UNIVERSITAT DE BARCELONA

Relación entre enfermedad de Parkinson y metabolismo de la glucosa: Estudio clínico de asociaciones bioquímicas y análisis epidemiológico de riesgo

Almudena Sánchez Gómez

RELACIÓN ENTRE ENFERMEDAD DE PARKINSON Y METABOLISMO DE LA GLUCOSA: ESTUDIO CLÍNICO DE ASOCIACIONES BIOQUÍMICAS Y ANÁLISIS EPIDEMIOLÓGICO DE RIESGO

Memoria de tesis doctoral presentada por **Almudena Sánchez Gómez** para optar al grado de doctora por la Universitat de Barcelona

Directores:

Yaroslau Compta Hirnyj

Mª José Martí Domènech

Filiación:

Hospital Clínic de Barcelona

PROGRAMA DE DOCTORADO DE MEDICINA E INVESTIGACIÓN TRASLACIONAL

Facultad de Medicina y Ciencias de la Salud. Universitat de Barcelona

Febrero/2022

INFORME DE LOS DIRECTORES DE LA TESIS

El Dr. *Yaroslau Compta Hirnyj*, Doctor en Medicina por la *Universitat de Barcelona*, y la Dra. *Mª José Martí i Domènech*, Doctora en Medicina y Cirugía por la *Universitat de Barcelona*.

CERTIFICAN: Que la memoria titulada *"Relación entre enfermedad de Parkinson y metabolismo de la glucosa: Estudio clínico de asociaciones bioquímicas y análisis epidemiológico de riesgo"*, presentada por Almudena Sánchez Gómez para optar al grado de *Doctora en Medicina e Investigación Traslacional* por la *Universidad de Barcelona* se ha realizado bajo su dirección y cumple todos los requisitos necesarios para ser defendida ante el tribunal correspondiente.

Dr. Yaroslau Compta Hirnyj

Dra Mª José Martí i Domènech

Barcelona, 8 de Febrero de 2022

AGRADECIMIENTOS

ÍNDICE

GLOSARIO

DM2: Diabetes mellitus tipo 2.

EP: Enfermedad de Parkinson.

MG: Metabolismo de la glucosa.

EA: Enfermedad de Alzheimer.

MDS: *Movement Disorders Society*

SNpc: Sustancia *nigra pars* compacta.

aS: α-sinucleína.

RI: Resistencia a la insulina.

IMC: Índice de masa corporal.

HbA1c: Hemoglobina glicada.

LCR: Líquido cefalorraquídeo.

EDI: enzima degradante de la insulina.

HOMA-IR: modelo homeostático de evaluación de la resistencia a la insulina.

ARNm: Ácido ribonucleico mensajero.

OR: *Odds ratio*.

IC: Intervalo de confianza.

HR: *Hazard ratio.*

RR: Riesgo relativo

UPDRS: *Unified Parkinson's Disease Rating Scale*

GLP-1: *Glucagon like-peptide 1*

PRESENTACIÓN

Esta Tesis Doctoral se presenta en formato de compendio de artículos que corresponden a la misma unidad temática: la relación entre alteraciones del metabolismo de la glucosa y la enfermedad de Parkinson, tanto de forma molecular como epidemiológica.

En esta tesis se plantean tres objetivos principales que se desarrollan en dos artículos científicos originales. El primer y el segundo objetivo de la tesis son determinar la presencia de alteraciones sanguíneas del metabolismo de la glucosa en la enfermedad de Parkinson y establecer la relación de éstas con los síntomas motores y no-motores de la enfermedad. Los dos primeros objetivos se abordan en el artículo 1: *Peripheral insulin and amylin levels in Parkinson's disease.*

Artículo 1: **Sánchez-Gómez A**, Alcarraz-Vizán G, Fernández M, Fernández-Santiago R, Ezquerra M, Cámara A, Serrano M, Novials A, Muñoz E, Valldeoriola F, Compta Y, Martí MJ. Peripheral insulin and amylin levels in Parkinson's disease. Parkinsonism Relat Disord. 2020 Aug 25;79:91-96. JCR Factor de Impacto 2020: 4.891. JCR Clasificación en la categoría de "Clinical Neurology": 51/208 (Q1).

El tercer objetivo de la tesis es determinar el riesgo relativo de desarrollo de enfermedad de Parkinson en relación a la presencia o ausencia de diabetes mellitus tipo 2 y/o prediabetes. El tercer objetivo se aborda en el artículo 2: *Prediabetes, type 2 diabetes mellitus and risk of Parkinson's disease: A population-based cohort study.*

Artículo 2: **Sánchez-Gómez A**, Díaz Y, Duarte-Salles T, Compta Y, Martí MJ. Prediabetes, type 2 diabetes mellitus and risk of Parkinson's disease: A population-based cohort study. Parkinsonism Relat Disord. 2021 Jun 8;89:22-27. JCR Factor de Impacto 2020: 4.891. JCR Clasificación en la categoría de "Clinical Neurology": 51/208 (Q1).

RESUMEN

Se considera que puede existir un vínculo entre la diabetes mellitus tipo 2 (DM2) y la enfermedad de Parkinson (EP) sustentado en la presencia de vías fisiopatológicas comunes en ambas entidades.

No obstante, los estudios de las alteraciones del metabolismo de la glucosa (MG) no han sido concluyentes en cuanto a la presencia de niveles de glucemia más elevados en la EP, así como en la asociación entre mayor resistencia a la insulina y mayor afectación motora y cognitiva. Diversos estudios epidemiológicos retrospectivos de cohortes realizados los últimos años, han sugerido que la presencia de DM2 conlleva un mayor riesgo de desarrollo de EP. Sin embargo, los estudios epidemiológicos de casos y controles han sido inconsistentes en cuanto a esta asociación.

Esta tesis doctoral pretende profundizar en el conocimiento tanto de las alteraciones del MG en los pacientes con EP y su relación con la afectación motora y no-motora de la enfermedad, como en el papel de la DM2 y prediabetes como factores de riesgo para la EP.

Nos hemos planteado las siguientes hipótesis: 1) los pacientes con EP presentan más alteraciones del MG que los sujetos controles sanos, 2) las alteraciones del MG se asocian a mayor afectación motora y no-motora (especialmente cognitiva) en los pacientes con EP y 3) la presencia no sólo de DM2 sino también de prediabetes comporta un mayor riesgo de desarrollo de la EP.

En consecuencia, los principales objetivos de esta tesis son: 1) determinar la presencia de alteraciones en sangre del MG en la EP, 2) establecer la relación de éstas con síntomas motores y no-motores y 3) estimar el riesgo relativo de desarrollo de EP en relación a la presencia o ausencia no sólo de DM2, sino también de prediabetes.

El primer y el segundo objetivo se han abordado en un estudio de una cohorte de pacientes con EP y un grupo control evaluados mediante escalas motoras, no-motoras y cognitivas y la determinación de niveles en

sangre y en condiciones de ayuno, de glucemia, insulina, hemoglobina glicada y amilina (proteína de depósito amiloide pancreática en la DM2). Al comparar a ambos grupos se han observado niveles significativamente más bajos de insulina en los pacientes con EP que en los controles, siendo los niveles de amilina significativamente más elevados en los sujetos con EP vs. el grupo control al limitar el análisis al subgrupo con edad superior a la mediana de la cohorte. Por otra parte, la ratio amilina/insulina también ha sido significativamente más alta en los pacientes con EP vs. sujetos control. Por último, se ha observado una modesta pero significativa correlación entre mayor resistencia a la insulina y mayor puntuación en la escala de síntomas no motores de la EP.

El tercer objetivo se ha evaluado en un estudio epidemiológico retrospectivo de cohorte en sujetos con DM2 y prediabetes. Al compararlos con la población control se ha observado un mayor riesgo de desarrollo posterior de EP no sólo en DM2 sino también en prediabetes, con predominio de esta asociación entre mujeres y personas menores de 65 años.

Estos estudios profundizan y aportan nuevos datos de las diferentes alteraciones del MG en la EP, así como del riesgo que la DM2 comporta en su desarrollo.

ABSTRACT

A link between type 2 diabetes mellitus (T2D) and Parkinson's disease (PD) is considered based on the presence of common pathophysiological pathways in both conditions.

However, to date studies on impaired glucose metabolism (GM) in PD have been inconclusive, both in terms of higher glucose blood levels in PD patients vs. controls, and of the association of the insulin resistance with greater motor and cognitive impairment. Moreover, a series of retrospective epidemiological cohort studies published in the last years have observed that T2D carries an increased risk of subsequent PD. Nevertheless, epidemiological case-control studies have yielded contradictory results.

This thesis aims to take further our knowledge of both GM alterations in PD patients and their relationship with motor and non-motor involvement of the disease, as well as the role of T2D as a risk factor for PD.

We hypothesized that 1) PD patients present more GM alterations than healthy control subjects, 2) GM alterations are associated with greater motor and non-motor (especially cognitive) involvement in PD patients and 3) the presence not only of T2D but also prediabetes implies a higher risk of PD development.

Accordingly, the main objectives of this thesis are: First, to determine the presence of blood disorders of GM in PD and, second, to establish the relationship of these with the motor and non-motor symptoms of the disease; third and final, to determine the relative risk of developing PD associated not only with T2D, but also with prediabetes.

The first and second objectives were addressed in the first study. A cohort of PD patients and a control group with motor, non-motor and cognitive scales were evaluated, and a fasting blood sample was performed to estimate glycemia, insulin, glycated hemoglobin and amylin (the T2D amyloid deposit protein in pancreas). Insulin levels were significantly lower in PD vs. controls, whereas amylin levels were significantly higher in

PD in analysis stratified for age greater than the cohort median. The amylin to insulin ratio was also significantly higher in PD vs. controls. Finally, greater insulin resistance modestly but significantly correlated with the scores of the non-motor symptoms in PD.

The third objective was evaluated in the second study. In this, a retrospective epidemiological cohort study of patients with T2D and another subgroup with prediabetes was carried out and compared with a control group to determine the relative risks of both metabolic conditions in the subsequent PD development. We observed a greater hazard of subsequent PD not only in T2D but also in prediabetes, with this association predominating in women and people under 65 years of age.

These studies delve into and provide new data on the different alterations of GM in PD as well as the risk that T2D carries in its development.

I. INTRODUCCIÓN

1.1 ENFERMEDAD DE PARKINSON: EPIDEMIOLOGÍA, DEFINICIÓN Y DIAGNÓSTICO, NEUROPATOLOGÍA Y FACTORES DE RIESGO ASOCIADOS.

La enfermedad de Parkinson (EP) es la segunda enfermedad neurodegenerativa más frecuente, únicamente precedida por la enfermedad de Alzheimer (EA), y es a su vez la enfermedad neurológica que mayor crecimiento ha experimentado en cuanto a prevalencia, discapacidad y fallecimientos [1,2]. En España y Europa se estima una prevalencia de entre 1.5-2% en mayores de 65 años y hasta un 3% en mayores de 80 años. Además, la incidencia en España se calculó de hasta 187 casos por cada 100.000 personas-año entre 65 y 85 años [3]. Se espera que duplique su prevalencia en el año 2030 respecto al 2010, debido fundamentalmente al envejecimiento de la población y al aumento en la esperanza de vida [4].

La EP es una enfermedad neurodegenerativa que produce un deterioro funcional progresivo y que se caracteriza clínicamente por la presencia de parkinsonismo. Además de los síntomas motores clásicos, la EP también puede presentar un amplio conjunto de síntomas no-motores. Algunos pueden llegar a preceder el diagnóstico de la enfermedad incluso en más de 15-20 años [5,6], y por lo tanto, se consideran síntomas prodrómicos de la EP [6,7].

El diagnóstico de la EP continúa siendo clínico debido a la ausencia de biomarcadores. Los últimos criterios diagnósticos consensuados por la *Movement Disorders Society* (MDS) en 2015 [8], establecen el diagnóstico en base a la presencia de parkinsonismo (definido por bradicinesia y su combinación con temblor de reposo y/o rigidez) e incluyen criterios de soporte tanto clínicos como paraclínicos. Además, se describen signos de alarma y criterios de exclusión para el diagnóstico.

La característica neuropatológica principal de la EP es la degeneración en núcleos del tronco cerebral, destacando la pérdida de neuronas dopaminérgicas en la sustancia *nigra pars* compacta (SNpc). También es una característica cardinal de la EP idiopática la presencia de depósitos de

α-sinucleína (aS). Alteraciones en el pliegue de esta proteína le confiere insolubilidad, facilitando su acúmulo, siendo el principal componente de los cuerpos de Lewy [9]. La patología Lewy no sólo se encuentra a nivel cerebral, sino también en el sistema nervioso periférico, como el plexo cardíaco y la inervación de las glándulas salivales entre otros [10,11].

Se han identificado múltiples factores asociados a mayor riesgo (edad e historia familiar de EP, entre otros) y otros relacionados con menor incidencia (como son por ejemplo el consumo de tabaco, alcohol y café) de desarrollo ulterior de EP [6, 12]. Existen, además, diversos factores de riesgo genéticos asociados al desarrollo de la EP [13-15].

Por lo tanto, se consideran como base de la patogenia de la EP la interacción de los factores ambientales y genéticos. La importancia de establecer y conocer todos estos factores implicados radica en la identificación temprana de los sujetos que puedan estar en riesgo de desarrollar la enfermedad, y que se encuentren por lo tanto en fases prodrómicas y/o premotoras de la EP [7, 16-18] en vista a posibles tratamientos modificadores del curso de la enfermedad.

En este sentido, otro de los factores de riesgo más estudiado en los últimos años por su posible relación con la EP es la diabetes mellitus tipo 2 (DM2) [19, 20].

1.2 ASOCIACIONES BIOQUÍMICAS ENTRE LA ENFERMEDAD DE PARKINSON Y LA DIABETES MELLITUS TIPO 2.

Diferentes mecanismos fisiopatológicos se han relacionado tanto a la EP como a la DM2. Entre ellos, destacan el depósito de proteínas amiloides, la resistencia a la insulina (RI), la disfunción mitocondrial, la inflamación crónica y el estrés oxidativo. El conocimiento de estas vías compartidas ha generado un incremento importante en el estudio de la relación epidemiológica y fisiopatológica de la EP y la DM2 en los últimos años.

1.2.1 DM2, EP Y LA HIPÓTESIS AMILOIDE.

La DM2 es una enfermedad poligénica y con una importante agregación familiar que ocurre por la disfunción de las células β pancreáticas. Esta alteración funcional pancreática comporta una deficiencia relativa en la secreción de la insulina y una resistencia a ésta en los órganos diana. Se considera que inicialmente se produce la RI y, como mecanismo compensatorio, se produce un estado de hiperinsulinemia para contrarrestar la situación de RI en los tejidos periféricos. La situación de diabetes (hiperglucemia) se manifiesta cuando la hiperinsulinemia compensadora es insuficiente, apareciendo entonces el déficit relativo de insulina [21]. Los factores de riesgo más destacados para su desarrollo son la edad, la obesidad y el sedentarismo, aunque también se han descrito otros como el tabaco [22].

Por otra parte, la prediabetes es un factor de riesgo conocido para el desarrollo de la DM2 [23]. Tanto la prediabetes como la DM2 se asocian con un aumento del índice de masa corporal (IMC), con la RI y con la disfunción de las células ß pancreáticas y su consiguiente disminución de la secreción de insulina [24]. La prediabetes está definida como el estado glucémico en el que los niveles de glucosa en sangre presentan niveles anormalmente elevados pero que aún no cumplen los criterios diagnósticos de diabetes.

El diagnóstico de la prediabetes se realiza mediante la presencia de unos valores de glucemia plasmática en ayunas entre 100 y 125 mg/dl, o bien con una hemoglobina glicada (HbA1c) entre 5.7% y 6.4% [25]. En el caso de la diabetes el diagnóstico es mediante la medición de la glucemia plasmática en ayunas $\geq$ 126mg/dL, $\geq$ 200mg/dL en cualquier momento y/o HbA1c $\geq$ 6.5%. También se pueden diagnosticar tanto la diabetes como la prediabetes mediante el test de tolerancia oral a la glucosa, pero su uso está mucho menos extendido en la práctica clínica habitual.

En 2019 se estimó que 463 millones de personas tenían diabetes (59 millones en Europa), siendo el 90% aproximadamente DM2. La estimación de la prevalencia de la diabetes es de 1 de cada 11 adultos entre 20-79 años, lo que comporta un 9% de población diabética aproximadamente. En mayores de 65 años esta estimación es de hasta 1 de cada 5 adultos, siendo un 20% de prevalencia en esta franja de edad. Se calcula que en el año 2045 puedan llegar a ser 700 millones de diabéticos [26].

En sujetos con DM2 se produce un depósito de amiloide en el páncreas, principalmente debido a la agregación de amilina o polipéptido amiloide de los islotes-IAPP (por sus siglas en inglés) [27,28]. La amilina es una hormona peptídica de 37 residuos co-secretada con la insulina en las células β pancreáticas. Ambas hormonas comparten funciones en la regulación de los niveles de glucosa en sangre. La amilina se ha implicado en diversas funciones, entre ellas la inhibición de la secreción de insulina [29, 30]. Se cree que los agregados de amilina conducen a una disfunción de las células β y promueven la progresión de la diabetes [27,28].

Se ha relacionado a la DM2 con diversas enfermedades neurodegenerativas [31].

En primer lugar, diferentes estudios han demostrado una asociación entre la amilina y la EA. Un estudio experimental con animales ha observado que la amilina puede desempeñar un papel en la eliminación de los péptidos β-amiloides en cerebros con EA [32]. Por otra parte, hay evidencia patológica de depósito de amilina agregada en el cerebro de pacientes con EA [31]. Además, se ha observado una interacción entre la amilina y las proteínas tau y β-amiloide tanto en el hipocampo como en

las células β pancreáticas [33]. En un estudio longitudinal, una concentración elevada de amilina plasmática se asoció con el riesgo de EA [34]. Sin embargo, los niveles de amilina en el líquido cefalorraquídeo (LCR) y en plasma no han diferido entre la EA y los controles [35].

En relación a la EP, como hemos comentado previamente, una de las características patológicas más destacadas además de la neurodegeneración, es la presencia de los cuerpos y las neuritas de Lewy, compuestos por agregados amiloides de aS mal plegada entre otras proteínas [36].

En cuanto al vínculo de la amilina con la EP, un estudio *in vitro* ha demostrado que la presencia de amilina acelera la formación de amiloide de aS, pero no a la inversa, lo que sugiere un vínculo unidireccional con una influencia de la DM2 sobre la EP [27]. Además, un estudio patológico encontró depósitos de aS fosforilada en células β pancreáticas de sujetos con un diagnóstico neuropatológico de α-sinucleinopatía, y también reveló una interacción directa entre la amilina y la aS [37].

Un aspecto no explorado hasta la fecha son los niveles de amilina plasmática en la EP.

Por otra parte, se ha demostrado que la enzima degradante de la insulina (EDI), que es una endopeptidasa que degrada la insulina y las proteínas amiloidogénicas en el páncreas y que a su vez está regulada por la propia insulina, interactúa con el ácido C-terminal de la aS [38]. Esta interacción, *in vitro*, se ha asociado a la inhibición de la aS, previniendo la formación de fibrillas de amiloide de aS [39].

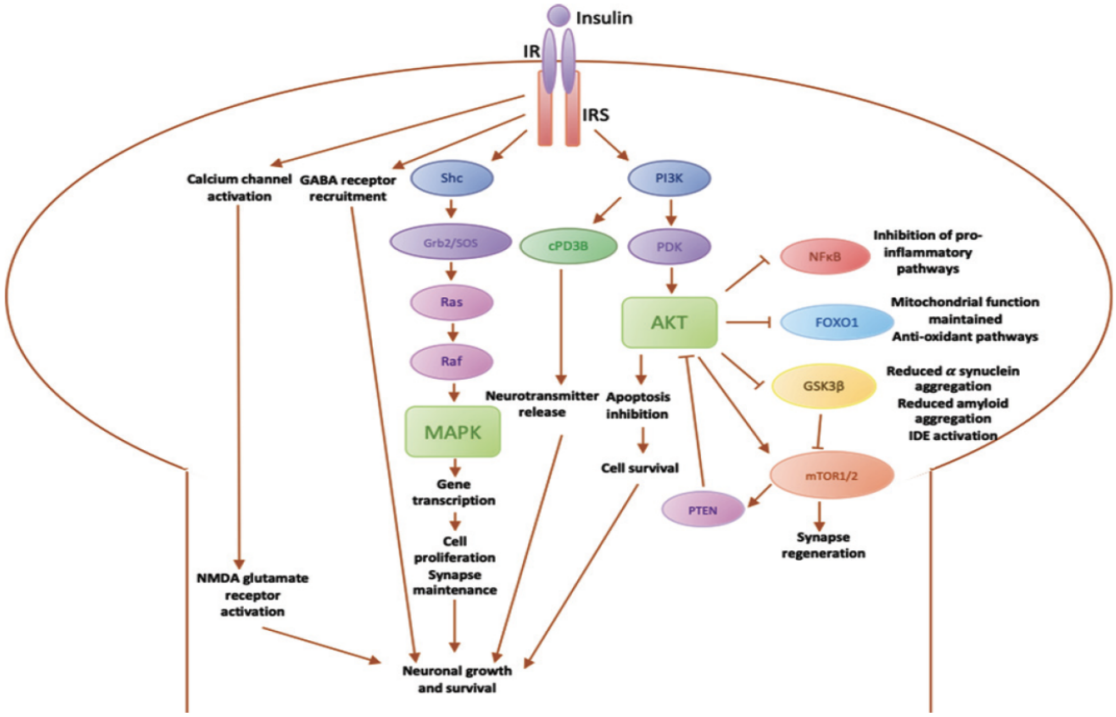
1.2.2 INSULINA: VÍAS DE SEÑALIZACIÓN A NIVEL CEREBRAL Y RESISTENCIA A LA INSULINA EN LA EP.

La insulina es una hormona de secreción principalmente periférica que regula la homeostasis de la glucosa estimulando su captación en el tejido muscular y adiposo. Además, inhibe la producción hepática de glucosa. Las funciones de la insulina a nivel cerebral son diversas, presentando un efecto homeostático al mantener las funciones fisiológicas cerebrales.

La mayor parte de insulina a nivel cerebral proviene de su producción en las células β pancreáticas, atravesando la barrera hematoencefálica [40, 41]. No obstante, también hay producción de insulina a nivel cerebral, principalmente en neuronas piramidales del córtex, hipocampo y bulbo olfatorio [42].

Existen múltiples vías implicadas en la señalización de la insulina en el cerebro. Debido al importante papel que estas desempeñan en la neuroprotección, las describimos brevemente a continuación y con el soporte esquematizado de la **figura 1** (figura de J.L.Y. Cheong et al. / *The association Between T2DM and PD* [43]). En esta figura se representan las principales vías de señalización de la insulina a nivel cerebral, como son la vía MAPK, involucrada en el mantenimiento y crecimiento celular y la plasticidad sináptica; la vía de mTOR que contribuye a la regulación del metabolismo celular y la regeneración sináptica en las neuronas; la vía PI3K/Akt, implicada en la inhibición de la apoptosis, que además da como resultado varios efectos posteriores, incluida la inactivación de la vía GSK3β, que se encuentra involucrada en la inactivación del EDI conduciendo a un aumento en la expresión de la aS y su agregación en fibras amiloides; así como otras vías como NFkB y FOXO1 que implican la inhibición de vías pro-inflamatorias y de mantenimiento en la función mitocondrial e implicaciones antioxidantes respectivamente.

FIGURA 1. VÍAS IMPLICADAS EN LA SEÑALIZACIÓN DE LA INSULINA A NIVEL CEREBRAL. *FIGURA DE J.L.Y CHEING ET AL 2020. THE ASSOCIATION BETWEEN TYPE 2 DIABETES MELLITUS AND PARKINSON'S DISEASE. 2020 [43].*



Por otra parte, la RI sistémica y cerebral es la respuesta alterada (disminuida) a la insulina en las células de los tejidos periféricos o en el cerebro respectivamente [41].

La RI periférica se puede medir mediante la ecuación del modelo homeostático de evaluación de la RI (HOMA-IR, por sus siglas en inglés) [44]. El cálculo se realiza con la división entre glucemia multiplicado por insulinemia y dividido por un factor de conversión (405 en el caso de presentar las unidades de glucemia en mg/dL y la insulinemia en mU internacionales por litro); glucosa x insulina / 405.

Debido a que la mayor parte de la insulina cerebral proviene de la producción pancreática, la RI periférica se asocia a la presencia de RI central [45]. La RI central por su parte, puede ser una causa o una consecuencia de la neurodegeneración [41].

En estudios previos al uso generalizado de los criterios diagnósticos estrictos de la EP, se llegó a estimar que entre un 50-80% de pacientes con

EP presentaban una intolerancia oral a la glucosa [46]. Estudios más recientes objetivaron como la RI periférica medida por HOMA-IR se encontraba alterada en sujetos con EP, pero con una correlación significativa con el sobrepeso y la obesidad. A pesar de ello, se estimó una prevalencia de intolerancia oral a la glucosa en pacientes con EP del 58.4% [47].

Mayores niveles de HOMA-IR se observaron en sujetos tanto con EP como con EA respecto a sujetos controles y éstos se correlacionaron con menores niveles de volumen de sustancia gris en la EA, pero no en la EP [48].

El envejecimiento se asocia a una disminución de la sensibilidad de los receptores de la insulina a nivel periférico y a menores niveles de ácido ribonucleico mensajero (ARNm) de los receptores de insulina en diversas áreas cerebrales [40, 47, 49]. En esta línea, se ha objetivado una pérdida de ARNm del receptor de la insulina en la SNpc de pacientes con EP en comparación a controles sanos apareados por edad [50].

También se ha observado una menor expresión de la insulina y de IGF-1 (*insulin like growth factor-1*) en la sustancia blanca frontal y en la amígdala de sujetos con EP y demencia por cuerpos de Lewy post-mortem. Estos hallazgos se asociaron, a su vez, con mayores niveles de aS y ubiquitina, entre otros [51].

Un estudio patológico recientemente publicado ha aportado nueva evidencia sobre la RI central en la EP [52]. Los autores han observado mayor intensidad por inmunofluorescencia de sustrato receptor de insulina tipo 1 fosforilado en los residuos de serina (marcador de RI) en las neuronas dopaminérgicas en la SNpc de sujetos con EP. Además, se ha observado una importante co-localización en los cuerpos de Lewy.

1.2.3 NIVELES PLASMÁTICOS DEL METABOLISMO DE LA GLUCOSA EN LA EP.

Los trabajos que han analizado distintas moléculas relacionadas con el metabolismo de la glucosa han evaluado tanto las posibles diferencias en los niveles de estas moléculas entre sujetos con EP vs. controles sanos como su correlación con la afectación motora y no-motora en la enfermedad.

En primer lugar, los niveles plasmáticos de glucosa han sido evaluados directa e indirectamente en algunos trabajos, obteniéndose resultados heterogéneos.

Un trabajo valoró sujetos con EP (con y sin deterioro cognitivo leve) y sin tratamiento dopaminérgico. Al compararlos con el grupo control se objetivaron niveles de glucemia en ayunas significativamente más elevados en los pacientes con EP. De hecho, tanto el grupo completo de EP como principalmente el subgrupo de EP con deterioro cognitivo, presentaron niveles >100mg/dL [53], definitorios de estado prediabético según los criterios diagnósticos de la asociación americana de diabetes [25].

Por otro lado, un estudio longitudinal de sujetos con EP de reciente diagnóstico no observó diferencias en los niveles de glucosa o HbA1c entre el grupo de sujetos con EP y un grupo de controles sanos a los cuatro años del seguimiento. Los pacientes con EP tampoco presentaron una diferencia en la incidencia de DM2 ni en el IMC. No obstante, una vez excluidos aquellos pacientes con DM2 de ambas cohortes, los niveles de glucemia en ayunas resultaron predictores a los cuatro años de deterioro cognitivo asociado a la EP, pero no de progresión motora [54].

Recientemente se han evaluado los niveles de glucosa plasmáticos en ayunas en una gran cohorte de sujetos con EP. El subgrupo de pacientes con EP menores de 65 años con mayor variabilidad de los niveles de glucosa plasmática en ayunas, mostraba un riesgo progresivamente creciente de presentar EP cuanto mayor era esta variabilidad. Los autores sugirieren que dicha variabilidad glucémica podía ser un factor de riesgo

para el desarrollo posterior de EP en sujetos más jóvenes (menores de 65 años). Estos resultados no se reprodujeron en los sujetos mayores de 65 años [55].

Otro estudio analizó la cinética de los niveles de glucosa y de insulina en sangre durante un test de tolerancia oral a la glucosa en sujetos con EP y controles apareados por edad, sexo e IMC [56]. Los niveles basales en sangre tanto de glucosa como de insulina no presentaron diferencias entre los grupos. Sin embargo, observaron que los niveles de glucosa a los 90 y 150 minutos eran significativamente mayores en el grupo de los sujetos con EP respecto a los controles. De la misma forma, fue significativamente mayor en los sujetos con EP el área total bajo la curva para las concentraciones de glucosa durante el tiempo analizado (180 minutos). En cambio, los sujetos con EP no presentaron el aumento consecuentemente esperado de los niveles de insulina durante el test de tolerancia a la glucosa. No se detectaron diferencias en los niveles de insulina en los diferentes tiempos analizados ni en su área total bajo la curva. Por este motivo, los autores concluyeron que las alteraciones que observaron en los niveles de glucosa podrían estar relacionadas con un déficit en la secreción de la insulina.

Diversos trabajos han evaluado la insulina y la resistencia a la misma en pacientes con EP. Como hemos comentado previamente, un estudio determinó el índice de RI a través del cálculo del HOMA-IR, hallándolo alterado en sujetos con EP. Pese a que en este trabajo la RI estaba relacionada a la obesidad y al sobrepeso, el porcentaje de RI en pacientes con EP e IMC normal también fue muy elevado (41%) [47].

En un estudio exploratorio con pacientes con EP *de novo* y sin tratamiento dopaminérgico y controles emparejados por edad, sexo, grasa y masa corporal magra, se evaluó la eliminación de glucosa corporal tras la estimulación con insulina [57]. Este método es uno de los más específicos para la determinación de la RI. Pese a detectar un índice de resistencia hepática a la insulina discretamente mayor en los sujetos con EP, no se obtuvieron diferencias respecto a la eliminación de glucosa corporal entre el grupo de pacientes con EP y los sujetos control.

Estos resultados tan heterogéneos ponen de manifiesto que aún queda por avanzar en el conocimiento de la asociación bioquímica entre ambas enfermedades.

1.2.4 OTROS MECANISMOS IMPLICADOS

1.2.4.1 Inflamación crónica y activación de la microglía

La inflamación es otro de los mecanismos que se ha asociado tanto a la DM2 como a la EP. En lo relativo a la EP, la activación de la microglía, mediante la liberación de citoquinas proinflamatorias, es uno de los mecanismos relacionados con la neuroinflamación [43, 58]. Un estudio preliminar con imagen PET (tomografía por emisión de positrones) con trazador [11C]-PK11195, un ligando periférico del receptor de benzodiacepinas, observó que los sujetos con EP presentaban mayores potenciales de unión para el marcador de activación de la microglía tanto en el putamen como en el mesencéfalo respecto a los controles. Además, los niveles de captación fueron también mayores en los sujetos con una EP más avanzada respecto a los sujetos con EP de más reciente diagnóstico. Por ello, se sugirió el papel de la neuroinflamación crónica como un mecanismo que podría incrementar el proceso neurodegenerativo [58].

Por otra parte, un estudio limitado a profesionales sanitarios de sexo masculino de Estados Unidos recogió muestras sanguíneas durante una década. Posteriormente, se analizó a los 84 sujetos que desarrollaron la EP. Se realizó un estudio comparativo con sujetos controles sin el desarrollo de la enfermedad. En él, se analizaron diferentes biomarcadores plasmáticos de inflamación como la proteína C reactiva (PCR), IL-6, receptor del TNF-α tipo 1 y 2, y fibrinógeno. Se observó cómo la IL-6, citoquina proinflamatoria expresada en neuronas, astrocitos y microglía, se asociaba con mayor riesgo de desarrollo de EP, persistiendo dicha asociación después de ajustar por potenciales factores de confusión [59].

1.2.4.2 Estrés oxidativo y disfunción mitocondrial

Se considera la disfunción mitocondrial como un punto común entre la EP y la DM2. Interviene en la RI por una reducción de la expresión de NRF1 y PGC1, reguladores importantes de las enzimas implicadas en la respiración mitocondrial. Por su parte, en la EP se ha objetivado una deficiencia del complejo I (enzima también implicado en la cadena respiratoria mitocondrial) en la sustancia *nigra* de pacientes la enfermedad [60,61].

1.3 RELACIÓN EPIDEMIOLÓGICA ENTRE LA DM2 Y LA EP.

En las últimas décadas, varios estudios han evaluado si la DM2 podría ser o no factor de riesgo para el posterior desarrollo de la EP. Estos estudios tienen diferencias importantes en cuanto a su diseño y metodología que deben tenerse en cuenta a la hora de analizarlos e interpretarlos.

1.3.1 ESTUDIOS DE CASOS Y CONTROLES

Se han realizado diversos estudios de casos y controles para evaluar la frecuencia relativa de la diabetes en la EP. Uno de los más destacables debido al número de sujetos con EP incluidos (*n*=1.931) es el de Schernhammer et al. [62]. En él se analizó la población de Dinamarca entre 2001 y 2006. Se consideró a la diabetes de forma genérica, incluyendo tanto la DM2 como la diabetes mellitus tipo 1 y otras formas de diabetes. En este estudio, el diagnóstico de diabetes se asoció a un mayor riesgo de EP reflejado en una odds ratio (OR) de 1.36 [95% intervalo de confianza (IC) 1.08–1.71], es decir, una frecuencia relativa (odds) entorno a un 36% mayor de EP entre diabéticos que no diabéticos.

Otros estudios de casos y controles no sólo no han objetivado esta asociación, sino que, al contrario, han observado una menor prevalencia de diabetes en la EP respecto a los controles. Un ejemplo es el estudio de D'Amelio et al. [63], donde reclutaron 318 sujetos con EP y los compararon con 318 controles apareados por edad y sexo. La presencia de diabetes previa a la EP se evaluó mediante cuestionarios y por revisión de la historia médica. Se observó una menor prevalencia de diabetes precediendo al diagnóstico de la EP.

Con el objetivo de dilucidar esta posible asociación (positiva o negativa) cuestionada hasta el momento, un meta-análisis realizado en 2014 por Lu et al. [64], englobó 14 estudios de casos y controles, incluyendo a 21.395 sujetos con EP y 84.579 controles. Los resultados mostraron una

asociación negativa, esto es, una prevalencia de diabetes menor en la EP (OR 0.75; 95% CI 0.58–0.98, p= 0.03).

Estudios de casos y controles posteriores a este meta-análisis han mostrado datos heterogéneos: algunos de ellos no han observado ninguna asociación entre ambas enfermedades [65, 66], otros han observado una asociación positiva, con mayor prevalencia de diabetes en la EP [67] y otros, por el contrario, han objetivado una asociación negativa entre la DM2 y la EP [68].

En la ***tabla 1*** se recogen resumidamente los resultados principales de los estudios de casos y controles enumerados.

Por último, describir un meta-análisis recientemente publicado por Komici et al. en el que, entre otros ítems, analizan la prevalencia de la diabetes en los sujetos con EP. Obtuvieron, de los 21 artículos analizados, una prevalencia de diabetes en la EP de un 10% [69], siendo similar a la prevalencia del 9% estimada en la población general en 2019 [26].

Debido a las diferencias estimadas e influidas sustancialmente por la heterogeneidad en la metodología de los múltiples estudios de casos y controles, se han postulado diferentes hipótesis para explicar la inconsistencia de estos resultados descritos. Así, Chong et al. [43], han mostrado que los OR en los estudios de casos y controles son más elevados (asociación positiva) a mayor edad media de los sujetos, y valores de OR menores a 1 (asociación negativa) en sujetos más jóvenes. Hipotetizan que el riesgo de la EP puede aumentar a mayor tiempo de exposición a la DM2 y destacan que, así como la DM2 suele presentarse a mediana edad y la EP generalmente en edades más avanzadas, puede deberse a la presencia de un sesgo de supervivencia (mayor mortalidad de los sujetos con DM2) y que estos sujetos con DM2 no alcancen a desarrollar la EP. Añaden también, que la relación negativa observada en algunos estudios con sujetos más jóvenes sea debido a la baja incidencia de la EP en edad más temprana.

Esto, sumado a una heterogeneidad metodológica, donde ya inicialmente no se realiza una diferenciación de los subtipos de diabetes, las diferencias en la recogida de datos mediante cuestionarios vs registros, diferentes

poblaciones y diferentes criterios diagnósticos de la EP entre otros, contribuyen a aumentar el riesgo de sesgos en las estimaciones del efecto. Ello conlleva que nuevos diseños orientados a evitar estos sesgos son necesarios para acabar de dilucidar la asociación epidemiológica entre la DM2 y la EP en la modalidad de estudios de casos y controles.

TABLA 1. RESUMEN ESTUDIOS CASOS Y CONTROLES DESCRITOS

ESTUDIO	PERIODO ANALIZADO	DIAGNÓSTICO DIABETES + *n*	DIAGNÓSTICO EP (+ *n)* Y CONTROLES	OR (95% IC)	VARIABLES DE AJUSTE	SUBANÁLISIS SEXO	SUBANÁLISIS EDAD	OTROS SUBANÁLISIS
Schernhammer 2011 [62]	2001-2006	DM TIPO 2, 1 Y OTRAS (ICD-10 E10-E14 + ICD-8 249, 250x)	ICD-10-G20. 1.931 EP/9.651 controles	**1.36 [1.08–1.71]**	Edad y sexo	**Femenino 1.50 [1.02-2.22]**	**EP <60 años 2.68 [1.04-6.91]**	
D'Amelio 2009 [63]	-	Cuestionario + revisión historia médica	Diagnóstico neurológico 318 EP/318 controles	**0.4 [0.2-0.8]**	Sexo, edad, IMC, consumo tabaco, alcohol, café, localidad, educación.	Iguales: **OR 0.4 [0.2-1.0]** en **masculino**; **0.4 [0.1-1.0] femenino**	**EP edad inicio ≥ 60.8 años OR 0.5 [0.2-1.0]**. No los <60.8 años.	**IMC≥26.1 menor riesgo: OR 0.4 [0.2-0.9]**, IMC <26.1 sin asociación.
Lu 2014, meta-análisis [64]	14 estudios (2001-2013)	Criterios diversos, diabetes sin diferenciar por subtipos	21.395 EP/ 84.579 controles	**0.75 [0.58-0.98]**	Sexo, geografía, fuente grupo control, tabaco, fármacos antidiabéticos y duración de DM.	Femenino 0.79 [0.41-1.49] Masculino 0.71 [0.40-1.23]	-	DM <10 años OR 0.90 [0.63-1.3] DM>10 años OR 1.27 [0.79-2.05]
Gupta 2014 [65]	2010-2013	Cuestionario, diabetes (general)	97 EP, 97 controles	4DM/97 EP 10DM/97 controles Test Chi2: 2.77 p=0.09	-	-	-	-
De Pablo-Fernández 2017 [66]	1994-2007	Diabetes (general). Cuestionario, fármacos antidiabéticos o historial médico.	Diagnóstico neurológico. 79 EP, 4.919 controles	1.89, [0.90-3.98]	Sexo, edad, FRCV, AVC, medicación antidiabética	-	-	**DM>10 años OR 3,27 [1.21-8.85]** DM<10 años OR 1.68 [0.7-4.03]
Wu 2017 [67]		Diabetes (general)	7.716 EP	**1.26 [1.26-1.41]**		-	-	-
Heilbron 2019 [68]	-	Plataforma *online*	13.196 EP/148.176 controles	Diabetes 0.91 [0.72-1.15] **DM2 0.79 [0.74-0.85]**	Sexo y edad	-	-	-

1.3.2 ESTUDIOS PROSPECTIVOS.

En los últimos quince años se han publicado diversos estudios prospectivos de cohortes evaluando si la DM2 supone un aumento de riesgo para el desarrollo de la EP. Inicialmente, los resultados también fueron contradictorios.

Uno de los primeros estudios con este diseño en realizarse fue el de Hu et al. en 2007 [70]. En él partieron de sujetos con DM2 y sujetos controles sin DM2 y realizaron un seguimiento promedio de 18 años. Un total de 633 sujetos desarrolló la EP, siendo el cociente de riesgo (HR, del inglés *hazard ratio*) mayor en el grupo de los sujetos con DM2 (HR 1.83, IC 95% [1.21–2.76]). Por lo tanto, en este estudio la DM2 se asoció a un mayor riesgo de desarrollo posterior de EP.

Posteriormente se publicaron diversos estudios prospectivos en los que se observaron datos contradictorios: en algunos de ellos el riesgo de EP no se asoció a la presencia previa de diabetes [71,73] mientras que en otros la diabetes sí supuso un aumento de riesgo de desarrollo posterior de EP [72, 74, 75].

Debido nuevamente a la presencia de estos resultados opuestos, en 2016 se realizó un meta-análisis de estudios prospectivos que evaluó el riesgo de EP en presencia de diabetes [76]. En este meta-análisis se incluyeron todos los estudios mencionados previamente. El resultado global de este trabajo fue un aumento del riesgo relativo (RR) de presentar EP debido a diabetes de un 38% (RR= 1.38 [95% IC: 1.18–1.62] $p<0.001$).

Estudios prospectivos realizados más recientemente, como el de Yang et al. [77] en 2017, encontraron que la diabetes aumenta el riesgo de desarrollo de EP. En este estudio aumentó el riesgo de EP en un 19% (HR de 1.19 [IC 95%: 1.08 a 1.32] $p<0.001$ en el análisis multivariado) en la cohorte de sujetos diagnosticados de diabetes de forma global.

En el trabajo prospectivo más reciente en el momento de escribir esta tesis, el estudio publicado por De Pablo-Fernández et al. en 2018 con

datos de 2 millones de pacientes con DM2 [78], se observó un aumento de riesgo de EP similar (32%) (HR=1.32).

En la ***tabla 2*** se recogen los resultados principales de los estudios prospectivos enumerados.

Por último, el meta-análisis de Komici et al. incluyó diez estudios de cohorte con el objetivo de analizar, además de la prevalencia de diabetes en la EP, el riesgo de desarrollo de EP en sujetos diabéticos. El resultado global es similar a los artículos descritos, con un aumento de riesgo del 34% [69].

La evidencia, por lo tanto, en los estudios de diseño prospectivo es más consistente que en los estudios de casos y controles. Cereda et al. [79] diseñaron un meta-análisis para diferenciar el efecto del riesgo asociado de EP en presencia de diabetes ajustando por el tipo de diseño. Los estudios prospectivos que se incluyeron presentaron un aumento de riesgo de EP posterior en los sujetos con diabetes, con un RR de 1.34 [1.14–1.58 p<0.001]. En cambio, no se observó una asociación significativa en los estudios de casos y controles (OR= 0.75 [95%CI 0.50–1.11] p=0.835).

TABLA 2. RESUMEN ESTUDIOS PROSPECTIVOS

ESTUDIO	PERIODO ANALIZADO	COHORTE (*n*)	DIAGNÓSTICO DIABETES + *n*	DIAGNÓSTICO EP + *n*	RR/HR (95% IC)	VARIABLES DE AJUSTE	SUBANÁLISIS SEXO	SUBANÁLISIS EDAD	OTROS SUBANÁLISIS
Hu 2007 [70]	1972-2002	51.552	Registros, historial medico. 1.098 DM2	Registros, historial medico, 633 EP	**1.83 [1.21-2.76]**	Edad, sexo, año de la recogida, IMC, TAS, colesterol, educación, actividad física, consumo de tabaco, café, té y alcohol.	**Femenino 1.91 [1.04-3.52] Masculino 1.78[1.01-3.12]**	-	-
Simon 2007 [71]	1976-2000	171.879 (121.046 mujeres + 50.833 hombres)	Registros de estudios de profesionales de la salud. Cuestionarios. Diabetes (general)	Diagnóstico neurológico / médico internista, 530 EP	1.04 [0.74-1.46]	Edad, sexo, consumo de tabaco, café, alcohol, IMC, actividad física.	-	-	-
Driver 2008 [72]	1982-2005	21.841 médicos hombres	Cuestionarios. Diabetes (general)	Cuestionarios. 556 EP	**1.34 [1.01-1.77]**	Edad + consumo de tabaco.	-	**Diabetes <5 años RR 7.17 [4.59–11.20] Diabetes 5-9 años RR 2.03 [1.22–3.36]** Diabetes >10 años sin asociación	**IMC <25 RR 1.88 [1.28-2.77].** IMC >25 sin asociación. **Diabetes sin complicaciones RR 1.63 [1.14–2.33]**; Diabetes con complicaciones sin asociación.
Palacios 2011 [73]	1992-2005	147.096	Cuestionarios, Diabetes (general)	Diagnóstico neurológico + historial médico. 656 EP.	0.88 [0.62, 1.25]	Edad, sexo, IMC, educación, consumo de café, alcohol, actividad física	Femenino 0.90 [0.48, 1.66] Masculino 0.87 [0.57, 1.33]	-	IMC sin asociación
Xu 2011 [74]	1995-2006	288.662	Cuestionarios, Diabetes (general)	Cuestionarios, 1.565 EP	**1.41 [1.20-1.66]**	Edad, sexo, raza, IMC, educación, actividad física, consumo de tabaco y café.	-	-	**Diabetes >10 años 1.75 [1.36–2.25]**

Sun 2012 [75]	2000-2008	472.188 controles	Códigos diagnósticos (ICD-9) 603.416 diabéticos	Códigos diagnósticos (ICD-9) 1.613 EP	**1.61 [1.56-1.66]**	Edad, sexo, área geográfica, HTA, DLP, enfermedad cardiovascular.	**Femenino 1.70 [1.63–1.77] Masculino 1.51 [1.44–1.57]**	**Mujeres 41-60 años el mayor riesgo 2.05 [1.82–2.30]**, <40 años sin asociación. **Hombre el mayor riesgo 21-40 años 2.10 [1.01–4.42]**	-
Yue 2016 meta-análisis [76]	2007-2014, 7 estudios	1.761.632 sujetos	Criterios diversos, diabetes sin diferenciar por subtipos	-	**1.38 [1.18-1.62]**	Variables confusoras (no especifican)	**Femenino 1.50 [1.07-2.11] Masculino 1.40 [1.17-1.67]**	**41-60 años 2.05 [1.50-2.25, >60 años 1.55 [1.41-1.70].** Menores de 40 años sin asociación.	**Diabetes <10 años 2.33 [1.25-4.34]**
Yang 2017 [77]	2000-2006	108.882 controles	36.294 diabéticos reciente diagnóstico (ICD-9)	Códigos diagnósticos (ICD-9) 1.782 EP	**1.19 [1.08-1.32]**	Edad, sexo, comorbilidades, tratamientos	**Femenino 1.29, [1.12–1.49]**	**>65 años 1.20, [1.06–1.35]**	-
De Pablo-Fernández 2018 [78]	1999-2011	6.173208 controles	Códigos diagnósticos 2.017.115 DM2 (ICD-10)	Códigos diagnósticos 14.252 EP en la cohorte de DM2	**1.32 [1.29-1.35]**	Edad, sexo, fecha de entrada a la cohorte, área geográfica, índice de deprivación por área	**Femenino 1.42 [1.37–1.47] Masculino 1.27 [1.23-1.30]**	**25-44 años 3.81 [2.84–5.11], a más mayores menor riesgo.**	**DM2 con complicaciones (órganos diana) 1.49 [1.42–1.56]**

1.3.3. POBLACIONES CON MAYOR RIESGO

En algunos de estos estudios epidemiológicos, tanto con diseño de casos y controles como prospectivos, se han realizado sub-análisis para valorar el riesgo en función de diferentes variables. Estas han sido principalmente el sexo, la edad, y la duración del diagnóstico de la diabetes (o DM2) previo al diagnóstico de la EP.

A nivel epidemiológico, en el estudio de casos y controles de Schernhammer et al. [62] destacaba la asociación de la diabetes con la EP con un OR de 1.36 de forma global, pero en el sub-análisis esta asociación global se asoció únicamente al sexo femenino, con un OR de 1.50 [IC 95% 1.02-2.22] mientras que en el masculino fue de 1.29 con un IC 95% de 0.97-1.72 (asociación no estadísticamente significativa).

Si bien otros estudios prospectivos han mostrado un mayor riesgo de EP debido a la presencia de diabetes en ambos sexos, el riesgo ha sido mayor en el sexo femenino [69,75-78]. No obstante, en el meta-análisis de Lu et al. [64], que mostró una asociación inversa entre la diabetes y la EP, al realizar la estratificación por sexo, tampoco se observaron asociaciones significativas entre la diabetes y la EP.

De forma opuesta, en un estudio se ha observado un mayor riesgo vinculado al sexo masculino, pero de forma dependiente de la variable duración de la diabetes (a menor tiempo de evolución de la diabetes, mayor riesgo de EP entre hombres) [72].

En relación a la edad ha habido más controversia. Estudios prospectivos como el de Sun et al. [75] mostraron un riesgo mayor en las franjas de edad entre 41-60 años para ambos sexos, y únicamente en hombres menores de 41 años (perdiendo esta significación asociada a la edad más joven, menores de 41 años, en las mujeres). Estos resultados de mayor riesgo en jóvenes también se observaron en el estudio de De Pablo-Fernández et al. [78]. Por contra, en el estudio taiwanés de Yang et al. [77], el riesgo de EP era únicamente significativo en los sujetos diabéticos mayores de 65 años.

Por último, en el estudio de casos y controles de Schernhammer et al. [62] observaron como en la EP temprana (diagnóstico previo a los 60 años) la diabetes presentó una mayor asociación, con un OR de 2.68 [1.04–6.91]) mientras que en la EP tardía (diagnosticada a partir de los 60 años) no presentaba una asociación estadísticamente significativa (OR= 1.16 [0.85-1.57]).

Respecto a la duración de la diabetes, disponemos de resultados más contradictorios. Mientras que tanto en el estudio de Xu et al. [74] como el de De Pablo-Fernández et al. de 2017 [66] relacionaron el aumento de riesgo de EP posterior a la presencia de diabetes con una duración mayor a los 10 años, en el estudio de Driver et al. [72] y el meta-análisis de Yue et al. [76] observaron totalmente lo opuesto, sólo una duración de DM2 menor a 10 años se asociaba a un mayor riesgo posterior de EP.

Un estudio reciente de una cohorte coreana observó un mayor riesgo de EP en sujetos con DM2 de más de 5 años de evolución respecto a los de menos de 5 años de evolución y también respecto a sujetos con una intolerancia oral a la glucosa. Los tres grupos presentaban asociaciones estadísticamente significativas de aumento de riesgo de EP durante el seguimiento [80].

Por otra parte, la relación del mayor riesgo de EP en DM2 con afectación de órganos diana se ha descrito en dos estudios prospectivos, observándose resultados opuestos. Un mayor riesgo se observó en los DM2 con afectación de los órganos diana en el estudio de De Pablo-Fernández et al. [78], mientras que en el de Driver et al. [72] el mayor riesgo de EP era en los sujetos con DM2 sin complicaciones asociadas. En este último trabajo los autores discuten que no explican la relación por un sesgo de supervivencia debido a que el seguimiento no difería, incluso era mayor, en los sujetos con DM2 que desarrollaron la EP.

1.3.4 ASOCIACIONES ENTRE LA DM2 Y LA SINTOMATOLOGÍA DE LA EP.

Estudios iniciales ya analizaron las asociaciones entre la DM2 y la EP. En varios estudios de gran tamaño muestral con sujetos con EP, la presencia de DM2 se asoció a una mayor afectación, y también a una mayor progresión de los síntomas tanto motores como cognitivos [46, 81, 82].

Un estudio posterior mostró que los pacientes con EP y DM2 tenían síntomas motores más graves, más puntuaciones en la escala motora habitualmente utilizada en la EP (UPDRS; *Unified Parkinson's Disease Rating Scale*), más severidad en el estadiaje de Hoehn & Yahr y requerían dosis más altas de levodopa [83].

En la misma línea, el síndrome metabólico (incluyendo la obesidad e intolerancia a la glucosa) se ha asociado con aumentos del riesgo de deterioro cognitivo en pacientes con EP [84]. También la RI y la DM2 se han asociado con demencia en pacientes con EP en estudios más recientes [85, 86], si bien en otro estudio la RI no se correlacionó con la afectación motora ni no-motora (ni específicamente con la cognitiva) [47].

Pacientes con EP y DM2 han presentado además una relación con el fenotipo de inestabilidad postural y dificultad de la marcha [87, 88].

En el meta-análisis de Komici et al. se ha evaluado de la misma forma, en base a diez estudios analizados, el impacto de la diabetes en la severidad y progresión de la EP. Observaron peores puntuaciones en los estadios de Hoehn y Yahr y mayores puntuaciones de UPDRS, así como peor función cognitiva [69].

Diversos estudios, por lo tanto, han objetivado la asociación entre la DM2 con una mayor afectación o progresión, tanto motora como cognitiva en la EP, aunque con algunas inconsistencias en la literatura publicada hasta el momento.

1.4 FÁRMACOS ANTIDIABÉTICOS EN LA EP.

Debido a las asociaciones positivas descritas entre la DM2 y la EP, otro enfoque ha sido evaluar el impacto de los fármacos antidiabéticos en la EP.

Diferentes estudios han analizado el efecto de los antidiabéticos orales en la EP. Estudios con fármacos del grupo de las tiazolidinedionas no mostraron un beneficio en la EP [89, 90]. Por otra parte, estudios con metformina han mostrado resultados opuestos sobre su posible papel neuroprotector en la EP [91, 92]. No obstante, modelos animales con EP tratados con metformina sí han sugerido un efecto neuroprotector disminuyendo la neurodegeneración y la agregación de aS [93].

Diversos estudios han valorado el uso de la exenatida, un agonista de GLP-1 (*glucagon like-peptide 1*) con el que se han observado discretas mejoras motoras en pacientes con EP [94-96], principalmente aquellos con un fenotipo tremórico dominante [97]. Además, un estudio epidemiológico ha asociado un menor riesgo de EP con el uso de agonistas de GLP-1 y también con el grupo de los inhibidores de DPP4 [98].

Por último, una de las hipótesis de la asociación bioquímica del MG y la EP es la posible secreción insuficiente de insulina [56], motivo por el cual se ha sugerido que fármacos liberadores de insulina deberían considerarse frente a los fármacos sensibilizadores de insulina.

Varios estudios con insulina intranasal en modelos animales con EP han observado un efecto neuroprotector reduciendo la muerte celular de neuronas dopaminérgicas y mejorando la función mitocondrial estriatal [99, 100]. Además, el tratamiento con insulina intranasal ha sugerido mejoras en la afectación motora y cognitiva en pacientes con EP [101],

En resumen, diversos tratamientos antidiabéticos están observando resultados prometedores en la EP. Deberá corroborarse en ensayos clínicos multicéntricos si pueden llegar a tener un papel neuroprotector o modificador de la enfermedad y con ello promover un cambio en el manejo de la EP, principalmente inicial e incluso prodrómica.

II. HIPÓTESIS

1. Los pacientes con enfermedad de Parkinson presentan más alteraciones del metabolismo de la glucosa en ayunas y de los niveles de amilina que los sujetos controles sanos.

2. Las alteraciones del metabolismo de la glucosa se asocian a mayor afectación motora y no-motora (especialmente cognitiva) en los pacientes con enfermedad de Parkinson.

3. La presencia no sólo de diabetes mellitus tipo 2 sinó también de prediabetes comporta un mayor riesgo de desarrollo de enfermedad de Parkinson.

III. OBJETIVOS

OBJETIVOS

1. Determinar indicadores del metabolismo de la glucosa y de los niveles de amilina en sangre en pacientes con enfermedad de Parkinson y sujetos controles.

2. Establecer la relación de indicadores de metabolismo de la glucosa con los síntomas motores y no-motores en la enfermedad de Parkinson.

3. Determinar el riesgo relativo de desarrollo de enfermedad de Parkinson en relación a la presencia o ausencia de diabetes mellitus tipo 2 y/o prediabetes.

IV. MATERIAL, MÉTODOS Y RESULTADOS

4.1

ARTÍCULO 1

PERIPHERAL INSULIN AND AMYLIN LEVELS IN PARKINSON'S DISEASE

Niveles periféricos de insulina y amilina en la enfermedad de Parkinson.

Resumen

Antecedentes: La diabetes tipo 2 (DM2) se ha postulado como un factor de riesgo potencial para la enfermedad de Parkinson (EP) en algunos estudios epidemiológicos. La evidencia de alteraciones del metabolismo de la glucosa en la EP a partir de estudios moleculares sigue siendo controvertida. La amilina, la proteína amiloide de la DM2, se ha implicado en la EP en estudios patológicos. Nuestro objetivo fue evaluar los niveles periféricos de amilina e insulina en pacientes con EP y en controles (Cs).

Métodos: Realizamos un estudio observacional transversal de 111 participantes: 73 EP y 38 Cs, similares en edad, sexo e índice de masa corporal. A todos se les realizaron escalas motoras (UPDRS-MDS-III), no-motoras (NMSS) y cognitivas (MDRS), así como se determinaron cuatro parámetros: glucemia en ayunas, hemoglobina glicada, insulina plasmática en ayunas (IPA) y amilina plasmática en ayunas (APA).

Resultados: IPA fue significativamente menor en los sujetos con EP que en los Cs (p = 0.034). En los participantes con una edad superior a la mediana de la cohorte, la APA fue mayor en la EP que en los C (p = 0,046). La ratio IPA/APA (RPIAA) fue significativamente mayor en los EP que en los Cs (p=0.024). En la EP, se encontró una correlación modesta entre una mayor resistencia a la insulina y las puntuaciones del NMSS.

Conclusiones: Los pacientes con EP tenían un IPA más bajo y un RPIAA aumentado. En el subgrupo de EP de mayor edad, se incrementó la APA. A mayores niveles de resistencia a la insulina, mayores son las puntuaciones no-motoras. Estos hallazgos proporcionan un vínculo adicional entre la fisiopatología de la DM2 y la EP. Esto podría estar relacionado con una secreción disociada de insulina y amilina en la EP, en línea con la evidencia reciente del papel del páncreas endocrino en la patogenia de la EP.

Parkinsonism and Related Disorders 79 (2020) 91–96

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Peripheral insulin and amylin levels in Parkinson's disease

Almudena Sánchez-Gómez [a,f], Gema Alcarraz-Vizán [c,d,e], Manel Fernández [b], Rubén Fernández [c], Mario Ezquerra [c], Ana Cámara [a], Mònica Serrano [a], Anna Novials [c,d,e], Esteban Muñoz [a,b,c,f], Francesc Valldeoriola [a,b,c,f], Yaroslau Compta [a,b,c,f,**], Maria José Martí [a,b,c,f,*]

[a] Parkinson's Disease and Movement Disorders Unit. Department of Neurology. Hospital Clinic of Barcelona, Spain
[b] Institut de Neurociències, Maeztu Center, University of Barcelona, Spain
[c] Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain
[d] Diabetes and Obesity Research Laboratory of IDIBAPS, Spain
[e] Spanish Biomedical Research Centre Diabetes and Associated Metabolic Disorders (CIBERDEM), Spain
[f] Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED: CB06/05/0018-ISCIII) Barcelona, Spain

ARTICLE INFO

Keywords:
Parkinson's disease
Insulin
Amylin
Glucose metabolic alterations

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.

1. 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

* Co-Corresponding author. Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Villarroel 170, 08036, Barcelona, Catalonia, Spain.
** Co-Corresponding author. Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Villarroel 170, 08036 Barcelona, Catalonia, Spain.
E-mail addresses: YCOMPTA@clinic.cat (Y. Compta), MJMARTI@clinic.cat (M.J. Martí).

https://doi.org/10.1016/j.parkreldis.2020.08.018
Received 4 April 2020; Received in revised form 26 June 2020; Accepted 12 August 2020
Available online 25 August 2020

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, cerebrospinal fluid (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.

2. Methods

2.1. 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 40–80 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).

2.2. Metabolic assessment

For all participants, the body mass index was calculated. A blood sample was collected after 8-hour fasting within 1–4 weeks of the inclusion visit. Blood samples were centrifuged at 1500×*g* for 15 min at 4° 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), fasting plasma insulin (FPI) and fasting plasma amylin (FPA). We calculated the HOMA-IR index (Homeostatic Model Assessment of Insulin Resistance) and fasting 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.

2.3. 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, MDS-UPDRS part III and MDRS scores respectively.

2.4. 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.

3. Results

3.1. 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 774 mg/day [23] (Table 1).

3.2. 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 Fig. 1a and b**).**

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: Tables 2 and 3).

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[a]
Age (years, median)	67 y (57–72)	65 y (53–72)	0,462[b]
Education level			
Basic	25 (35.2%)	3 (8.1%)	
Media	21 (29.6%)	16 (43.2%)	0,012[a,c]
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[a,c]
"Tremor" in family	4 (5.6%)	1 (2.6%)	
Cardiovascular risk factors			
Arterial Hypertension	19 (26.4%)	10 (26.3%)	0.993[a]
Dyslipidemia	7 (9.7%)	10 (26.3%)	0.022[a,c]
Ischemic Heart Disease	3 (4.2%)	1 (2.6%)	0.683[a]
BMI (median) (p25-p75)	25.2 (23.1–26.4)	24.8 (21.5–29)	0.495[b]
Clinical assessment			
NMSS median (p25-p75)	37 (19–65)	8 (2.8–15.3)	<0.001[b,c]
MDRS median (p25-p75)	140 (136–142)	141 (140–143)	0.015[b,c]
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[b]
HbA1c (%) median (p25-p75)	5.50 (5.3–5.7)	5.40 (5.2–5.7)	0.521[b]
FPI (mU/L) median (p25-p75)	6.9 (5–9.9)	9.10 (5.8–11.6)	0.034[b,c]
FPA (pM) median (p25-p75)	23.48 (17.41–40.78)	20.13 (15.13–36.49)	0.454[b]
HOMA-IR median (p25-p75)	1.6 (1.1–2.6)	2.08 (1.3–3.1)	0.081[b]
FPAIR median (p25-p75)	0.52 (0.32–0.89)	0.32 (0.24–0.66)	0.024[b,c]

[a] Pearson's chi-squared test.
[b] U Mann-Whitney.
[c] 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.

3.3. 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).

3.4. 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) (Fig. 2). Motor symptoms and cognitive assessment were not correlated with any of the metabolic parameters.

4. 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

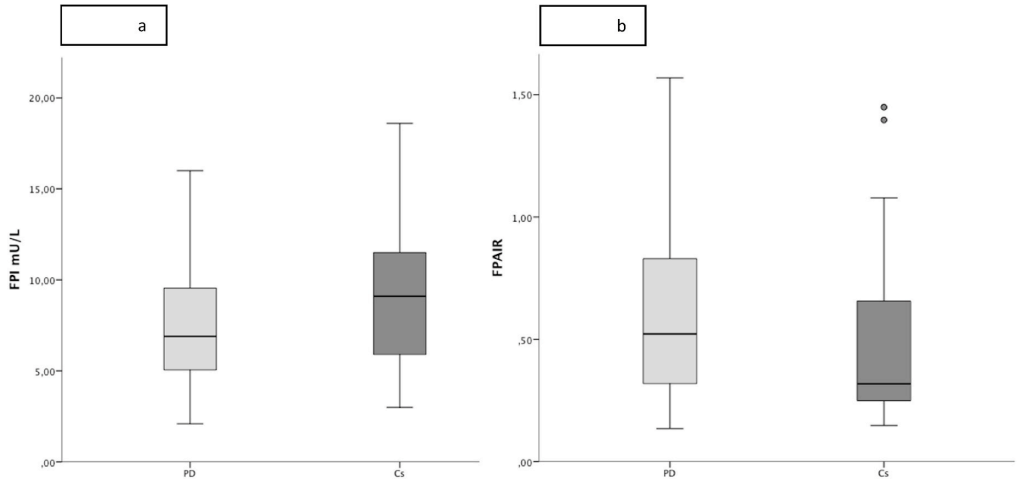


Fig. 1. Glucose metabolism results. Fig. 1a. Box plots of FPI levels [median (p25-p75)]. PD patients = 6.9 (5–9.9); Cs = 9.10 (5.8–11.6); p = 0.034. Fig. 1b: Box plots of FPAIR [median (p25-p75)]. PD patients = 0.52 (0.32–0.89); Cs = 0.32 (0.24–0.66). p = 0.024. PD: Parkinson's disease; Cs: Control subjects;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

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.

	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

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

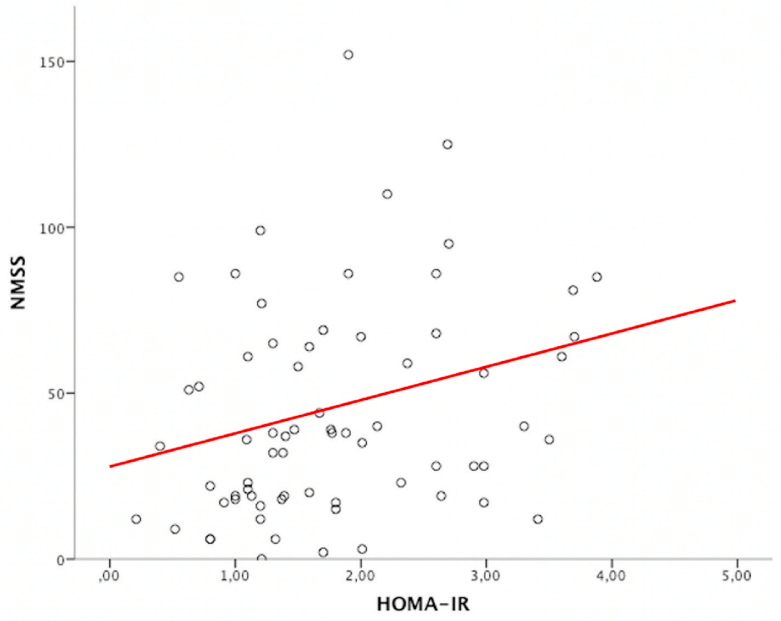


Fig. 2. Metabolic and clinical correlations. Growing HOMA-IR values were associated with increasing non-motor symptoms at NMSS (r = 0.328, p = 0.006). HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; NMSS: Non-Motor Symptom Scale.

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].

Relevant conflicts of interest/financial disclosures

Almudena Sánchez-Gómez: received an award from Hospital Clínic of Barcelona in 2017 (Emili Letang award) which supported this project.

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 Cámara 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).

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2020.08.018.

References

[1] E. De Pablo-Fernandez, R. Goldacre, J. Pakpoor, A.J. Noyce, T.T. Warner, Association between diabetes and subsequent Parkinson disease: a record-linkage cohort study, Neurology 91 (2) (2018 Jul 10) e139–e142, https://doi.org/10.1212/WNL.0000000000005771.

[2] J.A. Santiago, J.A. Potashkin, Blood biomarkers associated with cognitive decline in early stage and drug-naive Parkinson's disease patients, PLoS One 10 (11) (2015 Nov 13), e0142582, https://doi.org/10.1371/journal.pone.0142582.

[3] B. Mollenhauer, J. Zimmermann, F. Sixel-Döring, N.K. Focke, T. Wicke, J. Ebentheuer, M. Schaumburg, E. Lang, T. Friede, C. Trenkwalder, DeNoPa Study Group, Baseline predictors for progression 4 years after Parkinson's disease diagnosis in the de novo Parkinson cohort (DeNoPa), Mov. Disord. 34 (1) (2019 Jan) 67–77, https://doi.org/10.1002/mds.27492.

[4] D. Bosco, M. Plastino, D. Cristiano, C. Colica, C. Ermio, M. De Bartolo, P. Mungari, G. Fonte, D. Consoli, A. Consoli, A. Fava, Dementia is associated with insulin resistance in patients with Parkinson's disease, J. Neurol. Sci. 315 (1–2) (2012 Apr 15) 39–43, https://doi.org/10.1016/j.jns.2011.12.008.

[5] N.I. Bohnen, V. Kotagal, M.L. Müller, R.A. Koeppe, P.J. Scott, R.L. Albin, K.A. Frey, M. Petrou, Diabetes mellitus is independently associated with more severe cognitive impairment in Parkinson disease, Park. Relat. Disord. 20 (12) (2014 Dec) 1394–1398, https://doi.org/10.1016/j.parkreldis.2014.10.008.

[6] M. Giuntini, F. Baldacci, E. Del Prete, U. Bonuccelli, R. Ceravolo, Diabetes is associated with postural and cognitive domains in Parkinson's disease. Results from a single-center study, Park. Relat. Disord. 20 (6) (2014 Jun) 671–672, https://doi.org/10.1016/j.parkreldis.2014.02.016.

[7] V. Kotagal, R.L. Albin, M.L. Müller, R.A. Koeppe, K.A. Frey, N.I. Bohnen, Diabetes is associated with postural instability and gait difficulty in Parkinson disease, Park. Relat. Disord. 19 (5) (2013 May) 522–526, https://doi.org/10.1016/j.parkreldis.2013.01.016.

[8] I. Aviles-Olmos, P. Limousin, A. Lees, T. Foltynie, Parkinson's disease, insulin resistance and novel agents of neuroprotection, Brain 136 (Pt 2) (2013 Feb) 374–384, https://doi.org/10.1093/brain/aws009.

[9] S.E. Arnold, Z. Arvanitakis, S.L. Macauley-Rambach, A.M. Koenig, H.Y. Wang, R. S. Ahima, S. Craft, S. Gandy, C. Buettner, L.E. Stoeckel, D.M. Holtzman, D. M. Nathan, Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums, Nat. Rev. Neurol. 14 (3) (2018 Mar) 168–181, https://doi.org/10.1038/nrneurol.2017.185.

[10] D. Athauda, T. Foltynie, Insulin resistance and Parkinson's disease: a new target for disease modification? Prog. Neurobiol. 145–146 (2016) 98–120, https://doi.org/10.1016/j.pneurobio.2016.10.001.

[11] M.G. Spillantini, M.L. Schmidt, V.M. Lee, J.Q. Trojanowski, R. Jakes, M. Goedert, Alpha-synuclein in Lewy bodies, Nature 388 (6645) (1997 Aug 28) 839–840.

[12] I. Horvath, P. Wittung-Stafshede, Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 113 (44) (2016 Nov 1) 12473–12477.

[13] L. Haataja, T. Gurlo, C.J. Huang, P.C. Butler, Islet amyloid in type 2 diabetes,and the toxic oligomer hypothesis, Endocr. Rev. 29 (3) (2008 May) 303–316, https://doi.org/10.1210/er.2007-0037.

[14] M. Visa, G. Alcarraz-Vizán, J. Montane, L. Cadavez, C. Castaño, M.L. Villanueva-Peñacarrillo, J.M. Servitja, A. Novials, Islet amyloid polypeptide exerts a novel autocrine action in β-cell signaling and proliferation, Faseb. J. 29 (7) (2015 Jul) 2970–2979, https://doi.org/10.1096/fj.15-270553.

[15] J.N. Fawver, Y. Ghiwot, C. Koola, W. Carrera, J. Rodriguez-Rivera, C. Hernandez, K.T. Dineley, Y. Kong, J. Li, J. Jhamandas, G. Perry, I.V. Murray, Islet amyloid polypeptide (IAPP): a second amyloid in Alzheimer's disease, Curr. Alzheimer Res. 11 (10) (2014) 928–940.

[16] H. Zhu, X. Wang, M. Wallack, H. Li, I. Carreras, A. Dedeoglu, J.Y. Hur, H. Zheng, H. Li, R. Fine, M. Mwamburi, X. Sun, N. Kowall, R.A. Stern, W.Q. Qiu, Intraperitoneal injection of the pancreatic peptide amylin potently reduces behavioral impairment and brain amyloid pathology in murine models of Alzheimer's disease, Mol. Psychiatr. 20 (2) (2015 Feb) 252–262, https://doi.org/10.1038/mp.2014.17.

[17] I. Martinez-Valbuena, R. Valenti-Azcarate, I. Amat-Villegas, M. Riverol, I. Marcilla, C.E. de Andrea, J.A. Sánchez-Arias, M. Del Mar Carmona-Abellan, G. Marti, M. E. Erro, E. Martínez-Vila, M.T. Tuñon, M.R. Luquin, Amylin as a potential link between type 2 diabetes and alzheimer disease, Ann. Neurol. 86 (4) (2019 Oct) 539–551, https://doi.org/10.1002/ana.25570.

[18] H. Zhu, Q. Tao, T.F.A. Ang, J. Massaro, Q. Gan, S. Salim, R.Y. Zhu, V. B. Kolachalama, X. Zhang, S. Devine, S.H. Auerbach, C. DeCarli, R. Au, W.Q. Qiu, Association of plasma amylin concentration with alzheimer disease and brain structure in older adults, JAMA Netw Open 2 (8) (2019 Aug 2), e199826, https://doi.org/10.1001/jamanetworkopen.2019.9826.

[19] N. Schultz, S. Janelidze, E. Byman, L. Minthon, K. Nägga, O. Hansson, M. Wennström, Levels of islet amyloid polypeptide in cerebrospinal fluid and plasma from patients with Alzheimer's disease, PLoS One 14 (6) (2019 Jun 17), e0218561, https://doi.org/10.1371/journal.pone.0218561.

[20] I. Martinez-Valbuena, I. Amat-Villegas, R. Valenti-Azcarate, M.D.M. Carmona-Abellan, I. Marcilla, M.T. Tuñon, M.R. Luquin, Interaction of amyloidogenic proteins in pancreatic β cells from subjects with synucleinopathies, Acta Neuropathol. 135 (6) (2018 Jun) 877–886, https://doi.org/10.1007/s00401-018-1832-0.

[21] R.B. Postuma, D. Berg, M. Stern, W. Poewe, C.W. Olanow, W. Oertel, J. Obeso, K. Marek, I. Litvan, A.E. Lang, G. Halliday, C.G. Goetz, T. Gasser, B. Dubois, P. Chan, B.R. Bloem, C.H. Adler, G. Deuschl, MDS clinical diagnostic criteria for Parkinson's disease, Mov. Disord. 30 (12) (2015 Oct) 1591–1601, https://doi.org/10.1002/mds.26424.

[22] M.S. Kirkman, V.J. Briscoe, N. Clark, H. Florez, L.B. Haas, J.B. Halter, E.S. Huang, M.T. Korytkowski, M.N. Munshi, P.S. Odegard, R.E. Pratley, C.S. Swift, consensus development conference on diabetes and older adults, Diabetes in older adults: a consensus report, J. Am. Geriatr. Soc. 60 (12) (2012) 2342–2356, https://doi.org/10.1111/jgs.12035.

[23] C.L. Tomlinson, R. Stowe, S. Patel, C. Rick, R. Gray, C.E. Clarke, Systematic review of levodopa dose equivalency reporting in Parkinson's disease, Mov. Disord. 25 (15) (2010 Nov 15) 2649–2653, https://doi.org/10.1002/mds.23429.

[24] A. Marques, F. Dutheil, E. Durand, I. Rieu, A. Mulliez, M.L. Fantini, Y. Boirie, F. Durif, Glucose dysregulation in Parkinson's disease: too much glucose or not enough insulin? Park. Relat. Disord. 55 (2018 Oct) 122–127, https://doi.org/10.1016/j.parkreldis.2018.05.026.

[25] J. Sui, B. Shi, Y. Hu, M. Deng, Y. Wang, M. He, X. Zhao, Q. Ji, J. Ming, X. Yao, Islet function changes among the elderly population, Arch. Med. Res. 50 (7) (2019 Oct) 468–475, https://doi.org/10.1016/j.arcmed.2019.10.013.

[26] E. Hogg, K. Athreya, C. Basile, E.E. Tan, J. Kaminski, M. Tagliati, High prevalence of undiagnosed insulin resistance in non-diabetic subjects with Parkinson's disease, J. Parkinsons Dis. 8 (2) (2018) 259–265, https://doi.org/10.3233/JPD-181305.

[27] T. Sartorius, A. Peter, M. Heni, W. Maetzler, A. Fritsche, H.U. Häring, A. M. Hennige, The brain response to peripheral insulin declines with age: a contribution of the blood-brain barrier? PLoS One 10 (5) (2015 May 12), e0126804 https://doi.org/10.1371/journal.pone.0126804.

[28] S.K. Sharma, E. Chorell, P. Wittung-Stafshede, Insulin-degrading enzyme is activated by the C-terminus of α-synuclein, Biochem. Biophys. Res. Commun. 466 (2) (2015 Oct 16) 192–195, https://doi.org/10.1016/j.bbrc.2015.09.002.

[29] S.K. Sharma, E. Chorell, P. Steneberg, E. Vernersson-Lindahl, H. Edlund, P. Wittung-Stafshede, Insulin-degrading enzyme prevents α-synuclein fibril formation in a nonproteolytical manner, Sci. Rep. 5 (2015 Jul 31) 12531, https://doi.org/10.1038/srep12531.

[30] T. Sanke, T. Hanabusa, Y. Nakano, C. Oki, K. Okai, S. Nishimura, M. Kondo, K. Nanjo, Plasma islet amyloid polypeptide (Amylin) levels and their responses to oral glucose in type 2 (non-insulin-dependent) diabetic patients, Diabetologia 34 (2) (1991 Feb) 129–132.

[31] B.J. Edwards, H.M. Perry, F.E. Kaiser, J.E. Morley, D. Kraenzle, D.K. Kreutter, R. W. Stevenson, Age-related changes in amylin secretion, Mech. Ageing Dev. 86 (1) (1996 Jan 5) 39–51.

[32] C.J. Dechenes, C.B. Verchere, S. Andrikopoulos, S.E. Kahn, Human aging is associated with parallel reductions in insulin and amylin release, Am. J. Physiol. 275 (5) (1998 Nov) E785–E791, https://doi.org/10.1152/ajpendo.1998.275.5.E785.

[33] Thomas A. Lutz, Urs Meyer, Amylin at the interface between metabolic and neurodegenerative disorders, Front. Neurosci. 9 (16 Jun. 2015) 216, https://doi.org/10.3389/fnins.2015.00216.

[34] G. Llebaria, J. Pagonabarraga, J. Kulisevsky, C. García-Sánchez, B. Pascual-Sedano, A. Gironell, M. Martínez-Corral, Cut-off score of the Mattis dementia rating scale for screening dementia in Parkinson's disease, Mov. Disord. 23 (11) (2008 Aug 15) 1546–1550, https://doi.org/10.1002/mds.22173.

[35] D. Athauda, K. Maclagan, S.S. Skene, M. Bajwa-Joseph, D. Letchford, K. Chowdhury, S. Hibbert, N. Budnik, L. Zampedri, J. Dickson, Y. Li, I. Aviles-Olmos, T.T. Warner, P. Limousin, A.J. Lees, N.H. Greig, S. Tebbs, T. Foltynie, Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial, Lancet 390 (10103) (2017 Oct 7) 1664–1675, https://doi.org/10.1016/S0140-6736(17)31585-4.

Supplementary material

Panel a	PD ≥67 yo (n=39)	Cs ≥ 67 yo (n=18)	Significance (p) (U Mann-Whitney)
FPI mU/L median (p25-p75)	6.35(4.4-8.4)	9.1 (6.38-11.33)	**0.020***
	PD < 67 yo (n=33)	**Cs< 67 yo (n=20)**	**Significance (p)** (U Mann-Whitney)
	8.00 (5.55-11.40)	9.05 (5.45-13.30)	**0.521**
Significance (p) (U Mann-Whitney)	**0.063**	**0.784**	
Panel b	**PD ≥67 yo (n=40)**	**Cs ≥ 67 yo (n=18)**	**Significance (p)** (U Mann-Whitney)
FPA pM median (p25-p75)	24.9 (17-42.52)	17.87 (13.84-21.57)	**0.046***
	PD < 67 yo (n=33)	**Cs< 67 yo (n=20)**	**Significance (p)** (U Mann-Whitney)
	22.44 (17.96-35.75)	30.41 (18.23-74.50)	**0.271**
Significance (p) (U Mann-Whitney)	**0.817**	**0.030***	
Panel c	**PD ≥ 67 yo (n=39)**	**Cs ≥ 67 yo (n=18)**	**Significance (p)** (U Mann-Whitney)
FPAIR (p25-p75)	0.55 (0.39-0.95)	0.29 (0.25-0.43)	**p< 0.001***
	PD < 67 yo (n=33)	**Cs< 67 yo (n=20)**	**Significance (p)** (U Mann-Whitney)
	0.43 (0.25-0.77)	0.41 (0.23-1,00)	**0.956**
Significance (p) (U Mann-Whitney)	**0.104**	**0.228**	

Supplementary Table 1: Amylin and insulin levels in relation to age. Panel a: FPI levels had a significative difference between both groups in older subjects (PD ≥67 yo had lower FPI levels than Cs of the same age group; p=0.020). A trend in decline FPI levels in older PD subjects was observed (p=0.063). **Panel b:** FPA levels had a significative difference between both groups in older subjects (PD ≥67 yo had higher FPA levels than Cs of the same age group; p=0.046) but inside PD group we did not find differences by age in FPA value (p=0.817). Nevertheless, in Cs there were significative differences between ≥67 and < 67 yo age sub-groups (p=0.030). **Panel c**: FPAIR was significative higher in PD ≥67 yo than Cs of the same age group; p<0.001. A trend in increasing ratio in older age-subgroup occurred in PD, opposite to Cs where ratio declines in younger-subgroup. yo= years old. *Significant difference. PD: Parkinson's disease; Cs: Control subjects; FPI: Fasting plasma insulin; FPA: Fasting plasma amylin; FPAIR: Fasting plasma amylin/insulin ratio.

	Significance	Odds Ratio	95% CI for OR	
	(p)	(OR)	Lower limit	Upper limit
PD familiar history	0.013*	2.722	1.234	6.006
FPI levels (mU/L)	0.042*	0.886	0.789	0.995
Blood glucose levels (mg/dL)	0.106	1.038	0.992	1.086
BMI	0.979	0.998	0.888	1.122

Supplementary Table 2: Binary logistic regression model with PD familiar history, FPI levels, blood glucose levels and BMI. The significant values to predict being in PD group were lower FPI levels with an OR 0.886 and PD familiar history with OR 2.722. *Significant difference. PD: Parkinson's disease; FPI: Fasting plasma insulin; BMI: Body mass index.

	Significance (p)	Odds Ratio (OR)	95% CI for OR	
			Lower limit	Upper limit
Sex (female)	0.497	0.737	0.305	1.780
PD familiar history	0.014*	2.170	1.170	4.023
Blood glucose levels (mg/dL)	0.024*	1.055	1.007	1.104
FPI levels (mU/L)	0.012*	0.860	0.764	0.967

Supplementary Table 3: Binary logistic regression model with sex, PD familiar history, blood glucose levels and FPI levels. The significant values to predict being in PD group were lower FPI levels with an OR 0.860, PD familiar history with OR 2.170 and increasing blood glucose levels with an OR 1.055. *Significant difference. PD: Parkinson's disease; FPI: Fasting plasma insulin

	PD ≥67 yo (n=40)	Cs ≥ 67 yo (n=18)	Significance (p) (U Mann-Whitney)
Blood glucose levels mg/dL median (p25-p75)	97 (91-101)	95 (91.75-103.5)	**0.932**
	PD < 67 yo (n=33)	**Cs< 67 yo (n=20)**	
	95 (88-102)	92.5 (82.75-98.75)	**0.419**
Significance (p) (U Mann-Whitney)	**0.549**	**0.303**	

Supplementary Table 4: Blood glucose levels in relation to age. No differences were noted between PD and Cs neither between age-subgroups cohorts. PD: Parkinson's disease; Cs: Control subjects.

4.2

ARTÍCULO 2

PREDIABETES, TYPE 2 DIABETES MELLITUS AND RISK OF PARKINSON'S DISEASE: A POPULATION-BASED COHORT STUDY

Prediabetes, diabetes mellitus tipo 2 y riesgo de enfermedad de Parkinson: un estudio de cohorte basado en la población

Antecedentes: La asociación de la diabetes mellitus tipo 2 (DM2) con el posterior desarrollo de enfermedad de Parkinson (EP) ha respaldado el vínculo entre el metabolismo de la glucosa y la EP. Evaluamos el riesgo de EP no solo en la diabetes tipo 2 sino también en la prediabetes.

Métodos: Se realizó un estudio de cohorte retrospectivo de la población atendida en los centros de atención primaria del Instituto Catalán de la Salud en Cataluña entre 2006 y 2018. Los datos se obtuvieron del Sistema de Información para la Investigación en Atención Primaria (SIDIAP). Creamos una cohorte de pacientes con DM2 y prediabetes (HbA1c ≥ 5,7–6,4% sin fármacos antidiabéticos o diagnóstico previo de DM2) y se comparó con una cohorte de referencia. El resultado fue el diagnóstico de EP y se excluyó la EP antes o durante el primer año de seguimiento. Usamos modelos de regresión de Cox multivariados para calcular los cocientes de riesgo (HR) y los intervalos de confianza del 95% (IC del 95%). Se excluyeron sujetos con parkinsonismos atípicos y secundarios.

Resultados: Las cohortes expuestas comprendieron 281.153 pacientes con DM2 y 266.379 con prediabetes y una cohorte de referencia de 2.556.928 sujetos. La DM2 y la prediabetes se asociaron con un mayor riesgo de EP (HR ajustado 1,19; IC del 95%: 1,13-1,25 y 1,07; 1,00-1,14; respectivamente). En los análisis estratificados por sexo, la prediabetes solo se asoció con el riesgo de EP en las mujeres (1,12, 1,03–1,22 frente a 1,01, 0,99–1,10 en los hombres). Cuando el análisis se estratificó por edad, la DM2 y la prediabetes se asociaron con un mayor riesgo de EP tanto en mujeres (2,36, 1,96–2,84 y 2,10, 1,70–2,59 respectivamente) como en hombres (1,74, 1,52–2,00 y 1,90, 1,57–2,30 respectivamente) menores de 65 años.

Conclusiones: Informamos por primera vez que la prediabetes aumenta las probabilidades de una EP posterior y replicamos la asociación establecida con la DM2. Ambas asociaciones predominan en mujeres y sujetos jóvenes.

Parkinsonism and Related Disorders 89 (2021) 22–27

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Prediabetes, type 2 diabetes mellitus and risk of Parkinson's disease: A population-based cohort study

Almudena Sánchez-Gómez, MD [a,b,c,e], Yesika Díaz [d], Talita Duarte-Salles, PhD [d], Yaroslau Compta, MD, PhD [a,b,c,e,**], Maria José Martí, MD, PhD [a,b,c,e,*]

[a] Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Spain
[b] Institut de Neurociències, Maeztu Center, University of Barcelona, Spain
[c] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain
[d] Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol), Barcelona, Spain
[e] Centro de Investigación Biomédica en Red Sobre Enfermedades Neurodegenerativas (CIBERNED, CB06/05/0018-ISCIII), Barcelona, Spain

ARTICLE INFO

Keywords:
Diabetes mellitus
Prediabetes
Obesity
Parkinson's disease

ABSTRACT

Background: Association of type 2 diabetes mellitus (T2D) with subsequent Parkinson's disease (PD) has supported the link between glucose metabolism and PD. We assessed the risk of PD not only in T2D but also in prediabetes.

Methods: We conducted a retrospective cohort study of the population attended in primary care centres of the Catalan Health Institute in Catalonia between 2006 and 2018. The data were obtained from the Information System for Research in Primary Care (SIDIAP). We created a cohort of T2D and prediabetes patients (HbA1c $\geq$ 5.7–6.4% without antidiabetic drugs or previous T2D diagnosis) and compared to a reference cohort. The outcome was PD diagnosis and we excluded PD before or during the first year of follow-up. We used multivariate Cox regression models to calculate hazard ratios (HR) and 95% confidence intervals (95%CI). We excluded subjects with atypical and secondary parkinsonisms.

Results: The exposed cohorts comprised of 281.153 patients with T2D and 266.379 with prediabetes and a reference cohort of 2.556.928 subjects. T2D and prediabetes were associated with higher risk of PD (HRadjusted 1.19, 95%CI 1.13–1.25, and 1.07, 1.00–1.14; respectively). In analyses stratified by sex, prediabetes was only associated with PD risk in women (1.12, 1.03–1.22 vs. 1.01, 0.99–1.10 in men). When analysis was stratified by age, T2D and prediabetes were associated with a greater PD risk both in women (2.36, 1.96–2.84 and 2.10, 1.70–2.59 respectively) and men (1.74, 1.52–2.00 and 1.90, 1.57–2.30 respectively) below 65 years-old.

Conclusions: We report for the first time that prediabetes increases the odds of subsequent PD and replicate the association with established T2D. Both associations predominate in women and young individuals.

1. Introduction

Different studies have addressed the metabolic association between type 2 diabetes mellitus (T2D) and Parkinson's disease (PD), but the link among them remains unclear. Insulin resistance is one of the most relevant metabolic alterations found in the relationship between T2D and PD [1]. More recently, low insulin levels have also been postulated as one of the possible mechanisms involved in this association in conjunction with higher levels of amylin, the T2D amyloid deposit protein in pancreatic ß-cells, in older PD patients [2].

Studies aiming at addressing the epidemiological relationship between T2D and PD have shown conflicting results. A meta-analysis of case-control studies found a negative association between T2D and PD [3], but these results were opposed with a meta-analysis distinguishing between case-control and prospective studies. In prospective studies, T2D was found as a risk factor for subsequent PD development, but no association was found in case-control studies [4].

The latest studies with prospective design have been consistent,

* Corresponding author. Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Villarroel 170, 08036, Barcelona, Catalonia, Spain.
** Corresponding author. Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Villarroel 170, 08036, Barcelona, Catalonia, Spain.
E-mail addresses: YCOMPTA@clinic.cat (Y. Compta), MJMARTI@clinic.cat (M.J. Martí).

https://doi.org/10.1016/j.parkreldis.2021.06.002
Received 26 February 2021; Received in revised form 12 May 2021; Accepted 3 June 2021
Available online 8 June 2021

showing that T2D preceding PD increased the risk of its future development. Yang and colleagues found increased risk of PD with a hazard ratio (HR) of 1.19 (95% confidence interval-CI- 1.08-1.32) in the T2D cohort (36.294 subjects) in multivariate analysis. In women and older individuals (≥65 years old) the risk was greater (HR 1.29 and 1.20 respectively) [5]. In a more recent work, a similar risk was noted in 2 million T2D patients from London (HR = 1.32), predominantly in women, young patients and in those with complicated T2D (target organs affected) [6].

Recently, the use of two oral antidiabetic drugs (DPP4 inhibitors and GLP-1 receptor agonists) were associated with lower PD risk [7] and, in the case of exenatide (a GLP1 agonist), with a slight motor improvement in patients with PD [8].

To our knowledge, prediabetes as a risk factor for PD development has not been previously analyzed. Accordingly, we aimed at investigating the association between T2D and prediabetes on the risk of subsequent PD in a large population-based cohort.

2. Methods

2.1. Data source and study design

We conducted a retrospective cohort study using data from the Information System for the Development of Research in Primary Care (SIDIAP; www.sidiap.org) from January 2006 until December 2018 (Fig. 1). The SIDIAP includes information recorded in electronic health records by health professionals during routine visits at 287 primary health care centres from the Institut Català de la Salut (ICS, Catalan Health Institute) [9]. The SIDIAP has anonymized records for more than seven million people and is representative of the Catalan population in terms of age, sex, and geographic distribution [9]. It includes information on disease diagnoses (International Classification for Diseases, 10th revision [ICD-10]), drug prescriptions and dispensations in the primary care setting, and clinically relevant parameters (e.g., weight, blood pressure, laboratory tests). It is also linked to a hospital discharge database for patients who attend ICS hospitals (30% of the SIDIAP population) [10,11].

2.2. Type 2 diabetes mellitus, prediabetes, Parkinson's disease and covariates

The exposed cohort was constructed in two groups by identifying individuals with T2D diagnosis by an ICD-10 code E11 and/or glycated haemoglobin (HbA1c) ≥6.5%; and another group with prediabetic state defined by (HbA1c) levels between 5.7 and 6.4% without current or previous antidiabetic drugs neither T2D diagnosis code. The T2D group was defined first, so that a subject with prediabetes was not part of this second group if she/he developed T2D later during the study period. Subjects without T2D or prediabetes inclusion criteria constituted the reference cohort. Type 1 diabetes and other types of diabetes were exclusion criteria for enter into the study. Included individuals were aged between 40 and 80 years old. Individuals were excluded if they had a PD coded diagnosis (ICD-10 G20) (or they had prescribed antidopaminergics drugs) before or during the first year after entry into the study. Exclusion criteria for the entire cohort were subjects with a history of ICD-10 diagnostic categories of atypical and secondary (including pharmacological) parkinsonisms, G23 and G21 respectively (selected and excluded patients are detailed in Supplementary material, Fig. 1). All subjects were followed-up until a PD diagnosis, death, transferred out of SIDIAP, or end the study in December 2018.

PD, the study outcome, was defined by a PD diagnosis code registered in primary care (ICD-10 G20). In the MDS diagnostic criteria of PD [12] dementia is not considered an exclusion criterion of PD anymore, allowing for considering dementia with Lewy bodies (DLB) as PD with dementia of the Lewy-type. Hence, and to make sure that cases with PD with dementia were not omitted, we also included DLB diagnosis code (ICD-10 G31.8). In order not to miss cases with tremulous PD coded as tremor, we also included tremor diagnosis (ICD-10 R25.1) in combination with active prescriptions of levodopa and/or dopamine agonists for at least 1 year.

We evaluated other covariates such as age, sex, body mass index (BMI), cardiovascular risk factors (hypertension, dyslipidemia, ischemic heart disease by registered ICD-10 codes), smoking (never, past, current smokers) and the MEDEA deprivation index (socioeconomic level in quintiles). Covariates were identified during the year prior to PD diagnosis or study finalization.

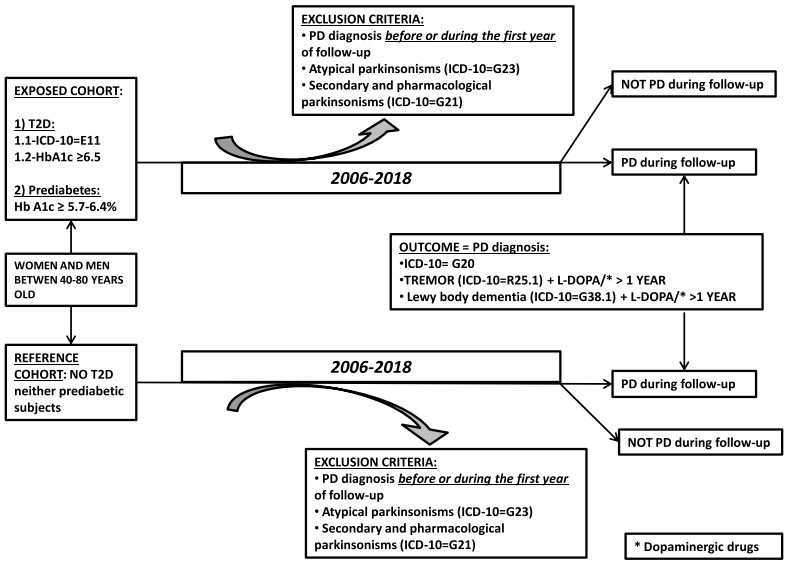


Fig. 1. Study Flow-chart. Abbreviations: T2D = Type 2 diabetes mellitus; ICD-10 = International Classification of Diseases, Tenth Revision; HbA1c = glycated haemoglobin; PD= Parkinson's disease. L-Dopa = levodopa.

BMI was calculated by body weight in kg divided by squared height in meters, and further categorised into BMI categories according to the World Health Organization (WHO) [13]: <25 kg/m^2 (underweight and normal weight; due to small sample size in the underweight category, we decided to combine the underweight and normal weight categories), ≥25–29.99 kg/m^2 overweight and ≥30 kg/m^{2}obesity.

2.3. Statistical analysis

We performed descriptive analysis of the studied cohorts (which are detailed in Supplementary material, Table 1), including the overall baseline characteristics.

We fitted Cox's proportional hazards model with the time metric to estimate HR and 95% CI for the association between T2D, prediabetes, and risk of PD. The first (crude) model included only age and sex, and the second (multivariable-adjusted) model was further adjusted for BMI, smoking status and socioeconomic status. We further stratifyed all models by sex and age (<65 and ≥ 65 years). We performed multiple imputations (using the fully conditional specification approach, with 20 imputed data sets created) to deal with missing values for covariates included in the models [14,15]. For all models, we checked the proportional hazard assumption by using the Schoenfeld test of proportionality and by visual inspection of the scaled Schoenfeld residuals [16].

We assessed effect modification by introducing interaction terms (one at a time) between T2D, prediabetes and BMI.

All statistical analyses were performed with R (version 3.6).

3. Ethical considerations

The project was approved by the Research Ethics Committee of the Jordi Gol University Institute for Research in Primary Care (IDIAP Jordi Gol).

Due to the study collected population-based data, the personal identification code was encrypted by the Catalan Health Institute, maintaining the anonymity of the study individuals. Therefore, the informed consent of the participants was not required and the research team worked with anonymized data.

4. Results

We included 3.104.460 people in the study. In the exposed cohort, there were 281.153 (9.1%) T2D patients and 266.379 (8.6%) subjects with prediabetes. The reference cohort included 2.556.928 (82.4%) people. The mean follow-up years of the study were 7.3. During the follow-up, a total of 13.715 people developed PD. In the reference cohort, 10.748 (0.42% of the entire reference cohort) subjects had a diagnosis of PD during follow-up. In the TD2 group, there were 1.789 (0.64% of T2D total group) subjects and in the prediabetic group 1.178 (0.44% of prediabetic total group) subjects with a PD diagnosis during follow-up (Table 1).

The mean age of the PD subjects were 68.4 years old, in the TD2 with PD and prediabetes with PD cohorts were 69.8 and 70.7 years respectively.

An increased risk of PD development was observed in the T2D cohort (adjusted HR of 1.19; 95% CI 1.13–1.25) and to a lesser extent but also statistically significant in the prediabetes cohort (adjusted HR of 1.07; 95% CI 1.00–1.14) when compared to T2D and prediabetes free individuals. Both associations were independent of other factors such as the BMI. The more age the higher risk for subsequent PD (Table 1).

Overweight and obese individuals had higher PD risk (adjusted HR of 1.21; 95% CI 1.15–1.27 and 1.19; 95% CI 1.13–1.26 respectively) compared to normal weight individuals independently of other factors such as T2D. Current and former smoking conferred lower PD risk development (adjusted HR of 0.70; 95% CI 0.66–0.74 and 0.80; 95% CI 0.75–0.85, respectively) when compared to never smokers.

In stratified analyses by sex, we have found that in women, the risk of PD development was higher than in men, both T2D (adjusted HR of 1.27; 95% CI 1.18–1.38 vs. adjusted HR of 1.11; 95% CI 1.04–1.20) and prediabetic group (adjusted HR of 1.12; 95% CI 1.03–1.22 vs. adjusted HR of 1.01; 95% CI 0.99–1.10). In fact, prediabetes did not confer an increased risk of subsequent PD in men (Table 2).

However, stratified analyses by both age and sex, the risk of subsequent PD increased substantially in young groups (<65 years old). It was in these age-subgroups, and mainly in women, that both T2D and prediabetes conferred a substantial increase in risk of subsequent PD (adjusted HR of 2.36; 95% CI 1.96–2.84 and HR of 2.10; 95% CI

Table 1
T2D and prediabetes cohorts with PD relative risk.

	Entire cohort (n = 3.104.460)	PD (n = 13.715)	HR	95% CI	p value
Reference cohort	2.556.928 (82.4%)	10.748 (0.42%)[a]	Ref	–	–
T2D	281.153 (9.0%)	1.789 (0.64%)[a]	1.19	1.13–1.25	<0.001
Prediabetes	266.379 (8.6%)	1.178 (0.44%)[a]	1.07	1.00–1.14	0.045
Sex					
Women	1.606.876 (51.8%)	6.608 (0.4%)[a]	Ref	–	–
Men	1.497.584 (48.2%)	7.107 (0.5%)[a]	1.54	1.49–1.60	<0.001
Age					
<65 yo	2.490.450 (80.2%)	3.915 (0.16%)[a]	Ref	–	–
≥65–74 yo	416.967 (13.4%)	5.896 (1.4%)[a]	7.74	7.42–8.07	<0.001
≥75 yo	197.043 (6.4%)	3.904 (2%)[a]	12.71	12.13–13.31	<0.001
BMI					
<25	963.576 (31.0%)	2.859 (0.3%)[a]	Ref	–	–
25–29.9	1.272.461 (41.0%)	6.496 (0.5%)[a]	1.21	1.15–1.27	<0.001
≥30	868.423 (28.0%)	4.360(0.5%)[a]	1.19	1.13–1.26	<0.001
Smoke					
Non smoker	1.828.719 (58.9%)	10.470 (0.6%)[a]	Ref	–	–
Current smoker	893.279 (28.8%)	1.909 (0.2%)[a]	0.70	0.66–0.74	<0.001
Former smoker	382.426 (12.3%)	1.336 (0.3%)[a]	0.80	0.75–0.85	<0.001
MEDEA U1	528.396 (17.0%)	2.732 (0.52%)[a]	Ref	–	–
MEDEA U2	508.518 (16.4%)	2.278 (0.45%)[a]	0.89	0.84–0.95	<0.001
MEDEA U3	498.594 (16.1%)	2.180 (0.44%)[a]	0.88	0.83–0.93	<0.001
MEDEA U4	485.009 (15.6%)	1.888 (0.39%)[a]	0.81	0.76–0.86	<0.001
MEDEA U5	449.055 (14.5%)	1.609 (0.36%)[a]	0.79	0.74–0.84	<0.001
MEDEA R	634.888 (20.4%)	3.028 (0.48%)[a]	0.89	0.85–0.94	<0.001

Abbreviations: PD= Parkinson's disease; HR= Hazard ratio; CI= Confidence interval; T2D = Type 2 diabetes mellitus; yo = years old; BMI= Body mass index; MEDEA = Socioeconomical index, U1 less deprive-U5 most deprive, R = rural.

[a] % = number of PD/total number of subjects in the cohort.

Table 2
T2D and prediabetes cohorts with PD relative risk in relation to sex.

	WOMEN with PD				MEN with PD			
	PD/*n total*	**HR**	**95% CI**	**p value**	**PD/*n total***	**HR**	**95% CI**	**p value**
Reference cohort	5.196/1.347.047 (0.39%)	Ref	-	-	5.552/1.209.881 (0.46%)	Ref	-	-
T2D cohort	779/116.527 (0.69%)	1.27	1.18–1.38	<0.001	1.010/164.626 (0.61%)	1.11	1.04–1.20	0.0027
Prediabetes	633/143.302 (0.44%)	1.12	1.03–1.22	0.0085	545/123.077 (0.44%)	1.01	0.99–1.10	0.91
Age								
<65 yo	1.688/1.267.174 (0.13%)	Ref	–	–	2.227/1.223.276 (0.18%)[1]	Ref	–	–
≥65 yo	4.920/339.702 (1.45%)	9.04	8.51–9.61	<0.001	4.880/274.308 (1.78%)	9.12	8.66–9.60	<0.001
BMI								
<25	1.507/574.134 (0.26%)	Ref	–	–	1.352/389.442 (0.35%)	Ref	–	–
25–29.9	2.741/565.673 (0.48%)	1.20	1.12–1.29	<0.001	3.755/706.788 (0.53%)	1.20	1.12–1.29	<0.001
≥30	2.360/467.069 (0.51%)	1.16	1.07–1.25	<0.001	2.000/401.354 (0.5%)	1.20	1.10–1.30	<0.001
Smoke								
Non smoker	6.027/1.132.638 (0.53%)	Ref	–	–	4.443/696.081 (0.64%)	Ref	–	–
Current smoker	410/349.736 (0.12%)	0.61	0.54–0.70	<0.001	1.499/543.543 (0.28%)	0.71	0.66–0.75	<0.001
Former smoker	171/124.502 (0.01%)	0.73	0.61–0.88	<0.001	1.165/257.960 (0.45%)	0.82	0.76–0.88	<0.001
MEDEA U1	1.350/287.988 (0.47%)	Ref	–	–	1.382/240.408 (0.57%)	Ref	–	–
MEDEA U2	1.103/269.926 (0.41%)	0.90	0.82–0.98	0.012	1.175/238.592 (0.49%)	0.88	0.81–0.96	0.003
MEDEA U3	1.023/260.739 (0.39%)	0.85	0.78–0.93	<0.001	1.157/237.855 (0.49%)	0.88	0.81–0.96	0.003
MEDEA U4	926/251.115 (0.37%)	0.83	0.76–0.91	<0.001	962/233.894 (0.41%)	0.77	0.70–0.84	<0.001
MEDEA U5	756/222.215 (0.34%)	0.78	0.71–0.85	<0.001	853/226.840 (0.38%)	0.77	0.70–0.84	<0.001
MEDEA R	1.450/314.893 (0.46%)	0.95	0.88–1.03	0.214	1.578/319.995 (0.49%)	0.85	0.79–0.92	<0.001

Abbreviations: PD= Parkinson's disease; HR= Hazard ratio; CI= Confidence interval; T2D = Type 2 diabetes mellitus; yo = years old; BMI= Body mass index; MEDEA = Socioeconomical index, U1 less deprive-U5 most deprive, R = rural.

1.70–2.59, respectively) (Table 3).

In women above 65 years old, neither T2D nor prediabetes, were associated with a higher risk of PD. In men above 65 years of age, no significant association between T2D and PD risk was found, whereas prediabetes was associated with a lower risk of subsequent PD (adjusted HR of 0.82; 95% CI 0.74–0.91; Table 3).

Prediabetes increased the risk of PD during a mean follow-up of 3.8 and 3.2 years in women and men under 65 years old respectively and 2.9 and 3 years in women and men above 65 years old respectively. T2D increased risk of PD during a mean follow-up of 5.3 and 4.9 years in women and men under 65 years old respectively and 3.4 and 4 years in women and men above 65 years old respectively.

Regarding BMI, we observed that both overweight and obesity conferred a higher risk of subsequent PD (Table 1). In women, the risk of PD development associated with both overweight and obesity was higher in those under 65 than in those over 65 years of age, where the association was lower or negative. In men, overweight and obesity were associated with a higher risk of subsequent PD in both age groups, with the youngest showing a slight increase compared to those over 65 years of age (Table 3). However, in an interaction model analysis, BMI did not influence the risk of PD associated with prediabetes neither T2D (analysis stratified by sex; Supplementary material, Table 2).

An association was also found with socioeconomic level too, in terms of lower rates of PD development among population with lower income (Tables 1 and 2).

5. Discussion

In this large cohort study, we found an overall higher risk of subsequent PD not only in T2D, but also in prediabetic subjects independently of other factors. When stratifying by sex, prediabetes was a risk factor for subsequent PD development only in women, and T2D conferred a higher risk in women relative to men. In the youngest age group (<65 years old) both T2D and prediabetes were associated with a substantially higher rate of subsequent PD in both sex categories. Besides, a higher rate of subsequent PD was observed in overweight and obesity subjects, but without interaction with either T2D or prediabetes.

Our results on the association between T2D and the subsequent PD risk were similar to previous prospective studies [4–6], which have led the Movement Disorders Society to consider T2D as a risk marker for PD, therefore highlighting its potential importance in the prodromal phase of PD [17]. T2D HRs in previous studies ranged from 1.19 to 1.32 [5,6]. The differences in relative risks, despite their narrow interval, may be due, as already postulated by De Pablo-Fernández et al. [6], to different populations (Taiwan, London, etc.), different study designs, and differences in the diagnosis definition of T2D and PD, as well as the covariates analyzed.

To the best of our knowledge, this is the first study to analyze prediabetes as a risk factor for PD development in a large cohort. A previous report links prediabetes with subsequent risk of PD in clinically suspected REM sleep behaviour disorder [18] and in LRRK2-PD, prediabetes was more prevalent than in idiopathic PD or GBA-PD in a small cohort [19]. Prediabetes is a known risk factor for the T2D development [20] and it is associated, as T2D, with increased BMI, insulin resistance, pancreatic ß-cell dysfunction and subsequent decrease in insulin secretion [21]. In spite of lower risk for subsequent PD (except in men <65 years) in prediabetes relative to T2D, it is expected that due to its high conversion rate to T2D [22] in the absence of healthy habits [23] prediabetes may be relevant for the risk of PD. Further studies to support this hypothesis are warranted.

In women above 65 years old, neither T2D nor prediabetes showed a difference in the risk rate for subsequent PD; and in prediabetic men above 65 years old, PD risk decreased. It is difficult to interpret this finding. It could be hypothesized that both metabolic conditions only can show its risk effect in younger people with longer time of exposition or that these elder cases might be somehow protected by other factors. This outstanding question warrants further research in larger prospective cohorts.

On the other hand, in our study we have observed a higher risk of PD in diabetic women compared to men. This finding is consistent with those from three previously published large cohorts [5,6,24]. These data contrast with the higher risk of PD classically related to the male sex [25]. Hormonal factors could be involved, but more research will be necessary to solve this pending issue.

We observed higher risk of PD in <65 years old both for T2D and prediabetic groups. Younger T2D individuals have previously been inconsistently associated with an increased risk for subsequent PD [5,6]. Young subjects with prediabetes are at increased risk of T2D. The difference in age has been related to greater insulin resistance in older subjects and to insulin deficiency in younger ones [26]. In turn, lower insulin values with higher fasting plasma amylin/insulin ratio have been observed in PD compared to healthy controls, hypothesizing a

Table 3
PD relative risk in relation to sex and age in T2D and prediabetes cohorts.

	WOMEN								MEN							
	<65 years old (n=1.267.174)				≥ 65 years old (n=339.702)				<65 years old (n=1.223.276)				≥ 65 years old (n=274.308)			
	PD/*n total*	HR	95% CI	p value	PD/*n total*	HR	95% CI	p value	PD/*n total*	HR	95% CI	p value	PD/*n total*	HR	95% CI	p value
Reference cohort	1.455/1.127.402 (0.13%)	Ref	–	–	3.741/219.645 (1.7%)	Ref	–	–	1.868/1.044.110 (0.18%)	Ref	–	–	3.684/165.771 (2.22%)	Ref	–	–
T2D	135/60.537 (0.22%)	2.36	1.96–2.84	<0.001	644/55.990 (1.15%)	1.09	1.00–1.19	0.06	241/104.804 (0.23%)	1.74	1.52–2.00	<0.001	769/59.822 (1.3%)	0.95	0.87–1.03	0.19
Prediabetes	98/79.235 (0.12%)	2.10	1.70–2.59	<0.001	535/64.067 (0.84%)	0.94	0.86–1.04	0.23	118/74.362 (0.16%)	1.90	1.57–2.3	<0.001	427/48.715 (0.9%)	0.82	0.74–0.91	<0.001
BMI																
<25	487/504.555 (0.1%)	Ref	–	–	1.020/69.579 (1.47%)	Ref	–	–	483/336.399 (0.14%)	Ref	–	–	869/53.043 (1.6%)	Ref	–	–
25–29.9	636/429.228 (0.15%)	1.36	1.19–1.56	<0.001	2.105/136.445 (1.54%)	1.10	1.01–1.2	0.026	1.126/568.422 (0.2%)	1.25	1.10–1.42	<0.001	2.629/138.366 (1.9%)	1.17	1.07–1.27	0.0035
≥30	565/333.391 (0.17%)	1.48	1.29–1.7	<0.001	1.795/133.678 (1.34%)	1.02	0.93–1.12	0.61	618/318.455 (0.19%)	1.23	1.07–1.42	0.004	1.382/82.899 (1.7%)	1.16	1.05–1.28	<0.001
Smoke																
Current smoker	206/334.364 (0.06%)	0.42	0.35–0.49	<0.001	204/15.372 (1.33%)	1.08	0.90–1.29	0.48	632/485.706 (0.13%)	0.61	0.55–0.68	<0.001	867/57.837 (1.5%)[1]	0.78	0.72–0.85	<0.001
Former smoker	73/113.463 (0.06%)	0.52	0.40–0.68	<0.001	98/11.039 (0.88%)	1.02	0.79–1.31	0.90	317/192.161 (0.16%)	0.77	0.67–0.88	<0.001	848/65.799 (1.3%)	0.84	0.78–0.92	<0.001
Non smoker	1.409/819.347 (0.17%)	Ref	–	–	4.618/313.291 (1.47%)	Ref	–	–	1.278/545.409 (0.23%)	Ref	–	–	3.165/150.672 (2.1%)	Ref	–	–

Abbreviations: PD= Parkinson's disease; HR= Hazard ratio; CI= Confidence interval; T2D = Type 2 diabetes mellitus; BMI= Body mass index.

dissociated insulin and amylin secretion in PD [2]. All this suggests that in younger subjects, metabolic factors such as lower insulin levels and possibly genetic factors along with the deposition of amyloid proteins (which occurs in both T2D and PD [27,28]), as well as a prolonged life expectancy allowing more time of exposure, can contribute to this association.

Overweight and obesity were associated with an increased risk for PD development independently of T2D or prediabetes diagnosis. Due to the association between overweight and obesity with T2D and prediabetes [29], a relationship between increasing BMI and PD can be expected. Nevertheless, both obesity and underweight have been associated with PD [30,31]. This contradiction deserves to be explored in depth in further studies specifically focused on it.

Although a higher incidence of PD has been related to lower incomes [32], other studies have shown inverse relationships as in our results [33] and/or minor effects between both associations [34]. Difficulties in evaluating socioeconomic status as well as inequalities in access to medical resources and specialized care, as well as to therapies, may contribute to these contradictory results.

The main limitations of the current study are the retrospective design (although data were collected prospectively) and their validity given the reliance on historical diagnostic codes and lack of complete medical history nor detailed neurological evaluation. Therefore, the risk of misdiagnosis was mainly with atypical parkinsonisms, especially in cases with shorter follow-up. We also emphasize the importance of case attrition during the first year of follow-up after T2D or prediabetes to minimize a chance effect regarding the risk factor that these metabolic alterations could involve in the subsequent PD diagnosis. We consider that the fact of excluding these patients may have minimized the diagnostic significance, but favors the notion of T2D and prediabetes as risk factors for the PD development.

These limitations notwithstanding, this is a large cohort study where the multiple imputations performed allowed for a more complete analysis. Besides, to our knowledge, this is the first time that BMI has been added as a confounding variable in the assessment of T2D as a risk factor for PD. BMI does not appear to modify the association between T2D (and prediabetes) with PD-risk.

The most noted strength and novelty of this study is the analysis of prediabetes as an additional glucose-related metabolism risk marker for PD not previously described in the literature. Furthermore, we confirm the data on T2D as a risk factor for subsequent PD in our population. Both metabolic conditions appear to increase the risk especially in women and in young people. Other strengths relate to the internal consistency of the different analyses along with the fact that BMI was also taken into consideration and controlled for, suggesting that our findings are specific to T2D and prediabetes as independent risk factors for subsequent PD.

As said above, these links could have a potential pathophysiological, diagnostic and, above all, therapeutic relevance in the prodromal phase of PD with regard to recent published studies in relation to the possible protective role of some oral antidiabetic drugs in PD [7,8].

Relevant conflicts of interest/financial disclosures

Almudena Sánchez-Gómez: received an award from Hospital Clínic of Barcelona in 2017 (Emili Letang award) which supported this project.

Yesika Díaz: nothing to declare.

Talita Duarte-Salles: nothing to declare.

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: No conflict of interest

Hospital Clínic de Barcelona receives support from the CERCA program of Generalitat de Catalunya.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.06.002.

References

[1] D. Athauda, T. Foltynie, Insulin resistance and Parkinson's disease: a new target for disease modification? Prog. Neurobiol. 145–146 (2016) 98–120, https://doi.org/10.1016/j.pneurobio.2016.10.001.

[2] A. Sánchez-Gómez, G. Alcarraz-Vizán, M. Fernández, R. Fernández-Santiago, M. Ezquerra, A. Cámara, M. Serrano, A. Novials, E. Muñoz, F. Valldeoriola, Y. Compta, M.J. Martí, Peripheral insulin and amylin levels in Parkinson's disease, Park. Relat. Disord. 79 (2020 Aug 25) 91–96, https://doi.org/10.1016/j.parkreldis.2020.08.018.

[3] L. Lu, D.L. Fu, H.Q. Li, A.J. Liu, J.H. Li, G.Q. Zheng, Diabetes and risk of Parkinson's disease: an updated meta-analysis of case-control studies, e85781, PLoS One 9 (1) (2014 Jan 21), https://doi.org/10.1371/journal.pone.0085781.

[4] E. Cereda, M. Barichella, C. Pedrolli, C. Klersy, E. Cassani, R. Caccialanza, G. Pezzoli, Diabetes and risk of Parkinson's disease: a systematic review and meta-analysis, Diabetes Care 34 (12) (2011 Dec) 2614–2623, https://doi.org/10.2337/dc11-1584.

[5] Y.W. Yang, T.F. Hsieh, C.I. Li, C.S. Liu, W.Y. Lin, J.H. Chiang, T.C. Li, C.C. Lin, Increased risk of Parkinson disease with diabetes mellitus in a population-based study, Medicine (Baltim.) 96 (3) (2017 Jan), https://doi.org/10.1097/MD.0000000000005921 e5921.

[6] E. De Pablo-Fernandez, R. Goldacre, J. Pakpoor, A.J. Noyce, T.T. Warner, Association between diabetes and subsequent Parkinson disease: a record-linkage cohort study, Neurology 91 (2) (2018 Jul 10) e139–e142, https://doi.org/10.1212/WNL.0000000000005771.

[7] R. Brauer, L. Wei, T. Ma, D. Athauda, C. Girges, N. Vijiaratnam, G. Auld, C. Whittlesea, I. Wong, T. Foltynie, Diabetes medications and risk of Parkinson's disease: a cohort study of patients with diabetes, Brain 143 (10) (2020 Oct 1) 3067–3076, https://doi.org/10.1093/brain/awaa262.

[8] D. Athauda, K. Maclagan, S.S. Skene, M. Bajwa-Joseph, D. Letchford, K. Chowdhury, S. Hibbert, N. Budnik, L. Zampedri, J. Dickson, Y. Li, I. Aviles-Olmos, T.T. Warner, P. Limousin, A.J. Lees, N.H. Greig, S. Tebbs, T. Foltynie, Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial, Lancet 390 (10103) (2017 Oct 7) 1664–1675, https://doi.org/10.1016/S0140-6736(17)31585-4.

[9] M.D.M. García-Gil, E. Hermosilla, D. Prieto-Alhambra, et al., Construction and validation of a scoring system for selection of high quality data in a Spanish population primary care database (SIDIAP), Inf. Prim. Care 20 (2) (2012) 1.

[10] Generalitat de Catalunya, Conjunt mínim bàsic de dades (CMBD). https://catsalut.gencat.cat/ca/proveidors-professionals/registres-catalegs/registres/cmbd/index.html#googtrans(ca%7Ces, 2017. (Accessed March 2019).

[11] Instituto Nacional de Estadística, Hospital Morbidity Survey: General Methodology, 2019. http://www.ine.es/en/daco/daco42/sanitarias/notaemh_en.htm. (Accessed March 2019).

[12] R.B. Postuma, D. Berg, M. Stern, W. Poewe, C.W. Olanow, W. Oertel, J. Obeso, K. Marek, I. Litvan, A.E. Lang, G. Halliday, C.G. Goetz, T. Gasser, B. Dubois, P. Chan, B.R. Bloem, C.H. Adler, G. Deuschl, MDS clinical diagnostic criteria for Parkinson's disease, Mov. Disord. 30 (12) (2015 Oct) 1591–1601, https://doi.org/10.1002/mds.26424.

[13] W.H.O. Managing, The global epidemic of obesity. Report of the World Health Organization (WHO) consultation on obesity, in: Paper Presented at the International Obesity Task Force, June 3Y5, Geneva, Switzerland, 1997.

[14] A.B. Pedersen, E.M. Mikkelsen, D. Cronin-Fenton, N.R. Kristensen, T.M. Pham, L. Pedersen, et al., Missing data and multiple imputation in clinical epidemiological research, Clin. Epidemiol. 9 (2017) 157–166.

[15] K.J. Lee, J.B. Carlin, Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation, Am. J. Epidemiol. 171 (5) (2010) 624–632.

[16] P. Grambsch, T. Therneau, Proportional hazards tests and diagnostics based on weighted residuals, Biometrika 81 (3) (1994 Sep 1) 515–526.

[17] S. Heinzel, D. Berg, T. Gasser, H. Chen, C. Yao, R.B. Postuma, MDS Task Force on the Definition of Parkinson's Disease. Update of the MDS research criteria for prodromal Parkinson's disease, Mov. Disord. 34 (10) (2019 Oct) 1464–1470, https://doi.org/10.1002/mds.27802.

[18] J.C. Wong, J. Li, M. Pavlova, S. Chen, A. Wu, S. Wu, X. Gao, Risk factors for probable REM sleep behavior disorder: a community-based study, Neurology 86 (14) (2016 Apr 5) 1306–1312, https://doi.org/10.1212/WNL.0000000000002414.

[19] A. Thaler, S. Shenhar-Tsarfaty, Y. Shaked, T. Gurevich, N. Omer, A. Bar-Shira, M. Gana-Weisz, O. Goldstein, M. Kestenbaum, J.M. Cedarbaum, A. Orr-Urtreger, N. Giladi, A. Mirelman, Metabolic syndrome does not influence the phenotype of LRRK2 and GBA related Parkinson's disease, Sci. Rep. 10 (1) (2020 Jun 9) 9329, https://doi.org/10.1038/s41598-020-66319-9.

[20] A.G. Tabák, C. Herder, W. Rathmann, E.J. Brunner, M. Kivimäki, Prediabetes: a high-risk state for diabetes development, Lancet 379 (9833) (2012 Jun 16) 2279–2290, https://doi.org/10.1016/S0140-6736(12)60283-9.

[21] K. Rett, U. Gottwald-Hostalek, Understanding prediabetes: definition, prevalence, burden and treatment options for an emerging disease, Curr. Med. Res. Opin. 35 (9) (2019 Sep) 1529–1534, https://doi.org/10.1080/03007995.2019.1601455.

[22] S. Ligthart, T.T. van Herpt, M.J. Leening, M. Kavousi, A. Hofman, B.H. Stricker, M. van Hoek, E.J. Sijbrands, O.H. Franco, A. Dehghan, Lifetime risk of developing impaired glucose metabolism and eventual progression from prediabetes to type 2 diabetes: a prospective cohort study, Lancet Diabetes Endocrinol. 4 (1) (2016 Jan) 44–51, https://doi.org/10.1016/S2213-8587(15)00362-9.

[23] A. Glechner, L. Keuchel, L. Affengruber, V. Titscher, I. Sommer, N. Matyas, G. Wagner, C. Kien, I. Klerings, G. Gartlehner, Effects of lifestyle changes on adults with prediabetes: a systematic review and meta-analysis, Prim. Care Diabetes 12 (5) (2018 Oct) 393–408, https://doi.org/10.1016/j.pcd.2018.07.003.

[24] C. Deischinger, E. Dervic, M. Kaleta, P. Klimek, A. Kautzky-Willer, Diabetes mellitus is associated with a higher relative risk for Parkinson's disease in women than in men, J. Parkinsons Dis. (2021 Jan 21), https://doi.org/10.3233/JPD-202486.

[25] G.F. Wooten, L.J. Currie, V.E. Bovbjerg, J.K. Lee, J. Patrie, Are men at greater risk for Parkinson's disease than women? J. Neurol. Neurosurg. Psychiatry 75 (4) (2004 Apr) 637–639, https://doi.org/10.1136/jnnp.2003.020982.

[26] L.N. Pani, D.M. Nathan, R.W. Grant, Clinical predictors of disease progression and medication initiation in untreated patients with type 2 diabetes and A1C less than 7%, Diabetes Care 31 (3) (2008 Mar) 386–390, https://doi.org/10.2337/dc07-1934.

[27] I. Horvath, P. Wittung-Stafshede, Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 113 (44) (2016 Nov 1) 12473–12477.

[28] I. Martinez-Valbuena, I. Amat-Villegas, R. Valenti-Azcarate, M.D.M. Carmona-Abellan, I. Marcilla, M.T. Tuñon, M.R. Luquin, Interaction of amyloidogenic proteins in pancreatic β cells from subjects with synucleinopathies, Acta Neuropathol. 135 (6) (2018 Jun) 877–886, https://doi.org/10.1007/s00401-018-1832-0.

[29] M.A. Laaksonen, P. Knekt, H. Rissanen, T. Härkänen, E. Virtala, J. Marniemi, A. Aromaa, M. Heliövaara, A. Reunanen, The relative importance of modifiable potential risk factors of type 2 diabetes: a meta-analysis of two cohorts, Eur. J. Epidemiol. 25 (2) (2010 Feb) 115–124, https://doi.org/10.1007/s10654-009-9405-0.

[30] J. Chen, Z. Guan, L. Wang, G. Song, B. Ma, Y. Wang, Meta-analysis: overweight, obesity, and Parkinson's disease, Internet J. Endocrinol. 2014 (2014) 203930, https://doi.org/10.1155/2014/203930.

[31] A.J. Noyce, D.A. Kia, G. Hemani, A. Nicolas, T.R. Price, E. De Pablo-Fernandez, P. C. Haycock, P.A. Lewis, T. Foltynie, G. Davey Smith, , International Parkinson Disease Genomics Consortium, A. Schrag, A.J. Lees, J. Hardy, A. Singleton, M. A. Nalls, N. Pearce, D.A. Lawlor, N.W. Wood, Estimating the causal influence of body mass index on risk of Parkinson disease: a Mendelian randomisation study, PLoS Med. 14 (6) (2017 Jun 13), e1002314, https://doi.org/10.1371/journal.pmed.1002314.

[32] L.M. Lix, D.E. Hobson, M. Azimaee, W.D. Leslie, C. Burchill, S. Hobson, Socioeconomic variations in the prevalence and incidence of Parkinson's disease: a population-based analysis, J. Epidemiol. Community Health 64 (4) (2010 Apr) 335–340, https://doi.org/10.1136/jech.2008.084954.

[33] J.C. Pressley, M.X. Tang, K. Marder, L.J. Cote, R. Mayeux, Disparities in the recording of Parkinson's disease on death certificates, Mov. Disord. 20 (3) (2005 Mar) 315–321, https://doi.org/10.1002/mds.20339.

[34] X. Li, J. Sundquist, K. Sundquist, Socioeconomic and occupational groups and Parkinson's disease: a nationwide study based on hospitalizations in Sweden, Int. Arch. Occup. Environ. Health 82 (2) (2009 Jan) 235–241, https://doi.org/10.1007/s00420-008-0327-z.

SUPPLEMENTARY MATERIAL

Table 1. Descriptive results of the entire cohort and non-PD and PD groups

	Total	No PD	PD
Entire cohort	3104460	3090745	13715
Reference cohort	2556928 (82.36%)	2546180 (82.4%)	10748 (78.4%)
T2D	281153 (9.06%)	279364 (9.04%)	1789 (13.0%)
Prediabetes	266379 (8.58%)	265201 (8.58%)	1178 (8.59%)
Sex			
Women	1606876 (51.8%)	1600268 (51.8%)	6608 (48.2%)
Men	1497584 (48.2%)	1490477 (48.2%)	7107 (51.8%)
Age			
<65 yo	2490450 (80.2%)	2486535 (80.5%)	3915 (28.5%)
≥65-74 yo	416967 (13.4%)	411071 (13.3%)	5896 (43.0%)
≥75 yo	197043 (6.35%)	193139 (6.25%)	3904 (28.5%)
BMI			
<25	963576 (31.0%)	960717 (31.1%)	2859 (20.8%)
25-29.9	1272461 (41.0%)	1265965 (41.0%)	6496 (47.4%)
≥30	868423 (28.0%)	864063 (28.0%)	4360 (31.8%)
Smoke			
Current smoker	893279 (28.8%)	891370 (28.8%)	1909 (13.9%)
Former smoker	382462 (12.3%)	381126 (12.3%)	1336 (9.74%)
Non smoker	1828719 (58.9%)	1818249 (58.8%)	10470 (76.3%)
MEDEA index			
U1	528396 (17.0%)	525664 (17.0%)	2732 (19.9%)
U2	508518 (16.4%)	506240 (16.4%)	2278 (16.6%)
U3	498594 (16.1%)	496414 (16.1%)	2180 (15.9%)
U4	485009 (15.6%)	483121 (15.6%)	1888 (13.8%)
U5	449055 (14.5%)	447446 (14.5%)	1609 (11.7%)
R	634888 (20.5%)	631860 (20.4%)	3028 (22.1%)

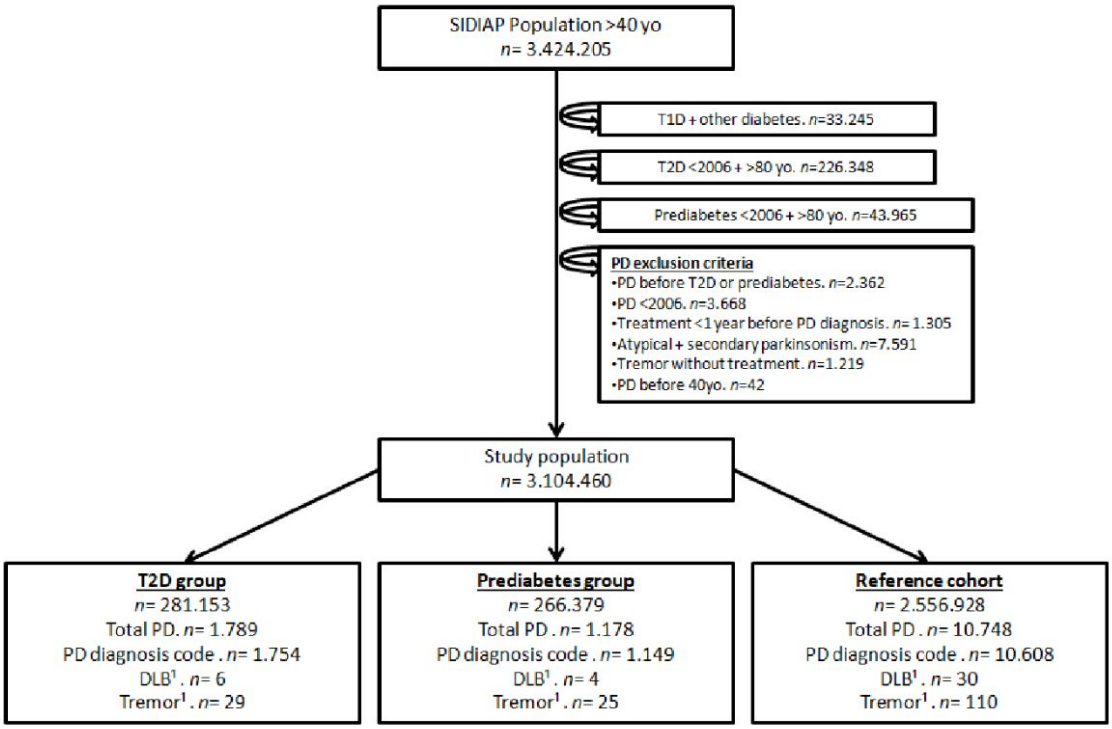
Abbreviations: PD= Parkinson's disease; T2D= Type 2 diabetes mellitus; yo= years old; BMI= Body mass index; MEDEA= socioeconomic level in quintiles, U1 less deprive-U5 most deprive, R= rural.

Table 2. Interactions of BMI with diabetes and prediabetes in PD relative risk.

	ENTIRE COHORT (*n*=13.715)			WOMEN (*n*=6.608)			MEN (*n*=7.107)		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
T2D-BMI <25	Ref	-	-	Ref	-	-	Ref	-	-
T2D-BMI 25-29.9	0.90	0.75-1.08	0.28	0.95	0.70-1.27	0.71	0.90	0.71-1.14	0.38
T2D-BMI>30	0.85	0.71-1.02	0.084	0.78	0.58-1.05	0.11	0.89	0.70-1.14	0.36
Prediabetes-BMI <25	Ref	-	-	Ref	-	-	Ref	-	-
Prediabetes-BMI25-29.9	0.84	0.70-1.02	0.074	0.82	0.63-1.06	0.14	0.89	0.67-1.17	0.40
Prediabetes-BMI>30	0.90	0.75-1.09	0.27	0.92	0.72-1.18	0.52	0.87	0.65-1.16	0.33

Abbreviations: PD= Parkinson's disease; HR= Hazard ratio; CI= Confidence interval; T2D= Type 2 diabetes mellitus; yo= years old; BMI= Body mass index.

Figure 1. The study population selection flow-chart.



Abbreviations: yo= years old; T1D= Type 1 diabetes mellitus; T2D= Type 2 diabetes mellitus; PD= Parkinson's disease; DLB: Dementia with Lewy bodies; [1] adding dispensing of levodopa or dopamine agonists for at least 1

V. DISCUSIÓN

Los trabajos presentados en esta tesis doctoral pretenden profundizar y aportar nueva información en la relación tanto clínica como epidemiológica de los diferentes indicadores del MG y de la propia DM2 en la EP. Evaluamos diferentes metabolitos relacionados tanto analítica como anatomopatológicamente con la DM2 para determinar cuáles se relacionan más estrechamente con la EP y valorar la correlación de éstos con los síntomas motores y no-motores de la enfermedad. Además, ampliamos el conocimiento sobre la asociación de la DM2, introduciendo el análisis del estado prediabético, en las fases pre-diagnósticas de la EP por su potencial papel como factor de riesgo para el desarrollo posterior de la enfermedad.

En el primer trabajo, evaluamos la presencia de diferencias en los niveles sanguíneos en ayunas de las moléculas principales del MG en la EP respecto a sujetos controles sin la enfermedad: glucemia e insulinemia, así como la HbA1c, y añadimos también el análisis de la amilina plasmática. Este es el primer estudio en el que se describe en la literatura el análisis de la amilina plasmática en sujetos con EP [47, 53-57]. Se ha dedicado una editorial sobre este primer artículo de la presente tesis doctoral que se adjunta en el anexo 1.

En primer lugar, los resultados de este trabajo sugieren que los sujetos con EP presentan unos niveles de insulina plasmática en ayunas inferior a los sujetos sin la enfermedad, habiendo excluido en todos ellos el diagnóstico de DM2 u otras formas de diabetes. Pese a que en la literatura se ha estudiado ampliamente la RI en la EP, hay pocos precedentes de valoración de los niveles de insulina plasmática en ayunas en sujetos con EP. Algunos trabajos previos no han observado diferencias en los niveles de esta hormona [53, 56], pero por contra, ha habido trabajos que han sugerido la presencia de un déficit de insulina en la EP relacionado con una ausencia del aumento de insulina esperado ante mayores niveles glucémicos [47, 56]. La mayoría de la insulina cerebral proviene de la periferia (producción pancreática) atravesando la barrera

hematoencefálica, y se han correlacionado los niveles plasmáticos de insulina con los niveles de ésta en LCR [45]. Por ello, a pesar de no disponer de los niveles en LCR, teniendo en cuenta esta correlación se podría hipotetizar que los menores niveles de insulina plasmática en la EP se asociarían también a menores niveles de insulina cerebral.

Como describimos anteriormente, la EDI que degrada a la insulina y a las proteínas amiloides, se encuentra regulada por la propia insulina. La EDI interacciona con la aS [38] y es capaz de prevenir su agregación [39]. Menores niveles de insulina traducirían una menor activación de la EDI con una consecuente menor degradación y por tanto mayor acúmulo de amiloide de aS.

En cuanto a los niveles de amilina plasmática, observamos niveles incrementados en los sujetos con EP de mayor edad respecto a los controles de la misma franja de edad. En la DM2 se han encontrado niveles mas bajos de amilina. No obstante, estos se han relacionado con la insulinoterapia. Los sujetos con DM2 y mayores niveles de amilina parecen ser los que no reciben insulina. Además, se han observado niveles más altos de amilina en sujetos sanos no diabéticos con intolerancia oral a la glucosa [102]. Dentro del grupo de los controles, observamos menor amilina en el subgrupo de mayor edad respecto a los controles más jóvenes.

Así como en el envejecimiento se han observado niveles más bajos de insulina por pérdida de la función de las células β pancreáticas [103], el efecto del envejecimiento sobre los niveles de amilina es controvertido [104, 105]. Dada la co-secreción pancreática de ambas hormonas y la interregulación entre ellas [29, 30], podríamos considerar por nuestros resultados que quizás la amilina sigue el mismo proceso que la insulina, es decir, disminuyendo sus niveles con el envejecimiento normal debido a una menor producción relacionada con la pérdida de células β pancreáticas.

Como se ha comentado previamente, estudios moleculares *in vitro* y patológicos han observado una interacción entre la amilina y la aS [37]

que podría ser unidireccional, favoreciendo la presencia de amilina la formación de amiloide de aS pero no a la inversa [27].

En esta línea, se ha documentado la capacidad de la amilina periférica de atravesar la barrera hematoencefálica y también la asociación entre hiperamilinemia con depósitos de amiloide de amilina cerebral en estudios de experimentación animal [106]. Además, se ha sugerido a la hiperamilinemia como factor de riesgo en el desarrollo de la EA [34]. Considerando estos hechos, podríamos hipotetizar que nuestros hallazgos de niveles de amilina plasmática más elevados en la EP podrían conducir a un mayor depósito de amilina a nivel cerebral y por tanto a una mayor interacción con la aS, promoviendo su agregación y progresión de la enfermedad.

Además de los menores niveles de insulina plasmática en ayunas en la EP, observamos una mayor ratio de amilina/insulina plasmática en ayunas la EP respecto a los controles. En conjunto con los resultados por separado ya descritos de los niveles plasmáticos de insulina y amilina, debido a la co-secreción de ambas hormonas en las células β pancreáticas, esta ratio podría ser una medida de disociación en la secreción de insulina y amilina en pacientes con EP.

La asociación de la DM2 con una mayor severidad de la EP había sido previamente documentada. Principalmente la DM2 se ha asociado a mayor afectación motora (con fenotipos más graves de inestabilidad postural y trastorno de la marcha) y cognitiva, asociándose a más prevalencia de demencia [46, 82, 83, 87, 88]. La RI, por su parte, se ha correlacionado con un mayor deterioro cognitivo de la enfermedad [84, 85]. En nuestra cohorte, consideramos no haber encontrado estas asociaciones de forma directa debido principalmente tanto a ser la DM2 un criterio de exclusión como a no haber presentado una RI los sujetos con EP (niveles de HOMA-IR normales).

No obstante, en nuestro trabajo observamos en los sujetos con EP una correlación modesta pero significativa en la asociación entre los niveles de HOMA-IR y una mayor afectación no-motora en base a mayores puntuaciones en la escala NMSS. Asociaciones similares, como el síndrome

metabólico y la propia RI, se han relacionado con una mayor afectación de los síntomas no-motores tanto en estudios experimentales con animales [107] como en humanos siendo los dominios anímicos, perceptivos, sexuales, gastrointestinales y miscelánea los asociados con el síndrome metabólico [108]. La confirmación del efecto negativo de la RI sobre los síntomas no-motores y su mecanismo subyacente deberá analizarse de forma específica.

En el segundo artículo, objetivamos un mayor riesgo de EP posterior no sólo en la DM2, sino también en sujetos prediabéticos independientemente de otros factores. Los sub-análisis tanto por edad como por sexo demostraron que las mujeres y los sujetos jóvenes menores de 65 años presentan un riesgo mucho más elevado de desarrollo de EP en relación tanto a DM2 como a prediabetes. Además, observamos un riesgo de desarrollo de EP posterior asociado al sobrepeso y la obesidad de forma independiente a la DM2 y a la prediabetes. Este segundo artículo de la presente tesis doctoral motivó una carta de otros autores en referencia a los resultados obtenidos. Nuestra correspondencia en respuesta a la carta la adjuntamos en el anexo 2.

La asociación entre la DM2 y el riesgo posterior de EP ha sido documentada previamente en la literatura con resultados similares a los obtenidos en nuestro estudio [70,72,74-79]. Estos resultados han llevado a la MDS a considerar la DM2 como un marcador de riesgo de EP, destacando así su potencial importancia en la fase prodrómica de la EP [7]. No obstante, este es el primer estudio en el que se describe el análisis de la prediabetes como un marcador adicional de riesgo en la fase prediagnóstica de la EP.

En la línea de la prediabetes como posible marcador de riesgo en la EP prodrómica, un estudio previo asoció el estado prediabético con el riesgo de EP posterior en el trastorno de conducta del sueño REM posible (evaluado por cuestionarios, no por polisomnografía) [109]. Y, aunque no como marcador de riesgo, se ha descrito previamente en la literatura que sujetos con la EP de reciente diagnóstico y sin tratamiento dopaminérgico, ya presentan niveles de glucemia en ayunas diagnósticos de prediabetes,

sugiriendo por lo tanto su posible relación en fases tempranas de la enfermedad [51].

El aumento de riesgo para el desarrollo posterior de EP debido a la presencia de DM2 en sujetos jóvenes había sido descrito previamente [62, 75, 78]. A nivel endocrinológico, se ha asociado a los sujetos jóvenes prediabéticos con un mayor riesgo de desarrollo de DM2 debido a déficits de insulina, contrariamente a lo que sucede en sujetos más mayores donde predomina la mayor RI [110].

Además, nuestros resultados muestran un mayor riesgo de EP en las mujeres tanto con DM2 como con prediabetes. Estos hallazgos son similares a los observados en la mayoría de la literatura en relación a la DM2 [62, 70, 75-78]. Es importante recordar que, clásicamente, se ha relacionado un mayor riesgo de EP en el sexo masculino. Por contra, la asociación de las alteraciones metabólicas y el riesgo que comportan en el desarrollo de EP posterior es más acusada en el sexo femenino. Por ello, deberían analizarse específicamente los factores fisiopatológicos implicados en el aumento de esta asociación epidemiológica en la mujer.

Así mismo, constatar que tanto el sobrepeso como la obesidad se asociaron en nuestro trabajo con un mayor riesgo de desarrollo de EP independientemente del diagnóstico de DM2 o prediabetes. La relación entre aumento de IMC y la EP ha sido ampliamente discutida, observándose resultados contradictorios [111, 112]. Debido al vínculo establecido entre el sobrepeso y la obesidad con la DM2 y la prediabetes, consideramos nuestros resultados concordantes en este aspecto [113].

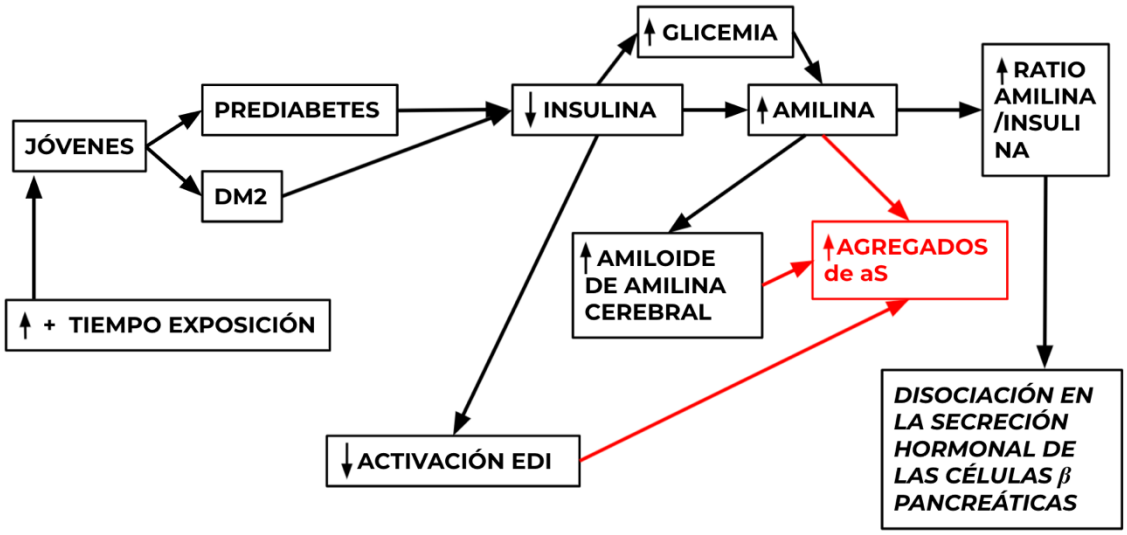
Como hemos visto a lo largo de la discusión, las alteraciones del MG están presentes en la EP, y los estados prediabéticos o diabéticos comportan un riesgo para el posterior desarrollo de la enfermedad. Las alteraciones analíticas más destacables son los menores niveles plasmáticos en ayunas de insulina junto con un aumento de amilina. Los pacientes con prediabetes o DM2, especialmente de sexo femenino y los jóvenes, asocian un mayor riesgo de desarrollo de EP posterior.

Una hipótesis plausible (representada esquemáticamente en la ***figura 2***) en base a los resultados obtenidos en los trabajos de la presente tesis

doctoral aunando la literatura previa en este campo, empieza al objetivar en nuestro medio que son los sujetos jóvenes con prediabetes y DM2 aquellos con un mayor riesgo de desarrollo posterior de EP. Los factores metabólicos como niveles más bajos de insulina y posiblemente factores genéticos junto con el depósito de proteínas amiloides [27,37], así como una esperanza de vida prolongada que permite más tiempo de exposición, podría contribuir a esta asociación. Consideramos, por lo tanto, en base a la literatura, que estos sujetos jóvenes presentan menores niveles de insulina que predisponen al desarrollo de la DM2 [21, 110].

Como se ha descrito en la literatura, los menores niveles de insulina conllevan a una menor activación de la EDI, pudiendo comportar a su vez una mayor agregación de aS [38, 39]. Los menores niveles de insulina junto con mayores niveles de amilina, como hemos descrito en nuestro trabajo, pueden estar íntimamente relacionados debido a la co-secreción de ambas hormonas en las células β pancreáticas con diversas funciones y, entre ellas, la del mantenimiento de la euglicemia [29, 30]. Estos mayores niveles de amilina junto con menores niveles de insulina comportan un aumento de la ratio amilina/insulina que traduciría una disociación en la secreción pancreática de ambas proteínas en la EP. También se considera que la amilina pueda llegar a inhibir indirectamente la secreción de insulina, autorregulándose entre ambas hormonas a nivel pancreático [114]. Por último, los mayores niveles de amilina comportarían también una mayor agregación de aS, influyendo de esta manera en la progresión de la EP [27].

FIGURA 2. HIPÓTESIS DE LA RELACIÓN ENTRE LA DM2 Y LA EP: LA TEORIA DE LAS PROTEINAS AMILOIDES Y EL DÉFICIT INSULÍNICO. DIAGRAMA PROPUESTO.



VI. CONCLUSIONES

CONCLUSIONES

1. En nuestra cohorte clínica, los pacientes con enfermedad de Parkinson presentan un metabolismo de la glucosa alterado respecto a los controles, destacando menores niveles plasmáticos de insulina y, en los sujetos de mayor edad, niveles más elevados de amilina plasmática.

2. Así mismo, hemos encontrado una asociación entre resistencia creciente a la insulina y mayor sintomatología no-motora en la enfermedad de Parkinson.

3. En nuestro estudio epidemiológico de la población catalana, tanto la diabetes mellitus tipo 2 como la prediabetes son factores de riesgo de la enfermedad de Parkinson.

4. Esta susceptibilidad al desarrollo de la enfermedad de Parkinson en presencia de diabetes mellitus tipo 2 y prediabetes es más acusada en mujeres y sujetos jóvenes.

VII. BIBLIOGRAFÍA

BIBLIOGRAFÍA

1. *GBD 2016 Parkinson's Disease Collaborators. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2018 Nov;17(11):939-953. doi: 10.1016/S1474-4422(18)30295-3. Epub 2018 Oct 1. PMID: 30287051*
2. *GBD 2015 Neurological Disorders Collaborator Group. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017 Nov;16(11):877-897. doi: 10.1016/S1474-4422(17)30299-5. Epub 2017 Sep 17. PMID: 28931491.*
3. *Benito-León J, Bermejo-Pareja F, Morales-González JM, Porta-Etessam J, Trincado R, Vega S, et al.; Neurological Disorders in Central Spain (NEDICES) Study Group. Incidence of Parkinson disease and parkinsonism in three elderly populations of central Spain. Neurology. 2004 Mar 9;62(5):734-41. doi: 10.1212/01.wnl.0000113727.73153.68. PMID: 15007123.*
4. *Wanneveich M, Moisan F, Jacqmin-Gadda H, Elbaz A, Joly P. Projections of prevalence, lifetime risk, and life expectancy of Parkinson's disease (2010-2030) in France. Mov Disord. 2018 Sep;33(9):1449-1455. doi: 10.1002/mds.27447. Epub 2018 Aug 25. PMID: 30145805.*
5. *Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015 Aug 29;386(9996):896-912. doi: 10.1016/S0140-6736(14)61393-3. Epub 2015 Apr 19. PMID: 25904081.*
6. *Noyce AJ, Lees AJ, Schrag AE. The prediagnostic phase of Parkinson's disease. J Neurol Neurosurg Psychiatry. 2016 Aug;87(8):871-8. doi: 10.1136/jnnp-2015-311890. Epub 2016 Jan 11. PMID: 26848171.*
7. *Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB; MDS Task Force on the Definition of Parkinson's Disease. Update of the MDS research criteria for prodromal Parkinson's disease. Mov Disord. 2019 Oct;34(10):1464-1470. doi: 10.1002/mds.27802. Epub 2019 Aug 14. PMID: 31412427.*
8. *Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015 Oct;30(12):1591-601. doi: 10.1002/mds.26424. PMID: 26474316.*
9. *Dickson DW. Neuropathology of Parkinson disease. Parkinsonism Relat Disord. 2018 Jan;46 Suppl 1(Suppl 1):S30-S33. doi: 10.1016/j.parkreldis.2017.07.033. Epub 2017 Aug 1. PMID: 28780180.*
10. *Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol. 1997;38 Suppl 2:2-7. doi: 10.1159/000113469. PMID: 9387796.*
11. *Del Tredici K, Hawkes CH, Ghebremedhin E, Braak H. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acta Neuropathol. 2010 Jun;119(6):703-13. doi: 10.1007/s00401-010-0665-2. Epub 2010 Mar 14. PMID: 20229352.*
12. *Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol. 2012 Dec;72(6):893-901. doi: 10.1002/ana.23687. Epub 2012 Oct 15. PMID: 23071076.*

13. *Winder-Rhodes SE, Evans JR, Ban M, Mason SL, Williams-Gray CH, Foltynie T, et al. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. Brain. 2013 Feb;136(Pt 2):392-9. doi: 10.1093/brain/aws318. PMID: 23413260.*
14. *Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al.; International LRRK2 Consortium. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol. 2008 Jul;7(7):583-90. doi: 10.1016/S1474-4422(08)70117-0. Epub 2008 Jun 6. PMID: 18539534*
15. *Edwards M., Stamelou M., Quinn N., Bhatia, K. Parkinson's Disease and other Movements Disorders. Oxford Specialist Handbooks in Neurology, 2016.*
16. *Stern MB, Lang A, Poewe W. Toward a redefinition of Parkinson's disease. Mov Disord. 2012 Jan;27(1):54-60. doi: 10.1002/mds.24051. PMID: 22252891.*
17. *Berg D, Postuma RB, Bloem B, Chan P, Dubois B, Gasser T, et al. Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease. Mov Disord. 2014 Apr;29(4):454-62. doi: 10.1002/mds.25844. Epub 2014 Mar 11. PMID: 24619848*
18. *Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, et al. MDS research criteria for prodromal Parkinson's disease. Mov Disord. 2015 Oct;30(12):1600-11. doi: 10.1002/mds.26431. PMID: 26474317.*
19. *Santiago JA, Potashkin JA. Shared dysregulated pathways lead to Parkinson's disease and diabetes. Trends Mol Med. 2013 Mar;19(3):176-86. doi: 10.1016/j.molmed.2013.01.002. Epub 2013 Jan 31. PMID: 23375873.*
20. *Athauda D, Foltynie T. Insulin resistance and Parkinson's disease: A new target for disease modification? Prog Neurobiol. 2016 Oct-Nov;145-146:98-120. doi: 10.1016/j.pneurobio.2016.10.001. Epub 2016 Oct 3. PMID: 27713036.*
21. *Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017 Jun 3;389(10085):2239-2251. doi: 10.1016/S0140-6736(17)30058-2. Epub 2017 Feb 10. Erratum in: Lancet. 2017 Jun 3;389(10085):2192. PMID: 28190580.*
22. *Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. PLoS One. 2018 Mar 20;13(3):e0194127. doi: 10.1371/journal.pone.0194127. PMID: 29558518.*
23. *Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. Lancet. 2012 Jun 16;379(9833):2279-90. doi: 10.1016/S0140-6736(12)60283-9. Epub 2012 Jun 9. PMID: 22683128.*
24. *Rett K, Gottwald-Hostalek U. Understanding prediabetes: definition, prevalence, burden and treatment options for an emerging disease. Curr Med Res Opin. 2019 Sep;35(9):1529-1534. doi: 10.1080/03007995.2019.1601455. Epub 2019 Apr 15. PMID: 30935247.*
25. *American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018 Jan;41(Suppl 1):S13-S27. doi: 10.2337/dc18-S002. PMID: 29222373.*
26. *International Diabetes Federation. IDF Diabetes Atlas, 9th edn. 2019. http://www.diabetesatlas.org/*

27. *Horvath I, Wittung-Stafshede P. Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease. Proc Natl Acad Sci U S A. 2016 Nov 1;113(44):12473-12477. doi: 10.1073/pnas.1610371113. Epub 2016 Oct 17. PMID: 27791129.*
28. *Haataja L, Gurlo T, Huang CJ, Butler PC. Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocr Rev. 2008 May;29(3):303-16. doi: 10.1210/er.2007-0037. Epub 2008 Feb 26. PMID: 18314421.*
29. *Visa M, Alcarraz-Vizán G, Montane J, Cadavez L, Castaño C, Villanueva-Peñacarrillo ML, et al. Islet amyloid polypeptide exerts a novel autocrine action in β-cell signaling and proliferation. FASEB J. 2015 Jul;29(7):2970-9. doi: 10.1096/fj.15-270553. Epub 2015 Mar 25. PMID: 25808537.*
30. *Fawver JN, Ghiwot Y, Koola C, Carrera W, Rodriguez-Rivera J, Hernandez C, et al. Islet amyloid polypeptide (IAPP): a second amyloid in Alzheimer's disease. Curr Alzheimer Res. 2014;11(10):928-40. doi: 10.2174/1567205011666141107124538. PMID: 25387341.*
31. *Bharadwaj P, Wijesekara N, Liyanapathirana M, Newsholme P, Ittner L, Fraser P, et al. The Link between Type 2 Diabetes and Neurodegeneration: Roles for Amyloid-β, Amylin, and Tau Proteins. J Alzheimers Dis. 2017;59(2):421-432. doi: 10.3233/JAD-161192. PMID: 28269785.*
32. *Zhu H, Wang X, Wallack M, Li H, Carreras I, Dedeoglu A, et al. Intraperitoneal injection of the pancreatic peptide amylin potently reduces behavioral impairment and brain amyloid pathology in murine models of Alzheimer's disease. Mol Psychiatry. 2015 Feb;20(2):252-62. doi: 10.1038/mp.2014.17. Epub 2014 Mar 11. PMID: 24614496.*
33. *Martinez-Valbuena I, Valenti-Azcarate R, Amat-Villegas I, Riverol M, Marcilla I, de Andrea CE, et al. Amylin as a potential link between type 2 diabetes and alzheimer disease. Ann Neurol. 2019 Oct;86(4):539-551. doi: 10.1002/ana.25570. Epub 2019 Aug 19. PMID: 31376172.*
34. *Zhu H, Tao Q, Ang TFA, Massaro J, Gan Q, Salim S, et al. Association of Plasma Amylin Concentration With Alzheimer Disease and Brain Structure in Older Adults. JAMA Netw Open. 2019 Aug 2;2(8):e199826. doi: 10.1001/jamanetworkopen.2019.9826. PMID: 31433485.*
35. *Schultz N, Janelidze S, Byman E, Minthon L, Nägga K, Hansson O, et al. Levels of islet amyloid polypeptide in cerebrospinal fluid and plasma from patients with Alzheimer's disease. PLoS One. 2019 Jun 17;14(6):e0218561. doi: 10.1371/journal.pone.0218561. PMID: 31206565.*
36. *Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28;388(6645):839-40. doi: 10.1038/42166. PMID: 9278044.*
37. *Martinez-Valbuena I, Amat-Villegas I, Valenti-Azcarate R, Carmona-Abellan MDM, Marcilla I, Tuñon MT, et al. Interaction of amyloidogenic proteins in pancreatic β cells from subjects with synucleinopathies. Acta Neuropathol. 2018 Jun;135(6):877-886. doi: 10.1007/s00401-018-1832-0. Epub 2018 Mar 13. PMID: 29536165.*
38. *Sharma SK, Chorell E, Wittung-Stafshede P. Insulin-degrading enzyme is activated by the C-terminus of α-synuclein. Biochem Biophys Res Commun. 2015 Oct 16;466(2):192-5. doi: 10.1016/j.bbrc.2015.09.002. Epub 2015 Sep 4. PMID: 26343304.*

39. *Sharma SK, Chorell E, Steneberg P, Vernersson-Lindahl E, Edlund H, Wittung-Stafshede P. Insulin-degrading enzyme prevents α-synuclein fibril formation in a nonproteolytical manner. Sci Rep. 2015 Jul 31;5:12531. doi: 10.1038/srep12531. PMID: 26228656*
40. *Havrankova J, Schmechel D, Roth J, Brownstein M. Identification of insulin in rat brain. Proc Natl Acad Sci U S A. 1978 Nov;75(11):5737-41. doi: 10.1073/pnas.75.11.5737. PMID: 364489*
41. *Athauda D, Foltynie T. Insulin resistance and Parkinson's disease: A new target for disease modification? Prog Neurobiol. 2016 Oct-Nov;145-146:98-120. doi: 10.1016/j.pneurobio.2016.10.001. Epub 2016 Oct 3. PMID: 27713036.*
42. *Warashina M, Terashima K, Sanosaka T, Nakashima K, Gage FH, Asashima M. Insulin biosynthesis in neuronal progenitors derived from adult hippocampus and the olfactory bulb. EMBO Mol Med. 2011 Dec;3(12):742-54. doi: 10.1002/emmm.201100177. Epub 2011 Oct 10. PMID: 21984534*
43. *Cheong JLY, de Pablo-Fernandez E, Foltynie T, Noyce AJ. The Association Between Type 2 Diabetes Mellitus and Parkinson's Disease. J Parkinsons Dis. 2020;10(3):775-789. doi: 10.3233/JPD-191900. PMID: 32333549*
44. *Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9. doi: 10.1007/BF00280883. PMID: 3899825.*
45. *Sartorius T, Peter A, Heni M, Maetzler W, Fritsche A, Häring HU, et al. The brain response to peripheral insulin declines with age: a contribution of the blood-brain barrier? PLoS One. 2015 May 12;10(5):e0126804. doi: 10.1371/journal.pone.0126804. PMID: 25965336.*
46. *Sandyk R. The relationship between diabetes mellitus and Parkinson's disease. Int J Neurosci. 1993 Mar-Apr;69(1-4):125-30. doi: 10.3109/00207459309003322. PMID: 8082998.*
47. *Hogg E, Athreya K, Basile C, Tan EE, Kaminski J, Tagliati M. High Prevalence of Undiagnosed Insulin Resistance in Non-Diabetic Subjects with Parkinson's Disease. J Parkinsons Dis. 2018;8(2):259-265. doi: 10.3233/JPD-181305. PMID: 29614702.*
48. *Morris JK, Vidoni ED, Perea RD, Rada R, Johnson DK, Lyons K, et al. Insulin resistance and gray matter volume in neurodegenerative disease. Neuroscience. 2014 Jun 13;270:139-47. doi: 10.1016/j.neuroscience.2014.04.006. Epub 2014 Apr 13. PMID: 24735819.*
49. *Zhao WQ, Chen H, Quon MJ, Alkon DL. Insulin and the insulin receptor in experimental models of learning and memory. Eur J Pharmacol. 2004 Apr 19;490(1-3):71-81. doi: 10.1016/j.ejphar.2004.02.045. PMID: 15094074.*
50. *Takahashi M, Yamada T, Tooyama I, Moroo I, Kimura H, Yamamoto T, et al. Insulin receptor mRNA in the substantia nigra in Parkinson's disease. Neurosci Lett. 1996 Feb 9;204(3):201-4. doi: 10.1016/0304-3940(96)12357-0. PMID: 8938265.*
51. *Tong M, Dong M, de la Monte SM. Brain insulin-like growth factor and neurotrophin resistance in Parkinson's disease and dementia with Lewy bodies: potential role of manganese neurotoxicity. J Alzheimers Dis. 2009;16(3):585-99. doi: 10.3233/JAD-2009-0995. PMID: 19276553*

52. *Bassil F, Delamarre A, Canron MH, Dutheil N, Vital A, Négrier-Leibreich ML, et al. Impaired brain insulin signalling in Parkinson's disease. Neuropathol Appl Neurobiol. 2021 Aug 17. doi: 10.1111/nan.12760. Epub ahead of print. PMID: 34405431.*
53. *Santiago JA, Potashkin JA. Blood Biomarkers Associated with Cognitive Decline in Early Stage and Drug-Naive Parkinson's Disease Patients. PLoS One. 2015 Nov 13;10(11):e0142582. doi: 10.1371/journal.pone.0142582. PMID: 26566043.*
54. *Mollenhauer B, Zimmermann J, Sixel-Döring F, Focke NK, Wicke T, Ebentheuer J, et al. DeNoPa Study Group. Baseline predictors for progression 4 years after Parkinson's disease diagnosis in the De Novo Parkinson Cohort (DeNoPa). Mov Disord. 2019 Jan;34(1):67-77. doi: 10.1002/mds.27492. Epub 2018 Nov 23. PMID: 30468694.*
55. *Chung HS, Lee JS, Kim JA, Roh E, Lee YB, Hong SH, et al. Fasting plasma glucose variability in midlife and risk of Parkinson's disease: A nationwide population-based study. Diabetes Metab. 2021 May;47(3):101195. doi: 10.1016/j.diabet.2020.08.006. Epub 2020 Oct 8. PMID: 33039669.*
56. *Marques A, Dutheil F, Durand E, Rieu I, Mulliez A, Fantini ML, et al. Glucose dysregulation in Parkinson's disease: Too much glucose or not enough insulin? Parkinsonism Relat Disord. 2018 Oct;55:122-127. doi: 10.1016/j.parkreldis.2018.05.026. Epub 2018 May 31. PMID: 29866628.*
57. *Aziz NA, Roos RAC, Pijl H. Insulin Sensitivity in De Novo Parkinson's Disease: A Hyperinsulinemic-Euglycemic Clamp Study. Mov Disord. 2020 Sep;35(9):1693-1694. doi: 10.1002/mds.28181. Epub 2020 Jun 30. PMID: 32602947.*
58. *Bartels AL, Willemsen AT, Doorduin J, de Vries EF, Dierckx RA, Leenders KL. [11C]-PK11195 PET: quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? Parkinsonism Relat Disord. 2010 Jan;16(1):57-9. doi: 10.1016/j.parkreldis.2009.05.005. Epub 2009 May 31. PMID: 19487152.*
59. *Chen H, O'Reilly EJ, Schwarzschild MA, Ascherio A. Peripheral inflammatory biomarkers and risk of Parkinson's disease. Am J Epidemiol. 2008 Jan 1;167(1):90-5. doi: 10.1093/aje/kwm260. Epub 2007 Sep 22. PMID: 17890755.*
60. *Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. Lancet. 1989 Jun 3;1(8649):1269. doi: 10.1016/s0140-6736(89)92366-0. PMID: 2566813.*
61. *Perier C, Tieu K, Guégan C, Caspersen C, Jackson-Lewis V, Carelli V, et al. Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. Proc Natl Acad Sci U S A. 2005 Dec 27;102(52):19126-31. doi: 10.1073/pnas.0508215102. Epub 2005 Dec 19. PMID: 16365298*
62. *Schernhammer E, Hansen J, Rugbjerg K, Wermuth L, Ritz B. Diabetes and the risk of developing Parkinson's disease in Denmark. Diabetes Care. 2011 May;34(5):1102-8. doi: 10.2337/dc10-1333. Epub 2011 Mar 16. PMID: 21411503*
63. *D'Amelio M, Ragonese P, Callari G, Di Benedetto N, Palmeri B, Terruso V, et al. Diabetes preceding Parkinson's disease onset. A case-control study. Parkinsonism Relat Disord. 2009 Nov;15(9):660-4. doi: 10.1016/j.parkreldis.2009.02.013. Epub 2009 Apr 7. PMID: 19356970.*
64. *Lu L, Fu DL, Li HQ, Liu AJ, Li JH, Zheng GQ. Diabetes and risk of Parkinson's disease: an updated meta-analysis of case-control studies. PLoS One. 2014 Jan 21;9(1):e85781. doi: 10.1371/journal.pone.0085781. PMID: 24465703*

65. *Gupta V, Garg RK, Pant KK, Khattri S. A study on risk factors for Parkinson's disease in Indian population. Bioinformation. 2014 Jun 30;10(6):342-6. doi: 10.6026/97320630010342. PMID: 25097376*
66. *De Pablo-Fernandez E, Sierra-Hidalgo F, Benito-León J, Bermejo-Pareja F. Association between Parkinson's disease and diabetes: Data from NEDICES study. Acta Neurol Scand. 2017 Dec;136(6):732-736. doi: 10.1111/ane.12793. Epub 2017 Jun 26. PMID: 28653373.*
67. *Wu MC, Xu X, Chen SM, Tyan YS, Chiou JY, Wang YH, et al. Impact of Sjogren's syndrome on Parkinson's disease: A nationwide case-control study. PLoS One. 2017 Jul 13;12(7):e0175836. doi: 10.1371/journal.pone.0175836. PMID: 28704397*
68. *Heilbron K, Noyce AJ, Fontanillas P, Alipanahi B, Nalls MA; 23andMe Research Team, Cannon P. The Parkinson's phenome-traits associated with Parkinson's disease in a broadly phenotyped cohort. NPJ Parkinsons Dis. 2019 Mar 27;5:4. doi: 10.1038/s41531-019-0077-5. PMID: 30937360*
69. *Komici K, Femminella GD, Bencivenga L, Rengo G, Pagano G. Diabetes Mellitus and Parkinson's Disease: A Systematic Review and Meta-Analyses. J Parkinsons Dis. 2021 Sep 1. doi: 10.3233/JPD-212725. Epub ahead of print. PMID: 34486987.*
70. *Hu G, Jousilahti P, Bidel S, Antikainen R, Tuomilehto J. Type 2 diabetes and the risk of Parkinson's disease. Diabetes Care. 2007 Apr;30(4):842-7. doi: 10.2337/dc06-2011. Epub 2007 Jan 24. PMID: 17251276.*
71. *Simon KC, Chen H, Schwarzschild M, Ascherio A. Hypertension, hypercholesterolemia, diabetes, and risk of Parkinson disease. Neurology. 2007 Oct 23;69(17):1688-95. doi: 10.1212/01.wnl.0000271883.45010.8a. Epub 2007 Aug 29. PMID: 17761552.*
72. *Driver JA, Smith A, Buring JE, Gaziano JM, Kurth T, Logroscino G. Prospective cohort study of type 2 diabetes and the risk of Parkinson's disease. Diabetes Care. 2008 Oct;31(10):2003-5. doi: 10.2337/dc08-0688. Epub 2008 Jul 3. PMID: 18599528.*
73. *Palacios N, Gao X, McCullough ML, Jacobs EJ, Patel AV, Mayo T, et al. Obesity, diabetes, and risk of Parkinson's disease. Mov Disord. 2011 Oct;26(12):2253-9. doi: 10.1002/mds.23855. Epub 2011 Jul 7. PMID: 21739472*
74. *Xu Q, Park Y, Huang X, Hollenbeck A, Blair A, Schatzkin A, et al. Diabetes and risk of Parkinson's disease. Diabetes Care. 2011 Apr;34(4):910-5. doi: 10.2337/dc10-1922. Epub 2011 Mar 4. PMID: 21378214*
75. *Sun Y, Chang YH, Chen HF, Su YH, Su HF, Li CY. Risk of Parkinson disease onset in patients with diabetes: a 9-year population-based cohort study with age and sex stratifications. Diabetes Care. 2012 May;35(5):1047-9. doi: 10.2337/dc11-1511. Epub 2012 Mar 19. PMID: 22432112*
76. *Yue X, Li H, Yan H, Zhang P, Chang L, Li T. Risk of Parkinson Disease in Diabetes Mellitus: An Updated Meta-Analysis of Population-Based Cohort Studies. Medicine (Baltimore). 2016 May;95(18):e3549. doi: 10.1097/MD.0000000000003549. PMID: 27149468; PMCID*
77. *Yang YW, Hsieh TF, Li CI, Liu CS, Lin WY, Chiang JH, et al. Increased risk of Parkinson disease with diabetes mellitus in a population-based study. Medicine (Baltimore). 2017 Jan;96(3):e5921. doi: 10.1097/MD.0000000000005921. PMID: 28099356.*
78. *De Pablo-Fernandez E, Goldacre R, Pakpoor J, Noyce AJ, Warner TT. Association between diabetes and subsequent Parkinson disease: A record-linkage cohort study.*

Neurology. 2018 Jul 10;91(2):e139-e142. doi: 10.1212/WNL.0000000000005771. Epub 2018 Jun 13. PMID: 29898968.

79. *Cereda E, Barichella M, Pedrolli C, Klersy C, Cassani E, Caccialanza R, et al. Diabetes and risk of Parkinson's disease: a systematic review and meta-analysis. Diabetes Care. 2011 Dec;34(12):2614-23. doi: 10.2337/dc11-1584. PMID: 22110170.*

80. *Rhee SY, Han KD, Kwon H, Park SE, Park YG, Kim YH, et al. Association Between Glycemic Status and the Risk of Parkinson Disease: A Nationwide Population-Based Study. Diabetes Care. 2020 Sep;43(9):2169-2175. doi: 10.2337/dc19-0760. Epub 2020 Jul 1. PMID: 32611610*
81. *SCHWAB RS. Progression and prognosis in Parkinson's disease. J Nerv Ment Dis. 1960 Jun;130:556-66. doi: 10.1097/00005053-196006000-00017. PMID: 14443930.*
82. *Pagano G, Polychronis S, Wilson H, Giordano B, Ferrara N, Niccolini F, et al. Diabetes mellitus and Parkinson disease. Neurology. 2018 May 8;90(19):e1654-e1662. doi: 10.1212/WNL.0000000000005475. Epub 2018 Apr 6. PMID: 29626177.*
83. *Cereda E, Barichella M, Cassani E, Caccialanza R, Pezzoli G. Clinical features of Parkinson disease when onset of diabetes came first: A case-control study. Neurology. 2012 May 8;78(19):1507-11. doi: 10.1212/WNL.0b013e3182553cc9. Epub 2012 Apr 25. PMID: 22539572.*
84. *Peng Z, Dong S, Tao Y, Huo Y, Zhou Z, Huang W, et al. Metabolic syndrome contributes to cognitive impairment in patients with Parkinson's disease. Parkinsonism Relat Disord. 2018 Oct;55:68-74. doi: 10.1016/j.parkreldis.2018.05.013. Epub 2018 May 16. PMID: 29908727.*
85. *Bosco D, Plastino M, Cristiano D, Colica C, Ermio C, De Bartolo M, et al. Dementia is associated with insulin resistance in patients with Parkinson's disease. J Neurol Sci. 2012 Apr 15;315(1-2):39-43. doi: 10.1016/j.jns.2011.12.008. Epub 2012 Jan 21. PMID: 22265943.*
86. *Bohnen NI, Kotagal V, Müller ML, Koeppe RA, Scott PJ, Albin RL, et al. Diabetes mellitus is independently associated with more severe cognitive impairment in Parkinson disease. Parkinsonism Relat Disord. 2014 Dec;20(12):1394-8. doi: 10.1016/j.parkreldis.2014.10.008. Epub 2014 Oct 15. PMID: 25454317.*
87. *Giuntini M, Baldacci F, Del Prete E, Bonuccelli U, Ceravolo R. Diabetes is associated with postural and cognitive domains in Parkinson's disease. Results from a single-center study. Parkinsonism Relat Disord. 2014 Jun;20(6):671-2. doi: 10.1016/j.parkreldis.2014.02.016. Epub 2014 Mar 5. PMID: 24685342.*
88. *Kotagal V, Albin RL, Müller ML, Koeppe RA, Frey KA, Bohnen NI. Diabetes is associated with postural instability and gait difficulty in Parkinson disease. Parkinsonism Relat Disord. 2013 May;19(5):522-6. doi: 10.1016/j.parkreldis.2013.01.016. Epub 2013 Feb 23. PMID: 23462483.*
89. *Connolly JG, Bykov K, Gagne JJ. Thiazolidinediones and Parkinson Disease: A Cohort Study. Am J Epidemiol. 2015 Dec 1;182(11):936-44. doi: 10.1093/aje/kwv109. Epub 2015 Oct 22. PMID: 26493264.*
90. *NINDS Exploratory Trials in Parkinson Disease (NET-PD) FS-ZONE Investigators. Pioglitazone in early Parkinson's disease: a phase 2, multicentre, double-blind, randomised trial. Lancet Neurol. 2015 Aug;14(8):795-803. doi: 10.1016/S1474-*

4422(15)00144-1. Epub 2015 Jun 23. Erratum in: Lancet Neurol. 2015 Oct; 14(10):979. PMID: 26116315.

91. *Wahlqvist ML, Lee MS, Hsu CC, Chuang SY, Lee JT, Tsai HN. Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with Type 2 diabetes in a Taiwanese population cohort. Parkinsonism Relat Disord. 2012 Jul;18(6):753-8. doi: 10.1016/j.parkreldis.2012.03.010. Epub 2012 Apr 10. PMID: 22498320.*
92. *Ping F, Jiang N, Li Y. Association between metformin and neurodegenerative diseases of observational studies: systematic review and meta-analysis. BMJ Open Diabetes Res Care. 2020 Jul;8(1):e001370. doi: 10.1136/bmjdrc-2020-001370. PMID: 32719079*
93. *Saewanee N, Praputpittaya T, Malaiwong N, Chalorak P, Meemon K. Neuroprotective effect of metformin on dopaminergic neurodegeneration and α-synuclein aggregation in C. elegans model of Parkinson's disease. Neurosci Res. 2021 Jan;162:13-21. doi: 10.1016/j.neures.2019.12.017. Epub 2019 Dec 24. PMID: 31881233.*
94. *Aviles-Olmos I, Dickson J, Kefalopoulou Z, Djamshidian A, Ell P, Soderlund T, et al. Exenatide and the treatment of patients with Parkinson's disease. J Clin Invest. 2013 Jun;123(6):2730-6. doi: 10.1172/JCI68295. PMID: 23728174*
95. *Aviles-Olmos I, Dickson J, Kefalopoulou Z, Djamshidian A, Kahan J, Ell P, et al. Motor and cognitive advantages persist 12 months after exenatide exposure in Parkinson's disease. J Parkinsons Dis. 2014;4(3):337-44. doi: 10.3233/JPD-140364. PMID: 24662192.*
96. *Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. Lancet. 2017 Oct 7;390(10103):1664-1675. doi: 10.1016/S0140-6736(17)31585-4. Epub 2017 Aug 3. PMID: 28781108*
97. *Athauda D, Maclagan K, Budnik N, Zampedri L, Hibbert S, Aviles-Olmos I, et al. Post hoc analysis of the Exenatide-PD trial-Factors that predict response. Eur J Neurosci. 2019 Feb;49(3):410-421. doi: 10.1111/ejn.14096. Epub 2018 Oct 6. PMID: 30070753.*
98. *Brauer R, Wei L, Ma T, Athauda D, Girges C, Vijiaratnam N, et al. Diabetes medications and risk of Parkinson's disease: a cohort study of patients with diabetes. Brain. 2020 Oct 1;143(10):3067-3076. doi: 10.1093/brain/awaa262. PMID: 33011770.*
99. *Pang Y, Lin S, Wright C, Shen J, Carter K, Bhatt A, et al. Intranasal insulin protects against substantia nigra dopaminergic neuronal loss and alleviates motor deficits induced by 6-OHDA in rats. Neuroscience. 2016 Mar 24;318:157-65. doi: 10.1016/j.neuroscience.2016.01.020. Epub 2016 Jan 14. PMID: 26777890*
100. *Iravanpour F, Dargahi L, Rezaei M, Haghani M, Heidari R, Valian N, et al. Intranasal insulin improves mitochondrial function and attenuates motor deficits in a rat 6-OHDA model of Parkinson's disease. CNS Neurosci Ther. 2021 Mar;27(3):308-319. doi: 10.1111/cns.13609. Epub 2021 Jan 26. PMID: 33497031*
101. *Novak P, Pimentel Maldonado DA, Novak V. Safety and preliminary efficacy of intranasal insulin for cognitive impairment in Parkinson disease and multiple system atrophy: A double-blinded placebo-controlled pilot study. PLoS One. 2019 Apr 25;14(4):e0214364. doi: 10.1371/journal.pone.0214364. PMID: 31022213*
102. *Sanke T, Hanabusa T, Nakano Y, Oki C, Okai K, Nishimura S, et al. Plasma islet amyloid polypeptide (Amylin) levels and their responses to oral glucose in type 2 (non-insulin-*

dependent) diabetic patients. Diabetologia. 1991 Feb;34(2):129-32. doi: 10.1007/BF00500385. PMID: 2065848.

103. *Sui J, Shi B, Hu Y, Deng M, Wang Y, He M, et al. Islet Function Changes Among the Elderly Population. Arch Med Res. 2019 Oct;50(7):468-475. doi: 10.1016/j.arcmed.2019.10.013. Epub 2020 Jan 17. PMID: 31958717.*
104. *Edwards BJ, Perry HM, Kaiser FE, Morley JE, Kraenzle D, Kreutter DK, et al. Age-related changes in amylin secretion. Mech Ageing Dev. 1996 Jan 5;86(1):39-51. doi: 10.1016/0047-6374(95)01664-3. PMID: 8866735.*
105. *Dechenes CJ, Verchere CB, Andrikopoulos S, Kahn SE. Human aging is associated with parallel reductions in insulin and amylin release. Am J Physiol. 1998 Nov;275(5):E785-91. doi: 10.1152/ajpendo.1998.275.5.E785. PMID: 9814997.*
106. *Lutz TA, Meyer U. Amylin at the interface between metabolic and neurodegenerative disorders. Front Neurosci. 2015 Jun 16;9:216. doi: 10.3389/fnins.2015.00216. PMID: 26136651*
107. *Bittencourt A, Brum PO, Ribeiro CT, Gasparotto J, Bortolin RC, de Vargas AR, et al. High fat diet-induced obesity causes a reduction in brain tyrosine hydroxylase levels and non-motor features in rats through metabolic dysfunction, neuroinflammation and oxidative stress. Nutr Neurosci. 2020 Oct 20:1-15. doi: 10.1080/1028415X.2020.1831261. PMID: 33078695.*
108. *Meléndez-Flores JD, Castillo-Torres SA, Cerda-Contreras C, Chávez-Luévanos B, Estrada-Bellmann I. Clinical features of metabolic syndrome in patients with Parkinson's disease. Rev Neurol. 2021 Jan 1;72(1):9-15. English, Spanish. doi: 10.33588/rn.7201.2020323. PMID: 33378074.*
109. *Wong JC, Li J, Pavlova M, Chen S, Wu A, Wu S, et al. Risk factors for probable REM sleep behavior disorder: A community-based study. Neurology. 2016 Apr 5;86(14):1306-1312. doi: 10.1212/WNL.0000000000002414. Epub 2016 Jan 27. PMID: 26819459*
110. *Pani LN, Nathan DM, Grant RW. Clinical predictors of disease progression and medication initiation in untreated patients with type 2 diabetes and A1C less than 7%. Diabetes Care. 2008 Mar;31(3):386-90. doi: 10.2337/dc07-1934. Epub 2007 Dec 14. PMID: 18083790.*
111. *Chen J, Guan Z, Wang L, Song G, Ma B, Wang Y. Meta-analysis: overweight, obesity, and Parkinson's disease. Int J Endocrinol. 2014;2014:203930. doi: 10.1155/2014/203930. Epub 2014 Feb 5. Erratum in: Int J Endocrinol. 2014;2014:306402. PMID: 24672544.*
112. *Noyce AJ, Kia DA, Hemani G, Nicolas A, Price TR, De Pablo-Fernandez E, et al. Estimating the causal influence of body mass index on risk of Parkinson disease: A Mendelian randomisation study. PLoS Med. 2017 Jun 13;14(6):e1002314. doi: 10.1371/journal.pmed.1002314. PMID: 28609445.*
113. *Laaksonen MA, Knekt P, Rissanen H, Härkänen T, Virtala E, Marniemi J, et al. The relative importance of modifiable potential risk factors of type 2 diabetes: a meta-analysis of two cohorts. Eur J Epidemiol. 2010 Feb;25(2):115-24. doi: 10.1007/s10654-009-9405-0. Epub 2009 Dec 13. PMID: 20012885.*
114. *Castillo MJ, Scheen AJ, Lefèbvre PJ. Amylin/islet amyloid polypeptide: biochemistry, physiology, patho-physiology. Diabete Metab. 1995 Feb;21(1):3-25. PMID: 7781840.*

VIII. ANEXOS

8.1

ANEXO 1

EDITORIAL DE OTROS AUTORES SOBRE EL PRIMER ARTÍCULO DE LA TESIS:

INSULIN RESISTANCE, DIABETES AND PARKINSON'S DISEASE: THE MATCH CONTINUES.

Parkinsonism and Related Disorders 80 (2020) 199–200

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Insulin resistance, diabetes and Parkinson's disease: The match continues

ARTICLE INFO

Keywords
Glucose metabolism
Insulin
Amylin

It is still a matter of debate whether diabetes increases the risk for Parkinson's disease (PD). Altogether, prospective cohort studies suggest an increased risk, while case-control studies have found the opposite [1,2]. Having said this, PD patients with concomitant diabetes show more severe disease and more rapid disease progression [3–5]. At the same time, epidemiological studies have reported a reduced incidence of PD in diabetes patients receiving dipeptidyl peptidase-4 inhibitors or thiazolidinediones for their diabetes [6,7]. In addition, a small, mono-centric, randomized, placebo-controlled treatment trial has shown a 3.5 point difference after 60 weeks on MDS-UPDRS motor OFF scores in favor of exenatide, a glucagon-like peptide 1 agonist that is approved for the treatment of diabetes [8].

Despite the increasing interest in the potential association between diabetes and PD in epidemiologic studies, not much is known about peripheral glucose metabolism in PD patients. Intriguingly, elevated plasma glucose concentrations predicted a lower risk for PD in one study [9]. Another study found insulin resistance as defined by a Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) value $\geq$ 2.0 or a glycated hemoglobin (HbA1c) concentration $\geq$ 5.7% in almost 60% of PD patients, with body mass index being a main driver of this observation [10]. Finally, a small, preliminary study using the hyperinsulinemic-euglycemic clamp technique, the gold standard to measure insulin sensitivity, found similar metabolic patterns in eight untreated de-novo PD patients compared to eight matched controls, except for slightly higher hepatic insulin resistance in PD patients.

In the October issue, Sanchez-Gomez et al. are reporting plasma levels of fasting glucose, fasting plasma insulin (FPI), fasting plasma amylin (FPA) and glycated haemoglobin in a cohort of 76 PD patients and 39 control subjects [11]. In addition, they have looked for potential associations between peripheral glucose metabolism with motor/non-motor symptoms and cognition in PD patients. None of the assessed markers was in favor of insulin resistance in PD patients, i.e. fasting glucose, glycated haemoglobin and HOMA-IR did not differ between groups. FPI levels were even lower in PD patients compared to controls, rather suggesting higher insulin sensitivity than insulin resistance. The HOMA-IR was moderately correlated to non-motor symptoms, with no association found with cognition or motor symptoms.

The authors further assessed blood amylin protein levels in PD patients. Indeed, amylin aggregates are found in the pancreas of diabetes patients [12] and an interaction between amylin and protein aggregation has been suggested in neurodegenerative diseases. For instance, amylin deposits have been found in Alzheimer's disease (AD) brains, co-localizing with amyloid beta and tau deposits [13,14]. An U-shaped relation has further been observed between plasma amylin levels and AD risk, with high amylin levels being protective compared to low and extremely high levels [15]. Another study found no differences in plasma and cerebrospinal fluid amylin levels between AD patients and controls, but observed distinct association patterns between amylin, tau and beta amyloid levels in AD patients compared to controls [16]. Pertaining to PD, preformed amylin amyloids promote the formation of α-synuclein amyloids in vitro and α-synuclein aggregates have been found in the pancreas of patients suffering from PD or Lewy body dementia, with some evidence suggesting a possible interaction between amylin and α-synuclein in patients bearing pancreatic phosphorylated α-synuclein inclusions [17,18]. While Sanchez-Gomez et al. have found no difference in FPA levels between controls and PD patients, the amylin/insulin ratio (FPAIR) was higher in the latter. The authors interpret this result as a dissociation between insulin and amylin secretion in PD, which are physiologically co-secreted by pancreatic beta cells [12]. Noteworthy, non-obese diabetes patients without insulin therapy show a lower FPAIR compared to controls and subjects with impaired glucose tolerance because of increased FPI levels [19]. Given the potential negative effect on α-synuclein aggregation in the pancreas and also in the brain of PD patients, it is worth to more closely explore the interaction between amylin and α-synuclein in future studies.

In conclusion, Sanchez-Gomez and colleagues provide here important data about peripheral glucose metabolism and plasma amylin levels in PD patients. They show some dysregulation without clear evidence for peripheral insulin resistance in PD patients. Altogether, the results are challenging those of previous studies and call for additional evidence to determine if peripheral insulin resistance is a relevant feature in PD

https://doi.org/10.1016/j.parkreldis.2020.10.013
Received 29 September 2020; Accepted 5 October 2020
Available online 6 October 2020

patients. Most importantly, the findings point to a relative upregulation of pancreatic amylin secretion with potential impact on the neurodegenerative process in PD.

References

[1] X. Yue, H. Li, H. Yan, P. Zhang, L. Chang, T. Li, Risk of Parkinson disease in diabetes mellitus: an updated meta-analysis of population-based cohort studies, Medicine (Baltim.) 95 (18) (2016) e3549.

[2] L. Lu, D.L. Fu, H.Q. Li, A.J. Liu, J.H. Li, G.Q. Zheng, Diabetes and risk of Parkinson's disease: an updated meta-analysis of case-control studies, PloS One 9 (1) (2014), e85781.

[3] G. Pagano, S. Polychronis, H. Wilson, B. Giordano, N. Ferrara, F. Niccolini, M. Politis, Diabetes mellitus and Parkinson disease, Neurology 90 (19) (2018) e1654–e1662.

[4] M. Ong, H. Foo, R.J. Chander, M.C. Wen, W.L. Au, Y.Y. Sitoh, L. Tan, N. Kandiah, Influence of diabetes mellitus on longitudinal atrophy and cognition in Parkinson's disease, J. Neurol. Sci. 377 (2017) 122–126.

[5] B. Mollenhauer, J. Zimmermann, F. Sixel-Doring, N.K. Focke, T. Wicke, J. Ebentheuer, M. Schaumburg, E. Lang, T. Friede, C. Trenkwalder, G. DeNoPa Study, Baseline predictors for progression 4 years after Parkinson's disease diagnosis in the de novo Parkinson cohort (DeNoPa), Mov. Disord. 34 (1) (2019) 67–77.

[6] P. Svenningsson, K. Wirdefeldt, L. Yin, F. Fang, I. Markaki, S. Efendic, J. F. Ludvigsson, Reduced incidence of Parkinson's disease after dipeptidyl peptidase-4 inhibitors-A nationwide case-control study, Mov. Disord. 31 (9) (2016) 1422–1423.

[7] Y. Zhu, J. Pu, Y. Chen, B. Zhang, Decreased risk of Parkinson's disease in diabetic patients with thiazolidinediones therapy: an exploratory meta-analysis, PloS One 14 (10) (2019), e0224236.

[8] D. Athauda, K. Maclagan, S.S. Skene, M. Bajwa-Joseph, D. Letchford, K. Chowdhury, S. Hibbert, N. Budnik, L. Zampedri, J. Dickson, Y. Li, I. Aviles-Olmos, T.T. Warner, P. Limousin, A.J. Lees, N.H. Greig, S. Tebbs, T. Foltynie, Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial, Lancet 390 (10103) (2017) 1664–1675.

[9] K. Saaksjarvi, P. Knekt, S. Mannisto, J. Lyytinen, M. Heliovaara, Prospective study on the components of metabolic syndrome and the incidence of Parkinson's disease, Park. Relat. Disord. 21 (10) (2015) 1148–1155.

[10] E. Hogg, K. Athreya, C. Basile, E.E. Tan, J. Kaminski, M. Tagliati, High prevalence of undiagnosed insulin resistance in non-diabetic subjects with Parkinson's disease, J. Parkinsons Dis. 8 (2) (2018) 259–265.

[11] A Sánchez-Gómez, G Alcarraz-Vizán, M Fernández, R Fernández-Santiago, M Ezquerra, A Cámara, M Serrano, A Novials, E Muñoz, F Valldeoriola, Y Compta, MJ Martí, Peripheral insulin and amylin levels in Parkinson's disease, Parkinsonism Relat Disord 79 (2020) 91–96.

[12] A. Kanatsuka, S. Kou, H. Makino, IAPP/amylin and beta-cell failure: implication of the risk factors of type 2 diabetes, Diabetol Int 9 (3) (2018) 143–157.

[13] K. Jackson, G.A. Barisone, E. Diaz, L.W. Jin, C. DeCarli, F. Despa, Amylin deposition in the brain: a second amyloid in Alzheimer disease? Ann. Neurol. 74 (4) (2013) 517–526.

[14] I. Martinez-Valbuena, R. Valenti-Azcarate, I. Amat-Villegas, M. Riverol, I. Marcilla, C.E. de Andrea, J.A. Sanchez-Arias, M. Del Mar Carmona-Abellan, G. Marti, M. E. Erro, E. Martinez-Vila, M.T. Tunon, M.R. Luquin, Amylin as a potential link between type 2 diabetes and alzheimer disease, Ann. Neurol. 86 (4) (2019) 539–551.

[15] H. Zhu, Q. Tao, T.F.A. Ang, J. Massaro, Q. Gan, S. Salim, R.Y. Zhu, V. B. Kolachalama, X. Zhang, S. Devine, S.H. Auerbach, C. DeCarli, R. Au, W.Q. Qiu, Association of plasma amylin concentration with alzheimer disease and brain structure in older adults, JAMA Netw Open 2 (8) (2019), e199826.

[16] N. Schultz, S. Janelidze, E. Byman, L. Minthon, K. Nagga, O. Hansson, M. Wennstrom, Levels of islet amyloid polypeptide in cerebrospinal fluid and plasma from patients with Alzheimer's disease, PloS One 14 (6) (2019), e0218561.

[17] I. Horvath, P. Wittung-Stafshede, Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 113 (44) (2016) 12473–12477.

[18] I. Martinez-Valbuena, I. Amat-Villegas, R. Valenti-Azcarate, M.D.M. Carmona-Abellan, I. Marcilla, M.T. Tunon, M.R. Luquin, Interaction of amyloidogenic proteins in pancreatic beta cells from subjects with synucleinopathies, Acta Neuropathol. 135 (6) (2018) 877–886.

[19] T. Sanke, T. Hanabusa, Y. Nakano, C. Oki, K. Okai, S. Nishimura, M. Kondo, K. Nanjo, Plasma islet amyloid polypeptide (Amylin) levels and their responses to oral glucose in type 2 (non-insulin-dependent) diabetic patients, Diabetologia 34 (2) (1991) 129–132.

Anna Delamarre[a,b], Vincent Rigalleau[c], Wassilios G. Meissner[a,b,d,e,*]

[a] *Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, 33000, Bordeaux, France*

[b] *CNRS, Institut des Maladies Neurodégénératives, UMR 5293, 33000, Bordeaux, France*

[c] *Service de Diabétologie, CHU Bordeaux, 33604, Pessac, France*

[d] *Service de Neurologie des Maladies Neurodégénératives, CHU Bordeaux, 33000, Bordeaux, France*

[e] *Dept. Medicine, University of Otago, Christchurch, and New Zealand Brain Research Institute, Christchurch, New Zealand*

[*] Corresponding author. Service de Neurologie des Maladies Neurodégénératives, Hôpital Pellegrin, CHU Bordeaux, Place Amélie Raba Léon, 33076, Bordeaux Cedex, France.

E-mail address: wassilios.meissner@chu-bordeaux.fr (W.G. Meissner).

8.2

ANEXO 2

CORRESPONDENCIA EN RESPUESTA A LA CARTA DE OTROS AUTORES EN REFERENCIA AL SEGUNDO ARTÍCULO DE LA TESIS:

INSULIN-RELEASING OR INSULIN-SENSITIZING DRUGS IN PARKINSON'S DISEASE? CHOOSING A PATHWAY.

ARTICLE IN PRESS

Parkinsonism and Related Disorders xxx (xxxx) xxx

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Insulin-releasing or insulin-sensitizing drugs in Parkinson's disease? Choosing a pathway

ARTICLE INFO

Keywords
Parkinson's disease
Type 2 diabetes mellitus
Insulin
Amylin

ABSTRACT

There are several reports in the literature showing an epidemiological and clinical association between type 2 diabetes mellitus and Parkinson's disease (PD). This notwithstanding, many aspects of the pathophysiological links between both diseases remain elusive. Filling this knowledge gap is important to address issues such as whether some antidiabetic drugs can be potential disease-modifying agents in PD.

Dear Editor,

We appreciate the comments made by Dr Cerasa and Dr Pagano on their letter *Sweet as Parkinson's disease: rethinking the impact of diabetes mellitus.* As these authors say, advances in research into the link between type 2 diabetes mellitus (T2D) and Parkinson's disease (PD) has gained growing interest in recent years.

In addition to the mechanisms previously shown to be shared by both conditions, such as inflammation, oxidative stress and dopaminergic dysfunction caused by chronic hyperglycemia, higher levels of fasting plasma amylin in old PD patients compared to controls of the same age might be new player [1]. Amylin is the protein of amyloid deposit of T2D and is co-secreted with insulin in the pancreatic β-cells. Both hormones share roles in regulating blood glucose levels, and amylin has been implicated also in the inhibition of insulin secretion. We have observed lower fasting plasma insulin levels in PD subjects compared to controls. The higher fasting plasma amylin/insulin ratio observed in PD subjects compared to controls therefore suggested a dissociation in insulin and amylin secretion in PD patients [1].

An *in vitro* work has shown that the presence of amylin accelerates the amyloid formation of α-synuclein (aS), but not the other way around, which suggests an *in vitro* justification of a unidirectional link with an influence of T2D on PD [2]. Besides, a pathological study found deposits of phosphorylated aS in pancreatic β cells from subjects with a neuropathological diagnosis of α-synucleinopathy, and also revealed a direct interaction between amylin and aS [3]. Therefore, a possible up-regulation of amylin could have an impact on neurodegeneration through the aS pathway.

Due to shared biological mechanisms between T2D and PD in addition to the positive clinical and epidemiological associations described, currently an interesting hypothesis is that of a potential neuroprotective role of antidiabetic drugs in PD.

Different studies have analyzed the effect of oral antidiabetics in PD. The thiazolidinediones group has been analyzed in a clinical trial following positive preliminary studies, where pioglitazone at different doses did not show a significant benefit on the motor symptoms of patients with early PD. With other oral antidiabetic drugs, in retrospective studies, the results have been contradictory: the combination of metformin and a sulphonylurea suggested a lower risk of PD, while metformin alone was associated with a higher risk of PD development. Despite this, animal models with metformin have suggested a neuroprotective effect by decreasing neurodegeneration and aS aggregation [4].

One of the antidiabetic drugs that has attracted attention in recent years is exenatide (glucagon agonist like-peptide 1), due to the positive result in a clinical trial where a slight motor improvement was observed in the OFF state [5]. In post hoc analyzes, the best motor response to exenatide occurred in subjects with a dominant tremor phenotype and lower scores on the MDS-UPDRS scale [6].

Regarding the possible insufficient secretion of insulin in patients with PD [1], another potential avenue in therapeutics is considering insulin-releasing drugs over insulin-sensitizing drugs. In preliminary studies conducted in animal models intranasal insulin treatment was observed to reduce motor impairment and cell death of dopaminergic neurons, and treatment was also associated with an improvement in striatal mitochondrial function. In 2019, a pilot clinical trial with 16 PD subjects evaluated the effects of insulin on daily intranasal administration for 4 weeks. Besides proving safe, intranasal insulin showed an improvement in motor impairment and a possible positive effect on cognitive function compared to baseline and placebo, respectively [7].

Further prospective multicenter clinical trials with metformin and insulin in prodromal and/or early PD might help to determine their potential neuroprotective effect in humans.

Declaration of competing interest

None.

References

[1] A. Sánchez-Gómez, G. Alcarraz-Vizán, M. Fernández, R. Fernández-Santiago, M. Ezquerra, A. Cámara, M. Serrano, A. Novials, E. Muñoz, F. Valldeoriola, Y. Compta, M.J. Martí, Peripheral insulin and amylin levels in Parkinson's disease, Park. Relat. Disord. 79 (2020 Aug 25) 91–96, https://doi.org/10.1016/j.parkreldis.2020.08.018. Epub ahead of print. PMID: 32911247.

[2] I. Horvath, P. Wittung-Stafshede, Cross-talk between amyloidogenic proteins in type-2 diabetes and Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 113 (44) (2016 Nov 1) 12473–12477, https://doi.org/10.1073/pnas.1610371113. Epub 2016 Oct 17. PMID: 27791129.

https://doi.org/10.1016/j.parkreldis.2021.10.017
Received 3 October 2021; Accepted 16 October 2021
Available online 20 October 2021

Please cite this article as: Almudena Sánchez-Gómez, *Parkinsonism and Related Disorders*, https://doi.org/10.1016/j.parkreldis.2021.10.017

[3] I. Martinez-Valbuena, I. Amat-Villegas, R. Valenti-Azcarate, M.D.M. Carmona-Abellan, I. Marcilla, M.T. Tuñon, et al., Interaction of amyloidogenic proteins in pancreatic β cells from subjects with synucleinopathies, Acta Neuropathol. 135 (6) (2018 Jun) 877–886, https://doi.org/10.1007/s00401-018-1832-0. Epub 2018 Mar 13. PMID: 29536165.
[4] N. Saewanee, T. Praputpittaya, N. Malaiwong, P. Chalorak, K. Meemon, Neuro-protective effect of metformin on dopaminergic neurodegeneration and α-synuclein aggregation in C. elegans model of Parkinson's disease, Neurosci. Res. 162 (2021 Jan) 13–21, https://doi.org/10.1016/j.neures.2019.12.017. Epub 2019 Dec 24. PMID: 31881233.
[5] D. Athauda, K. Maclagan, S.S. Skene, M. Bajwa-Joseph, D. Letchford, K. Chowdhury, et al., Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial, Lancet 390 (10103) (2017 Oct 7) 1664–1675, https://doi.org/10.1016/S0140-6736(17)31585-4. Epub 2017 Aug 3. PMID: 28781108.
[6] D. Athauda, K. Maclagan, N. Budnik, L. Zampedri, S. Hibbert, I. Aviles-Olmos, et al., Post hoc analysis of the Exenatide-PD trial-Factors that predict response, Eur. J. Neurosci. 49 (3) (2019 Feb) 410–421, https://doi.org/10.1111/ejn.14096. Epub 2018 Oct 6. PMID: 30070753.
[7] P. Novak, D.A. Pimentel Maldonado, V. Novak, Safety and preliminary efficacy of intranasal insulin for cognitive impairment in Parkinson disease and multiple system atrophy: a double-blinded placebo-controlled pilot study, PLoS One 14 (4) (2019 Apr 25), e0214364, https://doi.org/10.1371/journal.pone.0214364. PMID: 31022213.

Almudena Sánchez-Gómez
Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Spain
Institut de Neurociències, Maeztu Center, University of Barcelona, Spain

Yaroslau Compta
Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Spain
Institut de Neurociències, Maeztu Center, University of Barcelona, Spain
Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain
Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED: CB06/05/0018-ISCIII) Barcelona, Spain

Maria J. Martí*
Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Spain
Institut de Neurociències, Maeztu Center, University of Barcelona, Spain
Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain
Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED: CB06/05/0018-ISCIII) Barcelona, Spain

* Corresponding author. Parkinson's Disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic of Barcelona, Villarroel 170, 08036, Barcelona, Catalonia, Spain.
E-mail address: MJMARTI@clinic.cat (M.J. Martí).