Exenatide as a novel treatment for Parkinson's disease

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This dissertation is submitted for the degree of

Doctor in Philosophy

DECLARATIONS

I, Iciar Aviles-Olmos confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

This dissertation does not exceed the word limit for the Degree Committee.

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A Paco, Rosario y Carmen.

“La ciencia es un arma cargada de futuro”

Gabriel Celaya.

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THESIS SUMMARY

PD is a progressive neurodegenerative disease that results in intolerable disability for most patients despite the best current medical and surgical therapies. A treatment that slows, or stops clinical progression is the major therapeutic goal of current PD research.

Multiple avenues of research including epidemiology, molecular genetics and cell biology have identified links between Parkinson's disease (PD) and Type 2 diabetes mellitus (T2DM). Several recent discoveries have highlighted common cellular pathways that potentially relate neurodegenerative processes with abnormal mitochondrial function and abnormal glucose metabolism. In parallel with these advances, a treatment for insulin resistance (Exenatide) has been evaluated as a possible disease modifying drug in PD, which forms the core of my PhD.

Exenatide is the synthetic version of Exendin-4, confirmed to be an agonist of the Glucagon-like-peptide-1 (GLP-1) receptor, and resistant to the normal GLP-1 enzymatic degradation processes. The aim of this thesis is the evaluation of Exenatide's possible role as a potential neuroprotective/disease modifying agent in PD (with only preliminary insights regarding its mechanisms of action in neurodegeneration). This study presents the following data:

1. Methods used to obtained proof of concept data from patients with moderate PD treated with Exenatide to provide preliminary support for its further study.
2. An exploration of possible objective measures of differences resulting from Exenatide exposure in a PD biomarker- SPECT imaging, using

statistical parametric mapping of a subgroup of patients treated with Exenatide.

3. Prolonged follow up of these patients to further try and help distinguish placebo effects from possible biological effects of Exenatide in PD.
4. An attempt to identify a possible mechanism of action of Exenatide in our cohort of patients. Glucose tolerance tests were performed as an indirect measure of insulin resistance.

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LIST OF ABBREVIATIONS

PD Parkinson's disease

PGC1alpha PPAR gamma coactivator receptor alpha

PARIS Parkin Interacting Substrate

T2DM Type 2 diabetes mellitus

6OHDA 6-hydroxy-dopamine

MPTP 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine

IMPs Investigational medicinal products

GLP Glucagon like peptide

RN Red nucleus

NBM Nucleus Basalis of Meynert

PPN Peduncle Pontine Nucleus

LC Locus Coeruleus

DMV Dorsal motor nucleus of the vagus

ROS Radical oxygen species

DA Dopamine agonist

L-dopa Levodopa

UPDRS Unified Parkinson's Disease Rating Scale

NMS Non Motor symptoms

IR Insulin receptor

AD Alzheimer Disease

PUBLICATIONS ARISING FROM THIS THESIS

Chapter 1

Parkinson's disease, insulin resistance and novel agents of neuroprotection. Aviles-Olmos I, Limousin P, Lees A, Foltynie T. Brain. 2013 Feb;136

Chapter 2

Exenatide, and the treatment of patients with Parkinson's disease. Aviles-Olmos I, Dickson J, Kefalopoulou Z, Djamshidian A, Ell P, Soderlund T, Whitton P, Wyse R, Isaacs T, Lees A, Limousin A, Foltynie T . J Clin Invest. 2013 Jun 3;123(6):2730-6

Manuscripts In Preparation

The impact of Exenatide exposure in PD- Motor and non motor symptoms evolution over 2 year follow up

Exenatide as a potential treatment for patients with Parkinson's disease- first steps into the clinic

1 INTRODUCTION

1.1 SUMMARY

Parkinson's disease (PD) is a common neurodegenerative disease, affecting 1% of the population over age 65 (de Rijk et al. 2000; Kis et al. 2002). The PD research field is a rapidly moving one. As mechanisms underlying pathogenesis are being elucidated, novel therapies are making their way from the laboratory to the clinic. This chapter aims to highlight the most relevant updates in PD pathogenesis, especially recent discoveries related to the relationship between diabetes and the brain that have generated considerable recent research interest. A lot of research in recent years has focused on neurodegeneration/cell death associated with chronic Type 2 Diabetes Mellitus (T2DM). It has been widely proposed that neurodegeneration conditions may result from a complex interaction between T2DM and brain aging, as disruptions in shared molecular networks lead to both chronic diseases.

In parallel with these advances, Glucagon-like peptide 1 (GLP-1) is a hormone that facilitates insulin release under high blood sugar conditions. It is particularly noteworthy that GLP-1 also has a similar growth factor-like properties to insulin. Exenatide is a GLP-1 agonist used in T2DM.

Numerous pre-clinical studies utilising cellular and animal neurodegeneration models have established Exenatide's neurotrophic and neuroprotective properties. The aim of this chapter is to provide background on the potential benefit of using Exenatide as a possible disease modifying drug in PD, (with preliminary insights regarding its mechanisms of action in PD).

1.2 PD PATHOGENESIS

Today there is a well-described clinical and pathological phenotype of PD. There has been widespread adoption of the UK Brain Bank criteria (Hughes, et al. 1992a; Hughes et al. 1992b) as a reliable way of making the clinical diagnosis of PD. The cornerstone of these clinical criteria is that the patients exhibit "bradykinesia". PD is clinically characterized by the appearance of changes in motor function (bradykinesia, rigidity, postural instability and rest tremor) plus the increasingly recognized non-motor manifestations.

Neuropathologically, PD is defined as the selective degeneration of pigmented, dopaminergic neurons of the substantia nigra pars compacta (SNc) and other brainstem nuclei, with the presence of α-synuclein positive staining cytoplasmatic inclusions, (known as Lewy bodies) in the surviving neurons (Gibb & Lees 1988; Forno 1996; Spillantini et al. 1997; Bernheimer et al. 1973; Fearnley & Lees 1991).

The molecular mechanisms which lead to neurodegeneration are gradually being elucidated through the combined efforts of epidemiologists, geneticists and molecular and cell biologists. In recent years, several genes that cause certain forms of inherited PD have been identified, and great progress has been made in revealing their molecular mechanisms. However, most cases of PD appear to be sporadic, and are likely to represent interplay between both genetic and environmental factors (Warner & Schapira 2003). It is the current belief that the neurodegeneration in PD occurs in response to a mixture of deleterious mechanisms taking place both inside the degenerating neurons (oxidative stress, protein aggregation, defects in the ubiquitin-proteasome pathway, autophagy and alteration in mitochondrial function) and outside the degenerating neurons. Outside the neurons, mechanisms promoting

the spread of α-synuclein, inflammatory processes and loss of trophic support may contribute to progressive degeneration of dopaminergic and non-dopaminergic neurons, ultimately leading to progression of disease (Hirsch et al. 2013; Foltynie & Kahan 2013).

1.2.1 Determinants of vulnerability

There is lots of evidence to support that the pathology in PD is distributed across both the central and peripheral nervous system. There are several morphological and physiological features that appear to determine neural vulnerability. PD affects neurons with highly branched axons, which have a common physiological type (see section *1.2.1.2* below), Lewy pathology and neurons that synthesize a monoamine neurotransmitter. All of these factors are consistent with age being the strongest risk factor of PD. Based on the above body of information (discussed in detail below), genetic mutations, environmental toxins and proteostatic stress (affecting mitochondria) act synergistically in vulnerable cell types, to induce a bioenergetics crisis, that evolves into overproduction of misfolded proteins that might lead to aggregation and reduced dysfunctional protein degradation and cell degeneration that define the pathogenic scenario of PD.

1.2.1.1 A long, highly branched axon with multiple release sites.

The axons projecting from the Substantia Nigra pars compacta (SNc), Red Nucleus (RN), Pedunculo Pontine Nucleus (PPN), Nucleus Basalis of Meynert (NBM) and Locus Coeruleus (LC) are unmyelinated or thinly myelinated and highly branched (Orimo et al. 2011). There has been considerable speculation regarding the (initially) selective degeneration of dopaminergic neurons in PD.

Dopaminergic neurons in the SNc have an incredibly complex structure in terms of axon length and number of synapses. Mathematical modelling has shown that neuronal energetic demand rises exponentially with axon structure and arbour complexity (Bolam & Pissadaki 2012). This potentially makes dopaminergic neurons of the SNc more sensitive to energetic stress than other types of dopaminergic or indeed non-dopaminergic neurons (Moss & Bolam 2010; Matsuda et al. 2009; Bolam & Pissadaki 2012). Their large, complex axonal arbour puts them under such a tight energy budget that it makes them particularly susceptible to factors that contribute to cell death.

1.2.1.2 A common physiological phenotype.

PD is a disease of neurons. Therefore, it seems reasonable to think that features distinguishing neurons from other cell types may contribute to PD pathogenesis. One of the most characteristic properties of neurons is their excitability, which is energy dependent. To be electrically excitable, neurons maintain voltage gradients across their membranes by means of metabolically driven ion pumps, which combine with ion channels embedded in the membrane to generate intracellular-versus-extracellular concentration differences of ions such as sodium, potassium, chloride and calcium (John Hall 2011). During the waking state the SNc, RN, NBM, PPN, LC and Dorsal Motor Nucleus of Vagus (DMV) neurons spike continuously (Aston-Jones & Cohen 2005; McCann & Rogers 1990; Chan & Chan 1989). Furthermore, the SNc, LC, DMV and PPN neurons spike autonomously, i.e.in the absence of synaptic input. These neurons spend all their time at relatively depolarized membrane potentials (Beal 1998). These physiological features are responsible for a

sustained metabolic burden carried mainly by mitochondria in the neurons of SNc, LC, DMV and PPN (Nicholls 2009).

1.2.1.3 Lewy pathology.

Nearly all neurons that experience cell death in PD also display Lewy pathology. Despite this, Lewy pathology has been found in peripheral and central neurons without evidence of neuronal death. This fact has led to the hypothesis that Lewy pathology could reflect a neuroprotective response to detoxify forms of α synuclein (Conway 2001). Despite the fact that Lewy pathology might indicate a protective stress response, it might have deleterious effect on neuronal functioning, ie axonal transport (Dugger & Dickson 2010).

1.2.1.4 A common reactive neurotransmitter

SNc, LC, RN, enteric dopaminergic neurons, and sympathetic postganglionic neurons synthesize monoaminergic neurotransmitters. It seems that high levels of cytosolic monoamines could underlie the process of neuronal death (K. E. Larsen et al. 2002).

1.2.2 Defective glucose sensing and impaired insulin signalling in the brain

The classic idea that CNS was insulin-insensitive and the hormone was unable to cross the blood-brain barrier (BBB) has been challenged in the last decades, due to the increasing body of evidence showing that insulin can reach high levels in brain, and have long-term neuronal trophic effects (Salkovic-Petrisic & Hoyer 2007; Craft et al. 2000; Le Roith et al. 1983; Schulingkamp et al. 2000).

Insulin-induced neuronal *insulin receptor/insulin-growth-factor-1receptor* (IR/IGF-1R) activation under oxidative stress and subsequent *Phosphatidylinositide 3-kinases/Protein Kinase B* (PI3K/Akt) -mediated signalling prevented *Glucose transporter 3* (GLUT3) oxidation, restored hexokinase-II expression and glucose uptake, therefore recovering neuronal glycolysis and energy levels (Duarte et al. 2006). Therefore, it has been proposed that insulin may play other roles in the CNS rather than metabolic regulation. For example, evidence suggests it has a role in neurite outgrowth, regeneration of small myelinated fibers, maintenance of cortical, sympathetic and sensory neuronal survival during nervous system development, stimulation of neuronal protein synthesis, and improvement of synaptic activity and plasticity, memory formation, and storage, as well as neuroprotection (Moreira et al. 2009; Duarte et al. 2012).

The disruption of insulin regulation, inflammation, endoplasmic reticulum stress and mitochondrial dysfunction are candidates to play a role in the aetiology and/or progression of PD (Santiago & Potashkin 2013).

1.2.2.1 Insulin receptor (IR)

The IR is a trans-membrane receptor that belongs to a large class of tyrosine kinase receptors (Smith et al. 2010) and it is activated by insulin, insulin grow factor 1 (IGF-1) and IGF-II. Its functions are crucial to glucose homeostasis, a process that under degenerate conditions may result in a range of clinical manifestations including diabetes and cancer (McKern et al. 2006; Esposito et al. 2013). Insulin receptor (IR) and Insulin-like Growth Factor 1 Receptor (IGF1R) are hormone receptors made up of two α and two β subunits and are each the product of a single gene (IR located on chromosome 19 and IGF1R on chromosome 15 in humans)

(Werner et al. 1993). The binding of ligand to the α-chains of the IR ectodomain induces structural changes within the receptor leading to auto- phosphorylation of various tyrosine residues within the intracellular tyrosine kinase domain of the β-chain. The proteins that are phosphorylated by the IR include a protein called insulin receptor substrate 1 (IRS-1). The cascade of phosphorylation results in translocations of glucose transporters from sequestered sites within the cell to locations on the cell surface (Schulingkamp et al. 2000). Specifically, the glucose transporter 4 (GLUT4) is transported from cellular vesicles to the cell surface, where it then can mediate the transport of glucose into the cell.

1.2.2.1.1 JNK in Insulin Resistance

Although the mechanisms of insulin resistance are not fully elucidated, recent studies suggest that a complex interaction between inflammation, endoplasmic reticulum stress, oxidative stress, mitochondrial dysfunction and autophagy dysregulation plays an important role in insulin resistance. Obesity-associated insulin resistance is consistently associated with elevated levels of pro -inflammatory cytokines such as TNFα, IL-6, and IL-1β, and neutralization of TNFα improves insulin sensitivity in obese rodents (Hotamisligil 2006). These cytokines activate inflammatory pathways that terminate in activation of Jun N-terminal kinase (JNK1) and inhibitor of Kb kinase (IKKβ), the products of which alter signalling downstream of the insulin receptor and cause insulin resistance (Gao et al. 2002; Hirosumi et al. 2002). Knockout of JNK1 in non-hematopoietic cells protected mice from high fat diet-induced insulin resistance, in part through decreased adiposity (Hirosumi et al. 2002; Solinas et al. 2007). By contrast, mice with JNK1 knocked out of hematopoietic cells (macrophage-specific cells) became obese on high fat diet, with hepatic steatosis and increased intramuscular triglyceride content, but were still

protected against insulin resistance (Solinas et al. 2007). Protection against insulin resistance was conferred to these hematopoietic cell-specific Knocked out mice by a decrease in adipose tissue macrophages content and reduction in inflammatory pathway gene expression (Solinas et al. 2007). This experiment demonstrates that obesity and tissue lipid burden may not be sufficient to cause insulin resistance. Without the inflammatory component, obesity does not lead to appreciably impaired insulin action as demonstrated in macrophage-specific IKKβ and JNK1-knocked out mice (Arkan et al. 2005; Solinas et al. 2007).

The stress-activated c-Jun N-terminal kinase (JNK) has been increasingly recognized as a central mediator of insulin resistance and suppression of the JNK pathway has been shown to improve insulin resistance and glucose tolerance (Li & Yu 2013).

1.2.2.1.2. IR and Brain

Historically, skeletal muscle, adipose tissue, and liver were regarded as key insulin-sensitive organs involved in insulin-mediated regulation of peripheral carbohydrate, lipid, and protein metabolism. Insulin has conventionally been thought to act as the signal that tells these organs' cells to pull glucose in from the blood so it can be used to generate the energy the body needs.

The consequences of impaired insulin action in those organs were deemed to explain the functional and structural abnormalities associated with insulin resistance. In contrast, since the discovery of insulin in 1922 (Banting et al. 1922), the brain had been generally considered an insulin-insensitive organ. However, evidence of insulin actions in the brain emerged more than 30 years ago with the demonstration that i.c.v. infusion of insulin decreased food intake in baboons (Woods et al. 1979). This

discovery was followed by a number of studies reporting that the hypothalamic actions of insulin regulate peripheral energy homeostasis (Marino et al. 2011; Scherer et al. 2011). However, the effects of insulin in the brain are not restricted to the hypothalamus, as IRs are widely distributed throughout the encephalon (Zhao et al. 2004). The hippocampus, a region that is fundamentally involved in the acquisition, consolidation, and recollection of new memories, presents particularly high levels of IRs (Zhao & Alkon 2001), indicating that insulin might have additional targets in the CNS outside of the hypothalamus. Indeed, insulin has been shown to be neuroprotective (Bomfim et al. 2012; Plum et al. 2005; Ott et al. 2012) and to affect synaptic plasticity mechanisms (Wan et al. 1997). Insulin has been proposed to regulate neuronal survival and to act as a growth factor (Lin Li & Hölscher 2007), possibly by activating IGF receptors (Fernandez & Torres-Alemán 2012). IR signalling further regulates circuit function and plasticity by controlling synapse density (Chiu et al. 2008).

Nonetheless, knowledge of the precise roles of brain IRs is still limited. It is important to take into account that brain insulin receptors differ somewhat from their peripheral counterparts. It has been proposed that the role of these receptors exceeds mediation of insulin utilization. The distribution of the insulin receptors in the brain is neither homogeneous nor a simple function of glucose utilization pattern, and they have been shown to be present on synapses (Schulingkamp et al. 2000). Thus it has been proposed that Insulin receptors may play a role in CNS development, function, or pathophysiology (Schulingkamp et al. 2000).

1.2.2.1.3 Insulin Resistance & Alzheimer Disease

The idea that defective insulin signaling contributes to Alzheimer disease (AD) pathogenesis was first proposed by Hoyer more than 20 years ago (Hoyer & Nitsch 1989). Numerous clinical and epidemiological studies have since established that type 2 diabetes increases AD risk, and that targeting insulin levels or modulating sensitivity to insulin affects cognitive, imaging, and biochemical parameters in adults with AD or its presumed prodromal, amnestic mild cognitive impairment (MCI). Evidence regarding the mechanistic underpinnings of these relationships has been sparse however, as far fewer studies have systematically characterized insulin signaling in animal models of AD or in human brain tissue.

Two recent studies, published in the *Journal of Clinical Investigation*, have made important contributions in favor (Bomfim et al. 2012; Talbot et al. 2012). In one study, Talbot *et al*. showed that defective insulin signaling is a characteristic feature of the AD brain (Talbot et al. 2012). The authors demonstrate convincingly that postmortem brain tissue from patients with AD is less responsive to near-physiological doses of insulin than is tissue from non-AD cases. Neurotoxicity mediated by oligomeric amyloid-β has been proposed as possible mechanism underlying brain insulin resistance in AD and amyloid load in control, MCI and AD cases was negatively correlated with the level of tyrosine phosphorylation of the insulin receptor, and showed positive correlation with levels of IRS-1 serine kinases (Talbot et al. 2012). Importantly, high levels of insulin resistance markers were associated with poor performance on memory tests.

Bomfim *et al*. addressed possible therapeutic approaches to block the AD-promoting effects of insulin resistance. Patients with AD exhibited elevated levels of

IRS-1pSer and activated JNK (similar to what occurs in peripheral tissue in patients with DM). When neuronal cells from this group were taken in culture, both insulin and Exendin-4, prevented the increased serine phosphorylation and decreased tyrosine phosphorylation of IRS-1 caused by application of amyloid-β. *In vivo*, intraperitoneal injection of Exendin-4 in amyloid precursor protein/ presenilin 1 (APP/PS1) mice reduced hippocampal IRS-1 serine phosphorylation and amyloid burden, simultaneously improving spatial memory (Bomfim et al. 2012).

Additionally, intranasal insulin has shown therapeutic benefit in adults with early-stage AD in a phase II clinical trial (Craft et al. 2012).

However in a recent prospective cohort study with multiple assessments of glucose intolerance and insulin resistance, measures of glucose and insulin homeostasis are not associated with AD pathology (Thambisetty et al. 2013). Whether levels of glucose and insulin in peripheral blood parallel levels in the central nervous system is a question that should be clarified.

1.2.2.1.4 Insulin Resistance & Parkinsonism

Several studies have examined IR in the brain in relation to aberrant glucose metabolism associated with Parkinsonism (Peppard et al. 1992; Blum-Degen et al. 1995). Significant reductions (mean 20.7%) in the regional cerebral metabolic rate of glucose (rCMRG) have been found in Parkinson patients compared with the control group (Peppard et al. 1992).

In demented patients with PD, the mean rCMRG was 31.9% less than normal and 14.1% less than in non-demented patients. In patients with Parkinsonism there was a global decrease in glucose metabolism, with more severe abnormalities in the temporo-parietal regions. Insulin receptors distribution in midbrain regions of

Parkinsonism patients was examined by Moroo et al. (Moroo et al. 1994) using mono- and polyclonal anti-InsR antibody. The neurons in the substantia nigra pars compacta, parabrachial pigmental nucleus, tegmental pedunculopontine nucleus, supratrocheal nucleus, cuneiform nucleus, subcuneiform nucleus and lemniscus medialis, which were positive in non-neurological controls, were not stained by these antibodies in Parkinson brains. Specifically, the polyclonal InsR antibody recognized a major band of approximately 92 kDa, corresponding to that of the b subunit of the InsRb. The monoclonal antibody recognized two bands of 135 and 92 kDa corresponding to the α and β subunits, respectively. The results suggest that in PD a dysfunction of insulin or IR system might precede death of dopaminergic neurons. An upregulation of IR density has been reported for the occipital cortex of Parkinson patients (Blum-Degen et al. 1995) and selective neuronal loss in the substantia nigra is accompanied by decreased IR immunoreactivity (Takahashi et al., 1996). In the latter study, the authors performed semiquantitative mRNA analysis in the brain of 3 patients (82.0 ±9.5 years) and 6 controls (79.3±3.1years) by reverse transcription–polymerase chain reaction (RT–PCR) using specific primers for human IR exon 22, which encodes a region of the β subunit of the receptor serving as a tyrosine kinase domain. Levels of IR mRNA in patient tissues were markedly reduced compared with IR levels in the controls.

1.2.3 Inflammation

A growing body of evidence supports the hypothesis that inflammation plays an important part of the pathogenesis of PD. This is supported by consistent associations between the HLA locus and PD risk in meta-analyses of Genome wide association studies (IPDGC, WTCCC2 2011). Furthermore, epidemiological data reveal lower rates of PD among patients using non-steroidal anti-inflammatory

drugs, inflammatory pathways independent of HLA are associated with PD risk (Holmans et al. 2013), the presence of activated microglia seen in PD patients using PK11195 PET ligand (Gerhard et al. 2006) and the presence of pro-inflammatory mediators seen in the post mortem tissue of PD patients (Lee et al. 2009).

1.3 LINKS BETWEEN PD AND T2DM- EPIDEMIOLOGY

Evidence from prospective epidemiological studies has identified T2DM as an independent risk factor for multiple diseases of the nervous system such as diabetic neuropathy (Boulton et al. 2005), stroke (Hu et al. 2006b; Tuomilehto et al. 1996), and more recently Alzheimer's disease (Peila et al. 2002; Leibson et al. 1997; Ott et al. 1999) . There are also reports of varying associations between diabetes (or abnormal glucose tolerance) and sporadic forms of PD from both cross-sectional and cohort studies. Survey data reveals that diabetes is established in 8-30% of PD patients, consistently in excess of the prevalence found in non-PD individuals (Chalmanov & Vŭrbanova 1987; Pressley et al. 2003) . This might be readily explained by increased detection of hyperglycaemia through additional medical contact/-urine/-blood tests among PD individuals regularly attending hospital appointments. However, the association is greatly strengthened by reports that identified that 50-80% of PD patients have abnormal glucose tolerance when tested (Barbeau et al. 1961; Lipman et al. 1974; Sandyk 1993). This relationship is questionable and is explored further in Chapter 6. Nevertheless, in a series of 800 patients with PD, concurrent diabetes was also shown to accelerate progression of both motor and cognitive symptoms (Schaw 1960). In view of the possible confounding effects of PD treatment, newly diagnosed, never-treated adults with PD have also been studied and been shown to have reduced insulin-mediated glucose uptake (Van Woert & Mueller, 1971), inhibition of early insulin secretion, long-term hyperinsulinaemia, and hyperglycaemia after glucose loading (Boyd et al. 1971). This takes into account the effects of some drugs used to treat PD, such as levodopa, which induces both hyperglycaemia and hyperinsulinaemia, whereas others,

(including the ergot dopamine agonist- bromocriptine), may increase insulin sensitivity (Sirtori et al. 1972; Van Woert & Mueller 1971).

Neuropathological studies of patients with PD have shown that insulin receptors are densely represented on the dopaminergic neurons of the SNc (Unger et al. 1991), and loss of insulin-receptor immunoreactivity and mRNA in SNc of PD patients coincides with loss of tyrosine hydroxylase mRNA (the rate limiting enzyme in dopamine synthesis) (Moroo et al. 1994; Takahashi et al.1996). Indeed abnormal glucose utilization has been specifically shown in the brains of PD using magnetic resonance spectroscopy (Bowen et al. 1995) or fluorodeoxyglucose positron emission tomography (FDG-PET) (Hu et al. 2000), demonstrating increased lactate concentrations and glucose hypometabolism, supporting the hypothesis that PD is a systemic disorder characterized by an derangement of oxidative energy metabolism.

Further robust evidence for a positive association between PD and T2DM has been obtained from prospective cohort studies. A statistically significant direct association between triceps skin fold thickness and the risk of PD has been found in the Honolulu Heart Program (Abbott et al. 2012), and both excess weight (Hu, et al. 2006a) and T2DM itself (Hu et al. 2007) were associated with an increased risk of PD in a population- based prospective cohort of Finnish men and women. This association was independent of the known modifying factors such as smoking status, coffee and alcohol consumption and body weight, tempting speculation regarding common pathways underlying the development of these conditions. Nevertheless, a recent cohort study could not replicate an association between either T2DM or obesity and PD risk, although the authors acknowledge that diagnosis of T2DM was entirely based on self-report (Palacios et al. 2011).

1.4 INSULIN RESISTANCE AND MITOCHONDRIAL DYSFUNCTION

The development of insulin resistance in humans is closely correlated with immune cell infiltration and inflammation, with clear links between the development of obesity, T2DM and cardiovascular disease (Hotamisligil 2006). Explicit links have also been established between exercise, obesity, insulin resistance and levels of IL-6 (Kern et al. 2001). Evidence for mitochondrial dysfunction in the development of insulin resistance has been obtained through measuring rates of in vivo mitochondrial phosphorylation using proton 1(H) magnetic resonance spectroscopy in the relatives of patients with T2DM, finding that rates of mitochondrial ATP production are reduced by 30% in the muscle of lean, pre-diabetic insulin resistant subjects (Petersen et al. 2004). In addition, there are many specific examples of insulin resistance occurring due to mitochondrial mutations; it has been estimated that approximately 1.5% of T2DM is attributable to the mitochondrial A3243G mutation (the cause of Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes - MELAS (Gerbitz et al. 1995).

The more widespread development of insulin resistance has also been proposed to be mediated through mitochondrial dysfunction (Stark & Roden 2007), at least to a partial extent through muscle expression of transcriptional regulators including the PPARγ receptor coactivator (PGC1α), an important regulator of enzymes involved in mitochondrial respiration. Expression of PGC1α is rapidly induced following even a single bout of exercise, then reverts to baseline after cessation of exercise to enable fine control of the energy demands of skeletal muscle (Handschin & Spiegelman 2008). Individuals that undergo regular exercise have

chronically elevated levels of PGC1α, in association with a switch in muscle fibre type characterized by increased mitochondrial density and function (Lin et al. 2002).

Direct exploration of patterns of gene expression associated with insulin resistance has been studied in skeletal muscle in T2DM patients as well as non-diabetic individuals with and without a family history of T2DM. The earliest sign of insulin resistance was shown to be reduction in expression of PGC1α and the mitochondrial gene – nuclear respiratory factor 1 (NRF1) (Patti et al. 2003). In a whole genome methylation analysis of skeletal muscle from T2DM and control subjects, hypermethylation of PGC1α was found in association with reduced PGC1α mRNA and reduced mitochondrial DNA levels in T2DM subjects, suggesting a potential mechanism underlying the gene-environment interaction in T2DM risk (Barrès et al. 2009).

1.5 PD AND MITOCHONDRIAL DYSFUNCTION

Mitochondria are organelles present in all cells of the body (erythrocytes excluded), ranging from a few hundred to many thousands per cell, depending on cell type.

Maternally inherited, they are the locus for many of the body's "housekeeping" functions, including the biosynthesis of amino acids and steroids and the beta-oxidation of fatty acids; they also play a central role in apoptosis.

However, the function that sets this organelle apart, and which is responsible for the cliché that mitochondria are the "powerhouses of the cell", is the production of adenosine triphosphate (ATP) via the combined efforts of the tricarboxylic acid cycle and the respiratory chain/oxidative phosphorylation system (OxPhos). The respiratory chain is a set of biochemically linked multi-subunit complexes (complexes I, II, III, and IV) and two electron carriers (ubiquinone/coenzyme Q and cytochrome *c*). It uses the energy stored in carbohydrates to generate a proton gradient across the mitochondrial inner membrane, while at the same time transferring electrons to oxygen, producing water. The energy of the proton gradient drives ATP synthesis via ATP synthase (complex V), ATP is then distributed throughout the cell (Nelson & Cox 2013).

The earliest link between PD and mitochondrial dysfunction followed the observation that individuals injected with heroin contaminated with MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) acutely developed parkinsonism (Langston et al. 1983). Both MPTP and another environmental toxin, rotenone, were subsequently shown to cause degeneration of dopaminergic neurons in animal models by selective inhibition of complex I (Gerlach et al. 1991), suggesting that in humans, complex I

impairment may itself be sufficient to cause the disease. Complex I (NADH CoQ dehydrogenase) is the first enzyme of the mitochondrial respiratory chain, playing a crucial role in ATP generation.

Complex I dysfunction decreases ATP production, generates free radicals and sensitizes cells to the pro-apoptotic protein Bax, leading to apoptosis (Perier et al. 2005). A direct relation between mitochondrial dysfunction and sporadic PD has been demonstrated in post-mortem tissue, revealing complex I deficiency (Mann et al. 1994; Schapira et al. 1989) and damage (Keeney et al. 2006) in the substantia nigra of patients with PD. However it is likely that neurodegeneration provoked by Complex I pathology alone is rare.

Complex I toxins may however contribute to pathogenesis of PD, acting in tandem with other environmental influences and genetic causes (Schapira 2010). Although rare, there are numerous reports of parkinsonism occurring in association with mitochondrial mutations, including the G11778A mutation (associated with Leber's Hereditary Optic Neuropathy (Simon et al. 1999), polymerase γ mutations (Luoma et al. 2007), and more commonly in association with somatic mitochondrial deletions (Bender et al. 2006). A common mitochondrial haplotype has also been associated with sporadic PD (Pyle et al. 2005).

Converging evidence suggests that both environmental and genetic factors can interfere with the physiological removal of damaged or dysfunctional mitochondria known as mitophagy. Healthy mitochondrial turnover through mitophagy and mitochondrial biogenesis, as well as ongoing processes of fission, fusion, and mitochondrial budding, are essential to maintain normal cellular bioenergetic function. This relies on intact mitochondrial DNA (mtDNA), which is particularly

vulnerable to damage by free radicals due to its lack of a histone coat and limited facilities for repair. Furthermore, mtDNA mutations, whether induced by oxidative stress, or ageing, have a further effect on complex I and respiratory chain function leading to increased damage, again through the production of reactive oxygen species (Cooper et al. 1992). Normal human substantia nigra and striatum exhibit the greatest free radical-mediated mitochondrial damage with age (Kraytsberg et al. 2006). Other cellular pathways essential for normal mitochondrial turnover have been identified directly as a result of the identification of specific gene mutations causing Mendelian forms of PD (Schapira 2008).

1.5.1 Parkin

Parkin mutations have been found to cause juvenile parkinsonism. Intracellular localisation studies have reported the association of Parkin and mitochondria (Stichel et al. 2000). The function of Parkin is not completely clear but the protein has E3 ligase activity, and is thought to monitor the quality of mitochondria, and trigger mitophagy of dysfunctional mitochondria (Rakovic et al. 2010) by triggering the ubiquitin-proteasome system (Chan et al. 2011).

Parkin knockout mice have demonstrated alterations in abundance and/or modification of a number of proteins involved in mitochondrial function or oxidative stress, with reductions in several subunits of complexes I and IV, and functional assays showing reductions in respiratory capacity of striatal mitochondria isolated from Parkin-/- mice. Furthermore, these mice show a delayed rate of weight gain, suggesting broader metabolic abnormalities (Palacino et al., 2004). Mitochondrial function has also been shown to be decreased (complex I and IV activities) in peripheral blood from patients with Parkin mutations (Muftuoglu et al. 2004).

1.5.2 PINK1 (PTEN-induced putative kinase 1)

PINK1 (PTEN-induced putative kinase 1) mutations also cause autosomal recessive juvenile parkinsonism. The gene encoding PINK1 encodes a 63 kDa protein with an 8 kDa mitochondrial targeting sequence. In health, this protein is imported intact into the mitochondria, where it has been suggested that PINK 1 recruits parkin from the cytoplasm to the mitochondria to initiate the process of mitophagy (Vives-Bauza & Przedborski 2011).

1.5.3 DJ-1

DJ-1 is a 23 kDa protein that is expressed in peripheral tissues and parts of the brain, including the hippocampus, cerebellum, olfactory bulb, striatum, SNc, and Substantia Nigra pars reticulate (SNr), both in cells bodies and dendrites, localized to the mitochondrial matrix and intermembrane space (Zhang et al. 2005). One candidate pathophysiological mechanism is that the deletion or silencing of DJ-1 causes parkinsonism possibly by sensitising cells to oxidative stress, while over-expression of DJ-1 protects cells implying a protective role for the protein (Yokota et al. 2003). Substantia nigra neurons from *DJ-1* knockout mice have increased sensitivity to MPTP and oxidative stress (Kim et al. 2005), and cells derived from patients with DJ-1 mutations have abnormal mitochondrial morphology (Irrcher et al. 2010). DJ-1 has been shown to associate with the mitochondrial protein Bcl-XL, which has a role in down regulating apoptosis (Ren et al. 2011).

1.5.4 Alpha-synuclein (SNCA)

Alpha synuclein is the main component of the neuropathological hallmark of PD, the Lewy Body (LB). Alpha synuclein mutations can cause autosomal dominant PD. The pathological features of PD, can be replicated by simply overexpressing

SNCA in transgenic flies (Feany & Bender 2000), suggesting that one mechanism by which neurodegeneration occurs is through a toxic "gain-of-function". The exact function of alpha-synuclein remains unknown, and the relationship between SNCA oligomers and aggregates, their degradation by the ubiquitin-proteasome- and lysosomal systems, and neuronal toxicity requires further work (Wong & Cuervo 2010). There are nevertheless indications that SNCA affects various mitochondrial pathways (Poon et al. 2005). SNCA co-localises with cytochrome C forming hetero-oligomers which can prevent apoptosis, but in the process forms complexes with prolonged peroxidase activity that induces increased oxidative stress (Bayir et al. 2009). It has been also shown that phosphorylated SNCA (the dominant form in PD) influences normal protein-protein interactions including the "pull down" of protein complexes involved in mitochondrial electron transport (McFarland *et al.*, 2008). Over-expression of SNCA leads to the protein entering mitochondria and interfering with mitochondrial function (Devi et al. 2008; Chinta et al. 2010).

1.5.5 Leucine rich repeat kinase (LRRK-2)

Leucine rich repeat kinase (LRRK-2) mutations can also cause autosomal dominant PD. LRRK-2 has GTPase and kinase domains and study of its interaction with SNCA is ongoing (Greggio et al. 2011).Whether LRRK-2 plays a critical role in determining the phosphorylation status of SNCA remains to be determined, however it has recently been demonstrated that in the transgenic G2019S mutant LRRK-2 mouse, there is age-dependent degeneration of dopamine nigrostriatal neurons together with damaged mitochondria and an increase in mitophagy (Ramonet et al. 2011).

1.5.6 Glucocerebrosidase (GBA)

GBA mutations cause Gaucher's disease and have been identified as a risk factor for PD in both the homozygous and in the heterozygous state (E Sidransky et al. 2009). There has been shown to be a bidirectional link such that SNCA inhibits the lysosomal activity of GBA, and functional loss of GBA leads to accumulation of SNCA (Mazzulli et al. 2011), potentially explaining the risk of PD through a positive feedback loop. Other potential mechanisms also include lipid accumulation and impaired mitophagy or mitochondrial trafficking (Westbroek et al. 2011).

Researchers from UCL have recently sequenced the GBA gene in a series of early-onset PD patients. It has been showed that the frequency of GBA mutations is much higher in this series of patients than in typical late-onset patient cohorts, and that the most prevalent Parkinson's disease associated GBA mutation is E326K, a variant which does not, when homozygous, cause Gaucher's disease (GD) (Duran et al. 2013). This observation, supported by the presence of many other rare GBA variants not associated with GD in the PD group, dissociates the pathogenesis of GD from that of PD. This association with PD may not be caused by build up of glucosylceramide and consequent lysosomal disruption but rather by some more subtle and distinct effect on lysosomal biology.

In PD there is therefore ample evidence of mitochondrial dysfunction that includes complex I inhibition, oxidative stress, PINK1 and DJ1 dysfunction, and an interaction between Parkin and PINK1 that influences mitophagy & mitochondrial biogenesis. Complex I inhibition initiated by any of a range of environmental toxins can increase free radical generation, and thus initiate a vicious cycle of events that further impairs mitochondrial function, and may enhance any underlying genetic

defects and further impair neuronal activity. Neuro-inflammation leads to further mitochondrial stress through the production of reactive oxygen species as well as through the activation of microglia and subsequent release of pro-inflammatory cytokines such as Nitric Oxide (NO) and tumor necrosis factor α (TNFα) (Tansey et al. 2008; Whitton 2007). This can be readily detected through the identification of activated microglia on imaging (Gerhard et al. 2006) and neuropathology (McGeer et al. 1988). The role of a neuro-inflammatory process in PD is further supported by; (1) the association between the HLA locus and PD risk in a meta-analysis of genome wide studies (Nalls et al. 2011), and (2), the reported beneficial effects of anti-inflammatory agents on risk of PD (Wahner et al. 2007). It is likely that multiple pathways remain relevant with inter-related and common aspects involving mitochondrial function.

1.5.7 Converging evidence implicating PGC1α

Converging evidence suggests that cellular pathways leading to either insulin resistance or neurodegeneration involve mitochondrial mechanisms. It is well known that mutations in mitochondrial DNA can lead to a wide variety of phenotypes that commonly involve neurodegeneration and diabetes (Finsterer et al. 2008; Hara et al. 1994).

However, these mutations do not account for a significant proportion of cases of sporadic PD. The proteins of the mitochondrial respiratory chain are the product of a joint effort between the mitochondrial and nuclear genomes. Normal mitochondrial biogenesis, respiration, and metabolism of reactive oxygen species (ROS) requires intact expression of both nuclear and mitochondrial encoded genomes, now recognised as being regulated by the PPARγ receptor coactivator

(PGC1α) (Finck & Kelly, 2006; Lin et al. 2005; St-Pierre et al., 2006). It has previously been shown that PGC-1α has a powerful suppressive effect on ROS production, in parallel to its effects in elevating mitochondrial respiration. This occurs through the PGC1α-mediated expression of genes involved in ROS detoxification, as well as PGC1α expression, which is rapidly induced by these proteins following a single bout of endurance exercise in vivo (Handschin & Spiegelman, 2008). Insulin resistant patients show reduced expression of PGC1α and the mitochondrial encoded gene COX1 (Heilbronn et al. 2007) while reduction in PGC1α-responsive genes has been shown among patients with T2DM and their asymptomatic relatives compared with healthy controls (Petersen et al. 2004). Indeed polymorphisms in PGC1α have been associated with an increased risk for T2DM in diverse populations (Bhat et al., 2007; Ek et al., 2001; Hara et al., 2002).

PGC1α has also been implicated in having a major role in PD pathogenesis. Meta-analysis of gene expression data using microarrays has utilized post-mortem brain homogenates examining gene expression in the SNc of patients with confirmed SNCA positive Lewy body PD. Robust three tiered analysis from separate genome wide datasets have indicated that “gene sets” involved in mitochondrial electron transport, mitochondrial biogenesis, glucose utilization and glucose sensing were strongly associated with PD. Included amongst these gene sets were 10 PGC1α responsive genes. Furthermore, it was shown that over-expression of PGC1α was able to protect dopamine cell loss induced by the mitochondrial toxin rotenone (Zheng et al., 2010).

In parallel with these discoveries was the identification of a zinc finger protein, Parkin interacting substrate (PARIS), which is upregulated threefold in the nigra of

patients with both Parkin- related parkinsonism and sporadic PD, and is both necessary and sufficient for neurodegeneration associated in Parkin animal models (Shin et al., 2011). The same group further identified that PARIS suppresses the expression of PGC1α and PGC1α target genes, playing an important role in mitochondrial function including Nuclear Respiratory Factor 1 (NRF1), and the oxidative phosphorylation regulators ATP5b.The site of interaction between PARIS and PGC1α is a sequence that is involved in the regulation of transcripts involved in insulin responsiveness and energy metabolism (Mounier & Posner, 2006). Although other pathways are undoubtedly also relevant, it is clear that parkin, PARIS, PGC1α and NRF-1 contribute to the pathogenesis of PD. Loss of expression of PGC-1α controlled genes may therefore be a key link between abnormal mitochondrial function, abnormal glucose utilization and PD. Further hypermethylation of PGC1α during life may follow either genetic or environmental influences that promote accumulation of free fatty acids, TNFα and ceramides, (Staiger et al., 2006; Summers & Nelson, 2005), which might then lead to dysregulation of mitochondrial bioenergetics and the onset of PD. (See Figure 1)

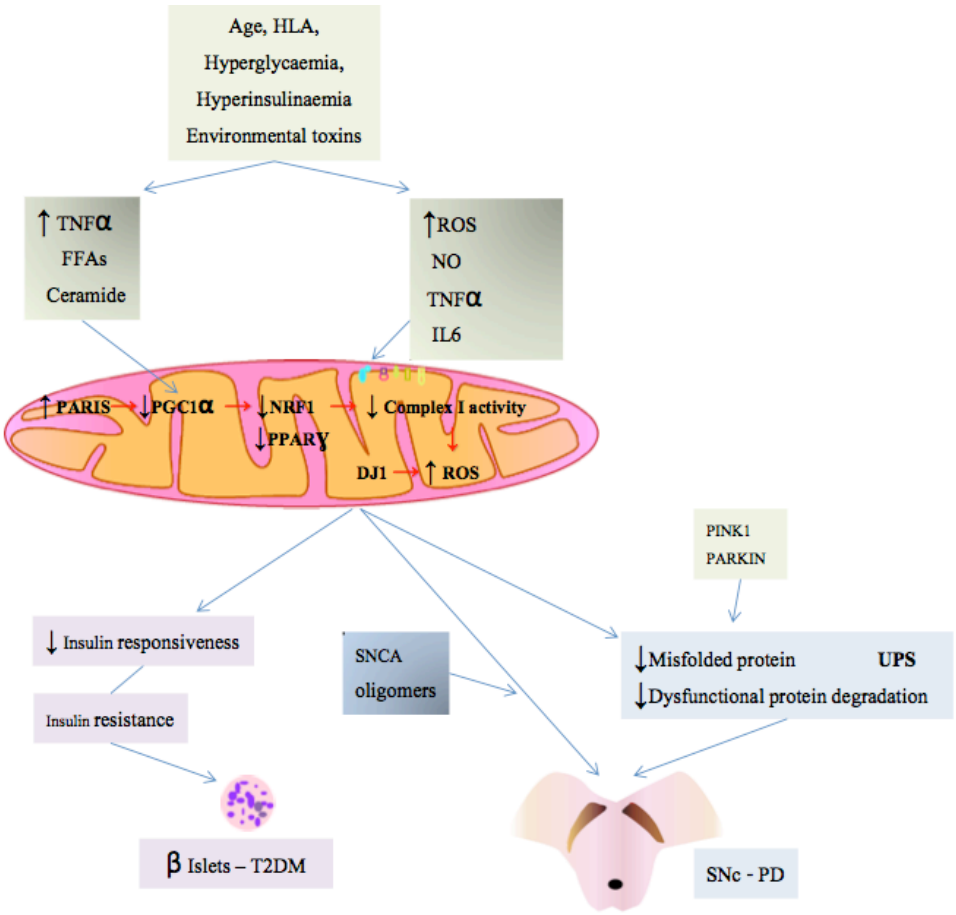


Figure 1 Schematic overview of emerging pathways linking insulin resistance and neurodegeneration.

FFAs= free fatty acids; HLA= human leucocyte antigen; IL-6= interleukin 6; LRRK-2= leucine rich repeat kinase 2; NO=nitric oxide; PGC1α= PPARɣ receptor coactivator; ROS= reactive oxygen species; UPS= ubiquitin proteasome system; SNCA= α synuclein.

1.5.8 Calcium conductance

Nigral neurons rely on calcium channels to drive their rhythmic pacemaking activity. Comparison of the two subpopulations of dopaminergic neurons from the ventral midbrain that are affected differentially inPD, namely, those in the SNc (severely affected) and those in the ventral tegmental area (VTA) (mildly affected) (Dauer & Przedborski 2003), reveals a compelling difference between these two groups of neurons. L-type calcium channel recruitment during normal autonomous pacemaking is associated with a high reactive oxygen species (ROS) signal in dopaminergic neurons of the SNc, but not in those of the VTA (Guzman et al. 2010). Based on recent findings, an intriguing hypothesis has emerged that suggests that the singular calcium-dependent pacemaking of nigral neurons is associated with increased mitochondrial ROS (Guzman et al. 2010).

1.5.9 Mitophagy

It is the current belief that PD pathogenesis might be linked to a defect in mitochondrial-control mechanisms. Defects in mitochondrial respiration had previously been proposed to contribute to the occurrence of many of the most common neurodegenerative disorders (Lodi et al. 2000). However, the discovery of genes mutated in hereditary forms of these enigmatic diseases has additionally suggested defects in mitochondrial dynamics (morphology and function i.e., organellar shape, size, distribution, movement, and anchorage). Such disturbances can lead to changes in mitochondrial quality control, mitochondrial trafficking, and inter-organellar communication. Mitochondria fuse and divide, fragment, swell and extend, and exist in clusters and as individual entities. Importantly, they travel within the cell. When static, they periodically anchor themselves on other organelles, such as the ER, endocytic vesicles, and the plasma membrane. In summary, mitochondria

are dynamic organelles that move from the cell body to regions of the cell to deliver ATP and other metabolites where they are most required. This process is seen most strikingly in highly elongated cells such as neurons; mitochondria are concentrated at presynaptic terminals at the ends of axons, and at postsynaptic terminals at the ends of dendrites, where bioenergetic demand is particularly high. When mitochondria reach the end of their life they are ultimately disposed of (and their component parts recycled) via autophagy ("mitophagy"), or via extrusion of "mitochondria-derived vesicles" (Braschi & McBride 2010). It is fully understandable that the inability of mitochondria to execute these functions would be expected to disrupt cellular physiology and viability. Consequently, the degree of impairment likely corresponds to that cell's requirements for well-functioning mitochondria positioned in the right place at the right time. PD mutations in either Parkin or PINK1 appear to impair the normal turnover of damaged mitochondria (Vives-Bauza et al. 2010). In short, it is the current belief that a loss of function of Parkin or PINK1 might prevent damaged mitochondria from being eliminated, leading to neuronal dysfunction and neuronal death. Thus, it is the current belief that defects in mitochondrial dynamics might play a pivotal role in the pathogenesis of neurodegenerative disorders.

1.6 THE GLUCAGON SUPERFAMILY OF PEPTIDE HORMONES. THE INCRETIN SYSTEM

The glucagon-like peptides include glucagon, GLP-1, and GLP-2, and exert diverse actions on nutrient intake, gastrointestinal motility, islet hormone secretion, cell proliferation and apoptosis, nutrient absorption, and nutrient assimilation. Normal regulation of insulin secretion and function is driven by the detection of glucose in the blood. Pancreatic beta cells detect the increase in blood glucose and secrete insulin in an attempt to regulate glucose levels by facilitating the transport of glucose into cells for cellular metabolic use. An alternative form of insulin regulation originates from gastrointestinal tract cells. Gut hormones facilitate the disposal of absorbed glucose through the stimulation of insulin secretion from the endocrine pancreas (Drucker & Nauck 2006). The incretin response accounts for approximately 70% of the total insulin secreted following the administration of oral glucose (Baggio & Drucker 2007).

Oral glucose administration enhances insulin secretion to a greater extent than that seen with isoglycemic intravenous (IV) loading (also known as the “incretin” effect), leading to the discovery of both glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (also known as glucose dependent insulinotropic peptide- GIP), as incretin hormones (Elrick et al. 1964). The incretin peptides: GIP and glucagon-like peptide-1 (GLP-1), integrate nutrient-derived signals to control food intake, energy absorption, and assimilation (Baggio & Drucker 2007; Holst et al. 2009; Nauck 2009).

Both peptides share common actions on islet beta-cells acting through structurally distinct yet related receptors. Therefore, incretin-receptor activation

leads to glucose-dependent insulin secretion, induction of beta-cell proliferation, and enhanced resistance to apoptosis in pancreatic cells (Drucker & Nauck 2006; Baggio & Drucker 2006; Lovshin & Drucker 2009). Both GLP-1 and GIP slow gut emptying and modulate postprandrial glucose disposal through glucose dependent inhibition of glucagon secretion (GLP-1) (Nauck et al. 2002; Holst et al. 2009), delayed gastric emptying (GLP-1) (Nauck et al. 1997), and potentially also through increased peripheral insulin sensitivity (GLP-1 and GIP) (Baggio & Drucker 2007). A key enzymatic regulator of these hormones is dipeptidyl peptidase-4, which rapidly inactivates/degrades the incretin hormones (Nauck, 2009).

Most GLP-1 is made in enteroendocrine L cells in the distal ileum and colon. Plasma levels of GLP-1, like GIP, increase within minutes of eating. The major physiological stimulus for GLP-1 and GLP-2 secretion is ingestion of a meal.

Despite the predominant localization of the L cell in the distal ileum and colon (Eissele et al. 1992), intake of either glucose or fat increases release of GLP-1 and GLP-2 within 15–30 min, with a second peak of secretion occurring at 90–120 min after a meal (Eissele et al. 1992). Hence a combination of endocrine and neural signals probably promotes the rapid stimulation of GLP-1 secretion before digested food can directly stimulate the Lcells in the small bowel and colon. (Drucker & Nauck 2006). A large number of studies in rodents have now demonstrated that placement of either glucose or fat directly into the upper gastrointestinal (GI) tract stimulates prompt rises in GLP release (Roberge & Brubaker 1993). Nutrient intake is also associated with release of two peripheral hormones with key roles in nutrient homeostasis; insulin and leptin. Interestingly, both of these hormones stimulate GLP-1 release upon activation of their respective receptors on the L cell (Anini &

Brubaker 2003). Consistent with these findings, leptin resistance has been demonstrated to decrease the effects of leptin on GLP-1 release (Anini & Brubaker 2003), and may therefore contribute to the reduced levels of GLP-1 that have been observed in obese individuals (Ranganath et al. 1996). Similarly, GLP-1 levels have been reported to be low in patients with insulin resistance or T2DM, independent of both obesity (Rask et al. 2001; Vilsbøll et al. 2001) and clearance rates (Vilsbøll et al. 2003).

The additional actions of GLP-1 appear to be largely beneficial to metabolic control; most notably inhibition of glucagon release, gastric emptying, gastrointestinal fat absorption, and appetite reduction (Holst et al. 2009; Nauck 2009).

Moreover, GLP-1 reduces glycaemia and haemoglobin A1c (HbA1c) levels in patients with T2DM, and the GLP-1 mimetics also reduce body weight (Näslund et al. 1999; Rodriquez de Fonseca et al. 2000). The glucagon-like peptides exert a wide array of biological actions including; (1) enhancement of digestion and absorption in the gastrointestinal (GI) tract, (2) facilitation of insulin secretion and nutrient disposal, and (3) promotion of growth of the intestinal epithelium and beta cells in the endocrine pancreas. These effects culminate in a positive energy balance.

The actions of these peptides are mediated by distinct members of the glucagon receptor superfamily of G protein-coupled receptors (GPCRs). These receptors exhibit unique patterns of tissue-specific expression, exhibit considerable amino acid sequence identity, and share similar structural and functional properties with respect to ligand binding and signal transduction (Brubaker & Drucker, 2002).

GLP-1 receptor (GLP-1R) belongs to the class B family of seven-trans-membrane spanning, heterotrimic GPCRs. The GLP-1R activation, causes activation of adenylylcyclase, leading to increased intracellular cAMP levels (Liu et al. 2011). Particularly exciting due to its potential implications to future therapeutic approaches, is the expression of GLP-1R in pancreatic α and β cells, heart, pituitary, kidney, lung, skin, ganglion of the vagus nerve, the central and peripheral nervous systems and gastrointestinal tract (Drucker & Nauck 2006; Baggio & Drucker 2007).

1.6.1 Exendin-4/Exenatide (GLP-1R agonist)

Exenatide is a synthetic form of Exendin-4 and is an agonist for the GLP-1 receptor. It has a circulating half-life of 60-90 minutes (Kolterman et al. 2005) with increases in plasma Exenatide concentrations lasting 4-6 hours after a single subcutaneous injection (Nielsen & Baron 2003).

1.6.1.1 The Discovery of Exendin

The glucagon superfamily consists of a diverse group of biologically active peptides that are structurally related, having an amino-terminal histidine residue (His^1) and a phenylalanine residue at position 6 (Phe^6), or one of several variant structures such as Tyr^1-Phe^6, His^1-Tyr^6, or His^1-Leu^5. An amino-terminal histidyl structure (His^1) is characteristic of most peptides in the glucagon superfamily. In 1990, an assay for amino-terminal amino acid sequencing for His^1 peptides was used as a chemical marker for biologically active peptides to screen venom from the Gila monster lizard, *Heloderma horridum* (Eng et al. 1990). Gila monster venom was chosen for screening because venoms from exocrine secretions of Heloderma lizards had been shown previously to contain two biologically active His^1-Phe^6 peptides, Helospectin (Exendin-1) and Helodermin (Exendin-2). A new His^1 peptide was

identified and named exendin-3 to indicate that it is the third peptide to be found in an exocrine secretion of Heloderma lizards. It has endocrine activity, acting as a pancreatic secretagogue (Eng et al. 1990; Vandermeers et al. 1987).

An amino acid sequencing assay for peptides containing an amino-terminal histidine residue (His^1) was used to isolate a 39-amino acid peptide, exendin-4, from *Heloderma suspectum* venom in 1992. Exendin-4 differs from exendin-3 by two amino acid substitutions, Gly^2-Glu^3 in place of Ser^2-Asp^3, at positions 2 and 3 from the amino terminus, but is otherwise identical (Eng, et al. 1992). Therefore both exendin-3 and exendin-4 are 39 amino acid peptides containing an amino-terminal histidine and a carboxyl-terminal serine amide, and are members of the glucagon superfamily of peptide hormones. In dispersed pancreatic acini, exendin-4 interacts only with the exendin receptor. The intrinsic biological activity of exendin-3 and exendin-4, in terms of increasing acinar cAMP, resides in the amino-terminal portion of the molecule. The amino acid sequence of exendin-3 and exendin-4 required for binding to exendin receptors resides in the middle and carboxyl-terminal portions of the molecule (Eng et al, 1992)

Table 1. Amino acid sequence of Exendin-4 and its comparison with peptide sequences of other members of the glucagon superfamily

EXENDIN-4	H	G	E	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S
EXENDIN-3	H	S	D	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	R	L	F	I	E	W	L	K	N	G	G	P	S	S	G	A	P	P	S
HELOSPECTIN	H	S	D	A	T	F	T	A	E	Y	S	K	L	L	A	K	L	A	L	Q	K	Y	L	E	S	I	L	G	S	S	T	S	P	R	P	P	S	S
HELODERMIN	H	S	D	A	I	F	T	E	E	Y	S	K	L	L	A	K	L	A	L	Q	K	Y	L	A	S	I	L	G	S	R	T	S	P	P	P			
GLUCAGON	H	S	Q	G	T	F	T	S	D	Y	S	K	Y	L	D	S	R	R	A	Q	D	F	V	Q	W	L	M	N	T										
GLP-1	H	A	E	G	T	F	T	S	D	V	S	S	Y	L	E	G	Q	A	A	K	E	F	I	A	W	L	V	K	G	R									

1.6.1.2 Exenatide and D.M

The appreciation that the incretin response is defective in T2DM (Nauck et al. 1986) led to the development of incretin-based therapies. GLP-1 is a naturally

occurring hormone that has an important role in insulin and glucose homeostasis but has a circulating half-life of only 1-2 minutes. Exenatide has confirmed beneficial effects on glucose control, thought to be mediated by β cell proliferation, glucose-dependent insulin production, decreased gluconeogenesis, and weight loss that follows chronic GLP-1 receptor stimulation in the GI tract. GLP-1 also stimulates the differentiation of ductal precursor cells into functional pancreatic beta cells. (Buse et al., 2004; DeFronzo et al., 2005; Drucker et al., 2008; Kendall et al., 2005).

Clinical studies utilizing the administration of Ex-4 in T2DM patients have found that patients experience decreases in caloric/food intake, hunger and body weight (Drucker & Nauck 2006; Lovshin & Drucker 2009; Vilsbøll et al. 2012). In addition, subjects had increased feelings of satiety/fullness and an increased resting metabolic rate (Bradley et al. 2010).

The starting dose of Exenatide is 5µgr twice daily for 4 weeks, followed by an increase to 10 µgr twice daily (Fineman et al. 2004). The most common adverse events with Exenatide in T2DM patients are gastrointestinal (nausea, vomitting and diarrhoea) (Buse et al. 2004; Kendall et al. 2005). In spite of gastrointestinal adverse effects, Exenatide is rarely discontinued because of them, and side effects subside with prolonged therapy (Buse et al. 2004; Kendall et al. 2005). GLP-1 receptors are also distributed throughout the brain, and stimulation of central receptors in the hypothalamus is responsible for early satiety. Exenatide was approved by the US Food and Drug Administration for the treatment of T2DM in April 2005, and it gained its indication as primary monotherapy approved by the FDA in October 2009.

(http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.DrugDetails)

The consensus algorithm for initiation and adjustment of therapy from the American Diabetes Association and European Association for the Study of Diabetes classifies the GLP-1agonist in "tier 2" category (less well-validated therapist) in 2009 (Nathan et al. 2009). Taking into account that long-term data regarding safety are lacking for incretin-based therapies, they suggest that GLP-1 agonists should be considered when weight gain or hypoglycaemia are particular concerns. The NICE guidelines suggest that Exenatide should be added as a third line therapy to first line metformin and a second-line sulfonylurea when control of blood glucose remains inadequate ($HbA_1C \geq 7.5\%$) and a body mass index ≥ 35.0 Kg/m^2 or ≤ 35.0 kg/m^2 and therapy with insulin would have occupational implications.

(http://www.nice.org.uk/nicemedia/live/12165/44318/44318.pdf).

A longer-lasting form of Exenatide (marketed as Bydureon) received Food and Drug Administration approval in January 2012.

1.6.1.3 Exendin-4 and neuronal cell model systems

The presence of GLP-1R in the brain suggests a possible role in the regulation of neuronal activity. A growing body of evidence now exists to support neuroprotective functions of GLP-1R stimulation. The fact that GLP-1R are located on neurons; pyramidal neurons in the hippocampus and neocortex, on dendrites and cell bodies suggests a likely role in neuronal activity and synaptic transmission (During et al. 2003; Alvarez et al. 1996; Calvo et al. 1995; Campos et al. 1994; Larsen et al. 1997; Hamilton & Hölscher 2009).

Particularly exciting is the realisation that similarities exist between cellular responses to incretins in pancreatic beta cells and neurons. The activation of GLP-1R

in neurons can produce cellular protection, proliferation and differentiation of precursor cells into neurons.

It is worth noting the promotion of cell proliferation in the dentate gyrus of the hippocampal formation of adult rodents after chronic *in vivo* treatment with Exendin-4, indicated by an increase in the marker of immature neuronal tissue, reflected on an increased BrdU and doublecortin (DCX) staining. Moreover, the proliferative properties of Exendin-4 in adult brain were supported by the elevation of the levels of gene transcripts for Ki-67 (Isacson et al. 2011). Furthermore, higher levels of proliferation (based upon Ki-67 staining) after long-term administration (21 days) of Ex-4 *in vivo* have been reported in the subgranular zone of adult mouse dentate gyrus (Li et al. 2009). Additionally, in mouse models of diabetes, chronic treatment with GLP-1 analogues for 4–10 weeks significantly increased the number of progenitor cells or DCX-positive young neurons in the dentate gyrus, as measured by BrdU or DCX immunohistostaining. This action was confirmed to be due to GLP-1R activation as GLP-1 receptor antagonist exendin (9–36) reduced progenitor cell proliferation in these mice (Hamilton et al. 2011).

PC12 cell lines (cells derived from a form of tumour located in the rat adrenal tissues), treated with GLP-1 did not show any significant levels of proliferation (Perry 2002b). However, the human neuroblastoma cell line, SH-SY5Y cells, treated with physiologically relevant doses of either GLP-1 or Exendin-4, showed evidence of proliferation (Li, Y et al. 2010)

In a rat striatal embryonic cell line (ST14A cells) in a primary rodent culture system, GLP-1R activation (Ex-4 and GLP-1) significantly increased mouse neural

stem cell numbers *in vitro*, as indicated by BrdU incorporation and ATP level measurements (Bertilsson et al. 2008)

1.6.1.4 Exendin-4 and neuronal cell differentiation

GLP-1R activators can induce the differentiation of neural stem cells into neurons. Immunohistochemistry studies in adult mice treated with Exendin-4 showed a 1.7-fold increase in DCX-positive cells in the medial striatum, and doubled the number of BrdU-positive cells in the subventricular zone, a known pool of neuronal stem cells (Bertilsson et al. 2008). The administration of Ex-4 for 2 weeks to adult rodent induced an elevation of markers of neurogenesis, e.g. DCX and Mash-1 gene transcripts, in the hippocampus (Isacson et al. 2011). In addition, chronic treatment of Exendin-4 has been proven to increase cell proliferation and neuroblast differentiation in the adult mouse hippocampal dentate gyrus, as mice treated with Exendin-4 showed a significant higher number of Ki-67, DXC and 5-bromo-2-deoxyuridine (BrdU) immunoreacive cells than the control group (Li H, 2010).

1.6.1.5 Exendin-4 and production of neurite outgrowth

Exendin-4 has been shown to increase the numbers of neurite-bearing cells in both human SH-SY5Y, and in PC12 cell models of neuronal differentiation (Luciani et al. 2010). In addition, GLP-1 and Exendin-4 induced neurite outgrowth in a manner similar to nerve growth factor (NGF), which was reversed by coincubation with the selective GLP-1 receptor antagonist Exendin (9-39). Furthermore, exendin-4 could promote NGF-initiated differentiation and may rescue degenerating cells after NGF-mediated withdrawal (Perry et al. 2002b).

1.6.1.6 Exendin-4 and synaptic plasticity

GLP-1 receptor stimulation has been shown to promote (long-term potentiation (LTP). LTP is defined as a long lasting enhancement in signal transmission between two neurons, considered as the cellular correlate of memory formation. Reference memory has been improved in rodents treated with Ex-4 when compared with controls using a radial maze paradigm (Isacson et al. 2011). Moreover, rats overexpressing GLP-1R in the hippocampus showed improved learning and memory with an improved fear learning performance on the Morris Water Maze (During et al. 2003).

1.6.1.7 Exendin and mitochondria

More recently, Exendin-4 has been shown to protect beta islet cells from apoptosis, to prevent damage to mitochondrial DNA encoded genes and to stimulate mitochondrial biogenesis (Fan et al, 2010). Fan and colleagues worked with clonal insulinoma (INS-1E) cells using transcriptional and translational assays to evaluate cell viability and cell mass as well as western blot to detect protein expression. The authors also used transfection of constitutively active protein kinase B (PKB/AKT) to examine the role of AKT, and quantification of mitochondrial biogenesis using mitogreen staining and RT-PCR.

To prove the potential protective action of Exendin-4 on beta cells apoptosis, the authors used human islet amyloid polypeptide (hIAPP), the major component of amyloid, which is 37-amino acid protein co-secreted with insulin by beta cells (Cooper et al. 1987). hIAPP spontaneously forms oligomers and causes beta-cell death, besides when beta cells are incubated with hIAPP, cell proliferation is

inhibited and apoptosis is induced. When given Exendin-4 to the INS-1Ecells, these cells were protected of the cell apoptosis induced by hIAPP-mediated AKT inhibition. Furthermore the mitogreen staining and RT-PCR revealed enhanced mitochondrial biogenesis.

The signalling pathways activated by GLP-1 in the beta cells are complex, and PKB/AKT is one of the key kinases involved (Wang et al. 2004; Li , L et al. 2005).

With this interesting work, the authors proved AKT involvement in downstream signalling pathways of GLP-1, proposing that the protective effects of Exendin-4 on beta cells could be facilitated somewhat by setback of impaired AKT activity and mitochondrial pathways.

1.6.1.8 Exendin-4 and animals models of neurodegeneration

GLP-1R stimulation has proven beneficial in neurotoxin-derived models of PD in terms of dopamine cell survival, cell functionality, and the resolution of abnormal behaviour. The use of GLP-1R agonists has been shown to protect animals against MPTP. The MPTP toxic insult was reversed by Exendin-4, which diminished the level of inflammation and increased the number of viable dopaminergic neurons (Kim et al. 2009; Li et al 2009). Exendin-4 induced elevation of endogenous TH levels (key enzyme for the production of dopamine, that converts tyrosine into L-DOPA, precursor of dopamine) in primary dopaminergic neurons (Li et al. 2009). Exendin-4 significantly elevates TH levels in catecholamine neurons in the area postrema, likely mediated by Exendin-4 induction of TH gene expression through the TH promoter (Yamamoto et al. 2003).

The 6 hydroxydopamine (6-OHDA) and Lipopolysaccharide (LPS) toxic insult was similarly reversed in rats treated with Exendin-4. In these rodents the apomorphine circling behaviour was alleviated by Exendin-4 in a dose-dependent manner (Harkavyi et al. 2008)

In pyridoxine (vitamin-B6) over-treated rats, which develop a form of peripheral neuropathy induced by over-consumption of vitamin B6, Exendin-4 has been found to improve functional and behavioural deficits in addition to morphological normalization of the sciatic nerve and dorsal root ganglia (Perry et al. 2007).

In the toxic model of Huntington Disease (HD), N171-82Q induced an improvement of abnormal blood sugar levels and reduced quantities of mutant huntingtin protein accumulations in pancreas and brain. Exendin-4-treated animals had lower motor function deficits and 18% higher animal survival time compared with control N171-82Q mouse life span (Martin et al. 2009).

Based on the fact that motor neurons express the GLP-1R, a recent study has focused on the potential benefits of GLP-1R activation in cellular and mouse models of Amyotrophic Lateral Sclerosis (ALS) (Li, Y et al. 2012). These actions largely translated to Superoxide dismutase-1 (SOD-1) (G93A) mice, in which subcutaneous (s.c). Exendin-4 mitigated the dysregulation of glucose evident in animals.

Exendin-4 was also neuroprotective at the level of the spinal cord, preserving neuron density and spinal cord structure with a clear decrease in loss of cholinergic markers and apoptosis (Li, Y et al. 2012).

Exendin-4 can protect neurons against metabolic and oxidative insults. It has been shown that administration of Exendin-4 reduced brain damage and improved functional outcomes in a transient middle cerebral artery occlusion stroke model (Li ,Y et al. 2009).

GLP-1R mRNA expression was detected in cultured embryonic primary cerebral cortical neurons, which are vulnerable to hypoxia. Interestingly it was found that GLP-1 and EX-4 conferred protection in these cells, but not in cells from GLP-1R knockout mice (Li, Y et al, 2009). Further effects from central GLP-1 stimulation are as yet unclear, but neurotrophic and neuroprotective properties have been identified in vitro (Perry et al. 2002a). Exenatide has been evaluated as a neuroprotective agent in multiple animal models of PD (Bertilsson et al., 2008; Harkavyi et al., 2008; Kim et al., 2009; Li et al., 2009), demonstrating consistent benefits. Its mechanism of action however remains unknown and could be due to anti-inflammatory effects (Harkavyi et al., 2008; Kim Chung le et al., 2009; S. Kim et al., 2009), or related to stimulation of neurogenesis (Belsham et al., 2009; Bertilsson et al., 2008; Li, Y et al. 2010). (For further description see section 1.6.1.9.4.2.Mechanism of action in neurodegenerative animal models).

1.6.1.9 Pharmacokinetic properties

1.6.1.9.1 Absorption

Following subcutaneous administration to patients with T2DM, at 0.02, 0.05, and 0.1 μgr/Kg Exenatide doses, Exenatide was detectable in plasma as early as 10-15 minutes post dose, demonstrating its rapid absorption after s.c. injection. The corresponding geometric mean AUC $_{0\text{-}5hr}$ values were 9.364, 23.224, and 1.387 pg*min/mL, respectively, and the corresponding geometric mean C_{max} values were

45.109 and 187 pg/mL, respectively. Exenatide reaches peak plasma concentrations in 2 hours. Exenatide exposure increased proportionally over the therapeutic dose range of 5 μg to 10 μg. Similar exposure is achieved with subcutaneous administration of Exenatide in the abdomen, thigh or arm. (Kolterman et al., 2005)

1.6.1.9.2 Metabolism and Elimination

Non-clinical studies have shown that Exenatide is predominantly eliminated by glomerular filtration, with subsequent proteolytic degradation (Copley et al. 2006). However, receptor mediated endocytosis and degradation may be responsible for the nonrenal clearance (Gao & Jusko 2012). Exenatide's elimination half-life ranged from 3.3 to 4.0 hours (Yoo et al. 2006). These pharmacokinetic characteristics of Exenatide are independent of the dose (Kolterman et al., 2005).

1.6.1.9.3 Exenatide and Blood Brain Barrier (BBB)

Up to date, using high-performance liquid chromatography Exendin-4 has been proved to cross the blood brain barrier in the mouse. Its high lipophilicity helps it to cross the BBB directly at a fast rate (Kastin & Akerstrom 2003). However, no data in humans are available so far.

An interesting approach is the one performed using a recombinant GLP-1-human serum albumin fusion protein (Albugon) in a combination of cell line studies in vitro and both wild –type and GLP-1R$^{-/-}$ (Baggio et al. 2004). Remarkably is the fact that GLP-1-albumin protein should exhibit a much more prolonged circulating $t_{1/2}$, given the long circulating $t_{1/2}$ of albumin linked drugs. This approach was taken into consideration when looking for strategies which allow an extension in the circulating $t_{1/2}$ of GLP-1. Albugon was proven to activate GLP-1receptor-dependent cAMP formation, decreasing glycaemic excursion and stimulate insulin secretion in

wild type but not in the GLP-1R$^{-/-}$ mice, as well as decreasing food intake after intracerebroventricular and intraperitoneal administration. What it is really outstanding is that after intraperitoneal injection of Albugon, there was an inhibition of gastric emptying and more importantly an activation of c-FOS expression in the area postrema, the nucleus of the solitary tract, the central nucleus of the amygdala, the parabrachial, and the paraventricular nuclei. Therefore this approach constituted a good approach to determine the relative importance of peripheral versus central GLP-1R networks for control of satiety and gut motility, supporting a model in which peripheral activation of GLP-1R-dependent vagal afferents is capable of activating CNS centres, consequently transducing the effects of GLP-1 in the brain (Wettergren et al. 1998; Imeryüz et al. 1997).

1.6.1.9.4 Pharmacodynamic properties

1.6.1.9.4.1 Mechanism of action in diabetic patients

See section 1.6.1.2

1.6.1.9.4.2 Mechanism of action in neurodegenerative animal models

1.6.1.9.4.2.1 Anti-inflammatory

In MPTP mouse models of PD, peripheral administration of Exendin- 4 boosts the survival of dopaminergic neurons by deactivating microglia, attenuating nigrostriatal dopaminergic neuron loss. It has been shown to inhibit microglial activation and to release microglia-derived pro-inflammatory mediators. The systemic administration of Exendin-4 significantly attenuates the loss of SNc neurons and the striatal dopaminergic fibres, as it has been seen in mice injected with exendin-4 thirty minutes prior to MPTP lesion. These mice preserved the number of TH cells and TH fibre density (Kim et al. 2009). Here in UCL, Exendin-4 has been

investigated in 2 separate animal PD models, LPS & 6OHDA, showing attenuation in the Apomorphine-induced circling behaviour, and higher TH activity in brain homogenates, suggesting protection against loss of TH+ cell (Harkavyi et al. 2008). Exendin-4 also appears to have neurotrophic properties in vitro: incubation with Exendin-4 with PC12 cells promotes neurite outgrowth in a similar manner to NGF, and may rescue degenerating cells after NGF withdrawal (Perry et al 2002b). It was also shown to protect hippocampal cells against glutamate-induced apoptosis, and protect against cholinergic cell degeneration in the rodent model using ibotenic acid injection into the basal forebrain cholinergic neurons (Perry et al. 2002a). Exendin-4 prevents MPTP-induced microglial activation in the SNc and striatum, and the expression of matrix metalloproteinase-3 (MMP-3). In addition, exendin-4 also suppressed MPTP-induced expression of pro-inflammatory molecules as TNFα and interleukin-1b (IL-1b) (Brouckaert et al. 2009; Li, Y et al. 2009). MPTP remarkably increases the number of activated microglia in both the SNc and striatum 24h after the injection. MMP-3, laminin and α-synuclein, released from damaged dopaminergic neurons, activate microglia to produce pro-inflammatory and neurotoxic molecules, resulting in neuronal death. This microglia-mediated self-perpetuating cycle of the neurotoxic activation of microglia in response to neuronal injury is one of the principal processes that drives progressive dopaminergic neurodegeneration (Liu et al. 2006). GLP-1 could inhibit LPS-induced IL-1b mRNA expression and IL-1g production in rat astrocytes (Iwai et al. 2006). Exendin-4 may function as a survival factor for dopaminergic neurons by preventing the morphological abnormalities induced by MPTP neurotoxicity.

1.6.1.9.4.2.2 Neurorestorative

Exendin-4 is able to promote adult neurogenesis in vitro and in vivo, normalize dopamine imbalance, and increase the number of cells positive for markers of dopaminergic neurons in the substantia nigra in a 6OHDA model of PD (Bertilsson et al. 2008). When Exendin-4 was given intraperitoneally to naıve rodents together with bromodeoxyuridine, a marker for DNA synthesis, both the number of bromodeoxyuridine-positive cells and the number of neuronal precursor cells expressing doublecortin were increased (Bertilsson et al. 2008). In the adult mammalian brain, new neurons and glial cells are continuously generated from a proliferating population of neural progenitor/stem cells (NSC) that become incorporated into the existing brain by a process, known as adult neurogenesis (Emsley et al. 2004). This process occurs mainly in the subventricular zone (SVZ)/olfactory bulb and hippocampal dentate gyrus (Palmer et al. 1995; Emsley et al. 2005). The existence of active, functional adult neurogenesis raises the possibility that pharmacological stimulation of endogenous neural stem/progenitor cells could lead to cell regeneration that may be beneficial in central nervous system disorders where cell loss occurs, e.g., Parkinson's disease (PD) (Emsley et al. 2005; Taupin 2005).

Exendin-4 was tested in the 6OHDA model of PD to investigate its possible functional effects in an animal model with neuronal loss. After unilateral lesion and a 5-week stabilization period, the rats were treated for 3 weeks with Exendin-4. Animals receiving Exendin-4 showed a reduction of amphetamine-induced rotations, that persisted for several weeks after drug administration had been terminated. Histological analysis showed that Exendin-4 significantly increased the number of both tyrosine hydroxylase (TH) and vesicular monoamine transporter 2 (VMAT-2) -

positive neurons in the substantia nigra. The improvement continued throughout the Exendin-4 treatment period and, remarkably, persisted for another 5 weeks in the absence of the treatment. Thus, this seems likely to be a plastic, rather than a transitory effect, and suggests that Exendin-4 is indeed triggering a stable change in the brain by improving the course of the pathology rather than just exerting a symptomatic and acute effect. The increase in the number of neurons positive for two DA markers, TH and VMAT2, reinforces the hypothesis that Exendin-4 is acting as a disease-modifying molecule (Bertilsson et al. 2008).

1.6.1.9.5 Possible expected serious adverse events

1.6.1.9.5.1 Exenatide and renal disease

Exenatide is not recommended for use in patients with end-stage renal disease or severe renal impairment (creatinine clearance <30ml/min). There have been reports of altered renal function, including increased serum creatinine, renal impairment, worsening chronic renal failure and acute renal failure, sometimes requiring haemodialysis. Some of these events occurred in patients with altered hydration states, (including nausea, vomiting and/or diarrhoea), and/or receiving pharmacological agents known to affect renal function/hydration status. Concomitant agents included angiotensin converting enzyme inhibitors, angiotensin-II anagonists, non-steroidal anti-inflammatory medicinal products and diuretics (López-Ruiz et al., 2010). Reversibility of altered renal function has been observed with supportive treatment and discontinuation of potentially causative agents, including Exenatide (Aroda & Ratner, 2011; Macconell et al. 2012).

1.6.1.9.5.2 Exenatide and gastrointestinal disease

Exenatide has not been studied in patients with severe gastrointestinal disease, including gastroparesis. Its use is commonly associated with gastrointestinal adverse reactions, including nausea, vomiting, and diarrhoea. Therefore, the use of Exenatide is not recommended in patients with severe gastrointestinal disease. (Aroda & Ratner, 2011).

The most frequently reported adverse reaction is nausea. In patients treated with 5µg or 10µg Exenatide, generally 40-50% reported at least one episode of nausea. Most episodes of nausea were mild to moderate and occurred in a dose-dependent fashion. With continued therapy, the frequency and severity decreases in most patients who initially experienced nausea (Macconell et al., 2012).

1.6.1.9.5.3 Exenatide and pancreatitis

There have been reported events of acute pancreatitis, although they are rare. Resolution of pancreatitis has been observed with supportive treatment, but very rare cases of necrotizing or haemorrhagic pancreatitis and/or death have been reported. If pancreatitis is suspected, Exenatide and other potentially suspect medical products should be discontinued (Dore et al., 2011; Wenten et al., 2012).

Cohort studies have found no increased risk of acute pancreatitis with current or recent use of Exenatide twice daily compared with use of other anti-diabetic drugs. In a follow-up study investigating the incidence of acute pancreatitis, after adjustments for (1) propensity score, (2) insulin and (3) use of medication potentially associated with acute pancreatitis, the odds ratio for Exenatide causing pancreatitis twice daily exposure was 0.95 (95% 0.65-1.38). A secondary analysis that examined current, recent and past medication exposure found no increased risk of acute

pancreatitis with Exenatide twice daily, regardless of exposure category. This study indicates that exposure to Exenatide twice daily was not associated with an increased risk of acute pancreatitis compared with exposure to other anti-diabetic medications (Wenten et al., 2012).

Exenatide has been shown to promote pancreatic duct hyperplasia in rats (Nachnani et al. 2010). Metaplasia and ductal proliferation are well-established features of human pancreatitis and risk factors for pancreatic cancer (Jura et al. 2005). It should be kept in mind that patients with T2DM have greater risk of pancreatitis than non diabetic patients (Girman et al. 2010), likely due to increased frequency of pancreatic duct replication (Jura et al. 2005). This makes more difficult to clarify possible links between increase incidence of pancreatitis and pancreatic adenocarcinoma and the use of Exenatide.

1.6.1.9.5.4 Exenatide and weight loss

Weight loss greater than 1.5kg per week has been observed in approximately 5% of clinical trials patients treated with Exenatide for glycemic control. Weight loss of this rate may have harmful consequences (Rosenstock et al., 2012; Tina Vilsbøll et al. 2012).

1.6.1.9.5.5 Exenatide and hypoglycemia

Studies in patients treated with Exenatide and a sulphonylurea (with or without metformin) have shown that the incidence of hypoglycaemia was increased compared to placebo (23.5% and 25.2% versus 12.6% and 3.3%) and appeared to be dependent on the dose of both Exenatide and sulphonylurea. Exenatide has a glucose level dependent hypoglycaemia effect, and hypoglycaemia in absence of other anti DM agent is very rare. Most episodes of hypoglycaemia were mild to moderate in

intensity, and all resolved with oral administration of carbohydrate (Aroda & Ratner, 2011; Macconell et al., 2012).

1.6.1.9.5.6 Injection Site Reactions

Injection site reactions have been reported in approximately 5.1% of subjects receiving Exenatide in long-term (16 weeks or longer) controlled trials. These reactions have usually been mild and usually did not result in discontinuation of Exenatide (Blevins et al., 2011).

1.6.1.9.5.7 Immunogenicity

As with all protein/peptide therapeutics, patients may develop anti-Exenatide antibodies following treatment with Exenatide. In most patients who develop antibodies, antibody titres diminish over time and remain low through 82 weeks. Patients who develop antibodies to Exenatide tend to have more injection site reactions (redness of skin and itching), but otherwise similar rates and types of adverse events as those with no anti-exenatide antibodies. Examination of antibody-positive specimens from one long-term uncontrolled study revealed no significant cross-reactivity with similar endogenous peptides (glucagon or GLP-1)(M S Fineman et al., 2012)

1.6.1.9.5.8 Exenatide and cancer

Concerns have been raised about GLP-1 trophic effects. Analyses of adverse events database suggest higher incidences of pancreatic and medullary thyroid carcinoma in patients treated with GLP-1 agonists. A recent article reviewed the literature available in the Medline database until March 2012 concluding that it is more likely due to stimulation of premalignant lesions, rather that new neoplasms induced. On the other hand, data have emerged suggesting beneficial effects of GLP-

1 on colon and breast cancer. Therefore GLP-1 agonists remain contra-indicated in patients with a personal history of multiple endocrine neoplasia type 2 or medullary thyroid cancer, but the data do not justify screening for those or other malignancies in patients treated with incretin-based therapies (Vangoitsenhoven et al. 2012).

1.6.1.9.5.9 Overall safety assessment

Exenatide has been the subject of phase III drug trials investigating the efficacy of subcutaneous administration (5 or 10μg twice daily) in the treatment of patients with Type 2 Diabetes (Buse et al., 2004; DeFronzo et al., 2005; Drucker et al., 2008; Kendall et al., 2005) and was approved by the FDA for the treatment of T2DM in April 2005. It received European marketing authorisation for the treatment of Diabetes in November 2006.

Subcutaneous administration of Exendin-4 at the doses used for Diabetes is thought to be very safe. The risk of hypoglycaemia among non-diabetic patients is negligible. The positive actions of Exendin-4 seen in the animal models of PD were achieved at doses equivalent to or lower than those used in the treatment of T2DM. Combined with an extensive impressive safety record, backed up by the FDA and EMEA regulator websites, a further advantage of Exendin-4 / Exenatide is the ability to readily cross the blood brain barrier after peripheral administration, due to its high lipophilicity (Banks, During, & Niehoff, 2004; Kastin & Akerstrom, 2003).

No drug has been shown to slow or reverse the neurodegenerative process of PD. All currently licensed therapies act as symptom relieving agents but have a limited lifespan of effectiveness because of continued brain cell loss. The preclinical work has shown that Exenatide may induce beneficial physiological effects on neuronal cell proliferation and neuronal stem cell differentiation. The vast amount of

work in laboratory models of PD suggest that Exenatide may work as a neuromodulatory agent that may slow or reverse the neurodegenerative process of PD. Positive results in these preclinical studies suggest that it will be highly effective in the treatment of patients with mild to moderate PD, assuming its physiological properties remain in pathological conditions in the human brain.

1.6.2 Liraglutide

Liraglutide is an anti-T2DM GLP-1R agonist that only requires once daily administration to improve glycaemic control (Campbell 2011). It can be used as monotherapy or in combination with metformin, a sulphonylurea but not with insulin (Campbell 2011). Interestingly, liraglutide was shown to be more effective than Exenatide in decreasing HbA_1C, with less nausea and hypoglycaemia episodes, although side effects were more severe (Campbell 2011). It has been shown to increase proliferation of progenitor cells in the subgranular zone of the dentate gyrus in high fat-fed mice and non-obese mice (Hamilton et al. 2011). Intra-hippocampal injection of liraglutide in rats also protects against Aβ induced impairment in learning and memory in a dose-dependent manner, supporting the idea that it could be a potential therapy for memory loss in AD patients (Han et al. 2013).

Hunter and Holscher have recently measured the kinetics of Liraglutide crossing the blood brain barrier (BBB), and effect on the GLP-1R, neuronal stem cell proliferation and neurogenesis (K. Hunter & Hölscher 2012). According to these authors, Liraglutide crossed the BBB at 25 and 250 nmol/kg ip, but no increase was detectable at 2.5 nmol/kg ip. 30 min post-injection, and at 250 nmol/kg ip. at 3 h post-injection and such transport may shutdown upon supraphysiologic doses (Hunter & Hölscher 2012).

1.6.3 Lixisenatide

Lixisenatide is another GLP-1R agonist. Lixisenatide is under development to be used as a once-daily treatment for T2DM (Christensen et al. 2011). Lixisenatide crossed the BBB at all doses tested (2.5, 25, or 250 nmol/kg bw ip.) when measured 30 min post-injection and at 2.5-25 nmol/kg bw ip. 3 h post-injection. Lixisenatide also enhanced neurogenesis in the brain (Hunter & Hölscher 2012). The main difference between the currently available incretin mimetics and those in clinical development besides lixisenatide seems to be in the pharmacokinetic profile (Christensen et al. 2010). With liraglutide, the large amount of bound drug confers a reservoir that provides stable plasma concentrations, whereas the short half-life of lixisenatide (and Exenatide) leads to plasma concentration that varies from very low to therapeutic values, leaving less room for development of tachyphylaxis to the gastrointestinal side effects (Christensen & Knop 2010).

1.6.4 Once-weekly dosing GLP-1R agonist

The novel long-acting incretin mimetics appear as promising antidiabetic drug candidates. However, these compounds are not one uniform group, and pharmacologic differences could influence the therapeutic achievements of these agents (Albiglutide/albugon, CJC-1131, CJC-1134-PC, Exenatide once weekly (Bydureon), and Taspoglutide). Only one head-to-head trial has been reported yet (Buse et al. 2010; Drucker et al. 2008) demonstrating that the longer acting agonists were superior with regard to glycaemic control and produced no difference in terms of body weight change. Notably, patient-reported treatment satisfaction and quality of life were also improved with the longer-acting agonist.

1.7 OTHER ANTI-T2DM WITH POSSIBLE NEUROPROTECTIVE FEATURES

1.7.1 PPAR gamma agonists; thiazolidinediones (rosiglitazone, pioglitazone)

The thiazolidinediones, (from the class of anti-T2D drugs, the peroxisome proliferator-activated receptor ɣ (PPARɣ) agonists) are insulin-sensitizing drugs that function by binding the PPAR ɣ in fat cells. Rosiglitazone and pioglitazone are known to control neuroinflammation and oxidative stress, and have been proved to prevent motor and olfactory dysfunctions in parkinsonian mice (Schintu et al. 2009). They were shown to decrease stroke-induced brain damage and neurobiological dysfunction in T2DM mice (Tureyen et al. 2007). However, rosiglitazone's and pioglitazone's side-effects in terms of cardiovascular risk led FDA to pose some restrictions for thiazolidinediones prescription Pioglitazone is a licensed treatment for patients with T2DM, and reduces insulin resistance via its action on the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ). It modulates the transcription of genes involved in insulin sensitivity. Of major interest is the recent observation that pioglitazone and other active thiazolidinedione compounds bind to the outer mitochondrial membrane protein (mitoNEET) with an affinity comparable to its binding to PPAR-γ and there has been a suggestion that many of the clinical effects of Pioglitazone are mediated by binding to mitoNEET (Colca et al. 2004; Paddock et al. 2007; Wiley et al. 2007). MitoNEET plays a key role in electron transport and oxidative phosphorylation, and Pioglitazone binding to MitoNeet has been shown to have positive regulatory effects on Complex 1 activity in neuronal cells (Ghosh et al. 2007). Pioglitazone has been shown to be protective

against neurodegeneration in MPTP mouse models of PD (Breidert et al. 2002). The neuroprotective properties of pioglitazone have been suggested to be mediated via a sequential action through PPAR activation, iNOS induction and NO-mediated toxicity (Dehmer et al. 2004). Pioglitazone has also been shown to be neuroprotective against the lipopolysaccharide model of PD through reduction of microglial activation and reduction of oxidative stress, enabling restoration of mitochondrial function (Hunter et al. 2007). Conflicting data has however suggested that the mechanism of action of Pioglitazone in protection against MPTP induced neurodegeneration is solely mediated through the inhibition of MAO-B, which prevents metabolism of MPTP to the toxic MPP+ (Quinn et al. 2008). In view of this uncertainty, but responding to the pressing need to evaluate this agent in patients with PD, a randomised trial has been initiated to explore possible neuroprotective effects of Pioglitazone among patients already on MAO-B inhibitors (Clinical Trials.gov Identifier NCT01280123).

1.7.2 Dipeptidylpeptidase-4 (DDP-4) inhibitors (Vildagliptin and Sitagliptin)

The Dipeptidylpeptidase-4 (DDP-4) inhibitors are incretin enhancers; improving endogenous GLP-1 levels to the upper limit of the normal physiological range (Drucker & Nauck 2006). They are not generally associated with a deceleration of gastric emptying or weight loss. Typically, they reduce serum DDP-4 activity by more than 80% with some inhibition maintained for 24h after one dose or with once daily treatment (Herman et al. 2005).

2 A TRIAL OF EXENATIDE FOR PD PATIENTS

2.1 INTRODUCTION

This study was designed to collect preliminary safety and efficacy data regarding the possible long-term biological effects of Exenatide in patients with treated PD, this factor being the main driver in the trial design.

2.1.1 Trial design

Several clinical trials of putative neuroprotective agents in PD have shown positive results. However, it could not be determined with certainty if the benefit was due to a neuroprotective effect because of potentially confounding pharmacologic or regulatory effects of the study agent. A major hurdle for the confirmation of any neuroprotective activity is the use of an appropriate clinical trial design.

2.1.1.1 Open label versus Placebo control

Having applied for funding to perform a large placebo controlled evaluation of Exenatide, and received feedback advising us to obtain open label data in view of the inevitable high expense of a placebo controlled design, because of pen style that needs good manufacturing practice (GMP) certification; we were compelled to choose an open label trial design for this “pilot” evaluation of Exenatide.

2.1.1.2 Randomised controlled parallel group versus delayed start versus crossover

There has been great interest in the use of novel trial designs such as the delayed start approach used in the TEMPO & ADAGIO trials (Parkinson Study Group 2002a; Olanow et al. 2009). These suggest that more prolonged therapy had a

positive effect on the underlying disease process. Despite encouraging findings from these trials there has been criticism of the delayed start approach. There is likely to be differential drop out in the two arms of a delayed-start trial, with more patients allocated to delayed treatment requiring symptomatic therapy (Clarke et al. 2011).

In a pharmacokinetic model of symptomatic and disease modifying effects in delayed-start and wash-out design studies using data from DATATOP and ELLDOPA, it has been concluded that delayed-start design is capable of

differentiating symptomatic from disease modifying effects with sufficient power only if the disease modifying effect was larger than 7 total UPDRS units per annum (Ploeger & Holford 2009). Such a difference has not been seen in any of the three delayed-start trials to date.

Given the open label nature of this project, and our wish to try and differentiate symptomatic from disease modifying effect, we chose a parallel group, randomised controlled design including a prolonged washout period to provide the possibility of distinguishing symptomatic from disease modifying effects.

2.1.1.3 Symptomatic versus Disease modifying

The 2 months washout period between 12 months follow up and 14 months follow up will provide some indication to differentiate symptomatic from disease modifying effects. As a substantial amendment to the initial trial design, an additional visit for all patients at 24 months was scheduled to collect further data to distinguish symptomatic from disease modifying effects (see Chapter 4).

2.1.1.4 Outcome measures- Clinical/biomarkers

Each of the outcome measures that have been used in clinical trials of interventions claiming neuroprotective effects in PD to date have been potentially confounded by symptomatic or pharmacological effects of the study intervention, so that if study results are positive, it cannot be unequivocally determined that the agent has an effect on disease progression. Options have been proposed, like the LS-1 study that enrolled a total of 1741 early treated PD patients that were randomized to either 10g of creatine/day or matching placebo. Thus participants were required to be receiving dopaminergic therapy before randomization, hoping to target participants who were at or near their maximum benefit from such therapy at the time of enrolment. Therefore the authors tried to avoid the variable improvement that occurs in patients when first being on dopaminergic therapy, and they followed them over a sufficiently long period of time, such that progression of features causing disability (i.e. balance impairment, and cognitive decline) could be observed (Elm 2012). The importance of assessing the natural evolution of treated PD from onset, so that relevant outcome measures can be identified for clinical trials, has been pointed out by Evans and colleagues. These authors followed a cohort of 132 PD patients from diagnosis for up to 7.9 years, finding that axial (gait and postural) symptoms evolved more rapidly than other motor features of PD and appeared to be the best index of disease progression. Consequently this group suggested that the efficacy of disease modifying therapies may be more meaningful assessed in terms of their effects in delaying the major milestones of PD, such as postural instability and dementia (Evans et al. 2011). Thus, it is very difficult to declare with certainty that an intervention is neuroprotective even if there are significant benefits in comparison with placebo in a clinical trial. Standard clinical scales including the motor section of

the UPDRS in the practically defined "Off" medication state as primary outcome measure, were chosen in our trial design. Although there is as yet no reliable and validated biomarker of PD progression, a small imaging arm was incorporated into the trial design to evaluate whether there was any evidence of a change in pre-synaptic dopaminergic integrity (DaT SPECT scans). There are well documented criticisms of DaT SPECT scans discussed in Chapter 3.

2.1.1.5 Study population of interest: Stage of disease

Careful consideration was given to the stage of PD targeted for recruitment. Evidence from various sources suggests that the rate of progression in early PD is faster than in later disease (Fearnley & Lees 1991; Hilker et al. 2005; Anette Schrag et al. 2007). Ideally the study of any novel drug would be performed in patients with early disease (de novo, untreated patients) but this approach is difficult as it can risk the inclusion of non PD patients, which, in a small study, can greatly limit the ability to distinguish positive effects. Given the need to collect useful pilot data in a timely fashion before embarking on a larger placebo controlled evaluation, and the longer follow up periods of early PD patients required to be able to see positive clinical effects in the presence of mild disability, a moderate PD group was chosen.

We recruited patients with mild-moderate PD who had already received L-dopa and were aware of "wearing off" effects which would therefore allow us to judge the effects of Exenatide on the "off phase" periods of PD (e.g. as in DBS trials and GDNF trials) as well as allowing us to judge effects on non-motor symptoms including cognition and gait problems. It was also felt that PD patients with a degree of disability would be more likely to maintain long term compliance, given that there are well recognised common side effects of Exenatide.

2.1.1.6 Duration

Beneficial effects in animal models have been seen over weeks however we decided that a minimum period of 12 months exposure to Exenatide would be necessary to judge any effect on the neurodegenerative process, in the absence of any symptomatic effects, as well as necessary to judge the tolerability of this drug in the PD. A washout visit at 14 months (after a 2 month washout period), again repeated at 24 months (a 12 month washout period) follow up also allowed us to collect long term follow-up data. The fact that longer term exposure to the drug if ineffective may reduce compliance was also taken into consideration.

2.1.1.7 Cost and Funding availability (MJFF/CPT)

A request was made to the Michael J Fox Foundation (MJFF) to support a double blind placebo controlled trial but the advice received was to collect open label pilot data to reduce risk and subsequently inform the design of a placebo controlled evaluation. This trial was finally supported by The Cure Parkinson's Trust (CPT).

2.1.1.8 Commercial versus academic led

This trial is non-commercially supported; it is entirely funded by the Cure Parkinson's Trust. The owners of the Intellectual Property of Exenatide (Amylin) were made aware of the trial and requested to be informed of the trial results.

2.1.1.9 Proof of concept

Preclinical animal testing of Exendin-4, is incapable of determining whether it will have an important clinical effect in PD. Therefore, there is a great need for early

studies that will confirm such an effect in a short time period, exposing as few patients as possible. Such studies are called “proof of principle trials” (B. Schmidt 2006). This step of proof-of-principle (or proof-of-concept) often links between Phase-I and dose ranging Phase-II studies. These small-scale studies are designed to detect a signal that the drug is active on a pathophysiologically relevant mechanism, as well as preliminary evidence of efficacy in a clinically relevant endpoint.

In recent years, drug development has been focused on early identification of the viability of candidate molecules for full clinical development to a commercially competitive product. The important step of proof-of-principle or proof-of-concept studies shifts decisions for continuing development to early, less costly stages of development, thereby reducing the cost of failures. Such studies may include proof of bioavailability in humans as well as an indication of tolerability and safety. Proof of concepts studies are small, brief, scientifically rather than regulatory driven designs.

The current trial design was conceived following feedback from both commercial and charitable organizations, which confirmed the impression that the risks associated with investment into potential neuroprotective agents need to be mitigated via the preliminary collection of cost-efficient (open-label) data in the first instance. In this context, the current study was designed as a proof of principle; i.e., with the aims of collecting rapid and cost-efficient data regarding the tolerability of Exenatide in patients with PD and providing preliminary indications whether the major neuroprotective and neurorestorative effects of Exenatide seen in the animal models might be replicable in human individuals with PD.

2.2 METHODS

2.2.1 Patients

All patients considered for the study had (at recruitment visit) a diagnosis of Idiopathic PD of moderate severity, with akinesia plus at least one of the following signs: muscular rigidity, tremor at rest and postural instability. All patients followed Queen Square Brain Bank criteria for the clinical diagnosis of PD (Hughes, Daniel, et al. 1992a; Hughes, Ben-Shlomo, et al. 1992b). Patients were between 45 and 70 years old, male or postmenopausal or surgically sterilised female, with disease onset after age 40 years and disease duration > 5 years.

Patients were on L-dopa treatment, with a history of wearing off phenomena and duration of action of single dose of L-dopa <6 hours.

All patients had improvement in response to levodopa, with improvement of at least 33% in the total Unified Parkinson's Disease Rating Scale (UPDRS) score, after a first morning dose of L-dopa.

The exclusion criteria were chosen to ensure that individuals at risk of the known adverse effects of Exenatide were excluded from the trial.

A full list of the Inclusion and the Exclusion criteria are documented in table 1.

2.2.1.1 Inclusion criteria

As stated in Table 1, we included patients with moderate severity of PD with UPDRS part III off medication at least 15 points, allowing us to appreciate a possible improvement along the 14 month follow up period, and avoiding recruitment of non-PD patients (e.g. Progressive Supranuclear Palsy (PSP), Multiple System Atrophy

(MSA) patients). There are no safety data of Exendin-4 on pregnancy so we included women only if sterilised or post-menopausal. We did not include very young PD patients to avoid over-representation of the genetic forms of PD, neither did we include very old patients who would be more likely to experience severe comorbidity. Our patients were all on L-dopa treatment as we wanted to ensure L-dopa responsiveness. They were also on a stable treatment regimen before inclusion to make sure that any changes were not due to delayed responses to medication adjustments made before baseline. They must have experienced wearing off when included so it would make easier to appreciate possible improvements. We anticipated that patients with disability would be more likely to maintain long term compliance of injectable treatment.

2.2.1.2 Exclusion criteria

We excluded patients that did not meet the Queen square brain bank PD diagnostic criteria or had a diagnosis or suspicion of other cause for Parkinsonism including Vascular parkinsonism, post traumatic parkinsonism, drug or toxin induced parkinsonism, or other neurodegenerative condition. We excluded patients having Parkinsonism possibly due to recent neuroleptic intake or having any major brain imaging abnormality. We did not include demented patients nor depressive patients as it will be more difficult for them and their families to maintain compliance. We excluded patients with previous brain surgery or in any other PD trial, to avoid confusing outcomes. We excluded diabetic patients or patients on diabetic treatment, to make sure any possible change in their PD signs were not related to any change in their diabetes control. And finally we excluded conditions that may increase the risk or trigger pancreatitis like history of alcoholism or

pancreatitis, as well as severe cardiac gastrointestinal or renal disease to minimize the associated risk of these with Exenatide.

Table 2. Inclusion and exclusion criteria

INCLUSION CRITERIA	*EXCLUSION CRITERIA*
Idiopathic PD of moderate severity. (Hoehn y Yard 2 to 2.5)	Other cause of parkinsonism
Male or postmenopausal or surgically sterilised female	Abnormality on CT or MRI brain imaging
45-70 years old	Dementia
Disease onset after 40 years old	Severe depression
Disease duration longer than 5 years	Neuroleptics intake within 6 months
On L-dopa	Intracerebral surgical intervention
History of wearing off	In a trial of a device, drug or surgery for PD
Stable PD medication (preceding 3 months)	Type 1 DM or type 2 DM on insulin
UPDRS part III of medication > 15	Ongoing treatment with sulphonylurea
L-dopa responsive	Severe cardiac disease
Able to consent	End stage renal disease
Able to comply protocol and o attend visit off medication	Alcoholism
	Pancreatitis
	Gastrointestinal disease (gastroparesis)

The protocol and a consent form describing the risks and potential benefits of the study were approved by REC, Joint Research office of University College London, ARSAC, and the MHRA. Written informed consent was requested from all the patients.

All patients had blood glucose, HbA1C and fasting Glucose tests to rule out concurrent presence of Diabetes Mellitus.

2.2.2 Schematic diagram of overall trial design

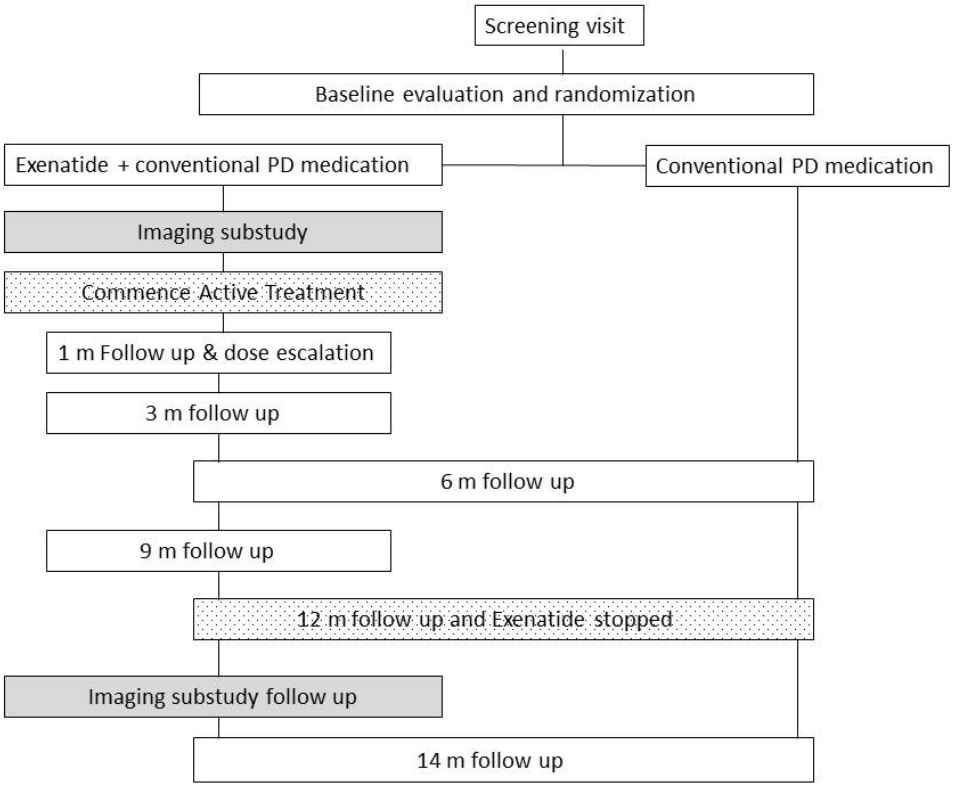


Figure 2. Schematic diagram of overall trial design

2.2.2.1 Randomization

Eligible individuals who have given informed consent were randomized to receive active drug (Exenatide on the top of their conventional PD medication) or to remain on their conventional PD medications and to act as a PD control. Block randomization were used with random block sizes. Separate randomization lists was generated for patients of greater (Hoehn & Yahr stage 2.5) or lesser (Hoehn & Yahr stage 2.0) disease severity to balance this as a possible prognostic factor, i.e. disease severity acts as a stratification variable. Randomization list was created prior to trial commencement, and stored by the trial pharmacists. The randomization list was

sufficiently long (N=50) to enable continued randomization should any patients drop out within the first 3 months. The patients and the examining physician were aware of treatment-group assignments throughout the study. The randomization outcome was 20 patients allocated in the Exenatide group and 24 patients in the control group.

2.2.2.2 Screening assessment

Each patient had a review of their demographics and data regarding their PD history, medication history, previous imaging, previous genetic test and previous drug compliance issues. Also an ECG, baseline blood test (including blood count, renal function, HbA_1C, serum amylase,) and clinical observations (Pulse, blood pressure and weight) were also performed.

2.2.2.3 Baseline visit

Each patient was sent the PDQ 39, NMS Quest, SCOPA Sleep, SCOPA AUT and Smell Identification test self-assessment forms to complete and bring with them to their baseline evaluation.

Baseline evaluations comprised the following:

Blood test to measure Blood count, renal function, liver function, serum amylase, HbA_1C, fasting serum glucose and lipid profile. The patient was allowed to eat and drink after it as they wish.

MDS-UPDRS, which is the standard validated tool for the assessment of patients with PD. This scale includes subsections collecting data regarding the impact of PD on a patient's mood and mental state, (UPDRS part 1), their activities of daily living (UPDRS part 2) and examination of the motor features of PD

(UPDRS part 3) and complications arising from the use of PD (UPDRS part 4) (C G Goetz 2010; Gallagher et al. 2012). Part 3 of the UPDRS was performed twice and video recording was taken on each occasion - first at a time when the patient has been free of all PD medications for at least 12 hours (including Exendin-4) and free of long acting dopamine agonists for at least 24 hours. The first UPDRS part 3 assessments took place as soon as the patient arrived to minimize the duration of any discomfort from not having taken medication. The UPDRS part 3 motor score was repeated 1 hour after the patient took his/her regular medication and confirmed that they had achieved their best medication response. The scores for the UPDRS part 3 range from score 0 (normal) to score 132 (worst possible).

For UPDRS part 3, the state of the patient was defined as "off medication" when testing was conducted before the patient had a first morning dose of levodopa and at least 12 hours after the administration of levodopa the previous day. The "on medication" scores refer to the best test scores recorded during the day while the patient was taking medication.

Assessment of PD severity using MDS-UPDRS part 3 off-medication was video recorded to allow objective rating of PD disability by observers blinded to randomization outcomes. Each patient video was rated by the same blinded clinician at each time point. All clinicians were experienced at evaluating PD patients and had successfully completed the official training module of the MDS-UPDRS.

Timed Motor tests include a hand tapping task to evaluate the number of hand taps that an individual can perform between 2 points 30 cm apart and a timed, sit, stand up, walk a distance of 7m as fast as possible, turn around and return to their chair and sit down task.

The Dyskinesia Rating Scale is an objective assessment of the severity of involuntary movements in 7 body regions with each region receiving a score of 0 (none) - 4 (extreme, no completion of the voluntary motor act) that was performed during patients on medication phase.

The PDQ39 is a 39 item quality of life assessment especially designed for patients with PD, by the Health Services Research Unit, Oxford 1998. Patients are given the questionnaire for self-completion. The completed questionnaire is designed to be deconstructed into a score for 8 separate dimensions: mobility, activities of daily living, emotional well-being, stigma, social support, cognition, communication and bodily discomfort. A summary score from 0 to 100 was calculated for the PDQ-39. This summary index (SI) is the arithmetic mean of the scores for the individual domains (Jenkinson et al. 1997).

2.2.2.4 One month assessment

At one month after baseline evaluation, each patient was telephoned to complete an adverse report and document their PD medication regime. Patients randomised to Exenatide were asked to attend the trial clinic taking their regular PD medication (and Exenatide). They were asked not to have eaten and to have drunk water only for 6 hours to enable fasting blood sample to be taken. Clinical observations including Pulse, Blood pressure and weight was measured and blood tests sent to measure renal function, liver function, serum amylase and fasting serum glucose. Each patient was given a 2 month supply of Exenatide 10 μg bd and supply of needles for the subcutaneous pen injection system.

2.2.2.5 Three month assessment

At three months after baseline evaluation, each patient was telephoned to complete an adverse events report and document their anti PD medication regime. Appointments were made for each patient on Exenatide to attend the trial clinic. They were once again asked not to have eaten and to have drunk water only for 6 hours to enable fasting blood sample to be taken.

Attending patients had blood tests to measure renal function, liver function, serum amylase and fasting serum glucose. An ECG, and clinical observations; weight, pulse, blood pressure was performed. Their current medication regime was noted. They were given a further 3 months' supply of Exenatide 10 μg bd and supply of needle for the subcutaneous pen injection system.

2.2.2.6 Six month assessment

At six months an appointment to attend the trial clinic for all the patients (Exenatide and conventional PD medication only) were made. The patients were asked to withhold all PD medications (including Exenatide) for at least 12 hours (overnight) and 24 hours for long acting dopamine agonists. They had video recording of UPDRS part III score, performed timed motor tests and they took their regular medications. The patient was asked to complete UPDRS part 1, 2, 4 and when they confirmed they their best on state was achieved, they had a further video of UPDRS part III score. During the on phase, the Dyskinesia rating Scale, MADRS and Mattis DRS was competed. (Non motor assessments are described in detail in Cahpter 5). They were given a further 3 months' supply of Exenatide 10 μg bd and

supply of needle for the subcutaneous pen injection system. (The same supply was given for the last time at 9months follow up).

2.2.2.7 Nine, twelve and fourteen month's assessments (As described in table 3)

Table 3. Nine, 12 and 14 month's assessment overview

Appointment	Control patients attend	Exendatide patients attend	Fasting blood	Clinical obs	UPDRS-3 off	UPDRS-3 on	Timed tests	UPDRS-1/2/4	Dyskinesia rating scale	MADRS	Mattis DRS	ECG	PDQ39	NMS	SCOPA	AUT	SIT
9 months	X	On medication	●	●													
12 months	Off medication	Off medication	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
14 months	Off medication	Off medication	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

2.2.3 Concomitant medication

Note was made of L-dopa equivalent doses, at each visit. No routine adjustment of PD medications was made at the trial clinic unless clinically urgent, under the premises of offering the best medical treatment to the patient. Patients were asked not to enroll in any other experimental treatment for PD until the end of this trial period. All of the patients were seen in the National Hospital of Neurology and Neurosurgery at University College London for three to four hours on five to eight occasions during the study: twice before the initiation of the treatment for screening and base-line assessments.

Because the doses of levodopa and other antiparkinsonian drugs are individualized for each patient, we are using levodopa equivalent doses (Tomlinson et al. 2010) to minimize changes in drug therapy which can confound the interpretation of outcomes.

2.2.4 Treatment procedures

Patients allocated in the Exenatide group were taught how to self-administer subcutaneous injections of the trial drug using the pre-filled pen devices. Patients randomised to active treatment were given supplies of 5 µg bd subcutaneous injections for 1 month. Further supplies of pre-filled pens, was given to the patient at each follow up visit.

They then returned for their 1 month assessment and were supplied with 2 months' supply of Exenatide 10 µg subcutaneous injections to be continued and replenished at subsequent follow up visits.

Exenatide was provided through the hospital pharmacy of the NHNN. It has a shelf life of 2 years if stored at 2-8 degrees. Each pre-filled pen can provide 60 doses of drug i.e. sufficient for 1 calendar month.

2.2.4.1 Starting dose and any dose escalation

Patients randomized to active treatment were given supplies of 5µg bd subcutaneous injections of Exenatide (manufactured as Byetta) for 1 month. They then returned for their 1 month visit and patients that tolerated the 5 µg dose were supplied with Exenatide 10µgr bd subcutaneous injections to be continued and replenished at subsequent follow up visits. Initial introduction of Exenatide using 5 µg dose prior to using the 10 µg dose has been associated with lower rates of nausea and vomiting than immediate introduction of 10 µgr dose (Fineman et al. 2004).

Patients with adverse reactions to the low dose injection e.g. nausea were given a supply of Domperidone 10 mg tablets to be taken orally up to three times daily as a

treatment to relieve nausea. No patient has been unable to tolerate the low dose injections.

Bioavailability of Exenatide is comparable following subcutaneous injection into abdomen, thigh or arm and the 10 µg dose compares favorably to the plasma levels in the rodent models namely 200 pg/ml plasma (Calara et al. 2005).

The dose of Exenatide chosen for this study has been based on 2 pieces of information.

A. There is extensive safety data regarding the use of Exenatide at a dose of 10 µg among patients with Diabetes. The key trials are outlined in the table 4. Hypoglycaemia among patients that are not on oral hypoglycaemic occurs at a frequency of 1 % (Drucker et al. 2008).

B. The 10 µg dose of Exenatide compares favourably to the plasma levels in the rodent models of Parkinson's disease namely 200 pg/ml plasma (Calara et al. 2005).

Table 4. Exenatide was granted a license for the treatment of patients with DM based on the following trials

Trial	Number of patients	Dose	Duration	Adverse events
Buse 2004	377	5 then 10 mcg bd Or 5 mcg bd Or placebo	30 weeks	Nausea 39-51% Hypoglycaemia 14-36% Diarrhoea 9-11%
De fronzo 2005	336	5 then 10 mcg bd Or 5 mcg bd Or placebo	30 weeks	Nausea 36-45% Hypoglycaemia 5% Diarrhoea 12-16%
Kendall 2005	733	5 then 10 mcg bd Or 5 mcg bd Or placebo	30 weeks	Nausea 39-49% Hypoglycaemia 19-28% Diarrhoea 10-17%
Drucker 2008	295	2mg once weekly Or 10 mcg bd	30 weeks	Nausea 26-34% Diarrhoea 13-14% Hypoglycaemia1% if not on sulphonylurea (15% if on sulphonylurea)
Moretto 2008	232	5 or 10 mcg bd	24 weeks	Nausea 3-13% Hypoglycaemia 4-5%

Patients were instructed that Exenatide could be administered at any time within 60-minute period before the morning and evening meal (or two main meals of the day, approximately 6 hours or more apart). Patients were told that it should not be administered after a meal. If an injection was missed, the patients were advised that treatment should be continued with the next scheduled dose.

Each dose was administered as a subcutaneous injection in the thigh, abdomen, or upper arm.

2.2.5 Assessment of compliance

Compliance was optimised by informing all patients randomised to receive Exenatide, of the most commonly experienced side effects and ways of minimising these. Patients were given adequate instruction regarding administration of injections and developing twice daily routine for their administration. Good relationship was established with all trial participants to maximise honest reporting of compliance.

Compliance was assessed by directly questioning patients, at each visit, with carers also asked to provide estimates of compliance. Patient estimates were checked against reports of how long each pen (with 60 pre-filled doses) lasted before it needed replacing. A compliance score was derived from the estimated number of injections actually administered each month and this figure recorded in the patient's clinical file at each visit.

2.2.6 Safety

Serious adverse events were defined as illnesses and incidents that necessitated prolong hospitalization or caused death. These events were reported to the monitoring board within 24 hours.

2.2.6.1 Serious Adverse Events (SAE), adverse reaction (AR)

All SAE and AR were defined and recorded according to standard GCP guidelines.

2.2.6.2 The Trial Management Group (TMG)

The TMG is a body that has been established to assess at intervals the progress of the trial, the safety data and the critical efficacy endpoints. The TMG recommend

to the sponsor whether to continue, amend or stop the trial. The TMG reviewed the summary of adverse event and the mean change in "off-medication UPDRS score". The TMG met initially at 3 months after the trial initiation and every 3 months after it.

The TMG was made up of; Thomas Foltynie, Iciar Aviles-Olmos, Patricia Limousin, Andrew Lees and Linda Taib.

2.2.7 Data handling and analysis

2.2.7.1 Data entry

Case report forms (CRF) were designed according to the sponsor's CRF template. All data were entered legibly in black ink with a ball-point pen. If an error were made, the error was crossed through with a single line in such a way that the original entry could still be read. The correct entry then was clearly inserted, and the alterations were initialled and dated.

At every visit, the patient CRF was completed, making sure the CRF review checklist was completed at the same time to avoid any missing data. Immediately after the visit was finished, the data were entered into a Microsoft Access database. Every 3 months the data-base was checked to make sure there was no missing data, nor data replication, to guarantee the completeness and accuracy of the data.

2.2.8 Endpoints

2.2.8.1 Primary endpoint

Change from baseline to 12 months and 14 months between patients on active Exenatide treatment and PD controls in respect of their part 3 UPDRS-off-

medication blind (excluding rigidity, as it cannot be assessed in a video) motor subscore.

2.2.8.2 Secondary endpoint

Adverse event profile among patients treated with Exenatide compared with matched PD controls. Change from baseline to 12 months/14 months between patients on active treatment and PD controls in respect of:

- Part III MDS UPDRS on medication motor subscore

-UPDRS ADL (MDS-UPDRS part 2) subscore

-Dyskinesia rating scale

-Timed motor test

-Mattis dementia rating scale (MDRS)

-Montgomery and Asberg depression rating scale (MADRS)

-PDQ39

-NMS Quest

-SCOPA Sleep scale _Scopa AUT scale _Smell identification test

-DAT SPECT images.

2.2.9 Statistical analysis

2.2.9.1 Statistical Considerations

Advice regarding sample size, endpoints and statistical analysis plans was sought from both Dr Foltynie & Dr Gareth Ambler of the UCL JRO Biostatistics Unit.

2.2.9.2 Sample size calculation

In previous studies involving patients on stable treatment regimens of either Pramipexole or L-dopa, there was a UPDRS decline of approximately 3 UPDRS points per year (SD of 6.8 points) (Holloway et al. 2004). PD trials that have used change in off-medication motor UPDRS scores as an outcome measure have shown similar rate of decline (1.7 points after 6 months) among patient on "best medical treatment" (Weaver et al. 2009; Follett et al. 2010).

Therefore it was considered that the group of conventional PD medication would decline at a rate of 3 UPDRS points over the course of the 12 month follow up. Using the formula, n=42 x (SD of difference/difference)2, the patient sample size (n=40) in this trial would have 90% power to detect a difference of 7 UPDRS points between the treated and untreated patients at a significance level of 5% assuming normality in distribution of response and a SD of 6.8 points. This translates to a mean improvement of 4 UPDRS points in the treated patients. This was considered a clinically important effect size. Less optimistically, this sample size would still have 80% power to detect a difference of 6.1 points, i.e mean improvement of 3.1 points in the treated patients. Whether the estimation of the possible magnitude of effect of Exenatide was realistic, was only established once pilot data were collected.

With 40 patients the treatment effect could be estimated to within +/- 4.2 units (1.96 x √(2 x sigma2 /n)). (Unit = UPDRS points). Patients that dropped out within

the first three months were replaced by further patients who underwent the same randomisation process.

2.2.9.3 Primary endpoint analysis

The difference between UPDRS part 3 off medication score at baseline and at 12 and 14 months was calculated for each patient. The mean difference and SD for patients randomised to treatment and for patients on conventional PD medication group was presented. It was anticipated that there would be high correlation between the baseline and follow up measures. The analysis was performed on an intention to treat basis including all patients who completed at least one follow up assessment. "Last observation carried forward" was used for participants with missing data.

Every effort was made to ensure that missing data was kept to a minimum. Patients dropping out prior to 3 months who refuse follow up assessments had their reason for dropping out reported. Additional patients were recruited and randomised to replace patients who drop out prior to their first follow up assessment.

The distribution of scores was checked for normality using the Schapiro-Wilk test. Differences between treated and untreated groups with respect to changes in scores from baseline to follow-up were analysed using 2-sided t tests. Data were analysed using IBM SPSS Statistics 20.

2.2.9.4 Secondary endpoints analysis

The difference between each of the secondary outcome measures at baseline and at 12 months was calculated for each patient. The mean difference and SD for patient randomised to treatment and for patients acting as controls was presented.

The analysis was performed on an intention to treat basis including all patients who complete at least one follow up assessment. The distribution of scores was checked for normality. As data were normally distributed, a paired t test was used to assess the difference between the means.

We performed an interim analysis of the primary outcome variable once every patient reached 6 months follow up assessment. Only the members of the performance and safety monitoring board were informed of the outcome, and they allowed the study to continue.

Exploratory subgroup analyses were performed to try and identify whether changes in part 3 MDS UPDRS were different in relation to younger age at baseline or lower score in Hoehn & Yahr scale at baseline; as a measure of disease severity, using correlation between our primary outcome and age at onset and Hoehn & Yahr to find out if younger age could influence a better response to treatment. Correlations were performed to explore any relationship between change in weight and change in part 3 MDS UPDRS off medication to find out if weight loss could contribute a better performance on motor improvement.

To explore differential effects in different UPDRS symptoms, UPDRS part 3 scores were subdivided into bradykinesia, tremor, axial signs (using blinded ratings) and rigidity (using open label ratings).

“Bradykinesia” was calculated as the sum of right and left finger tapping plus right and left hand movement plus right and left arm movements plus right and left foot tapping plus right and left leg movements plus facial expression and body bradykinesia.

"Rigidity" was calculated as the sum of rigidity on the neck plus right upper, left upper, right lower and left lower extremity.

"Tremor" was calculated as the sum of resting tremor on; the face, right arm, left arm, right and left leg and constancy of resting tremor plus postural tremor on the right hand and left hand plus kinetic tremor on the right and left hand.

"Axial signs" was the sum of speech, postural instability, gait, posture, rising from the chair.

2.2.9.5 Imaging analysis (See chapter 3)

2.2.10 Study Approval

The trial was conducted at the National Hospital of Neurology and Neurosurgery (London, United Kingdom), with trial sponsorship and monitoring by University College London. The protocol was approved by a research Ethics committee, the Administration of radioactive Substances Advisory Committee (ARSAC), and the UK Medicines Health Regulatory Agency, and all patients signed informed consent. The trial was registered at www.clinicaltrials.gov (trial ID NCT01174810).

2.3 RESULTS

Enrolment took place from July 2010 to March 2011. The period in which the treated patients started to inject themselves, began in September 2010 and finished by the end of March 2012.

Of the 45 patients recruited, one patient randomized to Exenatide dropped out before first follow up, and was therefore replaced per the study protocol and not

included in the final analysis (See figure 3). The baseline demographic data for the 44 participants included in the final analysis are presented in Table 5. Clinical data were missing for 2 patients (1 patient per group), both for their 14-month visit.

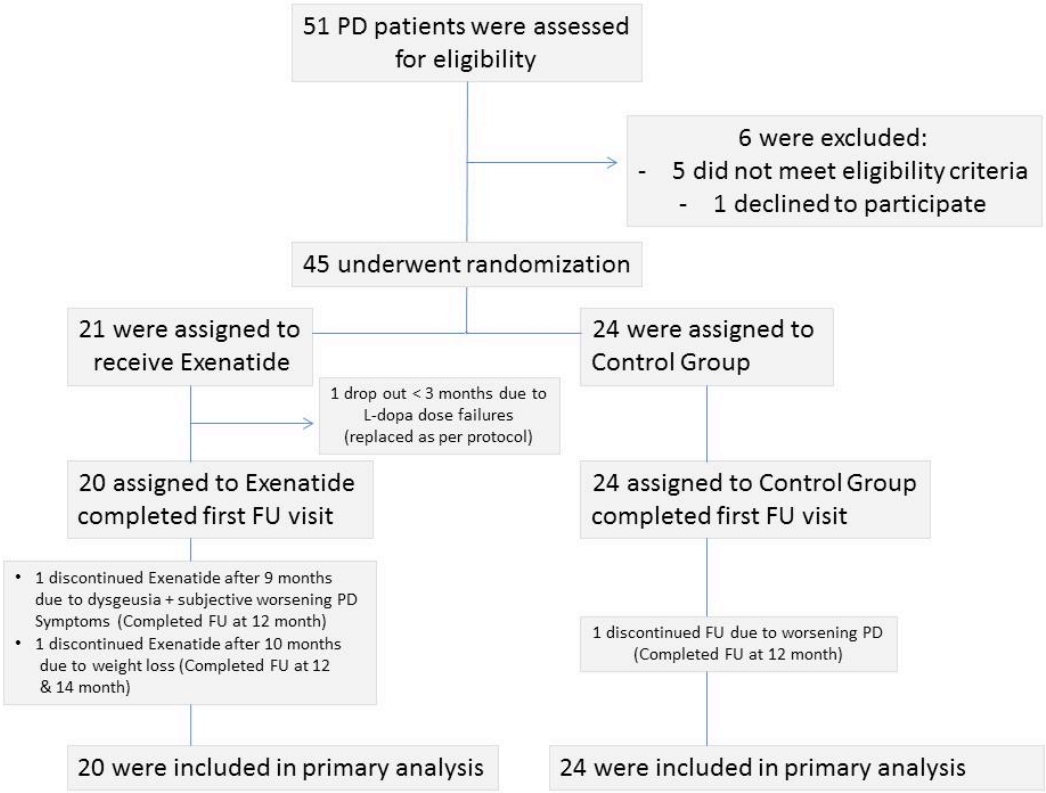


Figure 3. Random assignments to treatment, completion of the trial, and reasons for not completing it.

Patients withdrawing from the trial prior to the 3-month visit were replaced, and new recruits were randomly allocated to the 2 groups.

Table 5. Baseline characteristics of the study subjects

Randomization group	*n*	*Age at study enrolment, years Mean (SD)*	*Gender*	*Age at symptom onset, years Mean (SD)*	*Duration of symptoms at baseline, years Mean (SD)*	*LED, mg*
Exenatide	20	61.4 (6.0)	15M, 5F	51.6(7.8)	9.6(3.4)	973(454)
Conventional PD medication	24	59.4 (8.4)	20M,4F	48.4(7.4)	11.0(5.9)	977(493)

2.3.1 Clinical outcomes

2.3.1.1 Blinded video rating of MDS-UPDRS part 3 in the practically defined "off medication" condition

Patients allocated to the Exenatide group had a mean improvement at 12 months of 2.7 points (SD, 7.7) on the MDS-UPDRS part 3, while controls had a mean decline of 2.2 points (SD, 7.3) for a difference of 4.9 points (95% CI, 0.3-9.4; p=0.037; Table 7). At 14 months, the Exenatide group had a mean improvement of 1.7 points (SD, 7.4), while controls had a mean decline of 2.8 points (SD, 6.7), for a difference of 4.4 points (9.5% CI, 0.2-8.7; p=0.042). These scores did not include any changes detected in limb or neck rigidity, which cannot be reliably rated on video. Addition of open label rating of rigidity scores to the blinded data equated to an improvement of 1.8 points (SD,8.7) in the Exenatide group at 12 months, compared with a decline of 5.3 points (SD, 8.3) in the control group (difference, 7.0 points; 95% CI, 1.8-12.1; p=0.009), and an improvement of 0.8 points (SD, 8.7) in the Exenatide group at 14 months, compared with a decline of 6.4 points (SD, 7.8) in the control group (difference, 7.2 points; 95% CI, 2.1-12.2; p=0.006; Table 7).

UPDRS part 3 scores were then subdivided into bradykinesia, axial, rigidity and tremor. There was a significant improvement in bradykinesia subdomain of MDS UPDRS part 3 at 12 months in the Exenatide group in comparison with the control group, for a difference of 3.4 points (95% CI, 0.5-6.2, p=0.02) but this was less at 14 months, for a difference of 2.6 points (95% CI, 0.3-5.5, p=0.082).

Similarly, there was an improvement in the rigidity subdomain (open label data) favouring Exenatide, with a difference of 2.2 points (95% CI, -0.5-3.9, p=0.013) at 12 months and 2.7 points at 14 months (95% CI, -1.0-4.4 p=0.002)

As well as the axial subdomain with a difference of 1.4points favouring Exenatide (95% CI, -0.3-2.5) at 12 months and a significant trend at 14 months p=0.054.

There was no significant difference in tremor subdomains of MDS-UPDRS part 3 between Exenatide and control group at 12 months (p=0.467) neither at 14 months (p=0.447).

2.3.1.2 Secondary outcome measures

• Part 3 MDS UPDRS on medication motor subscore. Open label rating of MDS-UPDRS part 3 “on medication” showed a significant difference favouring patients treated with Exenatide compared with the control group (p=0.06 at 12 months, p<0.001 at 14months). The mean improvement in “on medication” scores in the Exenatide group (2.7 points) was unchanged between the 12- and 14-month visits. In contrast, individuals in the conventional PD medication group deteriorated over the total 14-month trial period by 7 points.

• There was a significant difference or non-significant trend favouring Exenatide in MDS-UPDRS parts 1, 2 (UPDRS ADL), and 4 at both the 12- and 14-month time points (Table 7).

• There were also general trends for improvement in the timed tests (Table 9 & 10) and L-dopa equivalent dose (LED) (Table 8).

• There was no improvement in PD-39 summary index between the 2 groups (Table 8).

2.3.1.3 Safety and tolerability

Four patients withdrew / dropped out from the study, 3 from the group randomized to Exenatide and one from the conventional PD medication group. Of the 3 patients in the Exenatide group, 1 withdrew due to worsening PD (recurrent L-dopa dose failures) prior to the first follow-up visit. Exenatide is known to cause slowing of gastric emptying and is the most likely explanation for this observation in this patient. Two further patients withdrew from treatment: the first at 9 months due to dysgeusia combined with subjective PD deterioration, and the second at 10 months due to excessive weight loss (despite reduction to the 5-µg dose). The last two were included in final analysis, using an intention to treat protocol. One patient randomized to the conventional PD medication group withdrew from the study at 12 months due to deteriorating PD and incapacity to attend the trial clinic in the "off medication" state. All serious adverse events, together with adverse events occurring in more than 1 patient, are listed in table 6. No clinically relevant changes in ECG, haematological, or biochemical indices were observed. Weight loss and nausea were more common in the patient group treated with Exenatide. Patients on Exenatide lost a mean of 3.2 kg (SD, 3.9; range, 3.5-kg increase to 12.3-kg decrease) over 12

months, necessitating 2 patients to reduce to the 5-µg dose. Control group patients lost a mean of 0.8 kg (SD, 3.3; range, 5.5-kg increase to 7.8-kg decrease) over 12 months, for a difference of 2.4 Kg (95% CI, 0.2-4.6; p=0.035). There was a greater increase in the mean dyskinesia rating scale score in Exenatide versus control patients at both 12 and 14 months. This necessitated lowering of LED in 5 Exenatide patients, whereas 8 Exenatide patients had unchanged dopaminergic medication and 7 patients had increased dopaminergic medication over the period of study.

2.3.1.4 Possible cofounders of clinical outcomes

To make a preliminary assessment of whether motor outcomes were confounded by other variables, simple correlations were sought. There were no correlations among change in UPDRS part III blind scores and change in body mass index at 12 months (p=0.812)

No correlation was found among patients suffering more dyskinesia at 12 months and change in body mass index at 12 months (p=0.119).

The improvement in MDS UPDRS part 3 at 12 months was not correlated with younger age at disease onset (p=0.678) nor younger age at start of the trial (p=0.211). There was a significant correlation between age at start of the trial and improvement in part 3 MDS UPDRS blind scores at 14 month (p=0.007), although age at symptoms onset was not correlated with changes in part 3 MDS UPDRS at 14 month (p=0.304).

Adverse events (AE)	*n*	*Serious Adverse events (SAE)*	*n*
Exenatide			
Weight loss	19	Sciatica and epidural injection	1
Constipation	18	Insomnia (admitted for	1
Nausea	13	polysomnography)	
Diarrhoea	7	Possible transient ischaemic	2
Abdominal pain	6	attack	
Back pain	5		
Other pain	7		
Loss of appetite	5		
Increase off time	4		
Increase dyskinesia	4		
Weight gain	3		
Hallucinations	2		
Injection bruising	2		
Memory impairment	2		
Viral upper respiratory	2		
infection	2		
Urinary infection	36		
Miscellaneous			
Conventional PD medication			
Constipation	14	Prostatectomy for prostate	
Increased Off time	12	Cancer	1
Weight gain	9	Lymph Node Dissection	1
Nausea	8	Anxiety requiring admission	1
Weight loss	8	Fracture radius	1
Abdominal pain	6		
Diarrhoea	5		
Memory impairment	5		
Back pain	4		
Other pain	8		
Impulsivity	4		
Falls	3		
Prostate cancer	3		
Freezing	2		
Hallucinations	2		
Viral upper respiratory	2		
infection	2		
Sleep disorder	46		
Miscellaneous			

Table 6. Adverse events reported by trial participants

	Baseline Mean(SD)	6months Mean(SD)	12months Mean(SD)	Difference Baseline to 12 months Mean(SD); 95% CI	P value	14months Mean(SD)	Difference Baseline to 14 months Mean(SD); 95% CI	P value
Blinded- MDS	***UPDRS Part III***	***"off meds" A***						
Exenatide	31.0(11.2)	25.2(9.0)	28.3(9.9)	-2.7(7.6);-6.3,0.9	P=0.037	29.3(8.5)	-1.7(7.4);-5.1,1.8	P=0.042
Conventional PD drugs	34.0(16.1)	34.4(15.0)	36.2(15.4)	2.2(7.3);-0.9,5.3		36.8(15.2)	2.8(6.7);-0.0,5.6	
MDS	***UPDRS III***	***"on meds" B***						
Exenatide	23.5(6.3)	22.9(7.4)	20.8(6.8)	-2.7(7.7);-6.3,0.9	P=0.006	20.7(8.1)	-2.8(7.0);-6.0,0.5	P=0.0003
Conventional PD drugs	25.3(10.7)	29.3(11.8)	29.0(11.0)	3.6(6.7);-0.8,6.5		32.3(12.5)	7.1(8.8);3.3,10.7	
MDS-UPDRS	***Part I***							
Exenatide	10.4(4.1)	8.8(2.8)	10.6(3.8)	0.2(4.4);-1.9,2.3	P=0.08	11.4(6.7)	1.0(7.6);-2.6,4.6	P=0.049
Conventional PD drugs	11.6(4.7)	11.5(6.3)	14.3(6.0)	2.8(4.7);-0.8,4.7		16.3(7.0)	4.7(4.3);2.9,6.5	
MDS-UPDRS	***Part II***							
Exenatide	10.2(5.2)	9.2(6.1)	9.6(6.0)	-0.6(3.9);-2.4,1.3	P=0.007	12.3(7.2)	2.1(6.3);-0.8,5.0	P=0.11
Conventional PD drugs	12.9(6.2)	14.1(6.6)	17.0(7.4)	4.1(4.4);2.3,6.0		17.9(8.1)	5.0(5.4);2.7,7.3	
MDS-UPDRS	***Part IV***							
Exenatide	6.3(2.4)	5.4(2.9)	5.8(3.3)	-0.5(2.5);-1.7,0.7	P=0.047	5.7(3.7)	-0.6(2.8);-1.9,0.8	P=0.16
Conventional PD drugs	6.3(3.4)	7.6(4.0)	7.4(3.5)	1.1(2.6);-0.0,2.2		7.0(3.2)	0.6(2.7);-0.5,1.8	

Table 7. Changes in MDS-UPDRS between baseline and month 14. A =blinded rating excludes rigidity. B =open-label rating includes rigidity scoring

	Baseline Mean(SD)	6months Mean(SD)	12months Mean(SD)	Difference Baseline to 12 months Mean(SD); 95% CI	14months Mean(SD)	P value	Difference Baseline to 14 months Mean(SD); 95% CI	P value
LED								
Exenatide	973(454)	1011(518)	997(446)	24.3(123.6);-37,78	1015 (467)	P=0.04	42.3(137);-22,107	P=0.08
Conventional PD medication	977(493)	1061(613)	1121(620)	143.7(223.6);49,238	1125(638)		148.0(232);50,246	
Dyskinesia Rating Scale	***(on medication)***							
Exenatide	2.3(2.8)	2.5(4.1)	3.3(4.5)	1.0(4.2);-1.0,3.0	3.2(4.0)	P=0.33	-1.0(3.8);-0.8,2.7	P=0.49
Conventional PD medication	2.6(2.9)	3.5(3.9)	2.5(2.7)	-0.04(2.7);-1.2,1.1	2.7(3.5)		7.10.1(4.3);-1.7,1.9	
PDQ39 summary index								
Exenatide	19.2(13.5)	18.1(13.4)	19.6(13.0)	-0.4(11.4);-4.9,5.7	21.5(19.6)	P=0.77	2.3(11.6);-3.1,7.7	P=0.27
Conventional PD medication	24.5(12.8)	25.2(15.8)	21.0(15.3)	1-0.6(11.2);-5.2,4.2	23.4(16.0)		-1.2(9.1);-5.0,2.7	

Table 8. Changes in the score on LED, dyskinesia rating scale and PDQ39 summary index between baseline and month 14

	Baseline Mean(SD)	6months Mean(SD)	12months Mean(SD)	Difference Baseline to 12 months Mean(SD); 95% CI	P value	14months Mean(SD)	Difference Baseline to 14 months Mean(SD); 95% CI	P value
R hand taps	***Off meds***							
Exenatide	43.5(10.8)	49.2(7.8)	52.6(8.3)	9.1 (8.0); 5.3, 12.9	P=0.02	52.5(8.4)	9.0(9.4);4.5, 13.4	P=0.08
Conventional PD medication	45.4(15.1)	42.3(13.9)	46.4(14.3)	1.1(13.0); -4.4,6.6		48.4(15.8)	3.1(11.8); 8.1,-2.0	
R hand taps	***On meds***							
Exenatide	53.3(10.8)	56.3(11.3)	60.2(14.2)	6.9(17.6);15.1, -1.4	P=0.07	63.3(12.9)	10.0(17.1); 18.0,1.9	P=0.03
Conventional PD medication	53.7(19.6)	49.8(15.2)	50.7(18.6)	-3.0(20.0.);5.5,-11.4		53.9(16.1)	0.2(16.8); 7.3,-6.8	
L hand taps	***Off meds***							
Exenatide	42.2(14.0)	46.5(9.4)	49.9(10.7)	7.8(8.9); 3.4, 12.0	P=0.21	49.8(10.9)	7.7(9.0); 3.5,11.9	P=0.15
Conventional PD medication	40.9(12.4)	39.9(10.6)	44.6(10.8)	3.7(11.3); -1.0. 8.5		44.4(11.2)	3.4(10.1); -0.8,7.7	
L hand taps	***On meds***							
Exenatide	50.7(14.5)	53.3(10.4)	56.6(14.0)	5.9(19.5); -3.3,15.0	P=0.012	58.5(12.1)	7.8(19.2); -1.2,16.8	P=0.008
Conventional PD medication	50.5(15.0)	48.6(13.8)	51.0(13.6)	-8.4(16.7);-15.5,-1.4		51.8(14.3)	-7.7(17.6);-15.2,-0.3	

Table 9. Change in the scores on the hand taps timed test between baseline and month 14

	Baseline Mean(SD)	6months Mean(SD)	12months Mean(SD)	Difference Baseline to 12 months Mean(SD); 95% CI	P value	14months Mean(SD)	Difference Baseline to 14 months Mean(SD); 95% CI	P value
Timed walk	***Off meds***							
Exenatide	17.3(5.2)	15.5(4.5)	15.5(4.3)	-1.7 (4.8);-4.0, 0.5	P=0.87	15.4(4.2)	-1.9(4.6); 4.0, 0.2	P=0.80
Conventional PD medication	23.8(22.4)	22.2(21.3)	21.6(17.1)	-2.2(12.3); -7.4, 3.0		21.3(18.6)	-2.5(10.1); -6.8,1.7	
Timed walk	***On meds***							
Exenatide	13.9(3.1)	13.3(2.3)	13.4(2.7)	-0.4(4.8);-2.7, 1.8	P=0.08	13.4(2.5)	-0.4(4.5); -2.5,1.7	P=0.08
Conventional PD medication	13.7(4.4)	13.8(3.4)	15.1(4.1)	1.4(4.8); -0.2, 3.8		14.7(4.0)	0.9(3.6); -0.1,2.9	

Table 10. Change in the scores on the walking timed test, between baseline and month 14

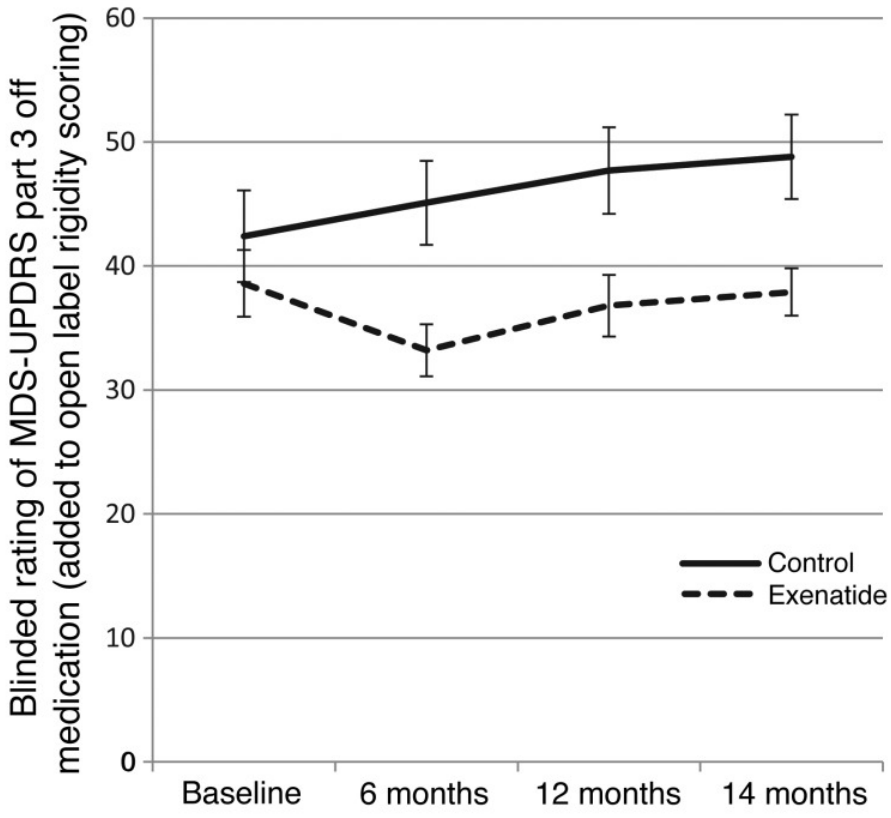


Figure 4. Change from baseline in the MDS-UPDRS part 3 score by study visit. Data present mean ± SEM

DISCUSSION

This is the first trial to report tolerability and pilot data of the possible biological effects of Exenatide in patients with a neurodegenerative disease. In view of the single-blind design, we cannot exclude placebo effects being responsible for the observed differences between patients treated with Exenatide and controls, and these data should not be interpreted as evidence of symptomatic efficacy or neuroprotection. Given the complex design of the Exenatide pen device, the cost of manufacture of a matched placebo, and the absence of commercial sponsorship, this

weakness was unavoidable, and these data should be considered as proof of principle/proof of concept only. Nevertheless, the trial design allowed the collection of data in a very cost-efficient manner, and the results demonstrated that Exenatide was generally well tolerated by PD patients and that clinically relevant differences emerged between the 2 groups that persisted beyond the 12-month exposure to the study drug, which may potentially reflect biological activity.

In an attempt to improve the cost-efficiency of trials of potential neuroprotective agents, previous investigators have used "futility" designs in order to compare disease progression in individuals assigned to experimental agents against the expected natural history of the disease, based on either historical or contemporary control data (NINDS NET-PD Investigators 2006; NINDS NET-PD Investigators 2007). These studies have highlighted the difficulties in selecting agents for major investment, given the presence of highly variable rates of placebo responses that can occur. The variable nature of the placebo response is a particularly important issue for complex or invasive interventions in which placebo versions of the licensed product are not readily available/represent additional significant expense, which can thus hinder the conduct and interpretation of even small phase 2 double-blind trials. In the current trial, we adopted a proof of principle approach to provide preliminary data regarding the tolerability of Exenatide in a small number of patients with PD, as well as to collect pilot data with respect to possible biological effects, in order to help justify the larger investment required to initiate a larger double-blind, placebo-controlled study and assist in sample size calculations. We chose not to set any futility threshold a priori, but instead chose to use a contemporary group of PD controls and to continue follow-up for a sufficiently long period to allow inevitable placebo effects to at least begin to diminish.

There are multiple issues in the optimal design of trials that aim to identify agents with neuroprotective effects that have been previously highlighted, including the prioritization of drugs for study, the optimal trial design and duration, the ideal outcome measures, and the optimal group of patients for study (Olanow et al. 2008). In the current study, the inclusion and exclusion criteria for patient selection were chosen on a pragmatic basis to minimize the risk of including patients with non-PD tremor or atypical Parkinsonism, with the understanding that patients with advanced disease would be likely to have fewer salvageable dopaminergic neurons. We also wished to determine whether this drug was tolerable in moderate-stage PD patients on L-dopa, given that it is unlikely that any neuroprotective agent will ever be able to entirely replace the need for symptomatic therapy. Since Exenatide is also being evaluated as an agent to influence cognitive decline, our inclusion criteria allowed us to evaluate the possible influence of Exenatide on cognition. Given that Exenatide has not previously been given to patients with PD, the design of this trial included a washout period to also allow for preliminary distinctions between possible symptomatic effects and possible disease-modifying effects.

However, the inclusion of a 2-month washout period provided information on short-term symptomatic effects only. Long-duration symptomatic effects may persist beyond this period and cannot be excluded using the current study design. This is particularly relevant given the increase in L-dopa–induced dyskinesia (LID) seen in the Exenatide-treated group, which necessitated reduction in L-dopa doses in 5 patients. The possibility of an interaction between Exenatide and conventional dopaminergic replacement must be considered in future study designs.

A further caveat must be made in the interpretation of these data given the small sample size. Despite randomization, minor differences in the baseline characteristics of the treated and control groups can influence subsequent disease progression. In a larger sample, randomization would be more likely to balance the treated and untreated groups. In the current trial, we attempted to minimize this chance variation by stratified randomization according to baseline disease severity. While there were no significant differences between the 2 groups at baseline, the control group had slightly longer disease duration than that of Exenatide-treated patients.

Weight loss is an important concern and prevented trial completion in 1 individual. This was fully reversible on cessation of the drug. Gastrointestinal symptoms are a common side effect of Exenatide and also in the PD population, but did not compromise trial participation in any individual. Patients generally tolerated the pen injection device well, and none of the serious adverse events observed were considered to be reactions to Exenatide. The frequency of the adverse events in the Exenatide group was similar to that seen in the previous clinical trials of Exenatide in diabetes patients (Buse et al. 2004; DeFronzo et al. 2005).

Bearing in mind these limitations, our data provided preliminary information about Exenatide tolerability in PD and allowed consideration of the size of the biological effects seen in comparison to previous trials of patients on placebo medications. The absolute size of the difference in PD severity between the Exenatide-treated and untreated groups using blinded rating was modest (4.9 points in MDS-UPDRS part 3), although this value excluded the additional effects on rigidity scores, which were only evaluated using open-label rating. Inclusion of the

rigidity scoring made by the unblinded investigator equated to a 7.0-point difference in MDS-UPDRS score at 12 months and a 7.2-point difference at 14 months.

Adding the blinded video rating of MDS-UPDRS part 3 to the differences seen in parts 1, 2, and 4 of the scale equated to a 13.8-point advantage in favour of Exenatide at 12 months and a 12.3-point advantage at 14 months. The changes detected in MDS-UPDRS scores were also largely reflected in the timed motor tests. However, there were no significant changes detected in depression or subjective ratings of quality of life (i.e., PDQ39 summary index).

The data presented herein demonstrated that clinically informative data can be obtained in a very cost-efficient manner as part of the process of selecting drugs for future study as potential neuroprotective agents in PD. These data support further, double-blind trials of Exenatide as a potential disease-modifying drug in PD, which will incur substantially greater costs than were required for the current study. It is arguable that prevention of deterioration is more achievable in the earlier stages of PD, when a greater number of dopaminergic neurons are still viable. While the major cohort of interest for future trials may be subjects with early PD, the data presented here indicate that further investigation of Exenatide as a treatment in later stages of PD may also be warranted.

3 ^{123}I FP-CIT SPECT in a subgroup of Exenatide treated patients.

3.1 INTRODUCTION

The mechanism of action through which Exenatide has beneficial effects in the animal models of PD is unproven. One hypothesis is that the drug leads to dopaminergic neurogenesis through GLP-1R, stimulating neuronal proliferation. In a human, such neuronal proliferation could be accompanied by increases in the activity measured by DaTSPECT scan. An alternative is that on-going inflammation leads to DA cells adopting a quiescent state and stopping DaT mRNA transcription. GLP-1 receptor stimulation may reduce inflammation. Another alternative is that GLP-1 stimualtion stops on-going cell death so there is less decline in DaTSPECT scan in patients v controls.

This hypothesis is based on the work done using immunohistochemical methods with stains that label different cell division markers. Different researchers have used this approach to study the effects of GLP-1R activation in rodent-based models of neuronal proliferation. In vivo work done with adult rodents chronically treated with Exendin-4 has shown an incremental cell proliferation in the hippocampus dentate gyrus of these animals as reflected by the elevation in BrdU and doublecortin (DCX) staining, a marker of immature neuronal tissue.

Testing Exendin-4 on primary adult mouse hypothalamic cultures, an elevated level of bromodeoxyuridine (BrdU) staining, an analog of thymidine/uracil that reflects the levels of nuclear DNA, was observed (Belsham et al. 2009). In addition, authors report an elevation in the levels of gene transcripts for Ki-67, a specific

nuclear protein found only in actively dividing cells (Isacson et al. 2011). Long term administration of Exendin-4 in vivo for 21 days induced an elevation of Ki-67 staining in the subgranular zone of adult mouse dentate gyrus, reflecting higher levels of proliferation (Li, H. et al. 2010).

It is worth noting that results reporting cellular proliferation in vitro appear to be dependent upon the model used. For example, on PC12 cell line, derived from a form of tumour located in rat adrenal tissue, it was not possible to prove any significant level of proliferation induced by GLP-1 treatment (Perry et al, 2002b). However, in SH-SY5Y cells derived from a human neuroblastoma cell line, evidence of proliferation was shown at physiologically relevant doses of GLP-1 and Exendin-4.

In primary rodent culture, the effect of Exendin-4 has been investigated on neural stem/progenitor cells in the subventricular zone of the adult rodent brain. Exenind-4 produced an increase in the number of neural stem/progenitor cells, and the number of cells expressing the neuronal markers microtubule-associated protein 2, beta-III-tubulin, and neuron-specific enolase. This same group of researchers gave Exendin-4 intraperitoneally to naïve rodents together with BrdU, showing an increase in the number of BrdU positive cells and in the number of neuronal precursor cells expressing DXC (Bertilsson et al. 2008).

3.1.1 Imaging biomarkers in PD

The well characterized pathophysiological feature of PD is the degeneration of dopamine neurons. Based on this, different imaging markers have been developed for evaluating PD. In an effort to demonstrate different aspects of dopaminergic neuronal function, presynaptic terminal have been targeted. The nigrostriatal

degeneration preferentially affects the putamen, which is in agreement with results from autopsy studies, which report a more severe depletion of DA in the putamen than in the caudate nucleus (Kish et al. 1988). This finding is likely due to the more extensive degeneration in PD of subpopulations of substantia nigra cells that project primarily to the putamen (Goto et al. 1989).

Radiolabelled tracer-based neuroimaging techniques, such as SPECT and PET, rely on infusing the patient with a radiolabelled molecule with an affinity to a molecule/complex that is relevant to the pathophysiology.

Three key molecular targets for neuroimaging biomarkers in PD are the dopamine transporter (DAT), the vesicular monoamine transporter (VMAT), and the enzyme dopa decarboxylase, all three targeting presynaptic dopamine functions with single photon emission computerized tomography (SPECT) and positron emission tomography (PET).

The detection of the small changes in the signal in a population of progressing PD patients demands more rigorous quantitative markers of disease (quantifiable and reproducible). Additionally, the most rigorous requirements of the imaging ligand are posed by studies which attempt to evaluate interruption in the normal progressive loss in PD, which could slow the rate of the expected signal loss of 6-13% per year, expressed as percent loss from baseline.

Radioligands such as 18F-DOPA, 11C-VMAT2, and dopamine transporter (DAT) ligands have been used as radiolabeled markers for the dopaminergic system to evaluate patients with PD (Morrish et al. 1996; Nurmi et al. 2000; Frey et al. 1996; Marek et al. 2001).

• 18F-DOPA: This ligand is a marker for dopamine synthesis in the neuron. Specific uptake with 18F-DOPA depends on the conversion of 18F-DOPA by the aromatic amino acid decarboxylase and uptake and trapping of 18F-dopamine into synaptic vesicles.

• 11C-VMAT2: The vesicular monoamine transporter sequesters newly synthesized or recovered monoamines (dopamine, norepinephrine, serotonin, and histamine) from the cytosol into the synaptic vesicles thereby protecting the neurotransmitters from catabolism by cytosolic enzymes and packaging them for subsequent exocytoxic release.

• DAT: The dopamine transporter (DAT) is a protein on the nerve terminal responsible for reuptake of dopamine from the synapse, thus is used as a marker of DA transport (Brooks 1997).

These three markers have demonstrated reduced uptake in the striatum, the location of the presynaptic nigral dopamine terminal projections. Therefore the striatum is a region that has a very high density of target sites, thus permitting high quality quantitative signal assessment.

A growing body of evidence has emerged from clinical research performed with 18F-DOPA- PET, demonstrating its feasibility of detecting changes in early PD, as well as in PD patients studied longitudinally, and interestingly, measuring the effects of possible disease-modifying therapies on the neurodegenerative process (Morrish et al. 1996; Nurmi et al. 2000).

Among the DAT tracers, ^{123}I-β CIT, ^{123}I altropane, ^{123}I FP-CIT and 99mTc-TRODAT have been the most widely used dopamine transporter agents for SPECT,

and 18F-CFT for PET (Fischman et al. 1998; Huang et al. 2001; Nurmi et al. 2000; Booij, Speelman, et al. 2001). A tropane derivative of cocaine (FP-CIT, DATSCAN®) is available as a ^{123}I labelled tracer.

^{123}-I-labelledN-(3-iodoprop-2E-enyl)-2-β-carbomethoxy-3β-(4-methylphenyl)

nortropane, named PE2I, is a relatively new radioligand that has about 10-fold higher in vitro selectivity for the DAT than for the serotonin transporter (SERT) compared ^{123}IFP-CIT (Ziebell 2011) .

Finally, in terms of the differences among scanners, PET scanners have better resolution than SPECT scanners, and may benefit from greater flexibility in the range of radiopharmaceuticals that can be tested. Nevertheless, SPECT studies may be technologically more feasible for clinical studies and in clinical practice, because they have the advantage of longer half-life radiopharmaceuticals and potentially lower cost.

3.1.2 Properties of imaging biomarkers

The imaging marker should be tailored accordingly to the research question. At this point, despite the fact that both 18F-dopa PET and ^{123}I B-CIT SPECT measure different features of dopamine function, they have shown a very good concordance of imaging findings. Both tracers have shown bilateral reductions in striatal tracer uptake in hemiparkinson patients with greater reduction on the site of the brain contralateral motor symptoms; around 50% signal loss for contralateral compared with 20-30% loss of ipsilateral relative to age-matched healthy control values.

Several studies using dopamine transporter (DAT) imaging agents in normal healthy subjects over a wide age range demonstrate age-related reductions in tracer

uptake(van Dyck et al. 2008; van Dyck et al. 1995). In studies of disease progression, both tracers consistently demonstrate that subjects imaged serially over several years, show a significant reduction of the imaging signal on the order of 5–11% per annum (Booij et al. 2001; Morrish et al. 1996; Brooks 2000a; Marek et al. 2001). Because the loss of striatal uptake is small and can vary both between patients and within patients over their course of disease, when the purpose of the imaging study lies in serially monitoring the progression of disease, the radiotracer should be amenable to accurate and reliable quantitation.

3.1.3 Imaging and clinical assessments of PD progression

Clinical trials designed to follow progression of disease or establish a disease-modifying effect of a treatment in PD are very difficult to design and execute. This difficulty is due to: (1) different rates of PD progression; (2) extended medication washouts necessary to remove symptomatic effects for purposes of accurate clinical assessment of patients; (3) duration of the trials need to be long enough to allow measures of disease progression or evaluation of potential disease-modifying interventions. Therefore biomarkers for disease progression that are unaffected by symptomatic treatments and can be assessed repeatedly throughout the duration of the study have become an increasingly crucial component for evaluations of potential disease-modifying drugs.

3.1.4 History of neuroimaging trials of PD progression

In longitudinal studies of PD progression using both PET and SPECT, the annual rate of reduction in striatal 18F-DOPA, 18F-CFT, ^{123}I β CIT, and ^{123}I FP-CIT uptake is about 6% to 13% in PD patients compared with 0% to 2.5% change in age-matched healthy controls (Morrish et al. 1998; Staffen et al. 2000; Nurmi et al. 2000;

Brooks 2000b; Brücke et al. 2000; Marek et al. 2001). Examples of which include the REAL-PET trial using 18F-dopa PET, and the CALMPD study with ^{123}I β-CIT SPECT, both of which followed PD patients over 2 and 4 years to study disease progression, looking at the effects of dopamine (DA) and levodopa (L-dopa) in early PD patients with imaging and clinical outcomes.

3.1.4.1 The CALM-PD trial

In the CALM-PD trial, the effects of initial treatment with dopamine agonist pramipexole were compared with levodopa, with regard to the development of major dopaminergic motor complications in patients with early PD (less than 7 years of disease duration)(Parkinson Study Group 2000). To compare the rates of dopamine neuron degeneration after initial treatment with pramipexole or levodopa, 82 out of the 301 patients entered an imaging study using the percentage change from baseline in striatal ^{123}I β-CIT uptake after 46 months, as the primary outcome measure. Patients initially treated with pramipexole showed a decline in mean (standard deviation) ^{123}I β-CIT striatal uptake from baseline of 20.7% (14.4%) at 46 months, compared to 25.5% (14.1%) decline seen in the levodopa group. The relative reduction in percent loss from baseline of ^{123}I B-CIT uptake in the pramipexole versus levodopa was 37% less at 46 months after initial treatment. A correlation of the ^{123}I B-CIT percent reductions with percent change in UPDRS scores was not demonstrated at 22 months follow-up, but significant correlation was shown at 46 months (Parkinson Study Group 2002b).

3.1.4.2 The REALPET trial

In contrast, the REAL-PET trial randomised 162 de novo PD patients with symptom duration of 2 years or less to have ropinirole or L-dopa, using reduction in putamen 18F-dopa uptake between baseline and 2 year as primary outcome measure (Whone et al. 2003). The group treated with the dopamine agonist ropinirole had a 13% reduction of 18F-dopa over two years, compared with a 20% reduction in the L-dopa cohort. This represents a relative reduction of 35% less signal loss in the ropinirole group than in the levodopa group. However it is important to point out that the reduction of 18F-dopa did not correlate with changes in UPDRS scores.

3.1.4.3 The ELLDOPA trial

The ELLDOPA trial evaluated a cohort of newly-diagnosed de novo PD patients, randomising them to receive either a placebo, or one of three doses of L-dopa (150mg, 300mg, or 600 mg) and followed over 9 months. The authors hypothesised that L-dopa is neurotoxic, therefore it was expected to enhance the rate of disease progression despite providing symptomatic benefit (Fahn 1999). After two weeks of medication washout, UPDRS scores in all the L-dopa groups increased, but did not return to the level of the placebo group, suggesting either that L-dopa is neuroprotective or that the two week washout was not long enough. The imaging data showed a near significant trend for increased percent loss of ^{123}I β-CIT SPECT in the high dose levodopa group compared with placebo.

Given the comparative design of the REAL-PET and CALM-PD studies, we cannot distinguish whether the DA agonists (ropinirole or pramipexole) were acting as neuroprotectors, slowing the rate of DA terminal loss, or whether L-dopa was

increasing the rate of terminal loss (or a combination of both effects). One possible conclusion that could be drawn from these imaging data is that L-dopa is neurotoxic. However, an alternative explanation is that the dopamine transporter is regulated by L-dopa treatment, causing the observed signal changes. Other issues are the lack of a placebo control in all these trials and the poor correlations among imaging and clinical scores. On one side this discordance could be explained by difficulty in achieving complete medication washout of PD patients, compromising the assessment of native disease status. However, the possibility that regulation of the imaging target site (dopa decarboxylase for 18F-dopa, dopamine transporter for ^{123}I β-CIT) may affect interpretation of neuroimaging data in long-term PD progression studies shouldn't be neglected.

In light of the recent use of functional imaging to monitor progressive dopaminergic degeneration, a small imaging arm was incorporated into the Exenatide trial design to evaluate whether there was any evidence of a change in pre-synaptic dopaminergic integrity (^{123} I-FP-CIT DaT SPECT scans).

3.2 METHODS

3.2.1 3.2.1. Patients

Due to budgetary constraints, not all trial participants could undergo imaging. As a pragmatic compromise, the first 10 (8 males, 2 females; mean age 60.3 years ± 4.8) of the 20 patients randomised to Exenatide treatment were invited to have a DaT-SPECT scan at baseline (before treatment initiation) and after 12 months of Exenatide therapy. The disease duration within the cohort ranged from 5 to 14 years (mean 8.3 ± 3.1), and the mean UPDRS-III off blind score was 29 ± 11.05. These 10 patients received an appointment to attend the Nuclear medicine department (UCLH)

for the injection of the radio-tracer, followed by the SPECT acquisition. All the patients received iodide capsules by post.

3.2.2 SPECT acquisition

Subjects received four 60mg oral capsules of potassium iodide; two were taken the evening before the tracer was administered, and two were taken in the evening following the scan, to block thyroid uptake of free radioactive iodine.

The scans were performed at the Institute of Nuclear Medicine, University College Hospital, London. The radiopharmaceutical was purchased commercially ^{123}I-FP-CIT, Amersham Health, GE Healthcare). On the day of scanning subjects were injected intravenously with a bolus of ^{123}I-FP-CIT (approximately 185 MBq, specific activity N 100 TBq mmol^{-1}). Subjects were scanned using a dual detector rotating gamma camera (GE Infinia Hawkeye) fitted with a low-energy high-resolution parallel hole collimators. The optimum time of acquisition was defined as the time at which specific radioactivity in the striatum (total striatal counts minus counts in the occipital cortex) was at a stable level (Booij et al. 1997); in our cohort, this meant scanning started between 180 and 240 minutes after radiotracer injection. One hundred twenty projections with a 128 x 128 image matrix were acquired at 40 seconds per view with the camera heads following a circular orbit, resulting in a total scan time of 43 minutes.

The SPECT data were reconstructed by OSEM iterative reconstruction using 10 iterations, 10 subsets and a Butterworth post filter cut-off of 0.55 cm^{-1} and power factor 10. The reconstructed images, uncorrected for gamma ray attenuation, were subsequently converted to Interfile format for further analysis. This same procedure

was repeated at 12 months follow up, the day after the last injection of Exenatide at month 12.

3.2.3 Analysis of SPECT data: Overview

SPECT data were scrutinised using two separate analysis methods. Data were first analysed using a region-of-interest-based approach, where the signal in each predefined region was extracted, and the statistical tests performed. The data were subsequently analysed using Statistical Parametric Mapping (SPM).

3.2.3.1 Analysis of SPECT data: Region-of-interest analysis

The data were first registered to a Tailairach and Tournoux based template using a modified version of the Brain Analysis Software (BRASS, version 3.4.4; Hermes Medical Solutions). An automated multistep procedure consisting of an initial nine-parameter linear fit was used (scale, translation and rotation in each the x, y and z planes), and subsequent fine adjustment of a standardized 3D volume-of-interest (VOI) map (Koch et al. 2005). We hypothesised a priori that we would be most likely to find changes within the striatum, as has been demonstrated in previous functional imaging studies monitoring PD progression. For this reason, our region of interest included the whole striatum. For completeness, the striatum was further subdivided in the caudate nucleus and putamen to assess whether changes were localised or diffuse. The occipital cortex served as a reference region given it is almost devoid of DA transporters (De Keyser et al. 1989). Any activity within this region was thus considered ‘non-specific’ activity (noise).

The volume of interest (VOI) map included the whole striatum, as well as a reference region within the occipital cortex. Sub-masks were able to isolate voxels of the caudate nucleus and the putamen separately in each hemisphere.

Mean counts per voxel within each VOI were calculated, as well as the 'uptake ratio', that is, the ratio of counts within the striatal regions (striatum, putamen and caudate nucleus) to the occipital region, as performed previously by (Koch et al. 2005).

The uptake ratio in each region of interest was recorded at each time point (baseline and 12 months). The striatal (and indeed caudate or putamen) uptake ratio is not a straight ratio. It is e.g. (striatal-occipital)/occipital. This means that the striatal region of interest counts has to be adjusted for non-specific binding within the region and then divided by the non-specific (occipital) uptake.

The absolute change in uptake ratio between the two time points were then calculated, as well as the percentage change. Correlations between SPECT uptake ratios and clinical rating scale scores (UPDRS and Mattis DRS-2- For full description of Non motor assessments see Chapter 5) were investigated using a Spearmen's statistical test. A P-value less than 0.05 was considered significant.

3.2.3.2 Analysis of SPECT data: Statistical Parametric Mapping

Data were formally interrogated using SPM, version 8 running in Matlab. Data were pre-processed in the normal way. Data were first realigned and the data from different time points were coregistered. Data were then spatially normalised to a standard MNI space, and then spatially smoothed using a Gaussian kernel (10mm full-width half maximum).

Once the images were spatially normalised and smoothed, the general linear model (Chatfield, C., Collins 1980) was employed to perform the appropriate voxel-wise univariate statistical tests. Image intensity was normalised between subjects to prevent inter-subject variability masking the regional changes. This was performed using proportional scaling (Frackowiak et al. 1997), which scales each image appropriately to a reference region. Total counts in the brain were used for the proportional scaling. So proportional scaling was performed by dividing the intensity values for each scan by the mean count per voxel for the scan.

The analysis threshold was set at the intensity (100%) of the occipital mean voxel count as this appeared to remove most of the regions containing little or no specific uptake, leaving the striatal uptake for analysis.

Statistical inference about regional effects was estimated from the distributional approximations using Gaussian random field theory (Friston et al.. 1994).

Brain regions with increased and decreased FP-CIT binding between subject groups were assessed using standard T-contrasts (contrast weights: increased = (1, -1); decreased = (-1, 1), and examined by superimposing the ensuing maps (SPMs) onto an anatomical template image. All statistical maps were threshold for peak height at the level $P \leq 0.05$. Correction for multiple comparisons was made using Family Wise Error rate (FWE).

3.3 RESULTS

3.3.1 Region of interest analysis

There were no changes in dopamine transporter availability pre- and post-Exenatide treatment using VOI analysis. Quantified uptake of ^{123}I-FP-CIT at baseline, together with absolute change and percentage change in ^{123}I-FP-CIT uptake at 12 months did not show difference for each sub-region of the basal ganglia for each individual, as presented in Table 12. All patients had profoundly abnormal scans at baseline with some variation in severity of pre-synaptic dopaminergic deficit (See Table 11). Two patients with severe baseline pre-synaptic deficits had minor improvement in ^{123}I-FP-CIT uptake in all basal ganglia sub-regions at 12 months (Patients 2 and 5. Figure 5). One person had deterioration in all sub-regions (Patient 9). Mean values for absolute and percentage changes in ^{123}I-FP-CIT activity showed minimal change in all basal ganglia sub-regions at 12 months. .

No correlations were found between SPECT uptake ratios and clinical rating scale scores (UPDRS and Mattis DRS-2).

3.3.2 Statistical parametric mapping

Statistical parametric mapping was performed using baseline UPDRS as covariate, no correlations among UPDRS and change in ^{123}I-FP-CIT uptake was found.

Exenatide patient number	Right Caudate Baseline	Left Caudate Baseline	Right Putamen Baseline	Left Putamen Baseline	Right Striatum Baseline	Left Striatum Baseline
1	1.05	0.97	0.58	0.47	0.78	0.69
2	0.75	0.73	0.19	0.26	0.43	0.46
3	1.65	1.31	0.76	0.50	1.14	0.85
4	1.04	0.92	0.32	0.37	0.63	0.61
5	0.45	0.46	0.12	0.22	0.26	0.32
6	1.02	1.05	0.35	0.49	0.64	0.73
7	1.51	1.61	0.37	0.92	0.86	1.22
8	0.45	0.79	0.23	0.29	0.33	0.50
9	1.09	1.42	0.44	0.67	0.72	0.99
10	0.90	1.37	0.46	0.68	0.65	0.98

Table 11. [123I]FP-CIT Uptake ratio of VOI to occipital cortex at baseline

Exenatide patient number	Right Caudate Absolute change % change	Left Caudate Absolute change % change	Right Putamen Absolute change % change	Left Putamen Absolute change % change	Right Striatum Absolute change % change	Left Striatum Absolute change % change
1	**0.045**	**0.14**	-0.09	-0.15	-0.03	-0.03
	4.3	**14.1**	-15.6	-31.5	-4.1	-3.7
2	**0.14**	**0.51**	**0.15**	**0.35**	**0.14**	**0.42**
	18.1	**69.9**	**78.0**	**133.3**	**33.0**	**90.0**
3	**0.006**	-0.22	-0.09	**0.02**	-0.05	-0.08
	0.38	-16.5	-11.8	**4.2**	-4.2	-9.7
4	-0.14	-0.21	**0.13**	-0.08	**0.01**	-0.14
	-13.6	-23.3	**41.8**	-22.8	**2.1**	-23.1
5	**0.20**	**0.21**	**0.11**	**0.08**	**0.15**	**0.14**
	45.3	**45.4**	**90.4**	**36.9**	**57.3**	**42.1**
6	-0.01	**0.005**	-0.21	-0.07	-0.13	-0.04
	-1.34	**0.43**	-61.7	-13.5	-20.0	-4.8
7	-0.24	-0.48	**0.05**	**0.04**	-0.07	-0.19
	-16.0	-30.1	**14.4**	**4.6**	-8.6	-15.2
8	**0.19**	-0.38	**0.06**	-0.04	**0.12**	-0.18
	42.2	-47.9	**25.4**	-13.1	**35.4**	-36.6
9	-0.28	-0.44	-0.12	-0.13	-0.19	-0.27
	-25.7	-30.9	-28.1	-19.9	-26.5	-26.7
10	**0.12**	-0.10	**0.07**	-0.12	**0.09**	-0.11
	13.3	-7.1	**14.3**	-18.2	**13.7**	-11.5

Table 12. Change in striatal [123I]FP-CIT Uptake between baseline and month 12 for each patient.

Absolute levels of [^{123}I] FP-CIT uptake are presented for each patient, together with absolute change and percentage change at 12 months scan (Bold type/positive values indicate improvement in [^{123}I]FP-CIT compared with baseline scan).

	Right Caudate	Left Caudate	Right Putamen	Left Putamen	Right Striatum	Left Striatum
Mean DATSCAN uptake at baseline (SD)	0.99 (0.39)	1.06 (0.35)	0.38 (0.19)	0.49 (0.22)	0.64 (0.26)	0.74 (0.28)
Mean of absolute changes in DATSCAN uptake at 12 m	0.002	-0.10	0.005	-0.01	0.004	-0.05
Mean of % change in DATSCAN uptake at 12 m	6.7	-2.6	14.7	6.0	7.8	0.09

Table 13. Mean (SD) of change in Striatal [123I]FP-CIT Uptake between baseline and Month 12

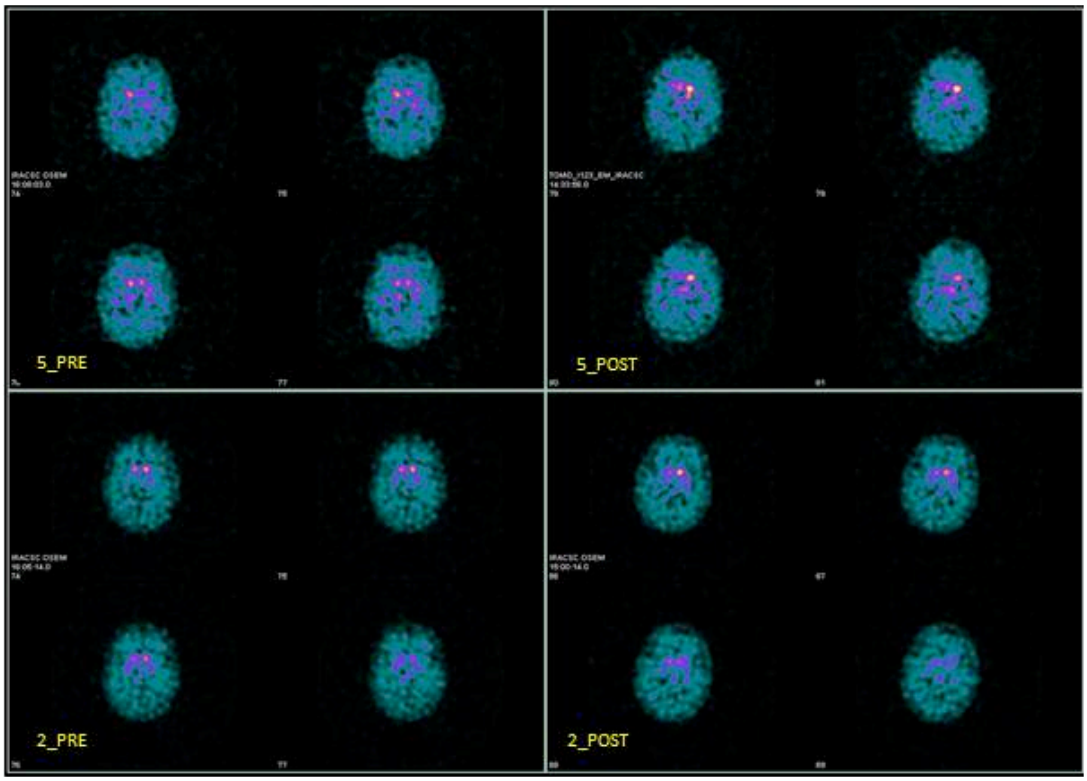


Figure 5. [123]FP-CIT SPECT pre and post-therapy of patient 2 and 5

3.3.2.1 DISCUSSION

In the absence of a placebo group, or any reliable measure that tracks the pathology of PD, our trial design incorporated a functional imaging arm that theoretically allowed us to get some indication of whether Dat SPECT scan might be a useful secondary outcome of effectiveness of Exenatide as disease modifying agent.

Different studies have utilised different functional imaging methods to image the dopaminergic pathway. There has been some controversy about the suitability of ^{123}I-FP-CIT- SPECT for assessing the progressive loss of striatal dopamine nerve terminal function (Morrish 2003). The choice of imaging modality is ultimately determined by the specific study questions and study design. 18F-dopa PET has been

shown to provide reliable measures over a short period of time, but it is extremely expensive, therefore it was unavailable due to budget restrictions (this trial was charity funded). Thus, our imaging arm subgroup was chosen using ^{123}I-FP-CIT - SPECT scans that may lack the sensitivity to detect gradual changes in the nigrostriatal pathway once the disease has started, in contrast to 18F-dopa PET images, but offer a cheaper and more feasible approach.

In prior imaging studies, the annual percentage loss of ^{123}I-FP-CIT striatal uptake of early PD patients has been reported as declining at 4.5% per year over the first 2 years then 3.0% per year over the subsequent 3 years (Pirker et al. 2003). However, a smaller reduction in DAT binding per year has been reported by a few studies on PD patients with a disease duration of more than 5 years (Marek et al. 2001; Colloby et al. 2004) suggesting a slowing down of the progression in the case of more advanced disease. On the other hand, in a cohort of 139 healthy controls for 13 different centres, an average age-related decline in [^{123}I]FP-CIT- SPECT availability of 5.5% per decade was found for both genders (Varrone et al. 2013). No differences were found in SPECT availability in our group when comparing images at baseline with the ones at month 12. Moreover, pre- and post-treatment DAT availability was assessed using SPM, but again no significant difference was found.

An interesting point that should be considered is that the majority of the patients in these studies were untreated at the time of the baseline scan and started antiparkinsonian medication before the follow-up scan. Results of the ELLDOPA trial suggest that levodopa may downregulate striatal dopamine transporter binding (Fahn 1999).This could be an explanation for the higher rates of progression that

have been found in imaging studies with de novo patients (Nurmi et al. 2000; Marek et al. 2001).

Moreover, important criticism have been raised in the past for the use of ^{123}I-FP-CIT- SPECT in neuroprotection studies, mainly driven by concerns about the very strong influence of scan to scan variability, as well as reproducibility of the ^{123}I-FP-CIT SPECT technique. It has been pointed out that considering that variability of SPECT measurement is substantially larger than the average annual decline of striatal binding, its suitability for measuring disease progression should be questioned.

The quantification of the radioligand binding in our study was performed automatically, using an automated three dimensional quantification on a voxel by voxel basis. This observer-independent automated system standardizes the semiquantification procedure, avoiding observer bias/dependency.

Another criticism that should not be forgotten, in progressive stages of parkinsonian syndromes, as it was the case of our imaged patients, is the little residual activity found in the caudate nucleus and almost none in the putaminal area, which might result in incorrect transformation of the data, introducing new sources of error. Errors can be introduced when imaging data are exported into mat lab software for analysis.

On the top of that, despite optimal standardization of manual ROI analyses, observer-independent automated systems are required to standardize the semiquantification process and to overcome observer dependency. The BRASS software allowed us to register the patients' studies to a tracer-specific template of

healthy control subjects and then apply a standardized 3D VOI map for semiquantification. Therefore objective and observer-independent semiquantification of the ligand binding in the striatum was performed.

Different outcomes have been published so far in relation to correlations in imaging outcomes and clinical outcomes. Longitudinal studies have shown only weak correlations at best between the change in imaging outcomes and the change in clinical outcomes. The REAL PET study did not find significant correlation, and the CALM-PD imaging study, with a much larger cohort, found significant correlation between the percentage reduction of striatal β-CIT binding and the change in total UPDRS, but only for the longest follow-up group at 46 months from baseline (Parkinson Study Group 2002b). Consequently it has been suggested that correlation between imaging and clinical outcomes can emerge in larger groups of patients followed up for longer enough periods (Pirker et al. 2003). In our group no correlations were found among the changes in part III UPDRS (Blinded scores) that improved significantly in Exenatide treated patients along the 12 months follow up and the difference in DAT availability in the imaged patients.

There are several explanations for the lack of correlation between [^{123}I]-β-CIT or [^{18}F]-dopa uptake and UPDRS in longitudinal studies. To start with, the UPDRS is confounded by the effects of the patient's anti-PD medications both acutely after initiation of therapy and with ongoing treatment. The UPDRS "off medication" state might be affected by prolonged washout medication period, consequently, some symptomatic effect might remain (Seibyl 2003). Another point to consider is that the rate of loss of dopamine terminals and the change of UPDRS may not share a simple linear relationship. This is reflected by the loss of 40-60% of striatal ^{123}I-β-CIT or

^{18}F-dopa uptake at the time of diagnosis (Tissingh et al. 1998), when UPDRS score is minimal. As UPDRS scores slowly increase later in the disease (at a rate of 1.34 – 1.58% a year) (Poewe 2009), imaging markers during this period show the expected continued loss of β-CIT or F-dopa uptake (Benamer et al. 2000), again indicating that clinical and imaging outcomes provide complementary but not necessarily correlative data.

Therefore DAT availability did not support a neuroprotective action of Exenatide in our group of patients. The SPM analysis was repeated using the baseline, pre-treatment UPDRS as a covariate, to see if different disease severity at baseline could influence the ^{123}I-FP-CIT striatal uptake at month 12, but no significant correlation were found either.

We acknowledge that a weakness of our imaging study is the lack of imaging data in the control group and sample size (only half the treated patients had scans). This was due to financial restrictions.

Although our results do not survive statistical testing, there appears to be improvements in more regions than decline. This is interesting and the mean change in activity and SD, allows future sample size calculations for subsequent studies.

4 TWO YEARS FOLLOW UP OF TRIAL PATIENTS

4.1 INTRODUCTION

Ahead of the last patient reaching the14 month visit, the idea of performing a prolonged open label follow up visit at 24 months emerged. This would potentially provide further information about the longevity of any effects of the 12 month period of Exenatide exposure (biological, placebo or otherwise) in our cohort. A substantial amendment of the protocol was REC/ MHRA approved to perform a further detailed assessment of all participants at 2 years post baseline assessment. The aim of this 24 month assessment was to further clarify whether any changes associated with Exenatide exposure at 12 and 14 months were sustained over this longer follow up period.

4.2 METHODS

(See chapter 2.2)

4.2.1 Patients

The 44 patients (Exenatide group: 20, Control group: 24) included in the final analysis at 12 and 14 months, all then underwent a 24 month clinic assessment using the same battery as previously performed.

4.2.2 Patients who had undergone DBS

Three out of the 44 patients followed up at 24 months, had clinically deteriorated and had needed Deep Brain Stimulation for the treatment of the PD symptoms between the 12 and 24 month follow up;

- One in the Exenatide group, 16months after baseline assessment.

• Two in the Control group.

- One at 14 months follow-up. This patient did not attend the trial clinic at 14 month due to worsening PD and inability to attend being off medication.

- One at 16 months follow-up.

Patients undergoing DBS frequently have substantial lowering of their conventional PD medication. This can mean that assessments in the Off medication and OFF stimulation setting can be associated with much more profound severity of "OFF" symptoms and signs, presumably because of the long duration response to L-dopa replacement that is not seen in the "practically defined "Off" state after a short overnight withdrawal (Piboolnurak et al. 2007). Therefore OFF assessments in these 3 patients would be a source of bias and not comparable with the rest of the cohort. Similarly their On drug and ON stimulation assessments would also be a potential bias. For this reason, the decision was taken to use "last observation carried forward" (LOCF) for these patients for their motor scores (UPDRS, Dyskinesia rating Scale and Timed Test) and L-dopa equivalent dose (LED) at the 24 month assessment. For 2 patients this was the data collected at 14 months, for 1 patient this was the data collected at 12 months. Non motor scales were collected at 24 months from all patients.

4.2.3 Schematic diagram of 2 years post baseline trial design

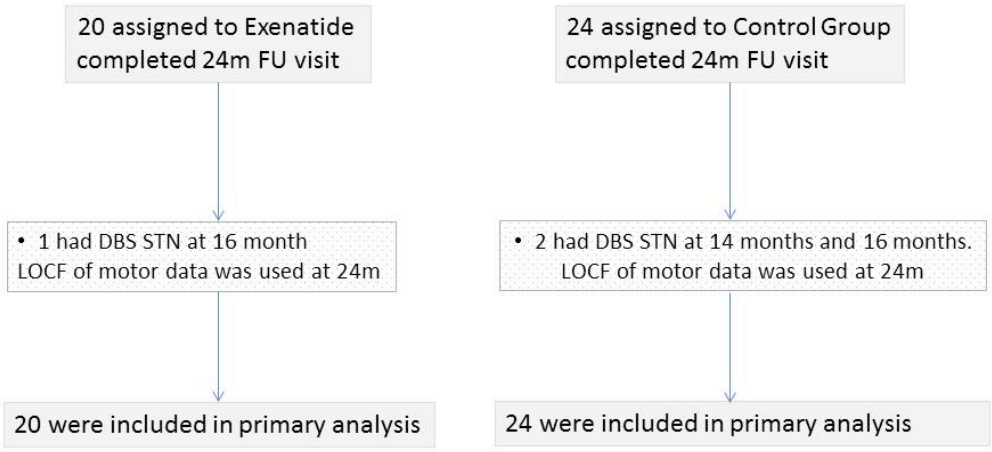


Figure 6. Schematic diagram of 2 years post baseline trial design

4.2.4 Twenty four month assessments

Patients were sent the PDQ39, to self-complete and bring to the clinic.

All patients withheld all PD medications as previously described, see section 2.2.2.iii. baseline visit. They were asked not to have eaten and to have only drunk water for 12 hours enable fasting blood samples to be taken.

Each patient had blood tests to measure fasting serum glucose.

All patients had a video recording of UPDRS part 3 score, performed timed motor test and then took their regular medication. The patients were asked to complete UPDRS part 1, 2, 4, and when confirmed that their best on state was achieved, they had a further video of UPDRS part 3 score. During their on phase, the Dyskinesia rating scale, MADRS, Mattis DRS was completed (Non-motor assessments are discussed in Chapter 5). Clinical observations (weight, pulse and blood pressure) were also collected and their current PD medication regime was noted. The adverse events along the previous year were recorded.

4.2.4.1 Blinded Video rating

Blinded rating of videos was performed by the same individuals trained and certified in MDS-UPDRS rating. Individuals were allowed to view videos and their previous scores at baseline, 12 months, 14 months while assessing the 24 month videos to ensure consistency across timepoints. The treatment status of 1 individual had become known to one of the blinded raters and therefore all their videos were assigned to a different blinded reviewer for re-scoring at each timepoint.

4.2.4.2 Analyses

Comparisons were made between the change at 24 month in primary outcome (Part 3 MDS UPDRS) and change at 24 month in secondary outcomes; MDS UPDRS part 1, 2 and,4 ,LED, Dyskinesia rating Scale, timed test and PDQ39 using 2-sided t test.

Appointment	Control patients attend	Exendatide patients attend	Fasting blood	Clinical obs	UPDRS-3 off	UPDRS-3 on	Timed tests	UPDRS-1/2/4	Dyskinesia rating scale	MADRS	Mattis DRS	ECG	PDQ39	NMS	SCOPA	AUT
24 months	Off medication	Off medication	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Table 14. Twenty four month's assessment overview

4.3 RESULTS

Twenty four months follow up visit started in August 2012, and last patient was seen for the 24 month assessment in March 2013.

The 44 patients (Exenatide group 20: Control group 24) included in the primary analysis at 12 and 14 month had a 24 follow up and were included in

primary analysis at 24 month. There were no missing data (allowing for the LOCF motor scores for the 3 patients who had undergone DBS surgery).

4.3.1 Clinical outcomes

4.3.1.1 Blinded video rating of MDS-UPDRS part 3 in the practically defined "off medication" condition

Patients allocated to the Exenatide group had a mean improvement at 24 months of 1.1 points (SD, 5.9) on the MDS-UPDRS part 3, while controls had a mean decline of 4.5 points (SD, 5.3) for a difference of 5.6 points (95% CI, 2.2-9.0; p=0.002; Table 15). These scores did not include any changes detected in limb or neck rigidity, which cannot be reliably rated on video. Addition of open label rating of rigidity scores to the blinded data equated to a decline of 0.5 points (SD,7.3) in the Exenatide group, compared with a decline of 8.5 points (SD, 6.3) in the control group (difference, 8.0 points; 95% CI, 3.8-12.2; p<0.001); (Table 15 & Figure 7)

There was a significant improvement in bradykinesia subdomain of part 3 MDS UPDRS at 24 months in the Exenatide group in comparison with the control group, for a difference of 4.1 points (95% CI ,1.6-6.6 , p=0.002). Similarly there was an improvement in the rigidity subdomain (open label data) favouring Exenatide, with a difference of 2.4 points (95% CI, 0.5- 4.2, p=0.014). There were no significant differences in the axial and tremor subdomains of MDS-UPDRS part 3 between Exenatide and control group.

4.3.1.2 Secondary outcome measures

• Part 3 MDS UPDRS on medication motor subscore. Open label rating of MDS-UPDRS part 3 “on medication” showed a significant difference ($p<0.001$) favouring patients treated with Exenatide compared with the control group. The mean improvement in “on medication” scores in the Exenatide group was 0.9 points, SD (6.8) at 24-month visit. In contrast, individuals in the conventional PD medication group deteriorated over the 24-month trial period by 7.8 points SD (6.7).

• There was a significant difference favouring Exenatide in MDS-UPDRS parts 1 ($p=0.049$) and 2 ($p=0.009$) (UPDRS ADL).

• There was an advantage in Exenatide group for MDS-UPDRS part IV, LED or Dyskinesia Rating Scale (Tables 15 and 16), although this did not reach threshold for statistical significance.

• There was significant improvement in Left hand tapping test on medication state ($p=0.026$). No further significant differences were found in the other tapping tasks or walking timed tests (Table 18 &19).

• There was no improvement in PD-39 summary index between the 2 groups (Table 16). There was an advantage in cognition subdomain with a mean improvement in the Exenatide group of 3.1points, SD (9.8) at 24-month visit. In contrast, individuals in the conventional PD medication group deteriorated over the 24-month trial period by 0.2 points SD (13.7), although this was not significant ($p=0.363$). There was also an advantage in the communication subdomain with an advantage of 2.9 points , SD(18.2) at 24 months in the Exenatide group and a deterioration of 2.8 points, SD (16.6)in the control group (Table 19 &20).

4.3.1.3 Adverse events

There were no serious adverse events at 24 months. No clinically relevant changes in haematological, or biochemical indices were observed.

Patients allocated to the Exenatide group had a mean weight loss at 24 months of 1.6 Kg SD(3.1), while controls had a mean weight loss of 1.7 kg SD(5.8) for a difference of 0.1 points (95% CI, 3.0-2.8; p=0.93) (Adverse events reported by trial participants at month 24 are presented in table 17).

4.3.1.4 Correlations of clinical outcomes

Change in MDS UPDRS part 3 at 24 months was not correlated with younger age at disease onset, or younger age at start of the trial. No correlation was found among change in MDS UPDRS part 3 at 24 months and change in weight at 24 months.

No correlation was found among Hoehn and Yahr state (2 vs 2.5) at baseline and change in MDS-UPDRS part 3.

4.4 DISCUSSION

There is a difference at baseline between groups in terms of age at study enrolment with the Exenatide group having an older age 61.4(6.0) years versus control group having 59.4 (8.4) years and disease duration with Exenatide having a shorter disease duration 9.6 (3.4) years than conventional PD medication 11.0 (5.9) years, that could be a source of confounding. It is important to take into account that on one hand older age of onset has been associated with more rapid progression (A Schrag et al. 2006) but on the other hand it has been proposed that the rate of progression in early PD is faster than in later disease (Fearnley & Lees 1991; Hilker et al. 2005; Anette Schrag et al. 2007)

Inclusion of the rigidity scoring made by the unblinded investigator equated to an 8.0 points difference in MDS-UPDRS part 3 score at 24 months. Adding the blinded video rating of MDS-UPDS part 3 to the differences seen in parts 1, 2, and 4 of the scale equated to a 14.5 points advantage in favour of Exenatide at 24 months. Significant differences in the primary outcome (part 3 MDS UPDRS) were found between groups, favouring Exenatide at 24 months. This advantage favouring Exenatide was accompanied by effects seen across a range of other measures; MDSUPDRS part 1, 2 and 4, LED and Dyskinesia rating scale. However, looking at the MDS UPDRS part 3 "subdomain", the advantage favouring Exenatide was restricted to bradykinesia and rigidity, and was not seen in the tremor or axial signs subdomains at 24 month. The possibility that these observations might be due to placebo effect cannot be completely ruled out. Due to the open label design of the trial is not possible to elucidate if the nature of this effect is real or placebo driven. Nevertheless no reports on placebo effect, lasting 24 months have been reported to date to my knowledge.

A decision of extending the follow up for a sufficiently long period was taken to allow inevitable placebo effects to at least begin to diminish. The initial improvement in the main outcome (MDS-UPDRS part 3 blinded ratings) was sustained in the long term follow up. Despite the fact that a possible placebo driven effect could not be completely ruled out, the 24 month data support the idea that perhaps a real biological effect of Exenatide should not be completely ruled out either, reinforcing the possibility that open label trial designs with long wash out periods may provide preliminary indications of clinically relevant biological effects and thus provide a cost efficient means of providing further support for future study of Exenatide in PD patients.

The adverse events at 24 months are well in keeping with what would be expected in moderate severity PD patients. Weight loss was reversible upon stopping Exenatide injections, and there were no difference in change in body mass index at 24 months among patients on Exenatide and control group. No correlation was found among change in part 3 MDS UPDRS at 12, 14 and 24 months and weight loss, making it less likely that Exenatide patients had improved motor scores as their BMI was reduced along the first 12 month trial period.

There is a discrepancy between the improvement in MDS UPDRS part 2; activities of daily living (ADL) and the lack of improvement in PDQ39 Summary Index and PDQ39 ADL subdomains separately. Surprisingly, there were quite marked differences between some of the PDQ39 subdomains comparing Exenatide patients with controls at baseline. These differences at baseline, despite the randomisation process can be seen in small trials and may contribute to observed differences over the subsequent 2 years. The relative sensitivity of, the MDS-UPDRS part 2 scale, in comparison to the PDQ39 ADL subdomain to detect change in ADL has not been directly compared.

The increase in Dyskinesia Rating Scale and reduction in LED seen at 12 and 14 months in the Exenatide group were no longer significantly different from controls at 24 months. This was explicable in part by 4 patients in the Exenatide group who had worsening of peak dose dyskinesia at 12 and 14 months that necessitated lowering the LED, subsequently went on to have their LED re-increased at 24 months. Although not reaching significance, Exenatide patients still had slightly more dyskinesias than controls despite lower increases in LED overall,

therefore it remains possible that Exenatide may worsen dyskinesia particularly during the period of exposure.

	Baseline Mean(SD)	24months Mean(SD)	Difference Baseline to 24 months Mean(SD); 95% CI	P value
Blinded- MDS UPDRS Part III. A	***"off medication"***			
Exenatide	31.0(11.2)	29.8(9.8)	-1.1(5.9);-3.9,1.6	P=0.002
Conventional PD medication	34.0(16.1)	38.5 (15.1)	4.6(5.3);2.2,6.7	
MDS UPDRS Part III.B	***"on medication"***			
Exenatide	23.5(6.3)	22.5(7.2)	-0.9(6.9);-4.2,2.3	P=0.000
Conventional PD medication	25.3(10.7)	33.2(11.4)	7.8(6.7);5.0,10.7	
MDS-UPDRS	***Part I***			
Exenatide	10.4(4.1)	12.4(5.0)	2.0(4.2);0.0,4.0	P=0.049
Conventional PD medication	11.6(4.7)	16.7(8.2)	5.1(5.5);2.8,7.4	
MDS-UPDRS	***Part II***			
Exenatide	10.2(5.2)	12.9(7.1)	2.7(5.4);0.2,5.3	P=0.009
Conventional PD medication	12.9(6.2)	19.9(7.2)	7.0(5.0);4.9,9.1	
MDS-UPDRS	***Part IV***			
Exenatide	6.3(2.4)	5.9(3.4)	-0.3(2.6);-1.6,0.9	P=0.107
Conventional PD medication	6.3(3.4)	7.3(3.5)	1.0(2.8);-0.1,2.2	

Table 15. Changes in MDS-UPDRS score between baseline and month 24.

A= Blinded rating, excludes rigidity. B = Open label, includes rigidity

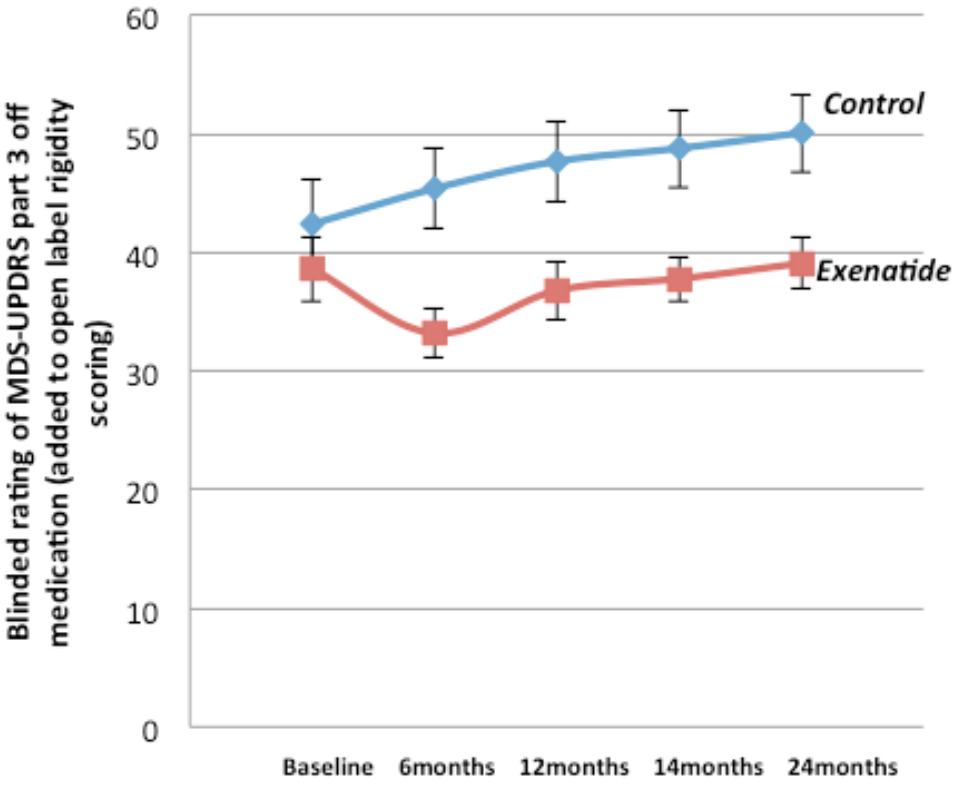


Figure 7. Change from baseline in the MDS-UPDRS part 3 score by study visit. (Data represent mean ± SEM)

	Baseline Mean(SD)	24months Mean(SD)	Difference Baseline to 14 months Mean(SD); 95% CI	P value
LED				
Exenatide	973(454)	1104.6 (506.5)	131.9(192.3);41.9,221.9	P=0.308
Conventional PD medication	977(493)	1189.1(679.5)	211.9(298.7);85.8,338.1	
Dyskinesia Rating Scale	***(on medication)***			
Exenatide	2.3(2.8)	3.5(4.2)	0.8(6.0);-2.0,3.6	P=0.328
Conventional PD medication	2.6(2.9)	2.0(2.5)	-0.6(3.0);-1.8,0.7	
PDQ39 summary index				
Exenatide	19.2(13.5)	19.1(13.5)	-0.1(12.3);-5.9,5.6	P=0.682
Conventional PD medication	24.5(12.8)	25.7(16.5)	1.2(9.3);-2.7,5.1	

Table 16. Changes in the score on LED, dyskinesia rating scale, and PDQ39 summary index between baseline and month 14

Adverse events (AE) ***Exenatide***	***n***	***Adverse events (AE)*** ***Conventional PD medication***	***n***
Exenatide			
Back pain	2	Other pain	10
Increase off time	5	Increase off time	3
Increase dyskinesia	2	Impulsive Compulsive Disorder	2
Memory/attention impairment	3	Sleep problems	3
Increase bradykinesia	2	Impaired balance	2
Cramps	3	Cramps	2
Urgency and bladder incontinence	4	Freezing on gait	4
Falls	2	Falls	3
Low mood	2	Low mood	3
Miscellaneous	21	Back pain	3
		Miscellaneous	25

Table 17. Adverse events reported by trial participants at month 24

	Baseline Mean(SD)	24months Mean(SD)	Difference Baseline to 24months Mean(SD); 95% CI	P value
R hand taps	***Off meds***			
Exenatide	43.5(10.8)	55.3(13.3)	**11.7** (9.9);2.9,16.9	P=0.347
Conventional PD medication	45.4(15.1)	47.2(17.3)	**1.8**(12.5);3.1,16.7	
R hand taps	***On meds***			
Exenatide	53.3(10.8)	61.4(13.9)	**8.1**(18.3);-0.5,16.6	P=0.226
Conventional PD medication	53.7(19.6)	53.7(19.7)	**0.0**(23.9);-10.1,10.1	
L hand taps	***Off meds***			
Exenatide	42.2(14.0)	52.4(16.0)	10.2(9.6); 5.7, 14.7	P=0.157
Conventional PD medication	40.9(12.4)	46.4(12.8)	5.5(11.7); 0.5,10.5	
L hand taps	***On meds***			
Exenatide	50.7(14.5)	56.2(14.7)	5.5(20.0);-2.9,14.8	P=0.026
Conventional PD medication	50.5(15.0)	51.9(13.9)	-7.6(17.5);-15.0,-0.2	

Table 18. Change in the score on hand tapping timed test between baseline and month 24

	Baseline Mean(SD)	24 months Mean(SD)	Difference Baseline to 24 months Mean(SD); 95% CI	P value
Timed walk	***Off meds***			
Exenatide	17.3(5.2)	17.1(7.1)	-0.2(6.6);-3.2,2.9	P=0.85
Conventional PD medication	23.8(22.4)	23.1(19.0)	-0.9(15.8);-7.5,5.8	
Timed walk	***On meds***			
Exenatide	13.9(3.1)	14.4(3.4)	0.5(5.0);(-1.8,2.8)	P=0.213
Conventional PD medication	13.7(4.4)	16.7(10.0)	3.4(9.3);(-0.5,7.4)	

Table 19. Change in the score on walking timed test between baseline and month 24

	Baseline Mean(SD)	24months Mean(SD)	Difference Baseline to 24 months Mean(SD); 95% CI	P value
PDQ39 summary index				
Exenatide	19.2(13.5)	19.1(13.5)	-0.1(12.3);-5.9,5.6	P=0.682
Conventional PD medication	24.5(12.8)	25.7(16.5)	1.2(9.3);-2.7,5.1	
Mobility subdomain				
Exenatide	18.6 (19.8)	19.9(20.1)	1.2(18.9);-12.1,6.8	P=0.582
Conventional PD medication	21.8 (19.9)	25.6(25.4)	3.8(12.0);-12.5,7.3	
ADL subdomain				
Exenatide	18.9(15.7)	18.5(16.5)	-0.4(13.5;-7.8,10.2	P=0.8
Conventional PD medication	30.0(21.1)	28.5(21.6)	-1.5(15.7);-7.7,10.0	
Emotion subdomain				
Exenatide	14.2(13.5)	15.6(15.7)	1.4(12.9);-6.6,10.2	P=0.669
Conventional PD medication	23.3(15.7)	22.9(22.1)	-0.3(14.4);-6.5,10.1	
Stigma subdomain				
Exenatide	19.1(22.3)	16.3(20.8)	-2.8(22.1); -14.3,9.2	P=0.664
Conventional PD medication	27.4(16.9)	27.1(16.9)	-0.3(16.5); -14.7,9.6	
Social subdomain				
Exenatide	10.4(22.7)	11.6(22.8)	1.2(17.8);-11.2,8.8	P=0.811
Conventional PD medication	10.4(13.1)	12.8(14.5)	2.4(15.0);-11.4,8.9	
Cognition subdomain				
Exenatide	25.0(17.2)	21.9(15.4)	-3.1(9.8);-10.8,4.0	P=0.363
Conventional PD medication	27.6(21.4)	27.8(21.4)	0.5(13.7);-10.6,3.8	
Communication subdomain				
Exenatide	21.2(20.1)	18.3(20.5)	-2.9(18.2);-16.3,4.9	P=0.285
Conventional PD medication	24.9(19.8)	27.7(23.0)	2.8(16.6);-16.4,5.0	
Body discomfort subdomain				
Exenatide	25.8(19.5)	29.9(25.9)	4.2(21.2);-9.7,13.1	P=763
Conventional PD medication	30.9(21.7)	29.9(33.3)	2.4(16.4);-10.0,13.5	

Table 20. Change in the PDQ39 summary index and subdomains between baseline and month 24

5 EXENATIDE AND NON MOTOR SYMPTOMS OF PD

5.1 INTRODUCTION

Patients with PD can present with a wide array of non-motor symptoms (NMS), in addition to the typical parkinsonian motor syndrome. NMS are mainly neuropsychiatric manifestations (e.g. depression, and apathy), cognitive impairment , sleep disorders (e.g. rapid eye movement (REM) behaviour disorder (RBD), and excessive daytime sleepiness), autonomic dysfunctions (e.g. intestinal constipation, postural hypotension and micturitional disorders) and others, such as fatigue and pain. In their various combinations, NMS may eventually become the main complaints of patients with PD, as NMS are frequently an untreated part of the disease.

NMS may be related to the neurodegenerative changes affecting several neural systems and/or caused by drugs employed in the treatment of PD. The main neural systems related to NMS are: (1) non-motor frontostriatal circuits, (2) serotoninergic nuclei of median raphe, (3) noradrenergic nuclei (locus coeruleus and subcoeruleus), (4) autonomic centres (hypothalamus and dorsal motor of vagus nucleus) and (5) olfactory system (Braak et al. 2006). The NMS in PD may be present in all the clinical phases of the disease: pre-motor phase, the initial phase (when the motor symptoms appear and the diagnosis is made), the honeymoon phase and in the advanced phase. In the pre-motor phase, the most common NMS symptoms are: hyposmia, depression, intestinal constipation and RBD. During the initial and honeymoon phases, several autonomic and neuropsychiatric manifestations may be

present, and in the advanced phase, the most important NMS is PD dementia (Chaudhuri et al. 2006).

The presence of NMS impacts on disability and quality of life, mainly in advanced disease and might limit the ability to prescribe other drugs (Chaudhuri et al. 2006) .

5.1.1 Measuring NMS

Full understanding of the patient's condition requires not only the consideration of symptoms and disabilities, but also patient's perspective and preferences, in what has been described as a holistic approach; trying to treat the whole person rather than just the physical symptoms of the disease (Martinez-Martin 2013). According to this, information coming from patient him/herself (questionnaires) should be considerably helpful in combination with rating scales to achieve a holistic assessment of PD.

5.1.1.1 Mattis dementia Rating Scale

The Mattis dementia Rating Scale is a widely used scale to evaluate cognitive functioning in older adults. A total score ranging from 0 to 144 is based on performance from five subscales; Attention (37 points), Initiation/Perseveration (37), Constructional ability (6), Conceptualisation (39) and Memory (25) with higher scores conferring better cognitive function (Schmidt, KS. et al. 2006).

5.1.1.2 Montgomery and Asberg Depression Rating Scale

A number of scales have been used to screen or measure the severity of depression. For screening purposes, The Montgomery and Asberg depression rating

scale (MADRS), has been shown to be valid for screening purposes and for measurements of severity of depressive symptoms (Aarsland et al. 2009).

The Montgomery and Asberg Depression Rating Scale is a 10 item physician rated depression severity scale constructed to be sensitive to change with treatment. Each question had six responses to rate severity from zero to six, thus the total score ranges from 0-60. The time frame is the past week, with higher scores representing worse depression scores (Montgomery & Asberg 1979).

5.1.1.3 NMS Quest

The NMS Quest is a 30-item self-completed screening tool, with a ‘‘yes/no’’ response to the questions about the presence of symptoms. These are grouped in nine domains:

Digestive/urinary/apathy/attention/memory/hallucinations/delusions/

depression/anxiety/sexual function/cardiovascular/sleep disorders/

and miscellany.

The sum of positive responses indicates the number of NMS perceived by the patient, and the time frame is the last month (Martinez-Martin et al. 2007) . To obtain a standardized ranking of prevalence for each domain, the sum of each item for every domain was transformed to percentage on the maximum possible number of “yes” responses in the domain.

5.1.1.4 SCOPA Sleep

The SCOPA Sleep is a 12 item questionnaire enquiring about day time (six items) and night time sleepiness (five items) plus a question related to the overall sleep quality. It has demonstrated internal consistency and test-retest reliability (Marinus et al. 2003; Martinez-Martin et al. 2008). Each question has four responses to rate severity from zero "not at all" to three "a lot", and total score ranging from 0-33. The time frame is the past month, with higher scores representing increased sleep dysfunction. It is recommended for rating overall sleep problems both to screen and to measure severity, and for rating daytime sleepiness in a recent review of scales to assess sleep impairment in PD (Högl et al. 2010). Thus the SCOPA Sleep includes three subscales: a night time scale (NS), a single-item quality of sleep scale and a daytime sleepiness scale (DS).

The NS is a five-item scale with four response options that address night time disturbances that occurred in the previous month. Subjects indicate the extent to which they were disturbed on a scale of 0 (not at all) to 3 (very much). The five items include sleep initiation, sleep fragmentation, sleep efficiency, sleep duration, and early wakening. The maximum score is 15, with higher scores reflecting more severe sleep problems.

In addition, quality of sleep is assessed using an additional question that evaluates overall sleep quality on a seven-point scale (ranging from slept very well to slept very badly). The score on this item is not included in the score of the NS but is used separately as a global measure of sleep quality.

The DS subscale evaluates daytime sleepiness in the past month and includes six items with four response options, ranging from 0 (never) to 3 (often). Subjects indicate how often they fell asleep unexpectedly, fell asleep in particular everyday

situations, how often they had difficulty staying awake, and whether falling asleep in the daytime was considered a problem. The maximum score is 18, with higher scores reflecting more severe sleepiness.

5.1.1.5 SCOPA AUT

The SCOPA AUT is a 25 item questionnaire enquiring about the existence of symptoms involving the autonomic nervous system often seen in PD patients, assessing gastrointestinal symptoms (7 items), urinary symptoms (6 items), cardiovascular symptoms (3 items), thermoregulatory function (4 items), pupillomotor symptoms (1 item), and sexual function (2 items for men and 2 items for women) giving a total of 23 items for men and women. Each question has four responses to rate frequency from zero "never" to three "often", and total score ranging from 0-69, with higher scores representing increased autonomic dysfunction. To obtain a standardized ranking of prevalence for each domain, the sum of each item of every domain was transformed to percentage on the maximum possible number of "often" responses in the domain.

5.1.1.6 Smell Identification Test (SIT)

The SIT is a standardized forced-choice test comprised of four booklets containing 10 odorants per booklet, (1 odorant per page). The stimuli are embedded in "scratch and sniff" microcapsules fixed and positioned on strips at the bottom of each page. A multiple-choice question with four response alternatives for each item is located above each odorant strip. The maximum score is 40 that equals normal smell sense (Doty et al. 1984)

5.1.2 Prevalence of NMS in PD

In a multicentre, international, cross-sectional study involving 545 PD patients (mean disease duration: 6.96 year) using the NMS Quest, it was reported 10.3 points (out of a maximum of 30) as the mean total NMS, and 98.4% of the cohort reported some NMS (Martinez-Martin et al. 2007).

In the PRIAMO study 1,072 patients with PD were assessed to determine the prevalence of NMS. It was found that 98.6% of patients with PD reported the presence of NMS and the mean number of NMS per patient was 7.8, ranging from 0 to 32. The frequency of NMS was found to increase along with the disease duration and severity, and cognitive dysfunction (Barone et al. 2009).

Consentino and colleagues determined the prevalence of NMS in a cohort of 300 Peruvian patients with PD patients using the Spanish version of NMS Quest. The mean total non-motor symptoms was 12.41, ranging from 0 to 27 of a maximum of 30. A progressive increase in mean total score was observed across each 5-year interval (Cosentino et al. 2013). Thus, the authors propose that the mean total NMS increases significantly with disease duration.

5.2 METHODS

5.2.1 5.2.1. Baseline visit

Each patient was sent the PDQ 39, NMS Quest, SCOPA Sleep, SCOPA AUT and Smell Identification test self-assessment forms to complete 1 week prior to their clinic assessment to be brought to the clinic with the patient for their baseline evaluation. Patients were requested to fill the smell identification test while being on medication. Questionnaires left at home were replaced and completed at the time of

the visit. During the patients on medication phase, Mattis DRS and MADRS were completed.

5.2.2 Flowchart of study assessments

Appointment	MADRS	Mattis DRS	NMS	SCOPA	AUT	SIT
Baseline	●	●	●	●	●	●
6 months	●	●	●	●	●	
12 months	●	●	●	●	●	●
14 months	●	●	●	●	●	
24 months	●	●	●	●	●	

Table 21. Flowchart of study Non-motor symptoms assessments

5.2.2.1 Endpoints

5.2.2.1.1 Secondary endpoint. Change from baseline to 12 /14 and 24 months between patients on active treatment and PD controls for the following scales:

-Mattis dementia rating scale (MATTIS DRS-2)

-Montgomery and Asberg depression rating scale (MADRS)

-NMS Quest

-SCOPA Sleep scale

-SCOPA Aut scale

-Smell identification test (SIT)

5.2.2.1.2 Statistical analysis

The difference between each of the secondary outcome measures (MATTIS DRS-2, MADRAS, NMS Quest, SCOPA Sleep, SCOPA AUT) at baseline and at 12, 14 and 24 months, and the difference between smell test at baseline and 12 month was calculated for each patient. The mean difference and SD for patients randomised to treatment and for patients on conventional PD medication group was analysed. It was anticipated that there would be high correlation between the baseline and follow up measures. The analysis was performed on an intention to treat basis including all patients who completed at least one follow up assessment. “Last observation carried forward” was used for participants with missing data.

The distribution of scores was checked for normality using the Shapiro-Wilk test. As data were normally distributed, a paired t test was used to assess the differences between treated and untreated groups with respect to changes in scores from baseline to follow-up. Data were analysed using IBM SPSS Statistics 20.

Correlations were used to generate hypotheses regarding the relationship between treatment effects and possible confounding factors like age and Hoehn & Yahr at baseline.

5.3 RESULTS

5.3.1 Patients

Retention of patients has been previously stated in the results of Chapter 2. In brief, of the 45 patients recruited, four patients withdrew / dropped out from the study, 3 from the group randomized to Exenatide and one from the conventional PD medication group. Of the 3 patients in the Exenatide group, 1 withdrew due to worsening PD (recurrent L-dopa dose failures) prior to the first follow-up visit; this

patient was replaced as per protocol and excluded for final analysis. Two further patients withdrew from treatment: the first at 9 months due to dysgeusia combined with subjective PD deterioration, and the second at 10 months due to excessive weight loss. These patients continued follow up assesstments and data collection as per protocol. Their data were included in final analysis, under our intention to treat protocol. One patient randomized to the conventional PD medication group withdrew from the study at 12 months due to deteriorating PD and incapacity to attend the trial clinic in the "off medication" state at 14 month. For this individual, last observation carried forward was used. The 44 patients (Exenatide group: 20, Control group: 24) included in the final analysis at 12 and 14 months underwent a 24 month visit assessment. NMS assessments were collected in parallel with motor assessments.

5.3.2 Clinical outcomes

5.3.2.1 Mattis DRS-2

At both 12 and 14 months, a significant advantage in the Mattis dementia rating scale-2 (Mattis DRS-2) was seen in patients treated with Exenatide, with a mean improvement of 2.2 points at 12 months compared with deterioration by mean of 2.8 points in the control patients (difference, 5 points; 95% CI, 5.2-0.8; p=0.006; and a mean improvement of 2.8 points at 14 months compared with deterioration by mean of 3.5 points in the control patients (difference, 6.3 points; 95% CI, 2.7-9.9; p=0.001) (Figure 8 and Table 22).

At 24 months there was a mean improvement of 1.8 points compared with deterioration by mean of 3.5 points in the control patients (difference, 5.3 points; 95% CI, 9.3-1.4; p=0.009).

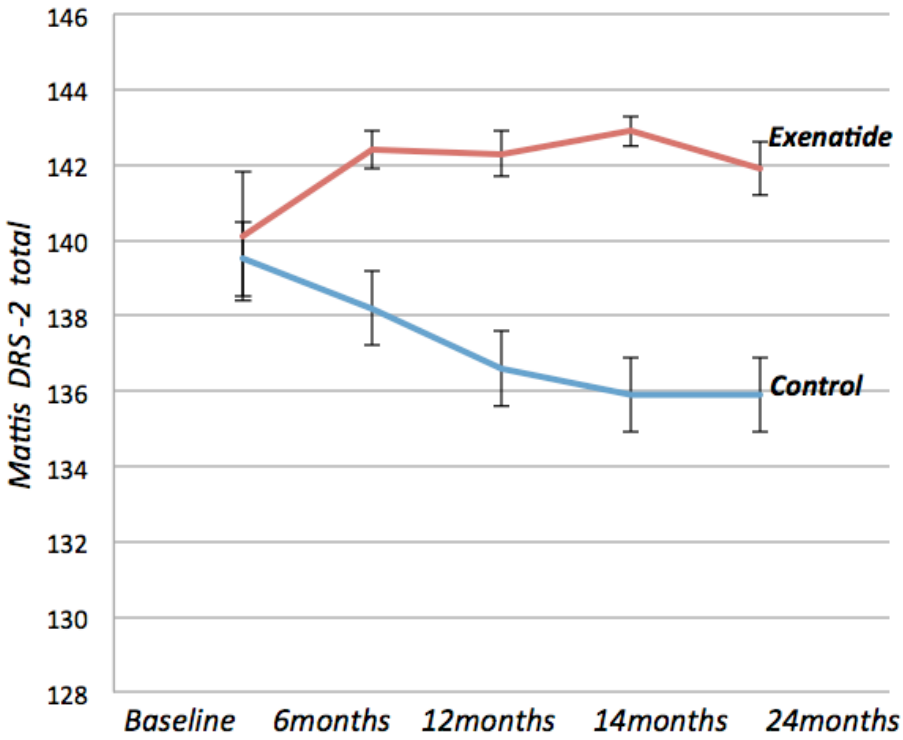


Figure 8. Change from baseline in the Mattis DRS-2 score by study visit. Data represent mean ± SEM

	Baseline Mean(SD)	6months Mean(SD)	12months Mean(SD)	Difference Baseline to 12 months Mean(SD); 95% CI	P value	14months Mean(SD)	Difference Baseline to 14 months Mean(SD); 95% CI	P value	24months Mean(SD)	Difference Baseline to 24 months Mean(SD); 95% CI	P value
Mattis DRS-2											
Exenatide	140.1(7.7)	142.2(2.3)	142.3(2.5)	2.2(6.4);-0.8,5.2	P=0.009	142.9(2.0)	2.8(6.0);-0.1,5.6	P=0.001	141.9 (3.1)	1.8(6.5); -1.2,4.8	P=0.006
Conventional PD medication	139.5(4.5)	138.2(5.1)	136.6(6.1)	-2.8(4.7);-0.6,-5.0		135.9(8.5)	-3.5(5.8);-1.1,-6.0		135.9 (7.3)	-3.5(6.4); -0.8,-6.3	
MADRS											
Exenatide	10.9(5.1)	8.6(4.2)	9.0(4.5)	-1.9(4.6);-4.1,0.3	P=0.094	10.2(6.7)	2.1-0.7(5.9);-3.5,2.1	P=0.184	9.0 (4.6)	-1.9 (5.2); 0.5,-4.3	P=0.79
Conventional PD medication	11.0(5.4)	11.3(6.5)	11.4(5.6)	0.5(4.5);-1.4,2.4		12.4(5.6)	5.0(1.5(4.7);-0.5,3.4		12.5(8.6)	1.5 (7.0);-1.4,4.4	

Table 22. Changes in the score on Mattis DRS-2 and MADRS between baseline and month 14

Changes in Mattis DRS-2 subdomains are represented in the following figures. (Figure 9 to 13). There were a tendency favouring Exenatide in all the subdomains but construction.

Figure 9. Changes from baseline in MEMORY subdomain by study visit. Mean ± SEM

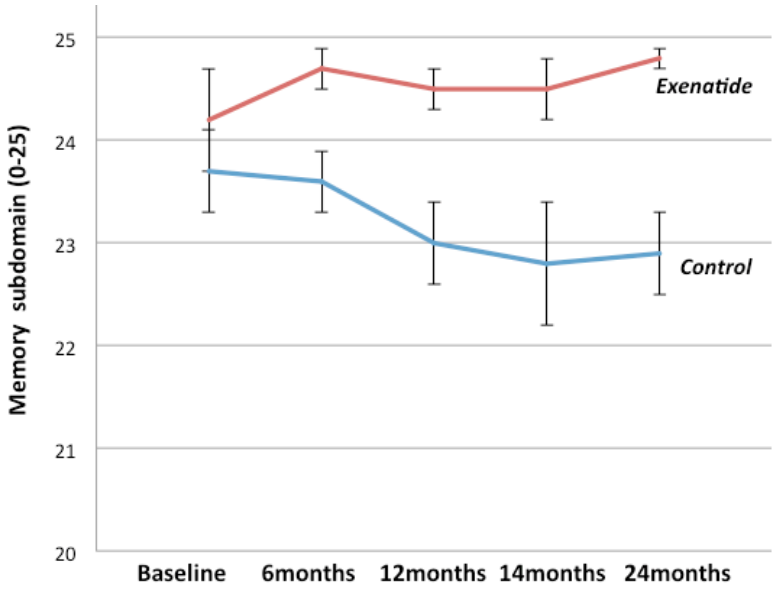


Figure 10. Change from baseline in CONSTRUCTION subdomain by study visit.

Mea± SEM

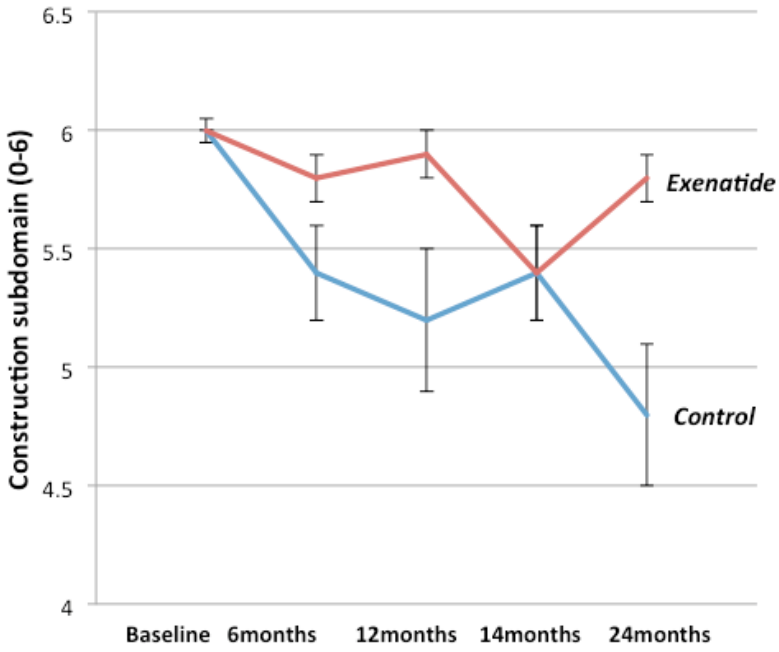


Figure 11. Change from baseline in CONCEPTUALIZATION subdomain by study visit. Mean ± SEM

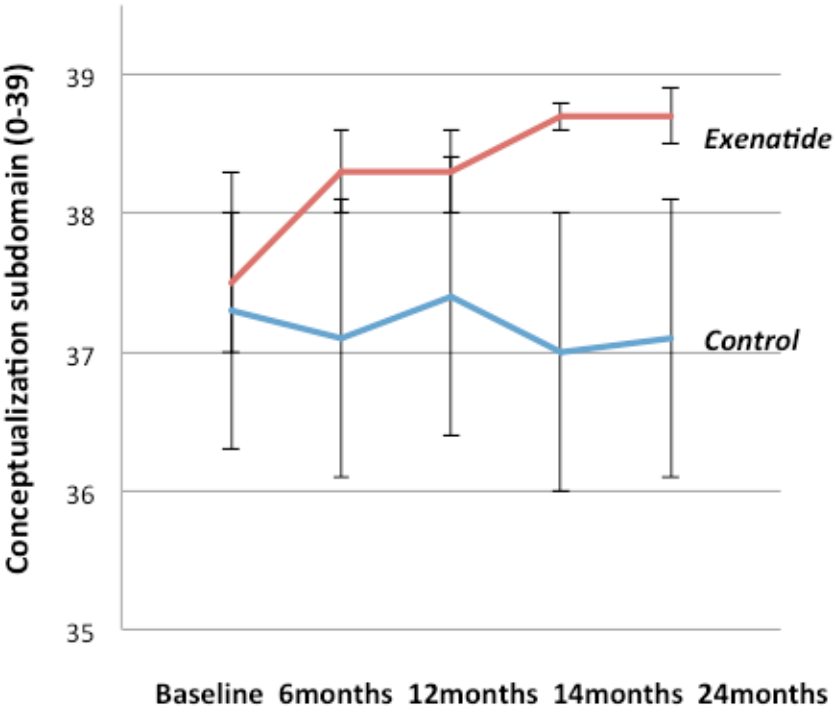


Figure 12. Change from baseline in INITIATION-PERSEVERATION subdomain.

Mean ± SEM

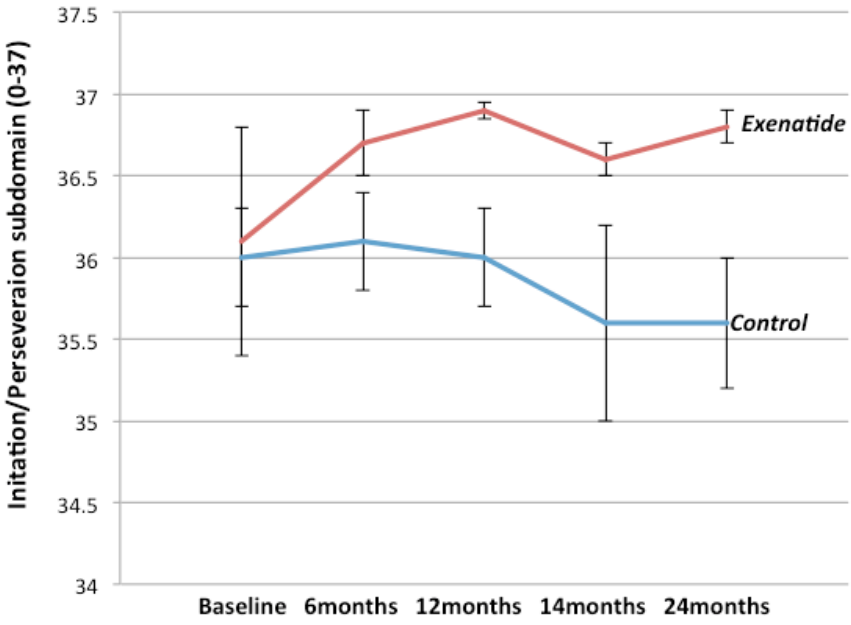
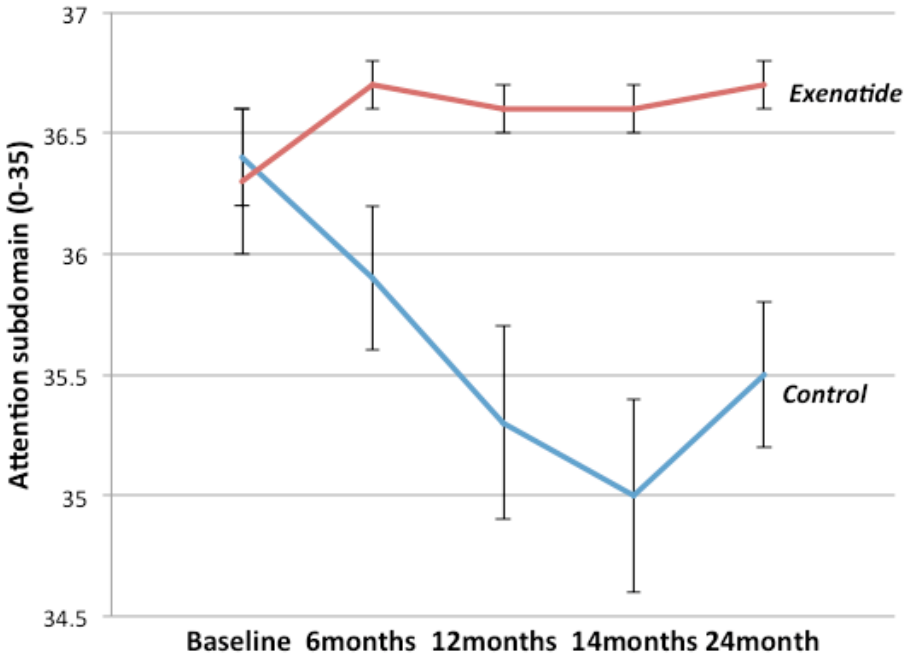


Figure 13. Change from baseline in ATTENTION subdomain by study visit. Mean ± SEM



5.3.2.2 MADRAS

There were no significant difference in Montgomery- Asberg depression rating scale (MADRS) score between baseline and 12 and 14 months. At 24 month there is a non-significant change favouring Exenatide, with a mean improvement of 1.9 points in the Exenatide SD (5.2), and a mean deterioration in the control group of 1.5 points SD (7.0) (difference, 3.4 points; 95% CI, 0.4, 7.2;p= 0.2). Table 22.

5.3.2.3 NMS Quest

Questions regarding sexual dysfunction were omitted by many of the female PD patients (44.4%). In total, 60% of the women in the Exenatide group and 25% of the women in the control group scored "not applicable" on these items, compared to 0% of the male patients in the Exenatide group and 5% of the men in the control group.

The mean total NMS at baseline was 10.4 ± 3.6 (SD), ranging from 4 to 17 of a maximum of 30 in the Exenatide group and 10.4 ± 5.5 (SD), ranging from 0 to 23 in the control group.

At 24 months there was a non-significant difference (p= 0.4) favouring Exenatide, with a mean improvement of 0.8 ± 3.8 (SD), at 24 month in the Exenatide group and a mean deterioration of 0.2 ± 4.3 (SD), in the control group.

The NMSQuest has nine domains and among these, the most frequents were digestive and miscellaneous. The mean of the digestive domain scored 0.19 ± 0.1 (SD), and the miscellaneous domain score 0.16 ± 0.0 (SD), in the Exenatide group at baseline. Similarly digestive 0.17± 0.01 (SD) and miscellaneous 0.13 ± 0.0 (SD), were most

prevalent in the control group. Therefore digestive and miscellaneous subdomains had the most "positive" answers at baseline in both groups.

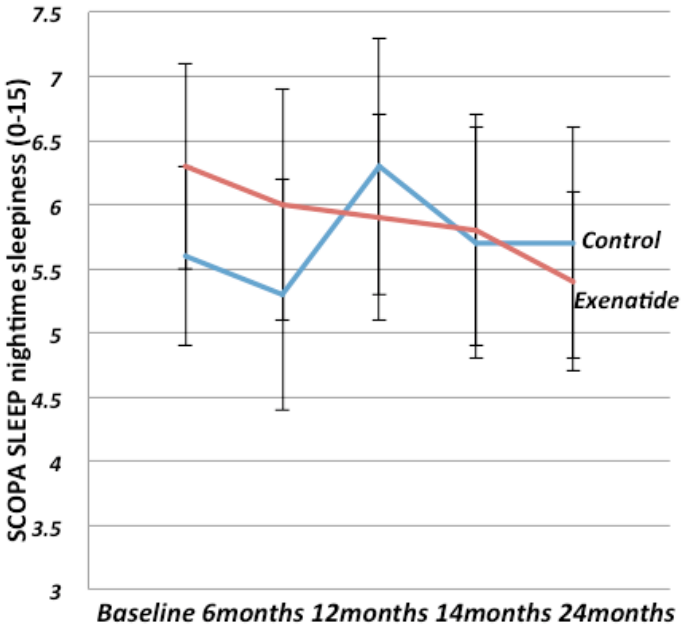
At 24 months the digestive domain was the one having the most prevalent complaint in both the Exenatide group 0.17 ± 0.1 (SD), and the control group 0.13 ± 0.1 (SD).

5.3.2.4 SCOPA sleep

The mean total sleep night time at baseline was 6.3 points ± 3.6 (SD), ranging from 0 to 14 of a maximum of 15 in the Exenatide group and 5.6 ± 3.6 (SD), ranging from 0 to 15 in the control group. At baseline the mean total sleep night time in the Exenatide group was 5.4 points ± 3.0 (SD), ranging from 1 to 13 and 5.7 ± 4.3 (SD), ranging from 0 to 15 in the control group.

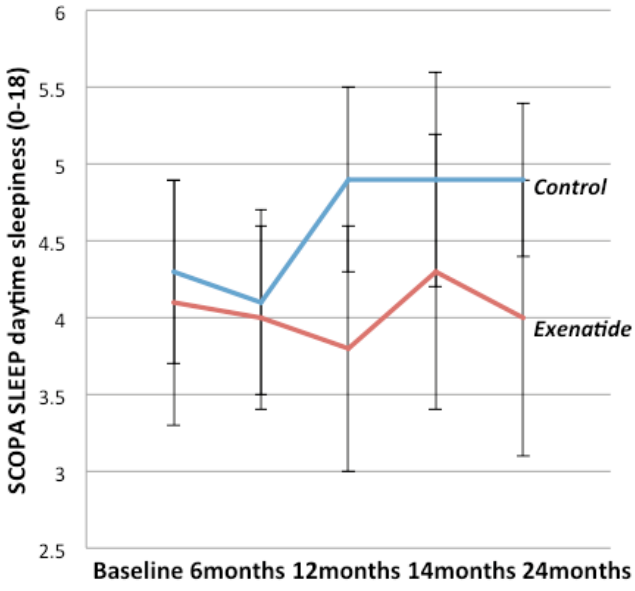
At 24 months, patients treated with Exenatide showed non-significant benefits in night time sleepiness, with a mean improvement of 0.9 points compared with deterioration by mean of 0.2 points in the control patients , although it was not significant (difference, 1.1 point; 95% CI, 0.8-2.9; p=0.8); and a mean improvement of 0.3 points at 12 months compared with deterioration by mean of 0.7 points in the control patients (difference, 1.0 points; 95% CI,1.1,3.3; p=0.3) . Figure 14.

Figure 14. Change from baseline in SCOPA sleep nightime sleepiness by study visit. Mean ± SEM



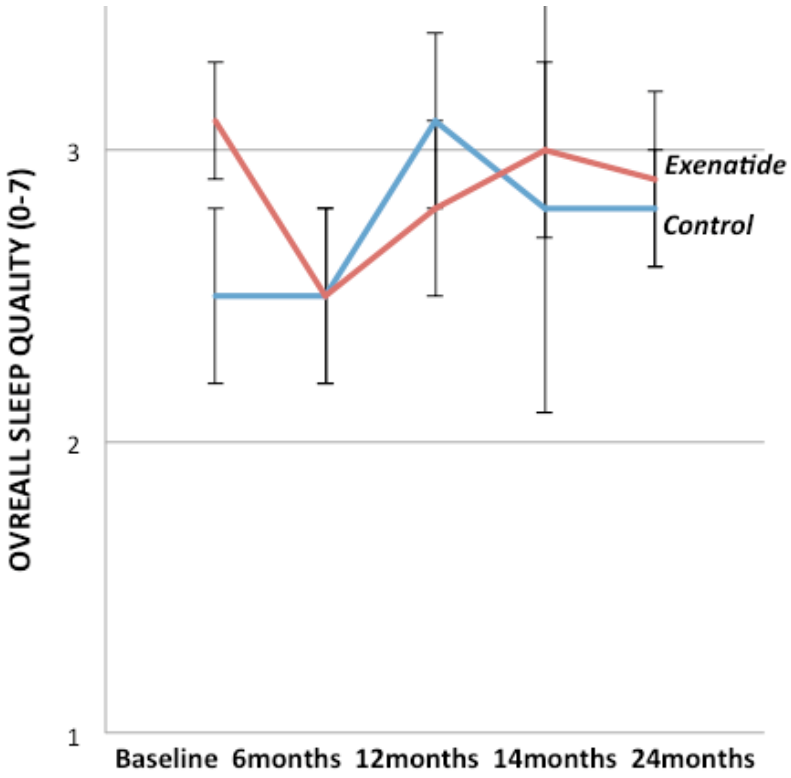
At 12 months, patients treated with Exenatide showed improvements in day time sleepiness, with a mean improvement of 0.3 points compared with deterioration by mean of 0.6 points in the control patients, although this too was not significant (difference, 0.9 point; 95% CI, 0.6-2.4; p=0.2); Figure 15.

Figure 15. Change from baseline in SCOPA sleep daytime sleepiness by study visit. Mean ± SEM



The overall sleep quality favoured Exenatide along the trial period, with a mean improvement of 0.1 points compared with a deterioration of 0.4 points in the control group at 24 months, although this was only trend significant (difference 0.5 points, 95% CI, 0.2,1.3; p=0.1). Figure 16.

Figure 16. Change in overall sleep quality by study visit. Mean ± SEM



5.3.2.5 SCOPA AUT

There were few missing data, except for the questions regarding sexual dysfunction, which had the most missing values, especially in female PD patients (44.4%). In total, 60% of the women in the Exenatide group and 25% of the women in the control group scored “not applicable” on these items, compared to 0% of the male patients in the Exenatide group and 5% of the women in the control group, paralleling the missing data in the NMSQuest.

At 14 months, an advantage in the total SCOPA-Aut score was seen in patients treated with Exenatide, with a mean improvement of 0.9 points at 14 months compared with deterioration by mean of 1.0 points in the control patients (difference, 1.9 points; 95% CI,-5.5,1.1; p=0.2); This effect was not present at 12months and it was not sustained at 24 months, with a mean deterioration of 1.0 points at 24 months in the Exenatide group compared with deterioration by mean of 0.5 points in the control group (difference, 0.5 points; 95% CI,-2.9, 3.9; p=0.7- Figure 17). The gastrointestinal domain deteriorated at 12 months, although it was nearly back to baseline levels at 14 months. The urinary domain got worse in both groups along the 24 months, with a slight improvement in both groups at 6 months. At 12 months the urinary subdomain deteriorated 1.1 points (SD 8.5) in the Exenatide group and 2.5 (SD 18.6) in the control group, although it was not significant p=0.754. The cardiovascular subdomain got better at 12 and 14 month in the Exenatide group, with an improvement of 0.7 points (SD2.8) favoring Exenatide and a deterioration of 0.6 (SD 4.0) in the control group although non-significant p=0.228. There was an advantage favoring Exenatide in the sexual dysfunction domain at 12 months, and a trend at 14 months. With an improvement of 7.5(SD 19.1) in the Exenatide group and a deterioration of 10.4 (SD25.0) in the control

group at 12 month p=0.12, and an improvement of 15.8 (SD 27.8) in the Exenatide group and a deterioration of 1.4 (SD 34.7) in the control group at 14 months p= 0.081, that was sustained at 24 months for a difference among both groups of 7.4 points p= 0.293.

Figure 17. Change in total SCOPA-AUT by visit time. Mean ± SEM

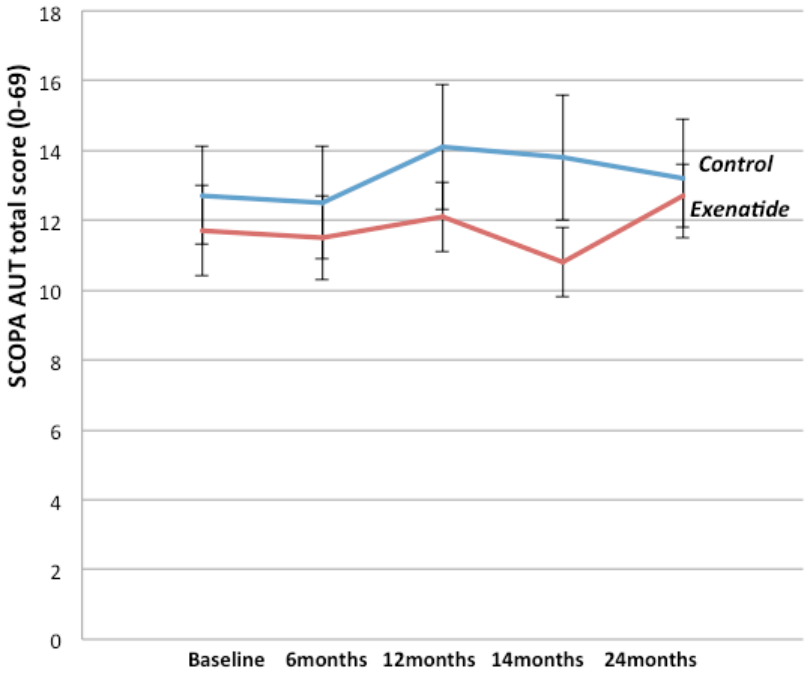
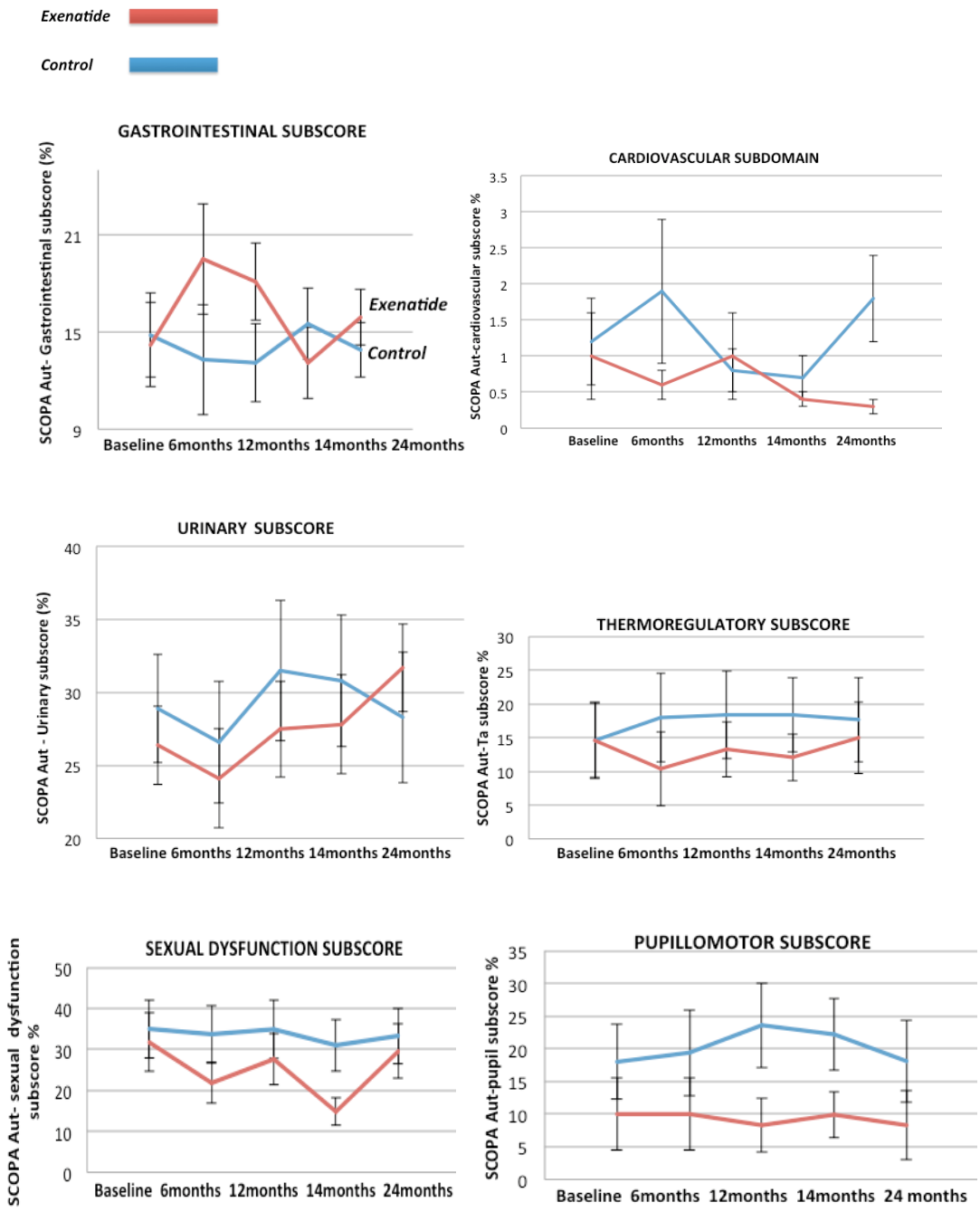


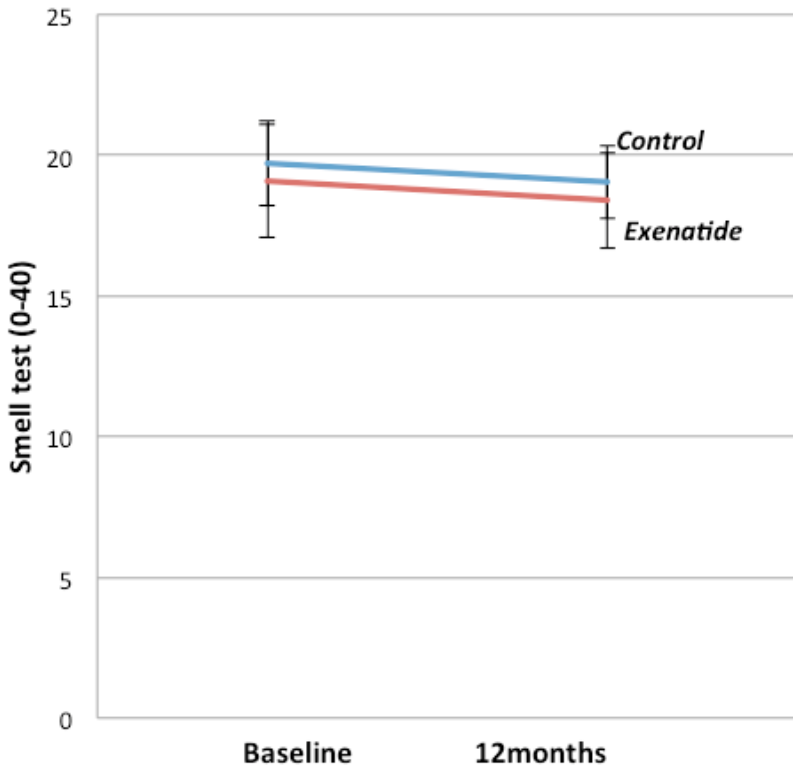
Figure 18. Change in score on SCOPA-AUT subdomains by study visit



5.3.2.6 Smell test

There was no significant difference in the smell test among both groups at 12 months

Figure 19. Change in smell identification test between baseline and month 12. Mean ± SEM



5.3.3 Correlations Exenatide group

NMS: There were no significant correlations between change in PDQ39 SI at all the time points and the NMSquest at the correspondence time point. There was no correlation between the prevalence of NMS measured with the NMSQuest at any time points and age of patients at disease onset nor with age at start of the trial.

SLEEP: There were no correlations between changes in LED and sleep scales (Neither Sleep night time, sleep daytime nor overall quality of sleep) at any time point along the trial. There were no correlation among change in sleep and change in MADRAS along the trial.

Mattis: There were no correlations between changes in Mattis DRS-2 and age at onset of the trial, neither age at symptoms onset. There was no correlation among changes in Mattis DRS-2 and Hoehn & Yahr state.

MADRAS: There were no correlations among changes in Mattis DRS-2 and MADRAS. No correlation was found among changes in activities of daily living UPDRS part1 and MADRAS.

5.4 DISCUSSION

5.4.1 Exenatide and Cognition. MATTIS DRS-2.

In the past 30 years, numerous studies that have explored in more detail the epidemiology of cognitive impairment and dementia in PD, have concluded that cognitive deterioration is a common, progressive and clearly disabling feature of Parkinson's disease. In an early review on the variable reported prevalence of Parkinson's disease dementia (PDD), among 4336 patients included from 27 studies, the mean prevalence was 40%. Aarsland et al. found a prevalence of PDD of 31.3% among 1767 patients included from 13 studies (Aarsland et al. 2005). These numbers indicate that PD patients have a 4- to 6-fold increased risk of developing dementia compared to age-matched general population (Aarsland et al. 2010).

Two longitudinal, community-based studies following PD patients for >15 years have reported very similar prevalences. In the Sydney study, that included 136 patients

with pathologically defined PD, the prevalence of dementia increased from 28% after 5 years of follow-up, to 48% at 15 years, and up to 83% after 20 years (Hely et al. 2008).

It has been proposed that the efficacy of disease modifying therapies may be more meaningfully assessed in terms of their effects in delaying the major milestones of PD, such as postural instability and dementia, since it is these that have the greatest impact on patients (Evans et al. 2011). Evans and colleagues proposed that understanding progression in PD enables the identification of relevant outcome measures for clinical trials of putative disease modifying agents. For that reason the authors followed a cohort of 132 PD patients from diagnosis for up to 7.9 years, finding that clinical presentation with a tremor dominant phenotype offered relative protection from progression, although the relevant factor was probably a lower burden of axial symptoms in these patients rather than the presence of tremor. In the "Milestone model" of PD progression proposed by Evans (Evans et al. 2011), the efficacy of disease modifying therapies is evaluated in their ability to postpone the onset of levodopa induced dyskinesia, reaching a higher H&Y state, developing Freezing or dementia. In this model the authors included olfactory dysfunction to illustrate that this concept can be extended to encompass premotor milestones.

To date, the design of trials of putative disease modifying agents has focused on the demonstration of sustained symptomatic effects (Olanow et al. 2009). However it has been proposed that identifying effects which translate into clinically meaningful benefits for patients would be a more useful approach. In our trial, cognitive decline was measured with Mattis DRS-2 scale during the on medication state at every time point. There was divergence in cognitive performance between the groups, with a 5-point advantage in the Mattis DRS-2 at 12 months that persisted as a 6.3-point advantage at 14

months and 5.3-points at 24 months. To date no reports have been found on Mattis DRS-2 being affected by placebo effect. We cannot however exclude placebo effect as a possible explanation for these observations. Nevertheless, an alternative interpretation is that the significant change in Mattis DRS-2 score favouring Exenatide that was sustained at 24 months, (12 months after stopping Exenatide injections) may be that the drug has modulated the natural history of the disease.

Muslimovic and colleagues followed 89 patients diagnosed at baseline with PD, 52 with established PD with a disease duration of 6.5 years and 64 healthy controls in a prospective study for 3 years, finding that the clinical features that best predicted cognitive change were age at disease onset and axial impairment (Muslimović et al. 2009).

In our trial there was a correlation between axial symptoms off medication (Blind assessment) at baseline and changes in Mattis DRS-2, although it was not significant; at 12 months ($p=0.072$) at 14months ($p=0.068$) and at 24months ($p=0.104$).

Fronto-striatal executive deficits with impairment of attention and memory search strategies, slower visuomotor processing, reduced verbal fluency, impairment of organizational and constructional strategies, and motor programming disturbances are characteristic and develop over the course of the disease (Pagonabarraga & Kulisevsky 2012). Up to 20% of non-demented PD patients also exhibit visuospatial and memory deficits more typical of posterior cortical functioning and fail performing tasks such as naming or copying. Major differences in the overall rate of cognitive decline among PD patients support the co-existence of at least two patterns of involution, differentiating a relatively slow (frontostriatal deficits) from a more rapid (posterior-cortical deficits) decline with different pathophysiological substrates, genetics, prognosis and response to

drugs used to treat the motor symptoms of PD. While frontostriatal defects appear more related to dopaminergic defects and reactive to dopaminergic manipulation, degeneration of cholinergic projection fibers from the basal forebrain is a highly probable correlate of posterior cortical defects and pivotal for the development of PDD (Kehagia et al. 2013).

The Mattis DRS-2 has high sensitivity (92.7%) and high specificity (91.4%) for a diagnosis of PDD versus PD cases without dementia (Docherty & Burn 2010). It involves 5 subdomains (Attention, Initiation/perseveration, Construction, Conceptualization and memory), most of them depending on frontostriatal related functions. Attention, construction and memory were the domains that had the most significant and sustained improvement along the 24-month trial period. However, no correlation was found among changes in these domains and changes in the DaT SPECT scans among the patients in the imaging substudy, as previously reported in Chapter 3.

Following the Braak hypothesis (Braak et al. 2003) to address the pathological correlates of cognitive dysfunction, a pivotal longitudinal clinico-pathological study that followed patients for >20 years (Halliday et al. 2008b) showed that cognitive pathological correlates are clearly different and dependent on the age of onset of PD. In patients with PD onset before 60 years, the progressive deterioration of cognitive function was seen to be associated with the progression of Lewy bodies from the brainstem to the limbic system, and finally to associative neocortical areas (Halliday 2008a). A trans-synaptic spread of the pathological process with presynaptic aggregation of α-synuclein, causing neurotransmitter deficiencies rather than Lewy bodies and cell death has recently been proposed to explain clinical-pathological inconsistencies in the progression of the neurodegeneration according to the Braak model (Schulz-Schaeffer 2010). In our group no correlations were found between Mattis DRS-2 baseline and age

at disease onset, or between change in Mattis DRS-2 at 12 (p=0.991), 14 (p=0.663) and 24 months (p=0.944) and age at disease onset.

Given the importance that synaptic dysfunction rather than neuronal loss seems to have in the development of clinical symptoms in PD (Plowey & Chu 2011), greater preservation of grey matter in PDD may indicate a potential reversibility of cognitive symptoms if the mechanisms leading to synaptic dysfunction could be treated. If the changes found in the Mattis DRS-2 in the Exenatide group are true biological effect rather than placebo effects, this might be an indication that Exenatide could play a role as stabiliser of synaptic function.

5.4.1.1 Exenatide and mood. MADRAS

Of the behavioural disorders in PD, mood disorders are amongst the most common and can occur in both early and late stages of PD (Tan 2012). Depression has been strongly associated with PD. The cause of depression in PD remains unknown. Two primary and not mutually exclusive hypotheses exist. The first argues that depression in PD represents a reactive state, resulting from the perception of progressive and social disabilities associated with the disorder. The other suggests that the cause of depression is intrinsic to PD itself, accounting for the occurrence of depressive PD patients prior to or at the onset of motor symptoms. In both instances, the induced neurochemical alterations are complex and probably include dopaminergic, serotonergic, and noradrenergic mechanisms. These findings have been supported by post-mortem studies, cerebrospinal fluid examinations and functional imaging (Aarsland, et al, 2009b). In our group there was a non-significant change in mood measure with MADRAS favouring Exenatide at 24 month. However, this change was not present at 12 or 14 months, thus there is little evidence from this data that Exenatide has a major effect on mood.

5.4.1.2 Exenatide and non-motor symptoms. NMSQuest

In previous studies describing the prevalence of non-motor symptoms, urinary symptoms have been among the most commonly present, scoring over 55%. Other symptoms particularly prevalent were cognitive, perceptual, autonomic (postural dizziness), sexual, and sleep problems all scoring over 30% (Martinez-Martin et al. 2007). In our Exenatide arm, gastrointestinal symptoms were the most prevalent in the first 12months, during the injections period. This is absolutely in keeping with the most frequent expected adverse event of Exenatide that is gastrointestinal disturbance.

5.4.1.3 Exenatide and sleep. SCOPA Sleep

With the intention of evaluating night time sleep problems and daytime sleepiness in PD patients, 420 PD patients (mean age (SD) 61.1 (11.5) years and 150 controls (mean age (SD) 60.9 (9.9) years) were assessed using SCOPA sleep questionnaire (Verbaan et al 2008). It was found that night time sleep problems were related to dopamine-agonist and levodopa dose, whereas daytime sleepiness was related to age, dopamine-agonist dose, and disease severity. The authors also reported that excessive daytime sleepiness (DS) is most commonly reported than excessive night time sleep problems (ENSP).

In our sample a non-significant difference in the NTSP was found favouring Exenatide throughout the trial period, while an improvement in the DS was just found at 12 months, but not sustained at 12 and 24 months. A significant correlation was found among changes in LED at 12 months in the Exenatide group and changes in EDS at 12 months ($p=0.02$), but no correlation was found with age at trial onset, or Hoehn & Yahr.

Thus it is likely that the lowering of LED in 5 Exenatide patients at 12 months (See chapter 2), explaining the proportional reduction in the LED in comparison with the patients in the control group (LED at 12 months (mean (SD)) was 997(446) in the Exenatide group versus 1121 (620) in the control group), could explain the improvement in the EDS at 12 months in the Exenatide group.

5.4.1.4 Exenatide and autonomic symptoms. SCOPA Aut

To date it has been concluded that SCOPA-AUT is a good tool for quantifying the dysautonomia in PD. However, the intensity of the autonomic symptoms as measured using this scale do not seem to correlate with any of the variables of clinical severity or progression of the disease (Berganzo et al. 2012). In our group there was a general improvement in the autonomic symptoms measured with total SCOPA-Aut, with modest non-significant improvement in the cardiovascular, temperature, pupillo motor and sex domain. A clear deterioration in the gastrointestinal domain at 12 month paralleled the NMSQuest scores and reflecting the main expected adverse event that was lessened once the injection period was finished. The urinary domain got worse along the trial period, but there were no differences among both groups during the 14 months.

5.4.1.5 Exenatide and smell sense. SIT

The purpose of this study was to determine if an association exists between performance on an odor identification task and subsequent risk of reaching milestones of clinically meaningful disease progression, particularly non motor complications including cognitive impairment (Evans et al. 2011). There was no significant change in the smell test between both groups at 12 months. Therefore perhaps a different pathology from motor and cognitive symptoms should be involved.

In conclusion, aside from the changes in MDS-UPDRS scores, there was also divergence in cognitive performance between the groups, with significant differences represented by advantage in the Mattis DRS-2, which were sustained along the trial period, beyond the injection period. Divergent results were found as well in the MADRAS, NMS Quest, SCOPA-Sleep and SCOPA-AUT favouring Exenatide along the 24 months trial follow up period, although these trends failed to reach significance. Despite the lack of change in the smell test at 12 month, the rest of non-motor symptoms scales and questionaires favoured Exenatide along the 24 months follow up period, further than the active drug injection period. Although it could not be completely rule out the possibility that this results could be placebo driven, the fact that the scores remained sustained in time are consistent with the possibility of a real disease modifying effect of Exenatide.

6 EXENATIDE AND ORAL GLUCOSE TOLERANCE TESTS

6.1 INTRODUCTION

6.1.1 Insulin resistance

Insulin resistance is a condition in which cells fail to respond to the normal actions of the hormone insulin, with reduced sensitivity or responsiveness to the metabolic actions of insulin; namely, insulin-mediated glucose disposal, and inhibition of hepatic glucose production. The body produces insulin, but the cells in the body become resistant to insulin, mainly due to changes in their surface receptors. One of insulin's main functions is to regulate delivery of glucose into cells, providing them with a source of energy. Therefore insulin resistant cells cannot take in glucose, amino acids and fatty acids. If insulin resistance exists, more insulin needs to be secreted by the pancreas as compensatory mechanism, finally evolving into the appearance of Type 2 DM.

At the same time, the liver that in health regulates glucose levels by reducing its secretion of glucose, continues secreting glucose to the blood stream, further contributing to hyperglycaemia. "Insulin responsiveness", is defined as the maximal effect of insulin, whereas "insulin sensitivity" is defined as the insulin concentration required for a half-maximal response (Muniyappa et al. 2008).

Insulin resistance normally refers to reduced insulin actions, such as glucose disposal and reduction of the inhibition of hepatic glucose production. However, other functions of insulin can also be affected. Insulin resistance in adipose cells results in reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides. Increased mobilization of stored lipids in these cells elevates plasma levels of free fatty acids, producing the "metabolic syndrome".

6.1.2 Impaired glucose tolerance

Impaired glucose tolerance is a pre-diabetic state of hyperglycaemia that is associated with insulin resistance and increased risk of cardiovascular pathology.

6.1.2.1 Epidemiology

The association among PD and DM is strengthened by reports suggesting that up to 50–80% of patients with Parkinson's disease have abnormal glucose tolerance when tested (although these figures are from non-contemporary papers and we are unaware of confirmatory data from more recent cohorts) (Barbeau et al. 1961; Elner & Kandel 1965; Lipman et al. 1974; Sandyk 1993). However, in a series of 800 patients with Parkinson's disease, concurrent diabetes was indeed shown to accelerate progression of both motor and cognitive symptoms (Schwab 1960). In view of the possible confounding effects of Parkinson's disease treatment, newly diagnosed, never-treated adults with Parkinson's disease have also been studied and been shown to have reduced insulin-mediated glucose uptake (Van Woert & Mueller 1971), inhibition of early insulin secretion and long-term hyperinsulinaemia and hyperglycaemia after glucose loading (Boyd et al. 1971). This takes into account the effects of some drugs used to treat Parkinson's disease, such as levodopa, which induces both hyperglycaemia and hyperinsulinaemia, whereas others (including the ergot dopamine agonist bromocriptine) may increase insulin sensitivity (Van Woert & Mueller 1971; Sirtori et al. 1972). Nevertheless, a recent cohort study could not replicate an association between either type 2 diabetes mellitus or obesity and Parkinson's disease risk although the authors acknowledge that diagnosis of type 2 DM was entirely based on self-report (Palacios et al. 2011). There has also been a review of cohort studies and case control studies that has looked at

relationship between PD and DM that did not found conclusive evidence on this association (Cereda et al. 2011).

6.1.3 Glucose tolerance test in Exenatide trial

PD patients with confirmed Type 2 DM, or previously unconfirmed Type 2 DM were excluded from participation in this Exenatide trial (n= 1 of a total of 51 screened), but at baseline, we had not previously formally quantified the frequency of impaired fasting glucose or impaired glucose tolerance. Based on the published epidemiology suggesting links between PD and glucose homeostasis, the decision was taken to further explore the frequency of impaired fasting glucose and impaired glucose tolerance in PD patients by performing oral Glucose Tolerance tests (OGTT) in several groups of individuals to crudely explore whether the high rates of abnormal glucose homeostasis previously reported (as an indirect measure of insulin resistance) also existed in our patients.

OGTT was performed at 24 months follow up visit in:

1. PD patients previously treated with Exenatide (n= 20).
2. PD patients recruited to the Exenatide trial but allocated to the control arm (n= 24). Data from these individuals was added to that from a random sample of PD patients not included in the trial and not previously diagnosed with DM (n=10).
3. Spouses of patients with PD and not previously diagnosed with DM (n=15).

The sample size was extended to PD patients not included in the trial and willing to take part and their spouses, to be able to increase the sample size. We included

spouses due to the higher prevalence of impaired glucoregulation with advanced age to be able to compare with a group of population of the same age without PD.

6.2 METHODS

The individuals were instructed not to have anything to eat or drink (other than plain water) after 22.00h on the evening prior to the test or on the day of the test, until it was completed. Individuals were informed what the GTT involves, and were given a patient information sheet at least 24 hours before the test. During the test, the subjects were at rest.

The first fasting venous blood test was performed immediately after the individual signed the consent form. This first fasting blood sample was labelled with number 1 with the individual's details and the time it was taken.

Immediately following the first sample, the individual was requested to drink 394 mL of new formulation of Lucozade Energy original (70 Kcal per 100ml), ensuring it was consumed within 5 minutes. The time the patient started to drink the Lucozade was noted. To avoid excessive fizziness, the bottle was opened 30 minutes before the test began. The second blood test was performed exactly 2 hours after the Lucozade was consumed. The 2nd venous blood sample was labelled with number 2 and the patient's details and time it was taken. The individuals were then advised to eat and drink as they wished. A test was considered as impaired fasting glucose when the level of glucose in the fasting blood test was higher than 6.0mmol/L. A test was considered as impaired glucose tolerance when the glucose level 2h after Lucozade intake was higher than 7.7mmol/L (Bartoli et al. 2011). A test was considered as T2DM when the glucose level 2h after Lucozade intake was higher than 11.1 mmol/L (American Diabetes Association 2013).

6.2.1 Analysis

Mean and standard deviation of the fasting glucose levels and the glucose levels 2 hours after the Lucozade intake were calculated for the Exenatide patients, PD patients never exposed to Exenatide and the spouses group.

6.3 RESULTS

6.3.1 Patients

Twenty PD patients in the Exenatide arm (age (SD), 63.2 (5.9)years), 34 PD patients receiving best medical therapy (59.1 (8.7) ears) and 15 healthy age-matched controls (60.7 (8.5) years had an OGTT.

6.3.2 GTT in Exenatide patients

The mean (SD) fasting glucose was 5.0 (0.5) mmol/L. The mean glucose level 2 hours later was 5.6 (1.4) mmol/L. One patient exhibited impaired glucose intolerance. There were no patients meeting criteria for DM. Normal range was considered 3.9-6.0 for the fasting test and 3.9-7.7 for the glucose tolerance sample.

6.3.3 GTT in PD patients

The mean (SD) fasting glucose was 5.2 (1.5) mmol/L. The mean glucose level 2 hours later was 5.6 (1.3) mmol/L. There were 2 patients with impaired fasting glucose, one of them with a fasting glucose in the diabetic range. There were three patients that were glucose intolerant.

6.3.4 GTT in spouses

The mean (SD) fasting glucose was 4.9 (0.4) mmol/L. The mean glucose level 2 hours later was 6.0 (2.0) mmol/L. There were two spouses that were glucose intolerant. There were no new cases of diabetes among the spouses.

6.4 DISCUSSION

Our data do not support the high prevalence of Glucose intolerance in PD patients previously described by other groups. We deliberately excluded patients with PD and previously confirmed DM (n=1 out of a total of 51 screened for inclusion). Furthermore no higher incidence of Glucose Intolerance was found in the Exenatide group in comparison with the other PD patients. While we acknowledge that we have only recruited small numbers of individuals that may be subject to several possible biases, our small sample does not lend any support to the previous observation that up to 80% of PD patients have impaired glucose tolerance.

The gradual decline in physiological reserve that is characteristic of aging results, in part, from insulin homeostasis. Impairment of gluco-regulatory mechanisms, which is characterized by altered glucose tolerance and features of insulin resistance, occurs frequently in the elderly and the prevalence of DM increases with age.

Approximately 40% of individuals 65 to 74 years old and 50% of individuals older than 80 years have impaired glucose tolerance or DM, and nearly half of elderly diabetes are undiagnosed (Harris 1990). For that reason our study performed OGTTs in spouses, which acted as a control match population, non-diabetic and without PD. In our spouses' sample, although the mean age was slightly younger than the Exenatide group we found a higher mean of glucose levels in the sample taken 2 hours after the Lucozade intake. Mean glucose levels 2hours after Lucozade in the Exenatide group was 5.6 (1.4) mml/L, almost identical to the figures in the PD group 5.6 (1.3) mmol/L, while in the spouses the mean glucose 2 hours after Lucozade was 6.0 (2.0) mmol/L.

This study used GTTs an indirect measure of insulin resistance. To date different methods have been used to measure insulin resistance.

Some methods rely on steady-state analysis of glucose and insulin, whereas others rely on dynamic testing. Each of these methods has distinct advantages and limitations, and optimal choice and employment of a specific method depends on the nature of the studies being performed. The hyperinsulinemic euglycemic glucose clamp and the insulin suppression test directly assess insulin-mediated glucose utilization under steady-state conditions that are both labour and time intensive. A slightly less complex indirect method relies on minimal model analysis of a frequently sampled intravenous glucose tolerance test. Finally, simple surrogate indexes for insulin sensitivity/resistance are available (e.g., Quantitative insulin sensitivity check index (QUICKI), Homeostasis model assessment (HOMA), 1/insulin, Matusda index) that are derived from blood insulin and glucose concentrations under fasting conditions (steady state) or after an oral glucose load (dynamic).

We elected not to use a glucose clamp approach because (1) it is an expensive test, and (2) requires an experienced operator to manage the process. Similarly, we did not use surrogate indexes due to budget limitations.

One important point to be considered is the plausible physiological role for dopamine in the regulation of insulin secretion. There is plenty of evidence suggesting that the components necessary for dopamine synthesis and secretion are all present in the β-cell (Lundquist et al. 1991; Lindström 1986; Saisho et al. 2008). More outstandingly, the presence of dopamine receptors has been found using reverse transcription-PCR in INS-1E insulin secreting cells as well as in rodent and human isolated islets (Rubí et al. 2005). These authors also showed that exogenous dopamine can inhibit glucose-stimulated insulin secretion from isolated cells likely due to distal steps in metabolism secretion coupling (Rubí et al. 2005).

It has been proposed that dopaminergic signalling can down-regulate glucose-stimulated insulin secretion from pancreatic islets. Activation of AKT/PKB mediated by GLP-1 was partially reversed by the addition of exogenous dopamine in the rodent β-cell line INS-SE (Ustione et al. 2013). In this model, dopamine would serve as an "antiincretin" signal that antagonises the stimulatory effect of GLP-1 (Ustione et al. 2013). This potential "antiincretin" effect of dopamine could be partially responsible for the difference incidence of DM and Glucose intolerance reported to date. With the intention of removing potential cofounding effects of dopamine, the OGTT was performed at 24 month visit when the patients were free of all PD medications for at least 12 hours (including Exendin-4) and free of long acting dopamine agonists for at least 24 hours. The rest of PD patients who took part in the OGGT attending the trial clinic also being off medication to be able to establish formal comparisons.

In conclusion our results do not confirm the high incidence of glucose intolerance reported in previous PD samples, although we have to acknowledge that our sample is small and the age is a bit younger than previously reported. Furthermore, we did not find evidence in our data that glucose tolerance is different in PD patients who received Exenatide for 12 months in addition to best medical therapy when compared to PD patients who only received best medical therapy, or when compared to a healthy age-matched control sample. Based on these results, we find little to suggest that any biological effects of Exenatide in PD are solely related to improvement in peripheral glucose metabolic control. Central mechanisms of glucose sensing and insulin signalling warrant further explorations.

7 SUMMARY & CONCLUSIONS

7.1 Summary of research findings

The management of PD consists of therapies that aim to relieve the symptoms of the condition at its various stages. There are no approaches universally accepted to modify the progressive course of the disease. A major obstacle to the development of a neuroprotective drug for PD is the duration and the cost of the development program. The average drug development program for a CNS drug is approximately 15 years from the laboratory until the time the drug is first introduced to the clinic then licensed, and the cost is approximately $1.2 billion (Sherer et al. 2012).

In the face of the obstacles outlined, it is reasonable to question why anyone would make the investment required for the development of a neuroprotective therapy for PD.

The increased risk of Alzheimer's disease, Parkinson's disease and stroke in people with Type 2 DM suggests that shared mechanisms/pathways of cell death, possibly related to insulin dysregulation, may underlie all of these disorders. It seems conceivable a wide range of genetic and environmental triggers result in activation of similar biochemical pathways in all of them, suggesting a complex network of biochemical events that feed in to a final common path towards cellular dysfunction and death. GLP-1 receptor agonist itself appears to be a promising strategy in the prevention and/or amelioration of a variety of neurodegenerative diseases. To date only preliminary in vitro and animal studies have investigated the potential neuroprotective functions of GLP-1-based therapies (Holst et al. 2011).

The current trial design was conceived following feedback from both commercial and charitable organizations, which confirmed the impression that the risks associated with investment into potential neuroprotective agents need to be mitigated via the

preliminary collection of cost-efficient (open-label) data in the first instance. In this context, the current study was designed as a proof of principle (Schmidt 2006); i.e., with the aims of collecting rapid and cost-efficient data regarding the tolerability of Exenatide in patients with PD and providing preliminary indications whether the major neuroprotective and neurorestorative effects of Exenatide seen in the animal models might be replicable in human individuals with PD.

Repurposing a drug for treating a novel disease is difficult, particularly with an injectable therapy. However, given the large body of safety data already gathered from its use in the DM patients, we moved to Phase II testing to also assess efficacy, and in the present study, we were challenged to explore whether Exenatide might modify the progressive course of PD, rather than simply have some direct symptomatic effect.

To date there is no reliable outcome measure that accurately reflects the underlying disease state and is not potentially confounded by symptomatic or pharmacological effects of the study intervention. Each of the outcome measures that have been used in clinical trials to date have been potentially confounded by symptomatic or pharmacological effects of the study intervention, so that even if study results are positive, it cannot be unequivocally determined that the agent has an effect on disease progression. For that reason we chose to include a longer follow up, 2 and 12 months after drug discontinuation to try to differentiate ongoing symptomatic effects and allow placebo effects to further diminish.

In chapter 2 and 4 our data provided preliminary information about the absolute size of the difference in PD severity between the Exenatide treated and untreated groups using blinded rating. At 12 month this difference was modest (4.9 points in MDS-UPDRS part 3), although this value excluded the additional effects on rigidity scores. These figures were sustained at 14 months (4.4 points) and at 24 months (5.6 points).

The study saw a sustained benefit twelve months after the Exenatide was discontinued, including in a number of motor measures as well as non-motor measures.

In chapter 5, cognitive scores were notably improved in the Exenatide group and this improvement remained sustained 12 month after drug discontinuation. Although a sustained symptomatic effect potentially could explain the improvement after Exenatide was discontinued, the observations are also consistent with the possibility that Exenatide may have slowed disease progression.

7.2 Main Criticisms

One criticism of this study is the possibility of placebo effect in the Exenatide group. The variable nature of the placebo response is a particularly important issue for complex or invasive interventions in which placebo versions of the licensed product are not readily available/represent additional significant expense, which can thus hinder the conduct and interpretation of even small phase 2 double-blind trials.

Any treatment (physical, pharmacological, or psychological) for a medical condition can potentially have a dual effect for the patient: that related to the treatment itself (eg, the intrinsic pharmacological property of an active drug) and that inherent in the perception that the treatment is being received. The latter is known as the placebo effect (Oh 1991).

Essentially, any sort of treatment can act as a placebo, but what determines whether there is a placebo effect is the response of the patient to the intervention. There is extensive clinical evidence for a prominent placebo effect in PD (Diamond et al. 1985; Shetty et al. 1999; Goetz et al. 2000; Hauser et al. 2007). The experience of being enrolled in a clinical trial includes numerous factors besides taking a new medication or undergoing a new surgical procedure. Added clinic visits, regular laboratory

assessments, increased contact with physicians and research staff, and the anticipation of change are experiences that may influence patient outcomes in both the study and control population.

Because of dopamine activation in neural pathways involved with motivation, reward and the response to novelty and change, attention has been particularly focused on changes in PD during placebo treatment (Schultz 1998).This effect is biochemically mediated by the activation of the nigrostriatal dopaminergic pathway, which leads to the release of dopamine in the striatum. Within [11C] raclopride PET scans of a patient with PD, the lower radioactivity observed in the striatum after placebo (saline injection) reflects increased occupancy of striatal D2 receptors by dopamine (ie, placebo-induced dopamine release).

The biochemical placebo effect in Parkinson's disease is as powerful as the effect of an active drug (apomorphine)(de la Fuente-Fernández et al. 2001), and also similar in magnitude to the effect of amphetamine in healthy people and patients with schizophrenia (Breier et al. 1997) .There is some indication that physical placebos may be even more powerful than oral placebos (Kaptchuk et al. 2000; Freeman et al. 1999).

PD is a disorder in which the response to treatment can be assessed directly by the examiner. This direct measurability might allow a better evaluation of the placebo effect. Objective changes in motor function during placebo treatment in PD were examined in a placebo treated group from a randomized, multicentre, placebo-controlled clinical trial of monotherapy with ropinirole in 105 patients. A prominent placebo effect was reported in all domains of parkinsonian disability (although there was a trend for a greater effect on bradykinesia and rigidity than on tremor and gait/balance)(Goetz et al. 2000). However, the clinical scales of motor function are also subjective measurements.

Rates and timing of placebo responses were examined using a strict definition of placebo-associated improvement in order to identify patient and study-based characteristics, predicting positive placebo response in several PD clinical trials (Goetz et al. 2008). Individual patient data from the placebo groups of 11 medical and surgical treatment trials involving PD patients with differing PD severities and placebo-assignment likelihoods were examined. They defined a positive placebo response as 50% improvement in total UPDRS motor score or a decrease by 2 points on at least two UPDRS motor score items compared to baseline. The overall placebo response rate was 16% (range: 0–55%). Patients with higher baseline UPDRS motor scores and studies that focused on PD with motor fluctuations, surgical interventions, or those with a higher probability of placebo assignment showed increased odds of positive placebo response. The greatest proportion of placebo-related improvements occurred in the patients with motor fluctuations. The only subject characteristic that increased the odds of a positive placebo response was the baseline UPDRS motor score, with higher scores associated with higher likelihood of placebo-related improvement. This association was driven primarily by tremor and bradykinesia scores and not by rigidity or gait impairments. The most likely driving factor for differences in placebo response rates across studies to be the greater overall severity of PD, as evidenced by the higher rates in the group with motor fluctuations and as demonstrated by the link between placebo-related improvement and baseline UPDRS motor score (Goetz et al. 2008).

Another studied looked at the placebo-treated group from a randomized, multicentre, placebo-controlled clinical trial of monotherapy with ropinirole in PD patients without motor fluctuations. In 105 patients, they evaluated placebo-associated effects on the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS), dividing the motor examination into four categories: tremor, bradykinesia, rigidity, and

gait/balance/midline functions. Again they applied a rigorous definition of placebo-associated improvement as an improvement over baseline score in motor UPDRS of at least 50% or a change in at least two motor items at any one visit by 2 points. During the 6-month study, 16% of subjects improved on placebo treatment. The prevalence of response was steady (8 to 9%) at any one visit without a predominance of an early effect. No patient showed a placebo-associated improvement on all visits. All domains of parkinsonian disability were subject to placebo-associated improvement, with a trend toward more response in bradykinesia and rigidity than in tremor or gait/balance/midline function. Gender, age, disease duration, and baseline disability score did not influence the likelihood of improvement in association with placebo treatment (Goetz et al. 2000). The sample size was relatively small in this study, but old and young patients, both men and women, experienced placebo-associated improvement (Goetz et al. 2000).

In another earlier an smaller double-blind trial of Pergolide for PD it was documented that both drug and placebo groups improved compared with baseline (Diamond et al. 1985).

Because placebo-associated improvements occur throughout a 6-month trial, placebo-controlled studies in PD should be at least 6 months to capture early as well as late improvements.

To date, no data on how the UPDRS motor scores vary in open-label assessments over 6 months in patients with mild PD, have been published to my knowledge. Furthermore, the natural progression of disability as measured by the UPDRS motor score over 6 months in open-label conditions compared with placebo treatment has not been determined.

The persistence of the response to placebo may be difficult to evaluate because the fear of exposing patients to placebo over long periods has often resulted in trial designs

that limit placebo periods. However, relatively long placebo periods have been infrequently used. For instance, in a long-term trial of placebo as the only antianginal medication for a cohort of 35 patients, the number of angina attacks decreased by 48 percent during titration (eight weeks), and by 77 percent during the entire six-month period (Boissel et al. 1986).The conflicting literature may be entirely expected considering the complex elements that make up the placebo response, including continued interaction between subjects and treatment team, monitoring of treatment effects by both subjects and researchers, and changes in disease processes over the course of the study. We have analysed the different subdomains of UPDRS, to look at differences in improvement in the gait and rigidity subdomain in comparison to tremor and bradykinesia subdomains, finding no improvements on tremor subdomain. No correlation was found among Hoehn and Yahr state (2 vs 2.5) at baseline and change in MDS-UPDRS part 3 in our trial.

In the current trial, we chose not to set any futility threshold a priori, but instead chose to use a contemporary group of PD controls and to continue follow up for a sufficiently long period to allow inevitable placebo effects to at least begin to diminish. However, without a placebo injectable study arm, one cannot entirely exclude that there was a protracted placebo effect after cessation of the drug therapy. Indeed, protracted placebo effects have been seen in some gene therapy trials while patients have still been blinded (Calara et al. 2005; Tomlinson et al. 2010). In the present study, one would predict that if a placebo effect played a major role, it ought to have waned during the final twelve months, when the patients were aware that they were no longer receiving injections of the active drug.

In the absence of a placebo group or any reliable peripheral measure that tracks the pathology of PD, the imaging substudy was incorporated to get some indication of

whether exenatide might have a disease-modifying effect. There was no change in [^{123}I]FP-CIT SPECT scan appearances over a 12-month period. Given the quite advanced stage of disease suggested by the baseline levels of [^{123}I]FP-CIT uptake, this may simply reflect a slower rate of decline of PD than occurs in the first few years. The small mean improvements in [^{123}I]FP-CIT uptake in 2 patients is out of keeping with the natural history of PD. These patients both experienced LID during the course of the trial that responded to lowering of LED. Whether this is due to a possible biological effect of Exenatide or not, could not be completely ruled out due to the lack of [^{123}I]FP-CIT SPECT scan in the control group.

At the same time, NMS were assessed looking for differences outcomes in cognition, mood, smell sense, sleep, and autonomic symptoms scales between both groups. Differing results were found in these tests between both groups, remarkable are the divergent result in cognitive tests and although could not be completely rule out the possibility that could be placebo driven, the sustainability of the results along the 24 month trial period is consistent with a possible real biological effect of Exenatide as disease modifying drug in PD.

Nevertheless we should take into account the possibility of further different biases affecting our results:

• *Randomization at baseline.*

Despite randomization, minor differences in the baseline characteristics of the treated and control groups can influence subsequent disease progression. In a larger sample, randomization would be more likely to balance the treated and untreated groups. In the current trial, we attempted to minimize this chance variation by stratified randomization according to baseline disease severity. While there were no significant

differences between the 2 groups at baseline, the control group had slightly longer disease duration than that of Exenatide-treated patients.

• *Weight loss influence in motor response*

No correlation was found among change in part 3 MDS UPDRS at 12, 14 and 24 months and weight loss, making it less likely that Exenatide patients had improved motor scores as their body mass index was reduced along the first 12 month trial period.

• *Blinded videos*

In an effort to reduce investigator bias, we used an established procedure in which participants are videotaped and assessed by a blinded third party (Galpern et al. 2012). Blinded rating of videos was performed by the same individuals trained and certified in MDS-UPDRS rating. Individuals were allowed to view videos and their previous scores at baseline, 12 months, and 14 months while assessing the 24 month videos to ensure consistency across time points. This was decided upon to reduce intraobserver variability however is a further potential source of bias given that raters were aware that the 12 month and 14 month data overall favoured Exenatide treated patients, they may have kept their scores in the same direction.

7.3 Interpretation of statistical tests

The aim of this trial was to collect pilot data upon which to design a further placebo control randomised trial, we analysed differences between treated and untreated groups with respect to change in scores from baseline to follow-up using 2-sided *t* tests. The statistical tests performed in this study have been provided to help in the judgement of which outcomes are most clearly changed in the context of this pilot trial, and thus to help guide planning of future placebo controlled trials and are thus restricted to the main outcomes of interest, not with the idea of proving efficacy. Multiple statistical tests have

been performed therefore the reader should not interpret a p value of 0.05 as evidence of efficacy.

7.4 Further work

A thorough understanding of how the brain integrates the feedback signals coming from the periphery to maintain normal brain function and metabolic homeostasis, as well as how this homeostasis is disrupted by dietary and environmental factors, will be key to better disease treatment and prevention. Our decision to perform OGTT in our trial patients and in PD patients not included in the trial, was an attempt to verify in our PD patients higher incidence of glucose intolerance, as previously described. However we did not see obvious evidence of impaired fasting glucose or impaired glucose tolerance in our small sample therefore the relationship between PD, dopamine, normal brain function, insulin signalling and peripheral metabolic homeostasis is clearly not a straightforward one. The interaction between peripheral and central effects of Exenatide in metabolism and neurodegeneration needs further clarification.

7.5 Concluding remarks

This is the first trial to report tolerability and pilot data of the biological effects of Exenatide in patients with a neurodegenerative disease. Our study clearly shows that the group receiving Exenatide improved over 24 months and that the patients not receiving the drug declined. This could be due to an acute treatment effect or, as discussed above, a placebo effect. Obviously, these data should not be interpreted as evidence of symptomatic efficacy or neuroprotection and can only be seen as providing initial pilot clinical data in order to help to justify the larger investment required initiating a larger double-blind, placebo-controlled study and assist in sample size calculations.

We decided to adopt a pragmatic approach to the problems of taking established drugs to new areas of therapy in a neurodegenerative disorder and have done so with a study design that is informative and affordable. In so doing we have provided further reassurance and to some extent mitigated against the risk associated with the sizeable investment required to further investigate the role of Exenatide in the treatment of PD.

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