Peripheral insulin and amylin levels in Parkinson's disease.

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Abstract word count: 224. **Main text word count**: 3262. **References**: 35.

Running title: Glucose metabolic alterations in Parkinson's disease.

Keywords: Parkinson's disease, insulin, amylin, glucose metabolic alterations

Relevant conflicts of interest/financial disclosures:

Almudena Sánchez-Gómez: received an award from Hospital Clínic of Barcelona in 2017 (Emili Letang award).
Gema Alcarraz-Vizán, Rubén Fernández, Mario Ezquerra, Mònica Serrano and Esteban Muñoz declare that they have no conflict of interest.
Manel Fernández is supported by: María de Maeztu Unit of Excellence (Institute of Neurociences, University of Barcelona) MDM-2017-0729, Ministry of Science, Innovation and Universities.
Ana Camara has received support from Medtronic, Abbvie and UCB and has a competitive PERIS grant (SLT008/18/00191; Health Department, Generalitat de Catalunya)
Anna Novials has competitive grants from Instituto de Salud Carlos III (ISCIII) / Spanish Ministry of Health (PI17/00447 and CIBERDEM PIE14/00061).
Francesc Valldeoriola received honoraria for conferences and advice from Boston Scientific and Medtronic.
Yaroslau Compta has served as a consultant for Abbvie and Zambon, and has received honoraria for scientific presentations from Abbvie, Alter, Bial, Medtronic, Merz, Teva, UCB, and Zambon. He is currently an associate editor of Parkinsonism & Related Disorders (Elsevier). He has competitive grants from Instituto de Salud Carlos III (ISCIII) / Spanish Ministry of Health (PI17/00096) and the European Commission H2020 program (lPI043760).
Maria José Martí: received honoraria for consulting and lectures from Merz Pharma and Allergan SA; grants from the MJ Fox Fundation (CP041639) and Fondo de Investigaciones Sanitarias (FIS)-ISCIII (PI17/00096).

ABSTRACT

Background: Type-2-diabetes (T2D) has surfaced as a potential risk factor for Parkinson's disease (PD) in some epidemiological studies. Evidence of glucose metabolism alterations in PD from molecular studies remains conflicting. Amylin, the T2D amyloid protein, has been implicated in PD in pathological studies. We aimed to assess peripheral levels of amylin and insulin in PD patients and control subjects (Cs).

Methods: We conducted an observational cross-sectional study of 111 participants: 73 PD and 38 Cs, similar in age, sex and body mass index. All underwent motor (UPDRS-MDS-III), non-motor (NMSS) and cognitive (MDRS) scales as well as determination of four parameters: fasting glycaemia, glycated haemoglobin, fasting plasma insulin (FPI) and fasting plasma amylin (FPA).

Results: FPI was significantly lower in PD than Cs (p=0.034). In participants with age above cohort-median-age, FPA was higher in PD than Cs (p=0.046). The FPA/FPI ratio (FPAIR) was significantly higher in PD than Cs (p=0.024). In PD, modest correlation was found between higher insulin-resistance and NMSS scores.

Conclusions: PD patients had lower FPI and increased FPAIR. In older PD subgroup, FPA was increased. The more the insulin resistance, the higher the non-motor scores. These findings provide an additional link between pathophysiology of diabetes and PD. This might be related to a dissociated insulin and amylin secretion in PD, in line with recent evidence of endocrine pancreas role in PD pathogeny.

Introduction

Recent epidemiological evidence has identified type-2 diabetes mellitus (T2D) as a risk factor for the subsequent development of Parkinson's disease (PD) suggesting a pathophysiological association between PD and T2D [1].

Glucose metabolism (GM) and T2D associations with PD remain controversial. Increased blood glucose levels in PD vs. controls have been reported in some, but not all studies [2, 3]. In a recent work, fasting glucose levels in PD appeared to be predictive of cognitive impairment, but not of motor progression [3]. Insulin resistance (IR) and T2D have been associated with dementia in PD patients [4, 5] and T2D also with the postural instability and gait difficulty phenotype [6, 7].

Systemic and brain IR are the impaired response to insulin in body general tissues or in brain respectively. Systemic and brain IR appear to be related in PD [8-10], but this association and their potential mechanistic relevance in PD is not well defined.

One potential rationale for the link between PD and T2D lies in their proteinopathic nature. Hence, the pathological hallmarks of PD are Lewy bodies and neurites, composed of amyloid aggregates of misfolded α-synuclein (aS) among other proteins [11]. T2D presents amyloid deposits too, chiefly due to the aggregation of amylin (or islet amyloid polypeptide [IAPP]) [12, 13]. Amylin is a 37-residue peptide hormone co-secreted with insulin in pancreatic beta-cells. Both hormones share functions in the regulation of blood-glucose levels. Amylin has been implicated in several functions such as the inhibition of insulin secretion [14, 15]. It is believed that amylin aggregates lead to beta cell dysfunction and promote diabetes progression [12, 13].

Different studies have shown an association between amylin and Alzheimer's disease (AD). An experimental animal study has found that amylin may play a role in the clearance of amyloid beta peptides in AD brain [16].There is neuropathological evidence of deposition of aggregated amylin in the brain of AD patients [17] and an interaction between amylin and tau and beta-amyloid proteins both in hippocampus and pancreatic beta-cells was noted [17]. In plasma, very high amylin concentration was associated with AD risk at follow-up in a longitudinal study [18]. However, CSF and plasma amylin levels have not differed between AD and controls [19].

In terms of the amylin link with PD, *in vitro* work has shown that the presence of amylin accelerates the formation of aS amyloid, but not in reverse, suggesting an *in vitro* justification of a one-direction link with a T2D influence over PD [12]. Moreover, a pathological study found phosphorylated aS deposits in pancreatic beta-cells of subjects with a neuropathological diagnosis of synucleinopathy and revealed a direct interaction between amylin and aS too [20]. However, to the best of our knowledge, plasma amylin levels have not been assessed in PD so far.

Accordingly, we decided to evaluate whether or not there are blood alterations of GM in PD as a potential peripheral reflection of what hypothetically occurs with amylin at cerebral and pancreatic level. Also, we tried to set out their relationship with motor and non-motor symptoms and cognitive impairment.

Methods

Study design and clinical assessment

This is an observational cross-sectional single-center study conducted at the Parkinson and Movement Disorders Unit of the Hospital Clínic in Barcelona. The protocol was approved by the Clinical Research Ethics Committee of our Hospital and all the participants signed a written informed consent.

PD patients who met the diagnostic criteria of the Movement Disorders Society (MDS) 2015 for clinically established PD [21] were invited to take part in the study. Control subjects (Cs) were relatives of PD subjects or patients with hemifacial spam. We recruited 73 PD consecutive patients and 38 Cs in the movement disorders

unit in their routine visits.

In both groups, patients were subjects aged 40 to 80 years from Spain. Exclusion criteria were presence of other central nervous system disorders and uncertain PD diagnosis. T2D and type-1 diabetes were exclusion criteria since they would make difficult the interpretation of GM alterations.

At inclusion, socio-demographics information, disease and family history and treatments were noted. The non-motor symptoms and cognitive status were graded using the Non-Motor Symptom Scale (NMSS) and Mattis Dementia Rating Scale (MDRS) respectively in both groups. In PD patients, the motor evaluation was ranked with part III of the MDS-UPDRS (MDS-Unified Parkinson Disease Rating Scale).

Metabolic assessment

For all participants, the body mass index was calculated. A blood sample was collected after 8-hour fasting within 1 to 4 weeks of the inclusion visit. Blood samples were centrifuged at 1500 x g for 15 minutes at 4 degrees and aliquots of 1 mL and 500 µL of serum and 500 µL of plasma were stored. All were deep-frozen at -80°C. We determined the concentrations of four fasting parameters: glucose, glycated haemoglobin (in fresh), plasma insulin (FPI) and plasma amylin (FPA). We calculated the HOMA-IR index (Homeostatic Model Assessment of Insulin Resistance) and plasma amylin/insulin ratio (FPAIR) too. HOMA-IR is a widely used method to quantify IR. Here we calculated HOMA-IR with this equation: glucose x insulin/405 (glucose in mg/dL and insulin in international mU per litre). Furthermore we calculated FPAIR to have one more marker of the possible alteration in amylin secretion relative to insulin production.

Glucose, glycated haemoglobin and insulin were analyzed at the central laboratory of Hospital Clínic of Barcelona. Amylin was analyzed in Diabetes and Obesity Research Laboratory of IDIBAPS by using the Human Amylin Elisa-Kits (96-well plate assay) of Merck-Millipore Company (Massachusetts, United States). All the samples were run in duplicate, the average coefficient of variability was 5.9% and all of them were under 20%. The median value was the final score of each patient in pM. Hexoquinase bichromatic procedure was performed for blood glucose levels in mg/dL. Glycated haemoglobin was determined by immunoassay in % like A1c. Insulin was determined with immunoanalysis in mU/L. To calculate the FPAIR we converted insulin values from mU/L to pM.

Outcomes measures

The primary objective was to evaluate the GM profile in PD *vs.* Cs group as reflected by four fasting parameters (glucose, glycated haemoglobin, FPI and FPA) analyzed or in the calculations derived from these (FPAIR and HOMA-IR index). The secondary objective was to assess the correlation between GM related markers and non-motor, motor or cognitive involvement in PD participants as indicated by the NMSS, UPDRS-MDS part III and MDRS scores respectively.

Statistical Methods

All qualitative variables are presented as absolute and relative frequencies, and the quantitative ones as medians and their respective interquartile ranges. The categorical variables of the subjects were compared by Fisher's test. Pairwise comparisons of quantitative variables between both PD *vs.* Cs and between dichotomized PD subgroups were performed by means of Mann Whitney's U test. Due to the evidence of high prevalence of T2D in ≥ 65-year-old [22], we decided to perform an age sub-group analysis and we dichotomized the PD group according to the cohort-median age for further statistical analyses.

Binary logistic regression models adjusted for different covariates were applied to calculate the corresponding Odds ratios and the respective confidence intervals of 95% (95% CI). The number of covariates was kept to a minimum considering the sample size in order to minimize the risk of overfitting.

In PD group, correlations between metabolic parameters and motor, non-motor and cognitive scores were

assessed with Spearman's coefficient.

The statistical analysis of clinical and biological results was carried out with the SPSS package for Windows (version 22 for Windows; IBM, NYC, USA). All analyses were two-tailed with significance threshold set at 0.05. Correction for multiple comparisons was not applied due to the hypothesis driven design.

Results

Demographic and clinical variables

PD and Cs did not significantly differ in age or sex. Cs had higher educational level and less family history of parkinsonism than PD subjects. Among cardiovascular risk factors only dyslipidemia was significantly more prevalent in Cs than in PD. We did not observe differences in body mass index (BMI) between both groups or between women and men. Patients with PD had greater NMSS scores and lower MDRS scores ***(table 1)***.

PD patients had a median of 8 years of disease duration at inclusion. PD group scored a median of 23 on the UPDRS-MDS-III and 2 at the Hoen & Yahr scale in ON state. Levodopa equivalent daily dose (LEDD) in PD patients was 774mg/day [23] ***(table 1).***

Glucose metabolism analysis

Univariate pairwise comparisons detected lower FPI levels in PD than Cs. Fasting blood glucose levels, glycated haemoglobin, FPA and HOMA-IR did not differ in the quantitative values between PD and Cs. However, the FPAIR was significantly higher in PD than Cs. ***(table 1 and figure 1a and 1b).***

In a subsequent multivariate binary logistic regression model, increasing FPI and blood glucose levels along with higher educational degree and positive PD familiar history significantly increased the risk of belonging to the PD group ***(table 2)***. Likewise, binary logistic regression including BMI or sex, with PD familiar history, blood glucose and FPI levels increased the risk of belonging to the PD group too ***(Supplementary material: Table 2 and 3).***

Insulin, amylin and blood glucose differences between PD and Cs in relation to the age

We explored the effect of age in the metabolic differences by dichotomizing the PD and Cs groups according to the cohort-median age: <67 vs. ≥67-year-old (yo). FPI levels were significantly lower in older PD patients than in older Cs (6.35 mU/L *vs.* 9.1 mU/L Cs median FPI levels; p=0.020) and tended to decrease in PD older-subgroup (6.35 mU/L in PD ≥67 yo *vs.* 8.00 mU/L in PD <67 yo; median FPI levels; p=0.063). FPI levels did not differ between younger PD and Cs subgroups (p=0.521), nor between Cs age-subgroups. (p=0.784) ***(Supplementary material: Table 1, panel a).***

FPA was significantly higher in older PD subjects than older Cs (24.9 pM *vs.* 17.87 pM median FPA levels; p=0.046), but not between PD and Cs younger age sub-groups (22.44 pM *vs.* 30.41 pM median FPA levels; p=0.271). In the Cs group, FPA was significantly higher in <67 yo than in ≥67 yo subjects (p=0.030). We did not find differences of FPA levels between PD age-subgroups (p=0.817) ***(Supplementary material: Table 1, panel b).***

FPAIR was significantly higher in PD subjects ≥67 yo than Cs ≥67 yo (0.55 *vs.* 0.29 median FPAIR; p <0.001). No other differences in FPAIR between groups and age-subgroups were found ***(Supplementary material: Table 1, panel c).***

Blood glucose levels did not differ between groups and the different age subgroups ***(Supplementary material: Table 4)***.

Metabolic and clinical correlations

Finally, in PD group, we found modest significant correlations between higher HOMA-IR levels with increased non-motor symptoms scores (NMSS; r= 0.328; p=0.006) ***(figure 2).*** Motor symptoms and cognitive assessment were not correlated with any of the metabolic parameters.

Discussion

In our study, we found GM impairment in PD patients relative to Cs. Our primary findings were decreased FPI levels and increased FPAIR in PD *vs.* Cs. We have also found that lower FPI levels, increasing glycaemia, lower educational level and PD familiar history in combination discriminated PD from controls. Modest correlations were found between rising HOMA-IR levels and non-motor symptoms.

Secondary findings were that in older age sub-groups, FPI was lower and FPA was higher in PD *vs.* Cs. Moreover, FPAIR showed a trend to be increased in older-PD-subgroup and the opposite in older-control-subgroup. HOMA-IR tended to be higher in Cs without significance between groups. No other metabolic parameters analyzed differed between PD and Cs*.* Blood glucose levels did not differ either between PD and Cs. Inconsistencies in previous literature and with our current report might be attributable to different population and overweight condition [2, 24]. In our case, we consider we did not find differences in blood glucose levels due to a normal BMI in our cohort and we believe also glycaemia could be less specific of GM alterations in PD in the absence of diabetes.

Multiple studies have analyzed the relationship between peripheral GM abnormalities and more specifically on IR and PD [10]. The GM alterations in PD based on differences in insulin in our cohort add to, and expand those from, previous studies. However, other metabolic works did not find differences in FPI levels in PD patients *vs.* Cs [2, 24]. Lower insulin values in older people may be attributable to aging and loss of beta cell function [25]. Insufficient insulin response has been suggested in PD patients in other works [24, 26] and our results with lower FPI levels would support this possibility. Most brain insulin derives from peripheral levels, since insulin crosses the blood-brain-barrier. Of note, in a cohort of 160 patients, FPI levels correlated with cerebrospinal fluid levels [27]. Despite we cannot correlate our data in PD at the peripheral level with what happens at brain level, our results with lower FPI levels in PD vs. C could suggest a lower insulin supply to the brain. Further studies to corroborate this hypothesis will be needed.

IR, measured by HOMA-IR, was found to be impaired in PD, but with a significant correlation with overweight and obesity condition [26]. In our work, HOMA-IR and BMI were normal in PD group without differences with Cs, and the binary logistic regression model including BMI did not show this parameter to be a significant predictor of PD. Thus, in our study the association of FPI with PD would appear to be independent of BMI.

The insulin degrading enzyme (IDE; an endopeptidase that can degrade insulin and amyloidogenic proteins in the pancreas and that it is up-regulated by insulin) has been shown to interact with the acid C-terminal of aS [28]. This interaction *in vitro* has been associated with aS inhibition by IDE and its subsequent prevention of aS amyloid fibrils formation [29]. Based on these results, we can hypothesize that the insulin pathway, including the IDE and lower FPI levels, has an important role in the relationship between T2D and PD. Further studies to support this hypothesis are warranted.

The potential link of amylin with PD is a new approach in the study of the association between PD and T2D, not previously described in the literature. Amylin has been found to be lower in T2D, but it seems to be dependent on insulin treatment, being higher in T2D patients without insulin treatment [30]. Higher amylin levels have been found also in non-diabetic healthy controls with oral glucose intolerance compared to those with normal glucose tolerance [30]. In our study, T2D was exclude and no other cardiovascular risk factors were different between PD and Cs.

In contrast to insulin trend with aging, amylin levels in older people have been inconsistent in the literature and these differences may be related with different measurement techniques and different population (small

groups, Asian population, etc.) [31, 32]. In our case FPA levels in healthy controls were lower in the older subgroup. We interpret this as, the same as insulin, amylin levels could tend to be lower in aging.

In our participants, older PD patients had higher FPA levels than elder controls, who in turn had lower FPA levels than younger controls. These results need to be taken with caution since FPA levels had not been previously studied in PD. Still, our findings suggest that while in controls FPA decreases with ageing, in PD its levels tend to keep the values.

In PD, an interaction between both amyloid proteins aS and amylin was observed in molecular and pathological studies [12, 20]. Likewise, amylin presence in brain of AD patients and an interaction between amylin and beta-amyloid and tau have been observed [17]. Peripheral amylin can cross the blood-brain barrier and, in experimental studies, humanized hyperamylinemia has been associated in rats with marked amylin amyloid deposits in brain [33]. Albeit we do not have previous data of amylin levels in PD, a recent longitudinal study has found that high amylin levels could increase AD risk [18]. This along with our findings in PD patients compared to controls, suggests that high peripheral amylin levels in PD could hypothetically lead to a greater deposit of amylin at brain level and to an interaction between both amyloid proteins (aS and amylin), promoting greater aS aggregation.

As discussed above, the literature on aging effect on amylin is quite inconsistent. Nevertheless, given the observed link between amylin and proteins that form aggregates in neurodegenerative diseases such as Alzheimer's and PD that are closely associated to aging (as is T2D), it could be speculated that aging in the setting of these conditions could promote increased levels and aggregation of these proteins. Future research should clarify if this is the case or not.

In light of all this and since amylin and insulin are co-secreted in pancreatic beta-cells, the FPAIR might be a measure of the regulatory function of beta cells that impacts on GM. In our cohort, the higher FPAIR in PD group at the expense of lower FPI (and higher FPA in the older age-group), could be due to a dissociation in insulin and amylin secretion in PD patients and the reflection of increased tissue amyloid deposition and interactions between both amyloid proteins (amylin and aS) with subsequent PD progression. The known colocalization of the two amyloid proteins in pancreatic and neuronal cells makes it plausible that they can have biological and pathophysiological interactions *in vivo* [12, 20].

Other authors found a link between greater PD motor symptoms and T2D; this association confers more severe motor involvement with postural instability and gait disorder phenotype [6,7]. We did not find a similar association perhaps our study was focused on metabolic and clinical associations in non-diabetic patients. Our findings of correlations between HOMA-IR with highest non-motor scores should be further explored to confirm or not this association. T2D and IR have been linked to dementia in PD [4,5]. Although the MDRS scores in our PD group were significantly lower than in Cs, the PD participants in this study were not particularly cognitively impaired [34]; This relative cognitive preservation could account for the lack of correlation between GM alterations and MDRS in our cohort.

Our study is not without limitations: first of all, the sample size was reasonable considering prior research but yet relatively small and perhaps some modest statistical differences indicate limited statistical power for part of the analyses. Second, our study is cross-sectional; it remains to be seen if a longitudinal study would show these GM markers to be predictors of PD diagnosis or progression. All subjects were from Spain; this fact limits the generalization of our results.

The most noted strength and novelty of this study is the analysis of amylin in a PD cohort. The research of amylin in PD cohort is a new approach of the study of the associations between PD and T2D, not described in the literature before. Hence, a future potential avenue of research in PD is measuring amylin in CSF from PD patients. Other strengths relate to internal consistency of the different analyses and the fact that BMI and other cardiovascular risk factors were also taken into consideration and controlled for, suggesting that our findings are specific of GM alterations and independent of other factors.

In conclusion, lower FPI and higher FPAIR in PD along with higher FPA levels in older PD patients indicate a moderate GM alteration in PD patients, suggesting a pathophysiological link between T2D and PD. Such a link might have potential pathophysiological, diagnostic and therapeutic relevance, in the current setting of great attention being paid to the potential neuroprotective effect of antidiabetic drugs [35].

Acknowledgements

We would like to thank the patients for their selfless collaboration in the study.
Hospital Clínic de Barcelona receives support from the CERCA program of Generalitat de Catalunya.

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Tables

Table 1. Comparison of clinical and biologic characteristics between PD and Cs groups

	PD patients (n=73)	Cs (n=38)	Significance (p)
Demographics data			
Sex (Women)	37 (50.7%)	23 (60.5%)	0,360[1]
Age (years, median)	67 y (57-72)	65 y (53-72)	0,462[2]
Education level			
Basic	25 (35.2%)	3 (8.1%)	
Media	21 (29.6%)	16 (43.2%)	0,012[1]*
Superior	25 (35.2%)	18 (48.7%)	
PD Familiar History			
No familiar history	44 (61%)	34 (89.5%)	
First Degree	12 (16.7%)	1 (2.6%)	
Other Degrees	12 (16.7%)	2 (5.3%)	0,019[1]*
"Tremor" in family	4 (5.6%)	1 (2.6%)	
Cardiovascular risk factors			
Arterial Hypertension	19 (26.4%)	10 (26.3%)	0.993[1]
Dyslipidemia	7 (9.7%)	10 (26.3%)	0.022[1]*
Ischemic Heart Disease	3 (4.2%)	1 (2.6%)	0.683[1]
BMI (median) (p25-p75)	25.2 (23.1-26.4)	24.8 (21.5-29)	0.495[2]
Clinical assessment			
NMSS median (p25-p75)	37 (19-65)	8 (2.8-15.3)	<0.001[2]*
MDRS median (p25-p75)	140 (136-142)	141 (140-143)	0.015[2]*
MDS-UPDRS-III median (ON state)	23 (17-40)	-	
Hoen & Yahr scale median (ON state)	2 (2-2)	-	
Disease duration median (years)	8 (5-15)	-	
LEDD median (mg/day)	774 (404-1230)	-	
Biological assessment			
Blood glucose levels (mg/dL) median (p25-p75)	96 (89.3-101.8)	94.00 (88.8-101.5)	0.448[2]
HbA1c (%) median (p25-p75)	5.50 (5.3-5.7)	5.40 (5.2-5.7)	0.521[2]
FPI (mU/L) median (p25-p75)	6.9 (5-9.9)	9.10 (5.8-11.6)	0.034[2]*
FPA (pM) median (p25-p75)	23.48 (17.41-40.78)	20.13 (15.13-36.49)	0.454[2]
HOMA-IR median (p25-p75)	1.6 (1.1-2.6)	2.08 (1.3-3.1)	0. 081[2]
FPAIR median (p25-p75)	0.52 (0.32-0.89)	0.32 (0.24-0.66)	0.024[2]*

[1]Pearson's chi-squared test; [2]U Mann-Whitney; *Significant difference. PD: Parkinson's disease; Cs: Control subjects; T2D: Type-2-diabetes; BMI: Body mass index; NMSS: Non-Motor Symptom Scale; MDRS: Mattis Dementia Rating Scale; MDS-UPDRS-III: Movement Disorders Society-Unified Parkinson Disease Rating Scale, part III; LEED: Levodopa equivalent daily dose; FPI: Fasting plasma insulin; FPA: Fasting plasma amylin; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; FPAIR: Fasting plasma amylin/insulin ratio.

	Significance (p)	Odds Ratio (OR)	95% CI for OR	
			Lower limit	Upper limit
Educational level	0.006*	0.398	0.206	0.772
PD familiar history	0.009*	2.466	1.255	4.848
Blood glucose levels (mg/dL)	0.008*	1.073	1.018	1.130
FPI levels (mU/L)	0.004*	0.826	0.726	0.939

Table 2: Binary logistic regression model covariate by educational level, PD familiar history, blood glucose levels and FPI levels. *Significant difference. FPI: Fasting plasma insulin.

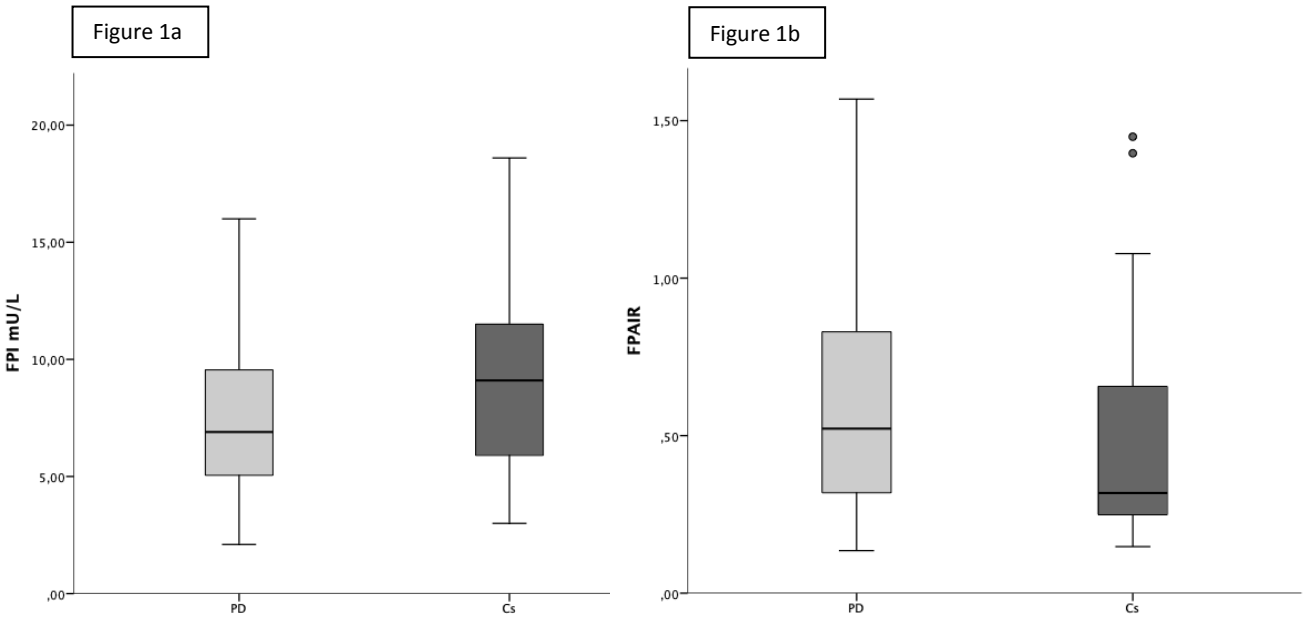

Figure 1: Glucose metabolism results. Figure 1a. Box plots of FPI levels [median (p25-p75)]. PD patients= 6.9 (5-9.9); Cs= 9.10 (5.8-11.6); p=0.034. **Figure 1b**: Box plots of FPAIR [median (p25-p75]). PD patients= 0.52 (0.32-0.89); Cs=0.32 (0.24-0.66). p=0.024. PD: Parkinson's disease; Cs: Control subjects; FPI: Fasting plasma insulin; FPAIR: Fasting plasma amylin insulin ratio.

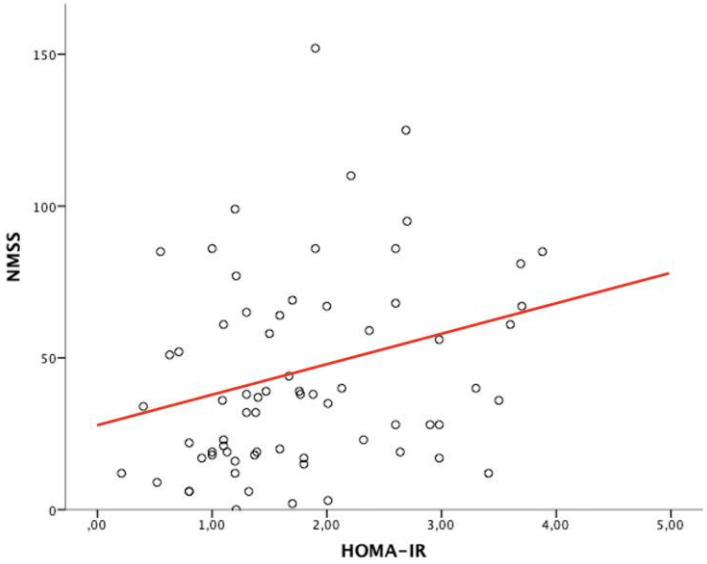

Figure 2: Metabolic and clinical correlations. Growing HOMA-IR values were associated with increasing non-motor symptoms at NMSS (r= 0.328, p=0.006). HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; NMSS: Non-Motor Symptom Scale.