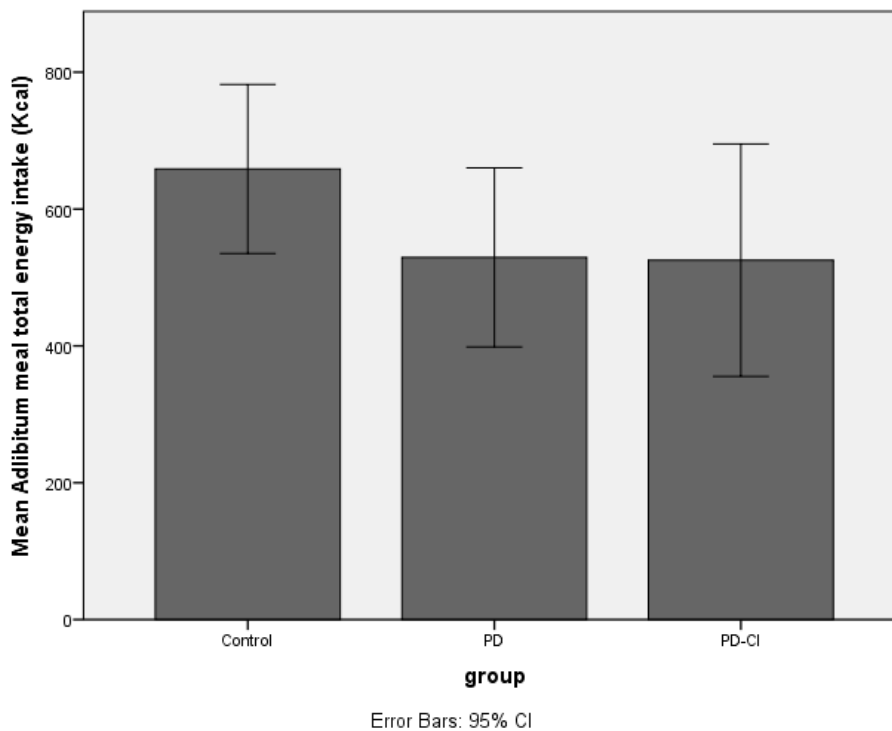


Figure 16. Total energy intake by group

Energy intake significantly negatively correlated with the satiety hormone PYY ($r = -0.29$, $p = 0.03$) and with AUC fullness ($r = -0.55$, $p < 0.001$). There was a positive correlation between energy intake and hunger ($r = 0.44$, $p = 0.001$) and energy intake and duration of the meal ($r = 0.39$, $p = 0.004$). There were no other significant correlations between other hormones of energy homeostasis and energy intake. There was no significant correlation between cognition (Log transformed reversed MoCA) and energy intake ($r = -0.06$, $p = 0.66$). Scatter plots are shown in Figure 17. Time taken to complete the *ad libitum* meal and PYY AUC were not significantly different between groups ($p = 0.15$ and $p = 0.38$ respectively).

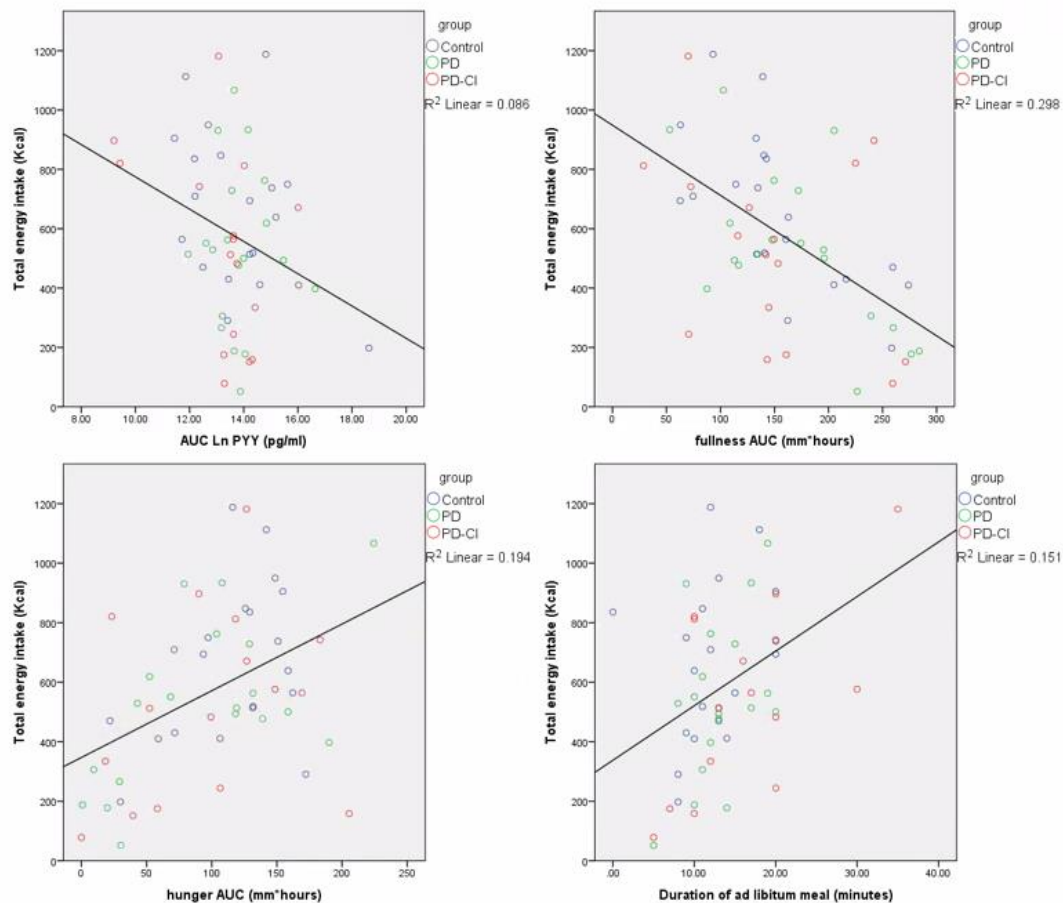


Figure 17. Scatter plots showing the relationship between energy intake (Kcal), Ln PYY, fullness, hunger and duration of ad libitum meal

In order to determine whether any of these factors predicted energy intake all variables with a significant correlation with energy intake were entered into a multiple linear regression model along with AG. Acyl ghrelin was included, despite no significant correlation with energy intake, in order to ensure balance in the model between factors promoting intake (AG and VAS hunger) and factors limiting intake (PYY and VAS fullness). This model predicted energy intake, accounting for 44.9% of the variance (F [4-48] 9.79, R squared=0.45). Fullness and PYY contributed significantly to the model (p=0.009 and 0.002 respectively) but hunger and AG did not (p=0.11 and 0.76 respectively).

Table 15. Multiple linear model regression for predictors of energy intake

Variable	Unstandardised coefficient B	Standard error of B	β	significance
AG AUC	-4.861	15.807	-0.036	P=0.760
PYY AUC	-64.532	19.892	-0.348	P=0.002
Hunger AUC	1.236	0.753	0.243	P=0.107
Fullness AUC	-1.722	0.637	-0.401	P=0.009

Dependent variable is energy intake

4.2 Discussion

4.2.1 Appetite

The majority of research into eating habits in PD has focused on oral intake, rather than patient reported measures of appetite. This study is primarily concerned with examining hormones of energy homeostasis in PD and PD-CI. These hormones profoundly affect eating behaviour through manipulating feelings of hunger and fullness generated with the arcuate nucleus of the hypothalamus (Cowley et al., 2001, Fan et al., 1997). Weight loss in PD has been paradoxically linked to increased energy intake in PD (Barichella et al., 2017). It has been suggested that appetite may be inadequate in PD, especially in those with a higher burden of non-motor symptoms (Politis et al., 2010). Given the link between weight loss, increasing numbers of non-motor symptoms and the development of cognitive impairment in PD (Lorefalt et al., 2004, Uc et al., 2006), we hypothesized that appetite may be diminished in PD-CI. Alternatively, appetite may be increased in keeping with the observed increase in energy intake in PD (Chen et al., 2003). To date there are no published studies examining appetite in PD-CI.

Appetite was assessed using VAS of hunger and fullness. We found that the pattern of hunger between meals was as expected for all groups, with a rapid decline after breakfast and recovery towards the *ad libitum* meal at 180 minutes (Figure 12). There was no significant difference in hunger AUC between groups. Similarly, we saw an attendant increase in fullness after eating in all three groups, which declined over 180 minutes (Figure 14). Again, there was no significant difference in fullness between groups. Our findings were similar to those seen in the original validation study carried out for VAS in 55 healthy men. Here participants were followed for 4.5 hours after a standardised breakfast and had VAS for hunger, fullness and a battery of other food preferences every 30 minutes. Hunger reached a nadir 30 minutes after breakfast and recovered back to baseline between 180 and 300 minutes. Similarly, fullness peaked at 30 minutes and returned to baseline at 240 minutes (Flint et al., 2000). It is possible that validity of VAS was inadvertently compromised in the PD-CI group by guidance from the nurse delivering the tool or from over-zealous carers. Against this, AUC was positively correlated with energy intake whilst fullness AUC was negatively correlated with energy intake

across the whole cohort. Moreover, confidence intervals for hunger and fullness AUC are also similar between groups. Our data are in keeping with previous research showing that energy intake at an *ad libitum* meal was positively predicted by hunger (measured using VAS) and negatively predicted by fullness (measured using VAS) in both younger and older adults (Parker et al., 2004a). Overall, our results suggest that VAS, whilst not validated for use in PD or PD-CI, were reasonably accurate in ascertaining feelings of hunger and fullness in this study.

Usual eating behaviour was assessed using the TFEQ. This is a 51 item questionnaire which evaluates three domains of eating behaviour; restraint; the extent to which a person is conscious of their eating habits and limits their intake; disinhibition, the degree to which they eat due to emotional cues; and hunger, how hungry they tend to feel. The TFEQ has mainly been used in research around obesity and has not been validated for use in a cognitively impaired cohort. In the context of obesity, higher levels of restraint can result in increased intake at a subsequent meal (Stunkard and Messick, 1985). A score of 11 or more in the restraint domain suggests high levels of restraint (Lesdéma et al., 2012). In this study of non-obese people it seems unlikely that restraint significantly impacted the eventual energy intake at the *ad libitum* meal. Only 2 participants, both controls, had restraint scores more than 11. If significant, this would have most likely resulted in greater intake in the control group, which was not observed (see below). Disinhibition and hunger were not significantly different between groups which is in keeping with results from hunger and fullness VAS.

There was no correlation between VAS AUC for hunger or fullness and MoCA, disease duration or motor disease severity. Taken together with the normal patterns of post-prandial hunger and fullness over time, these data suggest that sensations of hunger and fullness are intact in people with PD and PD-CI. Overall, our results do not support the hypothesis that appetite is disordered in PD-CI. This is perhaps unexpected since it is intuitive that the number of non-motor symptoms is likely to be higher in the PD-CI group. We did not, unfortunately, examine the burden of non-motor symptoms across our cohort so no further conclusions may be drawn regarding this.

4.2.2 Energy intake

Several studies suggest that energy intake is increased in PD (Barichella et al., 2017, Chen et al., 2003). This is the first study to look at energy intake in PD-CI. We found no correlation between energy intake and MoCA and there was no difference in energy intake between groups. This suggests that cognitive status did not alter feeding behaviour in our cohort. Furthermore, there were no significant correlations between energy intake and disease duration or disease severity. The time taken to complete a meal significantly correlated with energy intake across the cohort, even in those with more advanced motor PD. It is therefore unlikely that mechanical factors, such as bradykinesia and tremor, impacted energy intake in our cohort. Energy intake was predicted by PYY (a powerful anorexigenic hormone (Degen et al., 2005)) and fullness across the whole cohort, providing further evidence that appetite is not disrupted in our PD-CI group. Finally, no PD-related parameter predicted energy intake in the linear regression. Overall, we did not see the reported increase in energy intake in participants with PD (Barichella et al., 2017, Chen et al., 2003) or our proposed altered intake in PD-CI (see Chapter 2).

The design of our study may have confounded our results. Many of the studies demonstrating increased energy intake in PD have relied on indirect measures of food intake such as food diaries (Davies et al., 1994, Delikanaki-Skaribas et al., 2009), semi-quantitative food questionnaires (Logroscino et al., 1996) and food frequency questionnaires (Chen et al., 2003). Semi-quantitative food questionnaires and food frequency questionnaires reflect dietary habits over time, rather than discrete eating episodes, whilst food diaries capture a daily record of energy intake (Gibson, 1990). It is possible that energy intake in PD and PD-CI is increased through increased meal frequency rather than increased meal size. Alternatively, intake could be reduced in PD-CI due to cognitive difficulties affecting meal preparation. Our study was not designed to measure these factors. We directly weighed food eaten during an *ad libitum* meal in order to calculate energy intake. Whilst this allowed us to accurately record exactly what food was eaten during this study, the setting was artificial as participants in the PD and PD-CI groups were tested off their usual PD medications. Moreover, participants were offered a standardised range of foods, which may not have reflected their usual diet or food preferences. The *ad libitum* meal was quite

large and more than many people would eat in a normal lunch sitting (see appendix E). This may be important as there is evidence that greater portion size results in increased energy intake, irrespective of body habitus or appetite (Rolls et al., 2002). Another possible confounder could be medication as both the PD and PD-CI groups had relatively high rates of anorectic medication use. This could have masked the previously reported increased energy intake in PD (Chen et al., 2003). Unfortunately, our study was not powered to correct for this effect and we are therefore not able to draw further conclusions regarding the impact of medication on appetite or energy intake in PD and PD-CI. Finally, it has been suggested that weight-losing and weight stable people with PD may be phenotypically different (Sauerbier et al., 2016, Sharma and Turton, 2012). Our study excluded participants with recent weight loss. Our results are in keeping with a previous study that showed no difference in energy intake (measured by 3 day food diary) between controls and people with PD that also excluded participants with weight loss (Toth et al., 1997). As a result, we may not have been able to capture differences in appetite occurring in people with PD and weight loss, who may be at greater risk of more rapid cognitive decline than their weight stable counterparts (Kim et al., 2012). Despite these caveats, our study allows us to conclude that cognitive impairment in PD does not result in increased or reduced meal size in a supported research setting.

4.3 Summary and conclusions

We have found no evidence that appetite or energy intake are different in PD or PD-CI compared with controls. These results may be confounded by the fact that our participants were weight stable or as a result of artificial experimental conditions. Further studies into energy intake and cognition in PD should include weight-losing participants, measures of non-motor symptoms in PD and a mixture of direct observation and food diaries to accurately measure free living nutritional intake. There are a number of strengths and limitations of this study, which will be discussed in depth in chapter 7.

Chapter 5. Do hormones of energy homeostasis levels differ between people with PD, PD-CI and controls?

There is evidence that hormones of energy homeostasis such as AG, insulin and leptin may be neuroprotective in the context of neurodegenerative disease (see Chapter 2; Introduction and literature review). Moreover, total ghrelin levels have been shown to be low in PD patients who lose weight (Fiszer et al., 2010). This chapter explores the hypothesis that fasting and post-prandial ghrelin, GH, IGF-1, insulin, GLP-1 and leptin concentrations differ between controls, PD and PD-CI groups. Participants in the PD and PD-CI groups were tested “off” their usual PD medication. Our primary aim was to determine whether hormones of energy homeostasis could be used as biomarkers for cognitive decline in PD.

5.1 Results

5.1.1 Demographics and medication

These data are from the same study and participants as chapter 4. Demographic and medication data are therefore the same as reported there. Groups were matched for age and gender so these have not been corrected for in analysing our data. As in chapter 4, PD duration and GDS were included in ANCOVA comparing AUC.

5.1.2 Missing data

A number of participants were missing data regarding hormone levels, as some were undetectable. This was particularly the case for IGF-1 and GLP-1. A summary of missing data is outlined in Table 16.

Table 16. Number of participants missing data per analyte

Analyte	Control	PD	PD-CI	Comments
Acyl ghrelin	2	1	1	2 controls all values undetectable. 1 PD and 1 PD-CI participant missing data at 5 minutes due to difficult blood draw.
Total ghrelin	0	1	1	1 PD and 1 PD-CI participant missing data at 5 minutes due to difficult blood draw.
PYY	0	2	1	1 PD participant undetectable at baseline. 1 PD and 1 PD-CI participant missing data at 5 minutes due to difficult blood draw.
Leptin	0	1	2	1 PD and 1 PD-CI participant missing data at 5 minutes due to difficult blood draw.
Insulin	0	1	1	1 PD and 1 PD-CI participant missing data at 5 minutes due to difficult blood draw.
GLP-1	1	9	4	Variable number of undetectable results per participant.
IGF-1	3	8	6	Variable number of undetectable results per participant. 3 controls, 7 PD and 1 PD-CI had no recordable data for IGF.
GH	0	1	1	1 PD and 1 PD-CI participant missing data at 5 minutes due to difficult blood draw.

5.1.3 Hormones of energy homeostasis between groups

The following data were transformed to achieve normal distribution in order to aid statistical analysis. For clarity in the text below transformed variables will be referred to as follows;

Table 17. Shorthand nomenclature of transformed data

Transformed variable	Shorthand reference
Log base 10 reversed (1-score out of 31) Montreal Cognitive Assessment	Reversed MoCA
Log base 10 transformed glucose	glucose
Log base 10 transformed acyl-ghrelin	AG
Log base 10 transformed total ghrelin	TG
Log base 10 transformed growth hormone	GH
Log base 10 transformed insulin-like growth factor	IGF-1
Log base 10 transformed insulin	insulin
Log base 10 transformed glucagon-like peptide 1	GLP-1
Log base 10 transformed leptin	leptin
Log base 10 transformed peptide YY	PYY
Log base 10 transformed HOMA-IR	HOMA-IR

Relationships between variables were examined using scatter plots and Pearson correlations. Means were compared using ANOVA and ANCOVA with inclusion of disease duration and GDS as covariates as outlined above.

Variables were compared over time using a repeated measure multivariate linear model. Please see Chapter 3: Methods for further details.

The ghrelin IGF-1 axis

Fasting AG and AG AUC correlated negatively with age ($r=-0.39$, $p=0.004$ and $r=-0.386$, $p=0.004$, respectively), reversed MoCA ($r=-0.35$, $p=0.011$ and $r=-0.38$, $p=0.005$, respectively) and GDS ($r=-0.30$, $p=0.03$ and $r=-0.30$, $p=0.03$, respectively). Fasting AG significantly correlated with fasting TG ($r=0.78$, $p<0.001$) whilst AG AUC correlated significantly with TG AUC ($r=0.78$, $p<0.001$). There was a significant negative correlation between fasting AG and BMI ($r=-0.29$, $p=0.04$) but not with fat mass or motor UPDRS disease duration. Scatter plots are shown in Figure 18 and Figure 19. There was no demonstrable correlation between AG AUC and fat mass, BMI, motor severity of PD (measured by UPDRS), PD duration and energy intake or any other hormones tested. Visual inspection of scatter plots did not reveal any non-linear relationships between AG or TG and any of these variables.

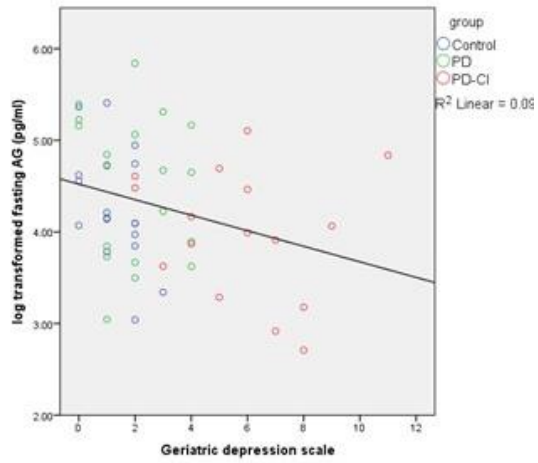
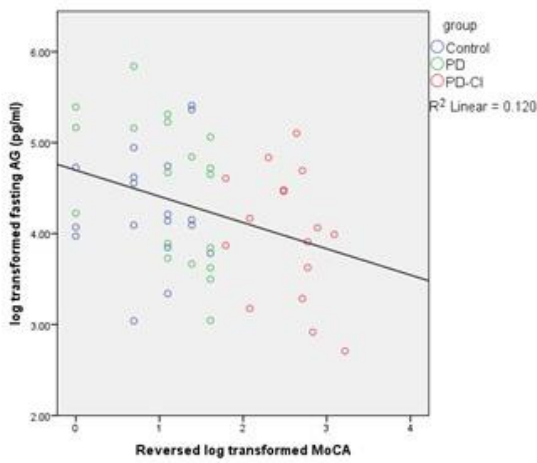
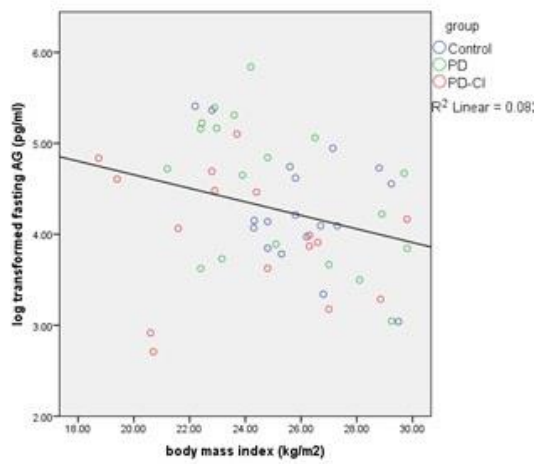
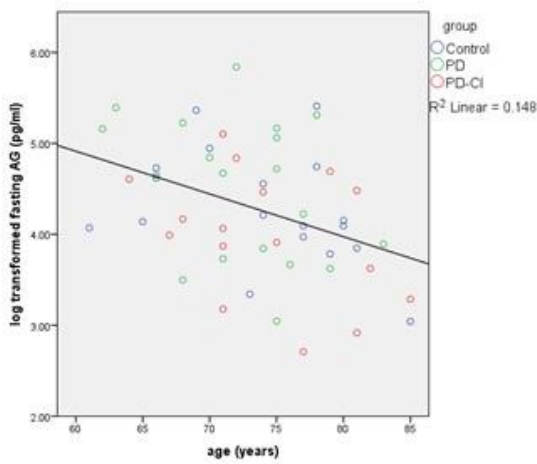
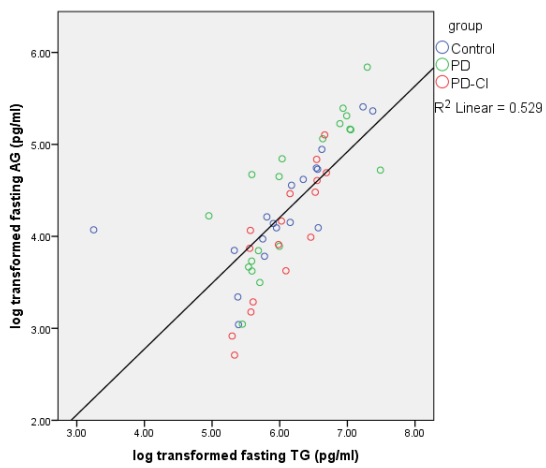


Figure 18. Scatterplots of fasting AG with TG, age, BMI, reversed MoCA and Geriatric depression scale

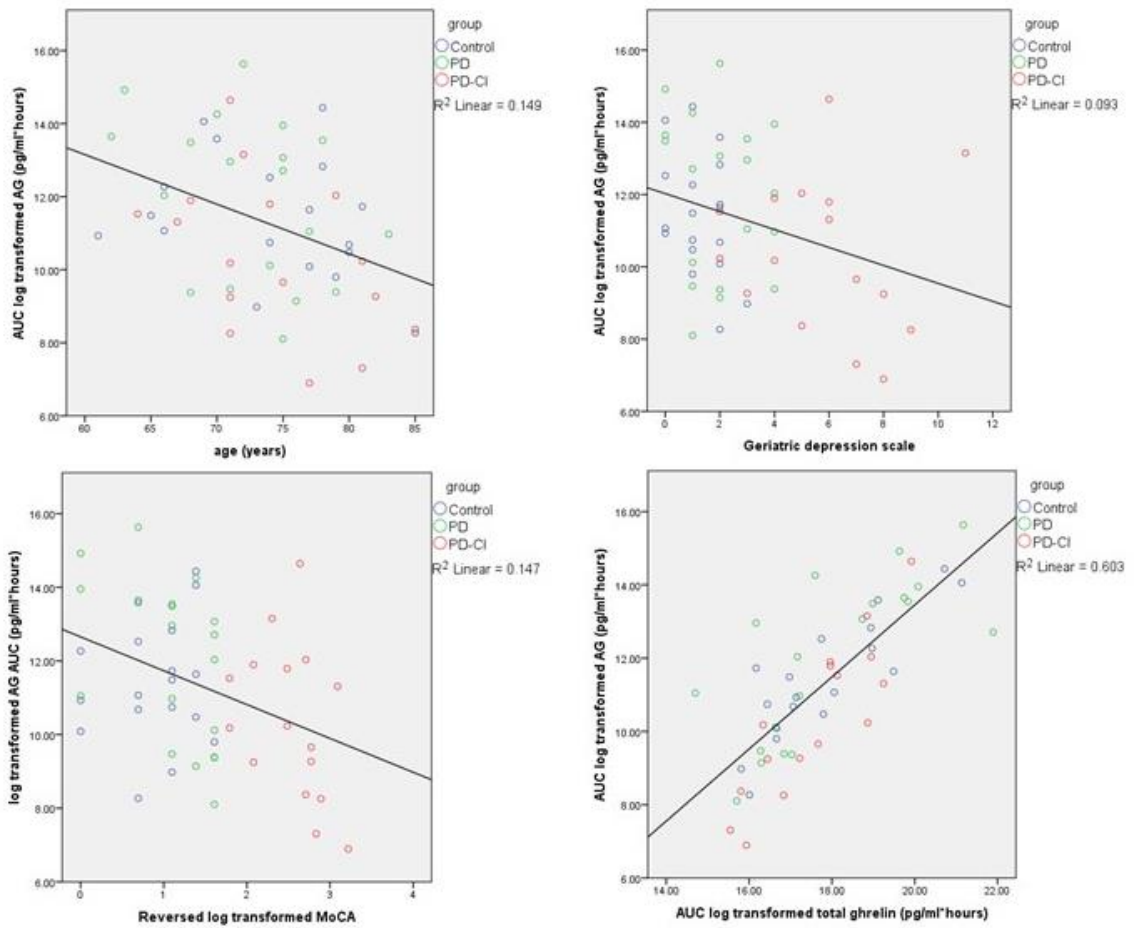


Figure 19. Scatter plots for AG AUC and age, GDS, reversed MoCA and TG AUC.

Repeated measures ANCOVA showed that change in AG over time was not significantly different between groups when corrected for PD duration ($p=0.99$). This is represented graphically in Figure 20.

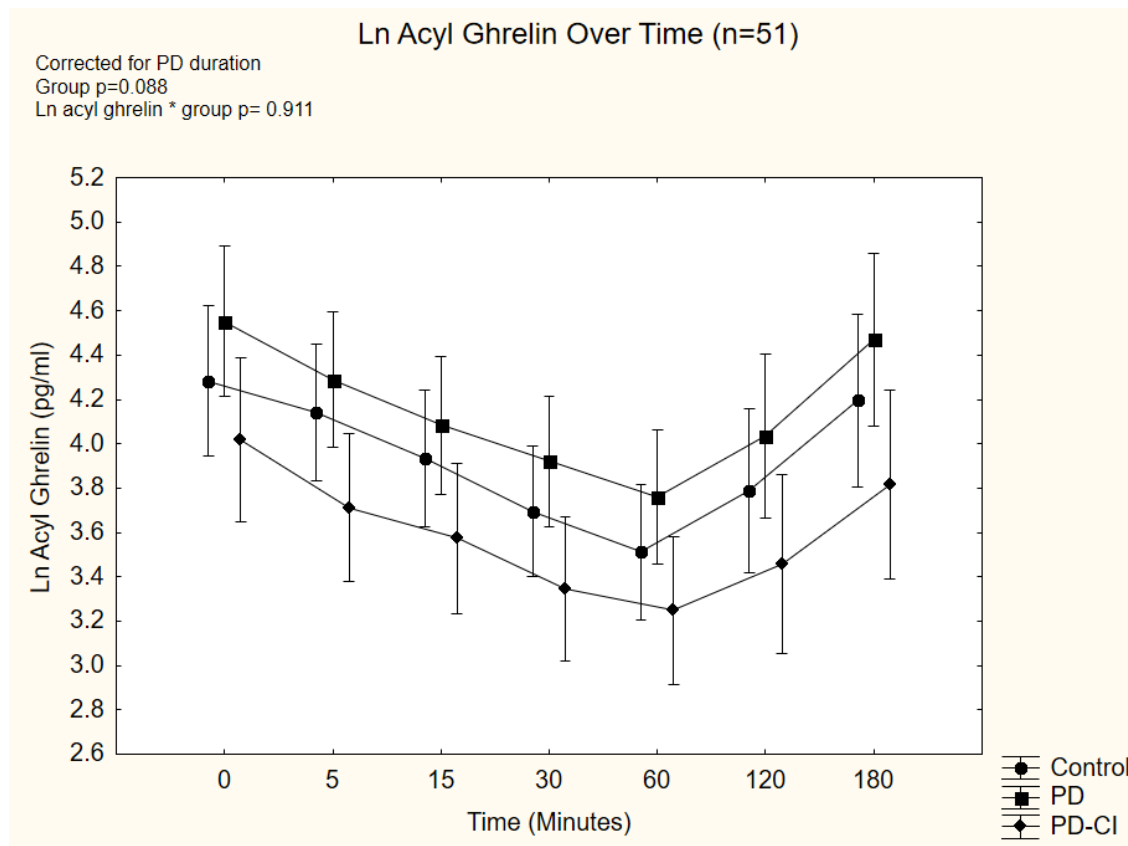
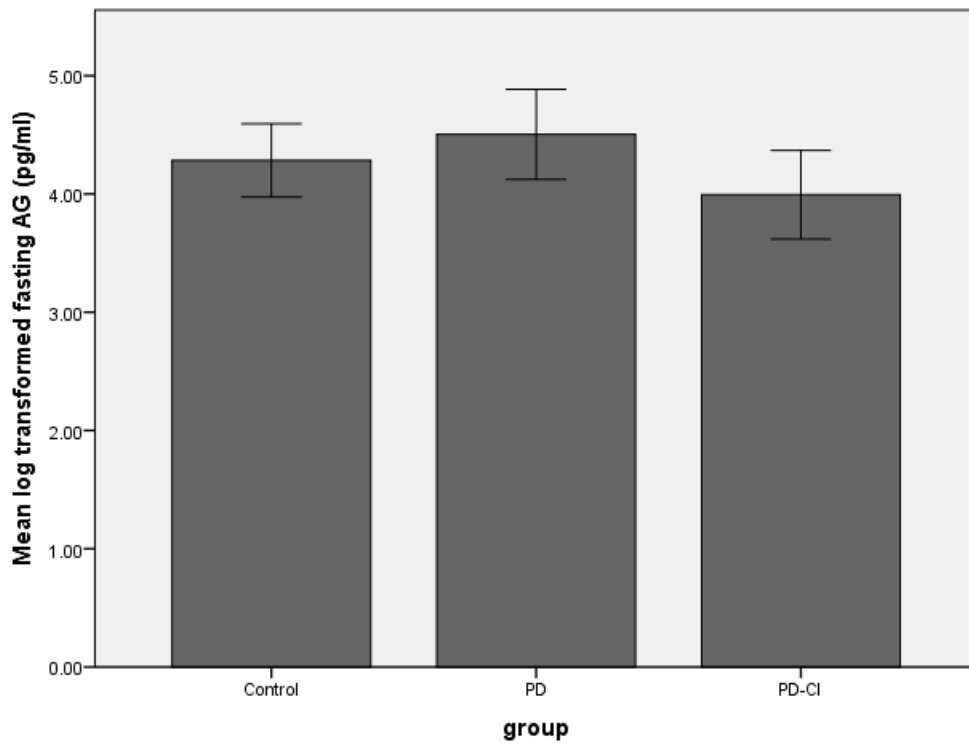


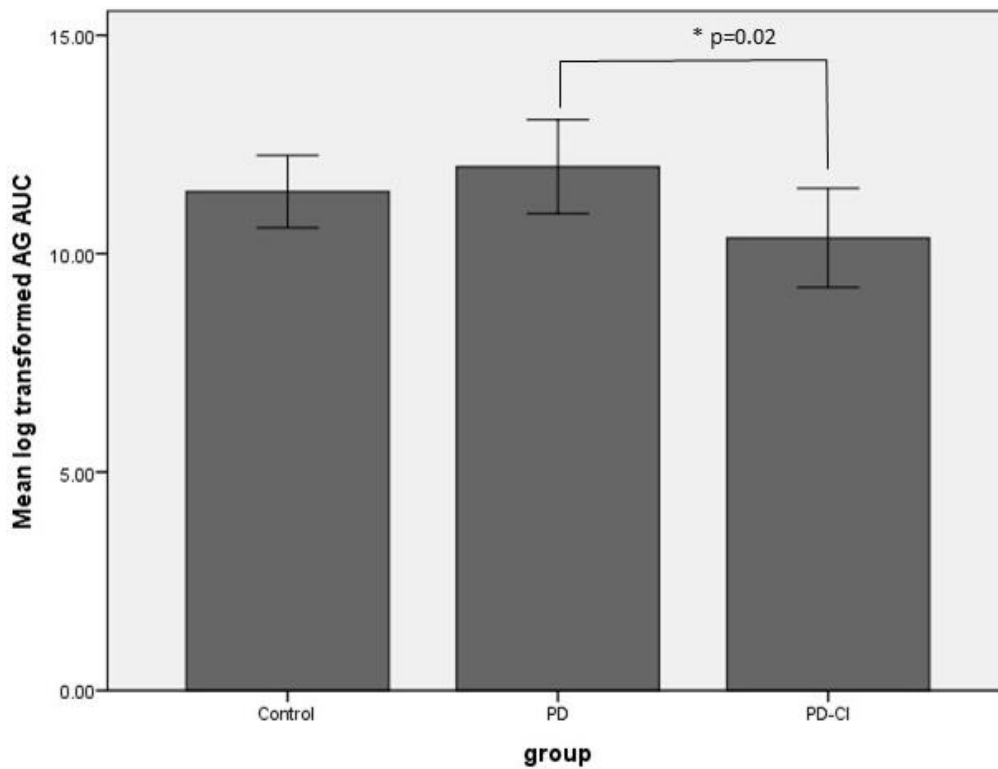
Figure 20. Acyl-ghrelin over time

Fasting AG was not significantly different between groups with or without inclusion of GDS and PD duration in the analysis ($p=0.12$ and 0.10 , respectively). There was a non-significant difference in AG AUC between groups ($p=0.07$). In view of the near significant difference post hoc analysis using the least significant difference test (LSD) was performed and a significant difference was seen between the PD and PD-CI groups, with AG AUC being lower in the PD-CI group ($p=0.02$) (Figure 21). There was no difference between groups when AG AUC was compared between groups using ANCOVA to account for PD duration and GDS ($p=0.16$).



Error Bars: 95% CI

Figure 21. Mean fasting AG by group



Error Bars: 95% CI

Figure 22. Mean AG AUC by group

One of our control subjects had a vagotomy in early adulthood. They did not have active gastrointestinal disease and were therefore included in the study. Ghrelin release is thought to be vagal-nerve dependent (Seoane et al., 2007). In order to ensure that their inclusion did not bias our results the data were re-analysed excluding this participant. The results were unchanged, with no significant difference in AG AUC between groups ($p=0.07$) but significantly lower AG AUC in the PD-CI group on post-hoc analysis ($p=0.02$).

Fasting TG and TG AUC negatively correlated with body mass index ($r= -0.33$, $p=0.02$ and $r=-0.40$, $p=0.003$, respectively). There was a near-significant negative correlation between age and TG AUC ($p=0.05$), which appears genuine on inspection of the scatterplot. Fasting TG did not correlate with age. There were no other significant correlations seen. Visual inspection of scatter plots between fasting TG and TG AUC against other variables did not reveal any non-linear relationships. Scatter plots are shown in Figure 23 and Figure 24.

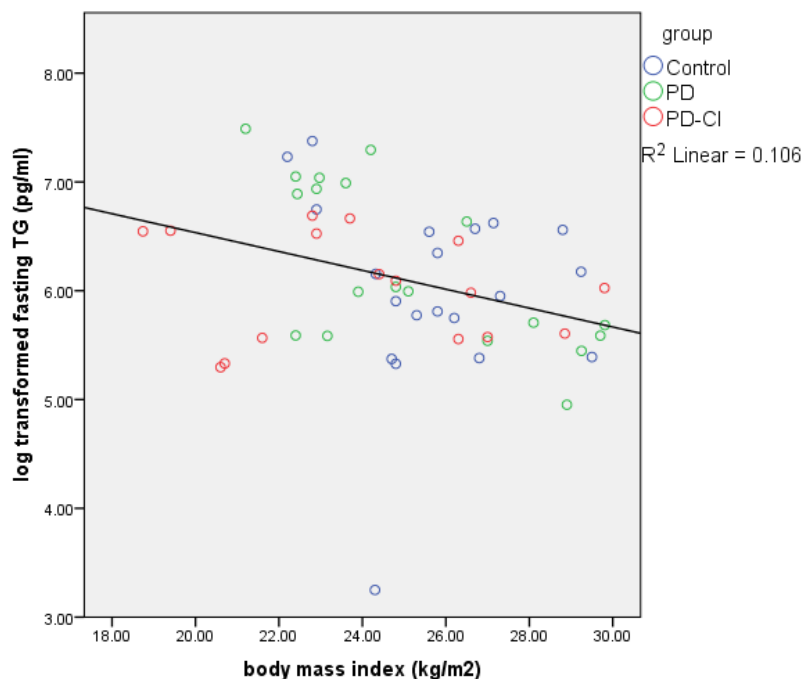


Figure 23. Scatter plot for relationship between fasting TG and BMI

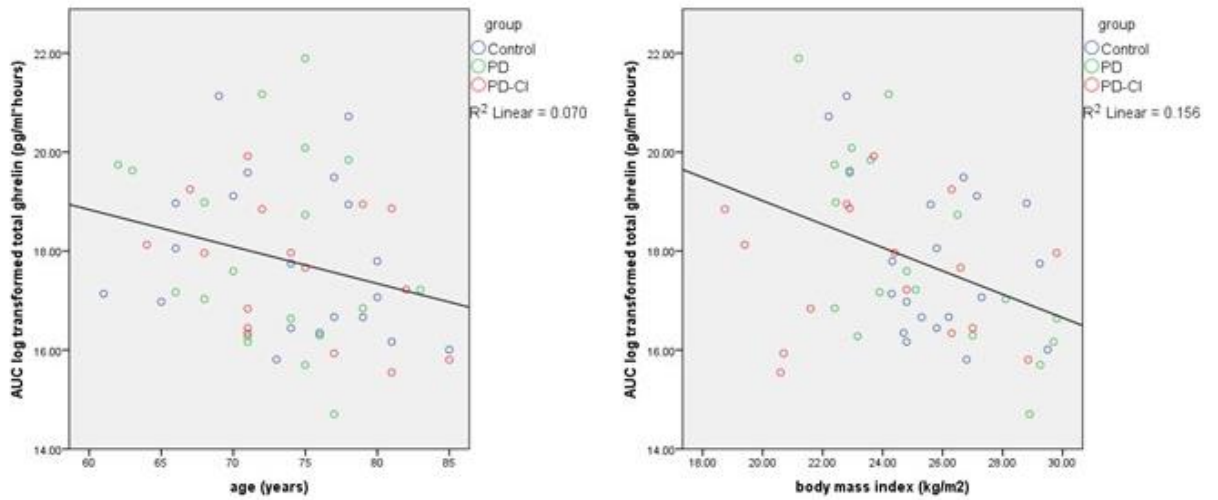


Figure 24. Scatter plots for relationships between TG AUC and age and BMI

There was no significant difference in TG over time between groups, even after correction for PD duration ($p=0.67$) (Figure 25). Comparison of fasting TG and TG AUC between groups showed no significant difference between groups, with or without correction for PD duration or GDS.

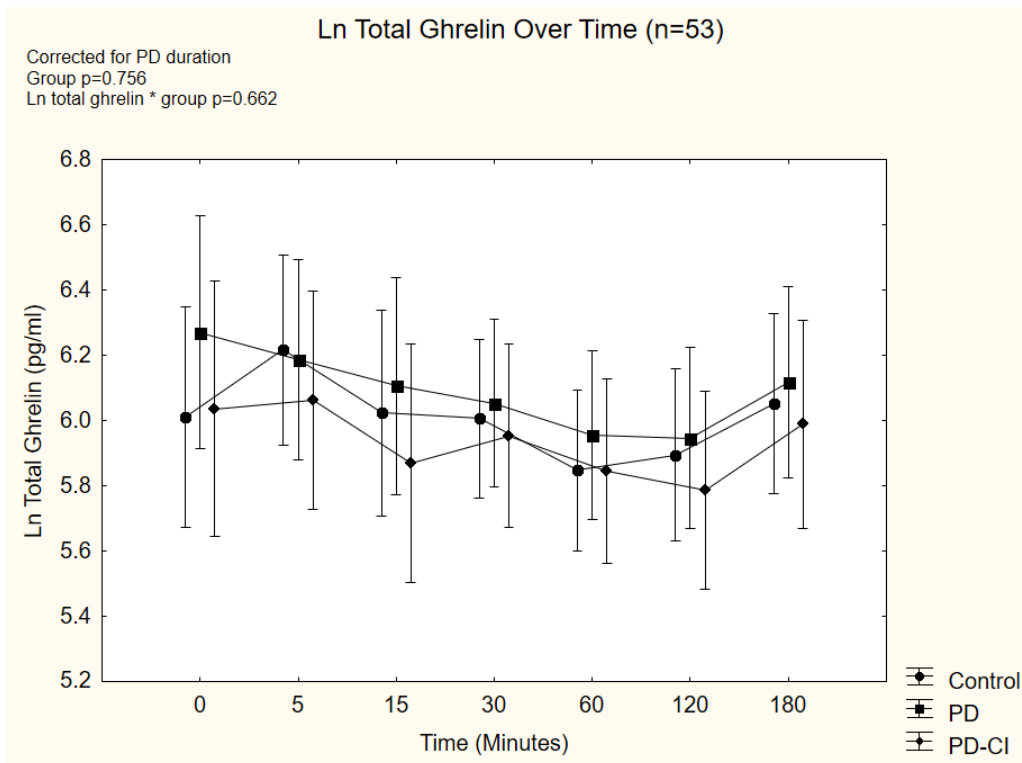
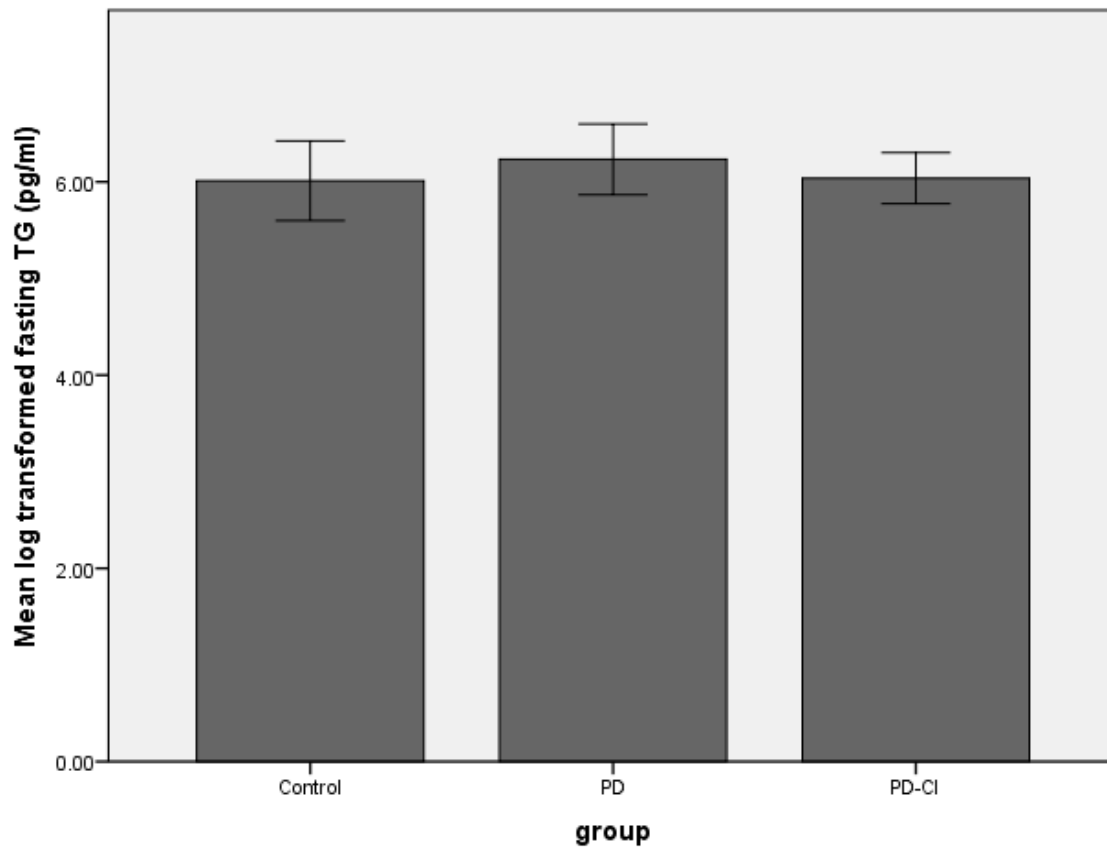


Figure 25. Total ghrelin over time

There were no significant differences between fasting TG or TG AUC compared between groups ($p=0.61$ and $p=0.80$, respectively) even with correction for PD duration and GDS ($p=0.58$ and $p=0.60$, respectively) (Figure 27 and Figure 28).



Error Bars: 95% CI

Figure 26. Fasting TG by group

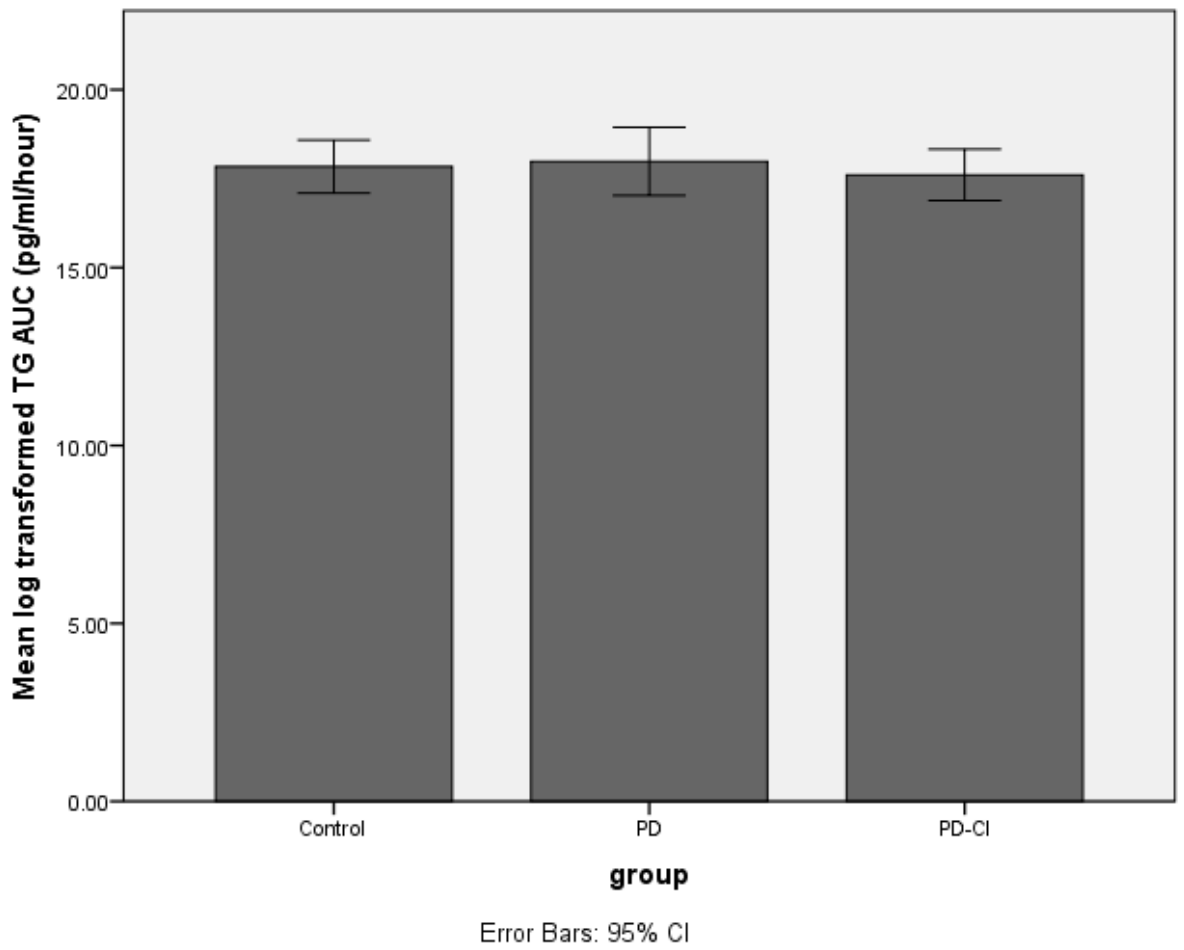


Figure 27. Total ghrelin AUC between groups

Both fasting GH and GH AUC significantly positively correlated with age ($r=0.31$, $p=0.02$ and $r=0.38$, $p=0.004$, respectively). Growth hormone AUC and IGF-1 AUC significantly correlated with each other ($r=0.45$, $p=0.003$) but no such relationship was seen between fasting GH and fasting IGF-1 (Figure 29 and Figure 30). There were no other significant correlations and visual inspection of scatter plots did not reveal any non-linear relationships between variables.

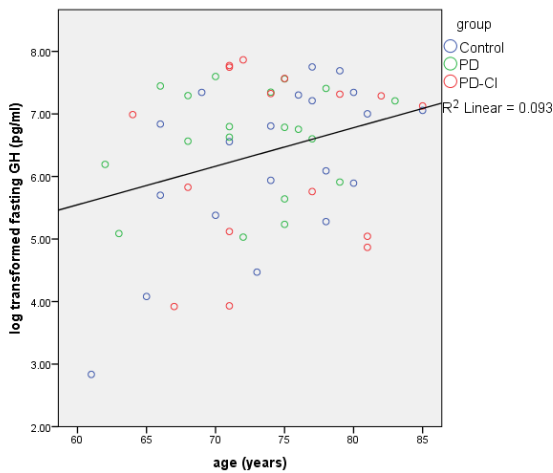


Figure 28. Scatter plot of fasting GH and age

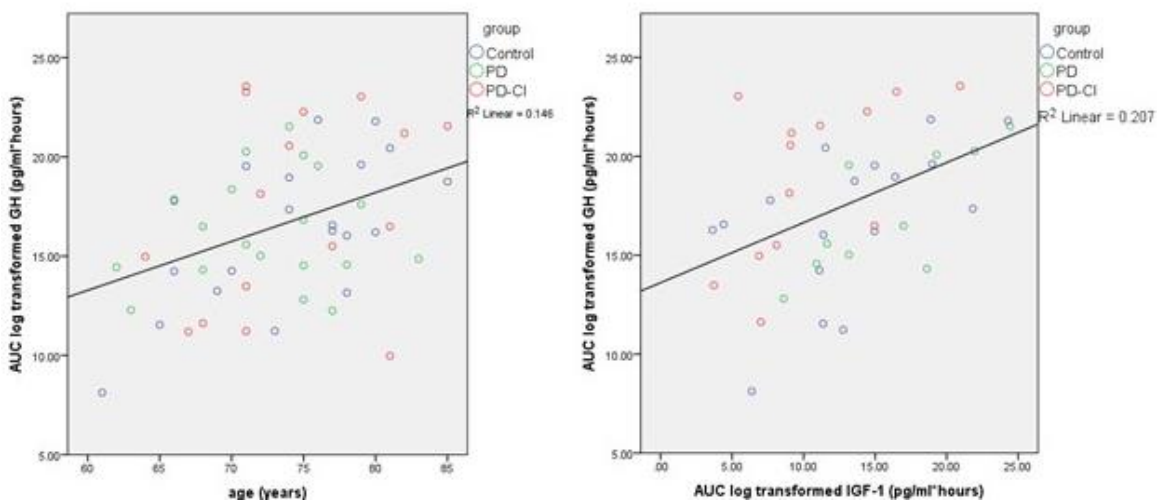


Figure 29. Scatter plot of GH AUC against age and IGF-1AUC

There was no significance difference between groups in GH levels over time, even after correction for PD duration ($p=0.10$) (Figure 31).

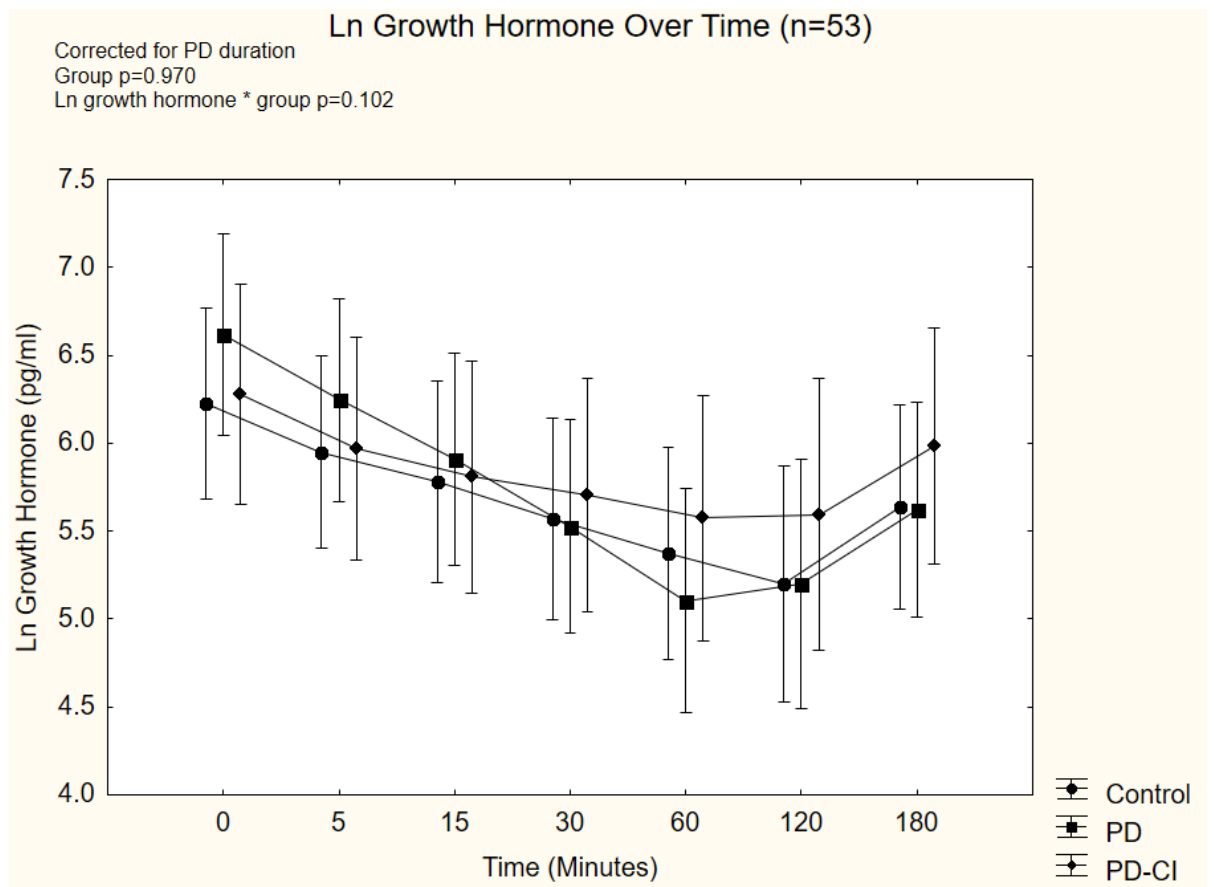
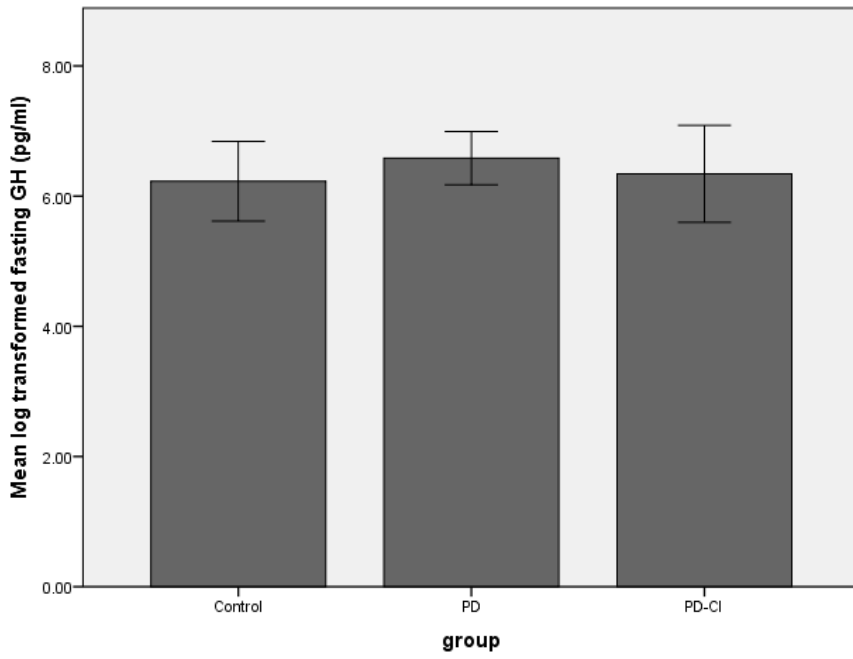


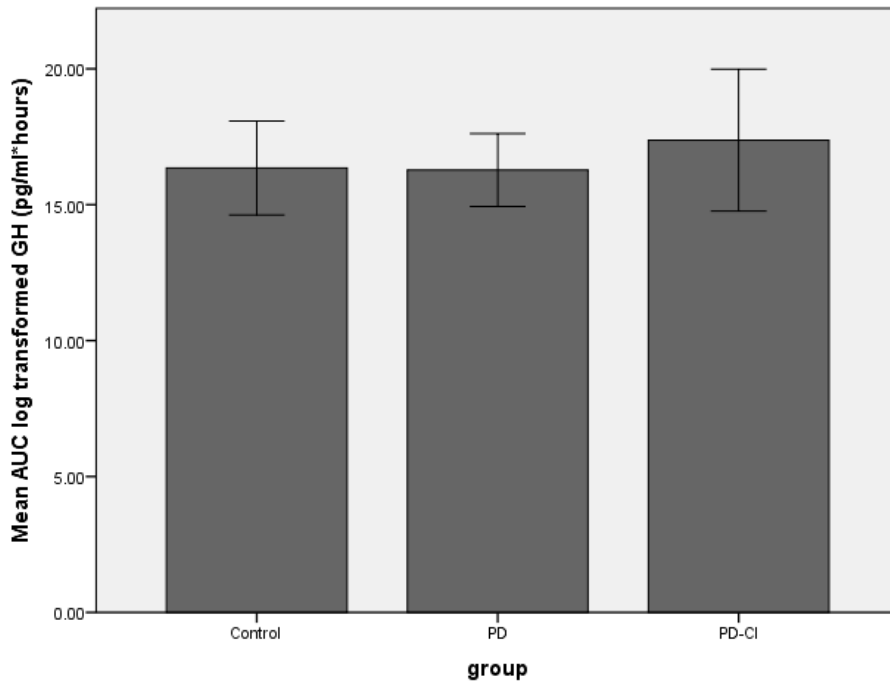
Figure 30. Growth hormone over time

There was no difference in fasting GH between groups, with or without including PD duration and GDS in the analysis ($p=0.65$ and 0.87 , respectively). A similar pattern was seen for GH AUC ($p=0.65$ and 0.91 , respectively) (Figure 32 and Figure 33).



Error Bars: 95% CI

Figure 31. Fasting GH by group



Error Bars: 95% CI

Figure 32. Growth hormone AUC by group

Insulin-like growth factor 1 AUC correlated significantly with growth hormone ($r=0.46$, $p=0.003$) as detailed above (Figure 30). There were no other significant correlations between fasting IGF-1 or IGF-1 AUC and any other variable. There was no significant difference in IGF-1 levels over time between groups ($p=0.96$) (Figure 34).

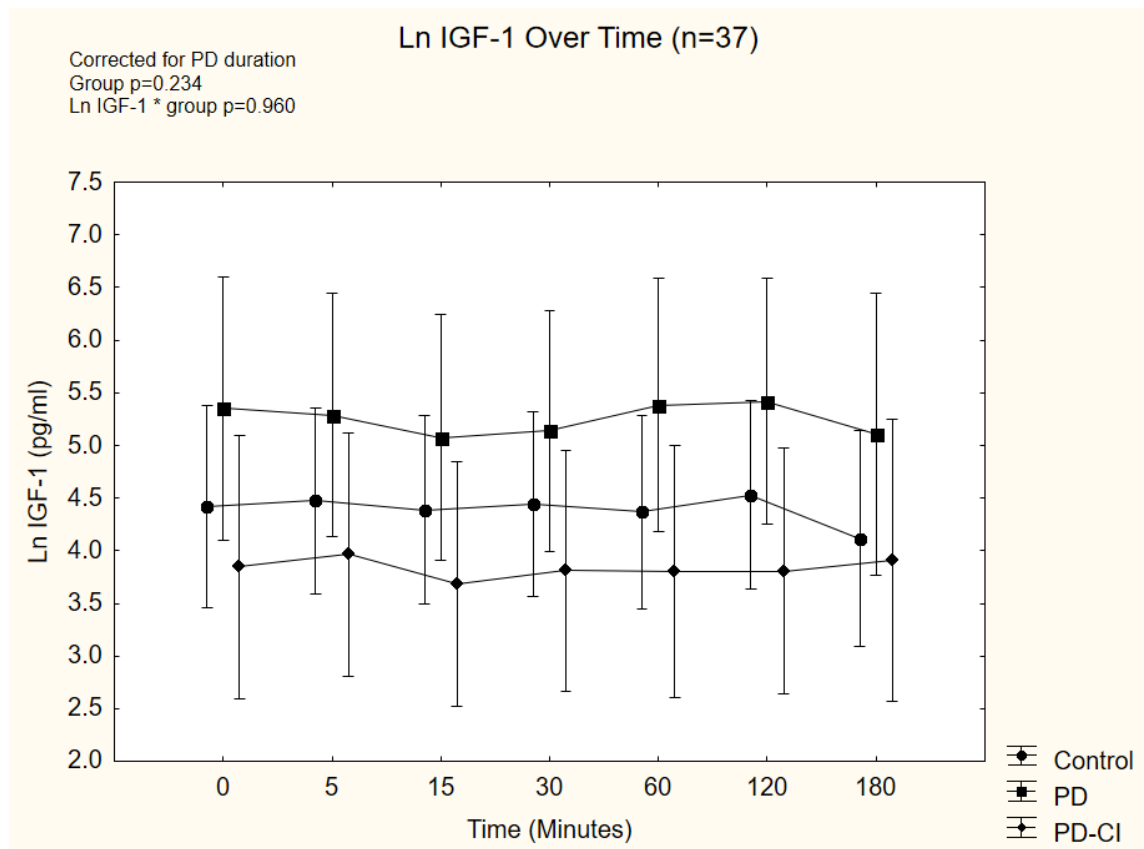
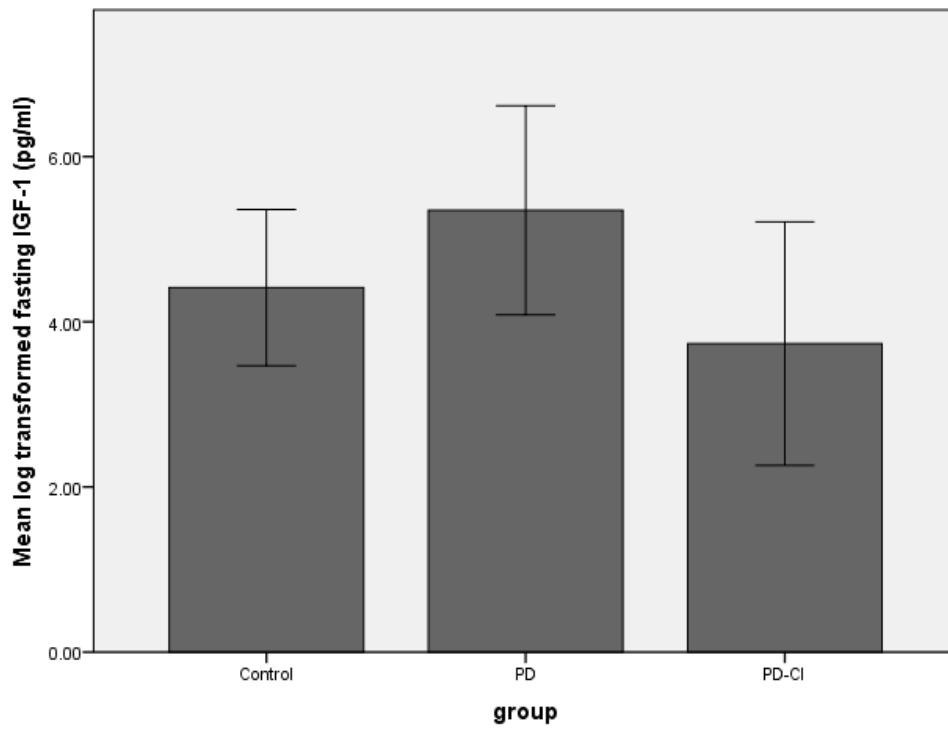


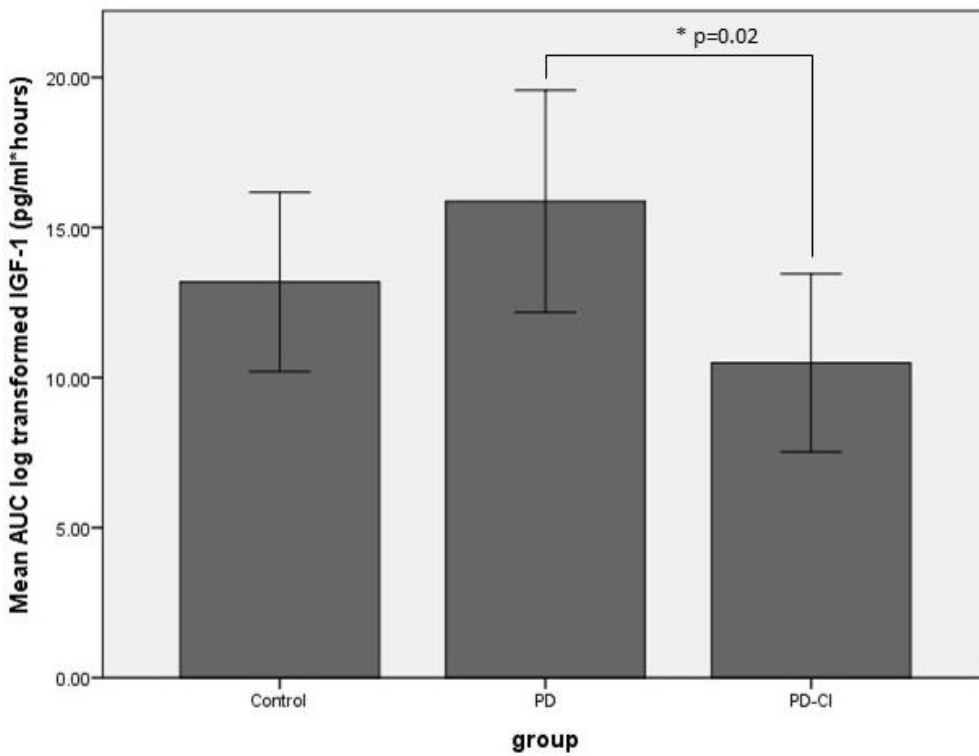
Figure 33. Insulin-like growth factor over time by group

There was a non-significant difference in IGF-1 AUC between groups ($p=0.07$). In view of the near significant difference post hoc testing with LSD was performed and a significant difference was seen between the PD and PD-CI groups, with IGF-1 AUC being lower in the PD-CI group ($p=0.02$) (Figure 36). There was no difference between groups when IGF-1 AUC was compared between groups using ANCOVA to account for PD duration and GDS ($p=0.24$). There was no significant difference in fasting IGF-1 between groups with or without the inclusion of covariates ($p=0.17$ and 0.44 , respectively) (Figure 35).



Error Bars: 95% CI

Figure 34. Fasting IGF-1 by group



Error Bars: 95% CI

Figure 35. Area under the curve of IGF-1 between groups

Insulin

Insulin AUC significantly correlated with glucose ($r=0.46$, $p=0.001$), fat mass ($r=0.279$, $p=0.039$), BMI ($r=0.476$, $p=0.000$) and leptin ($r=0.50$, $p<0.001$) There was a negative correlation between insulin AUC and motor disease severity as measured by motor UPDRS ($r=-0.36$, $p=0.04$) (Figure 38). Fasting insulin correlated with BMI ($r=0.41$, $p=0.002$), fasting leptin ($r=0.45$, $p=0.001$) and fasting glucose ($r=0.30$, $p=0.03$) but not motor UPDRS ($r=-0.32$, $p=0.06$) (Figure 37). There were no other significant linear relationships and no non-linear relationships were observed on inspection of the scatter plots.

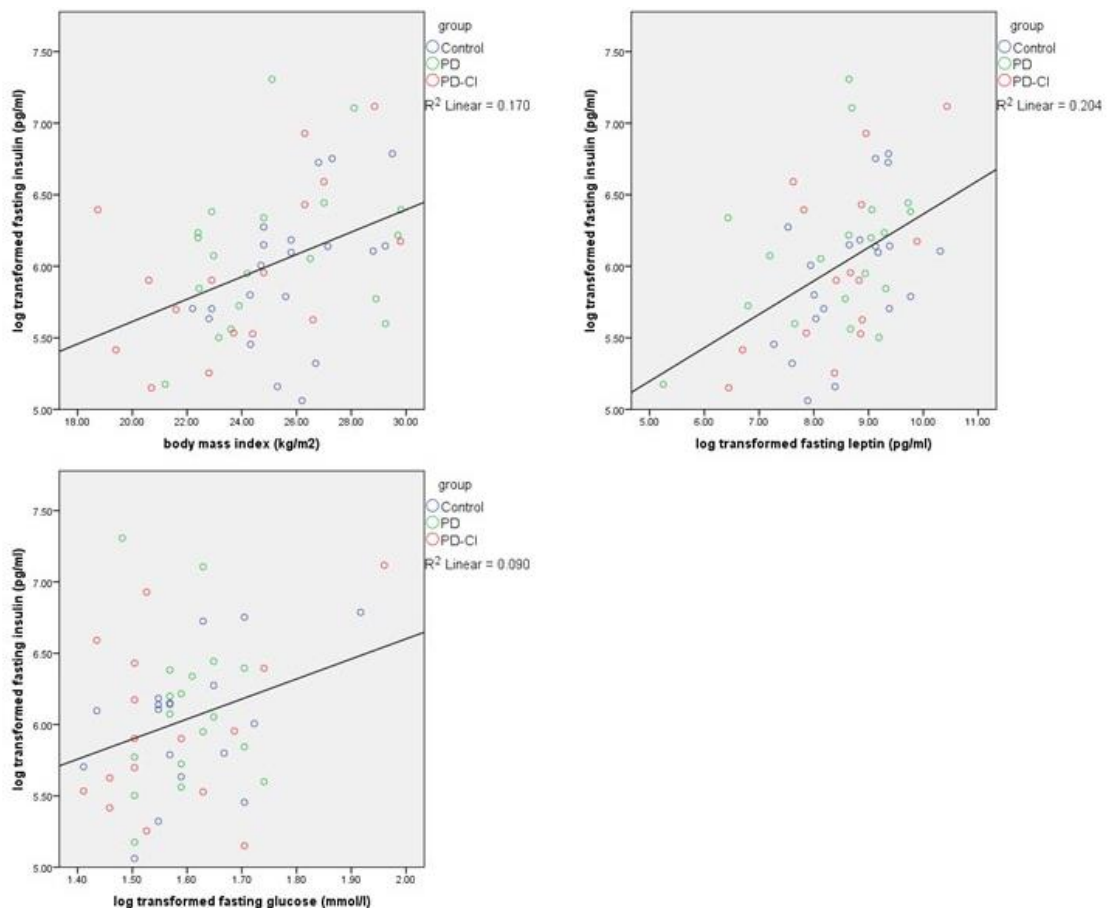


Figure 36. Scatter plots of fasting insulin and BMI, leptin and glucose

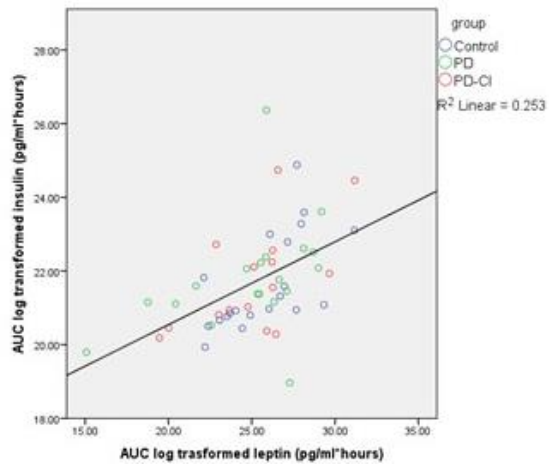
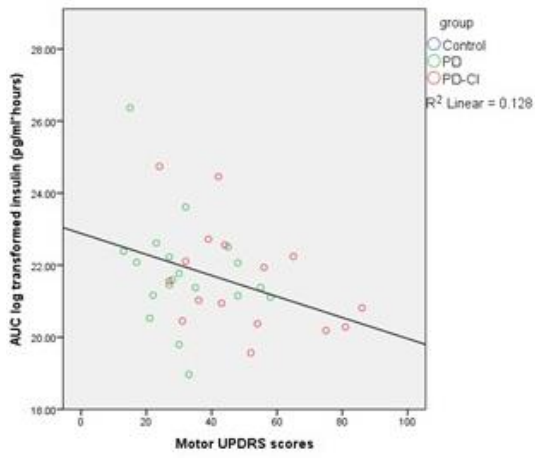
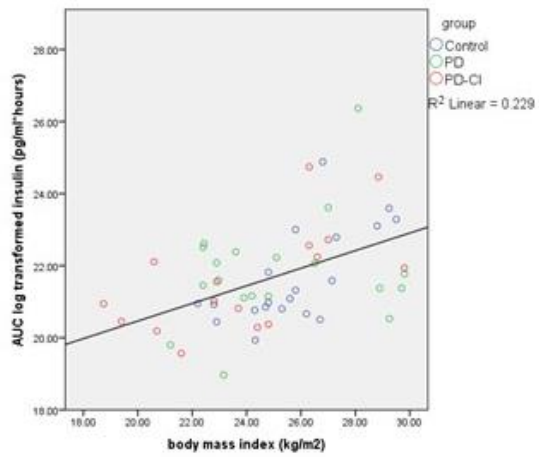
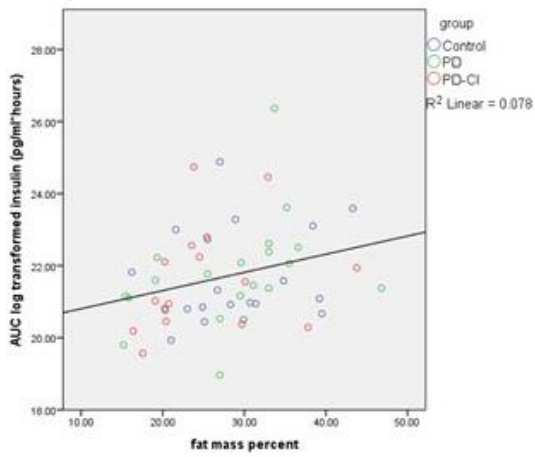
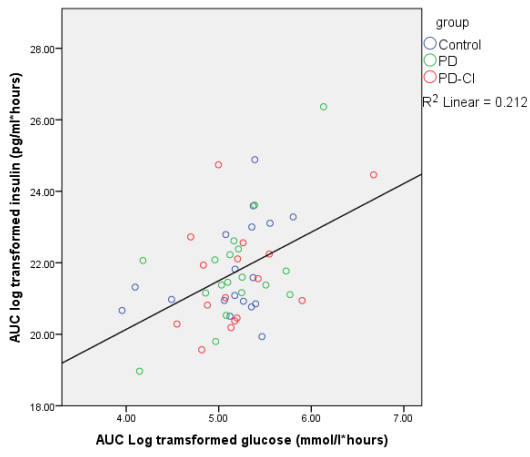


Figure 37. Scatter plots for relationships between insulin AUC and glucose AUC, fat mass, BMI, motor UPDRS and leptin

There was no significant difference between insulin levels over time between groups, with or without correction for PD duration ($p=0.76$) (Figure 39). There was no significant difference in fasting insulin or insulin AUC between groups ($p=0.65$ and 0.93 , respectively). This remained true after the inclusion of PD duration or GDS as covariates ($p=0.84$ and 0.995 , respectively) (Figure 40 and Figure 41).

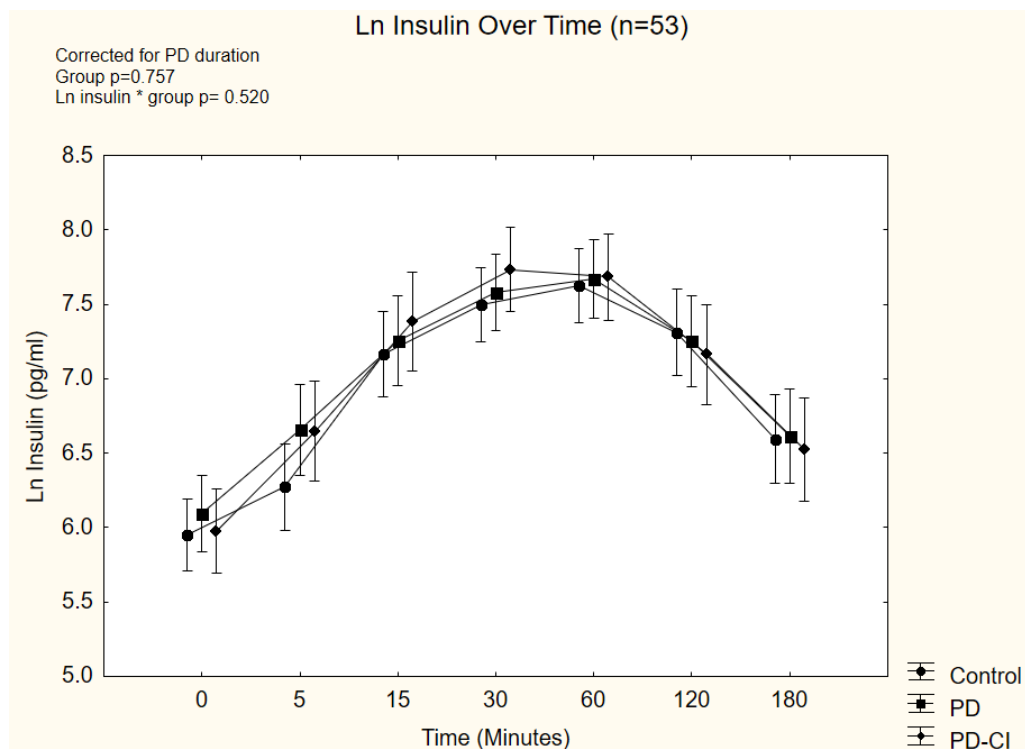
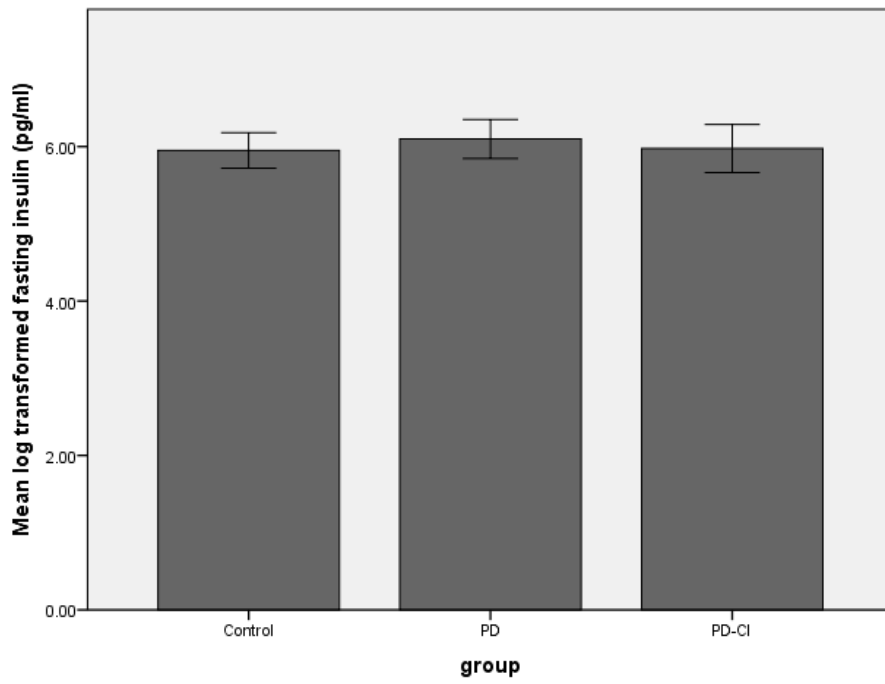
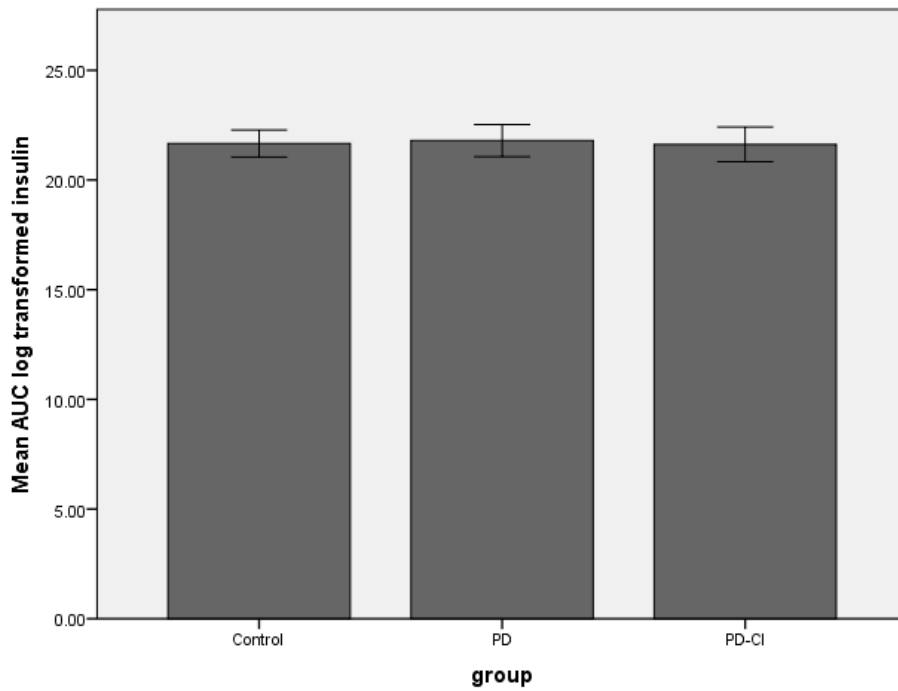


Figure 38. Log transformed insulin over time by group



Error Bars: 95% CI

Figure 39. Fasting insulin by group



Error Bars: 95% CI

Figure 40. Insulin area under the curve by group

Serum glucose AUC was not different between groups, with ($p=0.91$) or without inclusion of disease duration and GDS in the analysis ($p=0.96$) (Figure 43). No correlations were seen between glucose and any variable other than insulin ($r=0.46$, $p=0.001$). None of our participants had diabetes but 2 controls, 1 PD and 1 PD-CI had fasting glucose in the range of impaired glucose tolerance (5.6-7.0 mmol/l) (American Diabetes, 1997). One participant in the PD-CI group had a single fasting glucose of 7.1 mmol/l, which is in the diabetic range but not diagnostic of diabetes in itself. This participant's random glucose was, however, normal (6.8 mmol/l). Fasting glucose was not significantly different between groups ($p=0.92$) even after inclusion of covariates ($p=0.95$) (Figure 42).

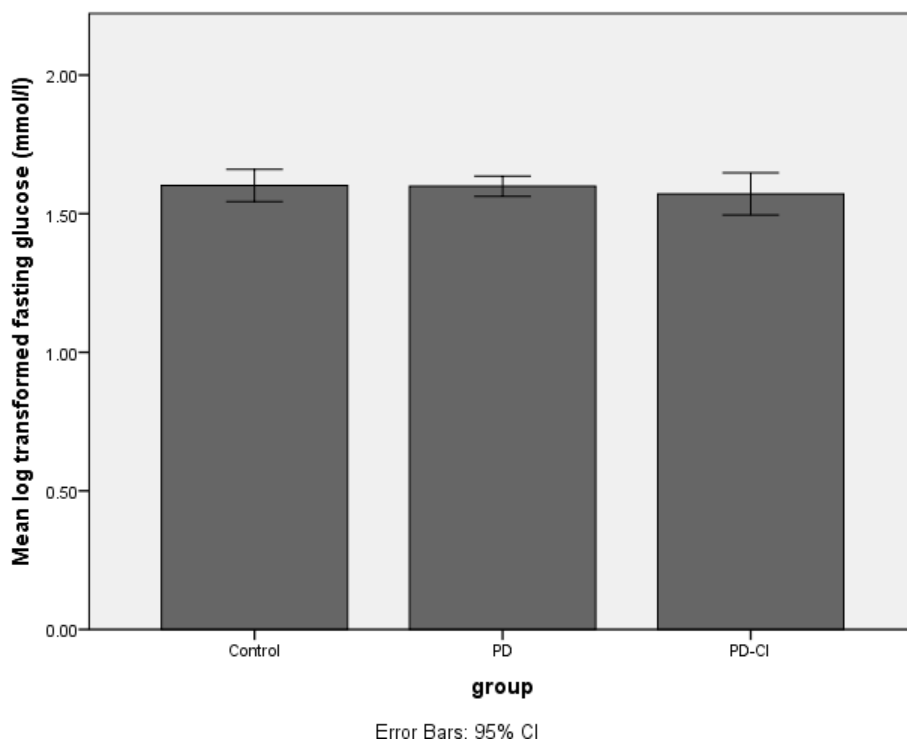


Figure 41. Mean fasting glucose by group

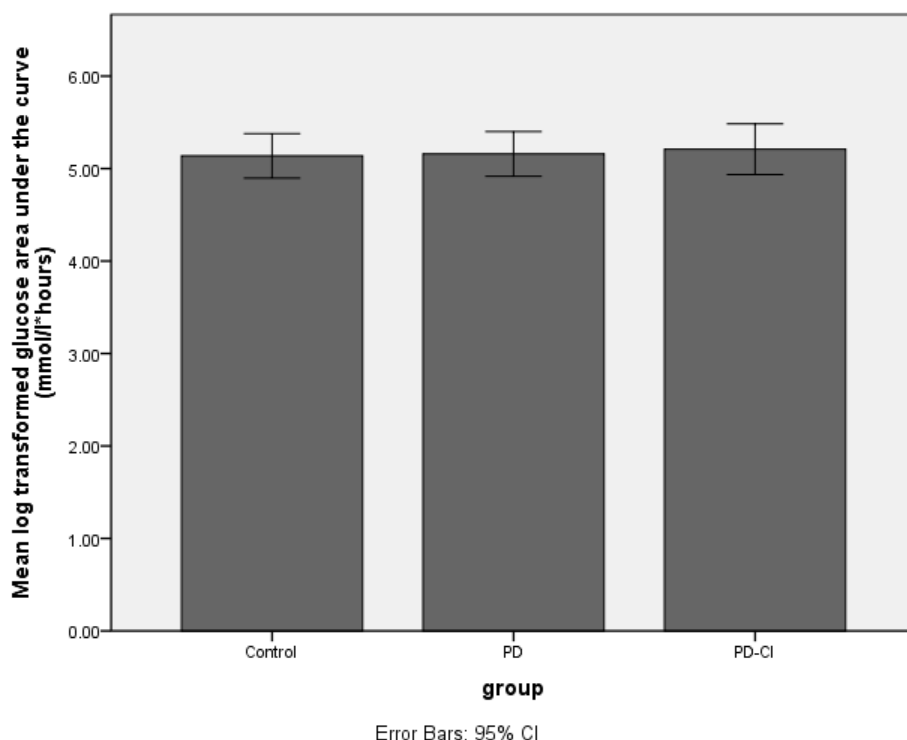
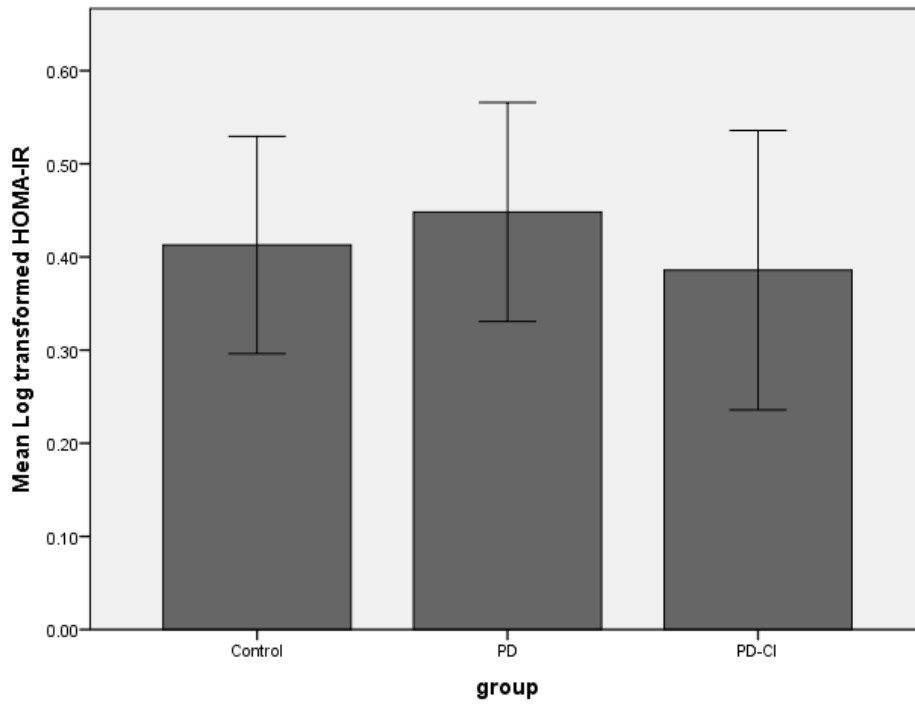


Figure 42. Glucose AUC by group

Insulin resistance measured by HOMA-IR was not significantly different between groups (Figure 44) with ($p=0.77$) or without correction for PD duration and GDS ($p=0.91$). Insulin AUC and HOMA-IR were highly correlated ($r=0.74$, $p<0.001$) (Figure 45). Visual inspection scatterplots suggested a positive correlation between HOMA-IR and motor UPDRS but this was not statistically significant ($r=-0.37$, $p=0.07$). There was no correlation between Insulin AUC and reversed MoCA ($r=-0.07$, $p=0.62$) or HOMA-IR and reversed MoCA ($r=-0.02$, $p=0.92$).



Error Bars: 95% CI

Figure 43. Log transformed HOMA-IR by group

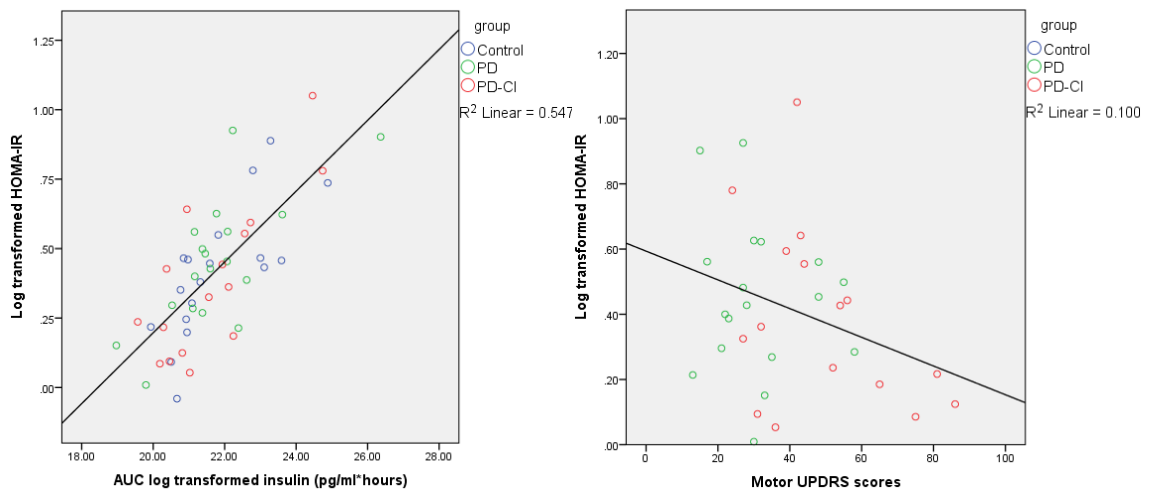


Figure 44. Scatter plots showing HOMA-IR against insulin AUC and Motor UPDRS scores

Glucagon-like peptide 1

Fasting GLP-1 and GLP-1 AUC both significantly correlated with PYY ($r=0.41$, $p=0.005$ and $r=0.46$, $p=0.001$, respectively) (Figure 46). There were no other significant correlations. There was no significant difference in GLP-1 levels over time between groups ($p=0.11$) (Figure 47) and no significant difference in fasting GLP-1 or GLP-1 AUC between groups with ($p=0.76$ and $p=0.97$) or without correction for PD duration or GDS ($p=0.54$ and 0.48 , respectively) (Figure 48 and Figure 49).

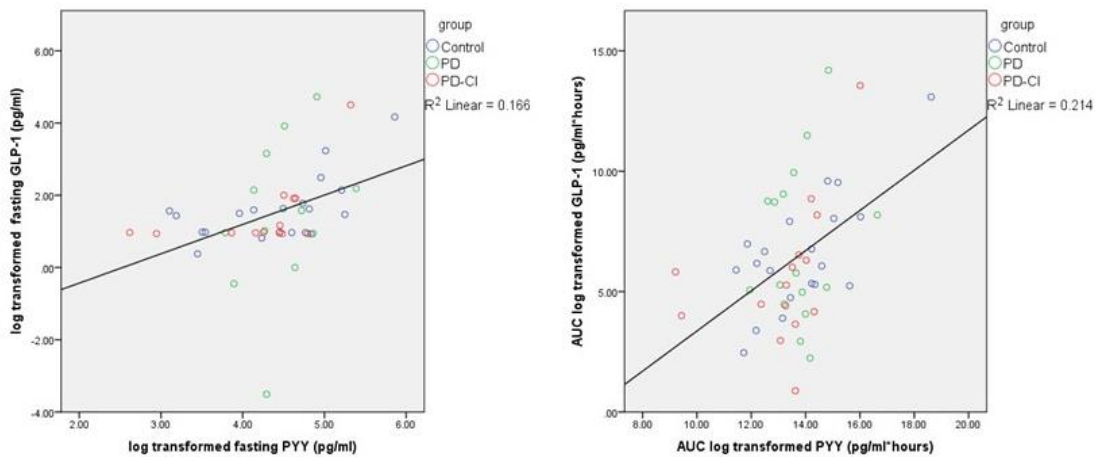


Figure 45. Scatter plots of GLP-1 and PYY

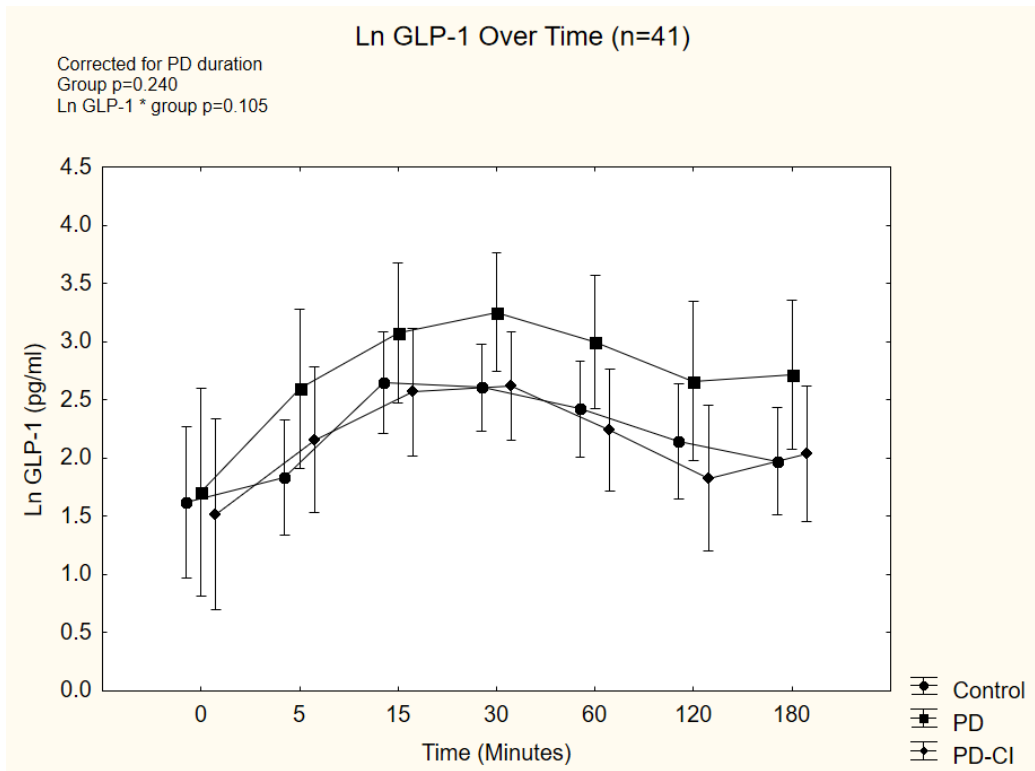


Figure 46. GLP-1 over time by group

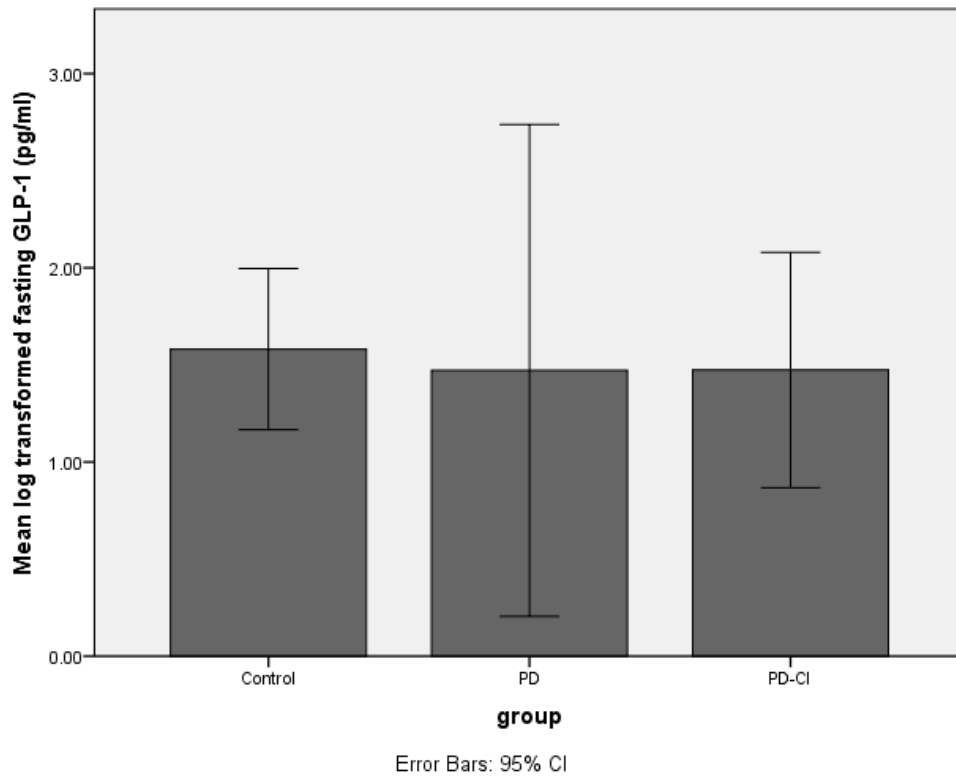


Figure 47. Fasting GLP-1 by group

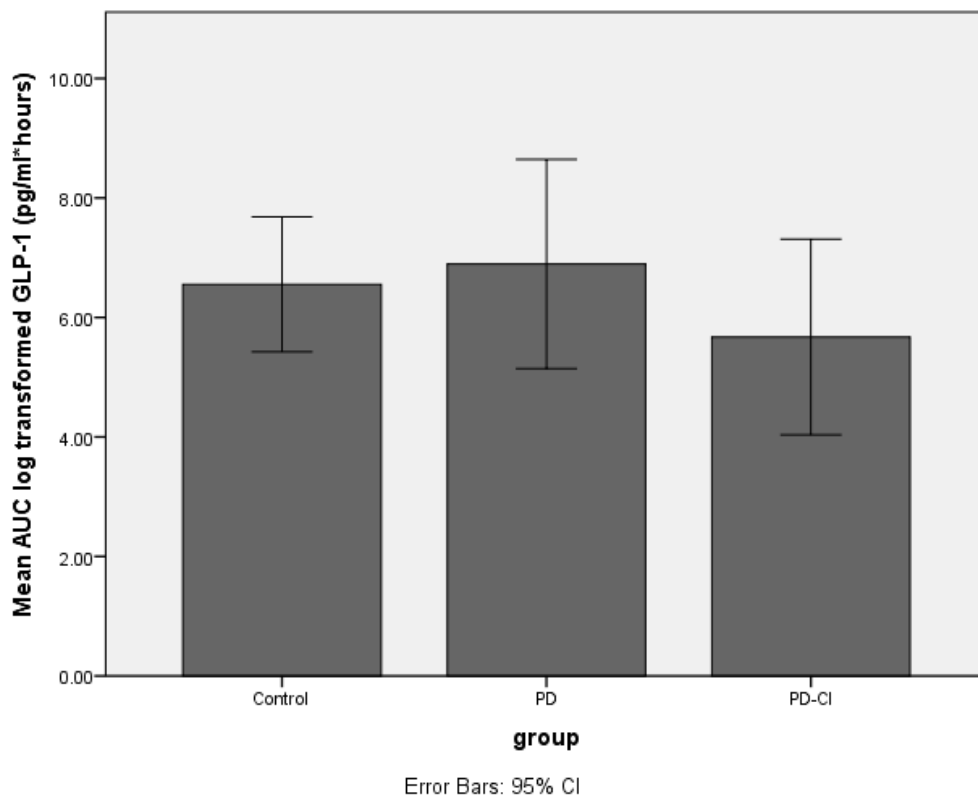


Figure 48. Area under the curve of GLP-1 between groups

Leptin

Fasting leptin significantly correlated with fat mass ($r=0.652$, $p=0.000$), BMI ($r=0.45$, $p=0.001$) and insulin ($r=0.45$, $p=0.001$). Leptin AUC showed the same results ($r=0.66$, $p<0.001$; $r=0.42$, $p=0.002$; and $r=0.50$, $p<0.001$ respectively). There was a negative correlation between leptin AUC and hunger ($r=0.38$, $p=0.005$) but not energy intake (Figure 51). There was no such relationship between fasting leptin and energy intake. There were no other significant correlations. There were no non-linear relationships between fasting leptin or leptin AUC and other variables seen on visual inspection of scatter plots. Scatter plots of significant correlations are shown in Figure 50 and Figure 51.

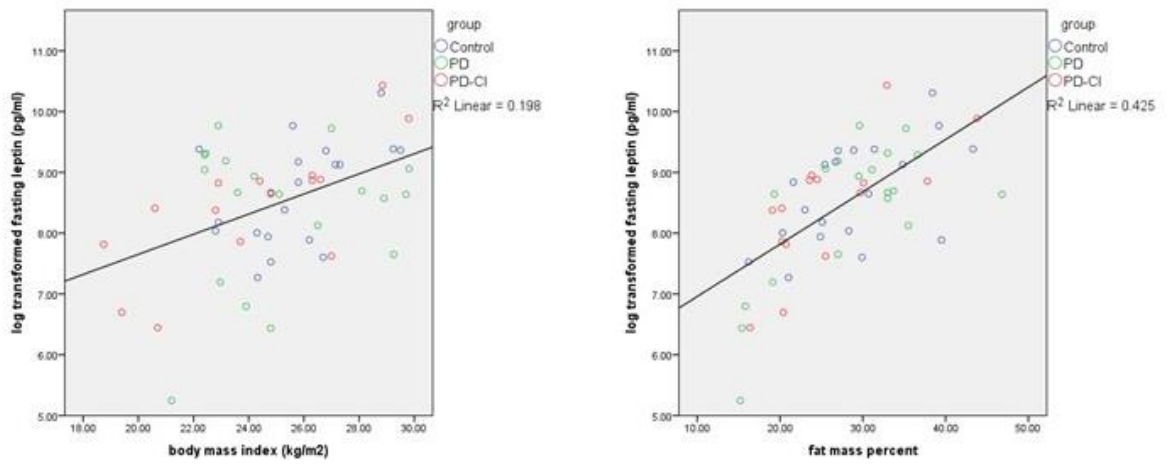


Figure 49. Scatter plots of fasting leptin and BMI and fat mass

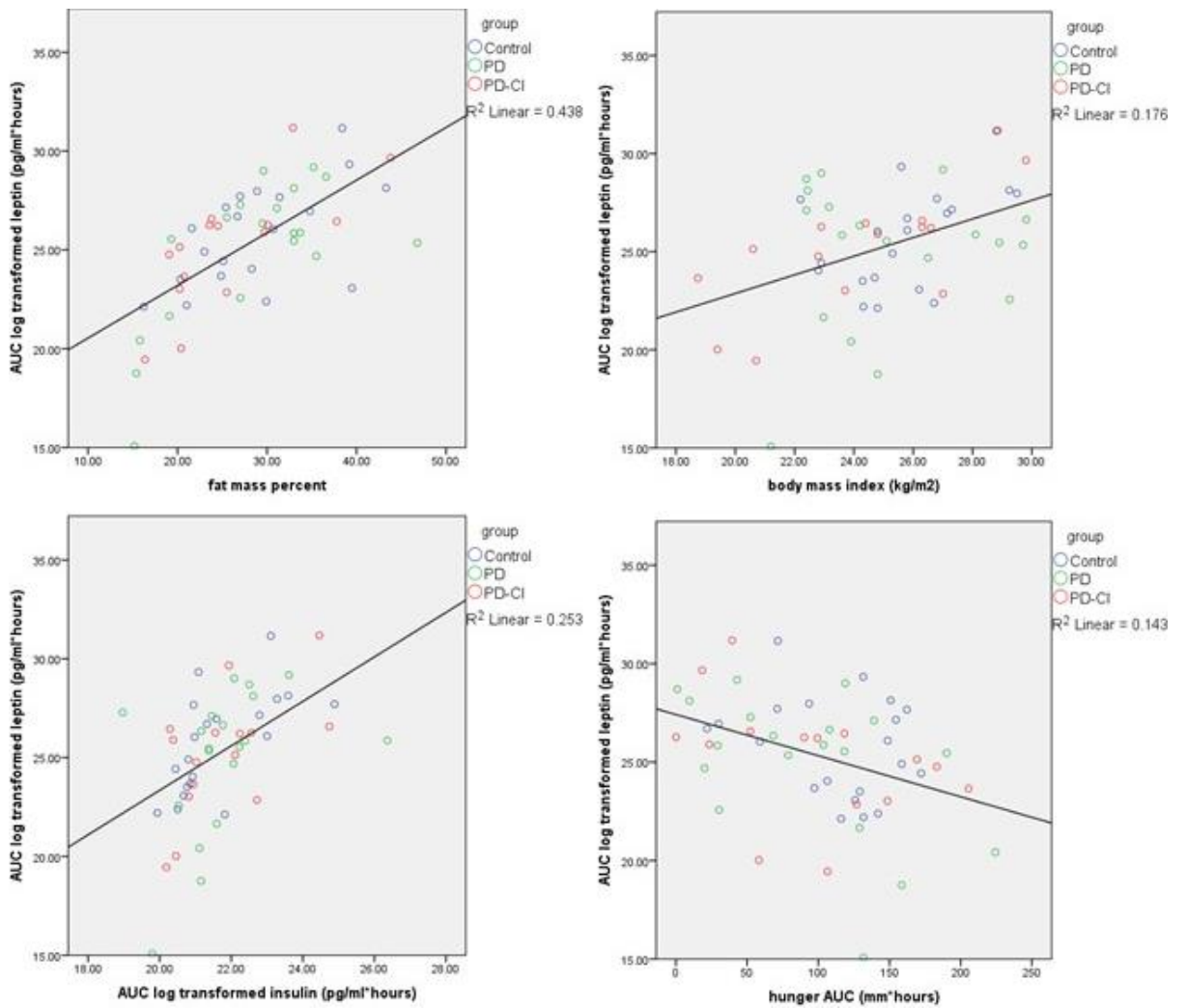


Figure 50. Scatter plots for relationships between leptin and fat mass, BMI, insulin and hunger

There was no significant difference between leptin levels over time between groups, with or without correction for PD duration ($p=0.41$) (Figure 52). There was no significant difference in fasting leptin or AUC between groups with ($p=0.70$ and $p=0.84$, respectively), or without the inclusion of PD duration or GDS as covariates ($p= 0.73$ and 0.70 , respectively) (Figure 53 and Figure 54).

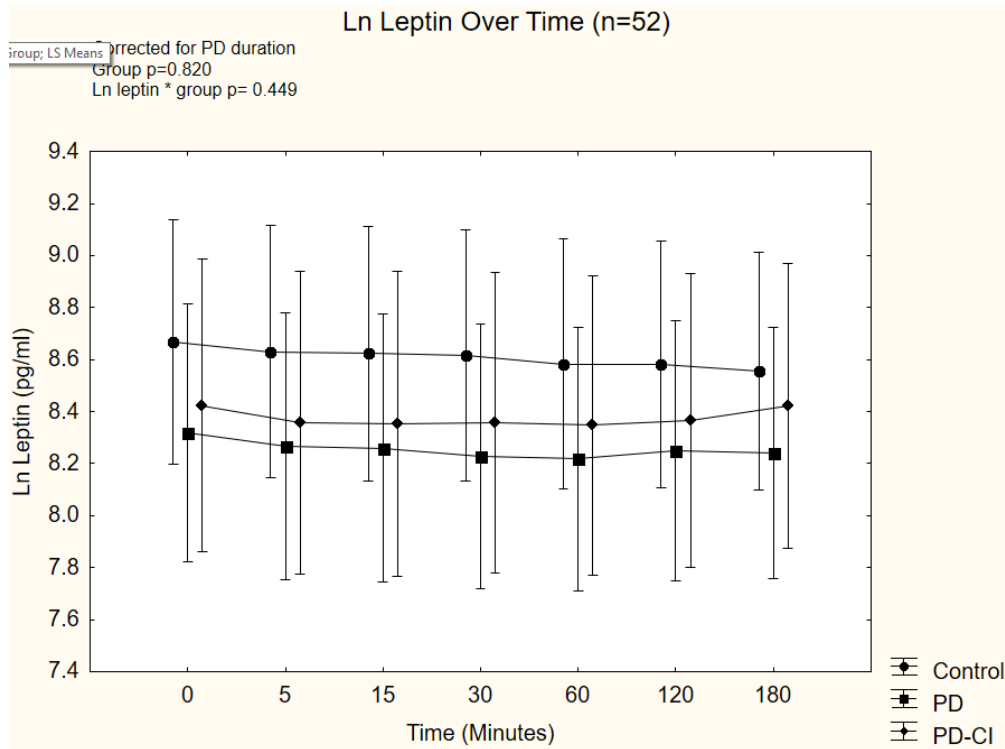


Figure 51. Leptin over time by group

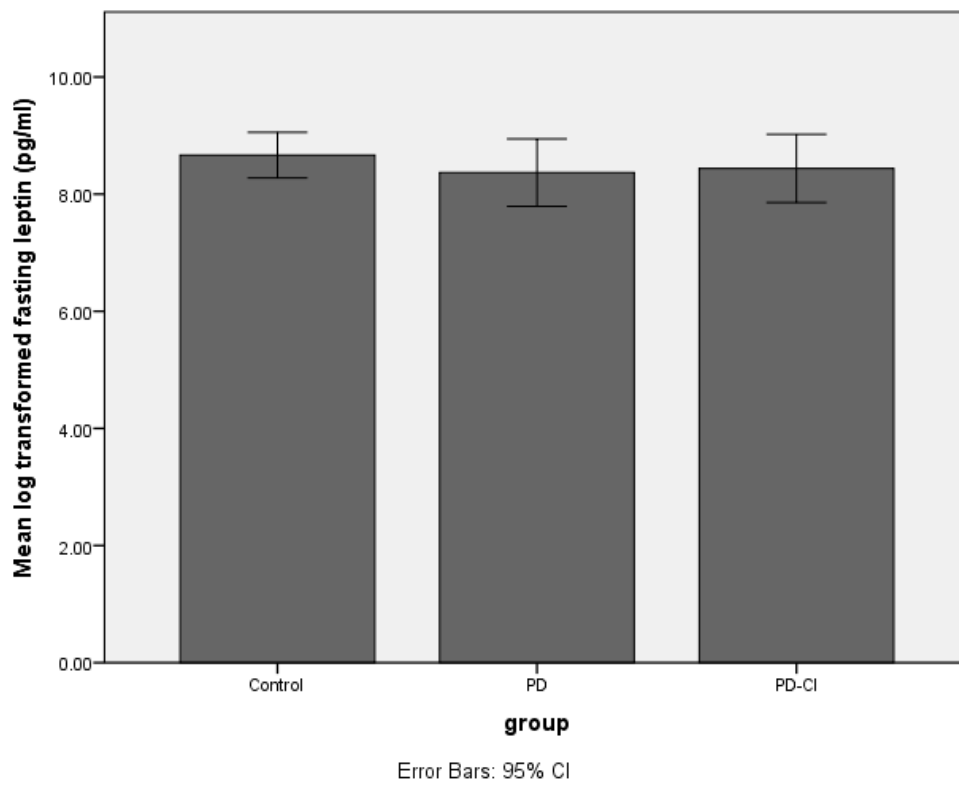


Figure 52. Fasting leptin by group

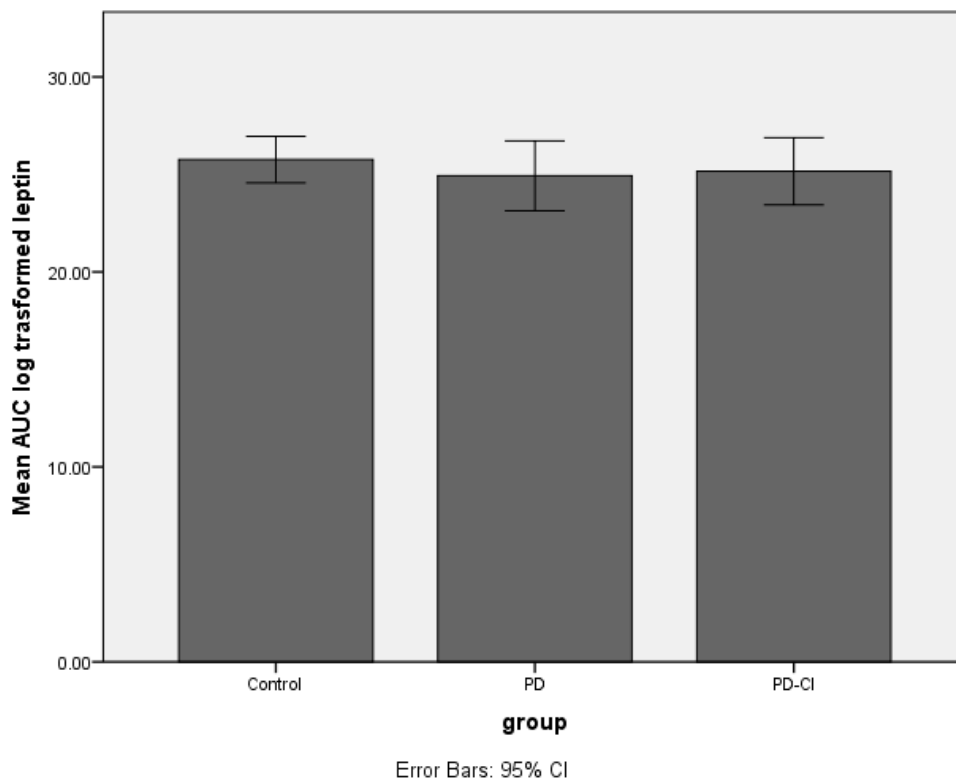


Figure 53. Leptin area under the curve by group

5.1.4 Hormones of energy homeostasis as predictors of cognition

Our aim in this pilot study was to explore whether hormones of energy homeostasis differ between patients with PD, PD-CI and controls, with the goal of designing a longitudinal study looking for hormonal predictors of cognitive decline in PD. We therefore examined predictors of cognition (MoCA) across the whole cohort using multiple linear regression. MoCA was reversed for this study to improve normality. Log transformed AG AUC negatively correlated with cognition ($r=-0.384$, $p=0.005$) whilst PD duration ($r=0.51$, $p<0.001$), motor UPDRS ($r=0.56$, $p<0.001$) and GDS ($r=0.68$, $p<0.001$) all positively correlated with reversed MoCA. These four variables were therefore included in the regression model. The model significantly predicted reversed MoCA accounting for 61% of the variance (R squared 0.61, $F_{11.03} [4,30]$, $p<0.001$). Acyl ghrelin AUC and motor UPDRS contributed significantly to the model ($p=0.003$ and 0.02 respectively). Individually, AG AUC accounted for 15% of the variance (R squared 0.15, $F_{8.81} [1,51]$, $p=0.005$).

Table 18. Multiple linear regression model for factors correlating with cognition

Variable	Unstandardised coefficient B	Standard error of B	β	significance
AG AUC	-0.151	0.047	-0.393	P=0.003
Duration of PD	0.000	0.002	0.017	P=0.898
GDS	0.102	0.050	0.316	P=0.51
Motor UPDRS	0.017	0.007	0.350	P=0.015

Dependent variable is LnMoCA (31-value)

5.2 Discussion

5.2.1 The Ghrelin-IGF-1 axis

The ghrelin-IGF-1 axis refers to ghrelin (total ghrelin and AG), GH and IGF-1. They are considered collectively here because they are intimately linked (Arvat et al., 2000, Nagaya et al., 2001b). Total ghrelin comprises active AG, an orexigenic hormone, and UAG, whose role is not yet well understood. Unacylated-ghrelin is converted to AG by the enzyme GOAT in the systemic circulation (Yang et al., 2008). Acyl-ghrelin then acts in the central nervous system to stimulate appetite, GH release and myriad other effects (Arvat et al., 2000). Growth hormone is produced by the pituitary in response to ghrelin and is important for normal growth and feelings of vitality (Carroll et al., 1998). Growth hormone also stimulates IGF-1 release from the liver (Chapman et al., 1996). Insulin-like growth factor-1 is a mitogenic hormone, which is to say that it promotes cell turnover through the birth of new cells, including neurons (Dyer et al., 2016). As such it is important for normal growth and tissue maintenance (Torres-Aleman, 2009).

Ghrelin BMI, fat mass and energy intake

Acyl ghrelin and total ghrelin increase as BMI decreases (Beberashvili et al., 2017, Tschöp et al., 2001b). Exogenous AG is a powerful appetite stimulant (Wren et al., 2001). Endogenous AG peaks prior to meal initiation and falls dramatically thereafter (Cummings et al., 2001). Together this suggests that AG maintains energy homeostasis by providing the stimulus to increase energy intake when total body energy stores are depleted. Our data demonstrated a negative correlation between TG AUC and BMI across the cohort ($r=-0.395$, $p=0.003$). Fasting AG and BMI were also significantly negatively correlated ($r=-0.287$, $p=0.037$). No correlations were seen between AG AUC and BMI ($r=-0.186$, $p=0.183$), TG AUC and fat mass ($r=-0.016$, $p=0.909$) or AG AUC and fat

mass ($r=0.410$, $p=0.318$). Overall, our results demonstrate a reduction in ghrelin (AG and TG) with increasing BMI as reported in the wider literature.

We did not observe increased intake, increased hunger or reduced fullness with increasing AG. There was no correlation between either AG AUC or TG AUC and total energy intake ($r=-0.145$, $p=0.292$ and $r=-0.070$, $p=0.617$ respectively), hunger ($r=0.146$, $p=0.296$ and $r=0.056$, $p=0.687$ respectively), or fullness ($r=0.171$, $p=0.220$ and $r=0.214$, $p=0.116$ respectively), across the whole cohort. Whilst it is intuitive that endogenous AG should have a role in appetite stimulation there is a lack of published evidence to support this in humans. In fact a small randomized control trial of physiological and low dose supraphysiological total ghrelin failed to alter feeding behaviour in 20 healthy young men of normal body weight (Lippl et al., 2012). Lack of evidence does not necessarily equate to lack of effect of course. There is a good scientific basis to believe that endogenous AG acts as an appetite stimulant. Pre- and post- prandial endogenous AG changes and a clear and consistent orexigenic effect of exogenous AG strongly support this. Moreover, it has been demonstrated that rodents treated with anti-ghrelin immunoglobulins have marked reduction in oral intake in the face of starvation (Nakazato et al., 2001). Overall, however, we did not demonstrate a relationship between endogenous AG and energy intake in our cohort.

Ghrelin and ageing

Acyl ghrelin AUC significantly negatively correlated with age across the whole cohort ($r=-0.386$, $p=0.004$). Results for TG AUC did not quite reach statistical significance but the scatter plot is suggestive of a genuine relationship ($r=-0.265$, $p=0.051$, see Figure 24). These data are consistent with previous studies showing that ghrelin levels decline with age as part of the somatopause (Akamizu et al., 2006, Nass et al., 2013, Rigamonti et al., 2002). Our study was not designed to identify frailty and therefore no conclusions can be drawn regarding the potential interplay between ageing, frailty and declining ghrelin levels suggested by some previous research (Schneider et al., 2008).

Ghrelin, Parkinson's disease and cognition

There is considerable evidence from animal and human studies that AG may have pro-cognitive (Carlini et al., 2002) and neuroprotective effects in neurodegenerative disease (Liu et al., 2006). Ghrelin secretion may be disordered in PD, especially in those patients that lose weight (Fischer et al., 2010, Song et al., 2017, Unger and Oertel, 2014). The nature of this relationship is not yet clear. A recent study showed no difference in AG or TG between groups of PD patients at different stages of disease (Song et al., 2017). Our data support these findings as there was no correlation between TG AUC or AG AUC and disease duration or motor disease severity as measured by the UPDRS. This is the first study to examine AG in people with PD-CI.

The pattern of AG and TG secretion over time was as expected in all three groups, which is to say there was a drop in levels immediately after eating with the nadir at 60 minutes followed by recovery towards baseline at 180 minutes. We did not see the failure of recovery in ghrelin over time in participants with PD that has previously been reported (Fischer et al., 2010, Song et al., 2017). There was a trend towards lower AG in the PD-CI group on visual inspection of our graph of AG over time by group. This difference did not reach statistical significance when AG AUC was compared across all three groups but there was a significant difference between the PD and PD-CI groups, with lower AG in the PD-CI group on post-hoc analysis. Significance was lost when PD duration and depression were included in the analysis. There was a negative correlation between AG AUC and reversed MoCA scores (*i.e.* the direction of correlation one would have predicted from previously published research). Log transformed MoCA was reversed in order to increase normality such that better performance results in lower transformed scores, which is to say that higher AG levels were associated with better cognitive performance on this screening tool for global cognition. Together these data suggest lower AG in cognitive impairment in PD.

We explored possible confounders that might lead us to falsely conclude that AG is lower in PD-CI than other groups. One of our control participants had previously had a vagotomy. We postulated that this may have biased results, as ghrelin modulation is thought to require an intact vagal nerve (Seoane et al., 2007). Acyl-ghrelin data were therefore re-analysed excluding this participant

and the results were unchanged, suggesting the difference between groups was not explained by failure of dynamic ghrelin release in this participant.

Another confounder could be differences in medications. The effects of dopamine on ghrelin release and signaling are not yet well understood, with dopaminergic medication causing ghrelin release *in vitro* (Iwakura et al., 2011), but dopamine receptor stimulation and blockade both attenuating ghrelin response to feeding in rats (Romero-Picó et al., 2013). All of our participants were tested “off” their usual PD medications in order to minimize confounding. It is possible that longer-lasting background effects of levodopa and longer lasting PD medications were still exerting an effect. Against this, the background LED was not significantly different between the PD and PD-CI groups. This suggests that anti-parkinsonian medication could account for a difference between controls and PD or PD-CI groups but not the observed reduction in the PD-CI group. Cholinergic medications may be relevant, however. Patients taking non-selective anticholinergics were excluded from taking part. There was no significant difference in selective anticholinergic medication use between groups, so this is unlikely to have biased our results. Seven of our PD-CI group were taking cholinesterase inhibitors, used for the symptomatic treatment of dementia and which augment acetylcholine in the brain. Acetylcholine increases ghrelin levels in humans whilst anti-cholinergics reduce levels overall (Broglio et al., 2004). Given the even spread of anticholinergic medications between groups and the high proportion of PD-CI participants taking cholinesterase inhibitors (43%) it seems implausible that these medications could account for the lower levels of AG in the PD-CI group. This is because cholinesterase inhibitor use would tend to increase ghrelin levels. It is more likely that the difference in AG between groups was blunted by cholinesterase inhibitor use in the PD-CI group. It would be neither medically appropriate nor ethical to discontinue these medications for research purposes, however, so future studies should be powered to allow statistical adjustment for this.

A final confounder to our observed results could be depression. Geriatric depression scale negatively correlated with AG AUC in our cohort. Moreover, GDS scores were significantly higher in the PD-CI group than other groups. This is important in the context of cognition in PD as depression in PD is associated with a greater risk of cognitive decline (Stern et al., 1993).

Conversely, the risk of depression increases with the development of cognitive impairment in PD (Lawson et al., 2014). In our study GDS correlated with disease duration UPDRS scores and reversed MoCA. None of our patients were clinically depressed, however, and GDS has not been validated for use in people with cognitive impairment. In fact, there is evidence that GDS validity declines in cognitive impairment (Kørner et al., 2006, Burke et al., 1989), which is a limitation of the study design. Ghrelin has been proposed to both ameliorate and exacerbate anxiety and depressive behaviour in rodents (See review by Steiger et al., 2011) and ghrelin levels do not differ between people with and without major depression (Kluge et al., 2009). We have included GDS and PD duration as covariates in our analysis in order to correct for potential bias. This resulted in loss of significance when AG AUC was compared between groups. It is not clear whether this is due to the relationship between AG UAG and depression scores confounding the original result (type 1 error) or loss of power with the inclusion of covariates due to our relatively small sample size (type 2 error).

In order to clarify this further we carried out multiple linear regression analysis for predictors of cognition across the whole cohort. We found that AG AUC significantly predicted cognition, accounting for 15% of the variance. This model was improved when other factors correlating with reversed MoCA (Parkinson's disease duration, motor UPDRS and GDS) were included. The final model significantly predicted reversed MoCA accounting for 61% of the variance. The significant contribution of AG in this model would suggest that the difference between groups is likely to be genuine. Overall, our results suggest that there is sufficient "signal" to warrant further studies into AG and cognitive impairment in PD. It appears likely that that AG levels are lower in the PD-CI group and that AG levels predict cognition but that our study is underpowered to detect this difference once depression and PD duration are included in the analysis.

This study was not designed to elucidate the basis of a correlation between increasing AG and better cognitive performance. Discussion around possible mechanisms responsible for this association is therefore speculative. Broadly, there are three main ways in which AG and cognition could be linked in this weight-stable cohort. First, AG levels could be reduced as a result of neurodegeneration resulting in reduced ghrelin secretion. As previously

discussed, cholinergic neurons have been implicated in the control of dynamic ghrelin secretion (Seoane et al., 2007). Loss of these neurons due to neurodegeneration could therefore decrease ghrelin secretion. This process could occur at the level of the DMNV or in the cortical areas contributing to higher control of ghrelin secretion (Seoane et al., 2007). The DMNV is usually damaged early in PD (Braak et al., 2003a) but we did not demonstrate any difference between the PD group and controls in our cohort, even when the control with vagotomy was excluded. All participants in the PD group had established PD with an average disease duration of nearly 6 years (69.53 months). It therefore seems unlikely that neurodegeneration at the DMNV is the primary cause of reduced AG in PD-CI. Cortical neurodegeneration resulting in changes in meal anticipation could be more likely. Animal studies show that tease feeding results in ghrelin suppression, suggesting cognitive processes are important in the control of ghrelin secretion (Seoane et al., 2007). Moreover, human studies in people with PD-MCI have shown that cortical thinning and hippocampal atrophy are associated with cognitive impairment in PD (Danti et al., 2015, Pereira et al., 2014). Taking these studies together it seems plausible that neurodegeneration in PD-CI could result in altered ghrelin secretion.

Second, a decline in AG as a result of α -synuclein deposition in the gut could lead to loss of neuroprotection and increase the likelihood of developing cognitive impairment. There is evidence of extensive α -synuclein in the enteric nervous system supplying the stomach (Braak et al., 2006). Acyl ghrelin is proposed to be pro-cognitive through promotion of LTP and neurogenesis at the hippocampus (Carlini et al., 2010b, Kent et al., 2015) and neuroprotective through mitochondrial stabilization, regulation of autophagy and anti-inflammatory actions (Ferreira-Marques et al., 2016, Moon et al., 2009). It is possible that gastric ghrelin secretion is lost and levels reach a threshold below which these protective effects are lost and cognitive impairment ensues. Finally, and perhaps most likely, both of the above mechanisms could be at play, resulting in a vicious cycle of neurodegeneration, loss of neuroprotection and accelerated cognitive decline in individuals at risk.

None of these potential explanations can account for normal total ghrelin across all three groups and a lack of relationship between total ghrelin and cognition in our study. Recent research suggest that acylation of UAG occurs in target

organs, not in the blood stream as previously thought (Hopkins et al., 2017). Our collaborator, Dr. Jeff Davies and his team, has examined the ratio of AG:UAG in our cohort and found that the AG:UAG ratio was significantly lower in participants with PD-CI than cognitively intact PD patients or healthy controls ($p=0.003$ and 0.001 , respectively). They did not include PD-MCI participants in their analysis (Horsby AKE *et al.* unpublished pers. com). It is possible that neurodegeneration results in reduced acylation of UAG to pro-cognitive and neuroprotective AG resulting in cognitive decline and accelerated neurodegeneration. Further research is needed to clarify the underlying processes and help to establish whether AG or AG:UAG ratio may be helpful in determining patients at risk of cognitive impairment in PD.

Growth hormone, IGF-1 and cognition in PD

Growth hormone followed a similar pattern to AG over time (Figure 31 and Figure 20), with a slight delay in the nadir (60 minutes for AG, between 60 and 120 minutes for GH). Our results are congruent with previous research as exogenous AG has been shown to result in GH secretion at around 15 minutes in rats (Wren et al., 2001). There was, however, no demonstrable correlation between GH AUC and AG AUC. Growth hormone AUC increased with age and increased with IGF-1 AUC (Figure 21). This was unexpected as most of the literature describes a decline in GH in older age. When the data were analysed by group the positive correlation between age and GH only persisted for healthy controls (see Appendix I). This may reflect reduced frailty in this cohort, with less frail individuals surviving for longer. This is highly speculative as relative GH deficiency, whilst associated with advancing age, has not been demonstrated to correlate with frailty (Morley, 2016). Moreover, we did not measure frailty in our cohort. The underlying cause of this unexpected positive correlation therefore remains unclear. There was no significant difference in GH between groups and no correlation with cognition. These data suggest that GH was not meaningfully disordered in PD or PD-CI. It is worth mentioning that nocturnal GH secretion has been shown to be disordered in previous studies of people with PD (Bellomo et al., 1991). Our study took place between the hours of 0730 and 1230 and we would not, therefore, have been able to detect this. There is some evidence that GH may be pro-cognitive, at least where there is

GH deficiency (Falletti et al., 2006) but the pulsatile nature of GH secretion makes it an unattractive target as a biomarker for cognitive decline overall.

The pattern of IGF-1 secretion did not follow the pattern of AG, TG or GH secretion over time (Figure 34). There are few data regarding the post-prandial secretion of IGF-1 available. A study in salmon reported that IGF-1 does not change with acute nutritional intake (Shimizu et al., 2009) and a study in cattle showed that pulsatile GH secretion was not accompanied by pulses in IGF-1 (Breier et al., 1986). This is unsurprising when one considers that IGF-1 is highly protein bound. It has been proposed that protein binding regulates the available IGF-1 resulting in a relatively steady state over time (Pan and Kastin, 2000). There was no correlation between IGF-1 AUC and AG AUC or TG AUC.

There is a large body of evidence to support a role for IGF in cognition (see table 4) and meta-analysis suggests that higher IGF-1 levels are associated with improved cognition in the elderly (Arwert et al., 2009). In our study there was no correlation between IGF-1 AUC and reversed MoCA. There is a suggestion in the literature that the relationship between cognition and IGF-1 may not be linear, with lower levels being associated with cognitive decline but no additional benefit above a certain threshold (Watanabe et al., 2005, Westwood et al., 2014). Visual inspection of the scatter plots did not reveal such a relationship across our cohort.

It has also been proposed that IGF-1 is neuroprotective in neurodegenerative disease through enhanced neurogenesis (Aberg et al., 2005), reduced apoptosis due to improved PI3K/AKT signalling (Quesada et al., 2008) and better A β clearance (Carro et al., 2002). Interestingly, a 2015 meta-analysis found that IGF-1 levels were increased in early PD, a phenomenon that has been proposed to be a compensatory response to neurodegeneration (Li et al., 2015b, Godau et al., 2009). As disease progresses, the picture is less clear with both increased (Picillo et al., 2014) and decreased (Suzuki et al., 2014) IGF-1 levels reported with increasing disease severity. Other studies have reported a decline in IGF-1 with PD duration but not disease severity (Godau et al., 2009). In our study IGF-1 AUC was not correlated with disease duration or with disease severity. Neither was it significantly different between groups. There appeared to be a trend toward lower IGF-1 AUC in the PD-CI group. In view of

this post hoc testing using LSD was carried out and the IGF-1 AUC was significantly lower in the PD-CI group compared to the PD group. Significance was lost with correction for disease duration and GDS. There was no significant difference between controls and the PD group, or between controls and the PD-CI group.

Our results appear somewhat incongruent. We found lower IGF-1 AUC in the PD-CI group but there was no demonstrable correlation between IGF-1 AUC and cognition, disease duration or disease severity. It may be relevant that large amounts of data were missing in our analysis of IGF-1 (table 16). Only 38 of 55 participants had complete data (17 of 20 controls, 11 of 19 PD and 10 of 16 PD-CI). The reason for this is unclear but may relate to practical limitations in processing samples on site. Insulin-like growth factor 1 samples required different handling than other samples (see appendix F). Only one centrifuge was available therefore IGF-1 samples were kept on ice and analysed after other hormonal assays. Our results should therefore be interpreted with caution. Insulin-like growth factor 1 is released from the liver in response to GH secretion (Torres-Aleman, 2009). Growth hormone AUC was not lower in the PD-CI group in this study. It therefore seems unlikely that there is a meaningfully deranged IGF-1 response in this group but further research is needed to clarify this. No firm conclusions may be drawn from our study as to whether or not IGF-1 is a useful biomarker for cognitive decline in PD.

5.2.2 Insulin

Insulin is a metabolic hormone whose primary action is to lower blood glucose and allow cellular glucose utilisation. Insulin insufficiency and resistance cause diabetes mellitus, a common chronic disease associated with excess mortality from cardiovascular disease and other complications (DeFronzo et al., 1985).

Insulin, BMI, fat mass and energy intake

Insulin has been demonstrated to increase with adiposity (Bagdade et al., 1967) and to suppress appetite and food intake through actions at the hypothalamus (Benoit et al., 2002). We found that insulin AUC showed the expected correlations with BMI, fat mass and leptin (Figure 38). There was no correlation with hunger, fullness or energy intake. The lack of correlation between insulin and intake may be due to our study being underpowered. This would be consistent with the literature. Numerous studies did not show a relationship between appetite and insulin levels until a meta-analysis pooling data from seven studies involving 180 participants was carried out in 2007 (Flint et al., 2007).

Insulin, Parkinson's disease and cognition

Determining the relationship between insulin levels, cognition and neurodegenerative disease, including PD, is challenging. This is because insulin deficiency results in diabetes which has an array of confounding consequences including vascular damage and nerve damage (Ribe and Lovestone, 2016). The picture is further complicated by the phenomenon of insulin resistance, in which chronic hyperinsulinaemia results in a reduced downstream response to circulating insulin levels. Where insulin resistance occurs peripherally it results in type 2 diabetes (Reaven, 1988). Where it occurs in the brain it has been linked with cognitive impairment, and in fact AD has been described as “type 3 diabetes” (Steen et al., 2005). Insulin resistance has been linked with PD in the literature and meta-analysis has confirmed an increased risk of PD in people with diabetes, even with correction for vascular disease (Cereda et al., 2011, Cereda et al., 2013).

Our data demonstrate a negative correlation between insulin AUC and motor disease severity, which is to say that as disease severity worsens (higher UPDRS), insulin levels also decline. We also calculated HOMA-IR for our cohort

retrospectively. Initial scatter-plots suggested a negative correlation between log-transformed HOMA-IR and disease severity, with greater disease severity associated with lower insulin resistance (Figure 45). This correlation did not reach statistical significance but is in keeping with the observed reduction in insulin with increased disease severity. There was no difference between groups with or without correction for PD duration and GDS. Interestingly, our data show no correlation between insulin AUC and reversed MoCA or HOMA-IR and reversed MoCA. This is in keeping with the conflicting published literature, which has shown both increased and decreased insulin sensitivity with cognitive impairment in PD (Schelp et al., 2017, Bosco et al., 2012). Unfortunately, our study was not prospectively designed to measure insulin resistance. There are therefore methodological limitations that make interpretation of retrospective HOMA-IR calculations unreliable in our study. The validity of HOMA-IR is reduced in people without diabetes and where fasting glucose is drawn prior to 15 minutes after cannulation due to the stress response to blood sampling (Matthews et al., 1985). Moreover, results for our cohort may not be generalisable as people with diabetes and obesity were excluded from the study. Nevertheless, our data suggest there are lower insulin levels and preserved insulin sensitivity in more advanced motor disease but no correlation between insulin and cognition. Decreased insulin with advancing motor disease could reflect homeostatic failure as a result of neurodegeneration. Our cohort was matched for BMI, age and gender, suggesting that the observed correlation is not due to differences in these parameters.

That there is a negative correlation between insulin and motor disease but no link between insulin and cognitive decline is surprising as advancing disease severity is a risk factor for cognitive decline in PD (Emre, 2003). Our data were in keeping with this as reversed MoCA and motor UPDRS were positively correlated in our cohort. A possible explanation for lower insulin with increasing disease severity but not with cognitive decline may be the different patterns of neuronal loss in motor PD and PD-CI. Motor symptoms of PD are associated with dopaminergic cell loss (Damier et al., 1999) whilst other neurotransmitters such as acetylcholine have been implicated in non-motor symptoms (Chaudhuri et al., 2006). Dopaminergic dysfunction is not a cardinal feature of AD. There is strong evidence for an interaction between insulin and dopamine signalling in

motor PD. Insulin receptors are found in high concentration in dopaminergic neurones (Unger et al., 1991). Moreover, IR stimulation has been shown to enhance dopaminergic cell function in the SNPC in animal models of PD. Insulin resistance is associated with lower dopamine levels and increased dopaminergic cell loss (Morris et al., 2011b) whilst DaT expression is increased with insulin administration (Finglewicz et al., 1994). In humans IR expression is reduced in nigral dopaminergic neurones in people with PD but not in those with AD or vascular disease (Moroo et al., 1994). Reduced or disrupted insulin signalling at the SNPC may therefore relate to reduced insulin and increased motor disease severity in PD.

An alternative mechanism by which lower insulin might relate to greater motor disease severity in PD is via IDE. Insulin degrading enzyme breaks down insulin in the CNS but also binds with α -synuclein *in vitro* (Sharma et al., 2015). On the one hand, lower insulin levels result in reduced IDE expression, which may exacerbate α -synuclein deposition. On the other hand, insulin directly competes with other substrates of IDE and so lower insulin may result in greater α -synuclein clearance as a compensatory response to greater disease severity. Our cross-sectional study cannot determine if lower insulin levels in more advanced disease are a cause of neurodegeneration, a negative decompensation due to neurodegeneration or a positive compensation in response to neurodegeneration in PD. Further longitudinal studies will be required to clarify this relationship. Our results support further investigation into CNS insulin as a possible disease-modifying agent in PD but do not support its use as a biomarker for cognitive decline.

5.2.3 Glucagon-like peptide 1

Glucagon-like peptide 1 is a short acting anorexigenic hormone produced mainly in the small intestine in response to vagal stimulation and nutrient ingestion (Hellstrom and Naslund, 2001). It increases insulin sensitivity during euglycaemia (Nauck et al., 2002) and GLP-1 analogues such as exendin-4 are therefore used in the treatment of type 2 diabetes (Brunton, 2014). Glucagon-like peptide 1 is rapidly broken down by DPP-4 in the blood stream (Alagiakrishnan et al., 2013) and DPP-4 inhibitors such as sitagliptin are also widely used in patients with diabetes (Brunton, 2014).

Glucagon-like peptide 1, BMI, fat mass and energy intake

Higher BMI is usually associated with a reduced post-prandial peak of GLP-1 (Nauck et al., 2011). There was no correlation between BMI or fat mass and GLP-1 in our cohort, which may reflect the narrow range of BMI and small sample size of our cohort. The post-prandial pattern of secretion was as expected in all three groups (Figure 47). There was no correlation between GLP-1 and energy intake at the *ad libitum* meal. This is unsurprising as GLP-1 is predominantly a satiety hormone that signals cessation, not initiation, of feeding. Intake at breakfast was standardised and we do not have data for GLP-1 during the *ad libitum* meal. There was a positive correlation with PYY, which is expected as they are both produced by L cells in the gastric mucosa in response to nutrient ingestion (Adrian et al., 1985). Overall, our results for GLP-1 and appetite are as expected across the cohort.

Glucagon-like peptide-1, Parkinson's disease and cognition

Pharmacological augmentation of GLP-1 has been shown to be neuroprotective in animal models of PD (Kim et al., 2009, Nassar et al., 2015). This may be through up-regulation of IDE (Athauda and Foltynie, 2016), increased neurogenesis (Bertilsson et al., 2008, Harkavyi et al., 2013), anti-inflammatory (Kim et al., 2009) and anti-apoptotic (Kim et al., 2009) effects and increased mitochondrial stability (Fan et al., 2010, Foltynie and Aviles-Olmos, 2014, Kang et al., 2015a, Li et al., 2015b). It is unclear, and perhaps irrelevant, if these effects are independent of the insulin sensitising and augmenting effects of GLP-1 or not. Human studies of GLP-1 agonists in PD are promising. An open label pragmatic trial of exenatide in the treatment of PD found improved motor and cognitive symptoms in the treatment group (Aviles-Olmos et al., 2014).

More recently, a randomized control trial reporting slower motor decline in the off state after 48 weeks of treatment. Cognitive and non-motor symptoms were not different between treatment and placebo groups (Athauda et al., 2017). Our study found no correlation between disease duration, disease severity or cognition and GLP-1. Moreover, there was no significant difference between groups with or without correction for PD duration or GDS. Given the role of the vagus nerve in control of GLP-1 secretion, a relationship between GLP-1 and some parameter of neurodegeneration in PD may have been expected. It is possible that the second wave of secretion caused by a direct response to nutrients in the small bowel (Hellstrom and Naslund, 2001) is sufficient to produce a normal response. Our results suggest that GLP-1 is unlikely to be a useful biomarker for cognitive decline in and of itself. Even if GLP-1 levels are not meaningfully disrupted in PD, GLP-1 agonists and DPP-4 inhibitors remain promising potential neuroprotective agents.

5.2.4 Leptin

Leptin is an anorexigenic hormone produced by adipose tissue (Friedman and Halaas, 1998). It does not show dynamic variation with meal ingestion but levels can be increased by prolonged fasting (Chan et al., 2003). Its actions at the hypothalamus are broadly opposite to those of AG (Cowley et al., 2001). Leptin levels are increased by insulin in humans under experimental conditions (Kennedy et al., 1997). Leptin resistance occurs in obesity, analogous to the insulin resistance seen in type 2 diabetes (Münzberg and Myers, 2005).

Leptin, fat mass, BMI and energy intake

As may be expected for a hormone that is secreted by fat cells, leptin typically increases with increasing BMI and adiposity (Mantzoros et al., 2001). Our study found a positive correlation between leptin and BMI and fat mass. Leptin and insulin were positively correlated whilst there was a negative correlation between leptin and hunger as measured by a visual analogue scale. These results are all expected and in keeping with the published literature (see chapter 2: Introduction and literature review). There was no correlation between energy intake and leptin. The relatively narrow range of BMI across our cohort and small numbers may have made our study underpowered to detect such a relationship. The negative correlation between leptin and hunger, which itself positively correlates with energy intake in this study (see chapter 4), suggests that leptin is anorexigenic in our cohort.

Leptin. Parkinson's disease and cognition

Like AG, leptin is pleiotropic and may be pro-cognitive in rodents through promotion of LTP (Shanley et al., 2001). Leptin is also neuroprotective in animal models of AD through altering A β handling. The result is lower A β due to reduced A β synthesis and increased A β clearance from the cytosol (Fewlass et al., 2004, Greco et al., 2009). Animal models of PD have also demonstrated a neuroprotective effect of exogenous leptin through mitochondrial stabilization (Ho et al., 2010) and anti-apoptotic effects (Weng et al., 2007). Moreover α synuclein over-expressing rodents are hypoleptinaemic (Rothman et al., 2013). Despite promising results in animal studies, there has not yet been conclusive proof of a role for leptin in cognition or neuroprotection in humans. It is true that learning difficulties occur in leptin deficient humans (Paz-Filho et al., 2008) but large numbers of studies examining leptin and cognition have failed to reach a

consensus as to whether leptin is pro-cognitive, negatively correlated with cognition or neutral (see tables 8 and 9 in Chapter 2). The true nature of the relationship between cognition and leptin in humans is unlikely to be determined without high quality meta-analysis.

Leptin levels may be lower in PD than in healthy older adults due to reduced BMI and adiposity from some other cause in PD (Folch et al., Davis et al., 2014). In keeping with this, we found no correlation between leptin and cognition, disease duration or disease severity in our BMI and fat-mass matched cohort. The relationship between leptin and cognition may be U shaped, with optimal performance in the mid-range of leptin expression (Oomura et al., 2011). Examination of scatter plots did not reveal such a relationship in our cohort. Moreover, there was no significant difference in leptin between groups, with or without correction for disease duration and GDS. It is possible that unintended weight loss results in lower leptin levels, which impairs neuronal maintenance and contributes to neurodegeneration and cognitive decline in PD. Our study is unable to identify such a relationship as it was cross-sectional and only included weight stable participants within a narrow range of BMI. Overall, our results would not support leptin as a biomarker of cognitive impairment in PD in the real world as it is likely to reflect weight loss that is easier and cheaper to measure.

5.2.5 Summary and conclusions

The main aim of this study and the focus of this chapter was to determine whether hormones of energy homeostasis warrant further investigation as putative biomarkers for cognitive impairment in PD. Our results support further research into AG and specifically the AG:UAG ratio as possible biomarkers for cognitive decline. Ghrelin dysfunction remains a plausible link between weight loss and cognitive impairment in PD. We were unable to draw conclusions about whether IGF-1 is a potential biomarker but this could easily be included in a further study assessing AG. It appears unlikely that insulin, GLP-1 or leptin will be useful biomarkers for cognitive decline in PD and research efforts should focus elsewhere. This study has a number of strengths and limitations that are discussed later in this thesis along with recommendations for future research.

Chapter 6. Does a bite counter predict energy intake in people with PD?

In this study we used a wrist worn device called a bite counter to record how many mouthfuls of food were eaten by participants. This has been shown to predict energy intake in healthy young adults (Scisco et al., 2011, Salley et al., 2016, Scisco et al., 2014, Dong et al., 2012) but has never been used in people with Parkinson's disease. This chapter explores the hypothesis that bite count measured by a bite counter predicts energy intake in controls, PD and PD-CI.

6.1 Results

6.1.1 Demographics

This was the same cohort as described in Chapter 4. Demographic data is as reported in that chapter.

6.1.2 Missing data

Missing data is outlined in the table below.

Table 19. *Missing data for bite count*

Ad libitum bite count	Control	PD	PD-CI	Comments
Manual	1	0	3	3 PD-CI participants were unable to feed themselves. Control participant missing data due to oversight.
Automated	0	1	3	3 PD-CI participants were unable to feed themselves. PD participant data missing due to bite counter malfunction

6.1.3 Manual and automated bite count

There was a strong correlation between manually and automatically recorded bite count ($r=0.74$, $p<0.001$) (Figure 55)

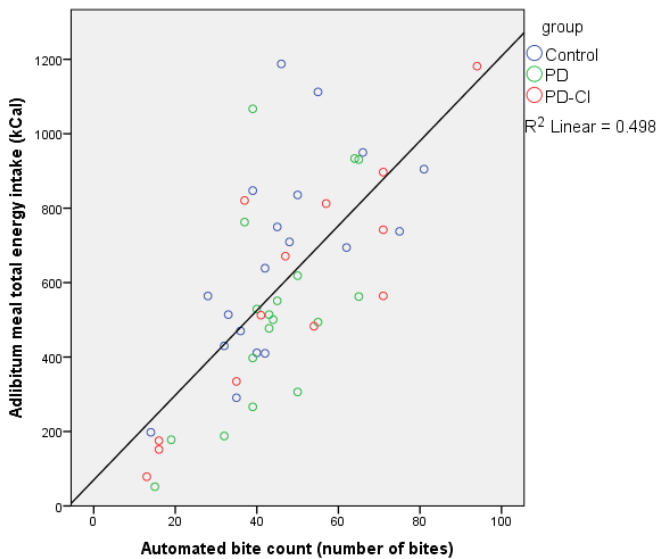


Figure 54. Scatter plot of manual and automatic bite counts at the ad libitum meal.

6.1.4 Bite count and energy intake

There was a strong correlation between automated bite count and energy intake at the *ad libitum* meal ($r=0.70$, $p<0.001$) (Figure 56).

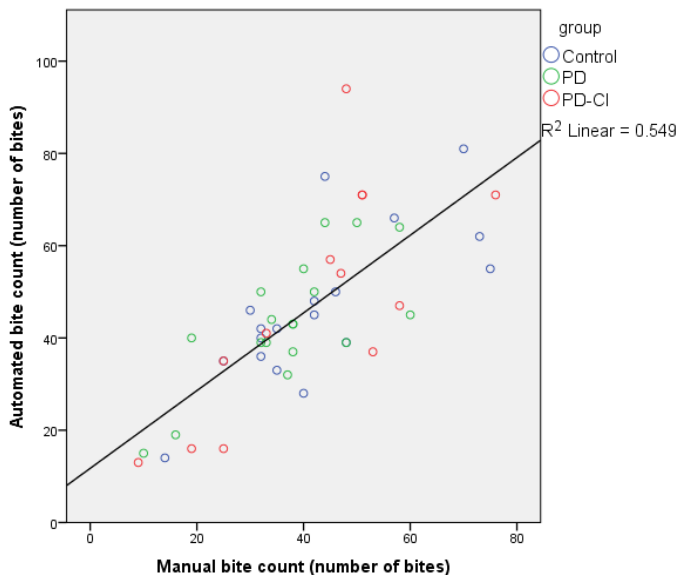


Figure 55. Scatter plot of total energy intake and automated bite count at the ad libitum meal.

6.2 Discussion

A bite counter is a wrist-worn accelerometer that records movements towards the mouth. It may be calibrated for an individual's particular bite size. In our study, this was not adjusted and we did not use the bite counter to calculate an estimated calorie intake. Despite this, our data demonstrate a strong correlation between bite count as measured by the bite counter and energy intake, even in people with PD and PD-CI. This relationship was actually stronger than that reported for healthy young people (Scisco et al., 2014) suggesting good predictive value. Although there was a strong relationship between manual and automated bite count, it was not a perfect correlation. This may be due to non-eating movements made with the hand, for example scratching the nose or touching the face. Importantly, the degree of correlation did not differ between groups (Figure 56), suggesting that tremor did not meaningfully impact upon results. Overall, our results show that a bite counter is able to predict energy intake in our cohort.

6.3 Summary and conclusions

Our results would support the use of bite counters as a proxy measure for energy intake in people with PD and PD-CI. This could be a valuable research tool in trying to clarify energy intake in free-living study participants. There are also potential clinical applications in monitoring intake in those at risk of malnutrition in the community.

Chapter 7. Strengths and limitations of this study

7.1 Overview

This was a cross-sectional pilot study examining hormones of energy homeostasis in a weight stable, normal BMI group without diabetes or significant other comorbidities. Our primary aim was to determine whether hormones of energy homeostasis warrant further investigation as putative biomarkers for cognitive impairment in PD. Our secondary aim was to explore whether appetite and food intake differ between patients with PD, PD-CI and controls

The main strength of this study is its novelty. To date this is the only study looking at appetite, energy intake and a comprehensive profile of hormones of energy homeostasis in people with PD-CI. This also is the only study to date to directly measure food intake in people with PD, which is both a strength and a limitation as the measurements are accurate but the environment is artificial. We were able to capture dynamic hormonal changes pre- and post-prandially up to 3 hours and to correlate our results with cognition, appetite and energy intake. In addition we were able to use robust techniques to prevent deacylation of AG *in vitro* (Delhanty et al., 2015), which has allowed us to accurately measure both AG and TG enabling more accurate understanding of results.

Limitations of our study include small sample size, strict inclusion criteria and the lack of validated tools with which to assess appetite in people with dementia. Our sample size was calculated using previous research into ghrelin in PD (Unger et al., 2011). We came close to the target sample size of 20 participants in each group but fell slightly short due to time pressure. Our study may therefore be underpowered to detect differences in some variables between groups. Despite this, we have demonstrated significant results that warrant further investigation.

7.2 Recruitment and selection

The narrow range of BMI and the exclusion of people with diabetes assisted analysis and prevented over-correction for multiple variables in this small study. This may have been at the expense of generalisability, however. The study tested a weight stable cohort and cannot, therefore, draw conclusions about hormone profile or appetite in weight losing people with PD. This could be relevant as people with PD who lose weight may be phenotypically different to their weight stable counterparts, with more non-motor symptoms and greater severity of disease than those who are weight stable (Sauerbier et al., 2016, Sharma and Turton, 2012). We also excluded participants at extremes of BMI. This may have affected the generalisability of our results. Against this, the number of people who failed screening for obesity was similar in the control and PD-CI groups and only one participant failed screening due to being underweight (**Error! Reference source not found.**).

Table 20. Screening failures by group

	BMI >30	BMI <18.5	MoCA >= 26	MoCA < 26	Other	Total
Controls n=6	4	0	0	1	1	6
PD n=4	1	0	0	2	1	4
PDD/PDMCI n=12	5	1	6	0	0	12
Total n=22	10	1	6	3	2	22

It is possible that people who agreed to take part were younger or fitter than those who declined. In keeping with good ethical practice we did not collect personal data for people who declined to take part. It seems unlikely that those who declined were significantly older than those who eventually took part, however, as our cohort had an average age of 73.58 and the oldest participants were 85 years old. Our cohort had an average of 2.96 comorbidities other than PD overall, with no significant difference between those who passed or failed screening ($p=0.72$). This is comparable to the general population, 50% of whom have 2 comorbidities at 70-74 years of age (Barnett et al., 2012). The average age and gender balance of those who failed screening were not significantly different to those who completed testing ($p=0.35$ and $p=0.10$ respectively). Moreover, we were able to recruit people with a wide spread of cognition, including participants with severe dementia. The lowest MoCA score recorded in our study was 6/30. Finally, all of our participants were Caucasian, which

clearly limits the generalisability of our study with regard to global populations. This was not intended at the outset of the study but reflects the ethnic mix of the North East of England, which has the highest proportion of people who identify as White British in England and Wales (93.6%) (Bradford-Ons, 2012). The older age of our cohort, their representative levels of comorbidity and the inclusion of people with severe dementia improves the generalisability of our study despite the strict inclusion criteria.

7.3 Missing data

As previously mentioned, the small sample size of our study may have rendered it under-powered to detect some differences in variables between groups. This was compounded by missing data. For most variables there was very little missing data (Table 21). Insulin-like peptide-1 and GLP-1 were exceptions to this, with undetectable hormone levels for multiple participants at multiple time points. Despite discussion with our biochemistry collaborators at Swansea, no clear cause for this has been identified. There were no known issues with transport or ELISA kits. Insulin-like peptide-1 and GLP-1 samples were handled differently to those for other analytes at the Newcastle site. They were centrifuged for less time and at a lower g-force in order to produce platelet-rich plasma according to a standard operating procedure (see appendix F). There was only one centrifuge of the appropriate size available so the IGF-1/GLP-1 samples were kept on ice and processed after the other samples. Although this protocol was agreed in advance with the team at Swansea, the difference in on site processing seems the most likely reason for the disproportionate amount of missing data for IGF-1 and GLP-1. A future study into IGF-1 and GLP-1 in PD-CI should have sufficient resources available to allow immediate processing on site.

Table 21. Number of participants with missing data by variable

Variable	Control	PD	PD-CI	Total
VAS Hunger	0	1	3	4
VAS fullness	0	1	3	4
Duration of <i>ad libitum</i> meal	1	1	1	3
Acyl ghrelin	2	1	1	4
Total ghrelin	0	1	1	2
PYY	0	2	1	3
Leptin	0	1	2	3
Insulin	0	1	1	2
GLP-1	1	9	4	14
IGF-1	3	8	6	17
GH	0	1	1	2
Manual bite count	1	0	3	4
Automated bite count	0	1	3	4

7.4 Assessment tools

Three of the assessment tools used in this study were not validated for use in cognitive impairment.

7.4.1 Geriatric depression scale-15

The geriatric depression scale has not been validated for use in dementia or cognitive impairment. There are different versions of this screening test available. The 15 item GDS (GDS-15) was used in this study and is widely used in clinical practice (D'Ath et al., 1994) (See appendix G). The longer 30-item GDS-30 is a valid tool to screen for depression in people with all cause MCI but not for people with AD (Debruyne et al., 2009). The GDS-15 has been found to be sensitive but not specific in detecting depression in cognitively impaired older people, with sensitivity dropping as cognitive impairment advances (de Craen et al., 2003, Kørner et al., 2006). Our study included people with severe dementia, none of whom were felt to be depressed by their clinician or their carers but yet scored highly on the GDS-15. A more appropriate depression screening tool for further studies would be the Cornell Scale of Depression in Dementia, which has been well validated in people with dementia (Kørner et al., 2006).

7.4.2 Visual analogue scales

Visual analogue scales (appendix G) measuring hunger, fullness and other aspects of appetite have been well validated in young and older adults (Parker et al., 2004b, Flint et al., 2000) but not in those with cognitive impairment. There has, however, been research into the use of VAS to assess pain in those with cognitive impairment and dementia. A study of 129 people with severe

dementia demonstrated that VAS for the assessment of pain had good reliability. This study included people with PDD and DLB (Warden et al., 2003). The authors do note that those with the most severe dementia had more difficulty understanding VAS compared to verbal or pictorial scales. To date there are no verbal or pictorial scales available for the assessment of hunger or fullness. Our study demonstrated a positive correlation between hunger and energy intake and a negative correlation between fullness and energy intake across all 3 groups. This suggests that, for our study at least, VAS has provided a reasonable measure of appetite in those with cognitive impairment. It is difficult, however, to fully exclude bias introduced by the research nurse delivering the tool or by the carers supporting participants in completing the VAS. Validity may have been improved by simplifying the tool, for example removing parameters that were not used in the analysis (e.g. would you like to eat something salty?). Other strategies could include presenting one scale per page, using larger font or spacing out text whilst maintaining a 10cm line. It would be desirable to validate this simplified VAS, or develop and validate a verbal scale for appetite for ongoing research in this area.

7.4.3 Three-factor eating questionnaire

The TFEQ (see appendix G) (Stunkard and Messick, 1985) is a 51-item questionnaire which assesses three aspects of eating behaviour; restraint, disinhibition and hunger. It has been used in obesity research (Karlsson et al., 2000). It is useful as it detects “latent obesity”, in which people who have high levels of restraint eat more at a subsequent meal (Stunkard and Messick, 1985). It therefore allows latent obesity to be accounted for in the analysis of data obtained for energy intake. The TFEQ has not been used in cognitively impaired populations to date. It was written in 1985 in America. Our experience of using this scale in a cohort of older British adults was that many found it difficult to understand, even if they did not have cognitive impairment. It was also time consuming to administer, taking around 30 minutes for each participant. Moreover, the prevalence of latent obesity in our cohort was very low (2/55 participants [3.6%]). Overall, the measurement of eating behaviour does not appear important in this group and the use of TFEQ in future studies of appetite in PD-CI would not be recommended.

7.5 Medication

We attempted to control for potential levodopa effects by asking participants with PD and PD-CI to attend the test visit without having taken their usual Parkinson's medications. We also excluded participants on non-selective anticholinergic medications to reduce confounding of cognition and ghrelin secretion. Despite these measures, we were not able to eliminate medication effects as potential confounders to our appetite or hormone data. This was because a washout period of 36 hours for dopamine agonists and MAOBs, which has been used in other studies (Athauda et al., 2017), was felt to be too burdensome. Moreover, we felt that it was neither ethical nor desirable to discontinue all potentially confounding medications on the day of the test visit. Longer acting dopaminergic medications such as MAOIBs and several psychiatric medications such as cholinesterase inhibitors and antidepressants may have therefore affected our results. Our study was not powered to control for these factors. Future studies should be powered to control for medication use without requiring drug discontinuation if possible. This may improve recruitment as attending "off" is likely to have been burdensome for our participants. Alternatively, a longer period of discontinuation for longer acting dopaminergic drugs should be used.

7.6 Summary

Despite the limitations outlined here, we believe we devised a well-conducted cross-sectional pilot study, which has been able to address its primary objective. We have identified acyl-ghrelin as a potential biomarker for cognitive decline in PD. We have also achieved our secondary aim of exploring appetite and food intake in people with PD, PD-CI and controls. Recruitment was close to target and comprehensive hormonal profiling over time was possible. This was the first study of its kind and provides an excellent springboard for further research. The lessons learned here will be invaluable for the development of a longitudinal study into hormones of energy homeostasis as biomarkers for cognitive decline in PD. This will be discussed further in chapter 8.

Chapter 8. Summary, avenues for future research and conclusion

8.1 Overview

This study was a pilot study with the primary objective of determining whether hormones of energy homeostasis could be putative biomarkers for cognitive decline in PD. This hypothesis was based on the observation that several such hormones are pro-cognitive or neuroprotective in animal models of PD and also influence body weight (see Chapter 2: Introduction and Literature Review). Disruption in the regulation of these hormones could potentially link the known association between unintended weight loss and dementia in PD. The secondary objective was to examine appetite and food intake in patients with PD, PD-CI and controls as the causes of weight loss in PD-CI are as yet unclear. Our participants were tested after an overnight fast and “off” their usual PD medication. There were no differences in BMI, fat mass, gender, degree of comorbidity or age between groups. Geriatric depression scale, motor UPDRS and disease duration were all greater in the PD-CI group than the PD group.

8.2 Appetite and energy intake

8.2.1 Main findings

There is evidence that energy intake is increased in people with PD and continues to increase, despite declining BMI as disease progresses (Barichella et al., 2017). This appears to occur in the face of reduced physical activity and resting energy expenditure, a phenomenon which has been attributed to increased motor fluctuations in more advanced disease (Barichella et al., 2017). People with PD are at increased nutritional risk compared to healthy older adults (Barichella et al., 2013a) and this carries an attendant increase in mortality (Walker et al., 2012). Moreover, those with dementia are especially in danger of weight loss (Lorefalt et al., 2004, Uc et al., 2006). Conversely, people with PD who lose weight are more likely to develop cognitive decline. Prior to this study energy intake in people with PD-CI had not previously been studied. We did not observe disordered appetite or a difference in energy intake between people with PD, PD-CI and controls. The pattern of hunger was as expected across the whole cohort, with a reduction after eating and recovery at

3 hours. The opposite pattern was seen for fullness in all three groups. Hunger and fullness demonstrated the expected relationships with energy intake across the cohort, with both greater hunger and lower fullness associated with greater intake. Energy intake was not different between groups at the *ad libitum* meal. Across the whole cohort energy intake positively correlated with meal duration suggesting that bradykinesia did not negatively impact intake. Moreover, wrist-worn accelerometers called bite counters, which measure movements towards the mouth, correlated with energy intake across all three groups, suggesting minimal if any interference due to tremor. We conclude that appetite and energy intake are unchanged in this cohort of weight stable people with PD and PD-CI. Weight-losing people with PD may be phenotypically different to those who are weight stable. We are unable to draw any conclusions about that group. Further research into energy intake in people with PD-CI is therefore warranted.

8.2.2 Future research

Understanding the relationship between cognitive decline, weight loss and energy intake may allow tailored interventions to improve outcomes. A future study should include free-living people with PD, PD-CI and healthy controls. This should be a pragmatic, low burden study carried out mainly at home over several days. Calorie intake could be approximated using a calibrated wrist worn bite counter and food diary. Food diaries have previously been successfully used in people with cognitive impairment with interviewer support (Gale et al., 1996). The study should not exclude people with recent weight loss and should aim to weigh people some months after the initial test period to identify people who have subsequently lost weight. The study should measure the burden of non-motor symptoms, especially gastrointestinal symptoms. Medications should be taken into account in the design and data analysis plan as participants will not be able to be “off” during the study. This is because levodopa may exacerbate gastrointestinal symptoms in PD (Shaw et al., 1980), which could confound results. Moreover, cholinergic and anticholinergic drugs may affect appetite through modulation of ghrelin (Broglia et al., 2004). Levodopa equivalent dose (LED) and anticholinergic burden should therefore be calculated and included in analysis along with cholinesterase inhibitor use.

8.3 Hormones of energy homeostasis

8.3.1 Main findings

Ghrelin-IGF-1 axis

We found that AG AUC showed a trend towards being lower in the PD-CI group. Post-hoc testing revealed this difference to be significant between PD and PD-CI groups. Significance was lost when GDS and PD duration were included as covariates in the analysis. This may be due to a genuinely confounding relationship between depression and AG or to our study being underpowered. None of our cohort were clinically depressed despite scoring highly on the GDS, however. Moreover, both fasting AG and AG AUC positively correlated with cognition across the cohort (negative correlation with reversed MoCA). Finally, AG AUC significantly predicted cognition in multiple linear regression, accounting for 15% of the variance. Taken together these data suggest sufficient “signal” to warrant further investigation of AG AUC, fasting AG or AG:UAG as putative biomarkers for cognitive decline in PD.

We did not find evidence that total ghrelin or GH were different between groups or predicted cognition in our cohort. There was a trend towards lower IGF-1 in the PD-CI group. There was a significant difference between the PD and PD-CI groups on post-hoc analysis. Unfortunately, IGF-1 was undetectable in a number of samples for reasons which have not fully been elucidated but which may have been due to sample processing. As a result we were unable to draw any conclusions regarding IGF-1 as a potential biomarker for cognitive decline in PD.

Insulin and GLP-1

Neither insulin, nor insulin resistance measured by HOMA-IR were different between groups. There was, however, a negative correlation between disease severity and insulin across the cohort and insulin resistance decreased with decreasing insulin levels. There was no correlation with cognition in this cohort, however. Similarly, no difference in GLP-1 was seen between groups and there was no correlation between GLP-1 and cognition in our cohort. Our results provide further support for the ongoing investigation of insulin sensitizing agents as possible disease modifying drugs in PD. However, there is no evidence from this study that insulin is a potential biomarker for cognitive decline.

Leptin

Leptin levels were not different between groups and there was no correlation between leptin and cognition in our cohort. It appears unlikely that leptin could be useful as a biomarker for cognitive impairment in PD and future research should be focused elsewhere.

8.3.2 Avenues for future research

It is possible that fasting AG, AG AUC over 180 minutes post-prandially or AG:UAG could be biomarkers for cognitive decline in PD. A future longitudinal study should be designed to further explore this. Such a study would require baseline fasting AG and AG:UAG ratio to be measured at baseline in controls and people with incident PD who are cognitively intact. Although AG AUC is more likely to yield a positive result, measuring the AUC may be too time consuming and cumbersome to be a useful clinical biomarker for cognitive decline. Baseline fasting IGF-1 could be included on an exploratory basis, to see if there is a genuine relationship with cognition or not in PD. Participants with weight loss and diabetes should be included to improve generalisability and to explore whether people with PD who lose weight are metabolically different from those who are weight stable. All participants should undergo baseline measurement of potential confounders such as LED, cholinergic burden, depression and level of education. The study should be powered to allow inclusion of these potential confounders in the final analysis. Follow-up should be at a sufficient interval to detect cognitive changes. The Sydney multicenter study found 20% of people with incident PD developed dementia over 4 years

(Hely et al., 2008). The optimal length of follow up will therefore depend on the sample size. Participants should be weighed at follow up to allow identification of participants with weight-loss. Permission should be sought to review the medical notes of participants lost to follow up for new diagnoses of dementia as some attrition is inevitable (Chatfield et al., 2005). Acyl-ghrelin, AG:UAG ratio and IGF-1 could be incorporated into any new prospective observational longitudinal study in PD examining cognition. Unfortunately, though several such studies have been carried out or are underway (Yarnall et al., 2014, Malek et al., 2015), it is not possible to test samples for AG retrospectively due to the need to stabilize samples with AEBSF at the time of blood draw (Delhanty et al., 2015).

Another interesting avenue of research could be to investigate whether ghrelin receptor agonists could be used as treatment for cognitive impairment in PD. There is a current stage two randomised controlled trial being carried out into the use of a ghrelin receptor agonist for the management of gastroparesis in PD (NCT01955616). Unfortunately, the treatment period of 28 days is too short to expect to detect effects on cognition in their cognitively intact cohort of 18 patients to date. It is feasible that a further phase 2 study using the same medication could be carried out in people with PDD to see if there is any benefit to cognition acutely, or after a period of more prolonged follow up.

8.4 Bite counter

Wrist worn bite counters have been used to calculate energy intake in healthy adults in the community (Scisco et al., 2011, Salley et al., 2016, Scisco et al., 2014, Dong et al., 2012) but had not previously been used in PD. We demonstrated a positive correlation between bite count and energy intake across the whole cohort, including in people with PD and PD-CI. Future research could use this tool as an adjunct to food diaries to more accurately assess nutritional intake in people with Parkinson's disease. Bite counters could also be used in clinical practice to help people with PD who are losing weight to track their energy intake. This could allow them to increase intake if needed to ensure that they meet their energy requirements and stave off malnutrition.

8.5 Conclusions

We have demonstrated that AG may be a potential biomarker for cognitive impairment in PD. This hormone could provide the mechanistic link between weight loss and cognitive decline in PD. Further research is needed to clarify this relationship and to determine if AG will become a clinically useful tool or treatment in the management of people at risk of cognitive decline. We have also demonstrated that a wrist-worn bite counter accurately counts bites in people with PD and PD-CI. This device could be used to estimate energy intake in community dwelling people with PD and could be a useful clinical and research tool.

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Appendix A. Ethical Approval



Health Research Authority

NRES Committee North East - Newcastle & North Tyneside 1

TEDCO Business Centre

Room 002

Rolling Mill Road

Jarrow

NE32 3DT

Telephone:

0191 428 3565 4 March 2014

Professor David Burn
Professor of Movement Disorders Neurology/
Honorary Consultant Neurologist
Clinical Ageing Research Unit
Campus for Ageing and Vitality
Newcastle University
Newcastle upon Tyne
NE4 5PL

Dear Professor Burn

Study title:	Plasma acyl-ghrelin: A biomarker for cognitive decline in Parkinson's disease?
REC reference:	14/NE/0002
Protocol number:	6883
IRAS project ID:	141456

Thank you for your letter of 21 February 2014, responding to the Committee's request for further information on the above research and submitting revised documentation

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Ms Gillian Mayer, nrescommittee.yorkshireandhumber-bradfordleeds@nhs.net

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **Favourable** ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Mental Capacity Act 2005

I confirm that the Committee has approved this research project for the purposes of the Mental Capacity Act 2005. The Committee is satisfied that the requirements of section 31 of the Act will be met in relation to research carried out as part of this project on, or in relation to, a person who lacks capacity to consent to taking part in the project.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

No non-NHS sites have been noted in the application, however if you decide to include a non-NHS site in the future, a Site Specific Information form must be submitted for ethical approval.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering Letter	Dr Fionnuala Johnston	03 December 2013
GP/Consultant Information Sheets		
Investigator CV	Prof David Burn	05 November 2013
Letter from Sponsor	Wendy Mitson	26 November 2013
Letter from Statistician	Dr. Shirley Coleman	30 January 2014
Letter of invitation to participant	Invitation and Brief Info Sheet, 1.1	18 November 2013
Other: Student CV - Dr Fionnuala Johnston		05 November 2013
Other: Funder Letter	Zurich Municipal	26 July 2013
Participant Consent Form: Consultee Declaration Form	1.1	17 December 2013
Participant Consent Form: Carers	1.1	05 February 2014
Participant Consent Form: Participants	1.2 (Clean and Tracked Copies)	05 February 2014
Participant Information Sheet: Information for consultees	1.1	18 November 2013
Participant Information Sheet: Participants with Dementia	2.0	03 February 2014
Participant Information Sheet: Carers	1.0	05 February 2014
Participant Information Sheet: Health Volunteers	1.0	03 February 2014
Participant Information Sheet: Participants	1.2 (Clean and Tracked Copies)	06 February 2014
Protocol	1.1	22 November 2013
Questionnaire: IQ Code Questionnaire		
Questionnaire: TFEQ Questionnaire		
REC application	3.5	29 November 2013
Referees or other scientific critique report		06 November 2013
Response to Request for Further Information	Dr. Fionnuala Johnston	21 February 2014
Summary/Synopsis		03 December 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "*After ethical review – guidance for researchers*" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

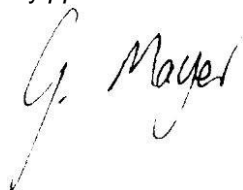
Further information is available at National Research Ethics Service website > After Review

14/NE/0002	Please quote this number on all correspondence
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We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

With the Committee's best wishes for the success of this project.

Yours
sincer
ely pp



Professor Philip Preshaw Chair

E-mail: nrescommittee.northeast-newcastleandnorthtyneside1@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Fionnuala Johnston - Clinical Ageing Research Unit, Newcastle
Newcastle University*

*Mr Andrew Johnston – Joint Research Office, Newcastle upon Tyne
Hospitals NHS Foundation Trust*

Appendix B. Patient information leaflets

Participant Information Sheet

“Ghrelin and Dementia in Parkinson’s disease”

Information for healthy volunteers

Date: 01.12.2014 Version: 3.0

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What is the purpose of the study?

Our study is looking into the differences between people with Parkinson's disease (PD), people with Parkinson's disease and memory problems and healthy older people (controls). PD causes stiffness, tremor, slow movements and balance difficulties. As well as these movement problems (motor symptoms) sufferers may develop memory problems, hallucinations or even dementia. Weight loss is also common in PD. People with PD who lose weight are more likely to develop memory problems than those who do not. The reasons behind this are not yet clear.

One possibility is that hormones (chemical messengers) which act on the brain to both make people hungry and help with memory don't work properly in some people with PD. One of these hormones is called ghrelin.

We would like to find out if people with Parkinson's disease and Parkinson's disease and memory problems have different ghrelin levels from each other and from healthy older people. We are interested in ghrelin because it has been shown to;

- Improve memory when given to mice that have too much amyloid β in their brains- a protein that is thought to be important in Parkinson's disease dementia.
- improve the ability of brain cells (neurones) to make connections with each other- a process needed for learning and to make memories
- Protect dopamine-containing neurones in mouse brains from poisons. These are the same cells damaged in Parkinson's disease.

This is the first time that a study has investigated the link between ghrelin, memory and weight loss. This is a small study designed to find out information to help us plan a bigger study in the future.

We would also like to find out if;

- people with low ghrelin feel fuller before eating, eat less food or choose different foods compared with people whose levels are normal
- people with PD, PDD and controls have different levels of hunger or food choices
- a “bite-counter” gives a good measure of how much people with Parkinson’s disease eat. This is worn like a wrist watch and counts the movements your arm makes to your mouth. It is completely safe and doesn’t cause any pain.

This will help us to understand the causes of Parkinson’s disease dementia better and may even lead to new medicines to treat PD and PDD in the future.

Why have I been chosen?

You have been invited to take part in this study because you are fit and well and aged 60-85.

This study is not suitable for people who;

- Have diabetes
- Smoke or chew tobacco
- Have recently lost or gained a lot of weight
- Have a serious illness
- Have severe depression

If you are not sure if any of these apply to you or if you have any questions please discuss it with us. Our contact details are at the end of this information sheet. We want to make sure you have all the information you need before deciding to take part.

Do I have to take part?

No. If you decide not to take part you do not need to give a reason. If you decide to take part but then change your mind you can stop anytime without giving a reason. Deciding not to take part will not affect any medical care you receive now or in the future.

What will happen to me if I take part?

If you decide you would like to take part we will arrange a screening visit at the Clinical Ageing Research Unit (CARU) at Newcastle University. Screening aims to make sure you are well and able to take part in testing. If you are you will be invited to a test visit at CARU.

Screening

This visit lasts about 2 hours. There are no special restrictions to your diet before this visit.

We will go through the study information with you and answer any questions you may have. If you are happy to go ahead we will ask you to sign a consent form. We will give you a copy to keep for your records.

You are under no obligation to take part and can withdraw from the study at any time and without giving a reason.

During the screening visit the doctor will ask you questions about your general health, previous illnesses, medications, smoking, alcohol intake and your home circumstances.

The team will measure your height, weight and blood pressure. The doctor will then examine you.

We will then measure how much body fat and muscle you have using “bioimpedance”. This is a pain free and harmless measurement of how electricity passes through the body. It is not recommended for people with pacemakers though so please let us know if you have one.

We will then carry out some paper based tests;

- A memory test which takes about 10 minutes to complete. We do not expect people to get full marks as it is deliberately quite difficult.
- A questionnaire to screen for depression.
- A questionnaire about your appetite which takes around 10 minutes.

During the visit we will take a blood sample to check your kidney, liver and thyroid function. We will also check for anaemia and diabetes.

We will ask you to provide a urine sample whilst you are with us which we will test for infection and blood.

Test Visit

This will last around 4 hours. For this visit you will need to come without having;

- Anything to eat for 12 hours before arriving at the research centre.
- Any sugary or milky drinks for 12 hours before your appointment. You can have plain water until 2 hours before the visit but nothing to drink after that.

The visit will involve blood tests, a “visual analogue scale” test to measure how full you feel, a test meal, a bite-counter and another meal called an “*ad libitum*” meal where a variety of foods will be on offer.

Blood tests

We will check active and inactive ghrelin levels along with a number of other hormones which may affect ghrelin results. These tests will be repeated during the morning. We will put an “intravenous cannula” into your vein. This means that we can take blood samples from this tube without having to put a needle in again. You will be able to move your arm without causing any harm. We will take about 200 mls of blood from you throughout the study (including screening and the test visit). This is about as much as a tea cup.

Visual Analogue Scale

This is a test of how hungry you are. It will look similar to this this;

	How hungry do	
I am not	you feel?	I have never
hungry at all	_____	been hungrier

We will ask you to mark the spot which best describes how you feel. This will be repeated at regular intervals throughout the morning.

Test meal (breakfast)

This will be a standard meal of around 300 calories and you will be required to eat all of the food. It will be given at around 9 am. It will include;

- One slice of white toast with butter
- One Activia Strawberry yoghurt
- Jam
- Short bread biscuit
- A glass of water

“Ad libitum” meal (lunch)

Ad libitum is Latin for “at your pleasure”. The *ad libitum* meal is designed to see how much you would like to eat and which foods. A choice of foods will be offered. You can eat as much or as little as you like. We will record how much you eat and which foods you choose. This meal will be at midday.

Bite counter

This is a wrist watch sized device which counts the movements your arm makes to your mouth. It is completely safe and doesn't cause any pain. It will be fitted for the test

meal and the *ad libitum* meal but can be taken off in between.

Telephone call

We will telephone you 7-10 days after the test visit to see if you have any questions after the study.

What will I have to do?

You will have to come to the test visit without having;

- Anything to eat for 12 hours before arriving at the research centre.
- Any sugary or milky drinks for 12 hours before your appointment. You can have plain water until 2 hours before the visit but nothing to drink after that.

During the test visit you will need to

- Sit down or lie in bed in between meals. This is because being active might affect our results. You will be able to get up to use the toilet.

Travel expenses:

We will arrange taxis to pick you up and take you home after each visit. If you would prefer to drive, or have a relative drive you, you will be able to use our car park for free. You will be able to claim back any travel expenses.

What are the possible disadvantages or risks of taking part?

Being involved in this study will not directly affect your health insurance but we may find a new health problem which could. You should consider this before deciding to take part. You may wish to seek advice from your insurer if you are unsure.

You will need to attend the test visit without having had anything to eat or drink that morning. Some people may find this uncomfortable.

The intravenous cannula that we take blood samples from can be uncomfortable when we put it in. You might get a bruise from this. This usually disappears after a few days.

What are the possible benefits of taking part?

The benefit of taking part is to help us to find out if the link between weight loss and Parkinson's disease and memory problems could be due to ghrelin. This might lead to new treatments in the long term. Taking part in this study will not help you, but might help people with Parkinson's disease in the future.

What if relevant new information becomes available?

Sometimes new scientific information may come to light during a study which will influence the way it is carried out. If this is the case the research team may change the study protocol. If a change is made you will be informed in writing and the new information will be discussed with you. No changes will be made once you have started the study.

What happens when the research study stops?

When the study has finished we will send you a summary of the results through the post.

What will happen if I don't want to carry on with the study?

You are under no obligation to take part and can withdraw from the study at any time and without giving a reason.

Will my taking part in the study be kept confidential?

Yes. If you decide to take part you will be given a unique code. The medical information we collect from you during this study will be entered into our computer system using this code. This anonymous information will be stored in our computer system for a minimum of 5 years.

We may find new information about your health during this study. If we do we will share it with you straight away. We may also write to your GP but only with your permission.

What if there is a problem?

If any problems come to light during this study, for example if we diagnose a new medical condition, we will tell you straight away.

Will my GP be told about the study?

With your permission, we will write to your GP and let them know you are taking part.

What will happen with any samples taken?

Your blood samples will be labelled with the same code as your other study information. They will not have any information on them which could identify you. The samples will be sent to Swansea for testing. This might be done either during the study or in the future. The samples will be stored for 6 months after the study.

What will happen with the results of the research study?

The results will be analysed by our research team at Newcastle University. We aim to distribute the results in scientific journals and at scientific meetings. Depending on the results we will use the study results to design a larger study looking at ghrelin levels in Parkinson's disease and how it relates to dementia.

Who is organising and funding the research?

This study is organised by Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. It is funded by the Newcastle NIHR Biomedical Research Unit in Lewy Body Dementia.

Who has reviewed the study?

This study has been reviewed and approved by the National Ethics Research Service.

Who should I contact if I have any questions?

If you would like to discuss this study further we would be happy to hear from you. We are available Monday to Friday 9am-5pm.

Study Doctor

Dr. Fionnuala Johnston. Tel; 0191 208 1278/1250

Study Nurse

.....Tel;.....
.....

Participant Information Sheet

Ghrelin and Dementia in Parkinson's disease

Date 18.11.2014 Version 3.0

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What is the purpose of the study?

Parkinson's disease (PD) is a complex illness causing stiffness, tremor, slow movements and balance problems. As well as these movement problems (motor symptoms) sufferers may develop problems such as memory problems, hallucinations or even dementia. Weight loss is also common in PD. People with PD who lose weight are more likely to develop problems with their memory than those who do not. The reasons behind this are not yet clear.

One possibility is that hormones (chemical messengers) which normally act on the brain to both make people hungry and help with memory don't work properly in some people with PD. One of these hormones is called ghrelin. There is evidence that ghrelin doesn't increase before eating as much in people with PD as it does in other people. We are interested in ghrelin because it has been shown to;

- Improve memory when given to mice that have too much amyloid β in their brains- a protein that is thought to be important in Parkinson's disease dementia.
- improve the ability of brain cells (neurones) in the memory centre of the brain (hippocampus) to make connections with each other- a process needed for learning and to make memories
- Protect dopamine-containing neurones in mouse brains from poisons. These are the same cells damaged in Parkinson's disease.

So far no studies have compared ghrelin levels in people with Parkinson's who have memory problems, PD with normal memory and healthy people. This study is designed to find out if ghrelin levels are low in people with memory problems in Parkinson's. It is a pilot study which means it is a small study designed to find out information to help us plan a bigger study in the future.

We would like to find out if;

- ghrelin levels are lower in people with Parkinson's disease and memory problems compared to people with PD who don't have memory problems and healthy people of similar ages (controls)
- people with low ghrelin feel fuller before eating, eat less food or choose different foods compared with people whose levels are normal

- people with Parkinson's disease with memory problems, without memory problems and controls have different levels of hunger, food intake or food choices
- A "bite-counter" gives a good measure of how much people with Parkinson's disease eat. This is worn like a wrist watch. It counts the movements your arm makes to your mouth. It doesn't cause any pain.

This will help us to understand memory problems in Parkinson's disease better and may even lead to new medicines to treat Parkinson's and Parkinson's disease dementia in the future.

Why have I been chosen?

You have been invited to take part in this study because you are aged 60-85 and have Parkinson's disease (PD) without memory problems.

Not everyone can take part in this study. Appetite, chemical messenger (hormone) levels, weight change and even smoking could affect our results and make them difficult to interpret. This study is not suitable for people who;

- Have diabetes
- Smoke or chew tobacco
- Have recently lost or gained a lot of weight
- Have another serious illness (other than PD)
- Have severe depression
- Have deep brain stimulation for Parkinson's disease

If you are not sure if any of these apply to you or if you have any questions please discuss it with us. Our contact details are at the end of this information sheet. We want to make sure you have all the information you need before deciding to take part.

Do I have to take part?

It is up to you whether or not you take part in this study. If you decide not to take part you do not need to give a reason. If you decide to take part but then change your mind you can stop anytime without giving a reason. Deciding not to take part will not affect the care you receive now or in the future.

What will happen to me if I take part?

If you decide you would like to take part we will arrange a screening visit at the Clinical Ageing Research Unit (CARU) at Newcastle University. Screening aims to make sure you are well and able to take part in testing. If you are you will be invited to a test visit at CARU.

Screening

This visit lasts about 2 hours. There are no special restrictions to your diet or medicines before this visit.

We will go through the study information with you and answer any questions you may have. If you are happy to go ahead we will ask you to sign a consent form. We will give you a copy to keep for your records.

You are under no obligation to take part and can withdraw from the study at any time and without giving a reason.

During the screening visit you will have a medical history, a physical examination, measurement of your body fat, paper-based tests, urine and blood tests.

Medical history

This will involve asking questions about;

- your general health and previous illnesses
- If you have Parkinson's disease, how long you have had it
- which medications you are taking
- whether or not you have any allergies
- Smoking and alcohol
- What kind of place you live in (is it your own home or a residential home for example) and who lives with you

Physical Examination

This will be similar to examinations you may have had from your doctor in the past but is likely to be more detailed. It will involve;

- Measuring your height, weight and blood pressure
- A neurological examination carried out by the research doctor. They will test;
 - The movements and feeling in your face, arms and legs

- Your eye movements.
 - The reflexes in your arms and legs
 - The coordination of your arms and legs
 - For stiffness in your arms and legs
- If you have Parkinson's disease, the "Movement Disorder Society United Parkinson's Disease Rating Scale Part 3" (MDS-UPDRS part III). This is a precise rating scale which
 - Rates how Parkinson's disease affects your movements
 - takes 5-10 minutes to do
 - Checks for slowness, problems with walking, balance stiffness, tremor and speech.
- Examination of your heart, lungs and abdomen. The doctor will;
 - listen to your heart, lungs and tummy (abdomen) with a stethoscope
 - Feel your neck, underarms, groins and abdomen for any lumps
 - Look for any leg swelling

Measurement of body fat

This is done by passing a very small electrical current through you and recording the time it takes to flow through your body. This is done either through attaching wires to your body with stickers or by standing on a special set of scales. The current used is harmless and cannot be felt so will not cause any pain. It is not recommended for people with pacemakers though so please let us know if you have one. Electricity moves more slowly through fat than muscle so we can calculate how much body fat you have by how quickly the electricity travels.

Paper-based tests and questionnaires

These include;

- Montreal Cognitive Assessment (MoCA). This is a memory test which takes about 10 minutes to complete. The study doctor will go through it with you. We do not expect people to get full marks as it is deliberately quite difficult.
- 15 item Geriatric Depression Scale (GDS 15). This asks 15 questions related to low mood and can be used to screen for depression.
- Three-Factor Eating Questionnaire. This asks questions about your appetite and takes around 10 minutes.

Blood tests and urine sample

We will take a blood sample to check your kidney, liver and thyroid function. We will also check for anaemia and diabetes.

We will ask you to provide a urine sample whilst you are with us which we will test for infection and blood.

Test Visit

This will last around 4 hours. For this visit you will need to come without having;

- Anything to eat or drink except plain water for 12 hours before.
- Any drinks for 2 hours before.
- Your usual Parkinson's disease medication the morning of the visit. You can take your other medicines as usual.

The visit will involve blood tests, a "visual analogue scale" test to measure how full you feel, a test meal, a bite-counter and another meal called an "*ad libitum*" meal where a variety of foods will be on offer.

You will be invited to take your Parkinson's disease medicines at around midday.

Blood tests

We will check active and inactive ghrelin levels along with a number of other hormones which may affect ghrelin results. These tests will be repeated at regular intervals throughout the morning. We will put an "intravenous cannula" into your vein. This means that we can take blood samples from this tube without having to put a needle in again. An intravenous cannula is a narrow plastic tube with a needle inside it. These are commonly used in hospital to give people fluids and medicines into the vein. Once it is in your vein the needle is taken out and only the soft plastic tube stays behind. You will be able to move your arm without causing any harm. We will take about 200 mls of blood from you throughout the study (including screening and the test visit). This is about as much as a tea cup.

Visual Analogue Scale

This is a test of how hungry you are. It will look similar to this this;

	How hungry do	
I am not	you feel?	I have never
hungry at all	_____	been hungrier

We will ask you to mark the spot which best describes how you feel. This will be repeated at regular intervals throughout the morning.

Test meal

This will be a standard meal of around 300 calories. It will be given at around 9 am. It will include;

- One slice of white toast with butter
- One Activia Strawberry yoghurt
- Jam
- Short bread biscuit
- A glass of water

“Ad libitum” meal

Ad libitum is Latin for “at your pleasure”. The *ad libitum* meal is designed to see how much you would like to eat and which foods. A choice of foods will be offered. You can eat as much or as little as you like. We will record how much you eat and which foods you choose. This meal will be at midday. You will be able to take your Parkinson’s tablets with this meal.

Bite counter

This is a wrist watch sized device which counts the movements your arm makes to your mouth. It doesn’t cause any pain. It will be fitted for the test meal and the *ad libitum* meal but can be taken off in between.

Telephone call

We will telephone you 7-10 days after the test visit to see if you have any questions after the study.

What will I have to do?

You will have to come to the test visit without having;

- Anything to eat or drink except plain water for 12 hours before.
- Your usual Parkinson's disease medication the morning of the visit. You can take your other medicines as usual.

During the test visit you will need to

- Delay your Parkinson's medications until around midday. This is because they could affect our results.
- Sit down or lie in bed in between meals. This is because being active might affect our results. You will be able to get up to use the toilet.

Travel expenses:

We will arrange taxis to pick you up and take you home after each visit. If you would prefer to drive, or have a relative drive you, you will be able to use our car park for free. You will be able to claim back any travel expenses.

What are the possible disadvantages or risks of taking part?

Being involved in this study will not directly affect your health insurance but we may find a new health problem which could. You should consider this before deciding to take part. You may wish to seek advice from your insurer if you are unsure.

You will need to attend the test visit without having had anything to eat or drink prior and without having your usual Parkinson's disease medication. Some people may find this uncomfortable.

Some people might find it upsetting to talk about memory problems. We are very used to looking after people with Parkinson's disease with and without dementia and will be there to answer any questions you may have.

The intravenous cannula that we take blood samples from can be uncomfortable when we put it in. You might get a bruise from this.

What are the possible benefits of taking part?

The benefit of taking part is to help us to find out if the link between weight loss and Parkinson's disease dementia (PDD) could be due to ghrelin. This might lead to new treatments in the long term. Taking part in this study may not help you, but might help people in the future.

What if relevant new information becomes available?

Sometimes new information may come to light during a study which will influence the way it is carried out. If this is the case the research team may change the study protocol. If a change is made you will be informed in writing and the new information will be discussed with you. No changes will be made once you have started the study.

What happens when the research study stops?

When the study has finished we will send you a summary of the results through the post.

What will happen if I don't want to carry on with the study?

You are under no obligation to take part and can withdraw from the study at any time and without giving a reason. This will not affect your care now or in the future.

Will my taking part in the study be kept confidential?

Yes. If you decide to take part you will be given a unique code. The medical information we collect from you during this study will be entered into our computer system using this code. We will not record any information that could identify you such as your name, address or date of birth. In other words it will be anonymous. This anonymous information will be stored in our computer system for a minimum of 5 years.

We may find new information about your health during this study. If we do we will share it with you straight away. We may also write to your GP but only with your permission.

What if there is a problem?

Other than a slight delay in taking your tablets there will be no changes to your usual care. If any problems come to light during this study, for example if we diagnose a new medical condition, we will tell you straight away.

Will my GP be told about the study?

With your permission, we will write to your GP and let them know you are taking part.

What will happen with any samples taken?

Your blood samples will be labelled with the same code as your other study information. They will not have any information on them which could identify you. The samples will be sent to Swansea for testing. This might be done either during the study or in the future. The samples will be stored for 6 months.

What will happen with the results of the research study?

The results will be analysed by our research team at Newcastle University. We aim to distribute the results in scientific journals and at scientific meetings. Depending on the results we will use the study results to design a larger study looking at ghrelin levels in Parkinson’s disease and how it relates to dementia.

Who is organising and funding the research?

This study is organised by Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. It is funded by the Newcastle NIHR Biomedical Research Unit in Lewy Body Dementia.

Who has reviewed the study?

This study has been reviewed and approved by the National Ethics Research Service.

Who should I contact if I have any questions?

If you would like to discuss this study further we would be happy to hear from you. We are available Monday to Friday 9am-5pm.

Study Doctor

Dr. Fionnuala Johnston. Tel; 0191 208 1278/1250

Study Nurse

.....Tel;.....
.....

Participant Information Sheet

Ghrelin and Dementia in Parkinson's disease

For Participants with Memory Problems

Date: 01.12.2014 Version: 4.0

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What is the purpose of the study?

Parkinson's disease is a complex illness causing stiffness, shaking, slow movements and balance problems. Patients may also develop memory problems. Patients with Parkinson's disease who lose weight are more likely to develop problems with their memory than those who do not. We don't know why this is. It may be because chemical messengers that work on the brain to make people hungry and help with memory aren't working properly. One of these chemical messengers is called ghrelin.

We would like to find out if;

- ghrelin levels are low in people with Parkinson's disease and memory problems
- people with low ghrelin eat less food or choose different foods
- people with Parkinson's disease and memory problems eat less food or choose different foods
- A "bite-counter" gives a good measure of how much people eat. This is worn like a wrist watch and counts the bites you take when you eat.
- This is a small study designed to find out information to help us plan a bigger study in the future. We hope that our study will help us to understand Parkinson's disease and memory problems better and may even lead to new medicines to treat memory problems in the future.



CARU Newcastle

Why have I been chosen?

You have been invited to take part in this study because you are aged 60-85 years and have Parkinson's disease and memory problems.

This study is not suitable for people with diabetes, serious illness or severe depression. It is also not suitable for people who smoke, have recently lost weight or who have deep brain stimulation.

If you are not sure if you have any of these please talk with us. We want to make sure you have all the information you need before deciding to take part.

Do I have to take part?

No. It is up to you whether or not you take part in this study. If you decide not to you do not need to give a reason. If you decide to take part but then change your mind you can stop anytime without giving a reason. Deciding not to take part will not affect the care you receive now or in the future.

What will happen to me if I take part?

If you decide you would like to take part we will arrange to see you at the Clinical Ageing Research Unit (CARU) at Newcastle University to make sure you are well and able to take part. This is called screening. If you are able to take part after screening you will be invited to a test visit at CARU. We ask you to bring a friend, relative or carer with you to both visits.



CARU inside

Screening

The screening visit lasts about 2 hours. You can eat and drink and take all of your medicines before this appointment.

We will go through the study with you and answer any questions you may have. If you are happy to take part we will ask you to sign a consent form. We will give you a copy to keep.

The doctor will ask you questions about your health and medicines, whether you smoke or drink alcohol, and your home and who lives with you.

We will measure your height, weight and blood pressure and the doctor will examine you.

We will then measure the amount of body fat you have. The machine we use to do this is painless and harmless. It can affect pacemakers so please let us know if you have one.

After this you will be asked to complete some questionnaires about your mood and appetite. These will take about 15 minutes in total.

We will then do a memory test which will take 10 minutes. We do not expect people to get full marks as it is quite difficult.

During the screening visit we will take a blood sample to check your general health. We will also ask you for a urine sample which we will test for infection and blood.

If you are able to take part after screening we will invite you for a test visit.

Test Visit

This will last around 4 hours. For this visit you will need to come without having;

- Anything to eat for 12 hours before arriving at the research centre.
- Any sugary or milky drinks for 12 hours before your appointment. You can have plain water until 2 hours before the visit but nothing to drink after that.
- Your Parkinson's disease medication the morning of the visit. You can take your other medicines as usual.

You will be able to take your Parkinson's disease medicines at around midday.



any harm once it is in.

We will take blood test to several times during the morning. We will put an “intravenous cannula” into your vein so that we can take lots of blood samples without putting a needle in again. You will be able to move your arm without causing

We will then fit a bite counter. This looks and feels like a wrist watch. It is completely safe and doesn't cause any pain. It will be fitted for breakfast and lunch but can be taken off in between.

Before breakfast we will ask you to fill in a short questionnaire about how hungry you feel. This will be repeated several times during the morning.

Breakfast

We will give you a breakfast of toast with butter and jam, a strawberry yoghurt, shortbread biscuit and a glass of water at around 9 am. You will need to eat all of this.

Lunch

Lunch will be at around midday. You will be able to eat as much or as little as you like. We want to see how much you eat and which foods you choose. You will be able to take your Parkinson's tablets with this meal.

Telephone call

We will telephone you 7-10 days after the test visit to see if you have any questions.

What will I have to do?

You will have to come to the test visit without having;

- Anything to eat for 12 hours before arriving at the research centre.
- Any sugary or milky drinks for 12 hours before your appointment. You can have plain water until 2 hours before the visit but nothing to drink after that.
- Your Parkinson's disease medication the morning of the visit. You can take your other medicines as usual.

During the test visit you will need to

- Delay your Parkinson's medications until around midday
- Sit down or lie in bed in between meals. You will be able to get up to use the toilet.

Some patients with Parkinson's and memory problems may have trouble remembering everything they need to decide whether taking part is right for them. If this is the case for you we will go through the information with a friend or relative who can help you decide.

Travel expenses

We will arrange taxis to pick you up and take you home after each visit. If you would prefer to drive, or have a relative drive you, you will be able to use our car park for free. You will be able to claim back any travel expenses.

What are the possible disadvantages or risks of taking part?

Being in this study will not affect your health insurance but we may find a new health problem which could. You should think about this before deciding to take part. You may wish to talk to your insurer, carer or relative if you are not sure.

You will need to come for the test visit without having had anything to eat or drink beforehand and without having your usual Parkinson's disease medicines. Some people may find this uncomfortable.

Some people might find it upsetting to talk about memory problems. We are very used to looking after people with Parkinson's and memory problems and will be there to answer any questions you may have.

Blood tests are sometimes uncomfortable. You might get a bruise from this. These usually disappear within a few days

What are the possible benefits of taking part?

The benefit of taking part is to help us to understand if ghrelin is linked to weight loss in Parkinson's and memory problems. Taking part in this study may not help you, but might help people in the future.

What if relevant new information becomes available?

Sometimes we find out things during a study which change the way it is carried out. If a change is made we will tell you about it. No changes will be made once you have started the study.

What happens when the research study stops?

When the study has finished we will send you a summary of the results through the post.

What will happen if I don't want to carry on with the study?

You don't have to take part and can stop the study any time without giving a reason. This will not affect your care now or in the future.

Will my taking part in the study be kept confidential?

Yes. If you decide to take part you will be given a unique code. We will record the information we collect from you in a computer system using this code. We will not record any information that could identify you such as your name, address or date of birth. The information will be stored in our computer system for a minimum of 5 years.

We may find new information about your health during this study. If we do we will tell you straight away. We may also write to your GP but only with your permission.

What if there is a problem?

Other than a slight delay in taking your tablets there will be no changes to your usual care. If any problems come to light during this study, for example if we diagnose a new medical condition, we will tell you straight away.

Will my GP be told about the study?

With your permission, we will write to your GP and let them know you are taking part.

What will happen with any samples taken?

Your blood samples will be labelled with the same code as your other study information. They will not have any information on them which could identify you. The samples will be sent to Swansea for testing. This might be done either during the study or in the future. The samples will be stored for 6 months after the study.

What will happen with the results of the research study?

The results will be analysed at Newcastle University. We want to present the results in scientific journals and at meetings. We want to use this study to design a larger study looking at ghrelin levels in Parkinson's and memory problems

Who is organising and funding the research?

This study is organised by Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. It is funded by the Newcastle NIHR Biomedical Research Unit in Lewy Body Dementia.

Who has reviewed the study?

This study has been reviewed and approved by the National Ethics Research Service.

Who should I contact if I have any questions?

Please telephone us if you would like to ask any questions. We are available Monday to Friday 9am-5pm and would be happy to hear from you.

Study Doctor; Dr. Fionnuala Johnston: 0191 208 1278/1250

Study Nurse;

.....
.....

Carer Information Sheet

“Ghrelin and Dementia in Parkinson’s disease”

Date; 01.12.2014 Version; 3.0

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What is the purpose of the study?

Our study is looking into the differences between healthy older people (controls), people with Parkinson's disease (PD) and people with Parkinson's disease and memory problems. Dementia is common in PD but not all sufferers develop memory problems. Those who lose weight are more likely to develop memory problems than others. The reasons behind this weight loss are not yet clear.

One possibility is that hormones (chemical messengers) which act on the brain to both make people hungry and help with memory don't work properly in some people with PD. One of these hormones is called ghrelin.

We would like to find out if healthy older people have different ghrelin levels in their blood compared with people with Parkinson's disease with normal memory and Parkinson's disease and memory problems. We are interested in ghrelin because it has been shown to;

- Improve memory when given to mice that have too much amyloid β in their brains- a protein that is thought to be important in Parkinson's disease dementia.
- improve the ability of brain cells (neurones) to make connections with each other- a process needed for learning and to make memories
- Protect dopamine-containing neurones in mouse brains from poisons. These are the same cells damaged in Parkinson's disease.

This is a pilot study which means it is a small study designed to find out information to help us plan a bigger study in the future.

We would also like to find out if;

- people with low ghrelin levels feel fuller before eating, eat less food or choose different foods compared with people whose levels are normal
- healthy older people, people with PD and PDD have different levels of hunger or food choices
- a "bite-counter" gives a good measure of how much people eat. This is worn like a wrist watch and counts the movements your arm makes to your mouth when you eat.

Why have I been chosen?

You have been chosen because you are a friend, relative or carer of someone with Parkinson's disease and memory problems and they have agreed to take part in this study.

Do I have to take part?

No. If you decide not to take part you do not need to give a reason. If you decide to take part but then change your mind you can stop anytime without giving a reason. Deciding not to take part will not affect the medical care of the participant now or in the future.

What will happen to me if I take part?

We would be very grateful if you could accompany your friend/relative to two study visits. These will take place at the Clinical Ageing Research Unit (CARU) at Newcastle University. This is to make the visits as comfortable as possible for participants with dementia. It also helps us to have someone on hand who knows the participant if they become disorientated or need reassurance. You will not be asked to provide any care whilst they are at CARU.

The first visit is called a screening visit and lasts about two hours.

The second visit is called a test visit. This will last around 4 hours. Your friend or relative will be asked to come without having had anything to eat for 12 hours before the visit. We also ask that they don't have any sugary or milky drinks for 12 hours before the visit. They can drink plain water until 2 hours beforehand. They will be given breakfast and lunch with us and will have their medication at lunchtime. We will be able to provide you with tea and coffee and a light lunch.

What will I have to do?

We will ask you to join your friend or relative for visits at CARU. You will not need to provide any care whilst you are at CARU.

Travel expenses:

We will arrange taxis to pick you up and take you home after each visit. If you would prefer to drive, you will be able to use our car park for free. You will be able to claim back any travel expenses.

What are the possible disadvantages or risks of taking part?

There are no major disadvantages or risks in taking part in this study. In the event of something unexpected happening there will be someone available for advice at all times. We will also be happy to answer any questions you might have.

What are the possible benefits of taking part?

The benefit of taking part is to provide reassurance for your friend/relative and to make their visit more comfortable. Taking part in this study will not help you, but might help people with Parkinson's disease in the future.

What if relevant new information becomes available?

Sometimes new scientific information may come to light during a study which will influence the way it is carried out. If a change is made you will be informed in writing and the new information will be discussed with you. No changes will be made once you have started the study.

What happens when the research study stops?

When the study has finished we will send you a summary of the results through the post.

What will happen if I don't want to carry on with the study?

You are under no obligation to take part and can withdraw from the study at any time and without giving a reason.

Will my taking part in the study be kept confidential?

Yes. We will store a copy of your consent form in the participant's confidential medical notes and in a confidential site file. These are kept securely and access to them is restricted.

What if there is a problem?

We hope that problems will be rare. If unexpected issues do arise a member of staff will always be available during test visits. We are also very happy to be contacted by telephone to offer advice during the study.

What will happen with the results of the research study?

The results will be analysed by our research team at Newcastle University. We aim to distribute the results in scientific journals and at scientific meetings. Depending on the results we will use the study results to design a larger study looking at ghrelin levels in Parkinson's disease and how it relates to dementia.

Who is organising and funding the research?

This study is organised by Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. It is funded by the Newcastle NIHR Biomedical Research Unit in Lewy Body Dementia.

Who has reviewed the study?

This study has been reviewed and approved by the National Ethics Research Service.

Who should I contact if I have any questions?

If you would like to discuss this study further we would be happy to hear from you. We are available Monday to Friday 9am-5pm.

Study Doctor

Dr. Fionnuala Johnston. Tel; 0191 208 1278/1250

Study Nurse

.....Tel;.....
.....

Appendix C. Consent and consultee forms

Centre Number:

Study Number:

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: **Ghrelin and Dementia in Parkinson's Disease**

Name of Researcher: **Prof. David Burn**

Please initial
all boxes

1. I confirm that I have read and understand the information sheet dated (version) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from Newcastle University, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
4. I agree to my GP being informed of my participation in the study.

5. I agree to take part in the above study.

Name of Participant

Date

Signature

Name of Person

Date

Signature

taking consent.

Information for Consultees Ghrelin and Dementia in Parkinson's Disease

Your friend/relative has been invited to take part in a research study. They have given us permission to involve you, as someone they trust, to help them decide whether or not to take part. Some people with dementia can find it difficult to remember all of the information that they need to decide whether a study is right for them. This may not be the case for your friend/relative but, if it is, we would be grateful if you could act as a spokesperson for them. This would mean giving permission on their behalf if you think that;

- taking part in this study is something is in keeping with your friend/relative's previous wishes
- taking part will not be too difficult for them

You are under no obligation to take on this responsibility.

If you would like to act as a spokesperson or "consultee" please read the Participant Information Leaflet included to see if you think this study might be suitable for your friend or relative.

Taking part in research is not for everybody. **If you feel that this study will not suit your friend/ relative then please say NO on their behalf.** This will not affect the care they receive in any way.

If you or your friend/ relative change your mind about participating during the study you can withdraw at any time, without giving a reason and without affecting their care.

The research team will also stop the study if your friend/ relative appears distressed or appears to object during the study.

If you have any questions please don't hesitate to contact us. We are available Monday to Friday 9am-5pm.

Study Doctor

Dr. Fionnuala Johnston. Tel; 0191 208 1297/1250

Study Nurse

..... Tel;

Date of issue: 18.11.2013
Version number: Version 1.1
Author Dr. Fionnuala Johnston

Appendix D. Power calculations

E-mail from Dr. Shirley Coleman

“Dear Fionnuala,

I have written a response below for the ethics committee. I hope this is OK. Any queries, please email me.

For Dr Fionnuala Johnston

Statistical aspects of proposal entitled Plasma acyl-ghrelin: A biomarker for cognitive decline in Parkinson’s disease?

The data analysis involves comparing the primary outcome of interest, fasting AG and UAG levels between PD, PDD and control groups.

A secondary outcome measure is the bite rate (frequency and count) and ad libitum food intake in PDD, PD and controls and there will also be a comparison between the bite rate measured manually and using a bite counter.

Sample sizes can be calculated using the mean ghrelin and standard deviation values found during previous studies (Unger et al., 2011).

In Unger et al, 2011, the largest observed group mean differences were for the increase in ghrelin serum concentrations in the late postprandial phase calculated by the ratio of individual ghrelin serum concentrations at 300 minutes divided by the individual ghrelin serum concentrations at 60 minutes. The difference between controls and the combined PD group was 0.21 with a pooled standard deviation of approximately 0.22.

The sample size in the proposed study needs to be large enough to detect a difference of this size at the 5% significance level with 80% power. Using standard methods, the sample size needs to be at least 19 in each of the control and PD groups to detect a difference of this size using t-tests. A sample size of at least 20 in each group is recommended. Assuming that the PDD group will be further from the control than the PD group, a sample size of 20 for the PDD group as well should be satisfactory to ensure that the difference between the largest and smallest values of the 3 groups will be detected as

significant at the 5% significance level with 80% power in an analysis of variance (ANOVA).

As indicated in the proposal the analysis will involve comparing demographic and clinical data between all 3 groups. The mean ghrelin plasma concentrations will be plotted over time for each group and the area under the curves will be compared between groups. Data for all groups will be controlled for relevant covariates such as BMI, age and gender using analysis of covariance (ANCOVA). Bonferroni correction will be used where multiple tests are carried out to ensure statistical significance.

Statistical support will be available throughout the study. A sample size of 20 in each of the control, PD and PDD groups is appropriate for the study. With this sample size, the study should be satisfactorily powered for the statistical analysis proposed.

With kind regards,

Shirley Coleman,

Principal Statistician,

ISRU, Newcastle University

www.isru.ncl.ac.uk

Appendix E. Standardised presentation of the *ad libitum* meal



Appendix F. Blood processing procedure

Blood collection protocol for subsequent ELISA quantification of hormone levels in human plasma

Hormones to be measured by ELISA include total ghrelin, acyl-ghrelin, PYY leptin, insulin and IGF-1.

The active ghrelin molecule is extremely unstable in plasma and should be rigorously protected during blood sample collection. For maximum protection, addition of AEBSF* should be performed on plasma samples that undergo total ghrelin, acyl-ghrelin and PYY ELISA. All samples should be processed as quickly as possible and kept on ice.

Preparation and storage of AEBSF

Product; AEBSF [4-(2-Aminoethyl)-benzenesulfonyl fluoride] (Cat no. A8456 Sigma), 500 mg vial.

1. Keep the bottle of lyophilised AEBSF (A8456-500mg) desiccated at -20°C (should be stable for ~3 years).
2. When ready to use, re-constitute AEBSF in 10mls sterile water. This will give you a clear colourless stock solution that is 50mg/ml (200mM). This stock solution will be stable for ~6 months if kept refrigerated (4°C).
3. 80ul of AEBSF stock solution (50mg/ml, or 200mM) should be in 2ml of blood (to give a final concentration of 2mg/ml, or 8mM)*

Sample preparation

1. Prepare microfuge tubes for AG/UAG samples by pipetting 80ul of 50mg/ml AEBSF stock solution into a 2ml microfuge tube for each time point (7 samples). Store in the fridge.
2. Make sure pre-treated microfuge tubes are clearly labelled.
3. Collect whole blood into Vacutainer® K2EDTA-plasma tubes (#VT-6450; can collect up to 7 ml blood/tube).
4. Gently rock the Lavender Vacutainer tubes several times immediately after collection of blood for anti-coagulation.
5. Place on ice and take to the lab immediately.
6. Immediately transfer 2 mls of the blood from the lavender vacutainer tubes to cold sterile centrifuge tubes containing AEBSF and gently rock several times to inhibit the activity of proteinases. (This fraction will be used for total ghrelin, acyl-ghrelin and PYY ELISA)
7. Pipette 2mls each into to untreated microfuge tubes. One of these will be used for leptin and insulin; the other will be used for IGF-1 and GH. Make sure they are clearly labelled.
8. Put the IGF-1 sample on ice.
9. Promptly centrifuge the AESBF sample and the leptin/insulin sample together at $2,000 \times g$ for 15 minutes at $4 \pm 2^{\circ}\text{C}$. This will result in the formation of three layers. First, the uppermost plasma layer (yellow) (should be free of signs of hemolysis and lipemia). Second, a very thin white layer containing leukocytes and platelets (white). Finally the bottom layer containing red blood cells (red).
10. Carefully transfer the supernatant (i.e uppermost layer of plasma) into separate sterile cryovial tubes (400ul aliquots).
11. Centrifuge the IGF-1 samples at $1,000 \times g$ for 10 mins
12. Date and identify each sample. Clearly label samples that underwent AEBSF pre-treatment).
13. Store samples at -80°C for later use.

Note that blood collection time points are baseline, 5, 15, 30, 60, 120 and 180 minutes; making immediate processing of all samples difficult. Therefore IGF-1 samples can be left on ice until 75 minutes to allow time for AG/UAG/PYY and leptin/insulin samples to be processed in a timely fashion.

Appendix G. Questionnaires

Geriatric Depression Scale -15 (GDS) Scoring Instructions

Instructions: Score 1 point for each bolded answer. A score of 5 or more suggests depression.

- | | | |
|---|------------|-----------|
| 1. Are you basically satisfied with your life? | yes | no |
| 2. Have you dropped many of your activities and interests? | yes | no |
| 3. Do you feel that your life is empty? | yes | no |
| 4. Do you often get bored? | yes | no |
| 5. Are you in good spirits most of the time? | yes | no |
| 6. Are you afraid that something bad is going to happen to you? | yes | no |
| 7. Do you feel happy most of the time? | yes | no |
| 8. Do you often feel helpless? | yes | no |
| 9. Do you prefer to stay at home, rather than going out and doing things? | yes | no |
| 10. Do you feel that you have more problems with memory than most? | yes | no |
| 11. Do you think it is wonderful to be alive now? | yes | no |
| 12. Do you feel worthless the way you are now? | yes | no |
| 13. Do you feel full of energy? | yes | no |
| 14. Do you feel that your situation is hopeless? | yes | no |
| 15. Do you think that most people are better off than you are? | yes | no |

A score of ≥ 5 suggests depression

Total Score

Ref. Yes average: The use of Rating Depression Series in the Elderly, in Poon(ed.): Clinical Memory Assessment of Older Adults, American Psychological Association, 1986

Three Factor Eating Questionnaire

1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
2. I usually eat too much at social occasions, like parties and picnics.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
3. I am usually so hungry that I eat more than three times a day.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
4. When I have eaten my quota of calories, I am usually good about not eating anymore.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
5. Dieting is so hard for me because I just get too hungry.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
6. I deliberately take small helpings as a means of controlling my weight.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
9. When I feel anxious, I find myself eating.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
10. Life is too short to worry about dieting.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
11. Since my weight goes up and down, I have gone on reducing diets more than once.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
12. I often feel so hungry that I just have to eat something	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
13. When I am with someone who is overeating, I usually overeat too.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
14. I have a pretty good idea of the number of calories in common food.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
15. Sometimes when I start eating, I just can't seem to stop.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
16. It is not difficult for me to leave something on my plate.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
17. At certain times of the day, I get hungry because I have gotten used to eating then.	
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
19. Being with someone who is eating often makes me hungry to eat also.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
20. When I feel blue, I often overeat.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
21. I enjoy eating too much to spoil it by counting calories or watching my weight.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
22. When I see a real delicacy, I often get so hungry that I have to eat right away.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
23. I often stop eating when I am not really full as a conscious means of limiting the amount I eat	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
24. I get so hungry that my stomach often seems like a bottomless pit.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
25. My weight has hardly changed at all in the last ten years.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>

27. When I feel lonely, I console myself by eating.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
28. I consciously hold back at meals in order not to gain weight.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
29. I sometimes get very hungry late in the evening or at night.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
30. I eat anything I want, any time I want.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
31. Without even thinking about it, I take a long time to eat.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
32. I count calories as a conscious means of controlling my weight.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
33. I do not eat some foods because they make me fat.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
34. I am always hungry enough to eat at any time.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
35. I pay a great deal of attention to changes in my figure.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>

36. While on a diet, if I eat a food that is not allowed, I often splurge and eat other high calorie foods.	TRUE <input type="checkbox"/> FALSE <input type="checkbox"/>
Please answer the following questions by filling in the circle on your answer sheet corresponding to the letter of the response that is appropriate to you.	
37. How often are you dieting in a conscious effort to control your weight?	rarely <input type="checkbox"/> sometimes <input type="checkbox"/> usually <input type="checkbox"/> always <input type="checkbox"/>
38. Would a weight fluctuation of 5 lbs. affect the way you live your life?	rarely <input type="checkbox"/> sometimes <input type="checkbox"/> usually <input type="checkbox"/> always <input type="checkbox"/>
39. How often do you feel hungry?	rarely <input type="checkbox"/> sometimes <input type="checkbox"/> usually <input type="checkbox"/> always <input type="checkbox"/>
40. Do your feelings of guilt about overeating help you to control your food intake?	rarely <input type="checkbox"/> sometimes <input type="checkbox"/> usually <input type="checkbox"/> always <input type="checkbox"/>
41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?	easy <input type="checkbox"/> slightly difficult <input type="checkbox"/> moderately difficult <input type="checkbox"/> very difficult <input type="checkbox"/>

42. How conscious are you of what you are eating?	not at all <input type="checkbox"/> slightly <input type="checkbox"/> moderately <input type="checkbox"/> extremely <input type="checkbox"/>
43. How frequently do you avoid “stocking up” on tempting foods?	almost never <input type="checkbox"/> seldom <input type="checkbox"/> usually <input type="checkbox"/> almost always <input type="checkbox"/>
44. How likely are you to shop for low calorie foods?	unlikely <input type="checkbox"/> slightly likely <input type="checkbox"/> moderately likely <input type="checkbox"/> very likely <input type="checkbox"/>
45. Do you eat sensibly in front of others and splurge alone?	never <input type="checkbox"/> rarely <input type="checkbox"/> often <input type="checkbox"/> always <input type="checkbox"/>
46. How likely are you to consciously eat slowly in order to cut down on how much you eat?	unlikely <input type="checkbox"/> slightly likely <input type="checkbox"/> moderately likely <input type="checkbox"/> very likely <input type="checkbox"/>
47. How frequently do you skip dessert because you are no longer hungry?	almost never <input type="checkbox"/> seldom <input type="checkbox"/> at least once a week <input type="checkbox"/> almost every day <input type="checkbox"/>
48. How likely are you to consciously eat less than you want?	unlikely <input type="checkbox"/> slightly likely <input type="checkbox"/> moderately likely <input type="checkbox"/> very likely <input type="checkbox"/>
49. Do you go on eating binges though you are not hungry?	never <input type="checkbox"/> rarely <input type="checkbox"/> sometimes <input type="checkbox"/> at least once a week <input type="checkbox"/>

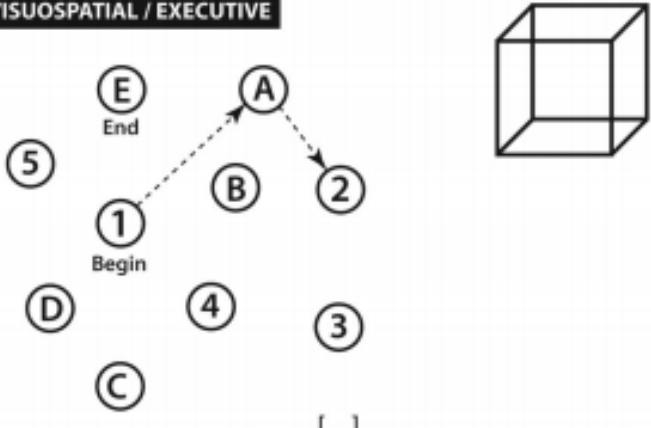
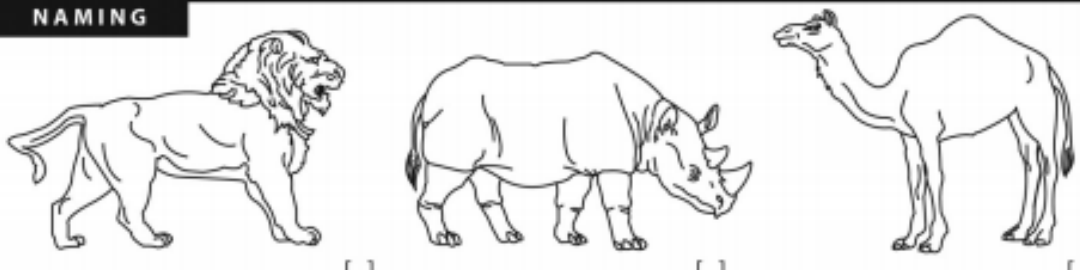
50. On a scale of 1 to 5, where 1 means no Restraint in eating (eating whatever you want, whenever you want it) and 5 means total Restraint (constantly limiting food intake and never “giving in”), what number would you give yourself?

- 1) usually or always eat whatever you want, whenever you want it
- 2) often eat whatever you want, whenever you want it
- 3) often limit food intake, but often “give in”
- 4) usually limit food intake, rarely “give in”
- 5) constantly limiting food intake, never “giving in”

51. To what extent does this statement describe your eating behavior? “I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.”

- not like me
- little like me
- pretty good description of me
- describes me perfectly

Montreal Cognitive Assessment

<p>MONTREAL COGNITIVE ASSESSMENT (MOCA) Version 7.1 Original Version</p>		<p>NAME : _____ Education : _____ Sex : _____ Date of birth : _____ DATE : _____</p>																			
<p>VISUOSPATIAL / EXECUTIVE</p>		<p>POINTS</p>																			
	<p>Copy cube</p>	<p>Draw CLOCK (Ten past eleven) (3 points)</p>	<p>___/5</p>																		
<p>NAMING</p>		<p>Contour Numbers Hands</p>																			
		<p>___/3</p>																			
<p>MEMORY</p>	<p>Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.</p>	<table border="1" style="width:100%; text-align: center;"> <tr> <td></td> <td>FACE</td> <td>VELVET</td> <td>CHURCH</td> <td>DAISY</td> <td>RED</td> </tr> <tr> <td>1st trial</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>2nd trial</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>		FACE	VELVET	CHURCH	DAISY	RED	1st trial						2nd trial						<p>No points</p>
	FACE	VELVET	CHURCH	DAISY	RED																
1st trial																					
2nd trial																					
<p>ATTENTION</p>	<p>Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [] 2 1 8 5 4 Subject has to repeat them in the backward order [] 7 4 2</p>	<p>___/2</p>																			
<p>Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors [] FBACMNAAJKLBAFAKDEAAAJAMOF AAB</p>		<p>___/1</p>																			
<p>Serial 7 subtraction starting at 100 [] 93 [] 86 [] 79 [] 72 [] 65 4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt</p>		<p>___/3</p>																			
<p>LANGUAGE</p>	<p>Repeat : I only know that John is the one to help today. [] The cat always hid under the couch when dogs were in the room. []</p>		<p>___/2</p>																		
<p>Fluency / Name maximum number of words in one minute that begin with the letter F [] _____ (N ≥ 11 words)</p>		<p>___/1</p>																			
<p>ABSTRACTION</p>	<p>Similarity between e.g. banana - orange = fruit [] train - bicycle [] watch - ruler</p>		<p>___/2</p>																		
<p>DELAYED RECALL</p>	<p>Has to recall words WITH NO CUE</p>	<table border="1" style="width:100%; text-align: center;"> <tr> <td>FACE</td> <td>VELVET</td> <td>CHURCH</td> <td>DAISY</td> <td>RED</td> </tr> <tr> <td>[]</td> <td>[]</td> <td>[]</td> <td>[]</td> <td>[]</td> </tr> </table>	FACE	VELVET	CHURCH	DAISY	RED	[]	[]	[]	[]	[]	<p>Points for UNCUED recall only</p>								
FACE	VELVET	CHURCH	DAISY	RED																	
[]	[]	[]	[]	[]																	
<p>Optional</p>	<p>Category cue</p>	<table border="1" style="width:100%; text-align: center;"> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>											<p>___/5</p>								
<p>ORIENTATION</p>	<p>[] Date [] Month [] Year [] Day [] Place [] City</p>		<p>___/6</p>																		
<p>© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30</p>		<p>TOTAL ___/30 Add 1 point if ≤ 12 yr edu</p>																			

Visual analogue scales

Participant number;

Date;

Please circle; Baseline / 5 / 15 / 30 / 60 / 120 / 180 minutes

Visual Analogue Scale

How hungry do you feel?

I am not
hungry at all

I have never
been more
hungry

How satisfied do you feel?

I am
completely
empty

I cannot eat
another bite

How full do you feel?

Not at all full

Totally full

How much do you think you can eat?

Nothing at all

A lot

Would you like to eat something sweet?

Yes very
much

No not at all

Would you like to eat something salty?

Yes very
much

No not at all

Would you like to eat something savoury?

Yes very
much

No not at all

Would you like to eat something fatty?

Yes very
much

No not at all

Flint A. *Et al* 2000 Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies *Int J Obes Relat Metab Disord* 24(1);38-48

Appendix H. Posters, presentations and publications

Posters

- **Johnston F**, Yarnall A, Davies J, Siervo M and Burn DJ. Pilot study protocol; Ghrelin and cognitive impairment in Parkinson's. Presented at Parkinson's UK conference 2014
- **Johnston F**, Lawson R, Khoo T, Yarnall A, Duncan G, Coleman S, Burn DJ. Leptin and insulin; biomarkers for cognitive impairment in Parkinson's disease? ICICLE-PD 36 month interim analysis. Presented 19th at the International Congress International Congress of Parkinson's Disease and Movement Disorders San Diego June 2015
- Lawson RA, Yarnall AJ, Duncan GW, **Johnston F**, Khoo TK, Collerton D, Taylor JP, Brittain KR and Burn DJ. The impact of cognitive impairment in people with Parkinson's disease and caregivers: a mixed methods study. Presented at the British Psychological Society Annual Conference, Nottingham, April 2016.
- Sleeman IJ, Lawson RA, Yarnall AJ, Duncan GW, **Johnston F**, Khoo TK, Collerton D, Taylor JP and Burn DJ. Urate and homocysteine: Changes in motor and cognitive symptoms in newly diagnosed Parkinson's disease. Presented at the 20th International Congress of Parkinson's Disease and Movement Disorders, Berlin June 2016
- **Johnston F**, Siervo M, Hornsby AKE, Davies JS, David J Burn DJ. Ghrelin and the IGF-1 axis in cognitive impairment in PD. Presented at the 21st International Congress of Parkinson's Disease and Movement Disorders, Vancouver June 2017

Presentations

- "Ghrelin and Dementia" delivered to a local Dementias and Neurodegenerative Diseases (DemaNDs) Research Group meeting 2015
- "Ghrelin and Parkinson's disease" delivered to the local Parkinson's Researchers Seminars series 2015
- "Appetite and cognition in Parkinson's Disease" delivered to the national British Geriatric Society Movement Disorder Section Meeting 2017

Publications

- Lawson, R.A., Yarnall, A.J., **Johnston, F.**, Duncan, G.W., Khoo, T.K., Collerton, D., Taylor, J.P. and Burn, D.J., 2016. Cognitive impairment in Parkinson's disease: impact on quality of life of carers. *International journal of geriatric psychiatry*
- **Under review by Journal of Neurology, Neurosurgery and Psychiatry.** Sleeman IJ, Lawson RA, Yarnall AJ, Duncan GW, **Johnston F**, Khoo TK, and Burn DJ. Urate and homocysteine: predicting changes in motor and cognitive symptoms in newly diagnosed Parkinson's

Appendix I. Supplementary data and analysis

Histograms showing normality of key transformed variables

All hormones measured in pg/ml

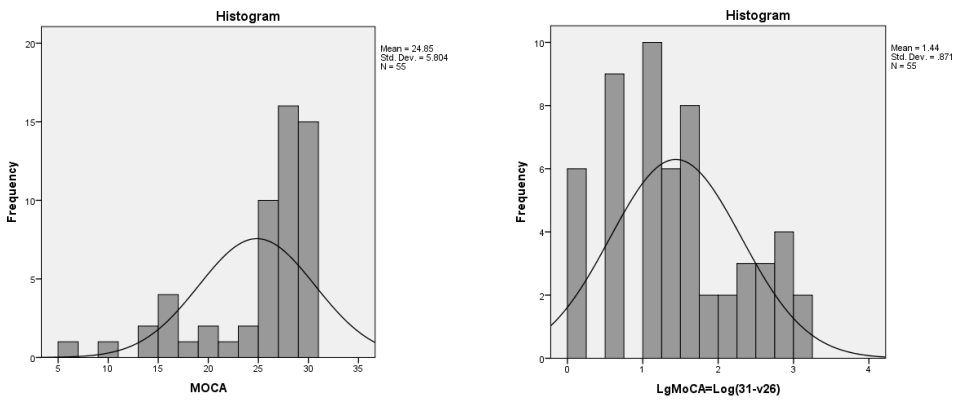


Figure 56. MoCA

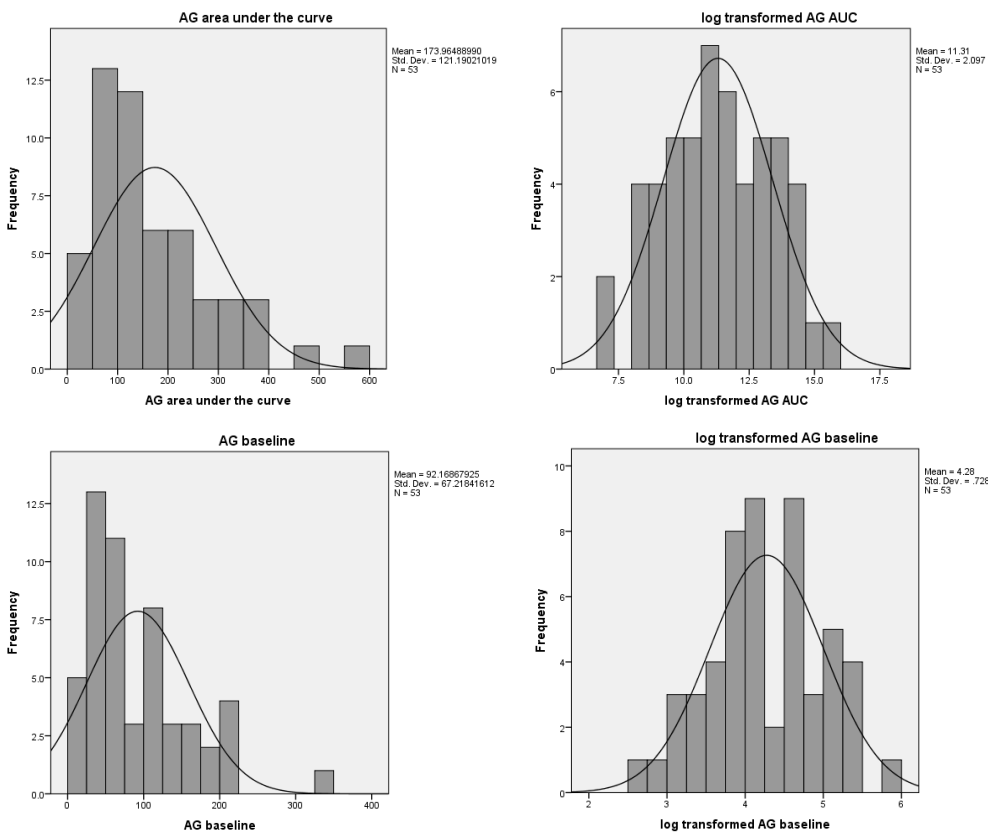


Figure 57. Acyl-ghrelin

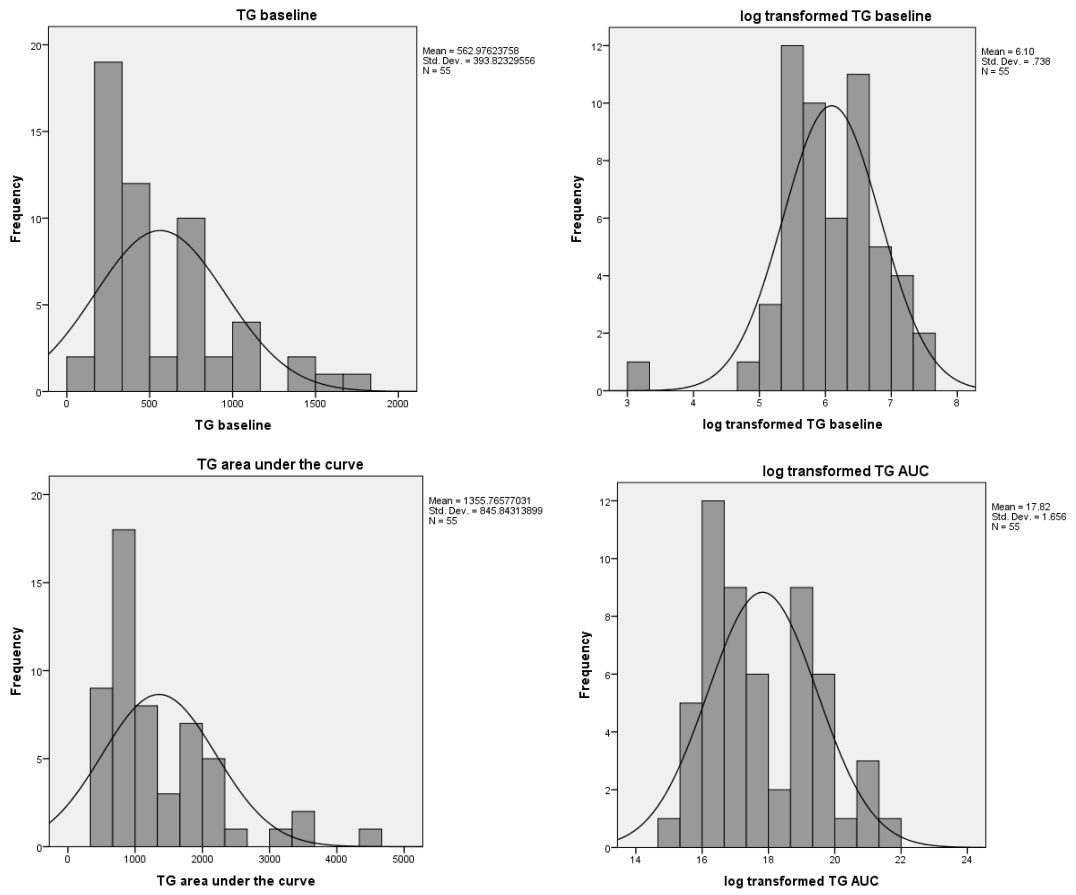


Figure 58. Total ghrelin

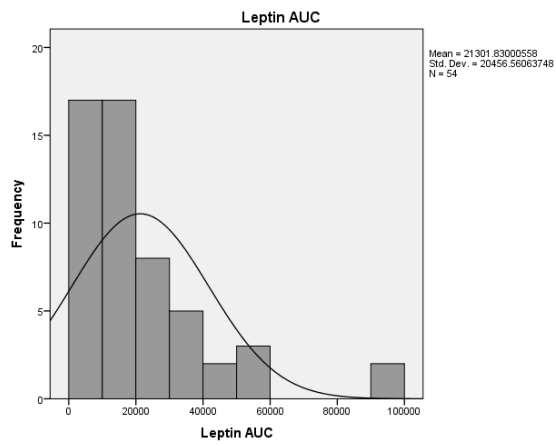
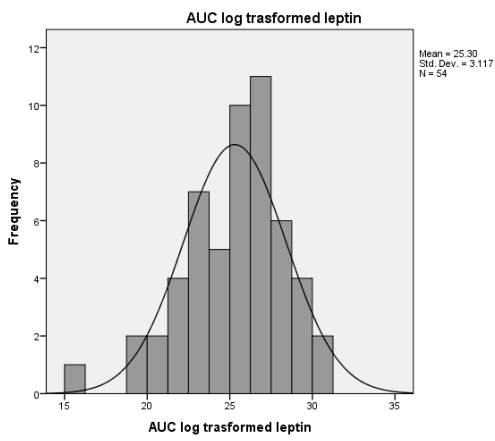
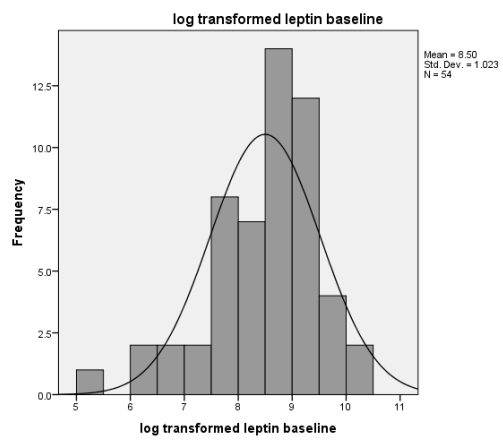
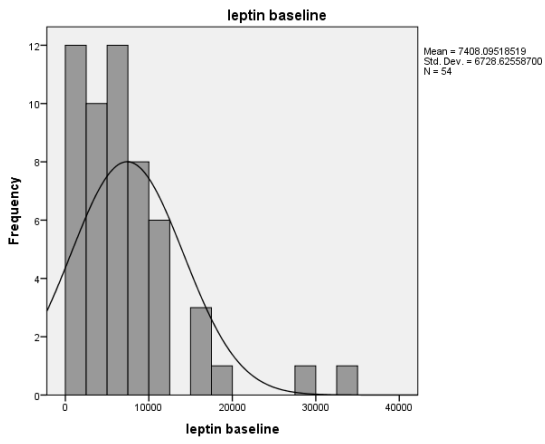


Figure 59. Leptin

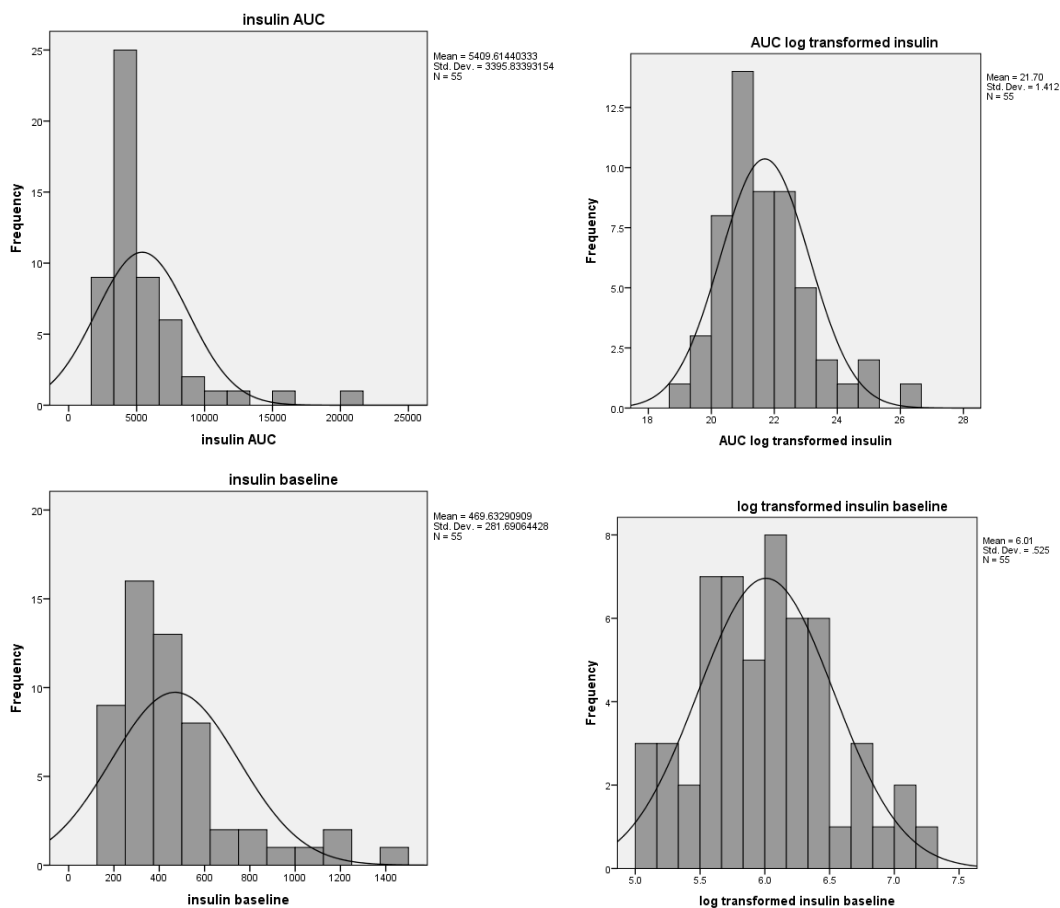


Figure 60. Insulin

Growth hormone analysis by group

Table 22. GH AUC against age by group

Group			Age	GH AUC
Control	age	Pearson Correlation	1	.506*
		Sig. (2-tailed)		.023
		N	20	20
	GH AUC	Pearson Correlation	.506*	1
		Sig. (2-tailed)	.023	
		N	20	20
PD	age	Pearson Correlation	1	.163
		Sig. (2-tailed)		.506
		N	19	19
	GH AUC	Pearson Correlation	.163	1
		Sig. (2-tailed)	.506	
		N	19	19
PD-CI	age	Pearson Correlation	1	.203
		Sig. (2-tailed)		.452
		N	16	16
	GH AUC	Pearson Correlation	.203	1
		Sig. (2-tailed)	.452	
		N	16	16

*. Correlation is significant at the 0.05 level (2-tailed).

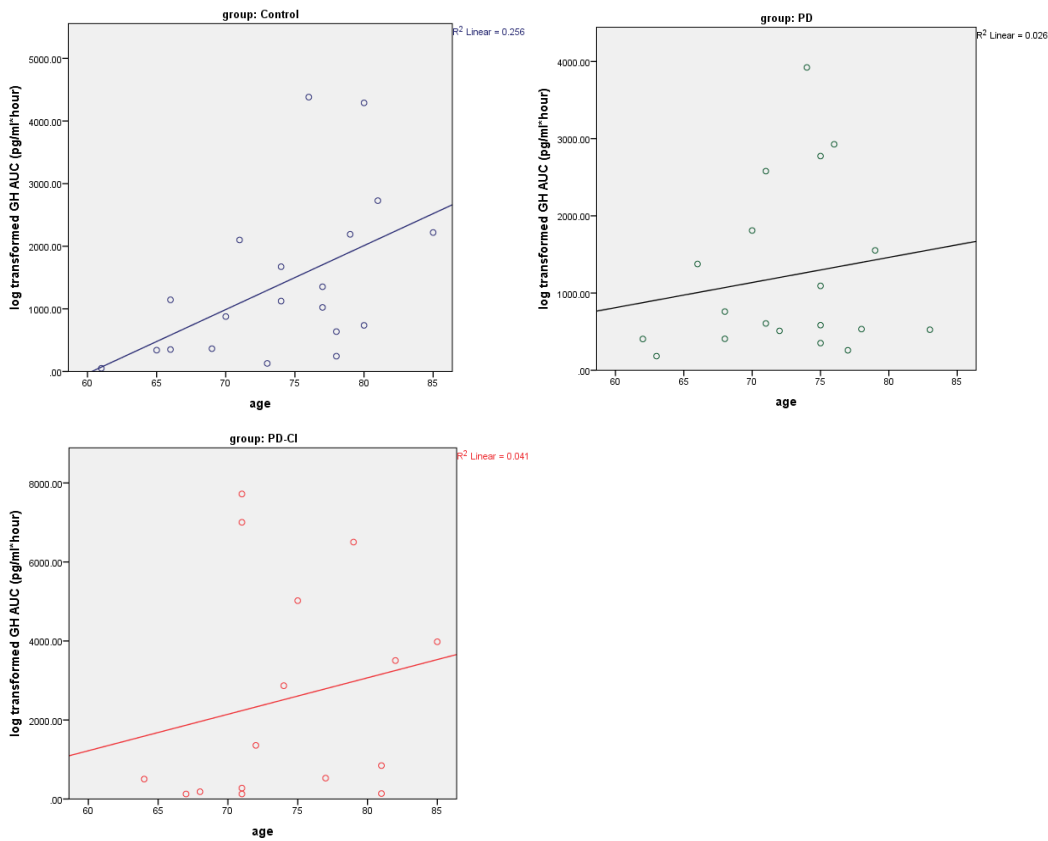


Figure 61. GH AUC against age by group

Table 23. Fasting GH against age by group

Group	Age	Fasting GH		
Control	age	Pearson Correlation	1	.452*
		Sig. (2-tailed)		.046
		N	20	20
	GH baseline	Pearson Correlation	.452*	1
		Sig. (2-tailed)	.046	
		N	20	20
PD	age	Pearson Correlation	1	.113
		Sig. (2-tailed)		.646
		N	19	19
	GH baseline	Pearson Correlation	.113	1
		Sig. (2-tailed)	.646	
		N	19	19
PD-CI	age	Pearson Correlation	1	-.009
		Sig. (2-tailed)		.973
		N	16	16
	GH baseline	Pearson Correlation	-.009	1
		Sig. (2-tailed)	.973	
		N	16	16

*. Correlation is significant at the 0.05 level (2-tailed).

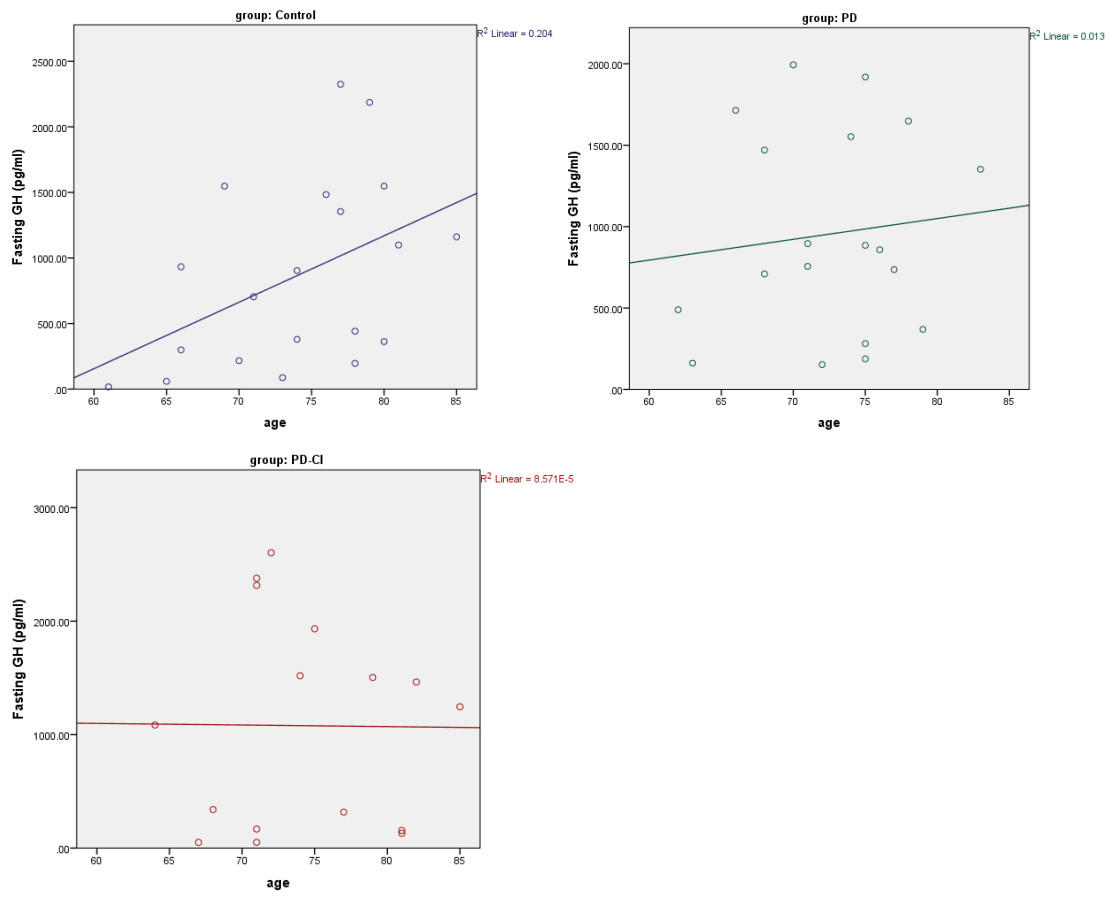


Figure 62. Fasting GH against age by group

Exploratory Pearson correlations

Table 24. *Pearson correlations duration ad libitum meal*

		Duration of ad libitum meal	Ad libitum meal total energy intake	Ad libitum meal bite counter count	Duration of PD	Motor UPDRS	LgMoCA=Log(31-value)	GDS
Duration of ad libitum meal	Pearson Correlation	1	.388**	.672**	.366**	.421*	.384**	.417**
	Sig. (2-tailed)		.004	.000	.007	.015	.004	.002
	N	53	53	48	53	33	53	53
Adlibitum meal total energy intake	Pearson Correlation	.388**	1	.706**	-.092	.215	-.061	.013
	Sig. (2-tailed)	.004		.000	.502	.214	.658	.923
	N	53	55	50	55	35	55	55
Ad libitum meal bite counter count	Pearson Correlation	.672**	.706**	1	.183	.208	.135	.305*
	Sig. (2-tailed)	.000	.000		.204	.261	.349	.031
	N	48	50	50	50	31	50	50
Duration of PD	Pearson Correlation	.366**	-.092	.183	1	.300	.510**	.621**
	Sig. (2-tailed)	.007	.502	.204		.080	.000	.000
	N	53	55	50	55	35	55	55
Motor UPDRS scores	Pearson Correlation	.421*	.215	.208	.300	1	.559**	.533**
	Sig. (2-tailed)	.015	.214	.261	.080		.000	.001
	N	33	35	31	35	35	35	35
LgMoCA=Log(31-v26)	Pearson Correlation	.384**	-.061	.135	.510**	.559**	1	.681**
	Sig. (2-tailed)	.004	.658	.349	.000	.000		.000
	N	53	55	50	55	35	55	55
Geriatric depression scale	Pearson Correlation	.417**	.013	.305*	.621**	.533**	.681**	1
	Sig. (2-tailed)	.002	.923	.031	.000	.001	.000	
	N	53	55	50	55	35	55	55

** . Correlation is significant at the 0.01 level (2-tailed). * . Correlation is significant at the 0.05 level (2-tailed).

rrrrr

Table 25. Pearson correlation fasting variables

		age	fat mass %	BMI	Duration PD	Motor UPDRS	LgMoCA=Lg(31-value)	GDS	IgAG	IgTG	IgPYY	IgIleptin	Iginsulin	Lg GLP-1	Lg IGF-1	IgGH	Ig glucose	energy intake	hunger	Fullness
age	Pearson Correlation	1	.066	.114	-.040	.046	.237	.196	.385*	-.095	.055	.090	.015	-.047	.060	.305*	.309*	-.126	.017	-.225
	Sig. (2-tailed)		.631	.406	.772	.792	.082	.152	.004	.490	.692	.518	.914	.758	.720	.024	.026	.359	.899	.099
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
fat mass %	Pearson Correlation	.066	1	.518*	-.254	-.042	-.264	-.240	.123	.056	.048	.652**	.120	-.216	-.181	-.058	.033	-.190	-.306*	.208
	Sig. (2-tailed)	.631		.000	.061	.810	.051	.078	.381	.683	.733	.000	.382	.150	.275	.673	.816	.164	.023	.128
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
BMI	Pearson Correlation	.114	.518*	1	-.253	-.007	-.237	-.231	-	-	.083	.445**	.412**	-.050	.108	-.075	.206	.173	-.099	.136
	Sig. (2-tailed)	.406	.000		.063	.969	.081	.090	.037	.015	.550	.001	.002	.741	.517	.586	.143	.208	.471	.322
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
Duration of PD	Pearson Correlation	-.040	-.254	-.253	1	.300	.510**	.621*	-.093	-.039	.050	-.163	.060	.153	-.006	.092	-.023	-.092	-.129	.085
	Sig. (2-tailed)	.772	.061	.063		.080	.000	.000	.509	.778	.720	.240	.664	.310	.970	.505	.873	.502	.348	.537
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
Motor UPDRS	Pearson Correlation	.046	-.042	-.007	.300	1	.559**	.533*	-.134	-.214	-.008	-.168	-.316	-.101	-.259	-.072	-.083	.215	-.174	.128
	Sig. (2-tailed)	.792	.810	.969	.080		.000	.001	.442	.216	.966	.341	.064	.625	.257	.680	.642	.214	.318	.463
	N	35	35	35	35	35	35	35	35	35	34	34	35	26	21	35	34	35	35	35
LgMoCA=Lg(31-value)	Pearson Correlation	.237	-.264	-.237	.510**	.559**	1	.681*	-	.002	-.131	-.145	-.097	-.122	-.074	.077	-.005	-.061	-.101	.094
	Sig. (2-tailed)	.082	.051	.081	.000	.000		.000	.011	.988	.344	.296	.481	.419	.660	.575	.970	.658	.463	.493
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
GDS	Pearson Correlation	.196	-.240	-.231	.621**	.533**	.681**	1	-	-.143	.057	-.130	.053	.018	-.170	.051	-.046	.013	-.111	.102
	Sig. (2-tailed)	.152	.078	.090	.000	.001	.000		.029	.297	.682	.349	.701	.904	.308	.713	.745	.923	.420	.460
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
LgAG fasting	Pearson Correlation	-	.123	-	-.093	-.134	-.346*	-	1	.727*	-.014	-.009	-.212	.015	-.115	.020	-.321*	-.090	.017	.138
	Sig. (2-tailed)	.004	.381	.037	.509	.442	.011	.029		.000	.919	.947	.127	.925	.504	.889	.022	.520	.905	.324
	N	53	53	53	53	35	53	53	53	53	52	52	53	44	36	53	51	53	53	53
Ig TG fasting	Pearson Correlation	-.095	.056	-	-.039	-.214	.002	-.143	.727*	1	-.076	-.055	-.201	.099	-.095	.178	-.278*	-.212	.050	.207
	Sig. (2-tailed)	.490	.683	.015	.778	.216	.988	.297	.000		.585	.692	.141	.512	.571	.193	.046	.120	.718	.129
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
Ig PYY fasting	Pearson Correlation	.055	.048	.083	.050	-.008	-.131	.057	-.014	-.076	1	-.104	.046	.407**	.451**	.158	.002	-.235	-.226	.067
	Sig. (2-tailed)	.692	.733	.550	.720	.966	.344	.682	.919	.585		.460	.739	.005	.005	.253	.991	.088	.101	.632
	N	54	54	54	54	34	54	54	52	54	54	54	54	45	37	54	51	54	54	54

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		age	fat mass %	BMI	Duration PD	Motor UPDRS	LgMoCA =Lg(31-value)	GDS	lgAG	lgTG	lgPYY	lgleptin	lginsulin	Lg GLP-1	Lg IGF-1	lgGH	lg glucose	energy intake	hunger	Fullness
lg leptin fasting	Pearson Correlation	.090	.652*	.445*	-.163	-.168	-.145	-.130	-.009	-.055	-.104	1	.452**	-.113	.062	-.156	.108	-.101	-.270*	.009
	Sig. (2-tailed)	.518	.000	.001	.240	.341	.296	.349	.947	.692	.460		.001	.461	.717	.261	.449	.466	.049	.950
	N	54	54	54	54	34	54	54	52	54	53	54	54	45	37	54	51	54	54	54
Lg insulin fasting	Pearson Correlation	.015	.120	.412*	.060	-.316	-.097	.053	-.212	-.201	.046	.452**	1	-.044	.211	-.130	.300*	.012	-.043	.043
	Sig. (2-tailed)	.914	.382	.002	.664	.064	.481	.701	.127	.141	.739	.001		.773	.203	.345	.031	.928	.753	.757
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
Lg GLP-1 fasting	Pearson Correlation	-.047	-.216	-.050	.153	-.101	-.122	.018	.015	.099	.407**	-.113	-.044	1	.262	-.212	-.126	-.128	-.095	-.001
	Sig. (2-tailed)	.758	.150	.741	.310	.625	.419	.904	.925	.512	.005	.461	.773		.112	.157	.420	.397	.528	.995
	N	46	46	46	46	26	46	46	44	46	45	45	46	46	38	46	43	46	46	46
lg IGF-1 fasting	Pearson Correlation	.060	-.181	.108	-.006	-.259	-.074	-.170	-.115	-.095	.451**	.062	.211	.262	1	.138	.135	.094	.037	-.230
	Sig. (2-tailed)	.720	.275	.517	.970	.257	.660	.308	.504	.571	.005	.717	.203	.112		.408	.434	.575	.825	.166
	N	38	38	38	38	21	38	38	36	38	37	37	38	38	38	38	36	38	38	38
lg GH fasting	Pearson Correlation	.305*	-.058	-.075	.092	-.072	.077	.051	.020	.178	.158	-.156	-.130	-.212	.138	1	.103	.020	.183	-.175
	Sig. (2-tailed)	.024	.673	.586	.505	.680	.575	.713	.889	.193	.253	.261	.345	.157	.408		.467	.887	.181	.200
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
lg glucose fasting	Pearson Correlation	.309*	.033	.206	-.023	-.083	-.005	-.046	-	-	.002	.108	.300*	-.126	.135	.103	1	-.081	.304*	-.022
	Sig. (2-tailed)	.026	.816	.143	.873	.642	.970	.745	.022	.046	.991	.449	.031	.420	.434	.467		.567	.029	.876
	N	52	52	52	52	34	52	52	51	52	51	51	52	43	36	52	52	52	52	52
Energy intake	Pearson Correlation	-.126	-.190	.173	-.092	.215	-.061	.013	-.090	-.212	-.235	-.101	.012	-.128	.094	.020	-.081	1	.341*	-.283*
	Sig. (2-tailed)	.359	.164	.208	.502	.214	.658	.923	.520	.120	.088	.466	.928	.397	.575	.887	.567		.011	.037
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
hunger baseline	Pearson Correlation	.017	-	-.099	-.129	-.174	-.101	-.111	.017	.050	-.226	-.270*	-.043	-.095	.037	.183	.304*	.341*	1	-.204
	Sig. (2-tailed)	.899	.023	.471	.348	.318	.463	.420	.905	.718	.101	.049	.753	.528	.825	.181	.029	.011		.135
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55
fullness baseline	Pearson Correlation	-.225	.208	.136	.085	.128	.094	.102	.138	.207	.067	.009	.043	-.001	-.230	-.175	-.022	-.283*	-.204	1
	Sig. (2-tailed)	.099	.128	.322	.537	.463	.493	.460	.324	.129	.632	.950	.757	.995	.166	.200	.876	.037	.135	
	N	55	55	55	55	35	55	55	53	55	54	54	55	46	38	55	52	55	55	55

Table 26. Correlations AUC values

		age	fat mass %	BMI	Duration PD	Motor UPDRS	LgMoCA= Lg(31-value)	GDS	Lg AG	Lg TG	Lg PYY	Ig leptin	Ig insulin	Ig GLP-1	Ig IGF-1	Ig GH	Ig Glu	Ad libitum bite count	energy intake	hunger	Fullness
age	Pearson Correlation	1	.066	.114	-.040	.046	.237	.196	-	-.265	.065	.104	.021	.027	.107	.382**	.138	.052	-.126	-.037	.058
	Sig. (2-tailed)		.631	.406	.772	.792	.082	.152	.004	.051	.636	.454	.881	.849	.510	.004	.330	.721	.359	.787	.672
	N	55	55	55	55	35	55	55	53	55	55	55	54	55	51	40	55	52	50	55	55
fat mass	Pearson Correlation	.066	1	.518**	-.254	-.042	-.264	-.240	.140	-.016	.103	.662**	.279*	.202	-.134	-.135	-.082	-.270	-.190	-.427**	.328*
	Sig. (2-tailed)	.631		.000	.061	.810	.051	.078	.318	.909	.454	.000	.039	.156	.410	.324	.563	.058	.164	.001	.014
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
BMI	Pearson Correlation	.114	.518**	1	-.253	-.007	-.237	-.231	-.186	-	.165	.419**	.479**	.262	.069	-.016	.163	-.021	.173	-.213	.040
	Sig. (2-tailed)	.406	.000		.063	.969	.081	.090	.183	.003	.230	.002	.000	.063	.674	.905	.248	.882	.208	.118	.772
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
Duration PD	Pearson Correlation	-.040	-.254	-.253	1	.300	.510**	.621**	-.107	-.063	-.048	-.154	-.014	-.014	-.096	.110	.048	.183	-.092	.125	-.035
	Sig. (2-tailed)	.772	.061	.063		.080	.000	.000	.447	.648	.726	.266	.921	.925	.555	.423	.736	.204	.502	.363	.798
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
Motor UPDRS	Pearson Correlation	.046	-.042	-.007	.300	1	.559**	.533**	-.091	-.137	-.047	-.160	-.358*	-.137	-.357	.087	-.186	.208	.215	.203	-.276
	Sig. (2-tailed)	.792	.810	.969	.080		.000	.001	.604	.433	.787	.365	.035	.463	.094	.618	.291	.261	.214	.243	.108
	N	35	35	35	35	35	35	35	35	35	35	34	35	31	23	35	34	31	35	35	35
LgMoCA= Lg(31-value)	Pearson Correlation	.237	-.264	-.237	.510**	.559**	1	.681**	-	-.087	-.200	-.133	-.068	-.190	-.128	.206	.110	.135	-.061	-.058	-.018
	Sig. (2-tailed)	.082	.051	.081	.000	.000		.000	.005	.526	.142	.337	.623	.182	.429	.132	.439	.349	.658	.676	.897
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
GDS	Pearson Correlation	.196	-.240	-.231	.621**	.533**	.681**	1	-.304*	-.193	.003	-.109	-.010	-.068	-.251	.119	.109	.305*	.013	.183	-.198
	Sig. (2-tailed)	.152	.078	.090	.000	.001	.000		.027	.159	.985	.433	.943	.638	.118	.388	.442	.031	.923	.182	.147
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
Lg AG	Pearson Correlation	-	.140	-.186	-.107	-.091	-.384**	-.304*	1	.777**	.004	-.055	-.171	.123	-.080	-.247	-.145	-.129	-.070	.146	.171
	Sig. (2-tailed)	.004	.318	.183	.447	.604	.005	.027		.000	.976	.696	.220	.400	.632	.074	.309	.380	.617	.296	.220
	N	53	53	53	53	35	53	53	53	53	53	52	53	49	38	53	51	48	53	53	53
Lg TG	Pearson Correlation	-.265	-.016	.395**	-.063	-.137	-.087	-.193	.777**	1	-.230	-.132	-.242	.022	-.125	-.169	-.090	-.103	-.145	.056	.214
	Sig. (2-tailed)	.051	.909	.003	.648	.433	.526	.159	.000		.091	.343	.076	.877	.440	.218	.525	.475	.292	.687	.116
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
Lg PYY	Pearson Correlation	.065	.103	.165	-.048	-.047	-.200	.003	.004	-.230	1	-.043	.008	.462**	.269	.035	.067	-.253	-.294*	.036	-.058
	Sig. (2-tailed)	.636	.454	.230	.726	.787	.142	.985	.976	.091		.758	.957	.001	.093	.799	.635	.077	.029	.794	.672
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55

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		age	fat mass %	BMI	Duration PD	Motor UPDRS	LgMoCA=Lg(31-value)	GDS	Lg AG	Lg TG	Lg PYY	Ig leptin	Ig insulin	Ig GLP-1	Ig IGF-1	Ig GH	Ig Glu	Ad libitum bite count	energy intake	hunger	Fullness
Lg Leptin	Pearson Correlation	.104	.662**	.419**	-.154	-.160	-.133	-.109	-.055	-.132	-.043	1	.503**	.135	.202	.004	.170	-.080	-.136	-.379**	.189
	Sig. (2-tailed)	.454	.000	.002	.266	.365	.337	.433	.696	.343	.758		.000	.351	.218	.976	.234	.587	.326	.005	.170
	N	54	54	54	54	34	54	54	52	54	54	54	54	50	39	54	51	49	54	54	54
Lg Insulin	Pearson Correlation	.021	.279*	.479**	-.014	-.358*	-.068	-.010	-.171	-.242	.008	.503**	1	.087	.079	-.102	.461**	-.034	-.028	-.211	.117
	Sig. (2-tailed)	.881	.039	.000	.921	.035	.623	.943	.220	.076	.957	.000		.545	.627	.458	.001	.816	.837	.123	.394
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
Lg GLP-1	Pearson Correlation	.027	.202	.262	-.014	-.137	-.190	-.068	.123	.022	.462**	.135	.087	1	.021	-.046	-.190	-.249	-.113	-.232	.237
	Sig. (2-tailed)	.849	.156	.063	.925	.463	.182	.638	.400	.877	.001	.351	.545		.898	.747	.196	.092	.432	.101	.094
	N	51	51	51	51	31	51	51	49	51	51	50	51	51	40	51	48	47	51	51	51
Lg IGF-1	Pearson Correlation	.107	-.134	.069	-.096	-.357	-.128	-.251	-.080	-.125	.269	.202	.079	.021	1	.455**	.264	.338*	.134	.052	-.153
	Sig. (2-tailed)	.510	.410	.674	.555	.094	.429	.118	.632	.440	.093	.218	.627	.898		.003	.109	.044	.411	.750	.346
	N	40	40	40	40	23	40	40	38	40	40	39	40	40	40	40	38	36	40	40	40
Lg GH	Pearson Correlation	.382**	-.135	-.016	.110	.087	.206	.119	-.247	-.169	.035	.004	-.102	-.046	.455**	1	.084	.170	.127	.021	-.111
	Sig. (2-tailed)	.004	.324	.905	.423	.618	.132	.388	.074	.218	.799	.976	.458	.747	.003		.553	.237	.355	.880	.421
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
Lg GLu	Pearson Correlation	.138	-.082	.163	.048	-.186	.110	.109	-.145	-.090	.067	.170	.461**	-.190	.264	.084	1	-.069	-.060	.155	-.047
	Sig. (2-tailed)	.330	.563	.248	.736	.291	.439	.442	.309	.525	.635	.234	.001	.196	.109	.553		.645	.670	.274	.740
	N	52	52	52	52	34	52	52	51	52	52	51	52	48	38	52	52	47	52	52	52
Ad libitum	Pearson Correlation	.052	-.270	-.021	.183	.208	.135	.305*	-.129	-.103	-.253	-.080	-.034	-.249	.338*	.170	-.069	1	.706**	.476**	-
	Sig. (2-tailed)	.721	.058	.882	.204	.261	.349	.031	.380	.475	.077	.587	.816	.092	.044	.237	.645		.000	.000	.000
	N	50	50	50	50	31	50	50	48	50	50	49	50	47	36	50	47	50	50	50	50
Energy	Pearson Correlation	-.126	-.190	.173	-.092	.215	-.061	.013	-.070	-.145	-.294*	-.136	-.028	-.113	.134	.127	-.060	.706**	1	.441**	-
	Sig. (2-tailed)	.359	.164	.208	.502	.214	.658	.923	.617	.292	.029	.326	.837	.432	.411	.355	.670	.000		.001	.000
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
hunger	Pearson Correlation	-.037	-	-.213	.125	.203	-.058	.183	.146	.056	.036	-	-.211	-.232	.052	.021	.155	.476**	1	.441**	-
	Sig. (2-tailed)	.787	.001	.118	.363	.243	.676	.182	.296	.687	.794	.005	.123	.101	.750	.880	.274	.000		.001	.000
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55
fullness	Pearson Correlation	.058	.328*	.040	-.035	-.276	-.018	-.198	.171	.214	-.058	.189	.117	.237	-.153	-.111	-.047	-	1	-.623**	1
	Sig. (2-tailed)	.672	.014	.772	.798	.108	.897	.147	.220	.116	.672	.170	.394	.094	.346	.421	.740	.000		.000	.000
	N	55	55	55	55	35	55	55	53	55	55	54	55	51	40	55	52	50	55	55	55

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).


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