Disrupted Mitochondrial and Metabolic Plasticity Underlie Comorbidity Between Age-related and Degenerative Disorders as Parkinson Disease and Type 2 Diabetes Mellitus

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Research article

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Abstract

Background. Idiopathic Parkinson's disease (iPD) and Type 2 Diabetes Mellitus (T2DM) are chronic, degenerative, multisystemic and age-related diseases, with eventual epidemiological co-morbidity and potential overlap in molecular basis. This study aims to explore if metabolic and mitochondrial alterations underlie the suggested epidemiologic and clinical co-morbidity at molecular level.

Methods. To evaluate the adaptation of iPD to a simulated pre-diabetogenic state, we exposed primary cultured fibroblasts from iPD patients and controls to standard (5mM) and high (25mM) glucose concentrations to further characterize metabolic and mitochondrial resilience.

Results. iPD fibroblasts showed increased levels of organic and amino acid levels related to mitochondrial metabolism with respect to controls, and these differences were enhanced in high glucose conditions (citric, suberic and sebacic acids levels increased, as well as alanine, glutamate, aspartate, arginine and ornithine amino acids; p-values between 0.001 and p=0.05). The accumulation of metabolites in iPD fibroblasts was associated (and probably due) to the concomitant mitochondrial dysfunction observed at enzymatic, oxidative, respiratory and morphologic level. Metabolic and mitochondrial plasticity of controls was not observed in iPD fibroblasts, that were unable to adapt to different glucose conditions. Impaired metabolism and mitochondrial activity in iPD may limit energy supply for cell survival. Moreover, deficient plasticity to adapt to disrupted glucose balance characteristic of T2DM may underlay the comorbidity between both diseases.

Conclusions:

Fibroblasts form iPD patients showed mitochondrial impairment resulting in the accumulation of organic and amino acids related to mitochondrial metabolism, especially when exposed to high glucose condition.

Mitochondrial and metabolic defects down warding cell plasticity to adapt to changing glucose bioavailability may explain the comorbidity between iPD and T2DM.

Background

In western countries, the increased life expectancy has led to an increment in chronic and age-related diseases, resulting in a decrease in quality of life of the elderly population and representing an important sociosanitary burden [1, 2]. Parkinson's Disease (PD) and Diabetes Mellitus (DM) are amongst the most prevalent 21st century epidemics. The world prevalence of PD is 1% of the population over 65 years, with an expected 50% increase for 2040 [3]. The world prevalence of diabetes was estimated to be 6.4% in 2010 and it is expected to rise to 10.4% in 2040, 90% represented by Type 2 DM (T2DM)[4].

Clinical manifestations in idiopathic PD (iPD) and T2DM are secondary to the decrement of biological products due to cell death in the target tissue of the disease. Both diseases affect multiple organs and molecular alterations appear years before clinical diagnosis is made [5–7]. Thus, for either iPD or T2DM, preventive measures and disease modifying therapies are a main target in research [6].

PD is characterized by the decrease of dopamine release resulting from the loss of dopaminergic neurons in the *substantia nigra*, associated to movement disorders. The main risk factor for PD is ageing, and, despite the small proportion of PD cases of genetic origin, 90% of PD is idiopathic.

T2DM is characterized by chronically elevated blood glucose concentration which arises from a combination of insufficient insulin secretion and a reduced sensitivity of target cells and tissues to insulin. Obesity and a low physical activity are the main risk factor for T2DM, and approximately 50% of diabetics show additional complications by the time they are diagnosed [8] .

The comorbidity of iPD and T2DM has been described in numerous epidemiological studies [9–11]. Despite an initial controversy [12, 13], the established risk for presenting iPD in T2DM patients is 38% [14]. It is also known that T2DM may predispose to a PD-like pathology and induce a more aggressive phenotype when coexisting with PD [15]. In fact, T2DM is a well known established risk factor for iPD development [16].

Recent evidences points towards shared deregulated molecular pathways between PD and DM such as protein misfolding and depot, endoplasmic reticulum stress or inflammation [17, 18]. Among these, growing evidences support metabolic and mitochondrial dysfunction to play a critical role in the development of both diseases [9, 11, 13, 17, 19–21]. Specifically, mitochondrial respiratory chain (MRC) dysfunction, oxidative stress, altered mitochondrial dynamics and morphology have been reported in iPD [22]. Similarly, insulin resistance is associated with increased oxidative stress, mitochondrial DNA mutations and mitochondrial dysfunction [17]. In fact, mitochondria are the focus of recent investigations as a pivotal player for T2DM and iPD preclinical therapies [23].

While metabolic and mitochondrial dysfunction clearly play a role in both iPD and T2DM, scarce studies have explored if such metabolic and mitochondrial deregulation may underlie the epidemiologic co-morbidity that exists between both diseases [10].

There is a crucial need to fully understand the molecular base of the epidemiologic comorbidity between iPD and T2DM and if it may be associated to a further worsening of the bioenergetic deficits characteristic of both diseases.

Taking advantage of the validation of fibroblasts as a reliable cell model for the study of PD [24–27] we have designed an *in vitro* model of T2DM-iPD comorbidity to assess metabolic and mitochondrial contribution to the reported concomitance.

Methods

Study design and population

A single-site, cross-sectional, observational study was conducted. Fourteen age and gender paired subjects were included: seven iPD patients and seven healthy unrelated controls (C). No significant differences in age and gender were observed between cases and controls (Table 1). All iPD patients met the UK Brain Bank Criteria for the diagnosis of PD [28]. Subjects with other comorbidities (including T2DM) were excluded from the study [29]. Both patients and controls signed the informed consent to participate in the study, previously approved by the Clinical and Research Ethical Committee of our institution, in accordance to the Declaration of Helsinki (code HCB/2015/0562).

Table 1
Epidemiological characteristics of patients and controls included in the study. No significant differences in age and gender were observed between cases and controls.

GROUP	N	GENDER		AGE		
		Male	Female	Range	Mean	SEM
iPD	7	4	3	46-66	58.57	3.08
CONTROL	7	2	5	41-69	54.86	4.00
TOTAL	14	6	8	41-69	56.71	2.48

Fibroblasts culture

Fibroblasts were obtained by a skin punch biopsy and mutation screening was performed, as previously described [30], to discard genetic contribution to disease.

Cells were grown in 25mM glucose DMEM medium (Gibco, Life Technologies) supplemented with 10% heat-inactivated foetal bovine serum and 1% penicillin-streptomycin at 37ºC, in a humidified 5% CO_2 air incubator, until 80% optimal confluence was reached. After cell expansion, cells were exposed for 10 days to 2 different glucose conditions to assess metabolic and mitochondrial contribution to disease: a 'pre-diabetogenic' high glucose (HG) environment containing 25mM of glucose (equivalent to 450mg/dl) or to a normoglycemic environment containing 5mM of glucose (equivalent to 90mg/dl; low glucose, LG). Fibroblasts were then harvested with 2.5% trypsin, (Gibco, Life technologies™) and centrifuged at 500g for 8 minutes for further analysis.

Metabolic and mitochondrial phenotyping was afterwards performed in iPD and C fibroblasts between passage 5 and 10. Some measures required fresh cell assessments such as oxygen consumption rate (OCR), which was performed in parallel including iPD and C in both glucose concentrations. Fixation of cells for immunofluorescent quantification of mitochondrial dynamics was also performed with fresh material at this time point. For the rest of experimental procedures, cell pellets were kept frozen at -80ºC until analysis.

Targeted metabolomic characterization

Fibroblast preparation for metabolomic assessments required a minimum of 1 million cells that were thaw, resuspended in 200 µl of PBS and centrifuged (1,500x g; 10 minutes) to collect the supernatant, were amino acids and organic acids were quantified.

- **Organic acids** were extracted in fibroblasts with ethyl acetate and diethyl ether and derivatized with bis(trimethyl-silyl)trifluoro-acetamide (BSTFA), as previously reported [31]. The trimethylsilyl derivatives obtained were separated by gas chromatography (Agilent 7890A) and detected in a mass spectrometer (Agilent 5975C). Results were expressed as nmol of organic acid/mg of protein.
- **Amino acids** were quantified in fibroblasts by ultra performance liquid chromatography (UPLC) coupled to tandem mass spectrometry, as previously reported [32]. Briefly, amino acids were separated in a Waters ACQUITY UPLC H-class chromatograph and quantified with a Waters Xevo TQD triple-quadrupole mass spectrometer using positive electrospray ionization conditions in the MRM mode. Results were expressed as nmol of amino acid/mg of protein.

Mitochondrial characterization

- **MRC and citrate synthase (CS)enzyme activities** were measured at 37ºC by spectrophotometry, following standardized procedures [33], as reported elsewhere [7,34]. All enzymatic activities were run in parallel with internal quality controls [58]. Complex II (CII), Complex IV (CIV) and glycerol-3-phosphate dehydrogenase (G3PDH) enzyme activities were then normalized by CS, to express enzymatic activities per mitochondrial content, as CS is widely considered as a reliable marker of mitochondrial mass. Changes in absorbance were registered in a HITACHI U2900 spectrophotometer through the UV-Solution software v2.2 and were expressed as nanomoles of consumed substrate or generated product per minute and milligram of protein and mitochondrial content (nmol/minute·mg protein.CS).
- **Mitochondrial respiration and oxygen consumption rates (OCRs)** were measured following the manufacturer's protocols with two different technologies: Oroboros^{TM} high resolution respirometry [35] and Agilent Seahorse^{TM} XF24 Analyzer using the Cell Mito Stress Test [36]. Punctual differences in the technical procedures were made, in order to adapt the experimental procedure to each methodology, which are furtherly described in supplementary material. OCR values were normalized to total cell protein content and mitochondrial mass, measured by CS enzymatic activity (pmol/min.ug protein.CS).
- **Lipid peroxidation** was measured by the spectrophotometric measurement of malondialdehyde (MDA) and 4-hydroxyalkenal (HAE) as indicators of reactive oxygen species (ROS) damage into cellular lipid compounds, as reported elsewhere [37]. Results were normalized per mitochondrial content (µM MDA and HAE/mg protein.CS).
- **Mitochondrial morphology** was assessed by immunocytochemistry using confocal microscopy, as previously described [7]. Briefly, a minimum of 3 fibroblasts from each subject were analysed using the Image J software [38] and quantified using a semi-automatic custom-made macro [39]. Mitochondrial network of each cell was subjected to particle analysis and the following parameters were assessed: aspect ratio (AR) (major axis/minor axis) and form factor (FF), which was calculated as the inverse of the circularity ($circ^{-1}$; 4π·area/perimeter2). AR and FF values correspond to mitochondrial length and branching, respectively, and are considered parameters of mitochondrial health. AR and FF values of 1 are indicative of circular, unbranched, isolated and pathologic mitochondria. As mitochondria elongate and become more branched, AR and FF values increase as a sign of mitochondrial health [40].

Statistical analysis

Statistical analysis was performed using two softwares: the GraphPad Prism Software (version 8.3.1 for Mac, San Diego, California USA, www.graphpad.com) and the Statistical Package for the Social Sciences (SPSS, version 21, IBM SPSS Statistics; SPSS Inc). Differences amongst groups were sought by non-parametric tests after filtering for outlier values in the datasets. Specifically, Kruskal-Wallis and Mann–Whitney U statistical tests for independent samples were used, when required, together with Holm-Sidak comparison. Significance was accepted for asymptotic 2-tailed p-values below 0.05 (for a confidence interval of α= 95%) and results were expressed as means ± the standard error of the mean (SEM).

Results

Targeted metabolomic characterization:

Measurement of organic acid and amino acid levels in iPD-fibroblasts showed unbalanced metabolic fluxes related to mitochondrial function.

- **Organic acids** related with mitochondrial energetic metabolism were increased in iPD patients, suggesting the deregulation of the intermediary metabolism (Figure 1).

Specifically, classical biomarkers of mitochondrial diseases as lactic acid, and the main components of the Krebs's cycle (citric, malic, succinic and 2OH-glutaric acid) showed trends towards increase in iPD samples at standard glucose concentration (5 mM). Similar trends were observed in the metabolites derived from Kreb's cycle related to amino acid or fatty acid metabolism (as ethylmalonic or glutaric acid) and the biomarkers from free fatty acid β-oxidation (including adipic, suberic and sebacic dicarboxylic acids). The accumulation of all these metabolites is frequently associated with MRC dysfunction and, specifically the increase in lactic acid levels, with the activation of anaerobic glycolysis in detriment of MRC function.

High glucose concentration (25mM) further accentuated such trends, as observed by the significant increase of citric, suberic and sebacic acids (p-value=0.01, p=0.03 and p=0.03, respectively). Such increment suggests a worsened phenotype for iPD-fibroblasts in case of high glucose exposition.

As a control, levels of organic acids non-related with mitochondrial energy metabolism (including uracil and pyroglutamic acid), were measured and found conserved among patients, controls and glucose conditions, suggesting that only mitochondrial-related organic acids were affected.

- Similarly, all **amino acids** related to mitochondrial function were increased in iPD patients, mimicking the same pattern than organic acids (Figure 2).

Specifically, alanine, glutamate, aspartate, arginine and ornithine, the classic amino acids related to mitochondrial metabolism, showed trends towards increase in iPD-fibroblasts at standard glucose concentration (5 mM), being statistically significant in the case of glutamate and aspartate (p-values=0.03 and p=0.008, respectively).

Exposure to a HG concentration (25mM) further confirmed such trends, as observed by the significant increase of all mitochondrially-related amino acids (p-values between 0.006 and 0.05 cut offs). Such increase suggests the worsening of the phenotype in case of high glucose exposition, as previously observed with organic acid metabolites.

As controls, levels of amino acids not related to mitochondrial metabolism (such as tyrosine or phenylalanine) were measured and found conserved among iPD patients, controls and glucose concentrations, suggesting that only mitochondrial-related amino acids were affected.

Overall, these findings show increased levels of organic acids and amino acids related to mitochondrial function in iPD-fibroblasts, especially when exposed to high glucose concentration, suggesting that impaired mitochondrial function is exacerbated in 'pre-diabetogenic' conditions.

Mitochondrial characterization:

Mitochondrial phenotyping at enzymatic, oxidative, respiratory and morphologic level confirmed such hypothesis.

- Specifically, **MRC enzymatic activities** from CII and G3PDH (fed by Kreb's cycle and b-oxidation pathways) tended to decrease in iPD-fibroblasts (Figure 3). Additionally, iPD cells manifested decreased metabolic plasticity than controls to adapt to changing glucose conditions. Interestingly, CIV activity trended to increase in iPD fibroblasts, significantly when exposed to HG (p=0.01), probably to overcome CII-G3PDH impairment.

As a result of CII-G3PDH MRC reduction in iPD-fibroblasts, **oxidative stress levels**, as a secondary product of MRC function, trended to decrease in iPD-patients (Figure 3).

- **Mitochondrial respiration**, measured by Oroboros™ and Seahorse™ technologies (Figures 4 and 5), confirmed the dysfunction of MRC previously observed at enzymatic and oxidative level.

OCR measures obtained by Oroboros™ technology (Figure 4) showed that basal (or routine) and maximal (ETC) respiration, reserve capacity and as ATP-linked respiration trended to decrease in iPD-fibroblasts.

Mitochondrial respiration measured by Seahorse™ technology (Figure 5) confirmed such trends by the decrease of basal respiration, coupling, maximal, spare and mitochondria working capacities in iPD-fibroblasts.

In all cases, iPD-fibroblasts showed reduced mitochondrial plasticity respect to controls to adapt to changing glucose conditions.

- Changes in **mitochondrial morphology** were expected after subjecting fibroblasts to different glucose concentrations. In line with this, increased glucose concentration significantly decreased aspect ratio and form factor from control fibroblasts (p-values=0.001 and p=0.05), accounting for less elongated and branched mitochondria. On the contrary, iPD-fibroblasts were unable to adapt to HG exposition (Figure 6) and showed conserved mitochondrial morphology, regardless the media, confirming their metabolic and morphologic rigidity.

Overall, fibroblasts from iPD patients showed a disarranged mitochondrial activity and morphology and manifested the inability to adapt to the different glucose conditions, in opposition to fibroblasts of controls, with preserved bioenergetic plasticity.

Discussion

Idiopathic PD and T2DM are increasingly prevalent diseases that are epidemiologically associated and share some altered molecular deregulated pathways [15],[9, 18]. This study aimed to explore if metabolic and mitochondrial complications are critical pathologic pathways that may explain this epidemiologic comorbidity.

Systemic effects of deregulated metabolism have been previously described by other studies [5, 41], and are relevant in the dissection of the molecular pathways that may lead to the development of treatment strategies [23]. Despite neural metabolism and mitochondrial function may be different from that of peripheral tissues, the study of metabolomic and mitochondrial function in fibroblasts is currently validated for the study of neurogenerative diseases, including iPD [42]. Fibroblasts have also been used as widespread cell model for the study of T2DM [43]. The

present work was performed in fibroblasts derived from iPD patients that were exposed to changing glucose concentrations, to mimic diabetogenic conditions and explore the potential worsening of the molecular phenotype.

In standard glucose conditions, fibroblasts from iPD patients showed a basal mitochondrial pathological phenotype that was accompanied by the accumulation of energetic metabolites related to organic and amino acid metabolism. These preexistent mitochondrial and metabolic dysfunction result in a defective capacity to adapt to stressful situations, such as the increase in glucose concentration, characteristic of T2DM [13, 17, 19]. In fact, the present findings unveil the poor resilience of iPD fibroblasts to adapt to changing glucose conditions, as opposed to controls, that showed wider plasticity in metabolic and mitochondrial performance. When exposed to HG concentrations, iPD fibroblasts worsened their basal pathologic metabolic and mitochondrial status.

Metabolic (including mitochondrial) flexibility is defined as the ability to perform efficient switches in metabolism, depending on the environmental demand (feeding/fasting cycles) [44]. Such capacity seems down warded in iPD fibroblasts, in concomitance with increased risk for T2DM development. Remarkably, iPD fibroblasts showed increased lactate production in detriment of decreased mitochondrial oxidative metabolism. Lactate is a classical marker for the diagnosis of primary mitochondrial diseases, but also a sign of upregulated glycolytic metabolism. Interestingly, T2DM patients also show increased levels of plasmatic lactate [45, 46], confirming both the rise of glycolytic metabolism and the metabolic co-morbidity between both diseases.

Whether metabolic alterations are causative, or a consequence of mitochondrial dysfunction, remains elusive. However, the specific accumulation of those organic acids and amino acids exclusively related to mitochondrial metabolism, supports the hypothesis that metabolite accumulation is triggered by deficient mitochondrial function, as described in the integrated mitochondrial stress response [47]. If mitochondrial dysfunction preludes metabolic disturbances, mitochondrial targets could set the path for novel therapeutics on iPD and T2DM management. For instance, mitochondrial dynamics has been recently proven to be one of the most important mechanisms for the rapid adaptation to the constant changes of environment, including nutrient bioavailability. In neurodegeneration, the capability of mitochondria to fuse with one another supporting the better performance of the MRC, is one of the new targets of early treatment [48, 49]. The present study shows an absence of mitochondrial adaptation to changes in glucose concentrations, including morphologic adaptations, which may be relevant in terms of efficiency of the MRC, mitochondrial DNA maintenance and mitophagy. Further studies and novel therapeutic options may address these questions.

Metabolic and mitochondrial alterations have already been reported in several tissues of iPD and T2DM patients [50, 51]. However, the present data demonstrates for the first time the aggravation of the metabolic and mitochondrial phenotype from iPD fibroblasts in high glucose environment, mimicking T2DM. A causal relationship between metabolic or mitochondrial alterations and iPD-T2DM comorbidity is not herein demonstrated, but its association is indirectly shown.

Metabolic and mitochondrial disturbances in iPD patients may limit energetic fuel to support the rest of biologic pathways essential for cell survival. Such bioenergetic failure in iPD seems to be aggravated by T2DM comorbidity, worsening cell fate and explaining the comorbidity between both diseases.

Of note, the present study contains some limitations. First, the model of study for iPD-T2DM comorbidity does not recapitulate some of the manifold molecular alterations that occur in iPD and T2DM, such as disrupted lipid metabolism or increment of inflammatory response. Second, fibroblasts are a validated model for the study of both iPD and T2DM, but we cannot discard the possibility of greater metabolic and mitochondrial extend of alterations in other tissues more dependent of aerobic metabolism or directly targeted to the present diseases such as dopaminergic neurons or β-pancreatic cells [52]. Similarly, the analysis of fibroblasts from T2DM patients or those presenting both iPD-T2DM diseases would be of high interest in further studies.

In summary, the present findings demonstrate that systemic metabolic and mitochondrial alterations are present in individuals with iPD accounting for a decreased capacity to successfully adapt to stressful events throughout life, including changing glucose environments. Advances on novel therapeutic targets and potential treatments for iPD and T2DM management would be of great value to face these challenging global health problems, to ameliorate the quality of life of patients and to reduce the associated sociosanitary burden.

Conclusions

- Fibroblasts form iPD patients showed mitochondrial impairment resulting in the accumulation of organic and amino acids related to mitochondrial metabolism, especially when exposed to high glucose condition.
- Mitochondrial and metabolic defects down warding cell plasticity to adapt to changing glucose bioavailability may explain the comorbidity between iPD and T2DM.

Abreviations

AR: aspect ratio

ATP: adenosine triphosphate

BSTFA: bis(trimethyl-silyl)trifluoro-acetamide

C: controls

CII: Complex II

CIV: Complex IV

CS: citrate synthase

DM: Diabetes Mellitus

DMEM: Dulbbecco's modified Eagle Medium

ETC: electronic transport chain

FF: form factor

G3PDH: glycerol-3-phosphate dehydrogenase

HAE: 4-hydroxyalkenal

HG: high glucose

iPD: Idiopathic Parkinson's Disease

LG: low glucose

MDA: malondialdehyde

MRC: mitochondrial respiratory chain

OCR: oxygen consumption rate

PBS: phosphate-buffered saline

PD: Parkinson´s Disease

ROS: reactive oxygen species

SEM: standard error of the mean

SPSS: Statistical Package for the Social Sciences

T2DM: Type 2 Diabetes Mellitus

UPLC: ultra performance liquid chromatography

Declarations

Ethical Approval and Consent to participate

Both patients and controls signed the informed consent to participate in the study, previously approved by the Clinical and Research Ethical Committee of our institution, in accordance to the Declaration of Helsinki (code HCB/2015/0562).

Consent for publication

All authors have read and approved the final version of the manuscript for publication.

Availability of supporting data

The availability of data and material is possible under request and, in case of samples, after the signature of the Material Transfer Agreement.

Competing interests

The authors declare no conflict of interest for the data contained in the present manuscript.

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Authors' contributions

Conceptualization, DLJ-F, FC, JMG, MJM, ET and GG.; methodology, DLJ-F, IG-C, CM, RF, AO, ET, J de la T, FV, EM, YC and GG; software DLJ-F, FJG-G and GG; validation DLJ-F, MG-M, MC, CR-G, JCF-C, RA and GG; formal analysis DLJ-F, ME, RF-S and GG; investigation DLJ-F and GG; resources DLJ-F, FC, JMG, MJM, ET and GG; data curation DLJ-F and GG; writing—original draft preparation DLJ-F and GG; writing—review and editing, all; visualization all; supervision GG; project administration GG; funding acquisition DLJ-F, FC, JMG, MJM, ET and GG. All authors have read and approved the final manuscript.

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Authors' information

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Figures

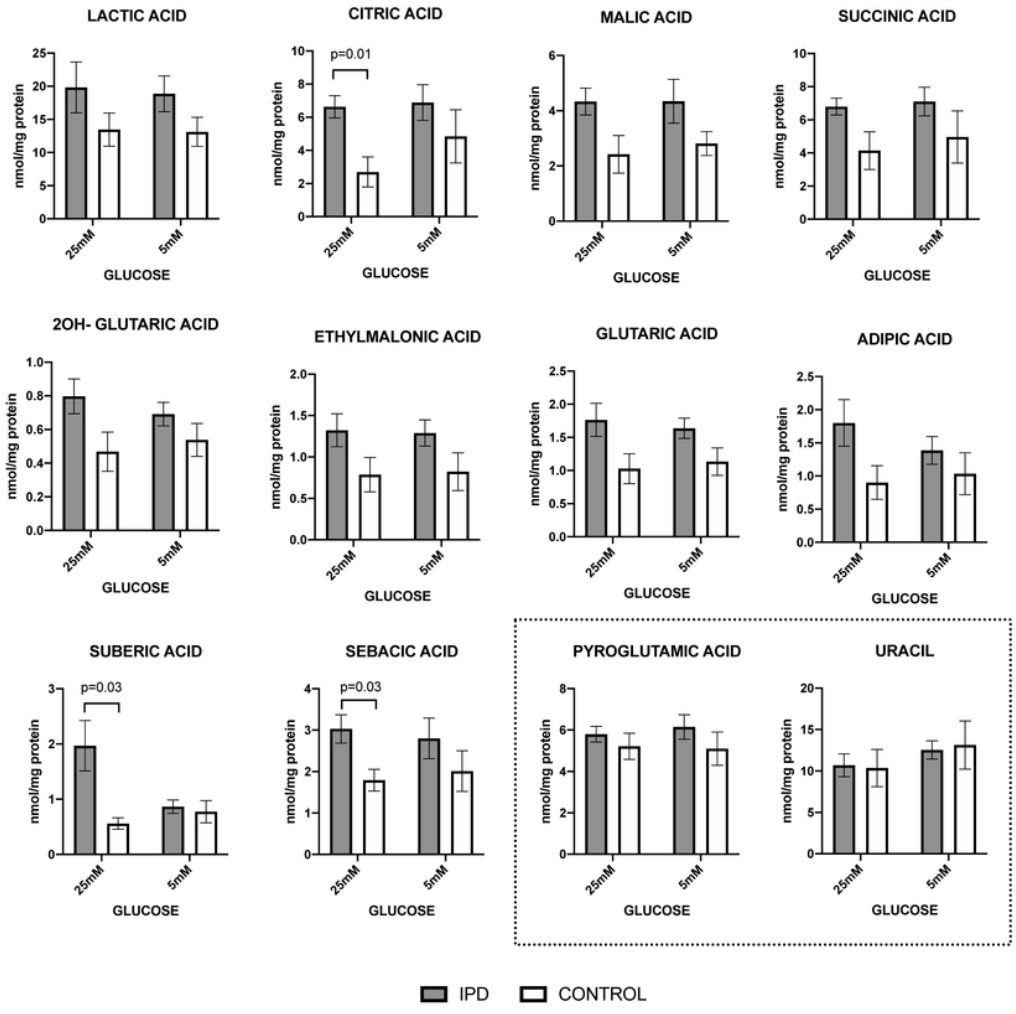

Figure 1

Levels of organic acids related to mitochondrial metabolism were increased in fibroblasts from idiopathic Parknson's disease patients (iPD) vs. controls (C), especially in high glucose exposition. At standard glucose concentration (5 mM) increases were observed in biomarkers of mitochondrial diseases as lactic acid, components of the Krebs's cycle (citric, malic, succinic and 2OH-glutaric acid), derived from Kreb's cycle related to amino acid or fatty acid metabolism (as ethylmalonic or glutaric acid) and biomarkers from free fatty acid β-oxidation (including adipic, suberic and sebacic dicarboxylic acids). High glucose concentration (25mM) accentuated such trends, as observed by the significant increase of citric, suberic and sebacic acids, suggesting worse phenotype in case of high glucose exposition. The accumulation of all these metabolites feeding mitochondrial metabolism is frequently associated with mitochondrial dysfunction and, specifically the increase in lactic

acid levels, with the activation of anaerobic glycolysis in detriment of mitochondrial function. As controls, the levels of organic acids non-related with mitochondrial energy metabolism (including uracil and pyroglutamic acid), were measured and found conserved among patients, controls and glucose conditions, suggesting that only mitochondrial-related organic acids were affected.

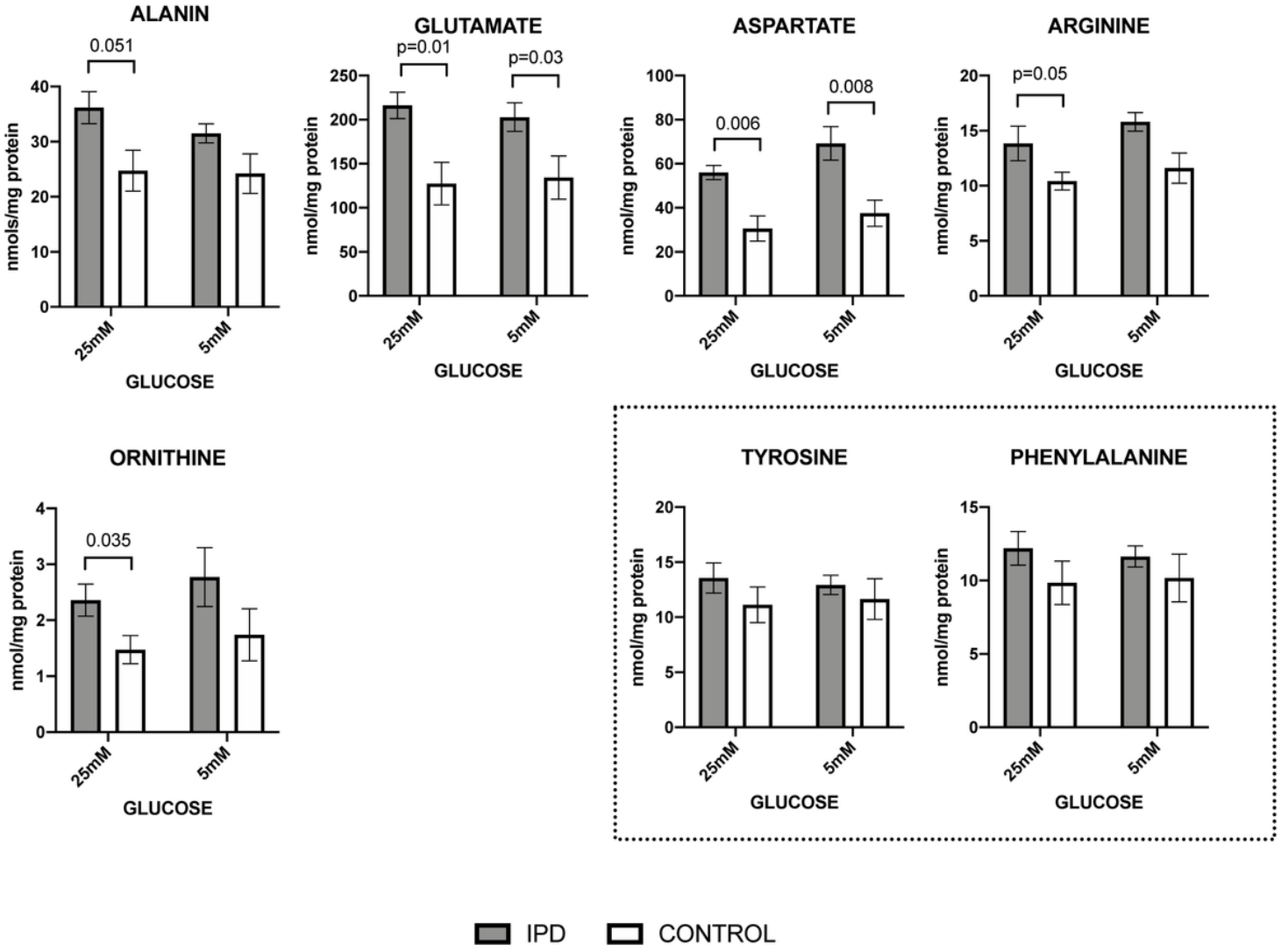

Figure 2

Levels of amino acids related to mitochondrial metabolism were increased in fibroblasts from idiopathic Parknson's disease patients (iPD) vs. controls (C), especially in high glucose exposition. At standard glucose concentration (5 mM) these increases were observed in alanine, glutamate, aspartate, arginine and ornithine. Exposure to a high glucose concentration (25mM) confirmed such trends, as observed by the significant increase of all these markers, suggesting worse phenotype in case of high glucoe exposition, as previously observed with organic acids. The accumulation of all these metabolites feeding mitochondrial metabolism is frequently associated with mitochondrial dysfunction. As controls, levels of amino acids not related to mitochondrial metabolism (such as tyrosine or phenylalanine), were measured and found conserved among iPD patients, controls and glucose concentrations, suggesting that only mitochondrial-related amino acids were affected.

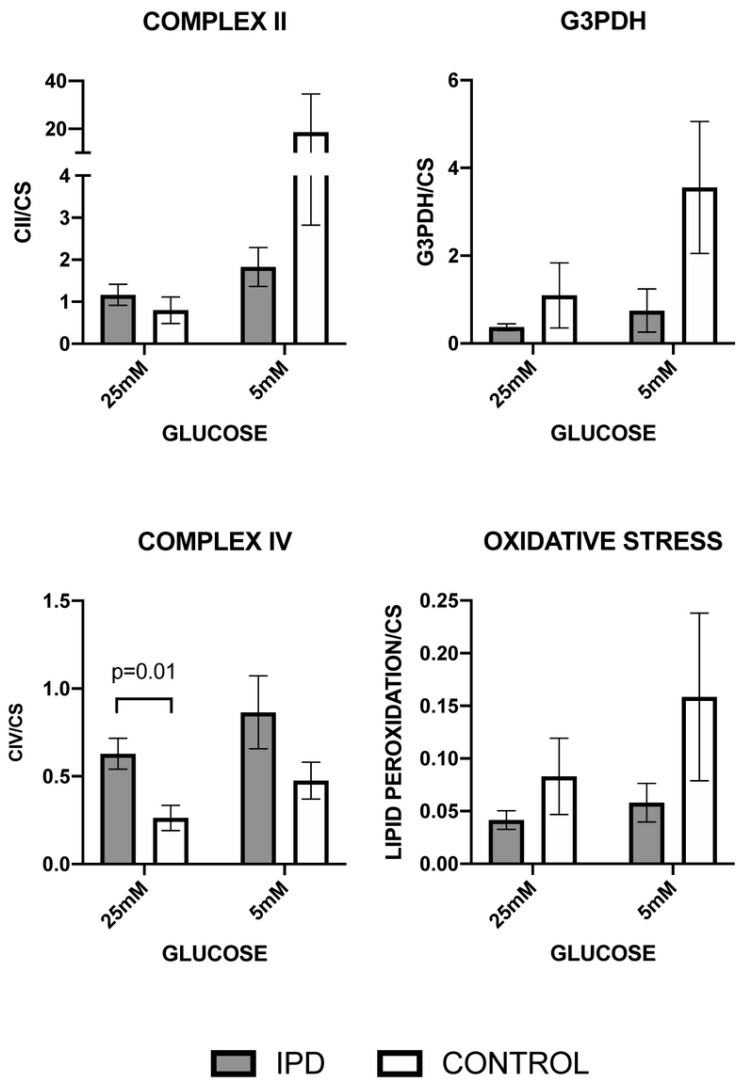

Figure 3

Mitochondrial respiratory chain (MRC) enzymatic activities were decreased in fibroblasts from idiopathic Parknson's disease patients (iPD) vs. controls (C). MRC complex II (CII) and glycerol-3-phosphate dehydrogenase (G3PDH) tended to decrease in iPD-fibroblasts probably generating the accumulation of organic acids and amino acids related to mitochondrial metabolism. Additionally, iPD cells manifested decreased metabolic plasticity than controls to adapt to changing glucose conditions. Interestingly, CIV activity trended to increase in iPD fibroblasts, significantly when exposed to HG, probably to overcome CII-G3PDH impairment. As a result of CII-G3PDH MRC reduction in iPD-fibroblasts, oxidative stress levels, as a secondary product of MRC function, trended to decrease in iPD-patients.

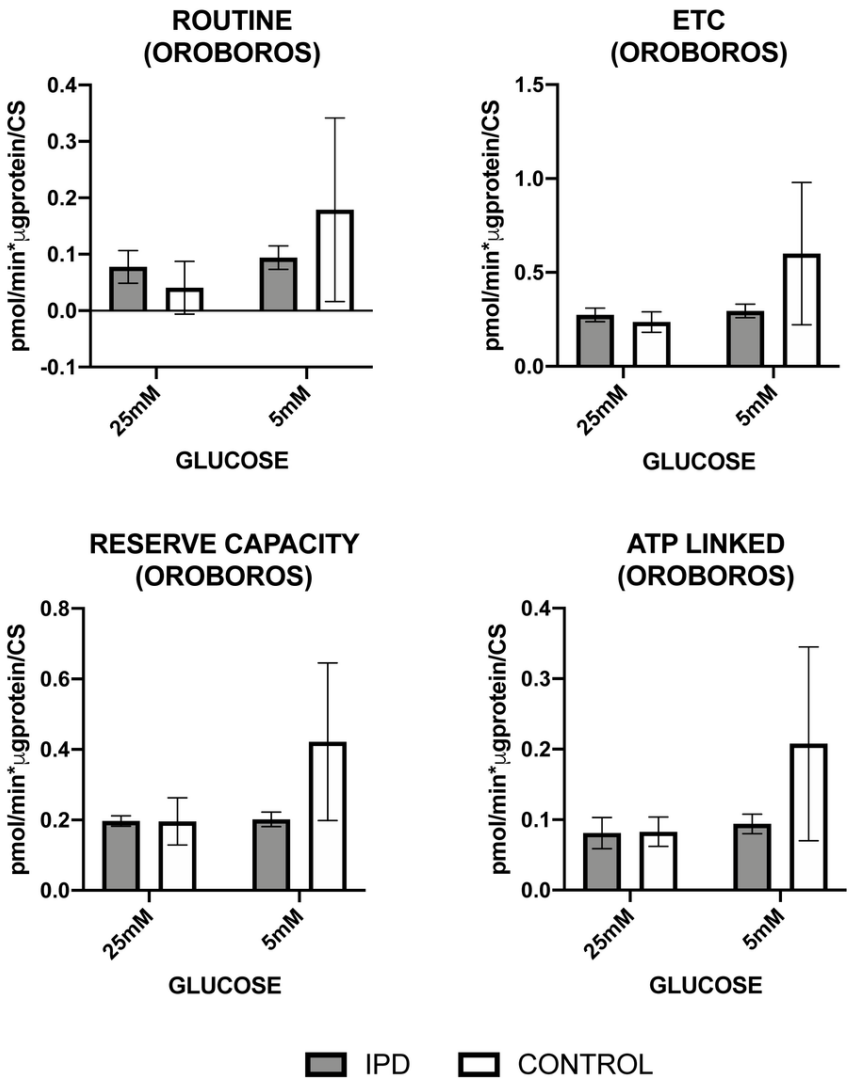

Figure 4

Mitochondrial respiration measured through the Mitostress test and OroborosTM technology confirmed decreased mitochondrial activity in fibroblasts from idiopathic Parknson's disease patients (iPD) vs. controls (C). Specifically, basal (routine) respiration, maximal respiration (after uncoupling), reserve capacity and ATP-linked respiration trended to decrease in iPD-fibroblasts, that additionally showed reduced mitochondrial plasticity respect to controls to adapt to changing glucose conditions.

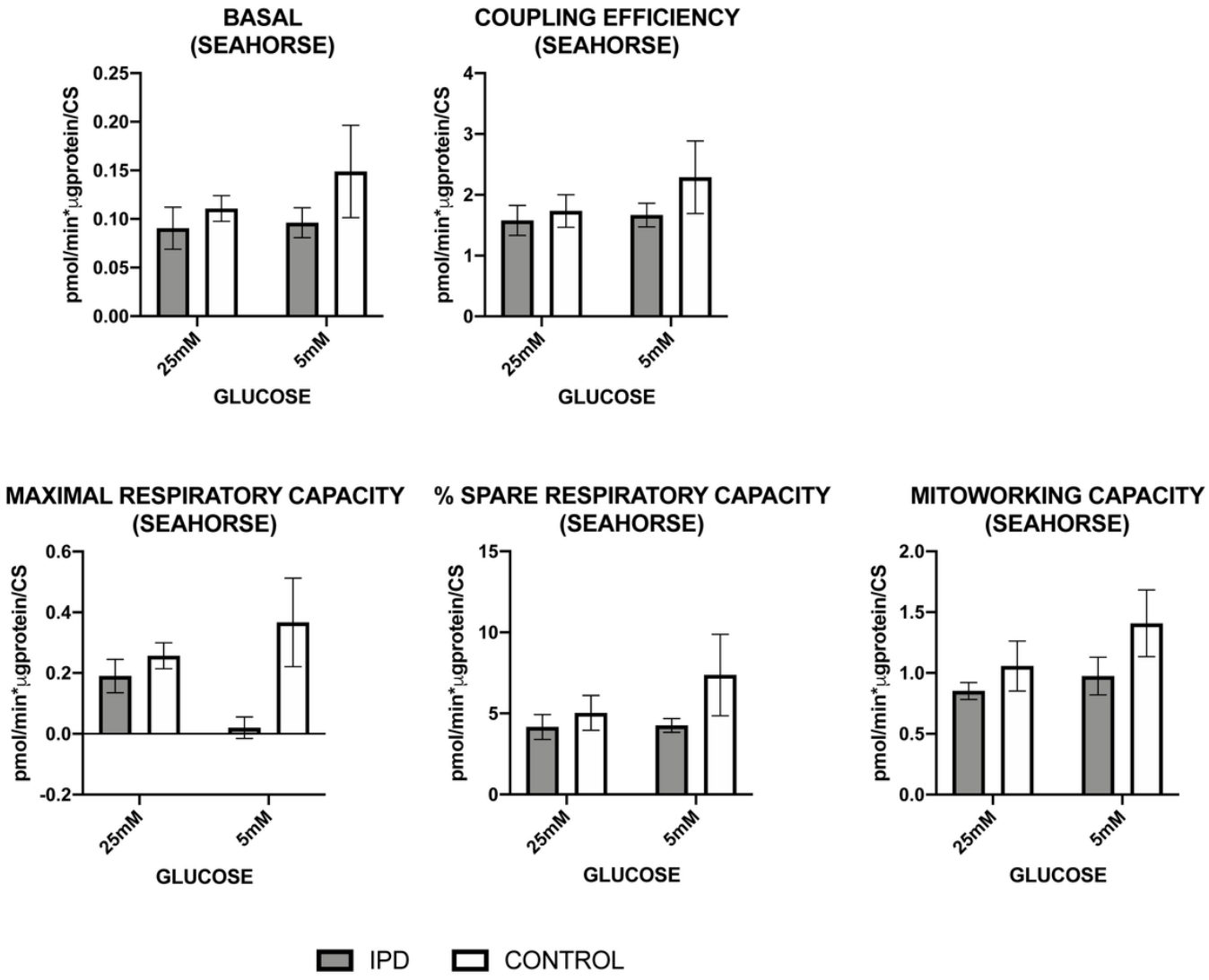

Figure 5

Mitochondrial respiration measured through the Mitostress test and SeahorseTM technology confirmed decreased mitochondrial activity in fibroblasts from idiopathic Parknson's disease patients (iPD) vs. controls (C). Specifically, basal respiration, coupling, maximal, spare and mitoworking capacities trended to decrease in iPD-fibroblasts, that additionally showed reduced mitochondrial plasticity respect to controls to adapt to changing glucose conditions.

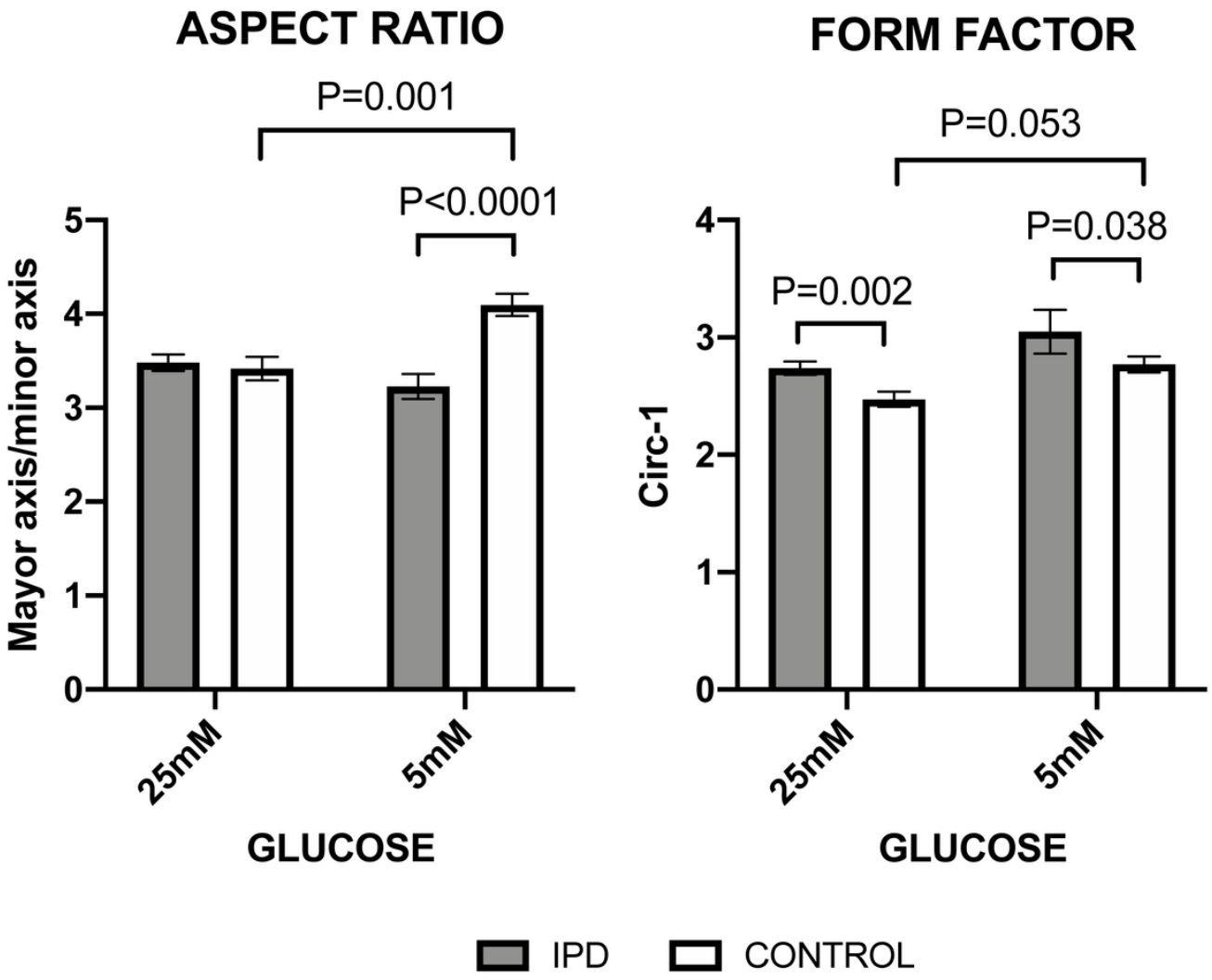

Figure 6

Mitochondrial morphology did not change in fibroblasts from idiopathic Parknson's disease patients (iPD) vs. controls (C) in accordance to glucose exposition, confirming their metabolic and morphologic rigidity. Specifically, high glucose (HG) concentration significantly decreased aspect ratio and form factor from control fibroblasts, accounting for less elongated and branched mitochondria, whilst iPD-fibroblasts were unable to adapt to HG exposition and showed conserved mitochondrial morphology, regardless the media.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx