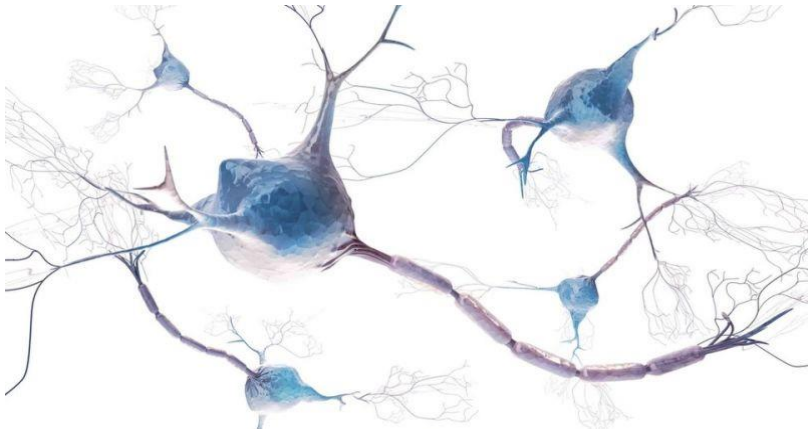
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1

Ignacio Pedrós Martín

TESIS DOCTORAL

ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1



UNIVERSITAT ROVIRA i VIRGILI

IGNACIO PEDRÓS MARTÍN

TARRAGONA 2016

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

UNIVERSITAT ROVIRA i VIRGILI

HACEMOS CONSTAR que el presente trabajo, titulado “Alteraciones metabólicas en el proceso de amiloidogénesis en el hipocampo de ratones app/ps1” que presenta Ignacio Pedrós Martín para la obtención del título de Doctor, ha sido realizado bajo nuestra dirección en la Facultat de Medicina i Ciències de la Salut de Reus de esta Universidad.

Reus, Enero de 2016

Los directores de la tesis doctoral

Jaume Folch López

Departament de Bioquímica i Biotecnologia

Francesc X. Sureda Batlle

Departament de Ciències Mèdiques Bàsiques

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

"Los científicos dicen que estamos hechos de átomos pero a mí un pajarito me contó que estamos hechos de historias"

Eduardo Galeano

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

AGRADECIMIENTOS

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

AGRADECIMIENTOS

Retrospectivamente todo empezó con Eduardo Punset, yo era un adolescente de 15 años que quería quedarse hasta tarde los domingos por la noche para ver REDES. Gracias a ese programa de televisión conocí a científicos como Luigi Luca Cavalli-Sforza, Richard Dawkins, Antonio Damasio o Lynn Margulis, entre muchos otros, que me ayudaron a comprender que la ciencia es un gran estimulante.

Con el paso del tiempo decidí hacer biotecnología una maravillosa carrera que te enseña conceptos sobre genética molecular y reglas nemotécnicas para recordar particularidades de microrganismos y aminoácidos. Durante esa aventura de 5 años conocí personas maravillosas a las que estaré profundamente agradecido: Patri, Husam, Eloy, Cris, Leo y nuestro maravilloso bull terrier Airon (con A sí), también Andrea y todos los compañeros de la Rovira i Virgili.

Mi primera oportunidad y contacto con un laboratorio y con la ciencia práctica fue gracias a la Dra. Rosa Cristòfol y la Dra. Coral Sanfeliu del grupo de neurodegeneración y envejecimiento del IDIBAPS; allí conocí a Yoelvis, Susana y Jessica que me enseñaron la praxis y la metodología para convertirme en doctor.

Al Dr. Francesc Sureda y al Dr. Jaume Folch les agradezco profundamente la oportunidad de aceptarme como su pipiolo. Soy muy consciente que toda esta etapa de mi vida no hubiera sido posible sin ellos; me hubiera perdido disfrutar del ambiente académico tan familiar de la Universidad de medicina de Reus y conocer a mis compañeras de laboratorio Mónica, Laura, Nuria, Vanesa, Jordi, Tania, Teresa, Merche y a los profesores el Dr Santafé, la Dra. Colominas, la Dra Nogués, la Dra Romeu y la Dra Giralt .

Al Dr. Antoni Camins estoy agradecido por ser mi jefe y compañero. Me gustaría recordar una frase de Charles Chaplin "*Nunca te olvides de sonreír porque el día que no sonrías será un día perdido*" Contigo, Toni, no he perdido ni un solo día, he sido muy feliz trabajando contigo porque me has permitido no solo convertirme en doctor sino también en científico. También gracias a ti, pude trabajar en la UB en tu grupo de investigación y tener a mi disposición todos los recursos necesarios. Te agradezco tu confianza y tu dedicación incondicional al proyecto común. También quiero agradecer a todo el equipo que ha formado parte en algún momento de este proyecto científico: gracias Dra. Auladell, Dr. Vázquez-Carrera, Dra. Verdaguer, Dr. Junyent, Dr. Petrov, Gonzalo, Michael, Melani, Nohora, Iván y Miren.

Quiero dar las gracias a mis compañeros de laboratorio de la Universidad de Barcelona que me han permitido vivir en un maravilloso ecosistema. Muchas gracias a Raúl, Sonia, Carla, David, Cristian, Gemma, Luisa, Miguel, Leti, Mario, Verónica, Emma, Xevi, Laia, Sergi, Aurelio, Patricia, Oriol y en especial a Andrés y Dimitri que han sido mis parejas de baile.

Grazie anche a Gaia che mi accompagna nella mia vita e non smette mai di stupirmi con la sua tenacia, forza e energia. Ti amo.

Por último y en ciencia siempre es lo más importante, quiero dar las gracias a mi familia. A Marisa mi coach personal, a mi padre al que debo gran parte de mi personalidad, a mi hermana Aida, por su apoyo incondicional y a Luís y Julia que hacen que seamos una gran familia, y por último a mi madre que es la persona más importante de mi vida. Sin ella nada de esto sería posible.

ÍNDICE

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

ÍNDICE DE CONTENIDOS

RESUMEN

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

RESUMEN

INTRODUCCIÓN

La prevalencia de la demencia, en términos de globalidad, presenta un crecimiento exponencial que se estima que crecerá hasta los 76,7 millones en 2030 y los 135 millones en 2050 en todo el mundo. Por otra parte, la *International Diabetes Federation* estima que, para el año 2030, la prevalencia será de más de 500 millones de diabéticos a nivel mundial. Ambas enfermedades suponen una pandemia en crecimiento exponencial y un importante problema de salud pública.

La Enfermedad de Alzheimer (EA) es el desorden neurodegenerativo más prevalente en la población de edad avanzada. Se define principalmente por un déficit progresivo de la memoria, acompañado de características neuropatológicas como la perdida neuronal, el incremento de la proteína precursora amiloidea (APP), la acumulación de placas β amiloide a partir de Aβ insoluble y la hiperfosforilación de la proteína citoesquelética tau.

La modulación de la presencia de estos marcadores en el cerebro ha sido insuficiente para frenar la enfermedad y por ello se continúan llevando a cabo nuevas investigaciones que ayuden a comprender la patología. En este trabajo nos hemos centrado en relacionar la pérdida de memoria con trastornos metabólicos como la diabetes y la obesidad, considerando la posibilidad que la EA sea una enfermedad metabólica ya que en el cerebro de nuestros ratones coexisten una incorrecta utilización de la glucosa y una producción energética deficiente.

Este enfoque viene desarrollándose a partir de estudios epidemiológicos que han demostrado que pacientes con intolerancia a la glucosa, con déficit de secreción de insulina o con diabetes tipo II (DM2) tienen incrementado el riesgo de desarrollar deterioro cognitivo leve (MCI) o EA (Janson et al. 2004; Mayeda et al. 2015). Otros estudios, en este caso longitudinales, correlacionan trastornos metabólicos como la DM2, la obesidad y dislipidemia con MCI y EA (Profenno et al. 2010; Gendron et al. 2013).

Los cambios metabólicos que produce la DM2 promueven alteraciones cerebrales. Así, la hiperglucemia y la resistencia a la insulina pueden acelerar la degeneración neuronal mediante la glicosilación no enzimática provocando un aumento del estrés oxidativo. Los trastornos metabólicos y energéticos han sido relacionados con un desequilibrio de la vía de señalización de insulina, del factor de crecimiento insulínico (Igf) y de la leptina (ob) en diferentes órganos del cuerpo, entre ellos el cerebro.

En el sistema nervioso central (SNC) estas hormonas participan en diferentes funciones fisiológicas como la ingesta de alimentos, la inhibición de la gluconeogénesis hepática, la modulación de la activación de la proteína Tau, el metabolismo de la Aβ-amiloide, la supervivencia neuronal y la memoria.

Tanto el receptor de insulina (RI), como el receptor de crecimiento insulínico (IGFR) y el receptor de leptina (OBR) son receptores de tipo tirosina cinasa. Cuando el mecanismo de acción es incorrecto, decrece la señal de substrato del receptor de insulina (Irs*), phosphoinositide 3-kinase* (PI3K) y *protein kinase b* (PKB/AKT) dando lugar a una reducción de plasticidad y supervivencia neuronal. Por otro lado, se activan diferentes proteínas como la *glycogen synthase kinase 3β* (GSK3β) o las *c-jun amino-terminal kinase* (JNKs) que regulan negativamente la señal de estos receptores. Esto conlleva un incremento de la fosforilación de la proteína Tau, estrés oxidativo y neuroinflamación.

En condiciones normales el exceso de Aβ puede ser eliminado a través de la proteína relacionada con el receptor de lipoproteína-1 (LRP1), o bien por un proceso de degradación en que interviene la enzima degradadora de insulina (IDE). En condiciones de hiperinsulinemia periférica crónica el transporte de insulina a través de la barrera hematoencefálica decrece, produciendo déficits en las acciones principales de la insulina a nivel central, como por ejemplo la memoria a largo plazo, la plasticidad neuronal y la expresión de la acetilcolintransferasa, enzima responsable de la síntesis de acetilcolina.

La insulina tiene la capacidad de activar IDE; por lo tanto, cuando los niveles de insulina efectiva a nivel central son insuficientes puede verificarse una menor activación de IDE y consecuentemente una mayor acumulación nociva de Aβ. Así, se definiría como diabetes tipo 3 la situación que se da cuando la hiperinsulinemia, en respuesta a la resistencia a la insulina, comporta una disminución de la insulina cerebral y una mala regulación de la funcionalidad de IDE con consecuente incremento en la acumulación de Aβ en el cerebro.

La hiperglicemia crónica además puede comportar un incremento del estrés oxidativo, disfunción mitocondrial y producción de productos finales de la glicación avanzada que aceleran la enfermedad.

Hipótesis general

La hipótesis general de la presente tesis doctoral es conocer si los niveles elevados de β-amiloide producidos por la mutación presente en los ratones APP/PS1 producen algún efecto metabólico a nivel central o periférico. Para evaluar esta hipótesis se han estudiado parámetros relacionados con el síndrome metabólico, como son la obesidad, la resistencia a insulina y la dislipemia en este

modelo animal. Al mismo tiempo, se ha querido evaluar el efecto de la alimentación rica en grasas sobre algunos parámetros cognitivos e histopatológicos característicos de la EA en el citado modelo animal.

Material y métodos

El ratón APPswe/PS1dE9 (APP/PS1) produce péptidos de Aβ debido a una doble mutación en los genes APP y PS1, y a partir del quinto mes se observan placas en cortes histológicos cerebrales. Se realizó una caracterización en dos estados temporales de su desarrollo. En una segunda fase experimental se administró una dieta rica en grasa durante 5 meses con el fin de observar como afectaba un cuadro clínico similar al síndrome metabólico en el desarrollo de la EA.

Objetivos

1. Caracterizar el fenotipo metabólico a nivel central y periférico en ratones APP/PS1 de 3 y 6 meses de edad sometidos a una dieta estándar.

2. Caracterizar el fenotipo metabólico a nivel central y periférico en ratones APP/PS1 de 6 meses de edad sometidos a una dieta rica en grasas.

Principales resultados

Se estudiaron las alteraciones metabólicas periféricas tras la formación de placas seniles en los ratones transgénicos. A nivel central, la vía de señalización de insulina estaba alterada antes y después de la formación de placas; también se detectaron deficiencias en la cadena de transporte de electrones y alteraciones en las cinasas responsables de la hiperfosforilación de tau.

Se evaluó el efecto de la obesidad y de la resistencia a insulina periférica, inducidas a través de una alimentación rica en grasas, en el proceso de amiloidogénesis que experimenta el ratón APP/PS1. Los resultados indican que el ratón salvaje alimentado con dieta grasa tiene un fenotipo similar al ratón transgénico alimentado con una dieta control. La dieta rica en grasa y la Aβ provocan resistencia a insulina central y alteraciones en la cadena de transporte de electrones de la mitocondria. Estas alteraciones parecen indicar que hay una estrecha relación a nivel molecular entre la EAf, la obesidad y la resistencia a insulina.

ACRÓNIMOS Y ABREVIATURAS

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

ACRÓNIMOS Y ABREVIATURAS

Aβ: péptido Aβ amiloide

ABCA1: ATP-binding cassette, sub-family A

AGRP: agouti related neuropeptide

AICD: activation-induced cell death

AMPK: adenosine monophosphate kinase

APLP 1/2: amyloid beta precursor-like protein 1/2

ApoE: apoliproteina E

APP: proteina precursora amiloide

APPSwe: APP Swedish mutation

Arc: activity-regulated cytoskeleton-associated protein

ATP: adenosín trifosfato

BACE: β-site APP cleaving enzyme

BHE: barrera hematoencefálica

BDNF: brain-derived neurotophic factor

CAMKKβ: calcio-calmodulina cinasa II

cAMP: cyclic adenosine monophosphate

CDC2: cyclin-dependent protein kinase Cdk1/Cdc2

CDK5: cyclin-dependent kinase 5

C-Fos: FBJ osteosarcoma oncogene

COX: clooxygenase

CREB: cAMP responsive element binding protein

CTF: carboxy-terminal fragment

Cyp46a1: cholesterol 24-hydroxylase

DM: diabetes mellitus

DM1/2: diabetes mellitus tipo I y II

DNA: ácido desoxirribonucleico

EA: enfermedad de Alzheimer

EAe: enfermedad de Alzheimer esporádica

EAf: enfermedad de Alzheimer familiar

ERK: extracellular regulated kinase

GLP-1: glucagon-like peptide I

GLUT: transportadores de glucosa

GSK3β: glycogen synthase kinase 3β

IAPP: islet Amyloid Polypeptide (Amylin)

IDE: enzima degradadora de insulina

IGF1: insulin growth factor-1

IGF2: insulin growth factor-2

IGFR1: insulin growth factor receptor 1

IκB: inhibitor of κB

IMC: índice de masa corporal

iNOS: inducible nitric oxide synthase

INS1: preproinsulina

IPGTT: intraperitoneal glucose tolerance test

ITT: insulin tolerance test

JAK2: janus kinase 2

JNK: c-jun amino-terminal kinase

LTP: potenciación a largo plazo

LCR: líquido cefalorraquideo

LRP-1: low density lipoprotein receptor- related protein 1

MCI: deteriro cognitive leve

mRNA: ácido ribonucleico mensajero

mTORC1: mechanistic target of rapamycin

NMDA: N-metil-D-aspartato

NPY: neuropeptide Y

NOS: *nitric oxide* synthase

NORT: novel object recognition test

NRF 1/2: nuclear respiratory factor 1/2

NTF: amino-terminal fragment

OB-R: receptor de leptina

ONF: ovillos neurofibrilares

OXPHOS: mitochondrial oxidative phosphorylation

PARP: poly EAP ribose polymerase

PEN-2: presenilin enhancer 2

PET: tomografía por emisión de positrones

PDPK: 3-phosphoinositide dependent protein kinase 1

PGC1α: peroxisome proliferator-activated receptor gamma coactivator 1-α

PHF: filamentos helicoidales apareados

PI3K: phosphoinositide 3-kinase

PKB/AKT: protein kinase b

PKC: protein kinase C

POMC: proopiomelanocortin

PPARα: peroxisome proliferator-activated receptor α

PSEN-1/2: presenilina 1/2

PSD-95: postsynaptic density protein-95

RE: retículo endoplasmático

RI: receptor de insulina

ROS: especies reactivas de oxígeno

SOCs: suppressor of cytokine signaling

SNC: sistema nervioso central

STATs: signal transducer and activator of transcription

SYP: synaptophysin

Tfam: transcription factor A, mitochondrial

WT: wild type

CAPÍTULO I. INTRODUCCIÓN

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

CAPÍTULO I. INTRODUCCIÓN

1. La enfermedad de Alzheimer

1.1 Historia

En 1901, en la clínica para enfermos mentales y epilépticos de Frankfurt, el Dr. Alois Alzheimer (1864-1915) evalúa y diagnostica una demencia a la paciente Auguste Deter (1850-1906). El Dr. Alzheimer describió que Auguste sufría un delirio celotípico, una rápida y progresiva pérdida de memoria, además de alucinaciones, desorientación temporo-espacial, paranoia, trastornos de la conducta y un grave trastorno del lenguaje (Guerra et al. 2009).

El 8 de abril de 1906, Auguste fallece y Alois Alzheimer solicita el cerebro y la historia clínica de la paciente para ser estudiados por sus colaboradores Perusini y Bonfiglio. Los resultados mostraron una atrofia de la corteza con citólisis generalizada, una patología extraña de las neurofibrillas, fuertes excrecencias de la neuroglia fibrosa y numerosas células gliales con forma de varilla, además de sedimentos de productos metabólicos en forma de placas en toda la corteza cerebral y signos leves de neovascularización.

El 4 de noviembre de 1906 presentó su observación anatomoclínica con la descripción de placas seniles, ovillos neurofibrilares y cambios arterioscleróticos cerebrales. El trabajo se publicó al año siguiente con el título *"Ueber eine eigenartige Erkrankung der Hirnrinde"* (Alzheimer et al. 1907) traducido al inglés en 1995 (Alzheimer et al. 1995). A pesar de esta publicación, fue solo en 1910 que Emil Kraepelin, una autoridad médica internacional, en la octava edición del Manual de Psiquiatría, se refirió por primera vez a la patología utilizando el epónimo de Enfermedad de Alzheimer (EA).

Alzheimer publicó la exposición completa del caso de Auguste en 1911 *"Über eigenartige Krankheitsfälle des späteren Alters"*. El artículo describía también a otro paciente, Johann F que había sido ingresado en la clínica de Múnich a la edad de 56 años, con síntomas clínicos muy parecidos a los que había observado en Auguste. El cerebro de Johann F se diferenciaba del de Auguste D. en un aspecto importante. Aunque sí exhibía las típicas placas amiloides, no presentaba signos de cambio de neurofibrillas (figura 1). Así pues, ya en esta fase inicial descriptiva de la EA se discutió la dificultad clínica que supone identificar los marcadores histopatológicos y clínicos inherentes a la enfermedad.

La histopatología del cerebro de Auguste D ha podido ser estudiada de nuevo (Graeber et al. 1998). En este estudio no se encontraron lesiones microscópicas vasculares, existiendo solo placas amiloideas y ovillos neurofibrilares (ONF).

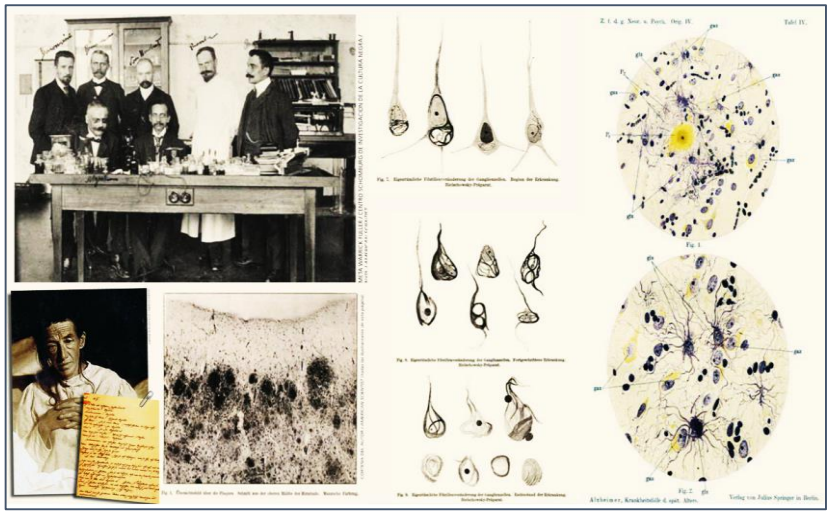


Figura 1. Legado fotográfico sobre el descubrimiento de la EA. Arriba a la izquierda: Grupo de psiquiatras del Hospital clínico de la Universidad de Múnich entre los que se encuentran Alois Alzheimer, Fuller, Baroncini, Von Norbert, Ranke y otros colaboradores no identificados que participaron en el descubrimiento y descripción de la enfermedad. Abajo izquierda: Auguste y los manuscritos del propio Alzheimer redescubiertos en Frankfurt en 1995 junto con todo el historial clínico de la paciente. Izquierda debajo: Corte histopatológico del cerebro de Johann F.; las manchas oscuras corresponden a placas amiloides. Zona central: Se muestran varios estadios en la formación de NTF en el cerebro de Auguste D. En la imagen superior se describen las fases iniciales del proceso. En la reproducción central e inferior se exponen, respectivamente, las fases intermedia y avanzada. Derecha: Dibujos de secciones procedentes de diferentes profundidades de la corteza de Auguste D. Se aprecian numerosas placas, así como células con neurofibrillas intensamente teñidas. Fuente: https://becker.wustl.edu/about/news/art-alois-alzheimer

1.2 Definición

La EA es una demencia neurodegenerativa primaria cortical; se caracteriza por la presencia de alteraciones en áreas del cerebro (hipocampo y corteza) que ocasionan la pérdida progresiva de las funciones intelectuales a causa de una disminución paulatina del número de neuronas.

Clínicamente, se define como un proceso degenerativo de evolución progresiva, que se caracteriza por deterioro cognitivo y por la presencia de dos marcadores histopatológicos: placas seniles y ovillos neurofibrilares intracelulares (figura 2).

En fases intermedias-moderadas, los pacientes están desorientados y desconocen incluso a sus familiares más próximos. En las últimas etapas (fase moderada-avanzada) el enfermo no es capaz de valerse por sí mismo y depende totalmente de sus familiares y/o cuidadores, que deben enfrentarse diariamente a problemas como la desorientación, agitación, agresividad, depresión, pérdida de conciencia, descoordinación motora y dificultades en el lenguaje tanto hablado como escrito.

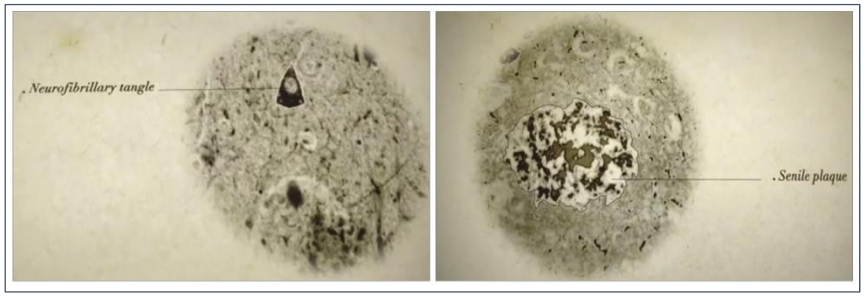


Figura 2. Ovillos neurofibrilares y placas β-amiloides. Los ovillos neurofibrilares son formaciones intraneuronales secundarias que mediante la hiperfosforilación de la proteína tau se forman filamentos helicoidales. Las placas seniles se forman en los espacios interneuronales de la sustancia gris del cerebro por el depósito de la proteína beta-amiloide.
Fuente: https://alzheimersdiseasebiol2095.wordpress.com/

La EA se puede clasificar en función de la edad de aparición de los síntomas o en función de la forma de herencia (tabla 1). En cuanto la edad, se distinguen entre forma precoz o presenil y forma tardía o senil. Según la forma de herencia, se distinguen la EA familiar (EAf), y la forma esporádica (EAe).

Tabla 1. Clasificación de la Enfermedad de Alzheimer.

CRITERIO	CARACTERÍSTICAS
Según edad de inicio de los síntomas	**Inicio precoz o presenil**: Comienza antes de los 60 años, es de curso más rápido y no es muy frecuente. Puede haber casos de aparición excepcionalmente precoz (40 o 50 años) y se suele asociar a factores hereditarios. Estos casos suponen un 1-5% del número total de enfermos de Alzheimer. **Inicio tardío o senil**: Aparece después de los 65 años, en la mayoría de las veces es esporádica, de curso lento y es también la más frecuente.
Según implicación del factores genéticos	**Familiar o de causa genética**: Se produce una alteración o mutación de tres genes: el gen de la Proteína Precursora Amiloide (APP) situado en el cromosoma 21; el gen de la Presenilina 1 (PS1) en el cromosoma 14; y el gen de la Presenilina 2 (PS2) en el cromosoma 1. Se heredan de forma dominante produciendo alteraciones similares en los descendientes que en la mayoría de los casos coinciden con la EA presenil (el 1% de los casos). **Esporádica**: Coincide generalmente con los casos de EA senil; comienza en personas mayores de 65 años y es el tipo más común (96%). No se conocen genes causales, estando implicados diversos factores genéticos de susceptibilidad.

1.3 Epidemiología

Según del *World Alzheimer Report 2014*, se cifran en 135 millones las personas que sufrirán demencia en el 2050 (Martin Prince et al. 2014). Es ampliamente aceptado que existe un aumento exponencial de las cifras de prevalencia e incidencia según la edad del paciente, siendo la edad el principal factor de riesgo para la EA (figura 3).

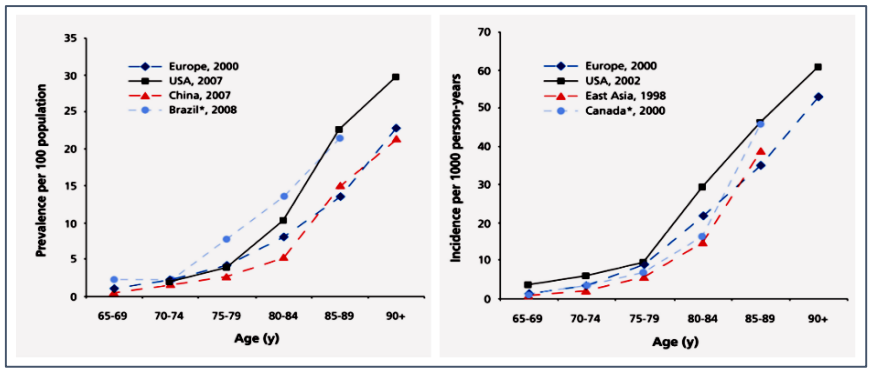


Figura 3. Prevalencia e incidencia de la EA en función de la edad. (Qiu et al. 2009)

Se ha descrito que los casos de EA constituyen el 50-75% de los casos totales de demencia (Martin Prince et al. 2014; Martin Prince et al. 2015). Teniendo en cuenta el creciente envejecimiento de la población mundial, la elevada prevalencia de EA se ha convertido en uno de los grandes problemas sanitarios de la sociedad actual. Con el incremento de la población se estima que el número de casos de demencia duplicará cada 20 años (Ferri et al. 2005).

En una enfermedad ligada al envejecimiento, es fundamental conocer el entorno demográfico en el que se enmarca. Según los datos del *National Institute of Aging,* la población mundial mayor de 65 años en 2010 era de 524 millones de personas (8% de la población); se estima que en 2050 sea de 1500 millones (16% de la población) (Hyman et al. 2012). Este incremento será debido a la mayor esperanza de vida de los países emergentes.

El Informe Mundial sobre la Enfermedad de Alzheimer publicado en 2013 indica que ese año había 44,3 millones de personas que padecían algún tipo de demencia en el mundo. Se calcula un aumento de 76 y 135 millones para los años 2030 y 2050 respectivamente (Prince et al. 2013) (figura 4). En el caso de la demencia tipo Alzheimer en 2013 se diagnosticaron más de 26,6 millones de casos a nivel mundial y se estima que el número de casos llegue a 100 millones para el año 2050 (Prince et al. 2013).

Algunos estudios muestran una incidencia de dos a tres veces superior en mujeres respecto a hombres, y parece que el aumento exponencial en la incidencia de la EA se producirá antes en la población femenina (Gao et al. 1998; Diaz Brinton & Yamazaki 1998). La explicación no está esclarecida, parece ser que existe una relación entre el gen *ApoE4* (factor de riesgo para la patología) y las hormonas sexuales femeninas (Duara et al. 1996; Payami et al. 1996). En esta dirección se ha descrito que la carencia de estrógenos en mujeres posmenopáusicas podría representar un factor de riesgo para el desarrollo de la EA (Schneider et al. 1996).

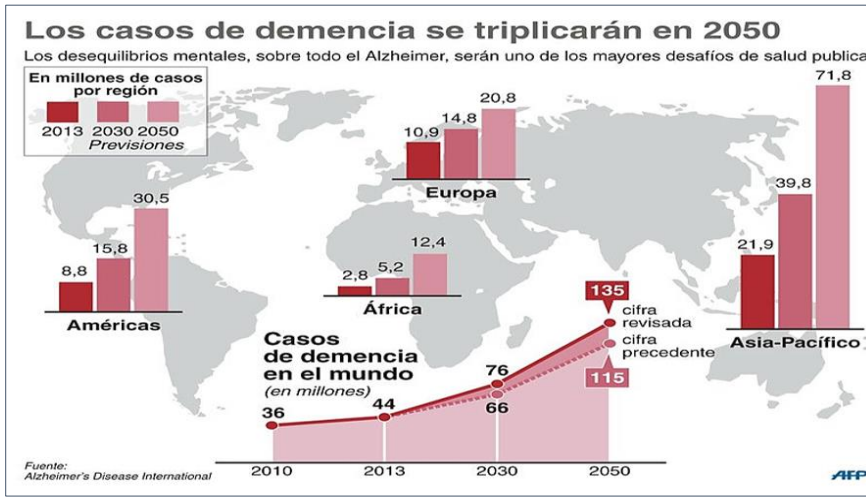


Figura 4. Incremento estimado de la demencia en cada continente.
Fuente: (Guerchet et al. 2013)

1.4 Áreas relevantes del cerebro

El sistema nervioso central (SNC) es el centro estructural y funcional de todo el sistema nervioso. Está formado por el encéfalo y la médula espinal. El encéfalo se divide en tronco del encéfalo, cerebelo y cerebro. El cerebro a su vez está formado por la corteza cerebral, los ganglios basales y el sistema límbico.

La corteza cerebral consta de dos hemisferios cerebrales unidos en la línea media a través de un tracto de fibras denominado cuerpo calloso. Cada hemisferio tiene una capa externa de sustancia gris de unos 2-4 mm de grosor denominada corteza cerebral. Esta corteza se encuentra extraordinariamente plegada formando las circunvoluciones que suponen un fuerte incremento de la superficie manteniendo el mismo volumen. En estos dos hemisferios distinguimos los lóbulos, que toman su nombre a partir de los huesos craneales

de los cuales están en contacto. Es decir lóbulo frontal, temporal, parietal y occipital.

- El **lóbulo frontal** se encarga de controlar la actividad motora, como la articulación del lenguaje, el estado de ánimo, el pensamiento y la planificación del futuro.
- El **lóbulo parietal** se encarga de interpretar las sensaciones que se reciben del organismo. También se encarga de controlar el movimiento corporal.
- El **lóbulo occipital** su principal función es integrar e interpretar la visión.
- El **lóbulo temporal** se encarga de la memoria y de las emociones.

El sistema límbico es responsable de la mayoría de los impulsos básicos y de las emociones que son importantes para la sobrevivencia del animal: miedo, furia, libido sexual, placer, dolor, angustia. Los principales componentes del sistema límbico incluyen las estructuras corticales (amígdala, hipocampo, cíngulo), el hipotálamo, algunos núcleos talámicos, los cuerpos mamilares y el septo pelúcido.

La formación hipocampal está compuesta por diferentes partes, entre las que se encuentran el giro dentado, las áreas divididas de CA1 a CA4, la corteza entorrinal y el subículo (Burwell et al. 1995) (figura 5). El hipocampo posee múltiples conexiones aferentes y eferentes con diversas áreas cerebrales, realiza funciones relacionadas con la memoria a corto y largo plazo así como con la memoria espacial. El hipocampo se encuentra en la base del lóbulo temporal y se conecta profusamente con otras estructuras corticales. Se ha visto que el hipocampo participa en funciones relacionadas con la memoria reciente y en el proceso de la información recién adquirida.

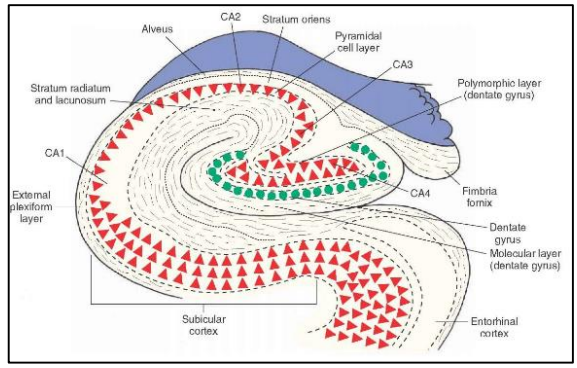


Figura 5. Anatomía de la formación hipocampal. El hipocampo se caracteriza por presentar una capa principal de neuronas, la capa de las células piramidales, que hace una trayectoria en forma de una C (rojo) y en la cual se distinguen tres regiones llamadas CA1, CA2, CA3 y giro dentado (verde) Fuente:http://what-when-how.com/neuroscience/the-limbic-system-integrative-systems-part-1/)

El cerebro de una persona que ha fallecido con EA tiene un peso inferior y presenta una atrofia en las circunvoluciones. Estas quedan estrechadas y los surcos ensanchados debido a una importante degeneración neuronal e hipofunción sináptica (Reiner et al. 2012). También se observa una atrofia en áreas cerebrales relacionadas con el aprendizaje y la memoria, como el hipocampo, la amígdala y las cortezas temporal, parietal y frontal (figura 6).

La atrofia es generalmente simétrica, pero también puede haber atrofia lobular, frontal, temporal u occipital asimétrica. Las lesiones en el hemisferio izquierdo se han asociado a dificultades para recordar información de tipo verbal, mientras que en el hemisferio derecho a dificultades para recordar patrones de información no verbal. Síntomas como la pérdida de memoria en pacientes con demencia están relacionados con la degeneración de neuronas del lóbulo temporal. La atrofia cerebral suele ser más grave en las formas familiares de inicio precoz que en las formas esporádicas de inicio tardío. El grado de atrofia cerebral se relaciona con el progreso de la enfermedad.

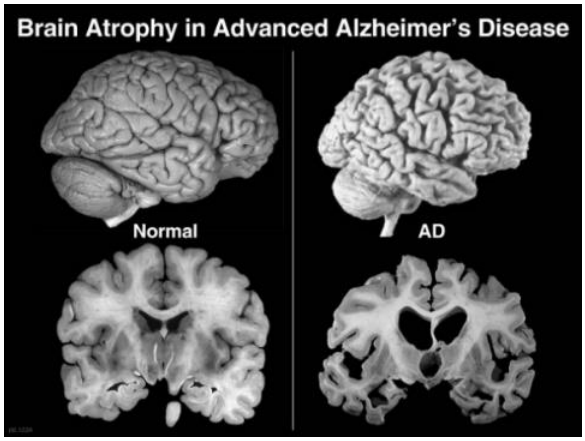


Figura 6. Atrofia de las circunvalaciones de una cerebro con la EA. Fuente: https://alzheimersdiseasebiol2095.wordpress.com/alzheimers-effect-on-the-brain/

Además de la afectación de la corteza entorrinal y del hipocampo, la EA cursa con pérdida neuronal en el locus cerúleo (pérdida de las conexiones noradrenérgicas con la corteza entorrinal y el hipocampo), en el núcleo dorsal del rafe y en el núcleo basal de Meynert (pérdida de conexiones colinérgicas) así como en la amígdala (Head et al. 2012).

Las zonas afectadas representan las áreas del cerebro implicadas en los procesos de memoria, pero no incluyen la corteza motora que mantiene intactas sus

funciones incluso en los estadios más avanzados de la enfermedad (Nieuwenhuis-Mark 2009).

1.5 Etiología

La etiología de la EA es desconocida. Sin embargo, en la EAf se han identificado más de 185 mutaciones en tres genes que contribuyen al desarrollo de la enfermedad: el gen que codifica la proteína precursora amiloide (APP) en el cromosoma 21, la presenilina 1 (PSEN1) en el cromosoma 14 y la presenilina 2 (PSEN2) en el cromosoma 1 (Tabla 2) (Fernández & Castro 2008). La función de estos genes y sus correspondientes proteínas están en continua revisión. En este trabajo se señalan las principales funciones.

Tabla 2. Principales genes implicados en la EAf. (Fernández & Castro 2008)

Gen	Mutación o ligamento	Proteina	Herencia	Efecto en patogénesis	Edad inicio
App	Cromosoma 21 21q21	Proteina precusrsa amiloide	Dominante	Altera la producción (ratio Aβ40 y 42↑) y agregación de Aβ amiloide	40-65 años
Ps1	Cromosoma 14 14q24	Presenilina 1	Dominante	Altera la producción (ratio Aβ40 y 42↑) del Aβ amiloide	25-60 años
Ps2	Cromosoma 1 1q31	Presenilina 2	Dominante	Altera la producción (ratio Aβ40 y 42↑) del Aβ amiloide	45-84 años
Apo E	Cromosoma 19 19q13	Apolipopro-teina E	Factor de riesgo	Desconocido ¿Agregación de Aβ amiloide? ¿Metabolismo lipidico?	>50 años

Gen App

El gen *app* codifica una proteína de transmembrana llamada APP que se encuentra en la mayoría de las células, incluidas las neuronas y las células gliales. La APP sufre una proteólisis por las secretasas α, β y γ-secretasa, lo que produce péptido Aβ amiloide de diferente tamaño.

La vía de la γ-secretasa produce los amiloides Aβ40 y Aβ42, que son más amiloidogénicos y que se depositan desde las etapas iniciales de la enfermedad. Al menos se han descrito 27 mutaciones del gen APP en 74 familias en todo el mundo. Las mutaciones representan menos del 1 % de los casos de EA, y poco más del 10 % del total de las EAf de inicio precoz.

La herencia es autosómica dominante, con una penetrancia casi completa, lo que supone que los portadores desarrollarán la enfermedad antes de los 60 años.

Gen Psen1

El gen *psen1* se traduce en una proteína transmembrana de 467 aminoácidos. Esta proteína se expresa en diferentes tejidos, incluido el cerebro. Se cree que la PSEN1 es un cofactor de la γ-secretasa y altera la vía de la APP. Al menos hay descritas 148 mutaciones de este gen en 82 familias en todo el mundo (Fernández & Castro 2008). La mayoría de las mutaciones son debidas a la sustitución de una base por otra y ocurren en el segundo dominio transmembrana codificado por el exón 5 y en el sexto dominio hidrofílico codificado por exones de 8 a 11. Aproximadamente entre el 30 y el 50 % de los casos de EAf de inicio precoz están relacionados con mutaciones de este gen.

Gen Psen2

El gen *psen2* se traduce a una proteína de 448 aminoácidos llamada PSEN2, homóloga a la PSEN1. La edad de inicio de los síntomas en el caso de EAf debido a una mutación en PSEN2 es más tardía, en torno a los 40 y hasta los 85 años, a veces sin mostrar penetrancia. Desde el punto de vista neuropatológico, esta demencia no puede distinguirse de la EAe clásica.

Las mutaciones de estos genes producen una mayor actividad γ-secretasa y β-secretasa dando lugar a una mayor formación de Aβ y por lo tanto, de placas seniles (Mattson 2004).

Gen Apoe

El gen de la apoliproteina E (*Apoe*) se encuentra en el cromosoma 19, y está implicado tanto en las formas familiares de comienzo tardío como en la EAe (Lovestone & McLoughlin 2002).

La ApoE es una proteína plasmática implicada en el transporte del colesterol y otros lípidos en diferentes tejidos. Es sintetizada por el hígado y el cerebro, y constituye la principal apoliproteina expresada en el tejido cerebral, de forma destacada en la glía.

El gen *Apoe* tiene 3 isoformas: ε2, ε3 y ε4. La más frecuente es la ε3 (constituye el 78% de los alelos presentes en población caucasiana) y la menos frecuente es la ε2 (constituye el 7%). Se ha observado que la isoforma ε4 aumenta el riesgo de padecer la EA mientras que ε2 probablemente reduce el riesgo o es un gen

protector. Sin embargo, muchas personas con la patología no tienen el gen tipo ε4.

En conjunto son muchos los esfuerzos en determinar las anomalías genéticas que causan la enfermedad, en el caso de la EAf se han podido encontrar los genes implicados pero en la EAe aún queda mucho por conocer e investigar.

Hoy en día los estudios sugieren que la EAe es un proceso fisiopatológico que comienza décadas antes de que surjan las primeras manifestaciones clínicas (figura 7) (Sperling et al. 2011; Pletnikova et al. 2015).

Un modelo hipotético sugiere que en las etapas preclínicas los factores de riesgo genéticos y ambientales modularían la producción de Aβ42 (Aido 2013). Actualmente está por identificar qué criterio diagnóstico utilizar para evaluar el deterioro cognitivo leve, la demencia y la EA (Jack et al. 2011; Montine et al. 2014).

Teniendo en cuenta que la EA incluye una fase asintomática, es fundamental identificar las características inherentes al envejecimiento fisiológico y las características patológicas. También es fundamental conocer los cambios moleculares que desencadenan un MCI y en qué nivel de organización celular actúan.

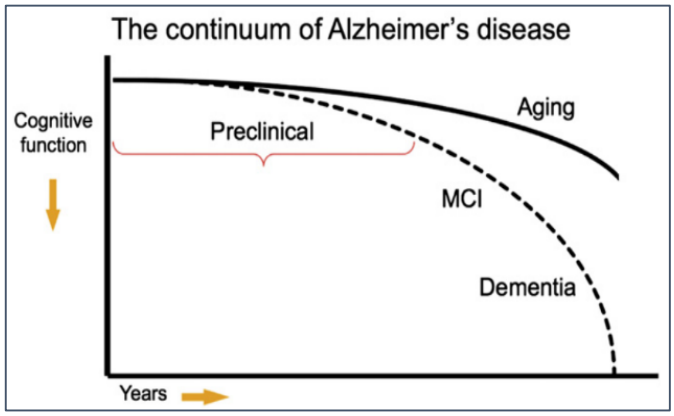


Figura 7. Modelo de la evolución clínica de la demencia tipo EA. El estado preclínico se ve directamente influenciado por las mutaciones autosómicas dominantes y los múltiples factores de riesgo. El estado preclínico evoluciona a deterioro cognitivo leve y en última instancia demencia (Sperling et al. 2011).

La investigación de los biomarcadores en la etapa preclínica es fundamental para conocer las causas que desencadenan la EA. Conocemos que los biomarcadores genéticos diagnostican la EAf pero no la EAe, por ello en la práctica clínica se usan dos tipos de biomarcadores:

1. De amiloidosis
2. De neurodegeneración

1. Marcadores de amiloidosis

Los marcadores de amiloidosis pueden observarse en el líquido cefalorraquídeo (LCR) o directamente en el cerebro mediante tomografía por emisión de positrones (PET).

- **Líquido cefalorraquídeo**

Dado que la patología de la EA está confinada en el cerebro, el LCR es un medio idóneo para el estudio de marcadores bioquímicos que reflejan la patología y el grado de la enfermedad.

El marcador de amiloidosis más utilizado para el diagnóstico de EA en LCR son los niveles de Aβ42, que en pacientes con EA se encuentran disminuidos. La disminución del Aβ42 se correlaciona con el deposito parenquimatoso del β-amiloide en forma de placas amiloideas en el cerebro, habiendo una correlación clara entre disminución del Aβ42 y la cantidad de placas amiloides (Strozyk et al. 2003; Buerger et al. 2006; Ossenkoppele et al. 2012)

Los niveles de Aβ 42 se encuentran ya disminuidos en el momento de la aparición de los síntomas tanto en casos esporádicos como en portadores de mutaciones (Fagan et al. 2007; Fortea et al. 2011).

- **Estudios PET con trazadores de β-amiloide**

El PET con trazadores para depósitos de amiloide fibrilar como el *Pittsburg Compound B* marcado con 11C-PIB, muestra un captación aumentada del trazador en zonas frontales, temporoparietales y estriatales en la inmensa mayoría de sujetos con EA respecto a controles (Klunk et al. 2004).

La técnica ha sido validada en estudios de correlación clínico-patológicos (Ikonomovic et al. 2008). Nuevos trazadores desarrollados y recientemente validados en cohortes patológicas (C. M. Clark et al. 2012) contribuirán probablemente en el futuro próximo a aumentar la accesibilidad a la prueba.

2. Marcadores de neurodegeneración

Los biomarcadores de neurodegeneración se evalúan en el LCR y en el cerebro mediante técnicas de neuroimagen como la resonancia magnética estructural y PET con trazadores de glucosa que evalúan el hipometabolismo cerebral.

- **Líquido cefalorraquídeo**

Los marcadores de neurodegeneración más validados en LCR en la EA son los niveles de t-tau y p-tau. En la EA, los niveles de t-tau y p-tau reflejarían el daño neuronal ya que esas proteínas se liberarían al espacio extracelular/LCR tras la muerte neuronal. Hay una correlación robusta entre el aumento de los niveles de t-tau y p-tau y la extensión de la pérdida neuronal y patología neurofibrilar en el estudio neuroanatómico (Strozyk et al. 2003; Buerger et al. 2006). La determinación de su isoforma fosforilada en el LCR se correlaciona de forma más específica con los ovillos neurofibrilares y permitiría aumentar la especificidad del diagnóstico (Koopman et al. 2009).

- **Resonancia magnética estructural**

Las técnicas de neuroimagen estructural han sido utilizadas para el diagnóstico clínico de la EA desde hace décadas para objetivar la atrofia cerebral secundaria a los cambios patológicos y para descartar diagnósticos alternativos.

Técnicas como la volumetría basada en vóxeles han permitido el uso de las técnicas de neuroimagen en la investigación, evidenciando el papel de la atrofia hipocampal en el diagnóstico de la EA y la correlación directa que existe entre el volumen del hipocampo y el rendimiento cognitivo en pacientes con y sin EA (Barnes et al. 2009).

Nuevas técnicas semiautomáticas puestas a punto en los últimos años, como la evaluación del grosor cortical, han permitido delinear un patrón de atrofia cortical característico de la EA que abarcaría zonas como la región temporal medial, temporoparietal lateral, precuneus o el cíngulo posterior y que permitiría discriminar con alta fiabilidad entre sujetos afectados y controles (Desikan et al. 2009). Este patrón cortical de atrofia es muy robusto y se ha podido identificar en sujetos en fase MCI y que posteriormente progresan a una demencia tipo EA (Dickerson et al. 2009).

Estudios longitudinales en portadores de mutaciones han demostrado que estos sujetos presentan cambios estructurales objetivables y característicos de la enfermedad (atrofia hipocampal, atrofia temporoparietal, cíngulo posterior y procuneus) hasta cinco años antes de la aparición del primer síntoma (Ridha et al. 2006). Este patrón de atrofia progresa y se generaliza a medida que la enfermedad va evolucionando.

- **PET con trazadores de glucosa**

El PET de fluorodeoxiglucosa (^{18}F-FDG PET) muestra en pacientes con EA un patrón característico de hipometabolismo cerebral temporoparietal bilateral, en cingulado posterior y en precuneus, que progresa a medida que la enfermedad avanza (Alexander et al. 2002)

La disminución del metabolismo regional cerebral se correlaciona con la progresión de los déficits cognitivos desde MCI hasta una fase de EA (Kim et al. 2005)

Los estudios llevados con biomarcadores podrían ayudar a comprender y a explicar la fase preclinica de la enfermedad. Sperling y colaboradores en el 2011 muestran un modelo que evalua los marcadores de la enfermedad teniendo en cuenta la evolución de la patología según los estadios preclinicos, MCI y clínico. El primer fenómeno es la acumulación del peptido Aβ 42, seguido de un hipometabolismo detectado con la fluorodeoxiglucosa; en los casos de portadores de gen APOE ε4 (alelo 34), este hipometabolismo es detectado incluso antes que la Aβ42 (línea naranja intermitente). Los marcadores de Tau están relacionados con el estadio preclínico así como los cambios estructurales del cerebro detectados mediante MRI. Los déficits cognitivos se manifiestan a partir de la fase MCI y se mantienen durante todo el desarrollo clínico de la EA (Sperling et al. 2011).

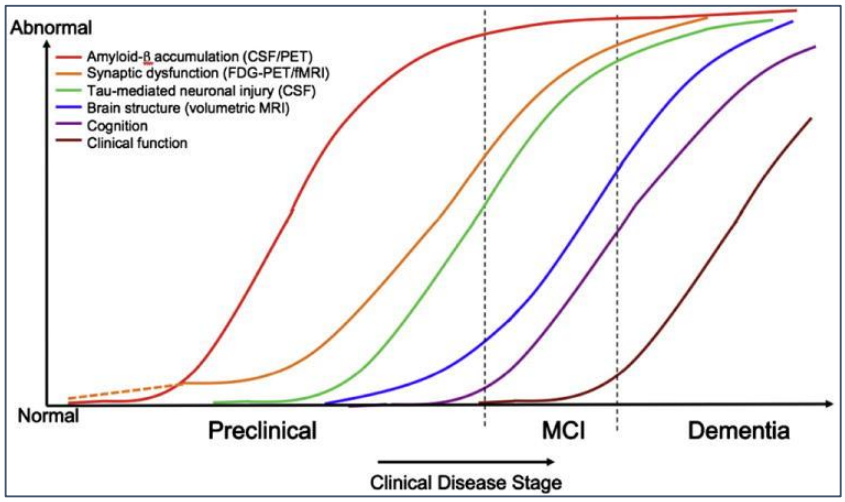


Figura 8. Modelo hipotético de biomarcadores dinámicos de la EA. (Sperling et al. 2011)

En los últimos años, la iniciativa ADNI (Alzheimer's Disease Neuroimaging Initiative) está realizando un estudio longitudinal multicéntrico a nivel mundial para la búsqueda de marcadores para el diagnóstico precoz y seguimiento de la

EA (Weiner et al. 2012; Srinivasa et al. 2015). Conocer los procesos y los estadios tempranos puede ayudar a diseñar una estrategia clínica enfocada a la prevención y la búsqueda de dianas terapéuticas multifuncionales.

1.6 Factores de riesgo

La etiología de la EAe es difusa y compleja y se han descrito múltiples factores de riesgo y/o protectores, de carácter genético o ambiental (Kalaria et al. 2008). Sobre la base de varias revisiones sistemáticas, estudios longitudinales y meta-análisis, se evalúan diferentes variables como los riesgos vasculares en la mediana edad (Meng et al. 2014), la obesidad (Beydoun et al. 2008), el colesterol (Studies 2013) o la diabetes (Profenno et al. 2010).

Hasta la fecha, nadie tiene duda que el principal factor de riesgo es la edad pero es evidente que interactúan muchas más variables. Con el fin de categorizar los factores de riesgo, éstos se clasifican en tres grandes grupos:

1. Factores genéticos (pequeño porcentaje sobre la incidencia total).
2. Factores vasculares y metabólicos.
3. Factores ambientales.

1. Factores de riesgo genéticos

El principal factor genético es el polimorfismo para ApoE (Saunders et al. 1993; Cramer et al. 2012). En concreto, la variante alélica ε4 es capaz de triplicar el riesgo de padecer la enfermedad en el caso de los heterocigotos y multiplicarlo por 15 en el caso de los homocigotos, respecto a los alelos ε2 y ε3 (Mahley et al. 2006).

2. Factores vasculares y metabólicos

Algunos de los factores de riesgo vascular que se han asociado a un mayor riesgo de desarrollar EA son la diabetes, la obesidad, la hiperlipidemia y la hipertensión.

- **Diabetes**

La asociación entre la diabetes y la demencia es fuerte, pero no concluyente (Lu et al. 2009; Profenno et al. 2010; Willette et al. 2015). Los individuos con Alzheimer muestran altos niveles plasmáticos de insulina y baja utilización de glucosa central, perfil que es característico de una resistencia a la insulina (Craft 2012). Además, un reciente meta-análisis demostró que las personas con

deterioro cognitivo leve (MCI) y diabéticos eran más propensos a progresar a demencia que los individuos con deterioro cognitivo leve no diabéticos (Cooper et al. 2015). La relación entre DM y el aumento de riesgo de padecer EA es motivo de discusión y estudio (Profenno et al. 2010; Mayeda et al. 2015).

La insulina no sólo modula el metabolismo de la glucosa periférica, sino también la función normal del cerebro. Recientemente, se demostró que el MCI y los niveles de insulina en ayunas están vinculados a alteraciones de las estructurales cerebrales (Raji et al. 2010).

Por lo tanto, los trastornos relacionados con la desregulación de la insulina, tales como la obesidad y la DM, pueden tener efectos nocivos en la función cerebral.

- **Obesidad**

Se ha demostrado la relación entre la obesidad, el deterioro cognitivo y la EA (Kiliaan et al. 2014). Un alto índice de masa corporal (IMC) en la mediana edad ha sido relacionado con un incremento del riesgo de padecer demencia en la vejez (Gustafson et al. 2003; Anstey et al. 2011; Tolppanen et al. 2014).

La edad es importante porque, como descrito en el estudio de Kiliaan et al 2014, y otros autores, un IMC superior a 30 en una edad que va de los 40 a los 45 años se asocia con un aumento de 3 veces el riesgo de desarrollar la EA (Anstey et al. 2011; Tolppanen et al. 2014).

El nivel de obesidad y el volumen de grasa blanca son el resultado de las desregulaciones metabólicas producidas por un incremento de adipocinas, también implicadas en procesos inflamatorios, trombosis e hipertensión (Kiliaan et al. 2014). Estas hormonas son las responsables del aumentado riesgo de demencia en pacientes que mantienen un IMC elevado durante gran parte de la vida. Por otro lado, en algunos casos esporádicos, cuando se observa una reducción drástica del IMC en pacientes ex obesos mayores de edad (alrededor de los 70 años), estas mismas hormonas podrían tener un papel neuroprotector, convirtiendo el sobrepeso en un factor positivo (figura 9).

La obesidad crónica tiene repercusiones a nivel central. Mediante una técnica de RMN denominada morfometría basada en tensores (Hua et al. 2008), la cual permite la observación simultánea tanto de la expansión como de la contracción de los volúmenes regionales cerebrales, se demostró que entre los individuos obesos (IMC > 30) había una disminución significativa del volumen cerebral. De hecho, un aumento de una unidad del IMC se asociaba a una disminución del 4% del volumen cerebral (Becker 2010).

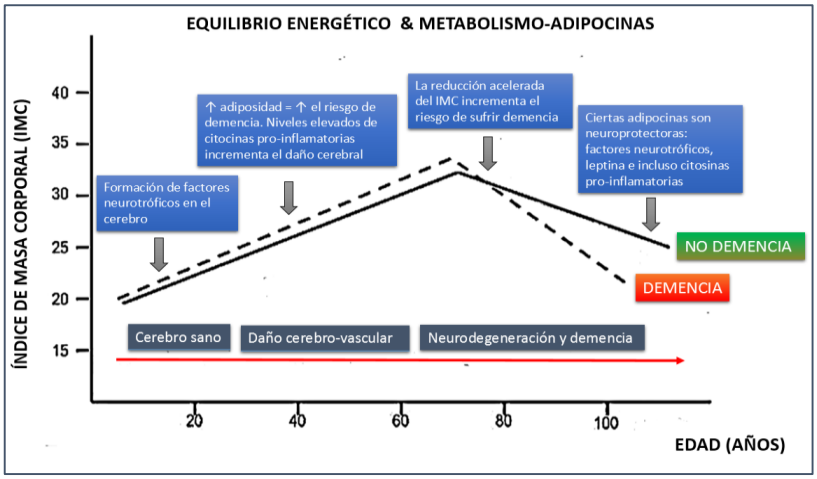


Figura 9. Relación entre la obesidad y la edad en la EA. Adaptación (Kiliaan et al. 2014). El tejido adiposo blanco está distribuido a lo largo de todo el organismo y secreta multitud de adipocinas para mantener la homeostasis celular. La cantidad de tejido adiposo se mide mediante el IMC. En promedio el IMC asciende durante la vida adulta y decrece en la vejez. Un IMC elevado se asociada a enfermedades cardiovasculares en la vida adulta, mientras que en la vejez una reducción del IMC pronunciada es un factor de riesgo para desarrollar EA. El mecanismo subyacente implica diferentes adipocinas proinflmatorias y factores neurotróficos.

- **Hiperlipidemia**

La hiperlipidemia e hipercolesterolemia son factores de riesgo metabólicos asociados a la enfermedad. Distintos estudios sugieren que la desregulación de las cascadas del metabolismo lipídico están implicadas en la génesis o desarrollo de la EA (Di Paolo & Kim 2011). Niveles elevados de colesterol hacia los 50 años de edad son un factor de riesgo pare el deterioro cognitivo leve en edades más avanzadas (Kivipelto et al. 2005).

Además, algunos estudios sugieren que las alteraciones del metabolismo del colesterol y la acumulación de algunos metabolitos como el 24S-hidroxicolesterol o el 27-hidroxicolesterol están asociadas con la neurodegeneración (Popp et al. 2013).

- **Hipertensión**

Existen estudios clínicos que sugieren la posibilidad de que pacientes con hipertensión tratada presenten menos lesiones neuropatológicas de EA respecto a pacientes no tratados (Hoffman et al. 2009). A su vez, los infartos cerebrales múltiples y recurrentes se han asociado a déficit cognitivo y a la aparición de EA (Newman et al. 2005; de Bruijn & Ikram 2014).

3. Factores de riesgo ambientales

- **Estrés crónico**

El estrés, considerado como la epidemia del siglo XXI en países de primer mundo, puede presentarse en épocas prenatales, neonatales o en edad adulta. Cada vez más estudios demuestran un posible rol crucial del estrés crónico en el desarrollo de enfermedades neuropsiquiátricas como depresión y ansiedad. Éstas a su vez han sido consideradas factores de riesgo para padecer EA. De hecho, el hipocampo, región cerebral muy afectada en la EA, es la estructura cerebral que más se ha relacionado con los efectos nocivos del estrés (Sapolsky 2000; Stratmann et al. 2014).

Además, la disminución de la neurogénesis, sobre todo en la zona subgranular del hipocampo, aparece como una alteración común en patologías asociadas al estrés (Snyder et al. 2011). La muerte neuronal en áreas implicadas en la neurogénesis desencadena atrofia hipocampal y deterioro cognitivo (Chadwick et al. 2011). Recientemente ha sido descrito que una situación de estrés crónico causa deterioro cognitivo (Cuadrado-Tejedor et al. 2012) y un aumento en la patología sináptica y amiloide, una situación muy similar a lo que ocurre en la EA.

- **Tabaco y alcohol**

El consumo de tabaco y alcohol también ha sido considerado como factor de riesgo de la patología. Un estudio reciente analiza 1629 individuos con el objetivo de evaluar si factores de riesgo metabólicos, como la diabetes y la obesidad, o ambientales, como el alcohol y el tabaco, afectan a áreas relacionadas con la memoria.

El estudio identificó que tanto el consumo de alcohol como la diabetes conllevaban un volumen total del cerebro más pequeño, mientras que el tabaquismo y la obesidad se relacionaban con volúmenes reducidos de la corteza cingulada posterior que representa el área del cerebro relacionada con la recuperación de la memoria (Srinivasa et al. 2015).

- **Dieta**

La dieta es una parte muy importante dentro de un estilo de vida saludable. La ingesta de alimentos poco saludables influye en el riesgo de padecer varias enfermedades y también afecta a la memoria y al aprendizaje hipocampal (Kanoski et al. 2011). El deterioro cognitivo podría provocar que el propio daño

hipocampal interfiriera con el control de la ingesta de alimentos, generándose una retroalimentación positiva (Kanoski et al. 2011).

En cuanto a los ácidos grasos, la ingesta moderada o elevada de grasas mono o poliinsaturadas se ha descrito como factor protector (Florent-Béchard et al. 2009), mientras que la ingesta moderada de grasas saturadas incrementa el riesgo de padecer EA (Morris & Tangney 2014), sobre todo en un fenotipo que presente la variante alélica *ApoE4*. Estos ácidos grasos podrían conducir al desarrollo de la enfermedad por diversos mecanismos como la aterosclerosis, el estrés oxidativo o la inflamación.

Las dietas hipercalóricas de mala calidad, con elevada presencia de grasas saturadas y azucares refinados, provocan en sujetos sanos una reducción en la memoria dependiente del hipocampo. (Francis & Stevenson 2011). Otro estudio indica que niños alimentados con dieta hipercalórica durante la adolescencia presentan un menor rendimiento académico (Nyaradi et al. 2014).

Los macronutrientes de la dieta y la composición en ácidos grasos son muy importantes para el correcto desarrollo cerebral. Los ácidos grasos saturados ingeridos durante la juventud o la mediana edad se asocian con una peor función cognitiva global, unas alteraciones en la memoria prospectiva y una mayor vulnerabilidad a los déficits relacionados con la vejez (Solfrizzi et al. 2011; Eskelinen et al. 2008; Tolppanen et al. 2014).

En contra, los ácidos grasos poliinsaturados (PUFA) se asocian con un incremento de la memoria y un menor riesgo de deterioro cognitivo (Solfrizzi et al. 2011; Eskelinen et al. 2008). Más en detalle, un mayor consumo de ácidos grasos tipo omega-3 se han relacionado con un menor riesgo de EA (Luchtman & Song 2013; Swanson et al. 2012).

La ingesta de antioxidantes puede reducir el riesgo de sufrir demencia, ya que disminuye la aparición de enfermedades cerebrovasculares, el estrés oxidativo y la inflamación (Morris 2004).

- **Lesión cerebral**

Existe evidencia sólida de que las lesiones cerebrales traumáticas aumentan el riesgo de desarrollar demencia. Los sujetos que han experimentado lesiones en la cabeza de forma repetida (boxeadores, futbolistas y veteranos de guerra) pueden tener un mayor riesgo de sufrir EA (Vanacore 2013; Baumgart et al. 2015), Si bien no se sabe qué aspecto específico de la lesión es más relevante (fuerza, repetición, etc.) y qué conduce a las alteraciones en la función cerebral.

En resumen, son muchos los estudios que se han realizado con el fin de evaluar varios factores de riesgo para la patología de la EA. Parece que en los estadios preliminares de la enfermedad los factores de riesgo más importantes sean las lesiones cerebrales, la obesidad, la hipertensión, el tabaquismo y la diabetes. En una una fase más avanzada, la lesión cerebral sigue siendo un factor de riesgo predominante, mientras que la obesidad, la hipertensión, el tabaquismo y la diabetes contribuyen en una menor medida (figura 10).

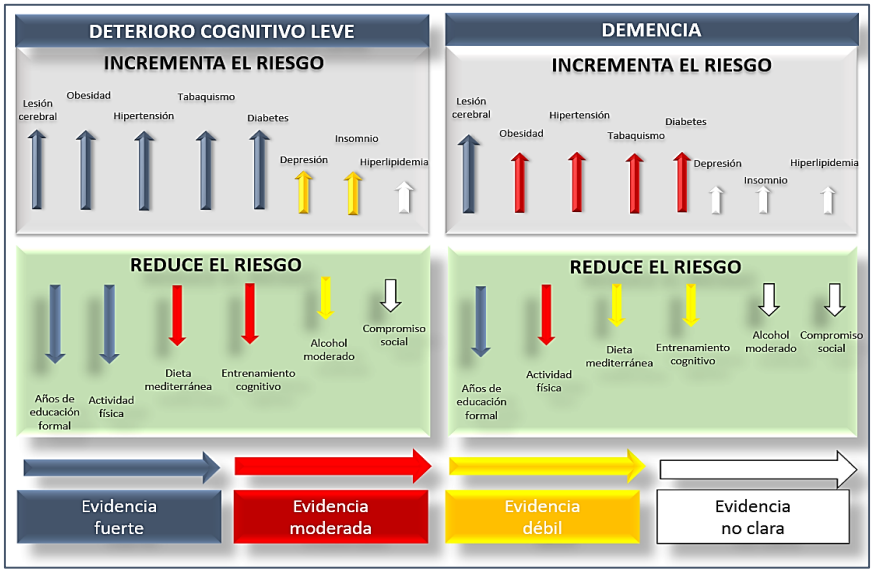


Figura 10. Factores de riesgo relevantes en el deterioro cognitivo y en la demencia (Baumgart et al. 2015).

1.7 Hipótesis sobre la etiopatogénia de la EA

En las últimas décadas se han formulado diferentes teorías que podrían explicar la aparición de la enfermedad. Inicialmente se analizó la estrecha correlación entre la acumulación a nivel cerebral de metales cuales el aluminio y el desarrollo de la patología (Terry & Pena 1965); más adelante se puso en relación la enfermedad con la reducción de los niveles del neurotransmisor acetilcolina (Davies & Maloney 1976). En los años 90 se publicaron varias hipótesis:

La hipótesis de la cascada Aβ y la proteína tau (Hardy & Allsop 1991); La hipótesis vascular (de la Torre & Mussivand 1993); La hipótesis de los canales iónicos (Pollard et al. 1995); La hipótesis del estrés oxidativo (Markesbery 1997); La hipótesis de la neurodegeneración excitotóxica (Olney et al. 1997);

Recientemente se han publicado nuevas hipótesis: La hipótesis de la neuroinflamación (Akiyama et al. 2000); La hipótesis de la cascada mitocondrial

(Swerdlow & Khan 2004a); La hipótesis de las disfunciones sinápticas (Snyder et al. 2005); La hipótesis de la diabetes tipo III o metabólica (de la Monte & Wands 2005); La hipótesis de las dendritas (Cochran et al. 2014).

Todas las teorías citadas siguen en continua revisión y, en conjunto, nos indican que la enfermedad es de origen multifactorial.

Los mecanismos que activan las desregulaciones homeostáticas no se conocen con precisión. En este trabajo se explican y se interconectan tres hipótesis con el fin de elucidar en la medida de lo posible la biología molecular de la EA en un modelo de ratón transgénico. Las hipótesis en que hemos trabajado son:

1. La hipótesis de la cascada del péptido Aβ amiloide y la proteína tau.
2. La hipótesis de la cascada mitocondrial.
3. La hipótesis metabólica.

1.7.1 Hipótesis de la cascada Aβ y la proteína tau

La hipótesis amiloide se formuló a principios de los años 90 (Hardy & Higgins 1992; Hardy & Allsop 1991) y considera que la producción patológica de los péptidos Aβ es la principal causa de la muerte neuronal y de las disfunciones centrales implicadas en la enfermedad.

El procesamiento defectuoso de la proteína precursora amiloide (APP) por acción de la β-secretasa produce un incremento de Aβ-42, que lleva a la formación de placas seniles. La formación de placas seniles activa la microglía y la astroglía, causando daño oxidativo, hiperfosforilación de tau y consecuentemente perdida neuronal y disfunción sináptica (Hardy & Higgins 1992) (figura 11).

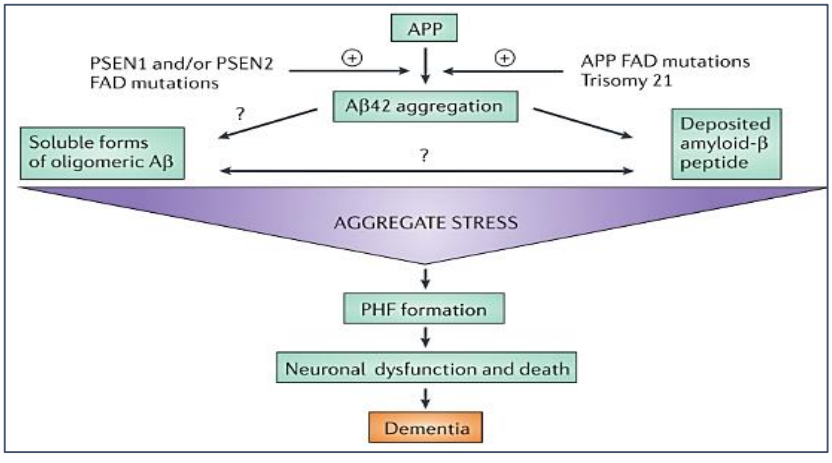


Figura 11. Hipótesis de la cascada amiloide. (Karran et al. 2011).

La proteína APP es una proteína transmembrana de tipo I con el extremo amino-terminal orientado hacia el espacio extracelular y el extremo carboxi-terminal hacia el citosol. Se localiza en todos los tipos celulares y su ARNm representa el 0,2% del total en neuronas.

Un gran número de funciones se han atribuido a la proteína APP y a sus derivados (Bossy-Wetzel et al. 2004). Por ejemplo, se ha descrito que el ectodominio amino-terminal soluble que se libera a partir de la APP como consecuencia de la acción proteolítica de la α-secretasa (α-APPs), actuaría como un factor autocrino y también tendría propiedades neurotróficas y neuroprotectoras (Jiang et al. 2013).

En 2004 el grupo de Herms desarrolló un modelo de ratón triple transgénico "knockout" para APP y dos proteínas similares, APLP1 y APLP2. Los autores observaron que este tipo de animal transgénico muere prematuramente y muestra síntomas de un extraño trastorno neurológico. A nivel morfológico, tales ratones sufren una gran displasia cortical (caracterizada por una fragmentación de la lámina basal). Los mecanismos moleculares implicados en estos procesos patológicos siguen actualmente desconocidos, aunque estas observaciones confirman la importancia del papel de la APP en los procesos de adhesión y migración celular (Herms et al. 2004).

La proteína APP es sintetizada a nivel del retículo endoplasmático y, después de su síntesis, es extensamente modificada a nivel postrasduccional mediante glucosilaciones complejas (N- u O- glucosilaciones). Sucesivamente, la APP es transportada al aparato de Golgi en la zona de la red del trans Golgi (TGN, trans-Golgi-network). La translocación de la APP desde la TGN hacia la superficie celular se verifica a través de vesículas secretoras. Durante este proceso, se producen ulteriores fosforilaciones y sulfataciones que conducen a la proteína final.

Una vez internalizada a nivel de la membrana celular, la APP puede sufrir una variedad de escisiones proteolíticas, actuadas por tres diferentes proteasas: α-secretasa, β-secretasa y γ-secretasa. Dichas escisiones se pueden agrupar en dos vías: la vía amiloidogénica y la vía no amiloidogenica (figura 12).

El fragmento Aβ es producido por la activación de la vía amiloidogénica, gracias a la acción consecutiva de la β- y de la γ- secretasa (Haass 2004). La actividad de la β-secretasa inicia el proceso liberando una parte del ectodominio de APP (APPsβ) y generando un fragmento de APP carboxi-terminal (β-CTF o C99), que seguidamente es escindido por la γ-secretasa. Este último corte proteolítico, realizado por la γ-secretasa, produce la liberación del fragmento Aβ que es soluble y, por lo tanto, se puede encontrar en fluidos extracelulares como el plasma o en el LCR (Seubert et al. 1992).

En la vía no amiloidogénica, la APP es escindida en la parte central mediante la actividad de la α-secretasa. Este procesamiento genera otro tipo de fragmento de APP carboxi-terminal (α-CTF o C83), que carece de la porción del dominio Aβ. Posteriormente C83 es escindido por la γ-secretasa, liberando un péptido llamado p3 (Haass et al., 1993), que es patológicamente irrelevante. La γ-secretasa no solo libera Aβ (a partir de C99) y p3 (a partir de C83), sino que también genera un dominio intracelular de APP (AICD, del inglés amyloid intracellular domain) (Radzimanowski et al. 2008) que es liberado al citosol y puede tener funciones a nivel de señalización nuclear (Cao & Südhof 2001; von Rotz et al. 2004).

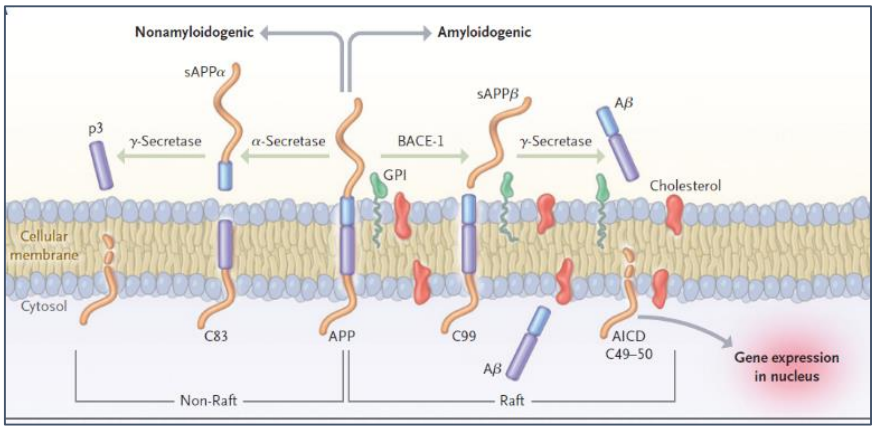


Figura 12. Procesamiento de la APP. A la izquierda se muestra el procesamiento por α-secretasa que forman C83 y sAPPα. Secuencialmente C83 es procesado por γ-secretasa, originando AICD y p3, que en su conjunto constituye la vía no amiloidogénica. A la derecha, el procesamiento por β-secretasa que origina C99 y sAPPβ Secuencialmente C99 es procesado por γ-secretasa, originando AICD con fragmentos Aβ, respectivamente, lo que constituye la vía amiloidogénica(Querfurth & Laferla 2010).

La vía amiloidogénica y la vía no amiloidogénica compiten entre ellas, al menos en algunos compartimentos subcelulares, ya que se ha descrito que, incrementando la actividad de la α-secretasa en modelos animales de EA o en cultivos celulares, es posible reducir la producción de Aβ y la formación de placas seniles (Postina et al. 2004).

Las placas seniles o agregados amiloides están formadas por depósitos amorfos de péptidos Aβ, cuyo tamaño puede variar de 39 a 43 residuos aminoacídicos, y se encuentran rodeadas de axones, dendritas distróficas, astrocitos y microglía activada. Este tipo de placas se localiza principalmente en regiones límbicas (hipocampo y amígdala) y también en regiones corticales y subcorticales, y genera alteraciones en la permeabilidad de la barrera hematoencefálica (BHE). Los agregados del péptido Aβ en el cerebro desencadenan la cascada de

neurotoxicidad que conduce a muerte celular por apoptosis, excitotoxicidad, inflamación, estrés oxidativo, desregulaciones en la homeostasis del calcio, pérdida de las sinapsis y reducción de ATP celular (LaFerla et al. 2007). Además, se ha descrito que niveles elevados del péptido Aβ activan la fosforilación de Tau a través de la glucógeno sintetasa cinasa 3β (GSK-3β), promoviendo la formación de los ovillos neurofibrilares (ONF) responsables del proceso neurodegenerativo a nivel intracelular.

Tau es una proteína altamente soluble presente en todas las células nucleadas cuya principal función es la estabilización de los microtúbulos, esenciales para el transporte de vesículas y orgánulos entre el soma y las neuritas (Caviston & Holzbaur 2006).

La proteína tau consta de dos dominios funcionales: el dominio de proyección y el dominio de unión a microtúbulos (MBD).

El dominio de proyección es así denominado porque se proyecta fuera de la superficie del microtúbulo. Representa los dos tercios de la molécula e incluye el extremo amino terminal (N-terminal); a su vez, se divide en dos regiones: la región N-terminal y la región rica en prolinas.

El dominio de unión a microtúbulos contiene dos regiones: la región de las repeticiones y la región carboxilo terminal (C-terminal). La región C-terminal también es rica en prolinas y contiene varios residuos susceptibles de fosforilación.

El balance fosforilación/defosforilación regula las funciones fisiológicas de la proteína tau (Martin et al. 2013). La hiperfosforilación de tau se produce como consecuencia del desequilibrio entre la acción de las cinasas y las fosfatasas.

Existen dos tipos de cinasas que fosforilan tau en residuos de serina o de treonina: las cinasas dirigidas por prolinas (PDPK) y las cinasas no dirigidas por prolinas (non PDPK).

Entre las dirigidas por prolinas se encuentran la tau proteína cinasa I o GSK3, la tau proteína cinasa II o Cdk5, la MAP cinasa o p38 MAPK, y otras cinasas dependientes de ciclina como Cdc2, JNK y SAPK. En el grupo de las no dirigidas por prolinas (non PDPK) están la proteína cinasa A (PKA), la proteína cinasa C (PKC), la calcio-calmodulina cinasa II (CaMKII), la MARK cinasa y la caseína cinasa II (CKII). Por último, existe un grupo de proteínas que fosforilan tau en residuos de tirosina: las tirosinas cinasas o TPK; entre estas se encuentran SFK y c-abl (Martin et al. 2013; Cárdenas et al. 2012).

Cuando la tau es hiperfosforilada en los residuos Ser-Pro o Thr-Pro pierde su actividad biológica a causa del cambio conformacional. Este fenómeno provoca la disociación de Tau de los microtúbulos y la consecuente translocación al

compartimento somatodendrítico. La proteína se vuelve resistente a la degradación proteolítica y padece ulteriores cambios conformacionales que la hacen insoluble. En consecuencia, tau polimeriza hasta formar los filamentos helicoidales apareados (PHF), que son el principal componente de los ONF (Metcalfe & Figueiredo-Pereira), (Figura 13).

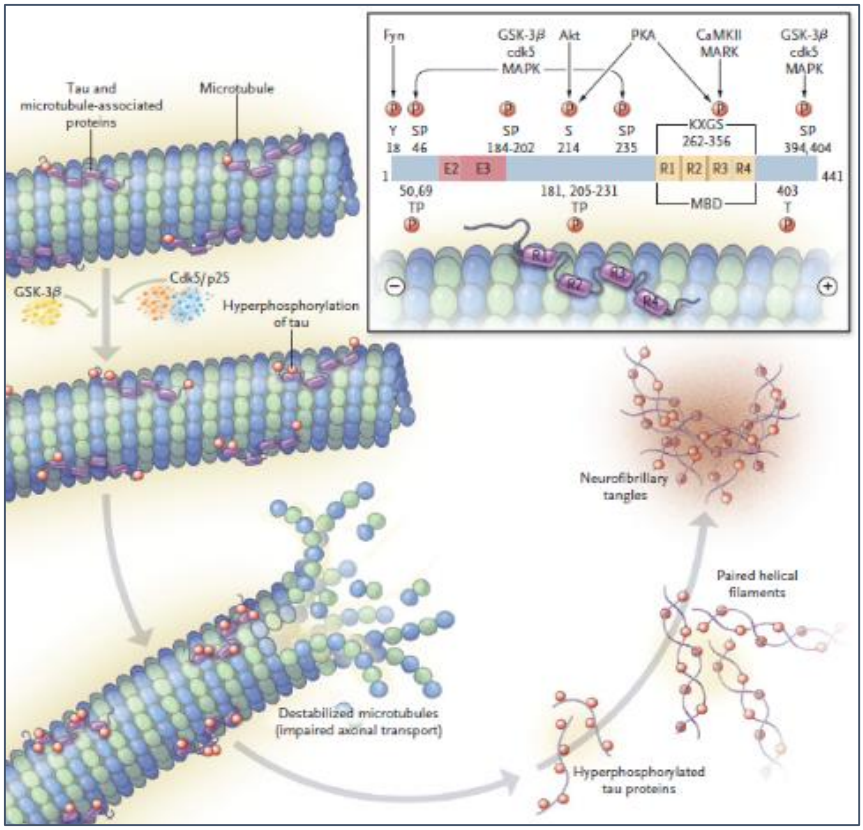


Figura 13. Estructura de Tau y función. La isoforma más larga de tau humana en el SNC, es de 441 aminoácidos y contiene 85 residuos susceptibles de fosforilación. La mayoría de los residuos susceptibles de fosforilación se sitúan en la región rica en prolinas o en el extremo carboxilo terminal, sin embargo, la desestabilización de los microtúbulos se produce en mayor medida cuando se fosforilan los sitios localizados en el dominio de unión a microtúbulos. La hiperfosforilación de tau se produce como consecuencia del desequilibrio entre la acción de quinasas y fosfatasas, de manera que se favorezca el estado fosforilado. En este sentido, en la EA, se ha descrito tanto una diminución de la actividad fosfatasa, principalmente de la PP2A y PP1, como un aumento de la actividad quinasa, concretamente de GSK3 y Cdk5. (Querfurth & Laferla 2010)

Existen múltiples evidencias que corroboran la hipótesis de la cascada del péptido Aβ y de la hiperfosforilación de tau. Uno de los primeros indicios para su formulación fue la observación de que un porcentaje importante de casos de EA

familiar de aparición precoz se producían por mutaciones de los genes app, ps1 y ps1 (Bekris et al. 2010). Por lo general, estas mutaciones conducen a un aumento en la producción del péptido Aβ y del cociente Aβ1-42/Aβ1-40 y probablemente a una pérdida de las funciones fisiológicas de APP y PS (De Strooper 2007). Asimismo, en base a las mutaciones de los genes *app*, *ps1 y ps2* asociados al desarrollo de EAf, se han generado multitud de modelos animales que desarrollan placas y muestran alteraciones en la memoria, entre ellos el ratón APP/PS1 utilizado para realizar este trabajo.

Además se ha observado que en cultivos neuronales de hipocampo de ratón la toxicidad del Aβ es dependiente de la proteína tau. Otros estudios relacionan cambios en el metabolismo de Aβ con el riesgo de la EA de aparición tardía (Hardy & Selkoe 2002).

1.7.2 Hipótesis de la cascada mitocondrial

El genoma humano está constituido por los miles de genes localizados en los 23 cromosomas heredados del padre y los 23 heredados de la madre. Todos ellos están localizados en el núcleo celular. Además, un pequeño número de genes, 37, se encuentran situados en un cromosoma circular y cerrado localizado en las mitocondrias y que se conoce como DNA mitocondrial (mtDNA). Estos genes mitocondriales codifican 13 proteínas del sistema de fosforilación oxidativa (OXPHOS) y los RNAs necesarios para su expresión, determinando el correcto funcionamiento de la mitocondria.

La hipótesis de la cascada mitocondrial se planteó para explicar la EAe (Swerdlow & Khan 2004a). El equipo de Swerdlow analizó el DNA mitocondrial (mtDNA) mediante cíbridos. Los cíbridos son líneas celulares (SH-SY5Y y NT2) en las que ha sido suprimido el mtDNA y que luego han sido fusionadas con plaquetas de donantes humanos con y sin EA. De esta forma se pudo comparar el DNA mitocondrial humano de pacientes con y sin EA de una forma rápida y segura. Los resultados mostraron que las células de pacientes con EA presentaban una reducción de la actividad de la citocromo oxidasa y del potencial de la membrana mitocondrial. En contra, las vías de señalización del estrés oxidativo, de la apoptosis, de la producción de Aß42 y también los niveles de radicales libres resultaron incrementados (Swerdlow et al. 2010).

Al día de hoy se discute sobre cuál son los principales desencadenantes de la enfermedad, si las mutaciones mitocondriales o la producción del fragmento Aβ (Mancuso et al. 2009). De todas formas, está consensuado que el conocimiento de la funcionalidad mitocondrial es fundamental para explicar el desarrollo de la EA.

Las neuronas contienen varios centenares de mitocondrias necesarias para cumplir las demandas energéticas. Las células neuronales requieren energía para un correcto gradiente iónico y para desarrollar los procesos sinápticos. Las mitocondrias neuronales producen el ATP en su mayor parte por fosforilación oxidativa, usando como combustible la glucosa que se capta del medio extracelular (las neuronas tienen una capacidad muy limitada de almacenamiento de glucosa, de manera que la reducción de glucosa disponible en el medio produce un detrimento del estado energético de la neurona). En ausencia de glucosa otras sustancias que quedan, como el piruvato o el lactato pueden ser usadas para la respiración mitocondrial, aunque la disponibilidad de estos metabolitos termina rápidamente y la producción de ATP desciende. La mayor parte del ATP producido (aprox. 60%) se consume por la actividad de una enzima localizada en la membrana plasmática, la ATPasa Na^+/K^+. Esta enzima es la encargada del mantenimiento del potencial de reposo de la membrana neuronal y evita que dentro de la célula haya un exceso de iones de Na^+ y que se produzca su despolarización.

La mitocondria es un orgánulo altamente dinámico. Su forma y biogénesis están controladas por fusión y fisión, mientras su estructura interna cambia en función de su estado fisiológico. La mitocondria participa en interacciones recíprocas con otros orgánulos, como el retículo endoplasmático.

La mitocondria constituye uno de los compartimentos celulares más susceptible a sufrir daño oxidativo. En particular, el mtDNA, por su proximidad a la cadena de transporte de electrones, y sobre todo por la carencia de histonas protectoras y de mecanismos de reparación eficientes, constituye un substrato ideal para el impacto de las especies reactivas de oxígeno y nitrógeno. Las mutaciones del genoma mitocondrial se acumulan durante el proceso natural del envejecimiento a causa de lesiones producidas por las especies reactivas de oxigeno (Schipper 2004). También se han observado altos niveles de mutaciones en el mtDNA del lóbulo temporal de pacientes con EA (Corral-Debrinski et al. 1992) que a la vez mostraban niveles elevados de daño oxidativo (Mecocci et al. 1994).

La toxicidad del péptido amiloide afecta directamente a la mitocondria e incrementa los niveles intracelulares de $Ca2^+$ y de NO en astrocitos y neuronas (Yan & Stern 2005). El péptido Aβ es un potente tóxico para la mitocondria; la exposición a Aβ inhibe las proteínas mitocondriales como el citocromo C, proteína esencial para el sistema de fosforilación oxidativa (Querfurth & Laferla 2010). En consecuencia, el transporte de electrones, la producción de ATP, el consumo de oxígeno y el potencial de membrana mitocondrial resultan alterados.

El estrés oxidativo producido por mutaciones o sustancias tóxicas interfiere en el control homeostático. En pacientes con EA se ha comprobado que hay un elevado ratio de fragmentación mitocondrial y se producen mitocondrias más pequeñas. (Cho et al. 2009). También la acumulación de Aβ y de la proteína tau hiperfosforilada afecta a homeostasis del calcio y produce un incremento de ROS y de NO (Brunden et al. 2009).

El NO es una molécula de señalización involucrada en muchos procesos fisiológicos de la célula, incluyendo la liberación de neurotransmisores y la plasticidad sináptica. Sin embargo, cuando los niveles de NO son excesivos, pueden contribuir a la toxicidad neuronal. Experimentalmente se ha demostrado que la exposición de neuronas al fragmento Aβ oligomerizado aumenta la nitrosilación en el residuo cisteína 644 de la proteína *dynamin-related protein* (Drp1), causando fragmentación mitocondrial (Cho et al. 2009; Nakamura et al. 2010).

La producción de energía en forma de ATP y la reducción de oxígeno a H_2O tienen lugar en la membrana interna de la mitocondria. El sistema OXPHOS, constituido por cinco subunidades o complejos, está implicado en estos procesos (DiMauro & Schon 2003) (figura 14).

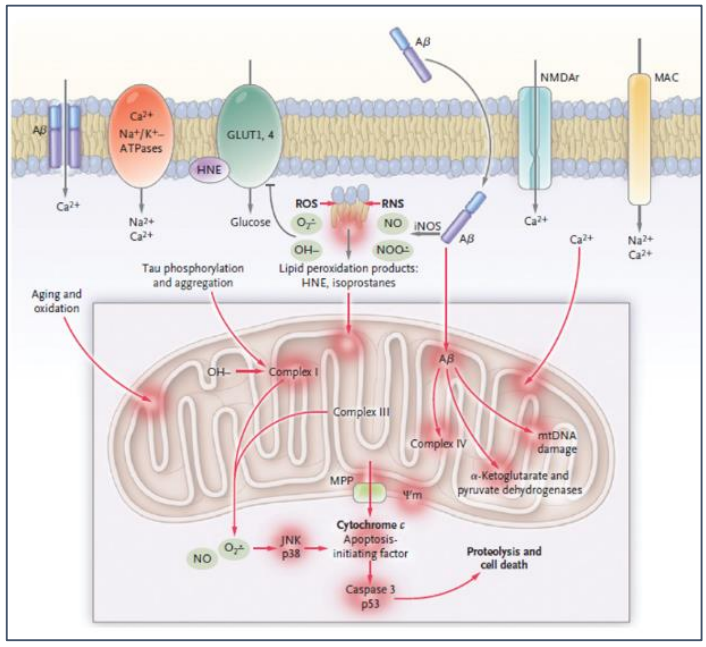


Figura 14. Efectos de la Aβ Estrés oxidativo y fallos mitocondria. (Querfurth & Laferla 2010).

Complejo I*:* también conocido como NEAH deshidrogenasa (ubiquinona oxidoreductasa) es un nucleótido con electrones de alta energía proveniente del ciclo del ácido cítrico. El complejo I transfiere electrones del NEAH a la ubiquinona o coenzima Q (CoQ) y luego al succinato, siguiente paso en la cadena de transporte. Al pasar de un transportador al siguiente, los electrones liberan energía que es utilizada por el complejo para bombear protones (H+) de la matriz al espacio de la intermembrana. Esto genera un gradiente transmembrana que termina activando a la enzima ATP sintetasa o ATPasa.

Complejo II*:* succinato-ubiquinona reductasa o succinato deshidrogenasa; en este complejo se transfiere electrones del succinato a la CoQ. En esta etapa no se produce traslocación de protones a través de la membrana; por lo tanto, el complejo II es un simple transportador entre los complejos I y III.

Complejo III*:* citocromo c, en este complejo se transporta electrones de la CoQ al citocromo C. En esta etapa hay traslocación de protones.

Complejo IV*:* constituido por la enzima citocromo C oxidasa (COX) que utiliza al citocromo C como sustrato. La enzima toma cuatro electrones del citocromo c y los transfiere a dos moléculas de oxígeno formando agua. Esta es la única circunstancia en que el oxígeno (que por su estructura atómica sólo puede tomar uno o a lo sumo dos electrones a la vez), adquiere en forma simultánea cuatro electrones. En esta etapa hay traslocación de protones.

Complejo V*:* Constituido por la enzima ATPasa (ATP sintetasa) que funciona en forma reversible. La enzima aprovecha la energía generada por la traslocación de protones en los complejos I, III y IV para sintetizar ATP que es el objetivo final de todo este mecanismo. Actuando en forma reversible, la ATPasa puede, a su vez, hidrolizar el ATP para bombear, contra gradiente, protones desde el espacio intermembrana hacia la membrana, con un mecanismo inverso al que se verificaba en los complejos I, III, y lV.

Recientemente se ha descrito la implicación de la los complejos OXPHOS en trastornos neurodegenerativos (Knott et al. 2008). También se conoce la relación entre el fragmento β-amiloide y la COX (subunidad IV) (Yao et al. 2009; Pickrell et al. 2009).

La biogénesis mitocondrial defectuosa y las anomalías en la respiración mitocondrial constituyen una característica del tejido cerebral en pacientes con EA. Estas alteraciones afectan a la vía de PKA/CREB/PGC1α (Castellani et al. 2002; Sheng et al. 2012). En tal sentido, estudios bioquímicos realizados *post-mortem* en cerebros de pacientes con EA han puesto de manifiesto deficiencias en la actividad de enzimas clave del ciclo de Krebs como la piruvato deshidrogenasa y la α-cetoglutarato deshidrogenada (Bubber et al. 2005).

El coactivador *transcriptional peroxisome proliferator-activated receptor-γ coactivator-1α* (PGC-1α), es un regulador de la biogénesis mitocondrial, termogénesis y homeostasis de lípidos y glucosa (St-Pierre et al. 2006).

PGC1α tiene como principal función la detoxificación de ROS (St-Pierre et al. 2006; Austin & St-Pierre 2012). Es un coactivador de moléculas que se encarga de ayudar determinados factores de transcripción, como PPAR γ y los *Nuclear Respiratory Factor-1 y 2* (NRF-1 y NRF-2), a unirse a regiones concretas de genes nucleares encargados de codificar proteínas mitocondriales como la *mitochondrial transcription factor A* (Tfam), incrementando su actividad.

En los procesos neurodegenerativos la actividad de PGC1α se reduce y por tanto disminuye la biogénesis mitocondrial y la detoxificación de ROS (Austin & St-Pierre 2012).

1.7.3 Hipótesis metabólica

Recientemente se ha planteado la hipótesis de que la EA sea de tipo metabólico. Muchos autores han utilizado la expresión diabetes tipo 3 para referirse a la patología (de la Monte & Wands 2005).

La hipótesis considera que los trastornos metabólicos periféricos provocan un incremento de los péptidos Aβ a nivel central (Steen et al. 2005; de la Monte, Suzanne M Wands 2008; Kroner 2009; Accardi et al. 2012).

La diabetes mellitus (DM) es una enfermedad crónica que se genera cuando el cuerpo no puede producir suficiente insulina o utilizarla de manera eficaz. La insulina es una hormona producida por las células beta del páncreas que permite la entrada de la glucosa a las células del cuerpo. Una vez en las células, la glucosa se convierte en la energía necesaria para que funcionen los músculos y los tejidos. Una persona que padece diabetes no absorbe adecuadamente la glucosa por lo que esta sigue circulando por la sangre. Con el paso del tiempo la glucosa circulante daña los tejidos del cuerpo.

Existen tres tipos de diabetes: diabetes mellitus tipo 1 (DM1), diabetes gestacional y diabetes mellitus tipo 2 (DM2). La DM1 es causada por una reacción autoinmune donde el sistema de defensa del cuerpo ataca a las células beta del páncreas, productoras de insulina. La diabetes gestacional se presenta en la mujer durante el embarazo alrededor de la semana 24 y normalmente desaparece después del nacimiento. En la DM2 el cuerpo produce su propia insulina pero no puede responder a sus efectos, dando lugar a la acumulación de glucosa en la sangre.

La relación entre la DM y la EA está publicada en varios estudios experimentales. En 2009, Ke y colaboradores estudiaron la posible relación entre la DM1 y la patología de la proteína tau implicada en el desarrollo de los ovillos neurofibrilares. Los autores examinaron los efectos de la DM1 en la en la cepa de ratón transgénico pR5 que presentaba patología tau preexistente. La DM1 experimental fue inducida mediante la administración de estreptozotocina, que causa la deficiencia de insulina. Se determinó la fosforilación de tau, mediante la inmunohistoquímica y *Western Blot*. Los resultados demostraron que la disminución de la insulina después de la inyección de estreptozotocina y el consecuente aumento de los niveles de glucosa en sangre causaban hiperfosforilación de tau (Ke et al. 2009)

Otro estudio determinó que la insulina participa en el mantenimiento de las funciones del SNC. Efectivamente, la expresión génica de numerosos genes relacionados con la secreción de insulina pancreática resultó alterada a nivel central en un modelo de ratón diabético; datos similares sugieren que esos genes podrían mediar la producción de insulina y su exocitosis en el cerebro (Abdul-Rahman et al. 2012).

Zhang y colaboradores investigaron el efecto del péptido Aβ en la sensibilidad a la insulina mediante la inyección *in vivo* de Aβ42 en ratones control. Los resultados demostraron que Aβ induce resistencia a la insulina hepática a través de la cinasa JAK2, lo que sugiere que la inhibición de la señalización de Aβ podría ser una nueva estrategia para tratar la resistencia a la insulina y la DM2 (Zhang et al. 2013).

La hipótesis de la diabetes tipo 3 considera que los desórdenes metabólicos (elevados niveles de glucosa en sangre, obesidad, esteatohepatosis o resistencia a insulina periférica) pueden producir efectos adversos sobre la memoria. Así, la hiperinsulinemia periférica prolongada disminuye los receptores de insulina a nivel de la BHE, alterando el transporte de insulina hacia el cerebro.

En resumen, la alteración de la vía de señalización de la insulina/IGF1 contribuye a la neurodegeneración debido al aumento de:

- Cinasas que fosforilan Tau.
- Síntesis y expresión de APP-Aβ.
- Estrés del retículo endoplasmático (ER).
- Generación de especies reactivas de oxígeno y especies reactivas de nitrógeno que dañan las proteínas, RNA y DNA.
- Disfunciones mitocondriales.
- Activación de mecanismos de inflamación y muerte neuronal.
- Desregulación de genes diana implicados en la homeostasis colinérgica.
- Metabolismo de lípidos alterado.

Todos estos efectos adversos afectan a la memoria, cognición y plasticidad cerebral (de la Monte 2012a; Adeghate et al. 2013).

Diversas evidencias muestran que los trastornos metabólicos, el estrés oxidativo, la neuroinflamación y la resistencia a la insulina/IGF participan en la EA. Las alteraciones en la vía de señalización de insulina y de IGF conducen a una mayor expresión del precursor de la proteína Aβ (AβPP) y a la consecuente acumulación de los péptidos AβPP-Aβ. Además, tales alteraciones promueven el estrés oxidativo e inhiben la ruta neuroprotectora AKT/GSK3β provocando neurodegeneración (figura 15).

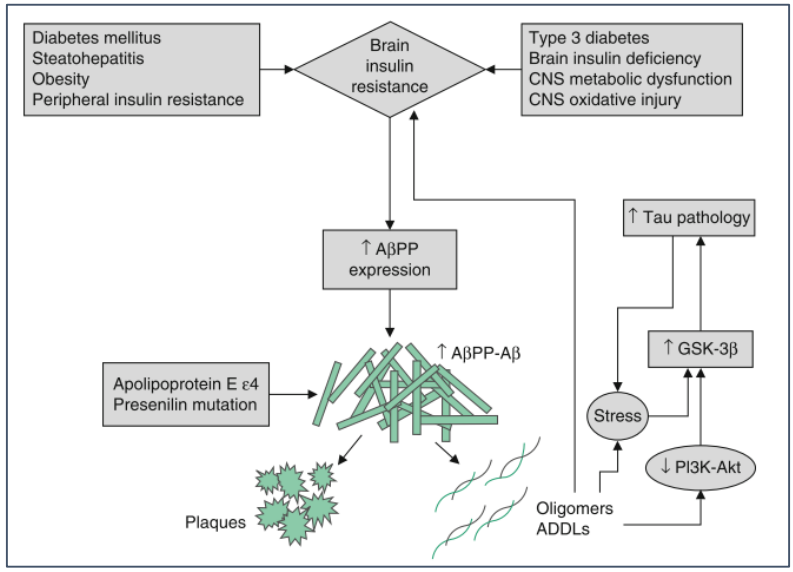


Figura 15. Alzheimer y diabetes tipo III. (de la Monte 2012b)

El proceso de envejecimiento y la esperanza de vida también están condicionados por la vía de la insulina. Un estado de resistencia a la insulina vuelve las neuronas más vulnerables al estrés oxidativos y altera la plasticidad sináptica. Además, el aumento de los niveles plasmáticos de glucosa, característico del envejecimiento, es responsable del daño a la estructura del hipocampo, de la hiperfosforilación de las proteínas tau y de la reducción en la actividad de la enzima degradadora de insulina (IDE).

La enzima degradora de insulina (IDE) es una Zn^{2+}-metaloproteasas de 113 KDa, altamente conservada, presente en el medio extracelular, en los peroxisomas y las mitocondrias. Los sustratos para las IDE son la cadena β de la insulina, lo péptidos Aβ, la amilina y el glucagón. En condiciones de resistencia a insulina IDE

reduce su actividad permitiendo un incremento de los péptidos Aβ, fenómeno que a su vez reduce la actividad de IDE provocando un circuito de retroalimentación positiva (Zhao et al. 2007).

A pesar de la estrecha relación entre las dos enfermedades, no queda claro si la aparición de resistencia a insulina tiene lugar antes o después de la acumulación del fragmento Aβ. Sin embargo, los pacientes con DM2 tienen mayor riesgo de desarrollar MCI, demencia y EA. Además, los pacientes con EA tratados con insulina intranasal mejoran sus puntaciones en test conductuales (Formiga & Pérez-Maraver 2014).

En cuanto a la administración intranasal crónica de insulina, se ha observado que mejora el rendimiento cognitivo en individuos no dementes y con EA (sin alterar los niveles de glucosa o insulina plasmática), y ha sido demostrado que la administración aguda de insulina mejora la memoria declarativa en pacientes con EA (de la Monte 2012b; I. Clark et al. 2012; Freiherr et al. 2013). Además la administración de antidiabéticos sensibilizadores de la insulina, como la rosiglitazona, ralentiza el declive cognitivo en pacientes con EA (Watson et al. 2005).

2. Metabolismo en el sistema nervioso central y en la EA

El cerebro humano representa el 2% del peso total del cuerpo y para su funcionamiento energético requiere hasta el 20% del oxígeno y el 25% de la glucosa totales, llegando en algunas ocasiones a gastar hasta el 50% (Peters et al. 2007).

Aproximadamente el 75% de la energía consumida por el cerebro es utilizada en procesos de señalización, mientras que el restante 25% es utilizado para actividades esenciales celulares como la síntesis y degradación de proteínas y de fosfolípidos (Attwell & Laughlin 2001; Alle et al. 2009).

La situación particular del cerebro respecto al resto de los órganos del cuerpo se caracteriza por su aislamiento químico mediante BHE. La BHE regula la permeabilidad del endotelio vascular del SNC. Solo pueden atravesarla el agua, gases como el oxígeno y el CO_2, determinadas moléculas liposolubles muy pequeñas (<400-600 Da) y moléculas orgánicas con sistemas de transporte específico y regulado.

La glucosa pasa la barrera mediante un transportador de glucosa GLUT-1 específicos de la familia (GLUTs); una vez dentro de las células interviene una hexoquinasa para producir glucosa-6-fosfato. Al igual que en otros órganos, la glucosa 6-fosfato puede ser procesada a través de diferentes vías metabólicas, (Bélanger et al. 2011), siendo las principales la glucólisis, el ciclo de ácidos tricarboxílicos (TCA) o ciclo de Krebs y la fosforilación oxidativa (cadena de transporte de electrones).

La captación de glucosa mediante transportadores pasivos es estimulada por la insulina. Concretamente, la insulina induce un cambio de ubicación celular del transportador que se transloca desde los compartimentos citoplasmáticos hasta la membrana superficial, lo que conlleva un aumento de la captación de glucosa plasmática.

En general, la glucosa en el cerebro se oxida casi en su totalidad a CO_2 y agua (Clarke & Sokoloff 1999). En las neuronas la glucosa es el principal sustrato para la producción de energía, aunque en determinadas circunstancias como el ayuno o el esfuerzo físico intenso se pueden utilizar otros substratos energéticos derivados de la sangre, como los cuerpos cetónicos (Bélanger et al. 2011) o el lactato (van Hall et al. 2009).

En situaciones patológicas de resistencia a la insulina o DM, las neuronas no disponen de glucosa suficiente para su metabolismo energético; por lo tanto se producen adaptaciones metabólicas que dan lugar a la producción de cuerpos cetónicos en las mitocondrias de los hepatocitos. Estos cuerpos cetónicos, cuyos

representantes principales son el acetoacetato y el 3-D-β-hidroxibutirato, van a ser el sustrato energético alternativo para las neuronas.

Esto significa que el principal papel fisiológico de los cuerpos cetónicos es transferir la energía derivada de los lípidos del hígado a los tejidos periféricos, de manera que en periodos de hipoglucemia o situaciones patológicas las células de los varios tejidos dispongan de un substrato alternativo para mantener la homeostasis energética. Bajo esas condiciones anómalas, la supervivencia cerebral será fuertemente (aunque no únicamente) dependiente de las reservas de triglicéridos periféricos (Peters et al. 2007).

Cabe destacar que en humanos, en periodos prolongados de ayuno o resistencia a insulina, los cuerpos cetónicos aportan aproximadamente el 75% de las necesidades energéticas del cerebro (Cahill 2006).

Los cuerpos cetónicos son sustancias hidrofílicas que no atraviesan fácilmente la BHE por lo que necesitan de proteínas transportadoras como los transportadores de monocarboxilatos. A largo plazo, la cetonemia causada por el ayuno prolongado o la cetoacidosis diabética induce un aumento de la actividad de esas proteínas transportadoras que conducen los cuerpos cetónicos hacia el cerebro. Aunque es asumido que es el hepatocito quien suministra los cuerpos cetónicos al resto de órganos, existen también ciertas evidencias que demuestran *in vitro* que los astrocitos proveen de cuerpos cetónicos *in situ* a las neuronas, y aumentan las posibilidades metabólicas de supervivencia neuronal (Guzmán et al. 2001). También se han encontrado indicios de que los astrocitos podrían producir cuerpos cetónicos in vivo a partir de ácidos grasos y ceramidas (Guzmán et al. 2001; Velasco et al. 2005).

La insulina regula la captación de la glucosa por parte del hipotálamo, por lo tanto una alteración en la vía de señalización de la insulina en esta zona del cerebro puede provocar un estado de resistencia a la insulina parecido a lo que se verifica en la DM2 y contribuir a largo plazo a la aparición de un déficit cognitivo (Gelling et al. 2006).

El control energético celular se produce en el hipotálamo gracias a equilibrios entre diferentes hormonas y adipocinas. Entre ellas las más importantes son la insulina y la leptina.

Entre la leptina y la insulina existe una retroalimentación negativa que permite que se regulen mutuamente (Morioka et al. 2007). Así, la leptina inhibe la producción de insulina en las células β del páncreas, mientras que la insulina estimula la producción de leptina en el adipocito. Sin embargo, en un estado de resistencia a leptina caracterizado por hiperleptinemia, esa regulación recíproca entre las dos moléculas se altera: la leptina deja de inhibir la producción de insulina en el páncreas induciendo una fase de hiperinsulinemia y resistencia a

esta hormona. Tanto la leptina como la insulina actúan centralmente para inhibir la ingesta de alimentos y para aumentar el gasto energético (Morton & Schwartz 2011) aunque no se conozcan los mecanismos.

Cuando este equilibrio homeostático se ve alterado, aumenta el riesgo de sufrir procesos neurodegenerativos. Se ha descrito que la leptina puede actuar disminuyendo la actividad de la β-secretasa, lo que disminuiría los depósitos de péptido β-amiloide y de tau (Bonda et al. 2014). Por otro lado, se ha descrito que la disponibilidad de leptina en el cerebro de individuos obesos estaría disminuida por dificultades en su transporte a través de la barrera hematoencefálica (Wang et al. 2001). En conjunto, estos datos indican un vínculo de tipo bioquímico y molecular entre obesidad y la EA.

2.1 Regulación metabólica en la EA

Existen evidencias que ponen de manifiesto la importancia de una correcta regulación metabólica en la evolución de la EA; algunos ejemplos son:

- La importancia de la dieta y la nutrición en la prevalencia de EA (Grant, 1999, 2004).
- Estudios en cultivos muestran que los lípidos activan las rutas amiloidogénicas (Puglielli y col., 2003).
- La mayoría de pacientes con EA sufren de alguna forma de resistencia a la insulina (Fishel y col., 2005).
- La hormona leptina esta atenuada en pacientes con EA (Power y col., 2001; Lieb y col., 2009).

Por estos motivos no es sorprendente que moduladores del colesterol como las estatinas (Sparks y col., 2006), o de la glucosa, como la rosiglitazona (Risner y col., 2006) o la insulina misma (Craft y col., 2012) se estén evaluando como potenciales agentes terapéuticos frente a la EA.

Las dos hormonas que se han estudiado en detalle en el presente trabajo son la insulina y la leptina (Pedros et al. 2016).

2.1.1 Insulina

La insulina es una hormona polipeptídica formada por 51 aminoácidos, producida y segregada por las células beta de los islotes de Langerhans del páncreas. El principal estímulo que desencadena la secreción de insulina es la glucosa; sin embargo, otros nutrientes, como aminoácidos, ácidos grasos y cuerpos cetónicos, también contribuyen a su liberación, sin olvidar la modulación producida por hormonas gastrointestinales y pancreáticas, así como por

neurotransmisores adrenérgicos, colinérgicos o excitatorios (por ejemplo el glutamato) (Gammelsaeter et al. 2011).

El origen de la insulina en el cerebro sigue siendo motivo de controversia. La mayoría de la insulina central deriva de la síntesis pancreática y es transportada al cerebro a través del LCR (Salkovic-Petrisic & Hoyer 2007; Bingham et al. 2002). Sin embargo algunas evidencias sugieren que una parte sea sintetizada *de novo* en el cerebro, ya que se ha descrito la existencia de mRNA de pre proinsulina I y II a nivel central (Schechter & Abboud 2001).

Se ha documentado que la insulina puede ejercer acciones pleiotrópicas en el cerebro (Cardoso et al., 2009). En otras palabras, además de ser el principal regulador metábolico, la insulina también posee funciones como neuromodulador y substancia neuroendocrina, desarrollando un papel importante en el crecimiento y la supervivencia neuronal (Candeias et al. 2012).

Efectivamente, datos emergentes sugieren que la vía de señalización de la insulina está implicada en la plasticidad sináptica mediante varios mecanismos como por ejemplo la modulación de la actividad de receptores excitatorios e inhibitorios (glutamato y GABA) y la activación de cascadas de transducción de señales que regulan la memoria a largo plazo (Zhao et al. 2004).

La insulina ejerce su acción uniéndose a su receptor específico, denominado receptor de insulina (RI). Este receptor está presente en prácticamente todos los tejidos de mamíferos, incluso en el cerebro. Los receptores de insulina son abundantes en muchas regiones del cerebro y su densidad es más elevada en las regiones del bulbo olfatorio, de la corteza cerebral, del hipocampo, del cerebelo y del hipotálamo.

Los RI pertenecen a la clase de receptores tirosina cinasa; cuando la insulina se une al receptor, se produce la fosforilación del dominio intracelular y se inicia la actividad tirosina cinasa del receptor. Tras esta autofosforilación, el receptor fosforila a su vez a un número de substratos intracelulares con residuos específicos de tirosina y, de este modo, desencadena la señalización intracelular. Esta familia de sustratos está compuesta por las proteínas IRS (Insulin Receptor Substrate). En el cerebro los más importantes son IRS1, IRS2 e IRS4. En este punto, el RI puede activar dos vías de señalización: la vía de la PI3K (fosfoinositol-3 cinasa) y la vía de las MAPK cinasas (figura 16).

La insulina promueve la actividad de las neuronas hipocampales piramidales y la utilización de glucosa en la corteza entorrinal y en el hipocampo, favoreciendo el crecimiento neuronal y aumentando la potenciación a largo plazo (LTP) a través de aumento en la actividad de receptores NMDA (fosforilación de subunidades GluN2A y GluN2B).

Además, se ha descrito que la inducción de LTP en el giro dentado de rata es inhibido por wortmannina, un potente inhibidor de PI3K (Kelly et al. 2000); esto confirmaría el papel de la vía de la insulina en este efecto. También la insulina puede inducir cambios en la transmisión sináptica esencial para formar la base de la memoria y el aprendizaje (van der Heide et al. 2005).

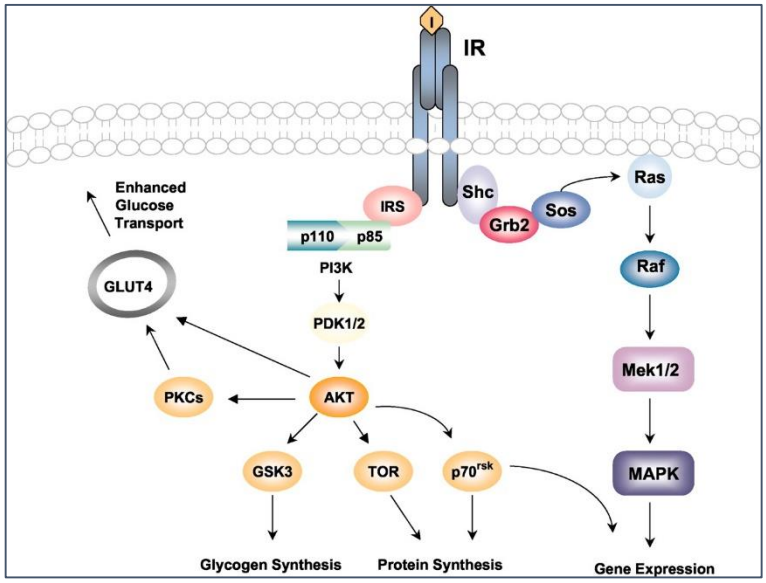


Figura 16. Representación esquemática del RI. La unión de la insulina a su receptor recluta proteínas Shc y sustratos de receptor de insulina (IRS). La fosforilación de IRS activa PI3K y ésta, tras una cascada compleja, activará fosforilando a AKT, con su implicación en distintos procesos mediante la activación de proteínas como mTOR, GSK3β, PKC. A su vez, un sustrato de AKT (AS160) es fosforilado para permitir la traslocación de glucosa a través de transportadores sensibles a insulina (GLUT4). Alternativamente, fosforilación de Shc activa la vía MAPK, implicada en proliferación celular. (Korc 2003)

La relación entre resistencia a la insulina en el cerebro y enfermedad de Alzheimer ha sido ampliamente descrita, aunque los mecanismos moleculares a la base de esa conexión no son del todo conocidos. La vía de señalización de la insulina a nivel central implica la activación de la PI3-K/Akt que a la vez fosforila la cinasa GSK3beta en un residuo de Serina (Ser9). La fosforilación en serina, en lugar de tirosina, inhibe la actividad de la enzima. En la enfermedad de Alzheimer resultan inhibidas tanto la isoforma α como la isoforma citosólica β de la GSK3β.

Se ha demostrado que la GSK-3α regula la formación de péptidos β- amiloide a nivel central. En consecuencia, la insulina también podría desarrollar un función importante en este proceso a través de la vía PI3-K dependiente. Efectivamente Solano y colaboradores describieron que la vía de la insulina afecta a la liberación

de β-amiloide soluble y especularon que el mecanismo podría implicar la regulación del tráfico vesicular (Solano et al. 2000).

Gasparini y colegas (2001) observaron que la insulina es capaz de disminuir la acumulación intraneuronal de beta-amiloide Aβ mediante la aceleración de tráfico de AβPP/Aβ desde la red trans-Golgi hacia la membrana plasmática (Gasparini et al. 2001).

Por otro lado, la isoforma GSK-3β está implicada en la fosforilación de la proteína tau. Hong y Lee utilizaron cultivos de células neuronales humanas para demonstrar que la GSK-3β es capaz de fosforilar tau y consecuentemente reducir su afinidad por los microtúbulos. Al contrario, la estimulación con insulina reduce la fosforilación de tau y promueve la unión de los microtúbulos; este efecto de la insulina está mediado por la activación de la cascada PI3-K/Akt que conduce a la inhibición de la GSK-3β (Hong & Lee 1997).

La función de la insulina en los procesos de aprendizaje y memoria no está del todo claro, aunque se ha descrito que las vías de PI3-K y de MAPK están implicadas. Más en específico, la cinasa ERK1/2 tiene un papel esencial en la supervivencia neuronal (Xia et al. 1995) y en la plasticidad sináptica relacionada con los proceso de aprendizaje y memoria (Davis & Laroche 2006).

En el cerebro de los mamíferos, la insulina tiene también efectos anorexigénicos, induce pérdida de peso corporal y regula el control hipotalámico sobre la ingesta de alimentos. Es capaz de regular la homeostasis de la glucosa periférica gracias a la estimulación de las neuronas productoras de proopiomelacortina (POMC) y de péptido relacionado con Agouti (AgRP). Estos efectos están mediados por la vía AKT/PI3K.

Por otro lado, un estado de hiperinsulinemia podría inhibir la degradación de Aβ, bloqueando competitivamente la IDE (Farris et al. 2003). Esto es precisamente lo que puede ocurrir en diabéticos o individuos resistentes a la insulina: la cantidad elevada de insulina presente en el cerebro compite con el Aβ en la unión al IDE (Qiu et al. 2006) y como consecuencia desaparece la protección ofrecida por esta enzima frente a la formación de placas seniles y frente a la acción tóxica de los oligómeros. Confirmando esta hipótesis, estudios histopatológicos han demostrado que en cerebros de pacientes con EA hay una menor expresión de IDE (McDermott et al. 1997; Vekrellis et al. 2000), fenómeno que provocaría un aumento del contenido de Aβ (Miller et al. 2003; Farris et al. 2003).

También se ha observado que ratones Tg2576 alimentados con dietas ricas en grasas se caracterizan por una menor actividad de IDE y por la acumulación del péptido Aβ (Zhao et al. 2004). Además en ratones knock-out para IDE hay una menor degradación de Aβ (Farris et al. 2003).

En resumen, la insulina es, sin duda, crucial para la función normal del cerebro. Por lo tanto, no es sorprendente que las perturbaciones sobre la función cerebral de la insulina y la transducción de señal de la insulina se hayan asociado a trastornos patológicos como la EA.

2.1.2 Leptina

La leptina es una hormona peptídica de 16 KDa producto del gen *ob*. La secuencia aminoacídica de la leptina, así como indican los datos cristalográficos (Zhang et al. 1997), adopta una estructura helicoidal de 3 dimensiones similar a la de algunas citoquinas.

La leptina es sintetizada principalmente por los adipocitos en el tejido adiposo blanco (Zhang et al. 1994), pero también se ha encontrado en la placenta (Senaris y col., 1997), en el estómago (Bado et al. 1998) y en el cerebro (Wiesner et al. 1999). La leptina modula la disponibilidad de energía metabólica (Schwartz et al. 2000), permite el almacenamiento o movilización de la grasa y es capaz de aumentar la sensibilidad a la insulina (Shimomura et al. 1999; Morton et al. 2005; Morton & Schwartz 2011; Amitani et al. 2013).

Se ha observado que los roedores obesos presentan resistencia a la leptina, en algunos casos debido al programa genético o perinatal (resistencia primaria), aunque normalmente esta resistencia aparece en respuesta a elevados niveles de leptina (resistencia secundaria). La resistencia secundaria causada por la leptina puede ser el resultado de un transporte reducido de la hormona al cerebro o de una disminución de su vía de señalización.

Existen cinco isoformas de receptores de leptina (Ob R, a-e). Los receptores Ob pertenecen a la familia de los receptores interleuquina-6 que a su vez proceden de la superfamilia de receptores de citoquinas clase I (Lee et al. 1996). Existen tres agrupaciones estructurales de los receptores: cortos (Ob-Ra, c, y d), largos (Ob Rb) y solubles (Ob-Re) (Hegyi et al. 2004).

Se cree que muchas de las acciones fisiológicas de la leptina se deben a la forma larga del receptor, debido a su mayor capacidad de activar cascadas de señalización. Las formas cortas están menos implicadas en la señalización intracelular. Parece que su función principal sea favorecer la transferencia de la leptina desde el torrente sanguíneo al interior del cerebro a través de la barrera hematoencefálica.

Aunque el ObRb se expresa en todo el SNC, es especialmente abundante en el hipotálamo, donde regula la homeostasis energética (reducción del apetito y aumento del gasto energético) y ejerce sus funciones neuroendocrinas.

A través de su receptor, la leptina actúa sobre ciertas neuronas hipotalámicas, en particular las neuronas del núcleo arcuato productoras de proopiomelanocortina (POMC) y las relacionadas con la proteína agouti (AgRP). Las neuronas POMC son anorexigénicas. La rotura proteolítica de la POMC da lugar a alfa-MSH que estimula los receptores de la melanocortina 3 y 4 (MC3R, MC4R), muy importantes en la homeostasis energética. Por el contrario, las neuronas AgRP son orexigénicas e inhiben la actividad de las neuronas POMC mediante la liberación del ácido gamma-amino-butírico; ademásesas neuronas secretan los neuropéptidos orexigénicos AgRP e Y (NPY), los cuales antagonizan MC3R y MC4R. La leptina inhibe la expresión de AgRP y NPY, reduce la excitabilidad de las neuronas AgRP, así como la liberación del ácido gamma-amino-butírico por las mismas. Por otra parte, en el hipotálamo, la leptina actúa sobre neuronas que directa o indirectamente regulan el nivel de otras hormonas circulantes (hormonas tiroideas, GH, hormonas sexuales)

La unión de la leptina al receptor altera la conformación del homodímero de Ob-Rb, permitiendo la trans fosforilación y activación de las JAK2 intracelulares asociadas al Ob-Rb. La molécula JAK2 activada fosforila entonces otros residuos tirosina del complejo Ob-Rb-JAK2 para mediar la cascada de señalización. Se ha descrito la existencia de 4 residuos tirosina (Tyr) en el dominio intracelular del Ob-Rb que son fosforilados y participan en la cascada de señalización de leptina: Tyr1138, Tyr985, Tyr974 y Tyr1077 (Myers et al. 2008).

Los receptores de leptina e insulina están estrechamente conectados por las mismas vías de señalización; de hecho, ambas controlan directamente la actividad de los circuitos neuronales implicados en los mecanismos de recompensa asociados a la ingesta de alimentos en el hipotálamo (Könner et al. 2009).

La activación de los receptores de insulina, leptina y otra adipocina (la prolactina) pone en marcha diversas vías de señalización intracelular que se describen a continuación:

- Vía de señalización JAK-STATs

La unión de leptina a Ob-Rb activa JAK2, ocasionando la fosforilación de residuos específicos. La fosforilación de Tyr1138 es fundamental en la señalización del receptor ya que recluta el transductor de señal y activador de la transcripción 3 (STAT3) hacia el complejo leptina-Ob-Rb-JAK2. De esta forma se genera la fosforilación y la posterior traslocación de STAT3 como dímero fosforilado al núcleo, para mediar la regulación de la transcripción de genes diana (Münzberg & Myers 2005). Entre los genes regulados por STAT3 está el supresor 3 de señalización de citoquinas (SOCS-3). La molécula SOCS-3 es miembro de la familia

de proteínas con dominios SH2 (dominios que contiene sitios específicos de unión para residuos de tirosina fosforilados) y está compuesta por una región amino terminal variable, un dominio central SH2 y un dominio carboxilo terminal llamado caja SOCS. La señalización de la leptina puede ser bloqueada por SOCS3. De hecho, la hiperleptinemia que acompaña la obesidad incrementa la expresión de SOCS-3 dando lugar a una reducción de la sensibilidad a la leptina.

Otra vía descrita es la que implica la fosforilación y la regulación transcripcional de STAT5 por la leptina; esta vía está mediada por el residuo Tyr1077, aunque el residuo Tyr1138 también contribuye a la activación de STAT5 (Myers et al. 2008). Se ha sugerido que la vía JAK2-STAT5 estaría relacionada con procesos de angiogénesis (Frühbeck 2006).

- Vía de señalización PI3K-AKT-GSK3β

El receptor de insulina es un dímero, unido por un puente disulfuro, que consta de una subunidad α y una subunidad β. Cuando éste se una a la insulina, el receptor se autofosforila creando un sitio de unión para el substrato del receptor de insulina (IRS) y este último activa a la fosfatidilinositol 3 cinasa (PI3K). La leptina puede mediar la fosforilación de IRS y regular la vía de señalización de PI3K (Niswender et al. 2004; Benomar et al. 2005). Muchos estudios han demostrado que la leptina induce la actividad de AKT mediante la fosforilación en el residuo Ser473.

La leptina e insulina también son responsables de la activación de la serina/treonina cinasa *mammalian target of Rapamycin* (mTOR) a través de la vía PI3K-AKT (Cota et al. 2008).

mTOR es una cinasa conservada a lo largo de la evolución que modula la traducción de varios transcritos de ARN implicados en el crecimiento y proliferación celular (Hay & Sonenberg 2004) también participa en la regulación energética del cerebro (Orr et al. 2014) y alteraciones en la actividad están vinculados a la hiperfosforilación de tau mediante PKA, AKT, GSK3β y CDK5.

- Vía de señalización ERK/MAPK

Las ERK (quinasas reguladas por señales extracelulares) son proteínas implicadas en la consolidación de la memoria hipocampal. Son estimuladas directamente por la vía IRS a través de la fosforilación del residuo de Tyr985 del Ob-Rb; también se ha descrito una activación de ERK independiente de la fosforilación del receptor de la insulina que parece ser inducida por la cinasa JAK2 (Bates & Myers 2003). La fosforilación del residuo de Tyr985 propicia la activación de SHP-2 que

posteriormente se enlaza a GRB-2 y promueve la cascada de señalización de ERK. Este es el primer paso de la vía de señalización canónica de las quinasas reguladas extracelularmente (ERK)-p21-ras. La activación de ERK por leptina ha sido relacionada con la fosforilación ribosomal de la proteína S6 y su consecuente traducción. A nivel periférico, esta vía es relevante para los efectos de la leptina y de la insulina en la función inmune (Frühbeck 2006); a nivel hipocampal la vía está implicada en los procesos de LTP que afectan a los receptores NMDA (Shanley et al. 2001).

CAPÍTULO II. HIPÓTESIS Y OBJETIVOS

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

CAPÍTULO II. HIPÓTESIS Y OBJETIVOS

El presente trabajo de tesis doctoral se enmarca en el contexto de lo que se conoce como hipótesis metabólica de la enfermedad de Alzheimer. En este contexto, las alteraciones en las vías de señalización de la insulina y de la leptina estarían implicadas en la etiología del proceso neurodegenerativo.

De acuerdo con ello, los principales objetivos de la presente tesis doctoral se proponen evaluar el efecto de la obesidad y de la resistencia a insulina inducidas mediante una dieta rica en grasas en el proceso de amiloidogénesis y analizar los mecanismos moleculares implicados.

En concreto:

Objetivo 1:

Caracterizar el fenotipo metabólico a nivel central y periférico en ratones APPswe/PS1dE9 de 3 y 6 meses de edad sometidos a una dieta estándar.

1.1. Determinar si el estado transgénico afecta al metabolismo periférico de los carbohidratos
1.2. Determinar si la vía de señalización de la insulina y de adipocinas como leptina están alteradas a nivel del hipocampo en ratones APP/PS1 de 3 y 6 meses de edad
1.3. Identificar los mecanismos moleculares que se encuentran alterados de forma temprana en un modelo animal de la enfermedad de Alzheimer, previamente a la aparición de placas y a la pérdida de memoria en hipocampo de ratones APP/PS1 de 3 y 6 meses de edad,
1.4. Determinar las alteraciones en el metabolismo periférico de colesterol y triglicéridos en animales APP/PS1 de 3 y 6 meses de edad.

Objetivo 2. Caracterizar el fenotipo metabólico a nivel central y periférico en ratones APPswe/PS1dE9 de 6 meses de edad sometidos a una dieta rica en grasas HFAT. En concreto:

1. Determinar si la dieta grasa afecta al metabolismo periférico de la glucosa en los ratones transgénicos y salvajes
2. Determinar si la exposición a una dieta grasa afecta a la memoria a corto plazo.
3. Determinar si la dieta rica en grasa acelera los marcadores de la enfermedad, en particular el péptido amiloide y la fosforilación de la proteína Tau.
4. Determinar si la exposición a la dieta grasa afecta a las vías de señalización de la vía de insulina y de adipocinas como leptina.
5. Estudiar si la dieta grasa afecta a la cadena de transporte de electrones y a la biogénesis mitocondrial.

CAPÍTULO III. MATERIAL Y MÉTODOS

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

CAPÍTULO III. MATERIAL Y MÉTODOS

3.1 Modelo experimental

En la presente tesis ha sido utilizada como control la cepa de ratón C57BL/6. Se trata de ratones que presentan homogeneidad genética y fenotípica y por lo tanto son una excelente herramienta para la investigación.

Los ratones transgénicos APP/PS1 se originan a partir de los C57BL/6 y han sido modificados para sobreexpresar dos proteínas responsables de la aparición del fenotipo característico de la enfermedad de Alzheimer familiar: una proteína quimérica ratón/humano de APP que contiene la mutación Sueca (K594M/N595L) y una proteína mutante humana presenilina PS1-dE9. Esta mutación de PS1 contiene una deleción del exón 9 que genera un aumento de la forma más agregante de Aβ, que consta de 42 residuos (Aβ42) (Citron et al. 1997), respecto a la forma de 40 residuos mucho menos agregante (Aβ40).

Los ratones a los 4 meses de edad comienzan a presentar placas seniles tanto en la corteza como en el hipocampo (Garcia-Alloza et al. 2006), y entre los 6 y los 15 meses presentan problemas de aprendizaje y memoria (Savonenko et al. 2005; Minkeviciene et al. 2008; Huang et al. 2011). Sin embargo, no sufren otras alteraciones de comportamiento, como la ansiedad (Webster et al. 2013). Estos animales presentan niveles elevados de algunos marcadores de la inflamación como IL-1β y TNFα (Babcock et al. 2015).

3.2 Genotipado

La confirmación del genotipo de los ratones APP/PS1 se realizó a partir de DNA genómico extraído de la cola del ratón. El tejido se almacenó a -20 ºC hasta su utilización para extraer el DNA. Sucesivamente el DNA obtenido se utilizó para realizar su análisis por PCR. La extracción del DNA a partir del tejido se realizó con DirectPCR lisis reagent (ViagenBiotech) y siguiendo el protocolo descrito por el productor.

Extracción de DNA

El primer paso en la extracción de DNA consistió en la obtención de tejido, a partir de un corte de 0,5-1cm de la cola del ratón. Posteriormente, este tejido se incubó con 100 µl de DirectPCR *lysis reagent* (ViagenBiotech) y 0,2 mg/ml de proteinasa K (Roche) a 55 °C durante toda la noche. A continuación el tejido se calentó a 85 °C durante 45 min y se centrifugó 3 min a 13000 g. Finalmente, se recuperó el sobrenadante y se guardó a -20 °C hasta su uso posterior.

Los reactivos no provistos con el kit fueron el agua Milli-Q estéril, el tampón TBE 0.5x estéril (0.045 M Tris-borato, 0,001 M EDTA en agua Milli-Q) y los oligonucleótidos (Sigma-Aldrich):

Para PS1:	5´-atagagaacggcaggagca-3´
	5´-gccatgagggcactaatcat-3´
Para APP:	5´-gactgaccactcgaccaggttctg-3´
	5´-cttgtaagttggattctcatatccg3´

Amplificación del DNA genómico mediante (PCR)

La reacción en cadena de la polimerasa (PCR) permite la amplificación de fragmentos de DNA hasta niveles que puedan ser fácilmente detectables. Para ello, se necesita el enzima DNA polimerasa y dos oligonucleótidos específicos complementarios a secuencias presentes en casa una de las cadenas de DNA, y que limitan el fragmento de DNA a amplificar.

La mezcla de reacción para la técnica de PCR se preparó siguiendo las instrucciones del kit Go Taq® Green Master Mix (Promega), para un volumen final de 20 µl de reacción.

La amplificación de la PCR se llevó a cabo en UN termociclador Veriti 96 Well Thermal Cycler (Applied Biosystems).

A continuación, se realizó el análisis de los fragmentos amplificados en un gel de agarosa al 2 % en tampón TBE (Tris 10,8 g/L, Ácido Bórico 5.5 g/L y 4 ml EDTA 0.5 M pH 8.0), que contenía RedSafe DNA Stain (ChemBio) a una dilución de 1:20000. Para ello, se cargó un volumen de 10 µl de muestra en cada pozo y se inició la separación de las muestras a 90 V, durante 30 min. Una vez acabada esta migración, se observó el gel mediante un transiluminador de luz ultravioleta (MiniBis Pro, Dnr), permitiendo visualizar las bandas de DNA resultado de la amplificación por PCR.

3.3 Dieta

Los ratones fueron destetados a las 3 semanas y alimentados, durante los siguientes 6 meses, con una dieta rica en grasas que consiste en 25% de grasa (45% kcal), aceite de coco principalmente hidrogenado, 21% de proteína (16 kcal%), y 49% de carbohidratos (39% kcal); Cat # D08061110 *(Research Diet Inc New Brunswick, EE.UU)*. La dieta control #2018 (Harlan Laboratories). En la tabla 3 podemos comparar las dos dietas.

Es importante remarcar que la dieta se administró justo en el momento del destete. El efecto nocivo la dieta rica en grasa no es el mismo si se administra en ratones adultos que en jóvenes, (Boitard et al. 2012). El peso de los ratones se registró semanalmente.

Tabla 3. Macronutrientes de las dietas administradas

Producto	Dieta control	Dieta grasa
	kcal %	kcal %
Proteína	24	16,4
Carbohidratos	58	38,6
Grasas	18	45,0
Total	100	100

3.4 Protocolos experimentales

Primer y segundo artículo

El diseño experimental de los dos primeros artículos es muy similar, porque persiguen el mismo objetivo: caracterizar y conocer los trastornos metabólicos implicados en el desarrollo del EA.

Se generaron cuatro grupos según las variables cepa y edad. Para el desarrollo experimental cada grupo contenía mínimo una N=10. En el siguiente esquema están representados los 4 grupos utilizados: *wild type* (WT) y APP/PS1 de 6 meses y WT y APP/PS1 de 3 meses.

Los ratones fueron genotipados en el momento del destete y repartidos en jaulas sin tener en cuenta el genotipo. Para el experimento se seleccionaron solo los que eran machos y hermanos de camada (*litter mice*)

Elegimos dos edades distintas (3 y 6 meses) porqué queríamos evaluar dos estadios diferentes de la enfermedad: el estadio precoz, anterior a la formación de placas amiloidogénicas (ratones de 3meses) y el estadio avanzado caracterizado por la presencia de placas (ratones de 6 meses).

Durante el transcurso de vida de los ratones se midió el peso una vez por semana, y un mes antes del sacrificio se realizaron el test de conducta, las curvas de glucosa e insulina y las lecturas de triglicéridos y colesterol. Cuando se sacrificaron los ratones se extrajeron los tejidos del córtex y del hipocampo para el sucesivo análisis genético y proteico.

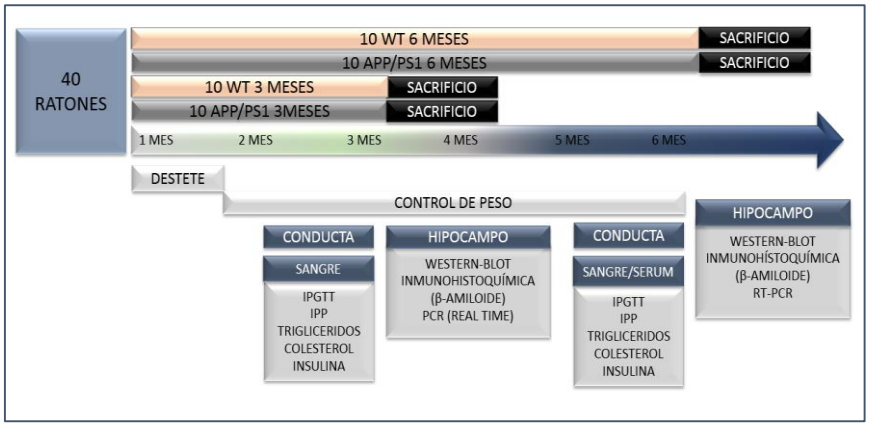


Figura 17. Diagrama experimental. Estudios 1 y 2

Tercer artículo

El diseño experimental varía respecto a los dos anteriores. Eliminamos la variable temporal, seleccionamos sólo ratones de 6 meses y añadimos la dieta HF. Por lo que tenemos los siguientes grupos: *wild type* (WT) y APP de 6 meses y WT HFAT y APP HFAT de 6 meses.

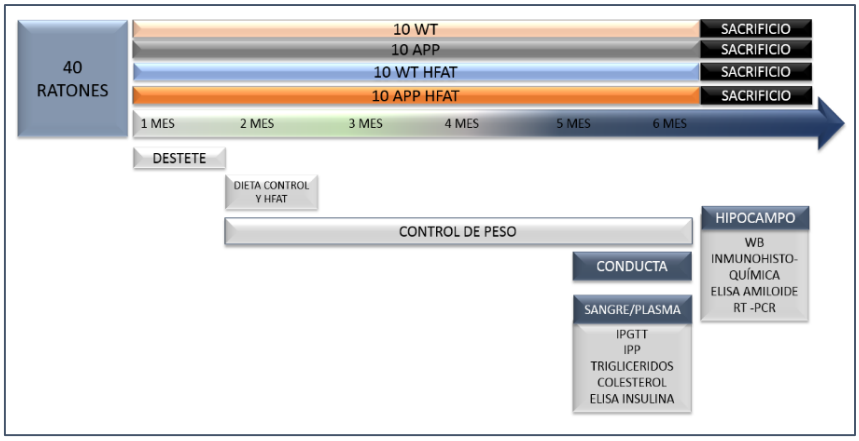


Figura 18. Diagrama experimental. Estudio 3

Durante el transcurso de vida de los ratones se midió el peso, y un mes antes de sacrificio se realizó el test de conducta, las curvas de glucosa e insulina y las lecturas de triglicéridos y colesterol. Cuando se sacrifican los ratones se extrajo el córtex y el hipocampo para el análisis genético y proteico.

3.5 Medidas de triglicéridos y colesterol

La determinación de las concentraciones de triglicéridos y colesterol se realizó mediante el detector Accutrend® Plus System (Roche Farma) utilizando las siguientes tiras reactivas:

- Triglicéridos: Accutrend Triglycerides; ref.11538144, Roche Farma; con un margen de detección de 70-600 mg/dl.
- Colesterol: Accutrend Cholesterol; ref.11418262, Roche Farma; con un margen de detección de 150-300 mg/dl

3.6 Test de conducta NORT

El *novel object recognition test* (NORT) es un test ampliamente descrito que se utiliza para evaluar el aprendizaje y la memoria (Aso et al. 2012; Antunes & Biala 2012). Las zonas del cerebro involucradas en este test son principalmente el hipocampo y la corteza perirrinal. Se trata de una prueba de conducta basada en el reconocimiento de objetos. Consiste en colocar un ratón en un laberinto en forma de L (Panlab, Barcelona, España) que contiene dos objetos idénticos en el extremo de los brazos y permitiendo que el ratón pueda explorar libremente el aparato durante 10 minutos. Transcurridas 24 h desde la sesión de entrenamiento, el animal se vuelve a colocar en el laberinto, pero esta vez uno de los dos objetos familiares ha sido reemplazado por un objeto nuevo. El tiempo que el animal dedica a explorar los dos objetos se registra y se cuantifica, mediante un índice de reconocimiento que depende del tiempo que interactúa y explora el objeto nuevo y el familiar. Los animales que exhiben alteraciones de la memoria puntúan un índice de reconocimiento menor.

3.7 Test de intolerancia a la glucosa y a la insulina

El test de intolerancia a la glucosa (IP-GTT) y el test de intolerancia a la insulina (ITT) son determinaciones que se realizaron con el objetivo de conocer el tiempo que los ratones tardan en metabolizar la glucosa y la insulina inyectada intraperitionalmente.

Estas determinaciones se realizaron una semana antes del sacrificio de los mismos, transcurrido un ayuno de 6 horas, se les inyectó glucosa por vía intraperitonealmente a razón de 1 gramo por kg de peso corporal, a partir de una solución de 0,4 g glucosa/ml preparada en NaCl 0,9% (p/v).

Se determinó la concentración de glucosa en sangre a los 0, 15, 30, 45, 60 y 120 minutos posteriores a la administración de glucosa. La sangre se obtuvo a partir de un pequeño corte en el ápice de la cola del animal y la concentración de

glucosa se determinó gracias al uso de tiras reactivas especiales para un glucómetro (Bayer Diagnostic)

Para la determinación de la curva de insulina, a los 0, 15, 30, 45 y 60 minutos posteriores a la administración de insulina 0,25 UI/kg de insulina humana (Lilly) diluida en solución salina.

3.8 Tinciones histológicas

Fijación tisular por perfusión cardiaca

Antes de proceder a la fijación del tejido, los ratones fueron anestesiados mediante la administración intraperitoneal de una mezcla de ketamina (Ketolar, Pfizer) a una dosis de 100 mg/Kg y xilacina (Rompun, Bayer) a una dosis de 10 mg/Kg. Posteriormente, se procedió a la fijación de los órganos mediante perfusión intracardiaca con una solución de paraformaldheido al 4 % (PFA) (Panreac) en tampón fosfato (PB) 0.1 M (K_2HPO_4 14 g/L, $NaH_2PO_4.H_2O$ 26.5 g/L).

Una vez finalizada la fijación intracardiaca, se extrajeron los cerebros de cada animal perfundido y se realizó una post-fijación en la misma solución de perfusión durante 24 h a 4 °C. A continuación, se llevó a cabo un proceso de crioprotección de 48-72 h en PFA 4 % y sacarosa 30 % en PB 0.1 M. Finalmente los cerebros se congelaron en hielo seco y se guardaron a -80 °C para la posterior realización de secciones coronales de 20 µm de grosor a una temperatura de -20 °C. Dichos cortes se realizaron con el criostato (Leica Microsystems, Wetzlar, Germany) y se guardaron en solución crioprotectora (glicerol 30 %, etilenglicol 30 % en PB 0.1 M) a -20 °C hasta el momento de su utilización.

Inmunoflorescencia de tioflavina S

La Tioflavina-S (TS) es una fluoresceína verde que se une de forma específica a las placas seniles que poseen una conformación de hoja β plegada y a los ovillos neurofibrilares.

Los cortes coronales se descongelaron y se rehidrataron en PBS 0,1 M durante 10 minutos. Se incubaron con tioflavina al 0,3% (Sigma-Aldrich) durante 20 min a temperatura ambiente en una sala oscura, para preservarlos de la luz.

Tras este paso, los cortes se lavaron tres veces en etanol al 80% durante 5 minutos y dos veces etanol 90%. Luego, se montaron usando Fluoromount®(EMS). Las imágenes se obtuvieron con un microscopio de fluorescencia (BX41; Olympus)

Tinción de Hoechst 33342

El colorante Hoechst 33342, también denominado de bisbenzimida H33342 (Sigma-Aldrich), es un colorante específico para regiones del DNA que son ricas en adenina y timina. Se puede utilizar tanto para la detección de DNA en muestras tisulares, como en células en cultivo. Es un fluorocromo permeable a la membrana plasmática que se excita a una longitud de onda de 343 nm (luz ultravioleta) y que emite fluorescencia azul correspondiente a una longitud de onda de 455 nm (Holmquist 1975).

Los cortes coronales se descongelaron y se rehidrataron durante 10 minutos en PBS 0,1 M. Tras tres lavados de 5 min con PBS, estas secciones se incubaron con la solución del colorante a una concentración de 5 µM en PBS durante 20 min en condiciones de oscuridad. Posteriormente, se realizaron tres lavados con PBS.

3.9 Determinaciones mediante ELISA

La técnica de ELISA (Enzyme-Linked ImmunoSorbent Assay: Ensayo de Inmunoadsorción Ligado a Enzima) es una técnica de ensayo inmunoenzimático que permite la detección tanto de antígenos como de anticuerpos (Engvall et al. 1971).

Se basa en la premisa de que un inmunoreactivo puede ser inmovilizado en una fase sólida y su reactivo recíproco puede unirse a una enzima; en ambos casos las sustancias retienen su actividad inmunológica donde la reacción inmunógeno-anticuerpo es monitorizada midiendo la actividad colorimétrica producida por launa enzima.

Mediante esta técnica se analizaron la insulina en suero, y los fragmentos Aβ40 y Aβ42 humanos y de ratón en sus formas solubles e insolubles aislados del de córtex de ratones con seis horas de ayuno.

Insulina

En el momento del sacrificio de los animales, la sangre obtenida a partir de la punción cardiaca se recogió en tubos colectores (Micro tube 1.1 ml Z-Gel, Sarstedt). Seguidamente, éstos se centrifugaron a 5000 *xg* durante 10 minutos a temperatura ambiente, para la obtención del plasma (sobrenadante), que se guardó a -80ºC hasta su posterior utilización. Los niveles de insulina se midieron con el kit comercial EZRMI-13K de MIllipore.

Aβ 40 y 42

Las muestras fueron procesadas con PBS más inhibidores de proteasas y fosfatasas (#539131, Calbiochem) y centrifugadas 10 min a 4000 *xg*. Se separó la fracción soluble mediante incubación de 3.5 horas con 5M de guanidina HCl/ 50 mM Tris HCl en un agitador orbital. Los niveles de Aβ40 y Aβ42 humano y ratón soluble e insoluble se determinaron mediante los siguientes kits comerciales: KMB3481, KMB3441, KHB3481 y KHB3441 (Invitrogen).

3.10 Western blot

La técnica de Western blot permite determinar los niveles de una proteína específica y combina un proceso de migración electroforética con una inmunodetección. La electroforesis permite la separación de las proteínas en un gel de acrilamida en función de su masa molecular. Posteriormente, se lleva a cabo una transferencia de las proteínas a una membrana sintética, que actuará como soporte para llevar a cabo la detección de los niveles de una determinada proteína mediante el uso de anticuerpos específicos.

Obtención de extractos totales de proteína

El tejido obtenido por decapitación de los animales y destinado para la valoración proteica, fue homogenizado con 500µl de tampón de lisis (Tris-HCl 50 mM pH 7.4, NaCl 150 mM, EDTA 5 mM, Triton X-100 1 % y cocktail de inhibidores de proteasas (Complete, Roche Diagnostics) mediante el homogeneizador (PT10-35, Metrohm). Posteriormente, los extractos homogenizados se centrifugaron a 13000 g durante 15 min a 4 °C y el sobrenadante se guardó a -80 °C para su posterior utilización. La concentración proteica del extracto se determinó siguiendo el ensayo del ácido bicinconínico (BCA).

Determinación de la concentración proteica: método del BCA

La determinación de la concentración proteica se realizó mediante el método del ácido bicinconínico (BCA) (Pierce Company). Para ello, se añadieron 48 µl H_2O MilliQ a 2µl de extracto proteico y a continuación se añadió 1 ml de reactivo de BCA (reactivo A + reactivo B, en proporción 50:1). Posteriormente, se incubó la mezcla durante 30 min a 65 °C y finalmente, tras dejarla enfriar 5 min, se procedió a la lectura de la absorbancia a 562 nm en un espectrofotómetro de microplaca (BioRad, Benchmark Plus). La concentración de proteína se determinó por interpolación de las lecturas de absorbancia obtenidas en una recta de calibrado

preparada con concentraciones conocidas de albúmina sérica bovina (BSA, 2 mg/ml, Pierce Company).

Preparación de las muestras

Para la preparación de las muestras, se tomaron volúmenes de proteína total equivalentes a 10 µg de proteína y se les añadió tampón de carga 2X (β-mercaptoetanol 100mM, Tris-HCl 50mM pH 6.8, Glicerol 10%, SDS (Dodecilsulfato sódico 2% y azul de bromofenol 0.05%) a una proporción 1:1 con el volumen proteico. A continuación las muestras fueron colocadas en un baño seco (Techne DRI-BLOCK DB-2A) a 95 °C, durante 5 min, con la finalidad de desnaturalizar las proteínas.

Electroforesis

Las muestras fueron cargadas en un gel de electroforesis, que consta de dos fases: el gel concentrador, que permite concentrar las proteínas cargadas en el gel, y el gel separador, en el cual se separan las proteínas en función de su masa molecular.

Una vez polimerizados los geles, éstos se colocaron dentro de una cubeta de electroforesis Miniprotean III (BioRad) con suficiente tampón de migración (Tris 125 mM, Glicina 1,25 M, SDS 0.5 %) para que ambos extremos del gel queden en contacto con el tampón y permitir de esta manera cerrar el circuito. Finalmente, se cargaron las muestras en el gel, junto con un marcador estándar de peso molecular (Precision Plus ProteinTM Dual Color Standards, 161- 0374; BioRad). La electroforesis se llevó a cabo a 90V-100 V el tiempo necesario para que la proteína se separara según su masa molecular.

Transferencia de las proteínas a la membrana

Una vez finalizada la electroforesis, se llevó a cabo la transferencia proteica del gel a una membrana de polivinilideno (PVDF, 162-0177; BioRad), utilizando el sistema Mini Trans-Blot® (Bio-Rad). Este proceso permite la transferencia de las proteínas a la membrana, donde se realizará posteriormente la inmunodetección de las proteínas de interés. La transferencia se realizó a una intensidad de corriente eléctrica constante de 200 mA en tampón de transferencia (Tris 25mM, Glicina 190mM, Metanol 20%) y manteniendo la cubeta de transferencia a 4 °C durante todo el proceso.

Inmunodetección

Para llevar a cabo la inmunodetección, se extrajeron las membranas de la cubeta de transferencia y se lavaron con TBS 1X-Tween® 0.1 % pH 7.4 (TBS-T: Tris 24,25g/L, NaCl 80g/L y Tween 20 1ml/L) durante 5 min. Posteriormente, se incubaron las membranas durante toda la noche a 4 °C en una solución bloqueadora (5% BSA en TBS-T) que contenía el anticuerpo primario de interés a la concentración correspondiente. Tras tres lavados de 5 min con TBS-T, se procedió a la incubación de las membranas con el anticuerpo secundario conjugado a un enzima peroxidasa, en TBS-T a temperatura ambiente durante 1 hora.

Como paso final de la inmunodetección, se lavaron las membranas con TBS-T para eliminar el exceso de anticuerpo secundario y se procedió a la detección del fragmento β-amiloidemediante una reacción de quimioluminiscencia. Para ello, se procedió al contacto de la membrana con la solución de detección (Immobilon Western HRP substrate Peroxide Solution®, Millipore) durante 1 min. El revelado de la señal quimioluminiscente se llevó a cabo mediante el aparato Chemidoc XRS Bio-Rad® y su cuantificación, mediante el empleo de un software específico para capturar imágenes digitales (ImageLab, Bio-Rad). Para normalizar los resultados se utilizó la proteína β-actina o GAPDH, inmunodetectada en la misma membrana que la proteína de interés.

3.11 Análisis de la expresión génica mediante PCR cuantitativa

Obtención de extractos de RNA

La extracción de RNA se realizó a partir de la región del hipocampo, que se obtuvo tras la decapitación del animal y la disección del cerebro.

Para la extracción de RNA, los dos hipocampos de cada cerebro de cada animal fueron homogenizados con 500 µl de TRIzol® (Invitrogen, Eugene, Oregon, USA). Posteriormente, se añadieron 200 µl de cloroformo y, tras agitación, se dejó reposar la mezcla 5 min en hielo. A continuación, se centrifugó dicha mezcla a 13000 g durante 15 min a 4 °C y se recuperó la fase acuosa superior. Con el fin de obtener un RNA más limpio, se repitió este proceso de extracción clorofórmica una vez más. Una vez obtenida la fase acuosa definitiva, se le añadió 200 µl de isopropanol. Tras una mezcla por inversión y una etapa de reposo de 10 min en hielo, la muestra se centrifugó a 13000 *xg* durante 15 min a 4 °C. Se descartó el sobrenadante y se lavó el precipitado con 1 ml de etanol al 70 %. Posteriormente se centrifugaron las muestras a 7500 *xg*, durante 5 min a 4 °C. Se repitió el lavado con etanol al 70 % una segunda vez y tras la segunda centrifugación, se descartó

el sobrenadante y se dejó secar el pellet mediante evaporación del etanol. Finalmente, se resuspendió el pellet con 30 µl de H_2O Milli-Q autoclavada.

Cuantificación del RNA

Una vez extraído el RNA, se procedió a la determinación de la concentración de RNA de las muestras obtenidas, así como del análisis de la pureza de éstas. Para ello, se utilizó el NanoDrop (ND-1000), mediante el cual se obtuvieron las lecturas de las absorbancias a 230, 260 y 280 nm. A partir de la lectura de 260 nm se obtuvo la concentración de RNA de la muestra, mientras que mediante las relaciones 260/280 nm y 260/230 nm se determinó la pureza de las mismas. Las muestras se consideraron puras cuando la primera relación era próxima a 2 y cuando la segunda era superior a 1.70. Tras la cuantificación de la concentración de RNA y de la pureza de las muestras, éstas fueron guardadas a -80 °C hasta su uso posterior.

La PCR en tiempo real (qPCR) o PCR cuantitativa es una variante de la PCR en la cual, el proceso de amplificación y de detección se producen simultáneamente. Para ello, se realiza una reacción de PCR convencional, pero en la que a cada ciclo de reacción, le acompaña una lectura de fluorescencia, mediante el uso de fluoróforos.

La fluorescencia liberada en cada ciclo es proporcional a la cantidad de DNA generado. La medición de esta emisión de fluorescencia y su representación gráfica se realiza en un detector, permitiendo así, la cuantificación de la expresión del gen de interés

Síntesis de cDNA: reacción de retrotranscripción

La reacción de retrotranscripción (RT), o transcriptasa inversa, permite sintetizar una cadena complementaria de DNA (cDNA) a partir de una molécula de mRNA, y es llevada a cabo por el enzima retrotranscriptasa inversa, un DNApolimerasa dependiente de RNA.

La síntesis de cDNA se realizó a partir de 1 µg de RNA que se llevó a un volumen de 10 µl, a estos 10 µl se les añadió 10 µl más de una mezcla de reacción, siguiendo el protocolo descrito por el Kit de Retrotranscription High Capacity cDNA reverse transcription (Allied Biosystems)

El proceso de retrotranscripción se llevó a cabo en el termociclador Veriti 96 Well Thermal Cycler (Applied Biosytems). Una vez obtenido el cDNA, las muestras se diluyeron H_2O Milli-Q (1:10) y se congelaron a -20 °C hasta su uso posterior.

PCR en tiempo real

Para la amplificación por la técnica de PCR en tiempo real, se llevaron a cabo dos métodos de detección diferentes: el SYBR Green® dye y Taqman® probe FAM dye.

Detección mediante SYBR Green® dye (Applied Biosystems).

Para este tipo de detección, se partió de 25 ng de cDNA obtenidos tras la retrotranscripción, y se llevaron hasta un volumen de reacción de 20 µl, en el que también estaban presentes, a una concentración de 1 µM, los cebadores forward y reverse de cada gen a amplificar, y 10 µl de SYBR Green PCR Master Mix (Applied Biosystems).

Los cebadores para PCR a tiempo real, se diseñaron con el software Primer Blast (http://www.ncbi.ulm.nih.gov/tools/primer-blast del NCBI). Dentro de los parámetros deseados para el diseño de estos cebadores se seleccionó, siempre que fuera posible, un amplicón de un tamaño de entre 70 y 150 bp y que la temperatura de *melting* fuera entre 59 y 61 °C. Además se procuró que dichos cebadores fueran multi-exonales

Detección mediante Taqman® probe FAM dye (Applied Biosystems): Para este tipo de detección se partió de 25 ng de cDNA obtenidos tras la retrotranscripción, y se llevaron hasta un volumen final de reacción de 20 µl, al que también se le añadió 1 µl de Taqman Gene Expression assay y 9 µl de Taqman Gene expression Master Mix (Applied Biosystems).

Para los dos tipos de detección utilizados, la reacción se llevó a cabo mediante el termociclador StepOne plusTM Real Time PCR system (Applied Biosystems) seleccionando el protocolo específico dentro del software StepOne software v2.2.2 (Applied Biosystems).

Para el análisis de los datos, se realizó una cuantificación relativa mediante el método de 2-ΔΔCt. Utilizando este método se determinó el cambio en la expresión del gen de interés en relación a la expresión de un gen constitutivo, cuya expresión no varía en las condiciones del experimento (control endógeno). En este caso, como gen constitutivo (también como housekeeping gene) se utilizó β-Actina o Gapdh

CAPÍTULO IV. RESULTADOS

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

CAPÍTULO IV. RESULTADOS

4.1 Publicación I

Early alterations in energy metabolism in the hippocampus of APP/PS1dE9 mouse model of Alzheimer's disease

Ignacio Pedrós, Dmitry Petrov, Michael Allgaier, Francesc Sureda, Emma Barroso, Carlos Beas Zarate, Carme Auladell, Mercè Pallàs, Manuel Vázquez-Carrera, Gemma Casadesús, Jaume Folch, Antoni Camins (2014). *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 1842:1556-1566.

Resumen

La resistencia a insulina y la diabetes son factores de riesgo en el desarrollo de EA. Este trabajo se propone como principal objetivo conocer si el proceso de amiloidogénesis que sufren los ratones APP/PS1 conlleva alteraciones metabólicas relacionadas con la glucosa, y - en caso afirmativo - si estas se producen antes o después de la aparición de placas β-amiloides. Otros objetivos fueron evaluar en nuestro modelo animal la biogénesis mitocondrial y la hiperfosforilación de tau y, por último, estudiar alteraciones en marcadores sinápticos.

Definimos dos estados fenotípicos: ratones jóvenes (3 meses de edad) que no presentaban placas β-amiloide y ratones mayores (6 meses de edad) con evidente presencia de placas. El diseño experimental se organizó de la siguiente manera; se repartieron los ratones en 4 grupos: grupo control de 6 meses, grupo transgénico de 6 meses, grupo control de 3 meses y grupo transgénico de 3 meses. Para reducir la variabilidad seleccionamos solo machos que eran hermanos entre ellos y cada semana medimos el peso. Las pruebas bioquímicas en sangre o plasma y el análisis conductual se realizaron dos semanas antes del sacrificio. Después se procedió con la disección el hipocampo

Los resultados muestran que los ratones transgénicos sufren una pérdida de memoria a los seis meses y presentan niveles elevados de β42 insoluble a los 3 y 6 meses. También se observan intolerancia a la glucosa y a la insulina a los 6 meses y desregulaciones en los transcriptos implicados en la vía de señalización de insulina, como por ejemplo el RI y el substrato del receptor de insulina, a los 3 meses.

Observamos que la vía implicada en la biogénesis mitocondrial AMPK/PGC1α resulta menos activa en los APP/PS1. Concretamente, observamos una reducción de PGC1α a 3 y 6 meses, y de los receptores nucleares NRF1 y 2 a 3 meses. También resulta alterada la cadena de transporte de electrones a nivel

mitocondrial, en particular observamos una reducción en los complejos I, II, III y IV a 3 meses y I, II y III a 6 meses.

La hiperfosforilación de la proteína citoesquelética tau fue observada en diferentes residuos tanto a 3 como a 6 meses. Además, evaluamos los niveles de las cinasas CDK5 y GSK3β y a 6 meses observamos una tendencia a la fosforilación inactivadora de GSK3β en serina 9. Por otro lado los niveles de CDK5 resultaron elevados en los ratones de 6 meses, sugiriendo un papel de esa cinasa en la fosforilación de tau. Respecto a las proteínas sinápticas no registramos cambios significativos.

Biochimica et Biophysica Acta 1842 (2014) 1556–1566

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease

Ignacio Pedrós [b,c,h,1], Dmitry Petrov [a,c,h,1], Michael Allgaier [a,c,h], Francesc Sureda [b,c,h], Emma Barroso [a,d,h], Carlos Beas-Zarate [f,g,h], Carme Auladell [e,h], Mercè Pallàs [a,c,h], Manuel Vázquez-Carrera [a,d,h], Gemma Casadesús [f,h], Jaume Folch [b,c,h,2], Antoni Camins [a,c,h,*,2]

[a] Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Institut de Biomedicina de la UB (IBUB), Universitat de Barcelona, Barcelona, Spain
[b] Unitats de Bioquímica i Farmacologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus, Tarragona, Spain
[c] Centros de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Spain
[d] Centros de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Spain
[e] Departament de Biologia Cellular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
[f] Department of Neurosciences, Case Western Reserve University, Cleveland OH USA
[g] Laboratorio de Neurobiología Celular y Molecular, División de Neurociencias, CIBO, IMSS, Mexico
[h] Laboratorio de Regeneración y Desarrollo Neural, Instituto de Neurobiología, Departamento de Biología Celular y Molecular, CUCBA, Mexico

ARTICLE INFO

Article history:
Received 7 February 2014
Accepted 20 May 2014
Available online 2 June 2014

Keywords:
APPswe/PS1dE9
Insulin receptor
Mitochondria
Hippocampus
Tau
Alzheimer's disease

ABSTRACT

The present study had focused on the behavioral phenotype and gene expression profile of molecules related to insulin receptor signaling in the hippocampus of 3 and 6 month-old APPswe/PS1dE9 (APP/PS1) transgenic mouse model of Alzheimer's disease (AD). Elevated levels of the insoluble Aβ (1–42) were detected in the brain extracts of the transgenic animals as early as 3 months of age, prior to the Aβ plaque formation (pre-plaque stage). By the early plaque stage (6 months) both the soluble and insoluble Aβ (1–40) and Aβ (1–42) peptides were detectable. We studied the expression of genes related to memory function (*Arc*, *Fos*), insulin signaling, including insulin receptor (*Insr*), *Irs1* and *Irs2*, as well as genes involved in insulin growth factor pathways, such as *Igf1*, *Igf2*, *Igfr* and *Igfbp2*. We also examined the expression and protein levels of key molecules related to energy metabolism (PGC1-α, and AMPK) and mitochondrial functionality (OXPHOS, TFAM, NRF1 and NRF2). 6 month-old APP/PS1 mice demonstrated impaired cognitive ability, were glucose intolerant and showed a significant reduction in hippocampal *Insr* and *Irs2* transcripts. Further observations also suggest alterations in key cellular energy sensors that regulate the activities of a number of metabolic enzymes through phosphorylation, such as a decrease in the *Prkaa2* mRNA levels and in the pAMPK (Thr172)/Total APMK ratio. Moreover, mRNA and protein analysis reveals a significant downregulation of genes essential for mitochondrial replication and respiratory function, including PGC-1α in hippocampal extracts of APP/PS1 mice, compared to age-matched wild-type controls at 3 and 6 months of age. Overall, the findings of this study show early alterations in genes involved in insulin and energy metabolism pathways in an APP/PS1 model of AD. These changes affect the activity of key molecules like NRF1 and PGC-1α, which are involved in mitochondrial biogenesis. Our results reinforce the hypothesis that the impairments in both insulin signaling and energy metabolism precede the development of AD amyloidogenesis.

1. Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia and the incidence rates of the disease are increasing exponentially due to the amount of aged population. AD diagnosis is based on the detection of senile amyloid-β (Aβ) plaques and neurofibrillary tangles in the brain [1]. Although the exact mechanisms triggering neurodegeneration in AD remain unclear, a number of hypotheses have been proposed [2–11].

In recent years, several studies have focused on the potential relationship between AD and metabolic disorders [14–17]. Obesity and diabetes significantly increase the risks of cognitive decline and AD, suggesting that brain glucose metabolism impairments [18–25] may be linked to AD pathogenesis [14]. Both the AD and type 2 diabetes mellitus (T2DM) are associated with peripheral and central insulin signaling abnormalities, including alterations in brain insulin and insulin-like

* Corresponding author at: Unitat de Farmacologia i Farmacognosia, Facultat de Farmàcia, Universitat de Barcelona, Avda/Diagonal 643, E-08028 Barcelona, Spain. Tel.: +34 93 4024531; fax: +34 934035982.
E-mail address: camins@ub.edu (A. Camins).
[1] These authors contributed equally to this work.
[2] Senior co-authors.

http://dx.doi.org/10.1016/j.bbadis.2014.05.025
0925-4439/

growth factor (IGF) levels [19–22]. Pathological changes in these signaling pathways affect neuronal survival, energy homeostasis, gene expression and memory processes [23–30]. For instance, insulin and IGF1 regulate the expression and phosphorylation of Tau protein through activation of kinases [26–36].

Therefore, AD could be considered as a "type 3 diabetes" metabolic disorder, with insulin providing the link connecting both chronic diseases [19–22]. Several studies have reported a role for insulin in the control of neuronal function in cortical and hippocampal areas, which are involved in memory processing and cognitive functions [24–26]. Thus, insulin directly influences neurons, modulates neurotransmitter release, neuronal outgrowth, neuronal survival, as well as synaptic plasticity [19]. Moreover, it has been demonstrated that soluble Aβ oligomers alter insulin signaling because they bind to insulin receptors in hippocampal neurons, thereby inducing receptor mobilization from the membrane and into the cell [15].

Another molecule implicated both in diabetes and AD is the insulin-degrading enzyme (IDE). IDE is capable of degrading both insulin and Aβ, however it binds insulin with a much higher affinity. In an animal model of T2DM, elevated levels of circulating insulin resulted in competitive inhibition of IDE, thus causing an increase in Aβ levels. Additionally, mice lacking IDE have lower rates of Aβ and insulin degradation, and develop hyperinsulinemia and Aβ deposits in the brain [19–21].

AD etiology is complex and Aβ by itself is unable to account for all aspects of AD [1–6,37–41]. In order to identify the underlying causes of the disease, it is of utmost importance to understand the potential correlations between Aβ oligomers and hippocampal metabolism in early disease-stages, prior to plaque deposition. Most of AD research is currently undertaken in animal models that have increased Aβ levels compared to controls, and while Aβ pathology is mimicked in these models, many other factors associated with AD are not. Transgenic mice that carry an APP and presenilin 1 (PS1) mutations show AD-like pathology and memory impairment, and are useful for studying AD and testing possible treatments [42]. The current study, carried out in an APP/PS1 model of AD, aimed to identify the metabolic pathways responsible for the onset of AD, with the main focus on the early disease-stages, prior to the formation of senile plaques and memory loss. For this purpose, we examined behavioral phenotype and mRNA expression and protein levels of genes related to insulin receptor and mitochondria signaling in 3 and 6 month-old APP/PS1 mice. Three month-old animals were chosen because at this age, neither significant cognitive loss, nor brain Aβ plaques are detectable compared to the six month-old mice, which exhibit high brain Aβ content and memory loss [12–14].

2. Materials and methods

2.1. Animals

Male APPswe/PS1dE9 and C57BL/6 mice were used in this study. APP/PS1 animals co-express a Swedish (K594M/N595L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9), allowing these mice to secrete elevated amounts of human Aβ peptide. Both mutations are associated with AD, are under control of the mouse prion protein promoter, directing both mutated proteins mainly to the CNS neurons, and result in age-dependent amyloid plaque depositions in mouse brain. The APPswe-mutated APP is a favorable substrate for β-secretase, whereas the PS1dE9 mutation alters β-secretase cleavage, thereby promoting overproduction of Aβ42. The animals were kept under controlled temperature, humidity and light conditions with food and water provided ad libitum. Mice were treated in accordance with the European Community Council Directive 86/609/EEC and the procedures established by the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Every effort was made to minimize animal suffering and to reduce the number of animals used. Fifty animals, divided into four groups, were used for the present study, with at least 10 wild-type and 10 APP/PS1 transgenic mice of 3 and 6 months of age, per group. Following in vivo testing, the animals were sacrificed and at least 6 mice in each group were used for RNA and protein extract isolation, with an additional 4 mice for immunohistochemistry.

2.2. Glucose and insulin tolerance tests

Intraperitoneal glucose tolerance tests (IP-GTT) and insulin tolerance tests (ITT) were performed in accordance with the previously published guidelines [72]. For IP-GTT, mice were fasted overnight for 16 h. The test was performed in a quiet room, preheated to +30 °C. The tip of the tail was cut with the heparin-soaked (Heparina Rovi, 5000 IU/ml; Rovi S.A.; Madrid, Spain) scissors, 30 min prior to 1 g/kg intraperitoneal glucose injection (diluted in H_2O). Blood glucose levels of the tail vein were measured at −30, 0, 5, 15, 30, 60 and 120 min after the glucose injection with the Ascensia ELITE blood glucose meter (Bayer Diagnostics Europe Ltd.; Dublin, Ireland). ITT was performed in similar conditions with the 0.25 IU/kg of human insulin, diluted in saline (Humulina Regular, 100 IU/ml/Lilly, S.A.; Madrid, Spain), except that the mice underwent a 5-hour morning fast. Blood glucose levels were measured at −30, 0, 15, 30, 45 and 60 min after the insulin administration. If, during this time, blood glucose levels dropped to below 20 mg/dl, 1 g/kg glucose was administered to counteract the effects of insulin, in order to reduce animal suffering.

2.3. Novel object recognition test

The test was conducted as described previously [43] in a 90° two arm, 25 cm long, and 20 cm high maze. The light intensity in the middle of the field was 30 lx. The objects to be discriminated were plastic figures (object A: 5.25 cm high, object B: 4.75 cm high). First, mice were individually habituated to the apparatus for 10 min a day, for two days. On the third day, they were submitted to a 10 min acquisition trial (first trial) during which they were placed in the maze in the presence of two identical novel objects (A + A, or B + B) placed at the end of each arm. A 10 min retention trial, with the objects (A + B) (second trial) occurred 2 h later. The amount of exploration time each animal spent on objects A and B during the acquisition trial varied between 5 and 20 s, depending on the individual mouse. Total exploration time between the 2 objects when calculated for each individual animal indicated the absence of the object preference bias (Fig. 1C) (n = 5–9 per group). During the retention trial, the times that the animal took to explore the new object (tn) and the old object (to) were recorded. A discrimination index (DI) was defined as (tn − to) / (tn + to). In order to avoid further object preference biases, objects A and B were counterbalanced so that half of the animals in each experimental group were first exposed to object A and then to object B, whereas the other half saw first object B and then object A. The maze, the surface, and the objects were cleaned with 96° ethanol between animals, so as to eliminate olfactory cues.

2.4. Immunohistochemistry

For detection of Aβ deposits, free-floating coronal sections, 20 μm thick, were rinsed with 0.1 mol/l PB, pH 7.2, and pre-incubated in 88% formic acid. Then, sections were treated with 5 ml/l H_2O_2 and 100 ml/l methanol in PBS and pre-incubated in a blocking solution (100 ml/l of FBS, 2.5 g/l of BSA and 0.2 mol/l of glycine in PBS with 5 ml/l of Triton X-100). After that, sections were incubated overnight (O/N) at 4 °C with the primary mouse anti-human beta-amyloid clone 6F/3D antibody (1:100; DakoCytomation, Denmark). Then, sections were incubated with the biotinylated secondary antibody (1:200; Sigma-Aldrich) followed by the avidin–biotin–peroxidase complex (ABC; 1:200; Vector, Burlingame, CA). Peroxidase reaction was developed with 0.5 g/l diaminobenzidine in 0.1 mol/l PB and 0.1 ml/l H_2O_2, and immunoreacted

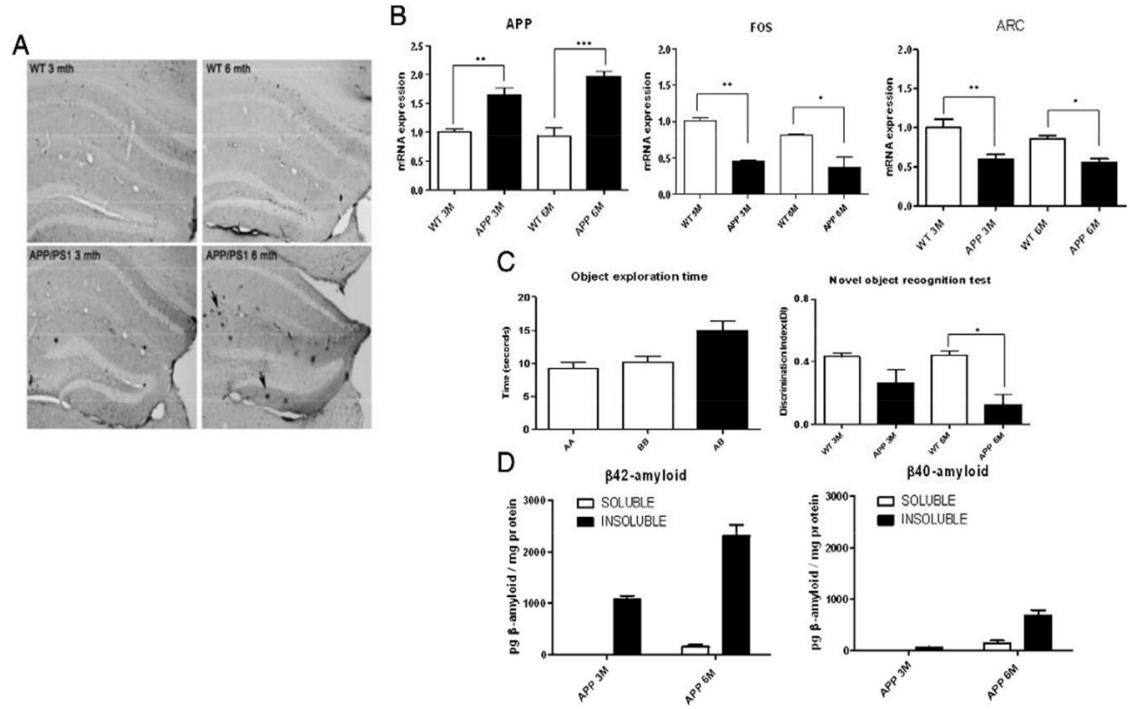


Fig. 1. Representative immunohistochemical staining with the Aβ-specific 6F/3D antibody, in 3 and 6 month-old mice, demonstrating Aβ plaque deposits in the hippocampus of 6 month-old APP/PS1 animals (A). mRNA expression profile of app, fos and Arc in the hippocampal extracts (n = 6) (B). The results of the 2 object novel object recognition test, demonstrating an absence of the object preference bias and a significant memory loss in 6 month-old APP/PS1 animals, compared to wild-type controls (n = 7–12) (C). Concentrations of the soluble and insoluble human Aβ (1–40) and Aβ (1–42) peptides in the cortical extracts in 3 and 6 month-old APP/PS1 mice, expressed as pg/mg of total protein as determined by ELISA (n = 5–6) (D). (Statistical analysis was performed with one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$.)

sections were mounted on gelatinized slides. Stained sections were examined under a light microscope (Olympus BX61).

2.5. Western blot analysis

Aliquots of hippocampus homogenate containing 15 mg of protein per sample were analyzed using the Western blot method. In brief, samples were placed in a sample buffer (0.5 m Tris–HCl, pH 6.8, 10% glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 0.05% bromophenol blue) and denatured by boiling at 95–100 °C for 5 min. Samples were separated by electrophoresis on 10–15% acrylamide gels. Following this, the proteins were transferred to PVDF sheets using transblot apparatus. Membranes were blocked overnight with 5% non-fat milk dissolved in TBS-T buffer (50 mM Tris; 1.5% NaCl, 0.05% Tween 20, pH 7.5). They were then incubated with primary antibodies, as detailed in Table 1. After O/N incubation, blots were washed thoroughly in TBS-T buffer and incubated for 1 h with a peroxidase-conjugated IgG secondary antibody (1:2000). Immunoreactive protein was detected using a chemiluminescence-based detection kit. Protein levels were determined by densitometry, using Chemi doc XRS + Molecular Imager detection system (Bio-Rad), with ImageLab image analysis software. Measurements are expressed as arbitrary units. All results are normalized to GAPDH, unless stated otherwise.

2.6. Serum insulin ELISA

Heart puncture was used to collect whole blood samples from 3 and 6 month-old wild-type and APP/PS1 mice, following a 5-hour morning fast, at the point of sacrifice. Blood samples were transferred to Serum-Gel Z microcentrifuge tubes (Sarstedt, Numbrecht, Germany), for serum separation. The samples were collected and kept at room temperature, and the serum was separated by centrifugation for 10 min at 5000 ×*g*. Serum insulin levels were measured with Rat/Mouse Insulin ELISA kit (Cat #: EZRMI-13K; EMD Millipore; St. Charles, MO, USA), according to manufacturer's instructions, utilizing 10 μl of mouse serum.

Table 1
A list of antibodies used for the immunoblotting experiments.

Protein	Antibody
pAMPK (Thr 172)	#2531 (Cell signaling)
AMPK	#2532 (Cell signaling)
PGC1A	101707 (Cayman chemical)
TFAM	DR1071b (Calbiochem)
NRF1	Sc-28379 (Santa Cruz biotech)
IDE	Ab32216 (Abcam)
pGSK3B (Tyr 216)	Ab74754 (Abcam)
pGSK3B (Ser 9)	#9336 (Cell signaling)
GSK3B	#9315 (Cell signaling)
pCDK5 (Tyr 15)	ab63550 (Abcam)
CDK5	Sc-173 (Santa Cruz biotech)
P35	#2680 (Cell signaling)
pTAU (Ser 199)	44734G (Life Technologies)
pTAU (Thr 205)	44738G (Life Technologies)
pTAU (Ser 396)	44752G (Life Technologies)
pTAU (Ser 404)	44748G (Life Technologies)
TAU5	AHB0042 (Biosource)
PSD-95	Ab18258 (Abcam)
SYP	M0776 (DakoCytomation)
OXPHOS	MS604 (MitoSciences)
GAPDH	MAB374 (Millipore)
2nd–ary Anti-Mouse	170-5047 (Biorad)
2nd–ary Anti-Rabbit	NA934V (GE Healthcare)

2.7. Measurement of Aβ peptides in brain tissues by ELISA

Aβ 1–40 and Aβ 1–42 were measured in cortical extracts according to a previously published procedure [44]. In brief, the samples were weighed and homogenized in a 8× volume of PBS with AEBSF protease inhibitor cocktail set (Cat # 539131; Calbiochem; La Jolla, CA, USA). The soluble fraction was separated by centrifuging the samples for 10 min at 4000 ×g. The pellets containing insoluble Aβ peptides were solubilized in a 5 M guanidine HCl/50 mM Tris HCl solution by incubating for 3.5 h on an orbital shaker at room temperature in order to obtain insoluble fraction. The levels of soluble and insoluble Aβ 1–40 and Aβ 1–42 were determined employing the commercially available human ELISA kits (Cat # KHB3481 and KHB3441; Invitrogen, Camarillo, CA, USA). Data obtained from the cortical homogenates are expressed as picograms of Aβ content per milligrams of total protein (pg/mg).

2.8. RNA extraction and quantification

Total RNA was isolated from the hippocampi of wild-type and APP/PS1 transgenic mice, as described previously [73]. Briefly, the tissue was homogenized in the presence of Trizol reagent (Life Technologies Corporation). Chloroform was added and the RNA was precipitated from the aqueous phase with isopropanol at 4 °C. RNA pellet was reconstituted in RNAse-free water, with the RNA integrity determined by Agilent 2100 Bioanalyzer.

2.9. Quantitative RT-PCR

First-strand cDNA was reverse transcribed from 2 μg of total RNA from the hippocampi of 3 and 6 month-old mice, using the High Capacity cDNA Reverse Transcription kit, according to manufacturer's protocol (Applied Biosystems). Equal amounts of cDNA of each individual animal were subsequently used for qRT-PCR, and each sample was analyzed in triplicate for each gene. TaqMan gene expression assays (Applied Biosystems) as detailed in Table 2 were used to determine transcription levels of individual genes. qRT-PCR was performed on the StepOnePlus Real Time PCR system (Applied Biosystems) and normalized to the average transcription levels of gapdh and tbp, using the delta–delta Ct method.

2.10. Statistical analysis

All data are presented as means ± SEM, and differences are considered significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$. Differences between samples/animals were evaluated using either one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$ and with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$ and $$$ denotes $p < 0.001$. Both the statistical analyses and the graphs presented here were created with the GraphPad InStat software V5.0 (GraphPad Sofware Inc., San Diego, CA, USA).

Table 2
A list of probes used for qRT-PCR analyses.

Gene	TaqMan probe
app	Mm01344172_m1
arc	Mm00479619_g1
fos	Mm00487425_m1
gapdh	Mm99999915_g1
igf1	Mm01228180_m1
igf2	Mm00439564_m1
igfbp2	Mm00492632_m1
igf1r	Mm00802831_m1
ins1	Mm01950294_s1
insr	Mm01211875_m1
irs1	Mm01278327_m1
irs2	Mm03038438_m1
nrf1	Mm01135606_m1
nfe2l2	Mm00477784_m1
Ppargc1a	Mm01208835_m1
prkaa1	Mm01296700_m1
prkaa2	Mm01264789_m1
tbp	Mm00446971_m1
tfam	Mm00447485_m1

3. Results

3.1. Early phenotypical signs of amyloidogenesis in APP/PS1 mice

In order to determine the extent of the amyloid deposition in the brains of APP/PS1 mice, hippocampal sections were stained with the 6F/3D monoclonal antibody, which is specific for the human form of the Aβ peptide. It was previously reported that the amyloid plaque deposits first start to appear at the age of 6 months in this model [13,14]. In agreement with the earlier studies, our immunohistochemical results demonstrated neither diffuse nor fibrillar plaque deposition in the brains of APP/PS1 mice at 3 months of age. In contrast, Aβ protein aggregates were clearly visible by the age of 6 months (Fig. 1A). In addition, a significant increase in the mRNA levels of the app (utilizing a probe which recognizes both human and mouse transcripts) was detected in hippocampal extracts of both 3 and 6 month-old animals. Likewise, we observed a significant reduction in the mRNA levels of *Arc* and *fos*, which play a role in memory function, in the brains of APP/PS1 mice [45] (Fig. 1B). Moreover, we have characterized the progression of cognitive impairment, utilizing a 2 object novel object recognition test. Our results showed a significant memory loss in 6 month-old APP/PS1 mice (Fig. 1C).

Interestingly, we have detected significant levels of the insoluble Aβ (1–42) peptides in the cortical homogenates of 3 month old APP/PS1 mice (>1000 pg/mg), at an age when Aβ plagues are not yet detectable by immunohistochemistry. The amyloid burden was further increased in 6 month old transgenic animals, with the detectable levels of both soluble and insoluble Aβ (1–40) (>140 and >680 pg/mg) and of soluble and insoluble Aβ (1–42) (>150 and >2300 pg/mg) (Fig. 1D).

3.2. Glucose and insulin tolerance tests and peripheral insulin levels

Since a connection between AD and T2DM has been established during the past decade, we intended to identify any metabolic perturbations in glucose metabolism in the APPswe/PS1dE9 strain [18]. In fact, as shown in Fig. 2, APP/PS1 mice exhibited impaired fasting glucose and insulin tolerance, following IP-GTT and ITT, respectively. Interestingly, the biggest differences in blood glucose levels, between the wild-type and transgenic animals, were detected from 30 to 120 min following i.p. glucose administration in IP-GTT or insulin administration in ITT. In addition, a slight increase in fasting peripheral insulin levels, determined by ELISA, was observed in 6 month-old APP/PS1 mice, compared to age-matched controls. Having confirmed the existence of the peripheral metabolic phenotype in the APP/PS1 mice, we then proceeded to study the expression of genes related to insulin metabolism in the brain, with a particular focus on hippocampal insulin receptor signaling pathway.

3.3. Identification of differentially expressed genes related to insulin receptor

Previous studies demonstrated alterations in brain insulin signaling in AD, but the onset and the severity of this impairment are unclear [43–46]. For this reason, we evaluated mRNA expression of preproinsulin 1 (*Ins1*), insulin receptor (*Insr*), insulin receptor substrates 1 (*Irs1*) and 2 (*Irs2*), insulin-like growth factors I (*Igf1*) and II (*Igf2*), IGF receptor (*Igfr*), as well as insulin-like growth factor-binding protein 2 (igfbp2), at 3 and 6 months of age (Fig. 3). We detected a small, but significant reduction

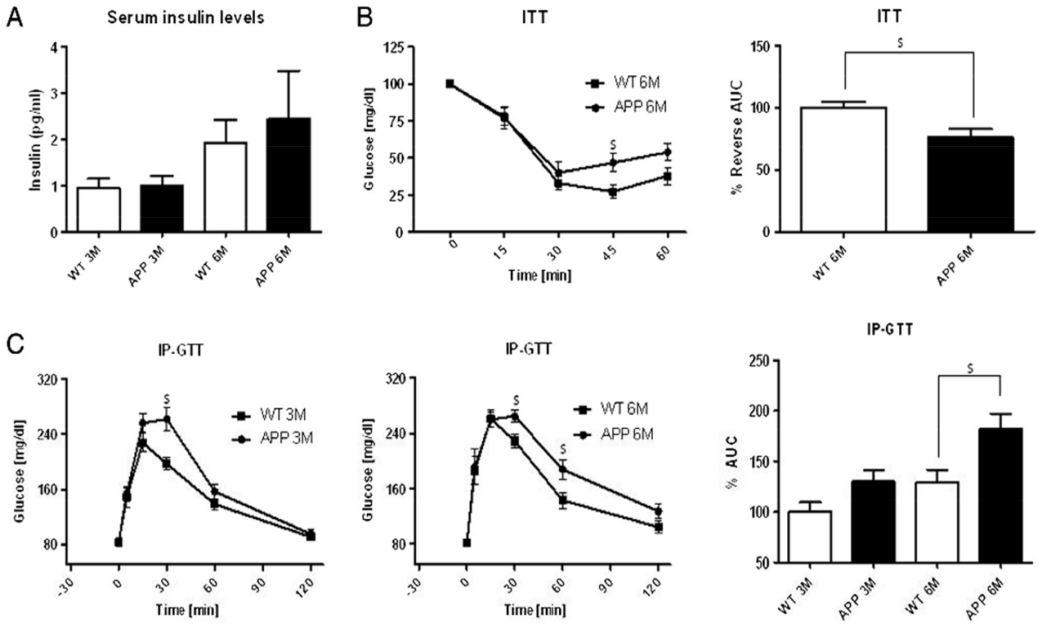


Fig. 2. Fasting serum insulin levels ELISA (n = 5–7) (A), insulin tolerance test (n = 5–7) (B) and intraperitoneal glucose tolerance test (n = 5–12) (C) in 3 and 6 month-old wild-type and APP/PS1 mice. For the ITT and the IP-GTT, AUC data were calculated from the timepoint 0 till the end of the experiment (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$.)

in *insr* and *irs2* transcripts in the hippocampal extracts of 3 month-old APP/PS1 mice, compared to age-matched controls. Interestingly, the expression of both *Igf2* and *Igfbp2* transcripts was significantly increased at 6 months in transgenic animals.

3.4. Energy metabolism is impaired in the early stages of the amyloidogenesis

AMP-activated protein kinase (AMPK) is a sensor of cellular stress that maintains energy homeostasis by promoting mitochondrial

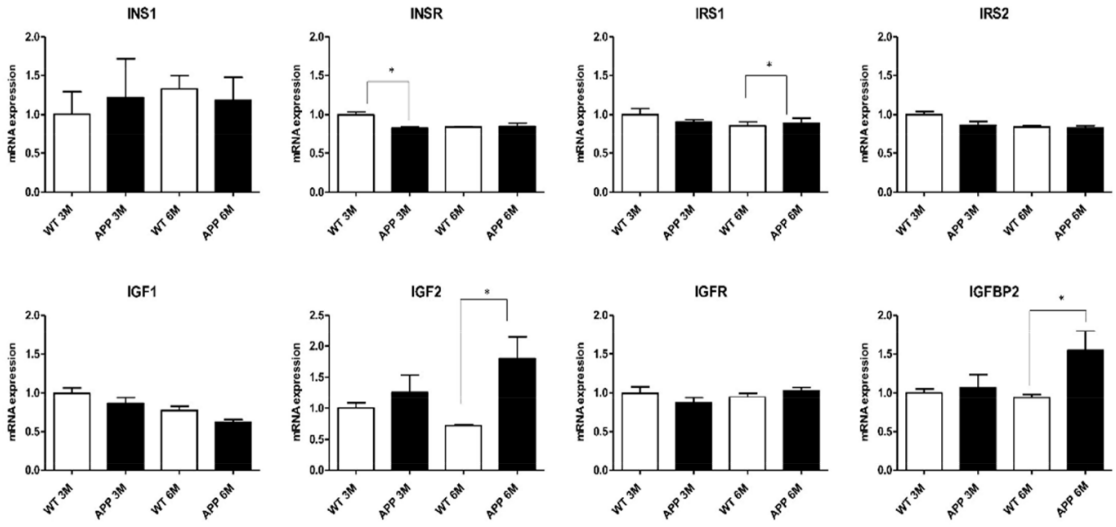


Fig. 3. mRNA expression profile of genes related to insulin signaling pathway in the hippocampal extracts of 3 and 6 month-old wild-type and APP/PS1 mice (n = 4–6). (Statistical analysis was performed with one-way ANOVA and with Tukey's post-hoc test, where * denotes $p < 0.05$.)

biogenesis through transcriptional coactivator peroxisome proliferator activated receptor-γ coactivator 1α (PGC-1α) signaling pathway [47–54]. We detected a significant reduction in the mRNA levels of the alpha 2 (*Prkaa2*), but not of the alpha 1 (*Prkaa1*) isoform of the catalytic subunit of AMPK, in the hippocampi of 3 month-old APP/PS1 mice, compared to wild-type controls (Fig. 4A). This observation is consistent with the slight reduction in the protein levels of phosphorylated AMPK (pAMPK (Thr172)/Total APMK ratio) (Fig. 4B). Thus, our data suggest alterations in a key cellular energy sensor that regulates the activities of a number of molecules involved in cellular metabolism.

PGC-1α is involved in energy homeostasis and glucose metabolism, as well as in mitochondrial metabolism and biogenesis [49,50]. In the current study, we detected a significant reduction in PGC-1α mRNA and protein (Fig. 4) levels in the hippocampi of 3 and 6 month-old APP/PS1 mice, compared to control animals. Since PGC-1α regulates the transcriptional activity of genes essential for mitochondrial replication and respiratory function, such as estrogen-related receptor α (ERR-α), nuclear respiratory factor (NRF) and mitochondrial transcription factor A (TFAM), we proceeded to study their mRNA and protein levels.

3.5. The impairment of mitochondrial biogenesis is involved in the early stages of the amyloidogenesis

It has been hypothesized that mitochondrial dysfunction could be a trigger of AD [49–54]. In fact, mRNA expression analysis of genes, downstream to PGC-1α, confirmed significant reductions in *Nrf1* and *Nrf2* transcripts in the hippocampi of 3-month old APP/PS1 animals, compared to controls. mRNA levels of *Tfam* were also slightly reduced at this age, although the observed changes did not reach statistical significance (Fig. 4A). These data suggest that reduced mitochondrial biogenesis is an early event in the hippocampus of APP/PS1 mice.

3.6. Mitochondrial OXPHOS expression

Deregulation in OXPHOS signaling is indicative of mitochondrial function impairment and has been previously reported in the brains of 3 and 6 month old APP mice [46,47]. In agreement with the above mentioned studies, we have detected a significant reduction in OXPHOS complexes I, II, III, and IV in the hippocampi of the 3 month old APP/PS1 mice (Fig. 5).

3.7. Tau phosphorylation and Tau kinase levels

Since Tau expression is regulated by insulin/IGF-I, and also by AMPK, increased Tau phosphorylation could be an early event in the brains of APP/PS1 mice. It is well-known that Tau is a microtubule-binding protein participating in neuronal cytoskeletal dynamics maintenance and axonal transport [55–59]. Evaluation of several Tau phosphoepitopes by Western blotting, revealed a global increase in Tau phosphorylation in the hippocampal extracts of APP/PS1 mice, both at 3 and 6 months of age (Fig. 6).

3.8. Involvement of GSK-3β and CDK5 kinases in the early stages of the amyloidogenesis and Tau phosphorylation

Insulin and IGFI signaling regulate the expression and phosphorylation of Tau proteins, as impaired insulin function leads to the overactivation of GSK-3β, a kinase capable of Tau phosphorylation. Our results show a significant increase in p35 content and pCDK5 (Tyr15)/CDK5 ratios in the hippocampus of APP/PS1 animals, compared to controls, both at 3 and 6 months of age. In contrast, the protein expression levels of GSK-3β, phosphorylated at Ser9 (inactive form), and Tyr216 (active form), as well as of IDE, remained unchanged (Fig. 7).

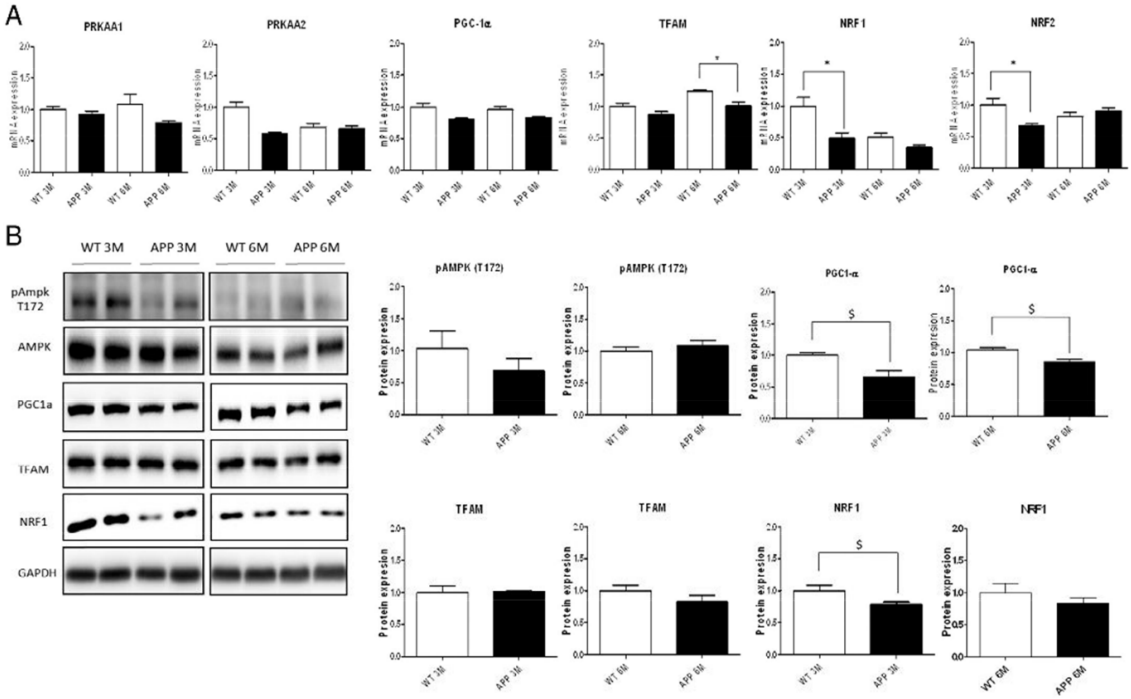


Fig. 4. mRNA expression profile (n = 4–6) (A) and representative immunoblot images and quantification (n = 4–6) (B) of the molecules related to energy metabolism and mitochondrial biogenesis in the hippocampal extracts of 3 and 6 month old wild-type and APP/PS1 mice. pAMPK (T172) is normalized to total AMPK levels, with the rest of the proteins normalized to GAPDH. (Statistical analysis was performed with one-way ANOVA and with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$, and with the student's t-test, where $ denotes $p < 0.05$).

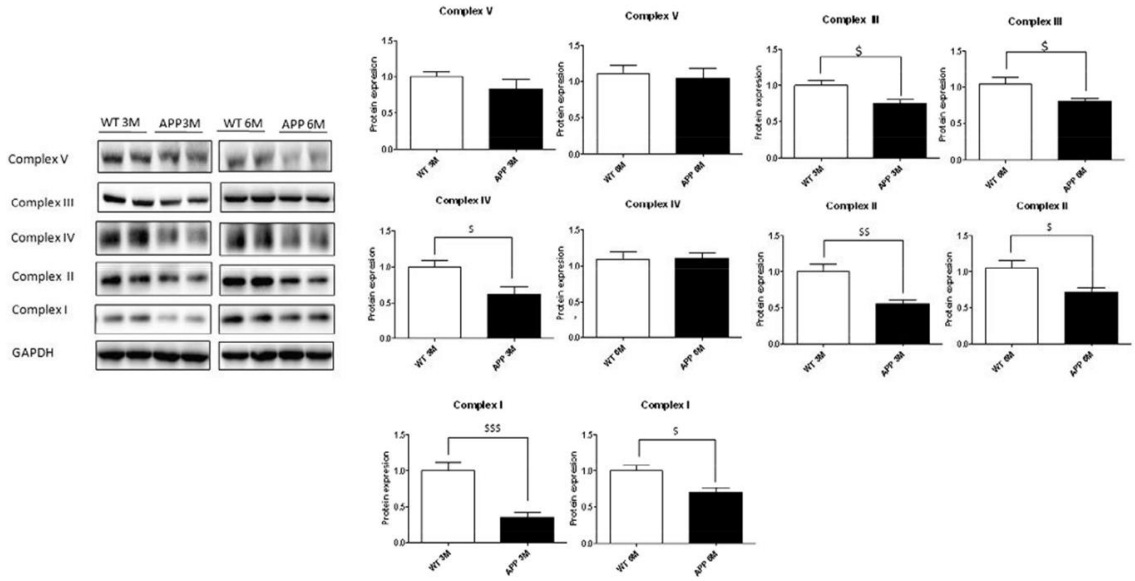


Fig. 5. Representative immunoblot images and quantification of various OXPHOS complexes, normalized to GAPDH protein levels, in the hippocampal extracts of 3 and 6 month old wild-type and APP/PS1 mice (n = 4–6). (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$, and $$$ denotes $p < 0.001$.)

3.9. Changes in synaptic protein levels

Because Aβ oligomers induce synaptic loss in AD we evaluated protein expression levels of representative pre- and post-synaptic proteins (synaptophysin (SYP) and PSD-95 respectively). As shown in Fig. 8, there were no significant changes in their levels in the hippocampus of APP/PS1 mice, relative to the control tissue. Our results are in agreement with a previous study by Minkeviciene et al. who did not detect any changes in synaptic protein levels in 17 month-old APP/PS1 mice [48].

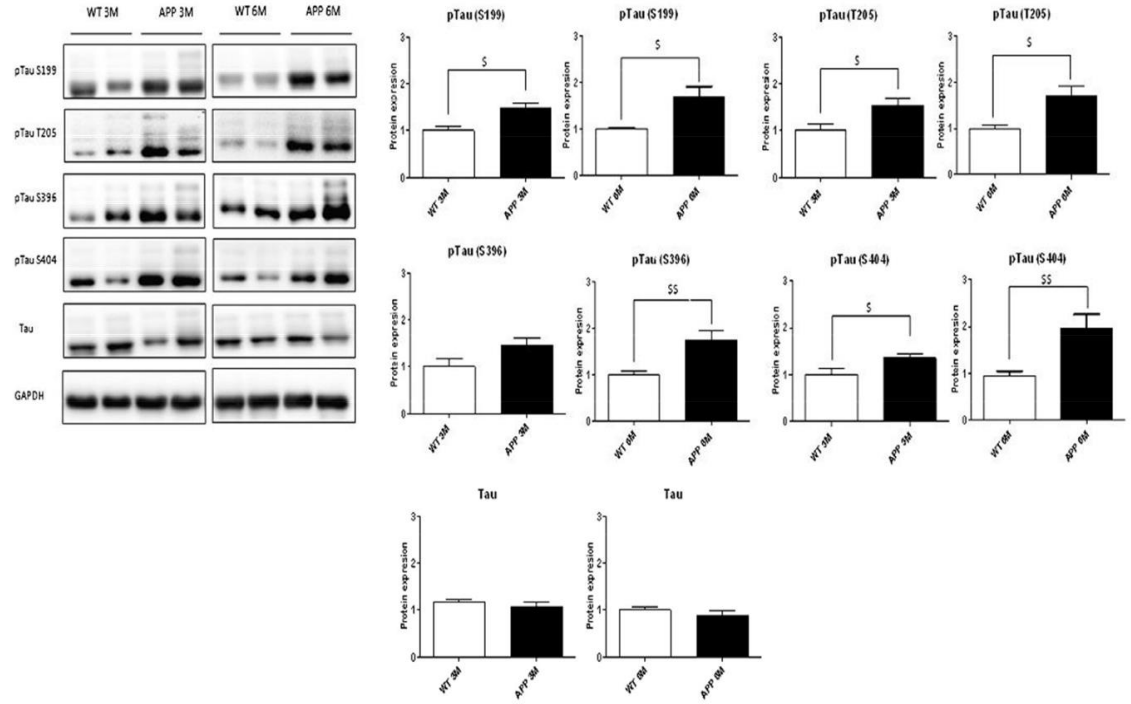


Fig. 6. Representative immunoblot images and quantification of various Tau phosphoepitopes, normalized to total Tau protein levels, in the hippocampal extracts of 3 and 6 month old wild-type and APP/PS1 mice (n = 4–6). (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$ and $$ denotes $p < 0.01$.)

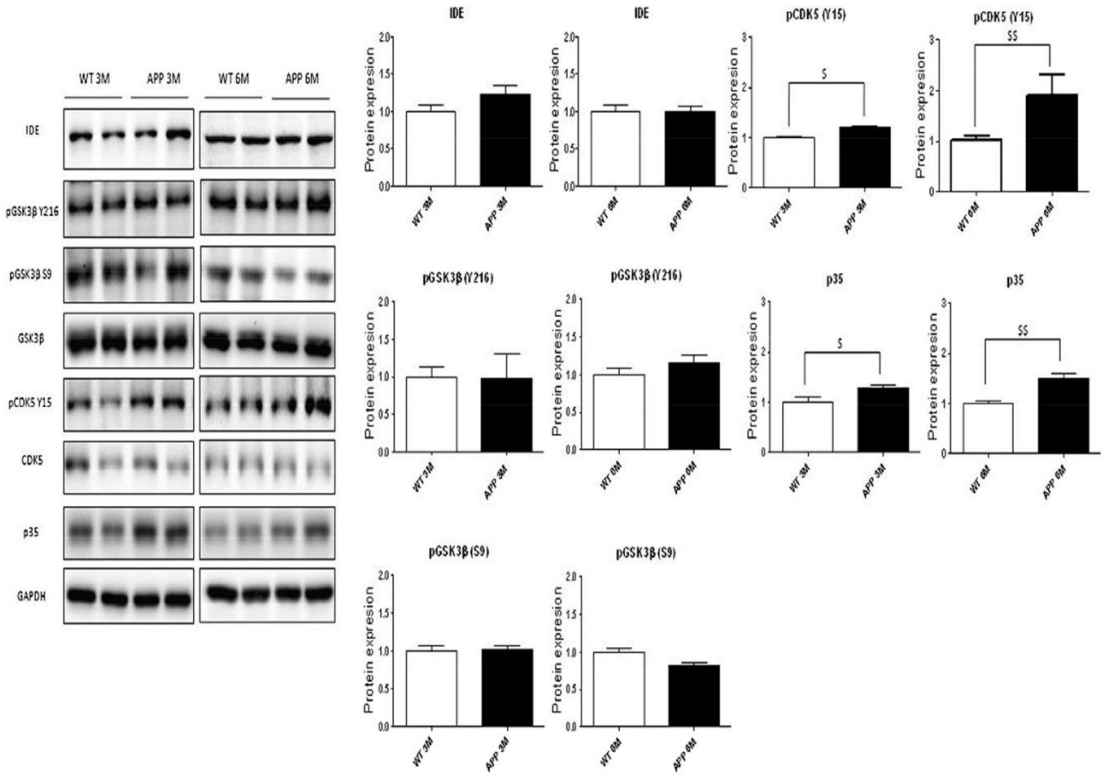


Fig. 7. Representative immunoblot images and quantification of molecules implicated in insulin signaling and Tau phosphorylation, in the hippocampal extracts of 3 and 6 month old wild-type and APP/PS1 mice (n = 4–6). pCDK (Y15), pGSK3β (Y216) and pGSK3β (S9) are normalized to their respective total unphosphorylated protein levels, whereas p35 and IDE are normalized to GAPDH. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$ and $$ denotes $p < 0.01$.)

4. Discussion

In the current study, we employed an integrated approach consisting of the analyses in both the periphery and at the CNS levels, in order to identify potential changes that occur in the early stages of the amyloidogenic process, prior to amyloid plaque formation in a mouse model of AD. We investigated several key metabolic routes related to glucose uptake and insulin signaling, cellular energy homeostasis, mitochondrial biogenesis and Tau phosphorylation.

In previous studies it was demonstrated that an increase in Aβ levels in APP/PS1 mice is accompanied by plaque deposition in the brain and memory loss, clearly evident at the age of 6 months [11–15]. Thus, APP/PS1 mice are commonly used in AD research [12,14,42,49]. In agreement with these studies, we detected a significant amyloid peptide deposition and the presence of Aβ aggregates in 6 month-old APP/PS1 mice, compared to age-matched wild-type controls. In addition, the 2 object novel object recognition test has revealed a significant cognitive impairment at the age of 6 months in this model. A novel finding in our study is the detection of the insoluble Aβ (1–42) in the brains of 3 month old APP/PS1 mice. This data is intriguing, as Aβ plaques were not detectable by immunohistochemistry at such an early age. Our results suggest that the formation of the insoluble Aβ "proto-fibrils" is

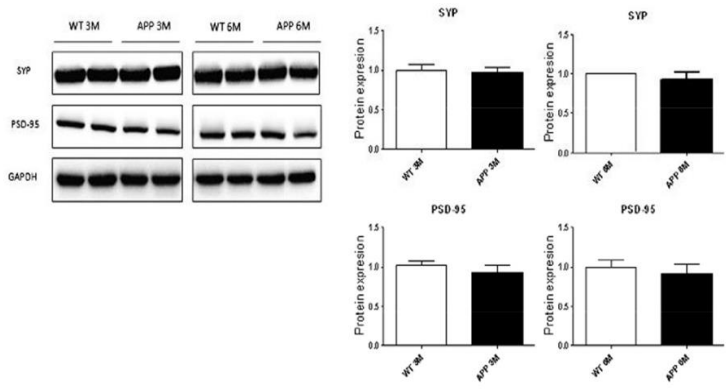


Fig. 8. Representative immunoblot images and quantification of synaptophysin and PSD-95, in the hippocampal extracts of 3 and 6 month old wild-type and APP/PS1 mice (n = 4–6).

an early event which leads to plaque formation by the age of 6 months. Elevated levels of the insoluble Aβ (1–42) were previously reported in brain homogenates of 6 month old APP/PS1 animals [50]. However, the authors of that study did not detect any Aβ in 4 month old mice. The discrepancy with this study could be explained by the differences in experimental methodologies. As expected, by the age of 6 months we have detected a further increase in the levels of insoluble Aβ (1–42) together with the appreciable levels of both soluble and the insoluble Aβ (1–40) as well as senile plaques,

Moreover, in the present research, we demonstrate early changes affecting insulin signaling in the preplaque APP/PS1 mice. These mice display impairment in genes involved in glucose metabolism and mitochondrial function, such as OXPHOS. Thus, as mentioned above, AD neuropathology can be explained, in part, through alterations in glucose metabolism. It was previously published that in 5–7 week-old APP/PS1 mice, glucose and insulin tolerance were not impaired [16]. In our study, we had used older animals and our results demonstrated a tendency towards altered glucose tolerance already at 3 months of age, with the 6-month-old animals exhibiting significantly impaired glucose and insulin tolerance. In the same model, but employing female mice, Hiltunen et al. reported significantly impaired glucose tolerance at 7 months of age in APP/PS1 animals, compared to wild-type controls. Interestingly, the authors did not detect any changes in ITT, but this could be explained by the fact that the ITT was terminated 20 min after the insulin injection. In our study, the biggest differences in ITT between the wild-type and transgenic animals occurred between 30 and 60 min, following i.p. insulin administration. In addition, Hiltunen and colleagues generated triple transgenic mice by cross-breeding APP/PS1 with the mice overexpressing pancreatic IGF2 [49]. While IGF2 single mutants showed impaired glucose and insulin tolerance, in triple APP/PS1/IGF2 animals, this phenotype was further exacerbated. In fact, our results clearly show a significant age-dependent increase in the IGF2 content in the hippocampus, supporting the role of IGF2 signaling in the metabolic perturbations affecting APP/PS1 mice.

Recent studies suggest that there is a close link between insulin-deficient diabetes and cerebral amyloidosis in the pathogenesis of AD [25,51–59]. Using a streptozotocin (STZ)-induced diabetic APP/PS1 mouse model, it has been shown that the diabetic condition promoted the processing of APP, resulting in increased Aβ generation, neuritic plaque formation, and spatial memory deficits [24]. Patients with AD show a remarkable deposition of Aβ peptide in the brain, whereas patients with T2DM present Islet Amyloid Polypeptide (IAPP) deposition in pancreatic β-cells [17]. Then, AD and T2DM share common key molecular alterations in Aβ peptide processing and insulin signaling, in a poorly understood interplay [15–17,51,52,60–65]. Chua and colleagues have suggested that an increase in brain Aβ42 levels in 15 month-old female APP/PS1 mice, may be dependent on impaired brain insulin signaling [53]. However, Sadowski and colleagues demonstrated a correlation between the hippocampal amyloid plaque levels and glucose utilization at 22 months of age. It is of note, that the majority of published studies focus on very late stages of the disease, when Aβ plaques are fully developed [66].

Therefore, the question would be: Are metabolic disorders the cause or the consequence of the AD? It is known that brain glucose metabolism defects are strongly associated with memory impairment in AD brain. Human brain imaging studies indicate that impaired glucose utilization precedes the onset of cognitive deficits in AD, suggesting causality [21]. In this context, the binding of insulin, IRS1 and IRS2 to the INSR, could modulate hippocampal synaptic plasticity and memory consolidation [19–22]. In agreement with this hypothesis, we detected a small, but significant reduction in the hippocampal *Arc*, *Fos*, *Insr* and *Irs2* transcripts in 3 month-old APP/PS1 mice, compared to wild-type controls. By the age of 6 months, APP/PS1 mice develop impaired glucose and insulin tolerance, accompanied by a significant increase in *Igf2* and *Igfbp2* transcripts. Interestingly, we did not detect any changes in the transcription of *Igf1*, which is involved in development, cognitive functions and aging processes, and the alterations of which had been linked to AD pathology.

Downstream of insulin signaling, we focused on mitochondrial markers. Structural and functional perturbations of mitochondria in AD have been recognized for some time, and led Swerdlow and Khan to propose the mitochondrial cascade hypothesis [5]. This hypothesis states that inherited mutations in mitochondrial DNA determine the basal functional ability of mitochondria to respond to, and to recover from stress-induced signaling. The physiopathology of AD develops when the mitochondria lose their functional capacity, and includes neuronal apoptosis, Aβ deposition, and neurofibrillary tangles [7,59–65]. Here, we report a significant downregulation in mitochondrial OXPHOS complexes in the brains of 3 month old APP/PS1 mice. Likewise, we detected reduced mRNA expression levels of genes related to mitochondrial biogenesis and the regulation of energy metabolism, including *Prkaa2* subunit of AMPK, *Pgc-1α*, *Nrf1* and *Nrf2*. NRF1, through its interaction with PGC-1α, regulates mitochondrial biogenesis directly, and is a key transcriptional regulator of IDE [62–64]. The vast majority of IDE protein is localized to the cytosol, with the small amounts present in the mitochondria, where it participates in Aβ degradation. Mitochondrial localization is dependent on the long isoform of ide mRNA transcripts, the expression of which was found to be positively correlated with *Pgc-1α* and *Nrf-1* transcripts in the brains of non-demented human patients. Interestingly, the correlation was weaker in the brains of AD patients, suggesting an impairment of this route [49]. The observed reduction of *Pgc-1α* and *Nrf-1*, both at the mRNA and protein levels, in the hippocampi of young APP/PS1 animals in our study, supports this hypothesis. The lack of changes in IDE protein levels can be explained by the phenomenon of eclipsed distribution [63]. As the dominant, short isoform of IDE is ubiquitously expressed in the cytosol, any changes at the mitochondrial level would be masked by this dominant isoform.

PGC-1α is a member of a family of transcriptional coactivators that plays a central role in the regulation of cellular energy metabolism. It stimulates mitochondrial biogenesis and participates in the regulation of both carbohydrate and lipid metabolism in peripheral disorders such as obesity and diabetes, however its role in the CNS is less clear [55–60]. In addition to the direct effects on mitochondrial gene expression, PGC-1α is also involved in the regulation of genes that protect neuronal cells from oxidative stress, such as mitochondrial superoxide dismutase. PGC-1α is regulated by several metabolism-responsive elements like AMPK which, when activated by elevated AMP/ATP ratios, can phosphorylate it directly [59]. Recent reports indicate that PGC-1α could be a potential biomarker of AD disease, as reduced PGC-1α mRNA and protein levels had been detected in AD brains [58–61]. In agreement with this, we detected significant reductions in PGC-1α mRNA and protein levels in APP/PS1 brains, compared to wild-type controls, at 3 and 6 months of age. We also observed a decrease in a ratio of activated pAMPK (Thr172)/Total APMK in 3 month old APP/PS1 mice, supporting the role of mitochondrial biogenesis impairment in the early stages of AD. PGC-1α remains an attractive target for AD therapeutic intervention [59].

AMPK is a cellular energy sensor conserved in all eukaryotic cells. It regulates the activities of a number of key metabolic enzymes and protects cells from stresses that cause ATP depletion, by switching off ATP-consuming biosynthetic pathways [58]. It AMPK can also phosphorylate substrates like Tau proteins, thereby causing their hyperphosphorylation. Tau hyperphosphorylation occurs both as a result of elevated levels of Aβ and genetic mutations in Tau proteins, and causes microtubule disassembly, which leads to the formation of neurofibrillary tangles and synaptic loss. In a mouse model overexpressing a P301L-mutated version of human Tau (rTgP301L transgene), Tau hyperphosphorylation resulted in its accumulation in the still functional dendritic spines. Significantly, these observations were reported in relatively young 4.5 month-old animals, at an age when cognitive impairments were already evident, but neither the neuronal, nor synaptic loss was detectable [61].

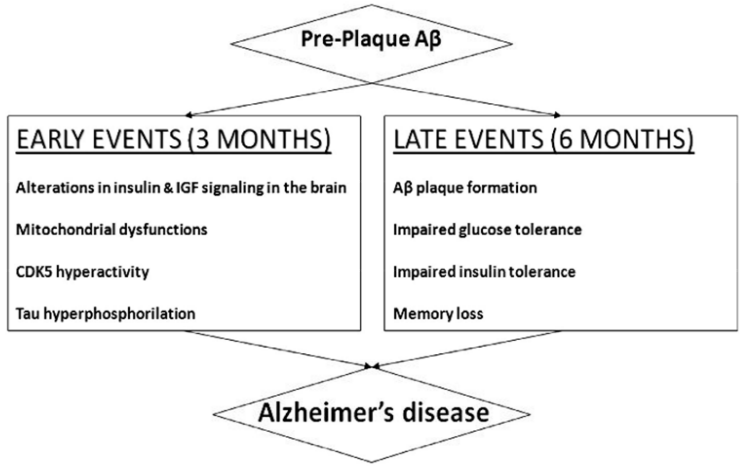


Fig. 9. Summary of the events, leading to the progressive amyloid plaque deposition and memory loss in an APP/PS1 mouse model of FAD.

We detected a significant increase in phosphorylation of several Tau phosphoepitopes in the hippocampi of APP/PS1 mice, compared to controls, including p-Tau (Ser199); p-Tau (Ser 205); p-Tau (Ser 396); and p-Tau (Ser404). At the same time, the protein levels of phosphorylated forms of AMPK and GSK-3β remained unchanged, suggesting that Tau phosphorylation occurs via an alternative pathway in our model. Several studies have suggested that AD and T2DM may share a common pathway to pathology: the hyperactivation of CDK5 [67–69]. CDK5 activation may cause aberrant phosphorylation of cytoskeletal components like Tau and neurofilaments. Results from our research demonstrated an increase in p(Y15) CDK5 phosphorylation in 3 and 6 month-old APP/PS1 mice, suggesting that CDK5 may be the kinase involved in Tau phosphorylation [62,64–71].

In summary, our results show an early downregulation of glucose, insulin signaling and energy metabolism pathways in an APP/PS1 mouse model of FAD. An overview of the key events, occurring between 3 and 6 months of age in our model, is presented in Fig. 9. These changes affect the activity of key molecules involved in memory processes (Arc, Fos) and mitochondrial regulation, such as OXPHOS, PGC-1α and NRF1, as well as Tau phosphorylation. The data presented here reinforces the hypothesis that the preceding events in the amyloidogenenic process in AD are related to both insulin signaling and energy metabolism impairment. Finally, we demonstrate an increase in the levels of pCDK5, which may be responsible for Tau phosphorylation and NFT formation in the hippocampi of the APP/PS1 mice.

Acknowledgments

This study was funded by grant 2009/SGR00853 from the Generalitat de Catalunya (autonomous government of Catalonia), by grants BFU2010-19119/BFI, SAF2011-23631, SAF2012-39852-C02-01 and SAF2012-30708 from the Spanish Ministerio de Ciencia e Innovación and grant 0177594 from the CONACYT (Mexico).

References

[1] M.A. Smith, Alzheimer disease, Int. Rev. Neurobiol. 42 (1998) 1–54.
[2] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[3] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, Science 256 (1992) 184–185.
[4] A. Erol, An integrated and unifying hypothesis for the metabolic basis of sporadic Alzheimer's disease, J. Alzheimers Dis. 13 (2008) 241–253.
[5] R.H. Swerdlow, S.M. Khan, A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease, Med. Hypotheses 63 (2004) 8–20.
[6] N. Bassil, G.T. Grossberg, Novel regimens and delivery systems in the pharmacological treatment of Alzheimer's disease, CNS Drugs 23 (2009) 293–307.
[7] R.H. Swerdlow, Mitochondria and cell bioenergetics: increasingly recognized components and a possible etiologic cause of Alzheimer's disease, Antioxid. Redox Signal. 16 (2012) 1434–1455.
[8] W. Zhang, J. Hao, R. Liu, Z. Zhang, G. Lei, C. Su, J. Miao, Z. Li, Soluble Aβ3 levels correlate with cognitive deficits in the 12-month-old APPswe/PS1dE9 mouse model of Alzheimer's disease, Behav. Brain Res. 222 (2011) 342–350.
[9] J.E. Selfridge, J. Lu, R.H. Swerdlow, Role of mitochondrial homeostasis and dynamics in Alzheimer's disease, Neurobiol. Dis. 51 (2013) 3–12.
[10] S.W. Pimplikar, Reassessing the amyloid cascade hypothesis of Alzheimer's disease, Int. J. Biochem. Cell Biol. 41 (2009) 1261–1268.
[11] S.W. Pimplikar, R.A. Nixon, N.K. Robakis, J. Shen, L.H. Tsai, Amyloid-independent mechanisms in Alzheimer's pathogenesis, J. Neurosci. 30 (2010) 14946–14954.
[12] W. Zhang, M. Bai, Y. Xi, J. Hao, L. Liu, N. Mao, C. Su, J. Miao, Z. Li, Early memory deficits precede plaque deposition in APPswe/PS1dE9 mice: involvement of oxidative stress and cholinergic dysfunction, Free Radic. Biol. Med. 52 (2012) 1443–1452.
[13] N. Sato, R. Morishita, Plasma β-amyloid: a possible missing link between Alzheimer disease and diabetes, Diabetes 62 (2013) 1005–1006.
[14] W. Zhang, M. Bai, Y. Xi, J. Hao, Z. Zhang, C. Su, G. Lei, J. Miao, Z. Li, Multiple inflammatory pathways are involved in the development and progression of cognitive deficits in APPswe/PS1dE9 mice, Neurobiol. Aging 33 (2012) 2661–2677.
[15] F.G. De Felice, Alzheimer's disease and insulin resistance: translating basic science into clinical applications, J. Clin. Invest. 123 (2013) 531–539.
[16] M. Jiménez-Palomares, J.J. Ramos-Rodríguez, J.F. López-Acosta, M. Pacheco-Herrero, A.M. Lechuga-Sancho, G. Perdomo, M. García-Alloza, I. Cózar-Castellano, Increased Aβ3 production prompts the onset of glucose intolerance and insulin resistance, Am. J. Physiol. Endocrinol. Metab. 302 (2012) E1373–E1380.
[17] L. Haataja, T. Gurlo, C.J. Huang, P.C. Butler, Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis, Endocr. Rev. 29 (2008) 303–316.
[18] Y. Zhang, B. Zhou, F. Zhang, J. Wu, Y. Hu, Y. Liu, Q. Zhai, Amyloid-β induces hepatic insulin resistance by activating JAK2/STAT3/SOCS-1 signaling pathway, Diabetes 61 (2012) 1434–1443.
[19] S.M. de la Monte, Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease, Curr. Alzheimer Res. 9 (2012) 35–66.
[20] M.C. Leal, N. Magnani, S. Villordo, C.M. Buslje, P. Evelson, E.M. Castaño, L. Morelli, Transcriptional regulation of insulin-degrading enzyme modulates mitochondrial amyloid β (Aβ3) peptide catabolism and functionality, J. Biol. Chem. 288 (2013) 12920–12931.
[21] S.M. de la Monte, Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's disease, Drugs 72 (2012) 49–66.
[22] de la Monte, J.R. Wands, Alzheimer's disease is type 3 diabetes—evidence reviewed, J. Diabetes Sci. Technol. 2 (2008) 1101–1113.

[23] A. Trueba-Sáiz, C. Cavada, A.M. Fernandez, T. Leon, D.A. González, J. Fortea Ormaechea, A. Lleó, T. Del Ser, A. Nuñez, I. Torres-Aleman, Loss of serum IGF-I input to the brain as an early biomarker of disease onset in Alzheimer mice, Transl. Psychiatry 3 (Dec 3 2013) e330.
[24] X. Wang, W. Zheng, J.W. Xie, T. Wang, S.L. Wang, W.P. Teng, Z.Y. Wang, Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model, Mol. Neurodegener. 2 (2010) 46.
[25] N. Sato, R. Morishita, Roles of vascular and metabolic components in cognitive dysfunction of Alzheimer disease: short- and long-term modification by non-genetic risk factors, Front. Aging Neurosci. 5 (2013) 64.
[26] E. van Exel, P. Eikelenboom, H. Comijs, D.J. Deeg, M.L. Stek, R.G. Westendorp, Insulin-like growth factor-1 and risk of late-onset Alzheimer's disease: findings from a family study, Neurobiol. Aging 35 (2014) 725.e7–725.e10.
[27] E. Steen, B.M. Terry, E.J. Rivera, J.L. Cannon, T.R. Neely, R. Tavares, X.J. Xu, J.R. Wands, S.M. de la Monte, Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J. Alzheimers Dis. 7 (2005) 63–80.
[28] W. Farris, S. Mansourian, Y. Chang, L. Lindsley, E.A. Eckman, M.P. Frosch, Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 4162–4167.
[29] S. Takeda, N. Sato, H. Rakugi, R. Morishita, Molecular mechanisms linking diabetes mellitus and Alzheimer disease: beta-amyloid peptide, insulin signaling, and neuronal function, Mol. Biosyst. 7 (2011) 1822–1827.
[30] S. Takeda, N. Sato, K. Uchio-Yamada, K. Sawada, T. Kunieda, D. Takeuchi, H. Kurinami, M. Shinohara, H. Rakugi, R. Morishita, Elevation of plasma beta-amyloid level by glucose loading in Alzheimer mouse models, Biochem. Biophys. Res. Commun. 385 (2009) 193–197.
[31] B.K. Binukumar, Y.L. Zheng, V. Shukla, N.D. Amin, P. Grant, H.C. Pant, TFP5, a peptide derived from p35,a CDK5 neuronal activator, rescues cortical neurons from glucose toxicity, J. Alzheimers Dis. 39 (2014) 899–909.
[32] J.L. Hallows, K. Chen, R.A. DePinho, I. Vincent, Decreased cyclin-dependent kinase 5 (CDK5) activity is accompanied by redistribution of CDK5 and cytoskeletal proteins and increased cytoskeletal protein phosphorylation in p35 null mice, J. Neurosci. 23 (2003) 10633–10644.
[33] M. Takahashi, E. Iseki, K. Kosaka, CDK5 and munc-18/p67 colocalization in early stage neurofibrillary tangles-bearing neurons in Alzheimer type dementia brains, J. Neurol. Sci. 172 (2000) 63–69.
[34] D. Alvira, I. Ferrer, J. Gutierrez-Cuesta, B. Garcia-Castro, M. Pallàs, A. Camins, Activation of the calpain/CDK5/p25 pathway in the girus cinguli in Parkinson's disease, Parkinsonism Relat. Disord. 14 (2008) 309–313.
[35] A. Camins, E. Verdaguer, J. Folch, A.M. Canudas, M. Pallàs, The role of CDK5/P25 formation/inhibition in neurodegeneration, Drug News Perspect. 19 (2006) 453–460.
[36] D.S. Smith, L.H. Tsai, CDK5 behind the wheel: a role in trafficking and transport? Trends Cell Biol. 12 (2002) 28–36.
[37] S. Bandyopadhyay, X. Huang, D.K. Lahiri, J.T. Rogers, Novel drug targets based on metallobiology of Alzheimer's disease, Expert Opin. Ther. Targets 14 (2010) 1177–1197.
[38] E.M. Blalock, K.C. Chen, A.J. Stromberg, C.M. Norris, I. Kadish, S.D. Kraner, N.M. Porter, P.W. Landfield, Harnessing the power of gene microarrays for the study of brain aging and Alzheimer's disease: statistical reliability and functional correlation, Ageing Res. Rev. 4 (2005) 481–512.
[39] A.L. Barabasi, Z.N. Oltvai, Network biology: understanding the cell's functional organization, Nat. Rev. Genet. 5 (2004) 101–113.
[40] E.M. Blalock, K.C. Chen, K. Sharrow, J.P. Herman, N.M. Porter, T.C. Foster, P.W. Landfield, Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment, J. Neurosci. 23 (2003) 3807–3819.
[41] J.T. Dou, M. Chen, F. Dufour, D.L. Alkon, W.Q. Zhao, Insulin receptor signaling in long-term memory consolidation following spatial learning, Learn. Mem. 12 (2005) 646–655.
[42] E. Aso, S. Lomoio, I. López-González, L. Joda, M. Carmona, N. Fernández-Yagüe, J. Moreno, S. Juvés, A. Pujol, R. Pamplona, M. Portero-Otin, V. Martín, M. Díaz, I. Ferrer, Amyloid generation and dysfunctional immunoproteasome activation with disease progression in animal model of familial Alzheimer's disease, Brain Pathol. 22 (2012) 636–653.
[43] E. Barroso, J. del Valle, D. Porquet, A.M. Vieira Santos, L. Salvadó, R. Rodríguez-Rodríguez, P. Gutiérrez, M. Anglada-Huguet, J. Alberch, A. Camins, X. Palomer, M. Pallàs, L. Michalik, W. Wahli, M. Vázquez-Carrera, Tau hyperphosphorylation and increased BACE1 and RAGE levels in the cortex of PPARβ/δ-null mice, Biochim. Biophys. Acta 1832 (2013) 1241–1248.
[44] E. Masliah, E. Rockenstein, I. Veinbergs, Y. Sagara, M. Mallory, M. Hashimoto, L. Mucke, Beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12245–12250.
[45] C.A. Dickey, M.N. Gordon, J.E. Mason, N.J. Wilson, D.M. Diamond, J.F. Guzowski, D. Morgan, Amyloid suppresses induction of genes critical for memory consolidation in APP + PS1 transgenic mice, J. Neurochem. 88 (2004) 434–442.
[46] S. Hauptmann, I. Scherping, S. Dröse, U. Brandt, K.L. Schulz, M. Jendrach, K. Leuner, A. Eckert, W.E. Müller, Mitochondrial dysfunction: an early event in Alzheimer pathology accumulates with age in AD transgenic mice, Neurobiol. Aging 30 (2009) 1574–1586.
[47] A. Eckert, K.L. Schulz, V. Rhein, J. Götz, Convergence of amyloid-beta and tau pathologies on mitochondria in vivo, Mol. Neurobiol. 41 (2010) 107–114.
[48] R. Minkeviciene, J. Ihalainen, T. Malm, O. Matilainen, V. Keksa-Goldsteine, G. Goldsteins, H. Iivonen, N. Leguit, J. Glennon, J. Koistinaho, P. Banerjee, H. Tanila, Age-related decrease in stimulated glutamate release and vesicular glutamate transporters in APP/PS1 transgenic and wild-type mice, J. Neurochem. 105 (2008) 584–594.
[49] M. Hiltunen, V.K. Khandelwal, N. Yaluri, T. Tiilikainen, M. Tusa, H. Koivisto, M. Krzisch, S. Vepsäläinen, P. Mäkinen, S. Kemppainen, P. Miettinen, A. Haapasalo, H. Soininen, M. Laakso, H. Tanila, Contribution of genetic and dietary insulin resistance to Alzheimer phenotype in APP/PS1 transgenic mice, J. Cell. Mol. Med. 16 (2012) 1206–1222.
[50] M. Garcia-Alloza, E.M. Robbins, S.X. Zhang-Nunes, S.M. Purcell, R.A. Betensky, S. Raju, C. Prada, S.M. Greenberg, B.J. Bacskai, M.P. Frosch, Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease, Neurobiol. Dis. 24 (2006) 516–524.
[51] M. Hokama, S. Oka, J. Leon, T. Ninomiya, H. Honda, K. Sasaki, T. Iwaki, T. Ohara, T. Sasaki, F.M. Laferla, Y. Kiyohara, Y. Nakabeppu, Altered expression of diabetes-related genes in Alzheimer's disease brains: the Hisayama study, Cereb. Cortex (2013) in press. http://dx.doi.org/10.1093/cercor/bht101 .
[52] J.J. Ramos-Rodriguez, O. Ortiz, M. Jimenez-Palomares, K.R. Kay, E. Berrocoso, M.I. Murillo-Carretero, G. Perdomo, T. Spires-Jones, I. Cozar-Castellano, A.M. Lechuga-Sancho, M. Garcia-Alloza, Differential central pathology and cognitive impairment in pre-diabetic and diabetic mice, Psychoneuroendocrinology 38 (2013) 2462–2475.
[53] L.M. Chua, M.L. Lim, P.R. Chong, Z.P. Hu, N.S. Cheung, B.S. Wong, Impaired neuronal insulin signaling precedes Aβ42 accumulation in female AβPPsw/PS1ΔE9 mice, J. Alzheimers Dis. 29 (2012) 783–791.
[54] U. Andersson, R.C. Scarpulla, Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells, Mol. Cell. Biol. 21 (2001) 3738–3749.
[55] B.N. Finck, D.P. Kelly, PGC-1 coactivators: inducible regulators of energy metabolism in health and disease, J. Clin. Invest. 116 (2006) 615–622.
[56] B. Sheng, X. Wang, B. Su, H.G. Lee, G. Casadesus, G. Perry, X. Zhu, Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease, J. Neurochem. 120 (2012) 419–429.
[57] L. Katsouri, C. Parr, N. Bogdanovic, M. Willem, M. Sastre, PARγ co-activator-1α (PGC-1α) reduces amyloid-β generation through a PPARγ-dependent mechanism, J. Alzheimers Dis. 25 (2011) 151–162.
[58] N.A. Shirwany, M.H. Zou, AMPK: a cellular metabolic and redox sensor. A minireview, Front. Biosci. (Landmark Ed.) 19 (2014) 447–474.
[59] D. Kim, M.D. Nguyen, M.M. Dobbin, A. Fischer, F. Sananbenesi, J.T. Rodgers, I. Delalle, J.A. Baur, G. Sui, S.M. Armour, P. Puigserver, D.A. Sinclair, L.H. Tsai, SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis, EMBO J. 26 (2007) 3169–3179.
[60] W. Qin, V. Haroutunian, P. Katsel, C.P. Cardozo, L. Ho, J.D. Buxbaum, G.M. Pasinetti, PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia, Arch. Neurol. 66 (2009) 352–361.
[61] L. Galluzzi, K. Blomgren, G. Kroemer, Mitochondrial membrane permeabilization in neuronal injury, Nat. Rev. Neurosci. 10 (2009) 481–494.
[62] B.R. Hoover, N.R.M. Reed, S. Jianjun, R.D. Penrod, L.A. Kotilinek, M.K. Grant, R. Pitstick, Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration, Neuron 6 (2010) 1067–1081.
[63] V. Shukla, S. Skuntz, H.C. Pant, Deregulated CDK5 activity is involved in inducing Alzheimer's disease, Arch. Med. Res. 43 (2012) 655–662.
[64] L. Zhang, D. Qingyang, W. Zhao, Nuclear respiratory factor 1 mediates the transcription initiation of insulin-degrading enzyme in a TATA box-binding protein-independent manner, PLoS One 8 (2012) e42035.
[65] C.X. Gong, K. Iqbal, Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease, Curr. Med. Chem. 15 (2008) 2321–2328.
[66] M. Jucker, L.C. Walker, Self-propagation of pathogenic protein aggregates in neurodegenerative diseases, Nature 501 (2013) 45–51.
[67] M. Sadowski, J. Pankiewicz, H. Scholtzova, Y. Ji, D. Quartermain, C.H. Jensen, K. Duff, R.A. Nixon, R.J. Gruen, T. Wisniewski, Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory impairment in APP/PS1 mice, J. Neuropathol. Exp. Neurol. 63 (2004) 418–428.
[68] N. Regev-Rudzki, O. Pines, Eclipsed distribution: a phenomenon of dual targeting of protein and its significance, Bioessays 29 (2007) 772–782.
[69] A. Vignini, A. Giulietti, L. Nanetti, F. Raffaelli, L. Giusti, L. Mazzanti, L. Provinciali, Alzheimer's disease and diabetes: new insights and unifying therapies, Curr. Diabetes Rev. 9 (2013) 218–227.
[70] Y. Yoshiyama, V.M. Lee, J.Q. Trojanowski, Therapeutic strategies for tau mediated neurodegeneration, J. Neurol. Neurosurg. Psychiatry 84 (2013) 784–795.
[71] K.J. Kopeikina, B.T. Hyman, T.L. Spires-Jones, Soluble forms of tau are toxic in Alzheimer's disease, Transl. Neurosci. 3 (2012) 223–233.
[72] J.E. Ayala, V.T. Samuel, G.J. Morton, S. Obici, C.M. Croniger, G.I. Shulman, D.H. Wasserman, O.P. McGuinness, NIH Mouse Metabolic Phenotyping Center Consortium, Dis Model Mech. 3 (2010) 525–534.
[73] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal Biochem. 162 (1987) 156–159.

4.2 Publicación II

Adipokine pathways are altered in hippocampus of an experimental mouse model of Alzheimer's disease

Ignacio Pedrós, Dmitri Petrov, Gonzalo Artiach, Sonia Abad, Carla Ramon-Duaso, Francesc Sureda, Mercé Pallàs, Carlos Beas-Zarate, Jaume Folch, Antoni Camins
(2015). *The Journal of Nutrition, Health & Aging* 19: 403–12

Resumen

En los últimos años se ha demostrado la presencia en el sistema nervioso central de un gran número de adipocinas y receptores correspondientes que regulan procesos como la inflamación, la ingesta de alimentos y la neuroprotección. Está descrito que estas citocinas están fuertemente relacionadas con las neurotrofinas bdnf, ngf y con los péptidos anorexigénicos (pomc, crh) y orexigénicas (npy).

La hipercolesterolemia también se puede considerar como un factor de riesgo en la EA. Efectivamente tratamientos con estatinas reducen los niveles de Aβ-40 y 42. (Review & Selection 2013). El exceso de colesterol en el cerebro es tóxico y la su eliminación parece estar alterada en la EA; moléculas como Cyp46a1 y Hmgcr tienen un peso relevante en el proceso catalítico a nivel central

La leptina y la prolactina son hormonas implicadas en la obesidad y en el control de la ingesta de alimentos y realizan sus funciones en el hipotálamo y en el hipocampo (Kanoski et al. 2011). Sus receptores son de tipo tirosina cinasa y desarrollan un papel importante en la neuroprotección y la memoria. El mecanismo de acción es complejo e incluye varias rutas y varios estímulos que actúan conjuntamente. Una de las vías implicadas es la vía de JAK/STAT.

El objetivo principal de este trabajo es identificar a nivel del hipocampo las posibles alteraciones que ocurren en las vías moleculares relacionadas con la ingesta de alimentos durante el proceso de amiloidogénesis en los ratones APP/PS1. Otros objetivos consisten en analizar la vía neuroprotectora de JAK/STAT y evaluar la presencia de alteraciones en marcadores implicados en el transporte y la eliminación del colesterol.

Los resultados obtenidos muestran ciertas alteraciones en moléculas relacionadas con la ingesta de alimentos y la obesidad. En concreto los receptores de leptina a 6 meses y prolactina a 3 meses muestran una reducción de expresión génica y proteica. No se observaron cambios en las neurotrofinas *bdnf* y *ngf*, pero sí en los péptidos que regulan el apetito (*npy* y *crh)*, tanto a 3 y 6 meses. Los niveles de pomc resultaron invariados

Los receptores de prolactina y el de insulina actúan activando *stat5* y *stat3*. Está ampliamente descrito que ratones con desregulaciones en *stat3* y *stat5* sufren obesidad y diabetes.(Gao et al. 2004; J. W. Lee et al. 2008; Nicolas et al. 2013). En nuestro modelo encontramos que las subunidades *stat5b*, *socs1*, *socs2* y *socs3* están reducidas a los 3 meses en el ratón APP/PS1. Los genes SOCS pertenecen a una familia de genes supresores de citoquinas, por lo tanto regulan negativamente esta vía de señalización.

A nivel periférico no observamos cambios significativos en los niveles plasmáticos de colesterol. Al contrario los niveles de triglicéridos resultaron aumentados. Se conoce que los polimorfismos del gen *apoE* tienen un papel clave en el desarrollo de la enfermedad de Alzheimer. Aun así, nuestros resultados no mostraron cambios en la expresión de *apoE* ni en la vía *lrp1*. En contra sí observamos un incremento en el gen del receptor de ldl y de Hmgcr, dos genes implicados en la síntesis de colesterol.

J Nutr Health Aging
Volume 19, Number 4, 2015

ADIPOKINE PATHWAYS ARE ALTERED IN HIPPOCAMPUS OF AN EXPERIMENTAL MOUSE MODEL OF ALZHEIMER'S DISEASE

I. PEDRÓS[2], D. PETROV[1], G. ARTIACH[1], S. ABAD[1], C. RAMON-DUASO[1], F. SUREDA[2], M. PALLÀS[1], C. BEAS-ZARATE[3,4], J. FOLCH[2], A. CAMINS[1]

1. Unitat de Farmacologia i Farmacognòsia Facultat de Farmàcia, Institut de Biomedicina (IBUB), Centros de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat de Barcelona, Barcelona, Spain; 2. Unitats de Bioquímica i Farmacologia, Facultat de Medicina i Ciències de la Salut, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat Rovira i Virgili, C./ St. Llorenç 21 43201 Reus (Tarragona), Spain; 3. Laboratorio de Neurobiología Celular y Molecular, División de Neurociencias, CIBO, IMSS, México; 4. Laboratorio de Regeneración y Desarrollo Neural, Instituto de Neurobiología, Departamento de Biología Celular y Molecular, CUCBA, México. Corresponding author: Antoni Camins PhD, Unitat de Farmacologia i Farmacognosia, Facultat de Farmàcia, Universitat de Barcelona, Spain. Avda/ Diagonal 643, E-08028 Barcelona, Spain. Tel: +34 93 4024531, Fax: +34 934035982, Email: camins@ub.edu

Abstract: A growing body of evidence suggests that β-amyloid peptides (Aβ) are unlikely to be the only factor involved in Alzheimer's disease (AD) aetiology. In fact, a strong correlation has been established between AD patients and patients with type 2 diabetes and/or cholesterol metabolism alterations. In addition, a link between adipose tissue metabolism, leptin signalling in particular, and AD has also been demonstrated. In the present study we analyzed the expression of molecules related to metabolism, with the main focus on leptin and prolactin signalling pathways in an APPswe/PS1dE9 (APP/PS1) transgenic mice model, at 3 and 6 months of age, compared to wild-type controls. We have chosen to study 3 months-old APP/PS1 animals at an age when neither the cognitive deficits nor significant Aβ plaques in the brain are present, and to compare them to the 6 months-old mice, which exhibit elevated levels of Aβ in the hippocampus and memory loss. A significant reduction in both mRNA and protein levels of the prolactin receptor (PRL-R) was detected in the hippocampi of 3 months old APP/PS1 mice, with a decrease in the levels of the leptin receptor (OB-R) first becoming evident at 6 months of age. We proceeded to study the expression of the intracellular signalling molecules downstream of these receptors, including stat (1-5), sos1, kras and socs (1-3). Our data suggest a downregulation in some of these molecules such as stat-5b and socs (1-3), in 3 months-old APP/PS1 brains. Likewise, at the same age, we detected a significant reduction in mRNA levels of lrp1 and cyp46a1, both of which are involved in cholesterol homeostasis. Taken together, these results demonstrate a significative impairment in adipokine receptors signalling and cholesterol regulation pathways in the hippocampus of APP/PS1 mice at an early age, prior to the Aβ plaque formation.

Key words: APP/PS1, leptin, hippocampus, prolactin, Alzheimer.

Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia in the world, followed by Parkinson's disease (1). AD progression is associated with the formation of senile β-amyloid (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau (1, 2). Currently it is widely accepted that Aβ is generated by a specific proteolytic clavage of the amyloid precursor protein (APP). In this amyloidogenic pathway, the β- and γ-Secretases cleave APP at the N- and C-termini of the Aβ peptide, respectively. The relationship between APP and Aβ caused the formulation of the amyloid cascade hypothesis that states that mutations in APP (or other genes) lead to an increase in Aβ, and that this in turn leads to disease progression (3, 4).

A number of animal models attempting to mimic the progression of the AD have been extensively investigated. APP/PS1 mice, which possess 2 of the more frequent mutations leading to familial AD (FAD) in humans, are commonly used in experimental animal studies of AD. One of the principal features of these mice is the development of memory loss and a significant Aβ plaque deposition in the hippocampus, clearly evident by 6 months of age (5-8). We have chosen to study 3 months-old animals which do not present brain Aβ deposits nor cognitive loss and we have compared them to the 6 months-old mice. The rationale behind this approach is to identify molecular events involved in the early pre-plaque stages of the AD-like pathology in this mouse model. In our opinion, this is especially relevant because despite the genetic and cell biological evidence that supports the amyloid cascade hypothesis, it is becoming increasingly clear that AD aetiology is more complex and that Aβ alone is unable to account for all the aspects of AD (9). Hundreds of genes have been identified as being involved in this neurodegenerative disease (10, 11). Recent studies suggest that metabolic alterations such as diabetes mellitus, cholesterol metabolism dysregulations and metabolic syndrome in general are strongly correlated with AD (11-17). Thus, a continuous effort should be made to identify components of the network involved in the progression of diseases like AD in order to develop more efficient and specific treatments (18-20).

Since the sporadic form of AD is a multifactorial disease influenced by several risk factors such as hypertension, diabetes, hypercholesterolemia, age, neuroinflammation, hypoxia and others, it is difficult to point out a single pathogenetic mechanism leading to the onset and progression

Received March 28, 2014
Accepted for publication June 5, 2014

J Nutr Health Aging
Volume 19, Number 4, 2015

of this devastating disorder (6-8, 14-20). For example, obesity significantly increases cognitive decline and AD risk, supporting the notion that molecular mechanisms of cellular energy homeostasis are linked to AD pathogenesis (15-20). Additionally, there is evidence of a relationship between adipokines and AD (21-24). The adipokines or adipocytokines are cytokines secreted by adipose tissue (21). These include leptin, adiponectin, tumor necrosis factor (TNF)-alpha, interleukins, including IL-6, and also molecules like prolactin (Prl), a well-known regulator of the lactating mammary gland, recently shown to be produced by human adipose tissue (21-27). Adipokines have come to be recognized for their contribution to the mechanisms by which obesity and related metabolic disorders influence diseases like cancer or AD (24-28). It has been observed that AD patients display increased circulating levels of anorexigenic adipokines that may contribute to the metabolic changes observed in AD patients (21).

Among the adipokine genes associated to AD, an adipostatic hormone leptin, coded by the ob or lep gene, stands out. Leptin is a hormone secreted by adipose tissue that acts to suppress appetite and regulates energy expenditure. In humans, a correlation between elevated leptin levels and reduced incidence of dementia and AD had been reported (28). In rodents, leptin modulates production and clearance of Aβ (29-31). Mice with leptin receptor disruption show impairments in long-term potentiation, synaptic plasticity and spatial learning, whereas treatment with leptin increases Aβ- and tau- clearance as well as ameliorates AD-like pathology (21, 25-31). Thus, in the context of the amyloid cascade hypothesis, leptin may interfere with the pathogenesis of AD in multiple ways: (a) by inhibiting the amyloidogenic process; (b) by decreasing the activity of glycogen synthase kinase-3β (GSK3β), causing a reduction in Tau protein phosphorylation; and (c) by improving cognitive function (25-27).

Beside the roles of adipokines per se, it has been shown that alterations in lipid metabolism can also promote the development of AD. The brain is rich in cholesterol and substantial evidence from in vitro and in vivo studies, as well as from human trials, indicates that cholesterol levels affect the synthesis, clearance, and the toxicity of Aβ (13, 14, 32). For example, elevated cerebral Aβ levels in living humans were found to be correlated with serum cholesterol fractions in a pattern analogous to that found in coronary artery disease (11).

In the current study we have focused on molecular mechanisms related to adipokine signalling, AD progression and memory loss in the hippocampus of an APP/PS1 mouse model of FAD at two time points: 1) at 3 months of age, prior to the plaque formation and memory loss, and 2) at 6 months of age, by which both cognitive decline and hippocampal Aβ deposits are clearly evident.

Materials and methods

Animals

Male APPswe/PS1dE9 and C57BL/6 mice were used in this study. APP/PS1 animals co-express a Swedish (K595M/N596L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9), allowing these mice to secrete elevated amounts of human Aβ peptide. Both mutations are associated with familial AD, are under control of the mouse prion protein promoter, directing both mutated proteins mainly to the CNS neurons, and result in age-dependent amyloid plaque depositions in mouse brain. The APPswe-mutated APP is a favourable substrate for β-secretase, whereas the PS1dE9 mutation alters γ-secretase cleavage, thereby promoting overproduction of Aβ42. Animals were kept under controlled temperature, humidity and light conditions with food and water provided ad libitum. Mice were treated in accordance with the European Community Council Directive 86/609/EEC and the procedures established by the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Every effort was made to minimize animal suffering and to reduce the number of animals used. Forty animals, divided into four groups, were used for the present study, with at least 8 wild-type and 8 APP/PS1 transgenic mice of 3 and 6 months of age, per group. Following in vivo testing, the animals were sacrificed and at least 6 mice in each group were used for RNA and protein extracts isolation.

Blood Cholesterol and Triglyceride measurements

Cholesterol and trygliceride levels were measured in the blood, collected from heart puncture, following a 5-hour morning fast, at the point of sacrifice with the Accutrend Plus meter (Roche; Mannheim, Germany).

Immunohistochemistry

For detection of Aβ deposits, free-floating coronal sections, 20 µm thick, were rinsed with 0.1 mol/L PB, pH 7.2, and pre-incubated in 88% formic acid. Then, sections were treated with 5 ml/L H2O2 and 100 ml/L methanol in PBS and pre-incubated in a blocking solution (100 ml/L of FBS, 2.5 g/L of BSA and 0.2 mol/L of glycine in PBS with 5 ml/L of Triton X-100). After that, sections were incubated for 10 minutes with Thioflavin S (Sigma T1892). Sections were mounted on gelatinized slides. Images were taken with a fluorescence laser and optic microscope (BX41, Olympus, Germany) and stored in tiff format. All images were acquired using the same microscope, laser and software settings.

Western blot analysis

Aliquots of hippocampus homogenate containing 15 mg of protein per sample were analyzed using the Western blot method. In brief, samples were placed in sample buffer (0.5 m Tris–HCl, pH 6.8, 10% glycerol, 2% (w/v) SDS, 5% (v/v) 2--mercaptoethanol, 0.05% bromophenol blue) and denatured

J Nutr Health Aging
Volume 19, Number 4, 2015

by boiling at 95–100ºC for 5 min. Samples were separated by electrophoresis on 10% acrylamide gels. Following this, the proteins were transferred to PVDF sheets using transblot apparatus. Membranes were blocked overnight with 5% non-fat milk dissolved in TBS-T buffer (50 mM Tris; 1.5% NaCl, 0.05% Tween 20, pH 7.5). They were then incubated with primary antibodies directed against the GAPDH (Mab374, Millipore), leptin (PA1-28843, Thermo Scientific) and prolactin (ab98015, Abcam) receptors. After O/N incubation, blots were washed thoroughly in TBS-T buffer and incubated for 1 h with a peroxidase-conjugated IgG secondary antibody (1:2000). Immunoreactive protein was detected using a chemiluminescence-based detection kit. Target protein levels were determined by densitometry, using Chemi doc XRS+ Molecular Imager detection system (Bio-Rad), with ImageLab image analysis software. Measurements are expressed as arbitrary units. All results are normalized to GAPDH, unless stated otherwise.

RNA extraction and quantification

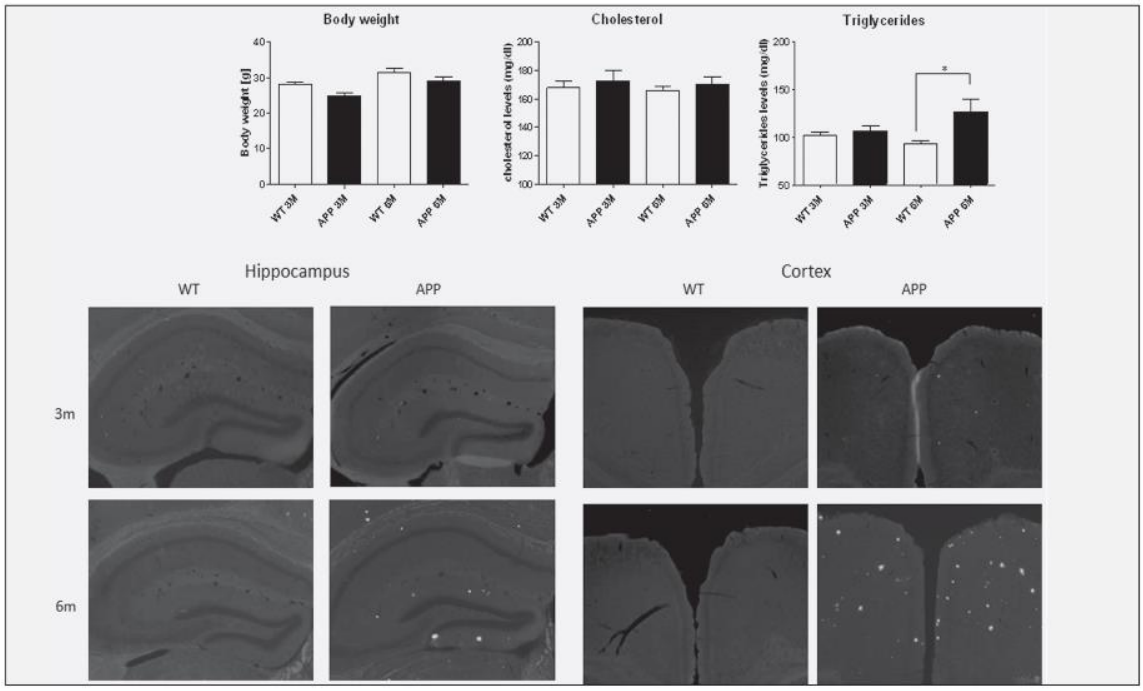
Total RNA was isolated from the hippocampi of wild-type and APP/PS1 transgenic mice, as described previously (Chomczynski and Sacchi, 1987). Briefly, the tissue was homogenized in the presence of Trizol reagent (Life Technologies Corporation). Chloroform was added and the RNA was precipitated from the aqueous phase with isopropanol at 4ºC. RNA pellet was reconstituted in RNase-free water, with the RNA integrity determined by Agilent 2100 Bioanalyzer.

Quantitative RT-PCR

First-strand cDNA was reverse transcribed from 2 μg of total RNA from hippocampi of 3 and 6 months-old mice, using the High Capacity cDNA Reverse Transcription kit, according to manufacturer's protocol (Applied Biosystems). Equal amounts of cDNA were subsequently used for qRT-PCR, and each sample was analysed in triplicate for each gene. TaqMan gene expression assays (Applied Biosystems) as detailed in Supplementary Table 1, were used to determine transcription levels of individual genes. qRT-PCR was performed on the StepOnePlus Real Time PCR system (Applied Biosystems) and normalized to the transcription levels of gapdh, actin and/or tbp, using the delta-delta Ct method.

Figure 1

Mean body weight (n=20), whole blood cholesterol and triglycerides levels in 3 and 6 months-old wild-type and APP/PS1 mice (n=7-12). (Statistical analysis was performed with one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$). (A). Representative immunofluorescent staining with the Thioflavin S, in 3 and 6 months-old mice, demonstrating Aβ plaque deposits in the hippocampus and cortex of 6 months-old APP/PS1 animals (B)



J Nutr Health Aging
Volume 19, Number 4, 2015

Statistical analysis

All data are presented as means ± SEM, and differences are considered significant at $p < 0.05$, $p < 0.01$. Differences between samples/animals were evaluated using either one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$, and with the student's t-test, where $ denotes $p < 0.05$.

Results

Physiological and metabolic parameters of APP/PS1 mice

No changes in either the body weight or blood cholesterol levels were detected between the 3 and 6 months old wild-type and APP/PS1 animals. A significant increase in blood triglycerides levels ($p < 0.05$) was observed in 6 months-old APP/PS1 mice, compared to respective controls (Fig.1A). As expected, Aβ plaque deposits were present in the hippocampal and cortical regions of 6 months-old APP/PS1 mice (Fig.1B).

Identification of differentially expressed genes related to food intake and obesity in the hippocampus

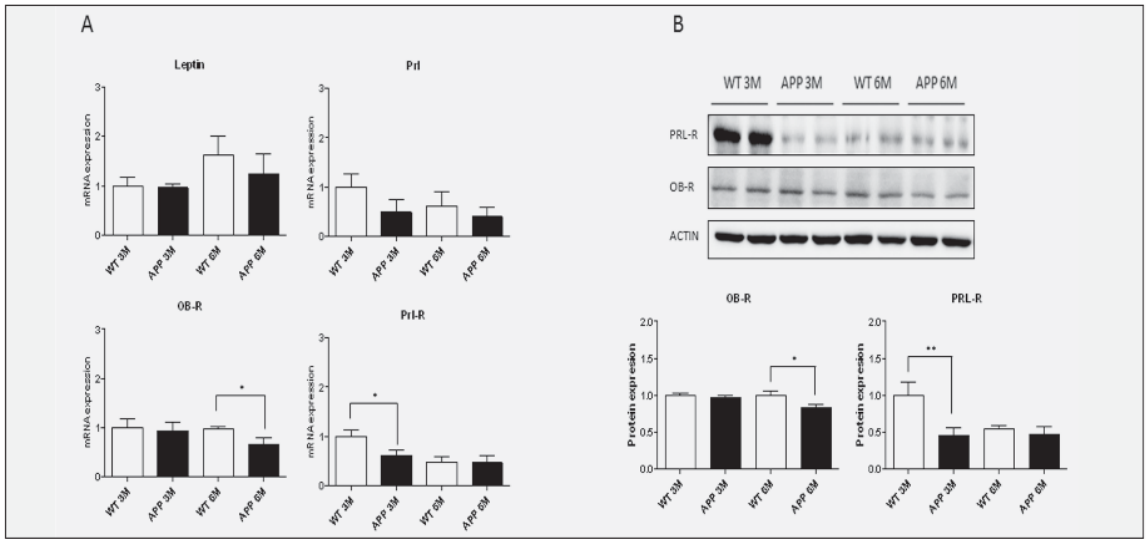
We did not detect significant differences in the mRNA expression of leptin, however a significant down regulation of the leptin receptor (OB-R), both at the mRNA (Fig. 2a) and protein (Fig. 2b) levels, was observed in the hippocampi of 6 months-old APP/PS1 mice compared to wild-type littermates. In contrast, we detected a significant reduction in the levels of the prolactin receptor (PRL-R), which is involved in the regulation of energy metabolism, already at 3 months of age in APP/PS1 brains (Fig. 2). The changes observed in the above mentioned molecules were related to the APP/PS1 phenotype (rather than age), as we did not detect statistically significant alterations when comparing 3- and 6- months-old APP/PS1 animals.

We determined mRNA expression profiles of neurotrophic factors and related receptors which play a role in food-intake regulation, including brain derived neurotrophic factor (bdnf), glucagon-like peptide 1 receptor (glp1r), insulin-like growth factor 1 (igf1), nerve groqth factor (ngf), and Neuropeptide Y (npy) (33-37) (Fig. 3). Of these, only npy was significantly downregulated in the hippocampi of the APP/PS1 mice, both at 3 and 6 months of age, when compared to wild-type littermates. Interestingly, there was a tendency towards the downregulation of the glp1r in 3 months-old animals, although this reduction did not reach statistical significance. Apart from the regulation of the food intake, GLP1 signalling has been implicated in the regulation of glucose metabolism, memory formation and may have neuroprotective effects against excitotoxic insults (33, 34).

Corticotropin-releasing hormone (CRH) is highly expressed in paraventricular nucleus neurons and is involved in the regulation of food-intake and body weight in rats (38, 39). It may also play a role in cognition and has been linked to neuroprotection in response to stress in the hippocampus (40). Semi-quantitative RT-PCR analysis showed a significant decrease in crh in hippocampi of 6 months-old APP/PS1 mice, compared to controls. We did not find significant changes in

Figure 2

mRNA expression profile (n=5-8) (A) and representative immunoblot images and quantification (n=5-8) (B) of the leptin and prolactin hormones and their respective receptors in hippocampal extracts of 3 and 6 months-old wild-type and APP/PS1 mice. Immunoblot images are normalized to beta actin. (Statistical analysis was performed with one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$, and with the student's t-test, where $ denotes $p < 0.05$)



J Nutr Health Aging
Volume 19, Number 4, 2015

Table 1
Probes and primers used for semi-quantitative RT-PCR. Gene ID# corresponds to the official classification of the Entrez Gene database (NCBI)

Name	Gene	ID	TaqMan Probe	Primers pairs (5'→3')
Actb	actin, beta	11461	Mm00607939_s1	
Apoc1	apolipoprotein C-I	11812	Mm00431816_m1	
Apoe	Apolipoprotein E	11816	Mm01307193_g1	
Bdnf	brain derived neurotrophic factor	12064	Mm04230607_s1	
Crh	corticotropin releasing hormone	12918	Mm01293920_s1	
Cyp46a1	cytochrome P450, family 46, subfamily a, polypeptide 1	13116	Mm00487306_m1	
Gapdh	glyceraldehyde-3-phosphate dehydrogenase		14433	Mm99999915_g1
Glp1r	glucagon-like peptide 1 receptor	14652	Mm00445292_m1	
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	15357	Mm01282499_m1	
Igf1	insulin-like growth factor 1	16000	Mm01228180_m1	
K-Ras	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	16653		F:AGACACGAAACAGGCTCAGGAGT, R:AGGCATCGTCAACAC CCTGTCTT
Ldl-R	low density lipoprotein receptor	16835	Mm00440169_m	
Lep	leptin	16846		F:CTCCAAGGTTGTCCAGGGTT, R:AAAACTCCCCACAGA ATGGG
Lrp1	low density lipoprotein receptor-related protein 1	16971	Mm00464608_m1	
Ngf	nerve growth factor	18049	Mm00443039_m	
Npy	neuropeptide Y	109648		F:CTCCGCTCTGCGACACTACA, R:AATCAGTGTCTCAGGG CTGGA
OB-R	leptin receptor	16847		F:CTGCACTTAACCTGGCATATCCA, R:GGCTCCAGCAGGTGA GAGAA
Pomc	pro-opiomelanocortin-alpha	18976	Mm00435874_m1	
Prl	prolactin	19109		F:GTATGTGCAAGACCGTGAGT, R:AGGGACTTTCAGGGC TTGTT
Prl-R	prolactin receptor	19116		F:ATCTGTGGGTAAAATGGTTGCC, R:GTTTGATGACCTGTGAA GTGGA
Socs1	suppressor of cytokine signaling 1	12703	Mm00782550_s	
Socs2	suppressor of cytokine signaling 2	216233	Mm00850544_g1	
Socs3	suppressor of cytokine signaling 3	12702	Mm00545913_s1	
Sos1	son of sevenless homolog 1	20662		F:TCCCCTAAAATCTCCTGGTGTTCGT, R:AGATGCTGTGCTTTC CGTCTCACT
Srebf1	sterol regulatory element binding transcription factor 1	20787	Mm00550338_m1	
Stat1	signal transducer and activator of transcription 1	20846	Mm00439531_m1	
Stat3	signal transducer and activator of transcription 3	20848	Mm01219775_m1	
Stat5a	signal transducer and activator of transcription 5A	20850	Mm03053818_s1	
Stat5b	signal transducer and activator of transcription 5B	20851	Mm00839889_m1	
TBP	TATA box binding protein	21374		F: ACCCTTCACCAATGACTCCTATG, R: TGACTGCAGCAAATCG CTTGG

the mRNA expression of a polypeptide hormone precursor pro-opiomelanocortin (pomc), mutations of which had been previously linked to the development of childhood obesity (Fig. 3) (41).

Leptin and prolactin signalling pathways

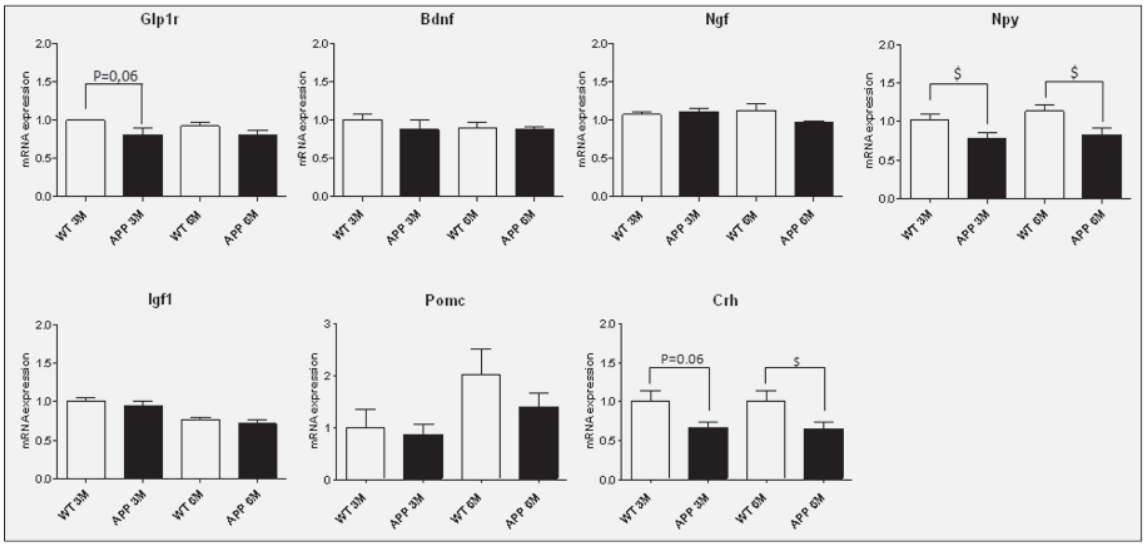
Because we detected significant alterations in the hippocampal expression of Prl-R at 3 months of age and of OB-R at 6 months of age in APP/PS1 animals, compared to controls, we proceeded to study the expression profiles of the downstream signalling molecules related to the physiological functions of both receptors.

It has been shown that the prolactin receptor activates Signal Transducer and Activator of Transcription (STAT-1), (STAT-3) and (STAT-5) (20). We did not detect any changes in the expression of stat-1 and stat-3, stat5a and k-ras, however mRNA levels of the 2b isoform of STAT-5 (stat5b) were significantly downregulated in the hippocampus of 3 months-

J Nutr Health Aging
Volume 19, Number 4, 2015

Figure 3

mRNA expression profile (n=5-8) of glp1r, bdnf, ngf, npy, igf1, pomc and crh in hippocampal extracts of 3 and 6 months-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$)



old APP/PS1 animals, versus wild-type animals (Fig. 4).

In addition, we identified a significant downregulation of the Son of Sevenless homologue 1 (SOS1) in 6 months-old APP/PS1 mice, compared to wild-type, which is another molecule downstream of the prolactin receptor (23, 24, 42). Moreover, suppressors of cytokine signaling (socs1), (socs2) and (socs3) were also downregulated at 3 months of age in APP/PS1 animals, with the mRNA expression levels of socs2 being just short of reaching statistical significance ($p = 0.06$) (Fig. 4). Taken together, our results clearly show a significative impairment in the adipokine receptors-related signalling pathways.

Changes in the transcripts involved in disorders of lipid metabolism in the early stages of amyloidogenesis

Impairment in cholesterol metabolism and biosynthesis, which may lead to neuronal damage, is thought to be a contributing factor to AD progression (43). We have detected a significant increase in the mRNA levels of the Low Density Lipoprotein receptor (Ldl-r) and 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase (hmgcr) transcripts in the hippocampi of the APP/PS1 mice at 6 months of age, compared to the control group (Fig. 5). In addition, in our study a decrease in lrp1 mRNA levels in 3 months-old APP/PS1 brains was found, which did not correlate to apoe and apoc1 mRNA levels, where no changes were detected (Fig. 5). Both apoE and Apolipoprotein C1 (apoc1) genes have been implicated in the development of sporadic AD (42, 43). Our data also indicate a significant downregulation in the mRNA of the cholesterol 24-hydroxylase enzyme (cyp46a1) that converts cholesterol to 24S-hydroxycholesterol, in the 3 months-old APP/PS1 mice.

Discussion

Recent evidence indicates that metabolic deficiencies may contribute to AD development and progression (43-50). Pathological changes in glucose and cholesterol metabolism, adipose tissue signalling, as well as in food-intake controlling neuropeptides have all been implicated (45, 46). At a peripheral level, we observed a significant increase in trygliceride levels in blood of 6 months-old APP/PS1 mice, when compared to wild-type littermates. Neither the peripheral cholesterol levels, nor the body weight were affected. At the CNS level, the role of feeding-regulatory peptides in the hypothalamus is well known, but their function in the hippocampus is less clear (31-33). As the hippocampus is involved in the processes of learning and memory formation, it has been proposed that these neuropeptides may take part in memory-related processes, and may also participate in neuroprotection (32).

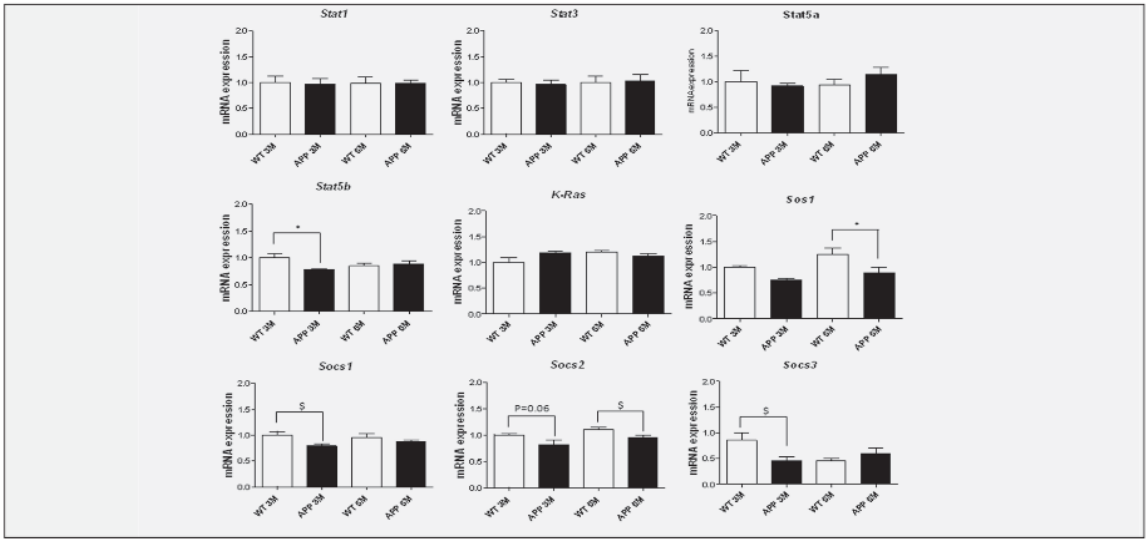
As one of the main purposes of our study was to examine potential changes at the central nervous system (CNS) level, we investigated gene expression profile of molecules related to adipokine, neuropeptide and cholesterol signalling in 3-6 months-old APP/PS1 transgenic animals.

It is becoming apparent that neuroendocrine hormones including oxytocin, progesterone and prolactin, apart from their roles in lactation, may also have neuroprotective effects on hippocampal neurons (45). Neuroprotective properties of PRL

J Nutr Health Aging
Volume 19, Number 4, 2015

Figure 4

mRNA expression profile (n=5-8) of stat1, stat3, stat5a, stat5b, K-ras, sos1, socs1, socs2 and socs3 in hippocampal extracts of 3 and 6 months-old wild-type and APP/PS1 mice. (Statistical analysis was performed with one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$, and with the student's t-test, where $ denotes $p < 0.05$)



are demonstrated in a Kainic acid (KA)-induced rat model of epilepsy, where the administration of PRL to ovariectomised rats significantly reduces seizures and KA-neurotoxicity in the hippocampus (51). Prolactin is also involved in immune regulation (52). In the brain Prl-Rs, which belong to the class I cytokine receptor superfamily, were detected in cortex, hypothalamus and hippocampus, and in astrocytes and glial cells (53, 54). This is noteworthy, as recent data suggest that prolactin may be a possible marker for obesity in humans (54) In the current study we observed a significant downregulation of the PRL-R mRNA and protein in the hippocampi of the 3 months-old APP/PS1 mice, when compared to a wild-type control group, indicating early perturbations in this particular biological route, at an age when both cognitive impairments and Aβ deposits have yet to develop.

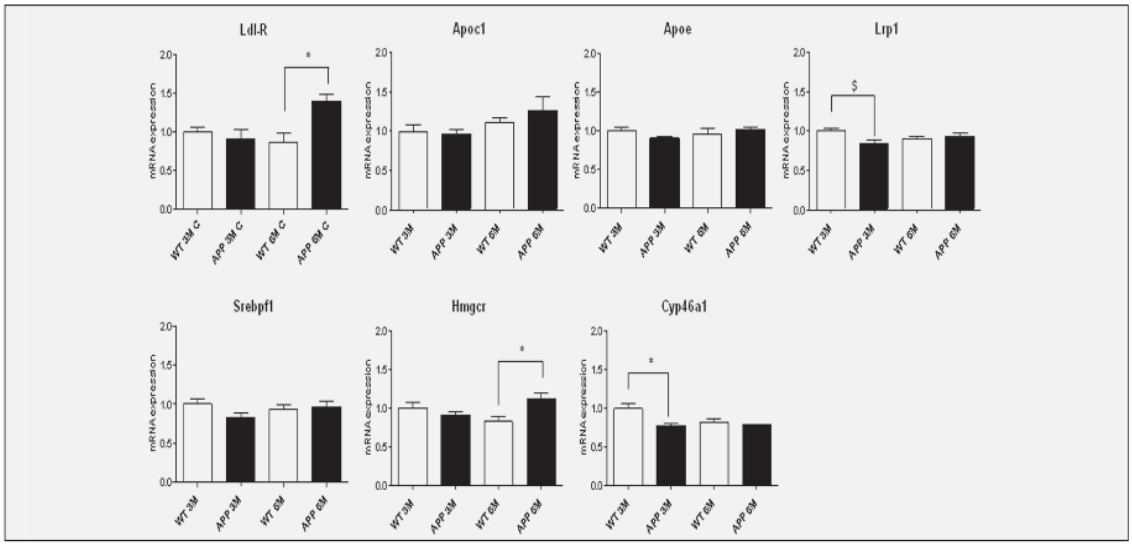
Leptin is an adipostatic hormone with a range of effects at the CNS level, and the distribution of the OB-R in the human brain is wide. In the hypothalamus, leptin signalling plays a prominent role in food-intake regulation (22-24). In the hippocampus, leptin has been implicated in the processes of learning and memory, neuroprotection, as well as synaptic plasticity (54). Leptin signalling alterations have recently been described in human patients with AD (55). In that study, authors detected a significant increase in leptin levels both in the cerebro-spinal fluid (CSF) and in the hippocampus of AD patients, compared to age-matched controls. Interestingly, an increase in circulating hormone levels was accompanied by a reduction in OB-R mRNA levels and the localization of the receptors to neurofibrillary tangles, hinting at the possibility of leptin resistance (55). In our study, we did not detect significant changes in the mRNA levels of leptin itself, however we observed a significant reduction in OB-R mRNA and protein in the hippocampus of 6 months-old APP/PS1 mice, compared to wild-type controls. On the other hand, targeted leptin delivery may in fact lead to a reduction in Aβ-load and to an improvement in the symptoms of AD-like pathologies, as described by Pérez-González et al., (2014). Authors demonstrated that the 3 months-long intra-cerebroventricular administration of a lentiviral vector expressing leptin protein resulted in the improvement of memory functions and a reduction in Aβ levels in APP/PS1 mice, compared to untreated controls (29).

Janus kinase (JAK)/STAT signalling pathways are among the principal transcriptional regulators in most cell types (56). Within the CNS, JAK/STAT signalling is activated in response to cytokines, growth factors and hormones, including leptin and prolactin (57-59). Perturbations in JAK/STAT signalling have been implicated in the processes of synaptic plasticity, neuroinflammation and the survival of both glia and neurons (59). Among the eight members of the STAT family of transcription factors, four have been identified as PRL-R transducer proteins: STAT1, STAT3, STAT5a and STAT5b. Upon activation of the PRL-R by JAK (which promotes dimerization, phosphorylation and the activation of the prolactin receptor), STATs are recruited, dimerized and phosphorylated, which results in nuclear translocation

J Nutr Health Aging
Volume 19, Number 4, 2015

Figure 5

mRNA expression profile (n=5-8) of ldlr, apoc1, apoe, lrp1, srebp1c, hmgcr and cyp46a1 in hippocampal extracts of 3 and 6 months-old wild-type and APP/PS1 mice. (Statistical analysis was performed with one-way ANOVA, with Tukey's post-hoc test, where * denotes $p < 0.05$, and with the student's t-test, where $ denotes $p < 0.05$)



of activated STATs, where they stimulate the transcription of the respective target genes (57). Moreover, three different classes of negative regulators of PRL-R have been identified, including protein tyrosine phosphatases (PTP), such as SH2 domain containing phosphatase-1 (SHP-1), protein inhibitors of activated STATs (PIAS) and suppressors of cytokine signalling (SOCS) proteins (59). Their expression is induced by PRL itself, constituting an auto-negative feedback loop. SOCSs (1 and 3 in particular) bind to the PRL-R or to JAKs and prevent the recruitment of JAKs, and thus the phosphorylation of STATs (57). In our model, we had detected a significant reduction in stat5b, socs1, socs2 (this being just short of reaching statistical significance with a p=0.06) and socs3 mRNA in the hippocampus of 3 months-old APP/PS1 animals, when compared to age-matched wild-type littermates. Interestingly, this reduction occurred at an early age, prior to the Aβ plaque formation. Thus, we demonstrate for the first time to our knowledge, a dysregulation of prolactin signalling, together with the alterations in the JAK/STAT pathways in the hippocampi of 3 months-old APP/PS1 animals.

The processes of learning and memory in the hippocampus are also regulated by NPY signalling (35-37). Several studies have reported reduced levels of NPY and its receptor densities in the brains of AD patients, as well as in rodent models of the disease (37). Moreover, a reduction in NPY levels had been detected in the plasma and the CSF of AD patients, compared to healthy controls (60). In a transgenic mouse model overexpessing APP, a decrease in NPY levels was observed in the hippocampus, while exogenous administration of NPY produced a neuroprotective effect (61, 62). Our data is in agreement with the above mentioned research, as we had detected a significant downregulation of NPY mRNA in the hippocampi of APP/PS1 animals, both at 3 and 6 months of age, when compared to the respective controls.

Moreover, we found a decrease in the mRNA transcripts of the crh in the hippocampus of 6 months-old APP/PS1 mice, compared to the control group. Interestingly, CRH protein has neuroprotective properties and also regulates APP processing, both via the CRH1 receptor activation (39). Previous studies suggest that the reduction of CRH signalling in the brains of AD patients and a decrease in CRH-levels in CSF could be a possible marker of the disease (39-40).

A number of studies have indicated that neurotrophins NGF and BDNF may promote survival and differentiation of neuronal cells in the CNS (62, 63). In the present study we did not find significant differences in either of the transcripts.

Since it is hypothesised that deregulation of cholesterol homeostasis could contribute to neurodegenerative diseases progression by provoking neuronal loss, as well as the formation of neuritic plaques and neurofibrillary tangles, we decided to focus on hippocampal expression of genes related to cholesterol metabolism (60-63. An increase in brain cholesterol levels augments Aβ deposition, which can regulate cholesterol homeostasis by itself, as demonstrated by Barbero-Camps et al., (2013). In a triple transgenic APP/PS1/SREBP-2 mice, which overexpress SREBP-2 in combination with APP/PS1 mutations,

J Nutr Health Aging
Volume 19, Number 4, 2015

authors detected an increase in mitochondrial cholesterol levels as well as an accelerated β-secretase activation and Aβ accumulation, when compared to age-matched APP/PS1 double transgenic controls (16). In our study, although we did not observe changes in blood cholesterol levels, we did detect a significant increase in LDLr mRNA in 6 months-old APP/PS1 animals, compared to wild-type littermates. The synthesis of LDLr in the cell is regulated by the levels of free intracellular cholesterol (65). These data is probably correlated with an increase in the levels of HMG-CoA reductase (hmgcr) in the hippocampus of APP/PS1 animals. HMG-CoA reductase is a rate-limiting enzyme involved in the cholesterol biosynthesis and it is affected by statins and cholesterol synthesis-inhibiting drugs, treatment with which correlates negatively with the incidence of AD (46-49). Besides, cholesterol is imported into the neurons by apoE, via LRP1 receptors on the cell surface, mRNA levels of which were significantly reduced in our model already at 3 months of age, compared to wild-type controls (67-69).

Significantly, cholesterol does not readily pass the blood-brain barrier (BBB), necessitating prior metabolism of the cholesterol molecule for the successful elimination of excess brain cholesterol (65). Cholesterol 24S-hydroxylase (CYP46A1) is an enzyme that converts cholesterol into the 24S-hydroxycholesterol (24OHC), one of the principal cholesterol metabolites in the brain, which easily crosses the BBB (65-69). A decrease in the brain 24OHC levels may thus be an indirect marker of elevated brain cholesterol levels. In our study, we have detected a significant reduction in the mRNA of cyp46a1 in the hippocampus of 3 months-old APP/PS1 mice, compared to controls. This is in accordance with previous reports indicating decreased hippocampal and plasma 24OHC levels in AD patients (67-69). Taken together, our results suggest that alterations in brain cholesterol metabolism pathways may be associated with the early signs of AD-like symptoms in an APP/PS1 mouse model.

In summary, we have detected disturbances in intracellular signalling pathways downstream of prolactin and leptin receptors, JAK/STAT signalling, as well as abnormalities in cholesterol metabolism in the hippocampi of APP/PS1 double transgenic mice between the 3 and 6 months of age. Significantly, a number of phenotypic alterations were observed in 3 months-old transgenic mice, at an age prior to the appearance of AD-like symptoms. The data presented here reinforces the hypothesis that AD may indeed be considered as a brain-type metabolic disorder.

Conflicts of interest: Authors declare no conflicts of interest

Acknowledgments: This study was funded by grant 2009/SGR00853 from the Generalitat de Catalunya (autonomous government of Catalonia), by grants SAF2011-23631, SAF2012-39852-C02-01 from the Spanish Ministerio de Ciencia e Innovación. Grant 0177594 from the CONACYT (Mexico).

References

1. Erol A. An integrated and unifying hypothesis for the metabolic basis of sporadic Alzheimer's disease. J Alzheimers Dis. 2008 ;13:241-53.
2. Pimplikar SW. Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 2009;41:1261–1268.
3. Pimplikar SW, Nixon RA, Robakis NK, Shen J, Tsai LH. Amyloid-Independent Mechanisms in Alzheimer's Pathogenesis. J. Neurosci. 2010;30:14946 -14954.
4. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. Science. 1992;256:184–185.
5. Zhang W, Bai M, Xi Y, Hao J, Liu L, et al. Early memory deficits precede plaque deposition in APPswe/PS1dE9 mice: involvement of oxidative stress and cholinergic dysfunction. Free Radic. Biol. Med. 2012;52: 1443-1452.
6. Sato N, Morishita R. Plasma β-amyloid: a possible missing link between Alzheimer disease and diabetes. Diabetes. 2013;62:1005-1006.
7. Zhang W, Bai M., Xi Y, Hao J, et al. Multiple inflammatory pathways are involved in the development and progression of cognitive deficits in APPswe/PS1dE9 mice. Neurobiol. Aging. 2012;33: 2661-2677.
8. Garcia-Alloza M, Robbins EM, Zhang-Nunes SX, Purcell SM, et al. Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. Neurobiol Dis. 2009;24:516-524.
9. Pimplikar SW Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 41:1261–1268.
10. Pimplikar SW, Nixon RA, Robakis NK, Shen J, and Tsai LH. Amyloid-Independent Mechanisms in Alzheimer's Pathogenesis. J. Neurosci 2010;30:14946-14954.
11. Budovsky A, Abramovich A, Cohen R, Chalifa-Caspi V, Fraifeld V. Longevity network: construction and implications. Mech Ageing Dev. 2007;128(1): 117–124.
12. Wolfson M, Budovsky A, Tacutu R, Fraifeld V. The signaling hubs at the crossroad of longevity and age-related disease networks. Int J Biochem Cell Biol ;2009;41:516-520.
13. Mendoza-Oliva A, Ferrera P, Arias C. Interplay between cholesterol and homocysteine in the exacerbation of amyloid-β toxicity in human neuroblastoma cells. CNS Neurol Disord Drug Targets. 2013;12:842-848.
14. Urano Y, Ochiai S, Noguchi N. Suppression of amyloid-β production by 24S-hydroxycholesterol via inhibition of intracellular amyloid precursor protein trafficking. FASEB J. 2013;27:4305-4315.
15. Reed B, Villeneuve S, Mack W, Decarli C, et al. Associations Between Serum Cholesterol Levels and Cerebral Amyloidosis. JAMA Neurol. JAMA Neurol. 2014;71:195-200.
16. Barbero-Camps E, Fernández A, Martínez L, Fernández-Checa JC, Colell A. APP/PS1 mice overexpressing SREBP-2 exhibit combined Aβ accumulation and tau pathology underlying Alzheimer's disease. Hum Mol Genet.; 2013;22:3460-3476.
17. Buchman AS, Wilson RS, Bienias JL, Shah RC, Evans DA, et al. Change in body mass index and risk of incident Alzheimer disease. Neurology. 2005; 65:892–897.
18. Haataja L, Gurlo T, Huang CJ, Butler PC. Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis. Endocr Rev 2008;29: 303–316, 2008.
19. Hiltunen M, Khandelwal VK, Yaluri N, Tiilikainen T, Tusa M, Koivisto H, et al. Contribution of genetic and dietary insulin resistance to Alzheimer phenotype in APP/PS1 transgenic mice. J Cell Mol Med. 2012;16:1206-1222.
20. Sadowski, M., Pankiewicz, J., Scholtzova, H., Ji, Y. Quartermain, D., Jensen, C. H., et al. Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory impairment in APP/PS1 mice. J Neuropathol Exp Neurol 2004;63, 418–428.
21. Intebi AD, Garau L, Brusco I, Pagano M, Gaillard RC, Spinedi E. Alzheimer's disease patients display gender dimorphism in circulating anorectic adipokines. Neuroimmunomodulation. 2002;10:351-358.
22. BrandebourgT, HugoE, Ben-Jonathan N. Adipocyte prolactin:regulation of release and putative functions. Diabetes Obes Metab. 2007;9:464-476.
23. Folch J, Pedrós I, Patraca I, Sureda F, Junyent F, et al. Neuroprotective and anti-ageing role of leptin. J Mol Endocrinol. 2012; 49:149-156.
24. Brandebourg TD, Bown JL, Ben-Jonathan N. Prolactin upregulates its receptors and inhibits lipolysis and leptin release in male rat adipose tissue. Biochem Biophys Res Commun. 2007;357:408-413.
25. Greco SJ, Bryan KJ, Sarkar S, Zhu X, Smith MA, et al. Leptin reduces pathology and improves memory in a transgenic mouse model of Alzheimer's disease. J Alzheimers Dis. 2010; 19:1155–1167.
26. Greco SJ, Hamzelou A, Johnston JM, Smith MA, Ashford JW, et al. Leptin boosts cellular metabolism by activating AMPK and the sirtuins to reduce tau phosphorylation and β-amyloid in neurons. Biochem Biophys Res Commun. 2011;414:170-174.
27. Harvey J, Solovyova N, Irving A. Leptin and its role in hippocampal synaptic plasticity. Prog Lipid Res. 2006;45:369–378.
28. Lieb W, Beiser AS, Vasan RS, Tan ZS, Au R, et al. Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. JAMA. 2009; 302:2565–2572.
29. Pérez-González R, Alvira-Botero MX, Robayo O, Antequera D, Garzón M, et al. Leptin gene therapy attenutes neuronal damages evoked by amyloid-β and rescues

J Nutr Health Aging
Volume 19, Number 4, 2015

ADIPOKINE PATHWAYS ARE ALTERED IN HIPPOCAMPUS OF AN EXPERIMENTAL MOUSE MODEL OF AD

memory deficits in APP/PS1 mice. Gene Ther. 2014;21:298-308.
30. Marwarha G, Dasari B, Prasanthi JR, Schommer J, Ghribi O. Leptin reduces the accumulation of Abeta and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. J Alzheimers Dis. 2010;19:1007-1019.
31. Marwarha G, Ghribi O. Leptin signaling and Alzheimer's disease. Am J Neurodegener Dis. 2012;1:245-265.
32. Luchsinger, J. A., Noble, J. M., Scarmeas, N. Diet and Alzheimer's Disease. Curr. Neurol. Neurosci. Rep. 2007;7: 366–372.
33. Femminella GD, Edison P. Evaluation of neuroprotective effect of glucagon-like peptide 1 analogs using neuroimaging. Alzheimers Dement. 2014;10:S55-S61.
34. Herzberg-Schäfer S, Heni M, Stefan N, Häring HU, Fritsche A. Impairment of GLP1-induced insulin secretion: role of genetic background, insulin resistance and hyperglycaemia. Diabetes Obes Metab. 2012;Suppl 3:85-90.
35. Rangani RJ, Upadhya MA, Nakhate KT, Kokare DM, Subhedar NK. Nicotine evoked improvement in learning and memory is mediated through NPY Y1 receptors in rat model of Alzheimer's disease. Peptides. 2012;33:317-328.
36. Ramos B, Baglietto-Vargas D, del Rio JC, Moreno-Gonzalez I, Santa-Maria C, Jimenez S, et al. Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1xAPP transgenic model of Alzheimer's disease. Neurobiol Aging 2006;27:1658–72.
37. Sperk G, Hamilton T, Colmers WF. Neuropeptide Y in the dentate gyrus. Prog Brain Res 2007;163:285–97
38. Meynen G, Unmehopa UA, Hofman MA, Swaab DF, Hoogendijk WJ. Relation between corticotropin-releasing hormone neuron number in the hypothalamic paraventricular nucleus and depressive state in Alzheimer's disease. Neuroendocrinology. 2007;85:37-44.
39. Bayatti N, Behl C. The neuroprotective actions of corticotropin releasing hormone. Ageing Res Rev. 2005;54:258-270.
40. Rehman HU. Role of CRH in the pathogenesis of dementia of Alzheimer's type and other dementias. Curr Opin Investig Drugs. 2002;3:1637-42.
41. Kuehnen P, Mischke M, Wiegand S, Sers C, Horsthemke B, et al. An Alu element-associated hypermethylation variant of the POMC gene is associated with childhood obesity. PLoS Genet. 2012;8:e1002543.
42. Ali S, Nouhi Z, Chughtai N, and Ali S.SHP-2 Regulates SOCS-1-mediated Janus Kinase-2 Ubiquitination/ Degradation Downstream of the Prolactin Receptor. J. Biol. Chem. 2003; 278: 52021–52031.
43. Kadish I, Thibault O, Blalock EM, Chen KC, Gant JC, et al. Hippocampal and Cognitive Aging across the Lifespan: A Bioenergetic Shift Precedes and Increased Cholesterol Trafficking Parallels Memory Impairment. J. Neurosci. 2009;29:1805–1816.
44. Rönnemaa E, Zethelius B, Vessby B, Lannfelt L, et al. Serum fatty-acid composition and the risk of Alzheimer's disease: a longitudinal population-based study. Eur J Clin Nutr. 2012;66:885-890.
45. Warren MW, Hynan LS, Weiner MF. Lipids and adipokines as risk factors for Alzheimer's disease. J Alzheimers Dis. 2012;29:151-7.
46. Rodríguez-Rodríguez E, Mateo I, Infante J, Llorca J, García-Gorostiaga I, et al. Interaction between HMGCR and ABCA1 cholesterol-related genes modulates Alzheimer's disease risk. Brain Res. 2009;1280:166-171.
47. Pierrot N, Tyteca D, D'auria L, Dewachter I, Gailly P, et al. Amyloid precursor protein controls cholesterol turnover needed for neuronal activity. EMBO Mol Med. 2013;5:608-625.
48. Lee EB, Mattson MP. The neuropathology of obesity: insights from human disease. Acta Neuropathol. 2014;127:3-28.
49. Orth M, Bellosta S. Cholesterol: its regulation and role in central nervous system disorders. Cholesterol. 2012:292598.
50. Sáez JM. Possible usefulness of growth hormone/insulin-like growth factor-I axis in Alzheimer's disease treatment. Endocr Metab Immune Disord Drug Targets. 12:274-286.
51. Morales T. Recent Findings on Neuroprotection Against Excitotoxicity in the Hippocampus of Female Rats J. Neuroendocrinol. 2011;23: 994–1001.
52. Goffin V, Binart N, Touraine P, Kelly PA. Prolactin: the new biology of an old hormone. Annu Rev Physiol. 2002;64:47-67.
53. Ma FY, Anderson GM, Gunn TD, Goffin V, Grattan DR, Bunn SJ. Prolactin specifically activates signal transducer and activator of transcription 5b in neuroendocrine dopaminergic neurons. Endocrinology. 2005;146:5112-5119.
54. Irving AJ, Harvey J. Leptin regulation of hippocampal synaptic function in health and disease. Philos Trans R Soc Lond B Biol Sci. 2013;2:369.
55. Bonda DJ, Stone JG, Torres SL, Siedlak SL, Perry G, et al. Dysregulation of leptin signaling in Alzheimer disease: evidence for neuronal leptin resistance.J Neurochem. 2014;128:162-172.
56. Nicolas CS, Amici M, Bortolotto ZA, Doherty A, Csaba Z, et al. The role of JAK-STAT signaling within the CNS. JAKSTAT. 2013;2:e22925.
57. Carré N, Binart N. Prolactin and adipose tissue. Biochimie. 2014;97:16-21.
58. Ignacak A, Kasztelnik M, Sliwa T, Korbut RA, Rajda K, Guzik TJ. Prolactin--not only lactotrophin. A «new» view of the «old» hormone. J Physiol Pharmacol. 2012;63:435-443.
59. Brooks CL. Molecular mechanisms of prolactin and its receptor. Endocr Rev. 2012;33:504-525.
60. Minthon L, Edvinsson L, Gustafson L. Correlation between clinical characteristics and cerebrospinal fluid neuropeptide Y levels in dementia of the Alzheimer type and frontotemporal dementia. Alzheimer Dis Assoc Disord. 1996;10:197-203.
61. Rose JB, Crews L, Rockenstein E, Adame A, Mante M, Hersh LB. Neuropeptide Y fragments derived from neprilysin processing are neuroprotective in a transgenic model of Alzheimer's disease. J Neurosci. 2009;29:1115-1125.
62. Croll SD, Chesnutt CR, Greene NA, Lindsay RM, Wiegand SJ. Peptide immunoreactivity in aged rat cortex and hippocampus as a function of memory and BDNF infusion. Pharmacol Biochem Behav. 1999;64:625–35.
63. Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther. 2013;138:155-175.
64. Moreira EL, de Oliveira J, Nunes JC, Santos DB, et al. Age-related cognitive decline in hypercholesterolemic LDL receptor knockout mice (LDLr-/-): evidence of antioxidant imbalance and increased acetylcholinesterase activity in the prefrontal cortex. J Alzheimers Dis. 2012;32:495-511.
65. Dietschy JM, Turley SD. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res. 2004;45:1375-1397.
66. Aqul A, Liu B, Ramirez CM, Pieper AA, Estill SJ, Burns DK, et al. Unesterified cholesterol accumulation in late endosomes/lysosomes causes neurodegeneration and is prevented by driving cholesterol export from this compartment. J Neurosci. 2011;31:9404-9413.
67. Heverin M, Bogdanovic N, Lütjohann D, Bayer T, et al. Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. J Lipid Res. 2004;45:186-193.
68. Maioli S, Båvner A, Ali Z, Heverin M, Ismail MA, et al. Is it possible to improve memory function by upregulation of the cholesterol 24S-hydroxylase (CYP46A1) in the brain? PLoS One. 2013;Jul 16;8(7):e68534.
69. Ali Z, Heverin M, Olin M, Acimovic J, Lövgren-Sandblom A, et al. On the regulatory role of side-chain hydroxylated oxysterols in the brain. Lessons from CYP27A1 transgenic and Cyp27a1(-/-) mice. Lipid Res. 2013;54:1033-1043.

4.3 Publicación III

High-fat diet-induced deregulation of hippocampal insulin signaling and mitochondrial homeostasis deficiences contribute to Alzheimer disease pathology in rodents

Dmitry Petrov, Ignacio Pedrós, Gonzalo Artiach, Francesc X. Sureda, Emma Barroso, Mercè Pallàs, Gemma Casadesús, Carlos Beas-Zarate, Eva Carro, Isidro Ferrer, Manuel Vázquez-Carrera, Jaume Folch, Antoni Camins (2015).
Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1852: 1687-1699

Resumen

La obesidad y la resistencia a insulina son factores de riesgo en la EA. La dieta rica en grasas (HFAT) provoca resistencia a insulina, obesidad, inflamación y pérdida de memoria. Rutas metabólicas como AKT-GSK3β y AMPK-PGC1α y moléculas como JNK1, IDE y ERK son claves para un correcto metabolismo energético.

El objetivo principal de este trabajo es conocer si la obesidad provocada por la dieta HFAT y el proceso de amiloidogénesis conllevan alteraciones en la vía de señalización de insulina. Otros objetivos son estudiar las cinasas implicadas en la hiperfosforilación de tau, la biogénesis mitocondrial y las proteínas implicadas en la degradación de β-amiloide e insulina.

El proceso de amiloidogénesis en el APP/PS1 se inicia alrededor de los tres meses mediante elevadas concentraciones de β-amiloide soluble e insoluble, pero no se consolida en la formación de placas hasta los 5 meses. Por esta razón elegimos 6 meses como marco temporal. La dieta HFAT fue subministrada durante los 6 meses de vida del ratón. Escogimos machos y en caso de que fuera posibles hermanos para reducir la viabilidad y cada semana medimos el peso. Las pruebas bioquímicas en sangre o plasma y el análisis conductual se realizó dos semanas antes del sacrificio y su correspondiente extracción del hipocampo.

Los resultados muestran una disminución significativa en la señalización de insulina y deficientes homeostasis de la glucosa en el hipocampo de ratones APP/PS1. También hemos detectado una regulación al alza significativa tanto de la CDK5 GSK-3β y cinasas en el hipocampo de ratones WT alimentados con HFAT. En el caso de la biogénesis mitocondrial observamos disminuciones significativas en la fosforilación oxidativa mitocondrial proteínas complejas, tanto en el HFAT alimentado ratones WT y en animales PS1/APP.

Según nuestros resultados, las reducciones en la vía de señalización de la insulina y la disfunción mitocondrial en el sistema nervioso central pueden ser consideradas como marcadores de un principio de enfermedad de Alzheimer.

Biochimica et Biophysica Acta 1852 (2015) 1687–1699

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

High-fat diet-induced deregulation of hippocampal insulin signaling and mitochondrial homeostasis deficiences contribute to Alzheimer disease pathology in rodents

Dmitry Petrov [a,c,1], Ignacio Pedrós [b,c,1], Gonzalo Artiach [a,c], Francesc X. Sureda [b,c], Emma Barroso [a,d], Mercè Pallàs [a,c], Gemma Casadesús [e], Carlos Beas-Zarate [f,g], Eva Carro [h], Isidro Ferrer [i], Manuel Vazquez-Carrera [a,d], Jaume Folch [b,c,2], Antoni Camins [a,c,j,*,2]

[a] Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Institut de Biomedicina de la UB (IBUB), Universitat de Barcelona, Barcelona, Spain
[b] Unitats de Bioquímica i Farmacologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Reus (Tarragona), Spain
[c] Biomedical Research Networking Center in Neurodegenerative Diseases (CIBERNED), Madrid, Spain
[d] Centros de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Spain
[e] Department of Biological Sciences Kent State University, Kent, OH, USA
[f] Laboratorio de Neurobiología Celular y Molecular, División de Neurociencias, CIBO, IMSS, México
[g] Laboratorio de Regeneración y Desarrollo Neural, Instituto de Neurobiología, Departamento de Biología Celular y Molecular, CUCBA, México
[h] Neuroscience Group, Instituto de Investigacion Hospital 12 de Octubre, Madrid, Spain
[i] Institute of Neuropathology, Bellvitge University Hospital-Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Spain
[j] Universidad Nacional de Loja, Department of Biotechnology, Ecuador

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 9 April 2015
Accepted 6 May 2015
Available online 21 May 2015

Keywords:
APPSwe/PS1dE9
Insulin receptor
Mitochondria
Hippocampus
TAU
Alzheimer disease

ABSTRACT

Global obesity is a pandemic status, estimated to affect over 2 billion people, that has resulted in an enormous strain on healthcare systems worldwide. The situation is compounded by the fact that apart from the direct costs associated with overweight pathology, obesity presents itself with a number of comorbidities, including an increased risk for the development of neurodegenerative disorders. Alzheimer disease (AD), the main cause of senile dementia, is no exception. Spectacular failure of the pharmaceutical industry to come up with effective AD treatment strategies is forcing the broader scientific community to rethink the underlying molecular mechanisms leading to cognitive decline. To this end, the emphasis is once again placed on the experimental animal models of the disease. In the current study, we have focused on the effects of a high-fat diet (HFD) on hippocampal-dependent memory in C57/Bl6 Wild-type (WT) and APPswe/PS1dE9 (APP/PS1) mice, a well-established mouse model of familial AD. Our results indicate that the continuous HFD administration starting at the time of weaning is sufficient to produce β-amyloid-independent, hippocampal-dependent memory deficits measured by a 2-object novel-object recognition test (NOR) in mice as early as 6 months of age. Furthermore, the resulting metabolic syndrome appears to have direct effects on brain insulin regulation and mitochondrial function. We have observed pathological changes related to both the proximal and distal insulin signaling pathway in the brains of HFD-fed WT and APP/PS1 mice. These changes are accompanied by a significantly reduced OXPHOS metabolism, suggesting that mitochondria play an important role in hippocampus-dependent memory formation and retention in both the HFD-treated and AD-like rodents at a relatively young age.

1. Introduction

Over the last couple of decades a global nutrition transition from undernourishment to overconsumption has taken place. Replacement of traditional diets with cheap and easily available processed foods rich in refined carbohydrates, animal fats and edible oils resulted in a global obesity pandemic. While usually considered the plight of the developed world, obesity is also an emerging public health concern among the growing middle classes in poorer countries [1]. Overweight and moderate obesity (defined as Body Mass Index (BMI) of between 25 and 35) may not have a major impact on life expectancy *per se* [2], however, excessive weight significantly increases the risks of developing a number of pathological conditions. These include metabolic syndrome, diabetes, non-alcoholic steatohepatitis, coronary heart disease, stroke, gallbladder disease, osteoarthritis, some types of cancers [3], cognitive decline and Alzheimer disease (AD) [4–7].

* Corresponding authr at: Unitat de Farmacologia i Farmacognosia, Facultat de Farmàcia, Universitat de Barcelona, Spain. Avda/ Diagonal 643, E-08028 Barcelona, Spain. Tel.: +34 93 4024531; fax: +34 934035982.
E-mail address: camins@ub.edu (A. Camins).
[1] These authors contributed equally to this work.
[2] Senior co-authors.

http://dx.doi.org/10.1016/j.bbadis.2015.05.004
0925-4439/

AD is the most common cause of senile dementia, accounting for between 60 and 80% of all dementias. According to recent estimates, the number of cases of AD worldwide is projected to rise from approximately 30 million in 2010 to 40 million by 2020 and to 100 million by 2050. Apart from the genetic component and old age, seven primary preventable environmental risk factors contributing to AD have been identified: diabetes mellitus, midlife hypertension, midlife obesity, depression, physical inactivity, smoking and cognitive inactivity [8,9]. Thus, it is becoming increasingly evident that most of the prognostic preventable AD risk factors may also be linked to obesity and resulting comorbidities, including metabolic syndrome and diabetes. Even though the epidemiological data suggest an existing relationship between AD and energy metabolism, molecular mechanisms behind this relationship are poorly understood. Because AD is a multifactorial disorder with complex etiology which takes decades to fully develop, it is especially challenging to identify the precise disease mechanisms. For example, in a patient with dementia it is not always easy to tell if the underlying pathology is that of a specific brain disease or whether it is also associated with vascular components, metabolic alterations or additional factors (ie. traumatism). Such difficulties notwithstanding, recent years saw a number of breakthroughs in AD research field which contribute to a greater understanding of the molecular dynamics of this devastating condition.

A classical, but currently hotly debated "amyloid cascade" hypothesis [10,11] states that cognitive decline and memory loss in AD are caused by the formation of large, insoluble beta amyloid plaques in the brain, which result in neuronal death and produce characteristic disease symptoms. However, it is necessary to differentiate between the insoluble plaques and soluble amyloid molecules. Recently emerged alternative theories suggest that the β-amyloid monomers, fibrils, or oligomers, and not the plaques, may in fact be the primary neurotoxic species in the brain, responsible for AD development and progression [12]. Apart from amyloid beta itself, mounting evidence suggests that impaired glucose and insulin signaling and metabolism in the brain play a key role in AD. The discovery of brain-specific insulin signaling deficiencies in the very early stages of AD pathogenesis has led some authors to propose that AD may be termed "type 3 diabetes" [13–15]. This hypothesis is further strengthened by a recent study of diabetes-related genes in the brains of post-mortem AD patients and in a mouse model of AD [16]. Microarray analysis has demonstrated significant alterations in the mRNA expression profiles of genes related to insulin signaling, obesity and diabetes in the frontal cortex, temporal cortex and hippocampus in both species. Interestingly, the biggest differences were observed in the hippocampus, a key area related to memory.

Deficiencies in Tau processing may provide yet another link between diabetes and AD. Hyperphosporylated Tau protein is a principle constituent of neurofibrillary tangles (NFT) [17] which, alongside amyloid beta plaques, have long been considered key histopathological hallmarks of AD. Abnormalities in Tau phosphorylation have been detected in cortex and the hippocampi of both type 1 (streptozotocin-induced) and type 2 (*db/db*) mouse models of diabetes [18,19].

Prior research has established a clear relationship between obesity, insulin resistance, diabetes and dementia (reviewed in [20]). Results from published research indicate that there is a close link between insulin deficient diabetes and cerebral amyloidosis in the pathogenesis of AD [21–24]. Epidemiological, clinical, and basic studies have shown a relationship between AD and Type 2 Diabetes Mellitus (T2DM), and that the main physiological link between both conditions is peripheral and central insulin signalling impairment [25,26]. In fact, results from the so called "Hysayama Study" indicate that altered expression of genes related to diabetes mellitus in AD brains is a result of AD pathology, which may thereby be exacerbated by peripheral insulin resistance or diabetes mellitus [16]. These cognitive deficits associated to T2DM have been argued to be due in large part to an impaired central insulin modulation in the hippocampus, which is a critical region for memory processing [27]. Furthermore, a number of recent pilot clinical trials have demonstrated an improvement in AD symptoms in patients upon administration of both the intranasal insulin and Glucagon-like peptide-1 (GLP1) analogues. It has been suggested that these compounds may affect synaptogenesis, neurogenesis, cell repair and inflammation processes, and may additionally help to reduce cerebral β-amyloid load (reviewed in [28]).

As it is especially difficult to study long-term effects of hypercaloric diet in human subjects, we have chosen a mouse model in order to further investigate the underlying molecular events linking brain energy metabolism to AD. A well-established experimental approach to induce insulin resistance in peripheral organs of rodents consists of a high-fat diet (HFD) treatment, which results in obesity [29–31]. We have characterized the neuropathological effects of a HFD in 6-months-old male APPswe/PS1dE9 (APP/PS1) mice in comparison to the nontransgenic C57BL/6 (non-Tg; WT) control animals.

2. Materials and methods

2.1. Animals

Male APPswe/PS1dE9 and C57BL/6 mice were used in this study. APP/PS1 animals co-express a Swedish (K594M/N595L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9), allowing these mice to secrete elevated amounts of human Aβ peptide. Both mutations are associated with AD, are under control of the mouse prion protein promoter, directing both mutated proteins mainly to the CNS neurons, and result in age-dependent amyloid plaque depositions in mouse brain. The APPswe-mutated APP is a favorable substrate for β-secretase, whereas the PS1dE9 mutation alters β-secretase cleavage, thereby promoting overproduction of Aβ42. The mice were fed for 5 months with a high-fat diet consisting of 25% fat (45 kcal %), mainly from hydrogenated coconut oil, 21% protein (16 kcal %), and 49% carbohydrate (39 kcal %); Cat# D08061110 (Research Diets Inc, New Brunswick, USA). Body weight was recorded weekly. The animals were kept under controlled temperature, humidity and light conditions with food and water provided *ad libitum*. Mice were treated in accordance with the European Community Council Directive 86/609/EEC and the procedures established by the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Every effort was made to minimize animal suffering and to reduce the number of animals used. Fifty animals, divided into four groups, were used for the present study, with at least 10 wild-type and 10 6-month-old APP/PS1 transgenic mice, per group. Following *in vivo* testing, the animals were sacrificed at the age of 6 months and at least 6 mice in each group were used for RNA and protein extract isolation, with an additional 4 mice used for immunofluorescence.

2.2. Total blood cholesterol and triglycerides measurements

Total blood cholesterol and triglyceride levels were measured following 4-hour-long fast at the point of sacrifice with Accutrend Plus meter (Roche Diagnostics, Switzerland).

2.3. Glucose and insulin tolerance tests

Intraperitoneal glucose tolerance tests (IP-GTT) and insulin tolerance tests (ITT) were performed in accordance with the previously published guidelines [32]. For IP-GTT, mice were fasted overnight for 16 h. The test was performed in a quiet room, preheated to +30 °C. The tip of the tail was cut with the heparin-soaked (Heparina Rovi, 5000 IU/ml; Rovi S.A.;Madrid, Spain) scissors, 30 min prior to 1 g/kg intraperitoneal glucose injection (diluted in H2O). Blood glucose levels in the tail vein were measured at −30, 0, 5, 15, 30, 60 and 120 min after the glucose injection with the Ascensia ELITE blood glucose

meter (Bayer Diagnostics Europe Ltd.; Dublin, Ireland). ITT was performed in similar conditions with the 0.25 IU/kg of human insulin, diluted in saline (Humulina Regular, 100 IU/ml/Lilly, S.A.;Madrid, Spain), except that the mice underwent a 4-5 hour-long morning fast. Blood glucose levels were measured at −30, 0, 15, 30, 45 and 60 min after the insulin administration. If during this time blood glucose levels dropped to below 20 mg/dl, 1 g/kg glucose was administered to counteract the effects of insulin, in order to reduce animal suffering.

2.4. 2-Object novel object recognition test (NOR)

The test was conducted as previously described by us and others [33]. In brief, a 90°, 25 cm long, and 20 cm high L maze was used. The light intensity in the middle of the field was 30 lx. The objects to be discriminated were plastic figures (object A: 5.25 cm high, object B: 4.75 cm high). First, mice were individually habituated to the apparatus for 10 min a day, for two days. On the third day, they were submitted to a 10 min acquisition trial (first trial) during which they were placed in the maze in the presence of two identical novel objects (A + A, or B + B) placed at the end of each arm. A 10 min retention trial, with the objects (A + B) (second trial) occurred 2 h later. The amount of exploration time each animal spent on objects A and B during the acquisition trial varied between 5 and 20 s, depending on the individual mouse. Total exploration time between the 2 objects when calculated for each individual animal indicated the absence of the object preference bias (Fig. 1C) (n = 5–9 per group). During the retention trial, the times that the animal took to explore the new object (tn) and the old object (to) were recorded. A discrimination index (DI) was defined as (tn − to) / (tn + to). In order to avoid further object preference bias, objects A and B were counterbalanced so that half of the animals in each experimental group were first exposed to object A and then to object B, whereas the other half were exposed to object B first, and then to object A. The maze, the surface, and the objects were cleaned with 96° ethanol between animals, so as to eliminate olfactory cues.

2.5. RNA extraction and quantification

Total RNA was isolated from the hippocampi of wild-type and APP/PS1 transgenic mice utilizing Trizol-based extraction (Life Technologies Corporation; Carlsbad, Ca, USA), as described previously [34]. Briefly, the tissue was homogenized in the presence of Trizol reagent (Life Technologies Corporation; Carlsbad, CA, USA). Chloroform was added and the RNA was precipitated from the aqueous phase with isopropanol at 4 °C. RNA pellet was reconstituted in RNAse-free water, with the RNA integrity determined by Agilent 2100 Bioanalyzer (Agilent Technologies; Santa Clara, CA, USA).

2.6. Real-time-PCR

First-strand cDNA was reverse transcribed from 2 μg of total RNA using the High Capacity cDNA Reverse Transcription kit, according to manufacturer's protocol (Applied Biosystems). Each sample was analyzed in duplicate for each target. TaqMan probes (Applied Biosystems), as detailed in Table 2, were used to determine transcription levels of individual genes. Reaction was performed on the StepOnePlus Real Time PCR system (Applied Biosystems; Carlsbad, CA, USA) and the values were normalized to *gapdh* and *tbp*.

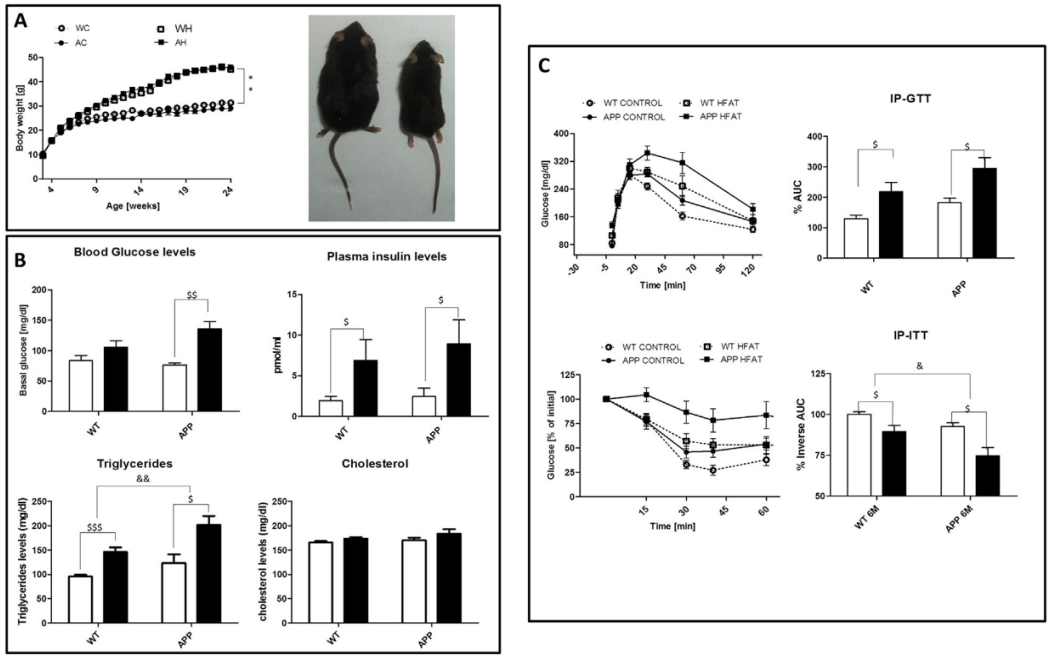


Fig. 1. Peripheral metabolic parameters in HFD-fed WT and APP/PS1 mice. (A) Body weight, (B) fasting blood glucose , fasting serum insulin levels ELISA, total blood triglycerides and cholesterol in 6-month-old animals (n = 5–12 independent samples per group). (C) Intraperitoneal glucose (16 hour fast) and insulin (4 hour fast) tolerance tests in 6-month-old mice (n = 5-12 independent samples per group). For the ITT and the IP-GTT, AUC data were calculated from the timepoint 0 till the end of the experiment. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$, $$$ denotes $p < 0.001$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$) *WC*: Wild-type control diet, *AC*: APP/PS1 control diet, *WH*: Wild-type high-fat diet, *AH*: APP/PS1 high-fat diet; open bar: chow, closed bar: high-fat diet.

Table 1
A list of antibodies used for immunoblotting and immunofluorescence.

Protein	Antibody
Akt	#9272 (Cell signaling)
pAkt (S473)	#4060P (Cell signaling)
CDK5	Sc-173 (Santa Cruz biotech)
pCDK5 (Y15)	ab63550 (Abcam)
ERK1/2	#9102 (Cell signaling)
pERK1/2 (T202/Y204)	#9101 (Cell signaling)
GSK3B	#9315(Cell signaling)
pGSK3B (Y216)	Ab74754 (Abcam)
IDE	Ab32216 (Abcam)
IRβ	Sc-20739 (Santa Cruz biotech)
pIRβ (Y1150/1151)	Sc-81500 (Santa Cruz biotech)
IRS1	#2382 (Cell signaling)
pIRS1 (S612)	#2386S (Cel signaling)
IRS2	Sc-1555 (Santa Cruz biotech)
pIRS2 (S723)	Ab 3690 (Abcam)
JNK	#9252 (Cell signaling)
pJNK (Y183/T185)	#9251 (Cell signaling)
Neprelysin	Ab951 (Abcam)
NRF1	Sc-28379 (Santa Cruz biotech)
OXPHOS	MS604 (MitoSciences)
p35	#2680(Cell signaling)
PGC1A	101707 (Cayman chemical)
PPARa	Ab8934 (Abcam)
PPARg	#2430 (Cell signaling)
TAU	AHB0042 (Biosource)
pTAU (S404)	44748G (Life Technologies)
TFAM	DR1071b(Calbiochem)
GAPDH	MAB374 (Millipore)
Thioflavin S	Thioflavin S (Sigma-Aldrich)
2nd –ary Anti-Mouse	170-5047 (Biorad)
2nd –ary Anti-Rabbit	NA934V (GE Healthcare)

2.7. Immunofluorescence, thioflavin S and Hoechst staining

Slides were allowed to defrost at room temperature and then were rehydrated with Phosphate-buffered saline (PBS) for 5 min. Later, the brain sections were incubated with 0.3% Thioflavin S (Sigma-Aldrich; St. Louis, MO, USA) for 20 min at room temperature in the dark. Subsequently, these were submitted to washes in 3-min series: with 80% ethanol (2 washes), 90% ethanol (1 wash), and 3 washes with PBS. Finally, the slides were mounted using Fluoromount (EMS), allowed to dry overnight at room temperature in the dark, and stored at 4C. Image acquisition was performed with an epifluorescence microscope (BX41; Olympus, Germany).For plaque quantification, similar and comparable histological areas were selected, focusing on having the hippocampus and the whole cortical area positioned adjacently [35].

Table 2
A list of Taqman probes used for real-time PCR analysis.

GENE	TaqManProbe
app	Mm01344172_m1
essra	Mm00433143_m1
gapdh	Mm99999915_g1
igf1	Mm01228180_m1
igf2	Mm00439564_m1
igfbp2	Mm00492632_m1
igf1r	Mm00802831_m1
ins1	Mm01950294_s1
insr	Mm01211875_m1
irs1	Mm01278327_m1
irs2	Mm03038438_m1
nrf1	Mm01135606_m1
nrf2 (nfe2l2)	Mm00477784_m1
Ppara	Mm00440939_m1
pparg	Mm01184322_m1
ppargc1a	Mm01208835_m1
prkaa1	Mm01296700_m1
prkaa2	Mm01264789_m1
tbp	Mm00446971_m1
tfam	Mm00447485_m1

2.8. Immunoblot analysis

Aliquots of hippocampal homogenates containing 15 mg of protein per sample were analyzed using the Western blot method. In brief, samples were placed in a sample buffer (0.5 m Tris–HCl, pH 6.8, 10% glycerol, 2%(w/v) SDS,5%(v/v) 2-mercaptoethanol, 0.05% bromophenol blue) and denatured by boiling at 95–100 °C for 5 min. Samples were separated by electrophoresis on 10–15% acrylamide gels. Following this, the proteins were transferred to PVDF sheets using transblot apparatus. Membranes were blocked overnight with 5% non-fat milk dissolved in TBS-T buffer (50 mM Tris; 1.5% NaCl, 0.05% Tween 20, pH 7.5). They were then incubated with primary antibodies, as detailed in Table 1. After O/N incubation, blots were washed thoroughly in TBS-T buffer and incubated for 1 h with a peroxidase-conjugated IgG secondary antibody (1:2000). Immunoreactive protein was detected using a chemiluminescence-based detection kit. Protein levels were determined by densitometry, using Chemidoc XRS+ Molecular Imager detection system (Bio-Rad Laboratories Inc.; Hercules, CA, USA)), with ImageLab image analysis software. Measurements are expressed as arbitrary units. All results are normalized to GAPDH, unless stated otherwise.

2.9. Measurement of β-amyloid peptides in cortical tissues by ELISA

Soluble and insoluble β-amyloid (βA) βA_{1-40} and βA_{1-42} were measured in cortical extracts employing the commercially available mouse and human ELISA kits (Cat # KMB3481, KMB3441, KHB3481 and KHB3441; Invitrogen, Camarillo, CA, USA) according to manufacturer´s guidelines. The soluble fraction was separated by centrifuging the samples for 10 minutes at 4000xg. The pellets containing insoluble Aβ peptides were solubilized in a 5 M guanidine HCl/50 mM Tris HCl solution by incubating for 3.5 hours on an orbital shaker at room temperature in order to obtain insoluble fraction. Data obtained from the cortical homogenates are expressed as picograms of Aβ content per milligrams of total protein (pg/mg).

2.10. Data analysis

All data are presented as means ± SEM, and differences are considered significant at $p < 0.05$. Differences between samples/animals were evaluated using student's t-test, and either one-way or 2-way ANOVA, with Tukey's post-hoc test. Both the statistical analysis and the graphs presented here were created with the GraphPad InStat software V5.0 (GraphPad Sofware Inc., San Diego, CA, USA).

3. Results

3.1. HFD treatment increases body-weight gain, insulin and triglycerides levels in blood and provokes impaired glucose and insulin tolerance in both WT and APP/PS1 mice

In order to determine the effects of a high-fat hypercaloric diet on metabolic parameters in a mouse model of AD, male WT and APP/PS1 animals were fed either standard chow or a HFD. Mice were divided into 4 groups (n = at least 10 per group): chow-fed WT control (WC), HFD-fed WT (WH), chow-fed transgenic APP/PS1 (TC) and HFD-fed APP/PS1 (TH). Treatment commenced at the time of weaning (21 days) and lasted until the animals reached 6 months of age. This specific time point was chosen as at six months-old APP/PS1 mice present with AD-like neuropathology, including readily detectable βA plaques and memory loss [33] (Fig. 2). As expected, HFD treatment produced progressive diet-induced obesity, with body weight at completion of the experiment reaching 144% in WH vs. WC ($P < 0.0001$),

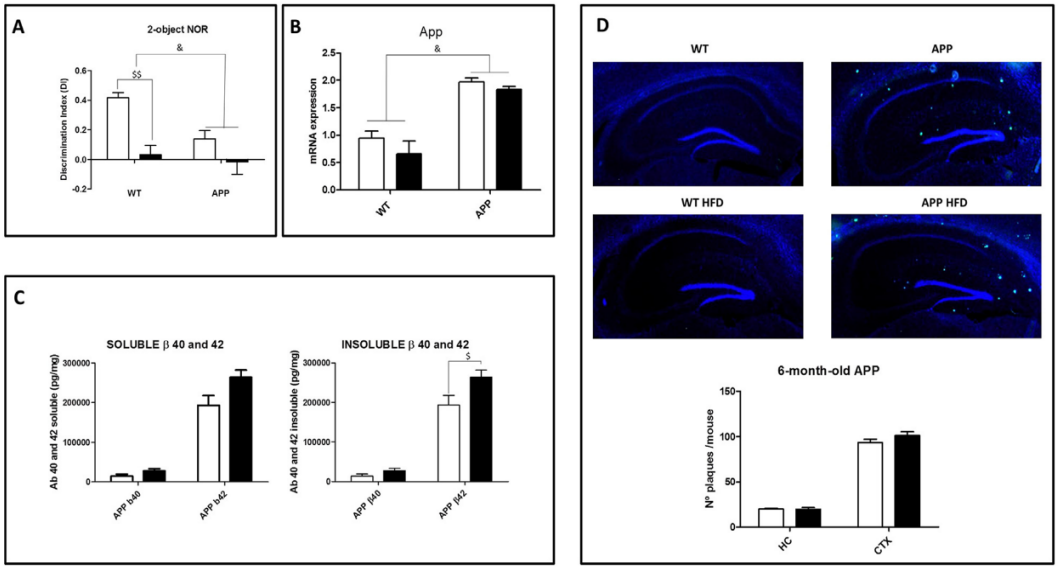


Fig. 2. (A) The results of the 2-object Novel Object Recognition test (NOR), demonstrating significant memory loss as a result of a HFD treatment in 6 month-old wild-type, as well as in chow-fed and HFD-fed APP/PS1 animals, compared to chow-fed wild-type mice (n = 7–12 independent samples per group). (B) Hippocampal mRNA expression of the *app* with the probe recognizing both mouse and human forms of *app* in 6-months-old mice (n = 10-12 independent samples per group, with 3 technical replicates per sample). (C) Concentrations of the soluble and insoluble human βA_{1-40} and βA_{1-42} peptides in the cortical extracts in chow- and HFD-fed 6-month-old APP/PS1 mice, expressed as pg/mg of total protein as determined by ELISA (n = 5–8 independent samples per group, with 3 technical replicates per sample). (D) Immunofluorescence analysis of β-amyloid plaque numbers in the brains of 6-month-old WT and APP/PS1 mice. No plaques were observed in WT animals (n = 4–6 independent samples per group, with at least 5 slices analyzed per sample). Hoechst staining in blue / Thioflavin S staining in green (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

and 158% in TH vs. TC ($P < 0.0001$) (Fig. 1A). This weight increase was accompanied by fasting hyperinsulinemia, with plasma insulin concentrations of 6.88 pM/ml in WH (1.92 in WC; $P = 0.0484$) and 8.92 pM/ml in TH (2.44 in TC; $P = 0.0332$) (Fig. 1B). An additional sign pointing to the possible presence of metabolic syndrome in 6-months-old HFD-fed mice was an increase in fasting triglycerides levels with 146 mg/dl in WH (96 in WC; $P < 0.001$) and 202 mg/dl in TH (123 in TC; $P = 0.0119$). Interestingly, a 2-way ANOVA demonstrated significant differences between WT and APP/PS1 groups as a whole, with higher triglycerides concentrations in transgenic animals, suggesting exacerbated phenotype in AD-like mice (Fig. 1B). Fasting blood glucose levels support this observation, as the HFD treatment resulted in a significant increase of this parameter in TH vs. TC group (136 vs 76 mg/dl; $P = 0.0046$), but not in WH vs. WC (106 vs 84 mg/dl; $P = 0.1452$). Fasting blood cholesterol levels were not affected in any of the groups (Fig. 1B).

As our initial screening has not only demonstrated clear alterations in peripheral glucose metabolism in response to HFD, but also significant differences between the WT and APP/PS1 animals, we performed additional glucose and insulin tolerance tests. Predictably, IP-GTT has shown impaired glucose tolerance in 6-months-old WH and AH groups, when compared to their respective controls ($P = 0.02$ and $P = 0.0436$) (Fig. 1C). The results of the ITT were intriguing. While the test results indicated impaired insulin tolerance in both WH and AH ($P = 0.0106$ and $P = 0.0346$) we, once again, detected a more severe phenotype in an APP/PS1 model ($P = 0.0239$, with 2-way ANOVA) (Fig. 1C). Taken together, our data indicate a possible acceleration of a HFD-induced peripheral metabolic phenotype in APP/PS1 animals compared to control mice. In the following steps, we proceeded to study the effects of HFD on CNS and attempted to identify molecular pathways related to insulin metabolism in the brain, with a particular focus on hippocampal metabolic and insulin signaling.

3.2. High-fat diet contributes to increased cerebral β-amyloid levels and memory loss

We have employed a 2-object Novel Object Recognition (NOR) test as a means of evaluating the impact of HFD on cognitive performance. Interestingly, our results demonstrate that HFD treatment has a significant impact on memory function in both the WT and transgenic animals (Fig. 2A). In order to determine if the resulting memory loss is dependent on the increased cerebral βA load, we have measured hippocampal expression of the APP, cortical levels of the βA_{1-40} and βA_{1-42} and assessed the numbers of senile plaques in the brain.

Because APP/PS1 mouse model expresses a human form of the APP and the βA, it is necessary to quantify the combined expression of both endogenous and transgenic protein. At the mRNA level, a probe recognizing both human and mouse versions of *app* was selected for Real-time PCR analysis. We have detected approximately a 2-fold increase in *app* transcripts in the hippocampal extracts of APP/PS1 mice, compared to WT controls (Fig. 2B). Hypercaloric diet did not influence mRNA expression of this target in either group. Elevated levels of soluble and insoluble forms of βA_{1-40} and βA_{1-42} peptides were detected in cortical homogenates of APP/PS1 animals. However, HFD treatment resulted in a significant increase in insoluble βA_{1-42} levels only, and only in TH versus TC group (~266 compared to ~195 ng/mg) (Table 3 and Fig. 2C). Surprisingly, this increase did not have an effect on the total number of plaques in the hippocampal and cortical areas of the brain (Fig. 2D). Furthermore, there appeared to be an increase in the concentrations of the soluble βA_{1-42} in WH animals, but it only affected a subset of this group, rendering the data unsuitable for statistical analysis (Table 3). Thus, our results suggest that alterations in cerebral amyloid levels do not play a critical role in HFD-induced memory loss in 6-month-old mice.

Table 3
Concentrations of the soluble and insoluble mouse and human βA_{1-40} and βA_{1-42} peptides in the cortical extracts in chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice, expressed as pg/mg of total protein as determined by ELISA (n = 5–8 independent samples per group, with 3 technical replicates per sample). (Statistical analysis was performed with the Student's t-test; ± is S.E.M.). *WC*: Wild-type control diet, *AC*: APP/PS1 control diet, *WH*: Wild-type high-fat diet, *AH*: APP/PS1 high-fat diet, *m*: mouse, *h*: human, *t*: total (mouse + human), *p*: p value (t-test) *N/A*: not applicable, *N/D*: not detected.

Sol. (pg/mg)	m βA 40	h βA 40	t βA 40	m βA 42	h βA 42	t βA 42
WC	N/D	N/A	N/A	10 ± 10	N/A	10 ± 10
WH	N/D	N/A	N/A	1132 ± 632	N/A	1132 ± 632
AC	N/D	649 ± 262	649 ± 262	1120 ± 706	1088 ± 168	2208 ± 863
AH	N/D	879 ± 260	879 ± 260	1976 ± 1121	1244 ± 266	3220 ± 1346
WC vs WH (p)	N/A	N/A	N/A	0.1431	N/A	0.1431
AC vs AH (p)	N/A	0.558	0.558	0.5215	0.588	0.5305
WC	460 ± 158	N/A	460 ± 158	6 ± 6	N/A	6 ± 6
WH	331 ± 17	N/A	331 ± 17	270 ± 270	N/A	270 ± 270
AC	1276 ± 320	14075 ± 5195	15352 ± 4231	1722 ± 655	193310 ± 24590	194688 ± 24396
AH	1350 ± 190	27338 ± 5954	28688 ± 6138	2502 ± 633	263797 ± 18173	265882 ± 18194
WC vs WH (p)	0.3878	N/A	0.3878	0.3024	N/A	0.3024
AC vs AH (p)	0.8467	0.1476	0.1114	0.4245	0.043*	0.041*

3.3. HFD affects expression of genes involved in insulin signaling in the hippocampus

We evaluated mRNA expression profiles of preproinsulin 1 (*Ins1*), insulin receptor (*Insr*), insulin receptor substrates 1 (*Irs1*) and 2 (*Irs2*), insulin-like growth factor I (*Igf1*), and IGF receptor (*Igfr*) in the hippocampus of 6 months-old mice (Fig. 3A). A modest increase in *ins1* transcripts was accompanied by a small but significant reduction in *insr* and *igfr* levels in the TH vs. TC group, while HFD treatment did not have an effect on these molecules in WT brains. Conversely, *igf1* was upregulated in WH vs. WC group, but remained unchanged in transgenic animals. We did not detect significant differences in total IRS1 and IRS2 expression in any of the groups, both at the mRNA and protein levels (Fig. 3A, B). As post-translational modifications and especially ligand-mediated tyrosine$^{1150/1151}$ autophosphorylation of the signal-transducing catalytic β-subunit of IR play a major role in receptor activation, we have measured protein levels of both total IRβ and pTyr$^{1150-1151}$-IRβ. Immunoblotting analysis revealed no changes in the ratios between the phosphorylated and total IRβ protein in the hippocampal extracts at 6 months of age (Fig. 3B), suggesting that receptor functionality is unaffected. Functional IR is necessary for downstream signaling which is controlled in large part by IRS1 and IRS2 adaptor molecules. Autologous (insulin-mediated) and insulin independent Ser/Thr phosphorylations of IRS may both potentiate and attenuate IR signaling. Mouse pSer^{612}-IRS1 and pSer^{723}-IRS2 (corresponding to human pSer^{616}-IRS1 and pSer^{731}-IRS2) are amongst the better known negative regulators of the IR-IRS pathway. Thus, an insulin-dependent increase in the phosphorylation state of these residues may lead to

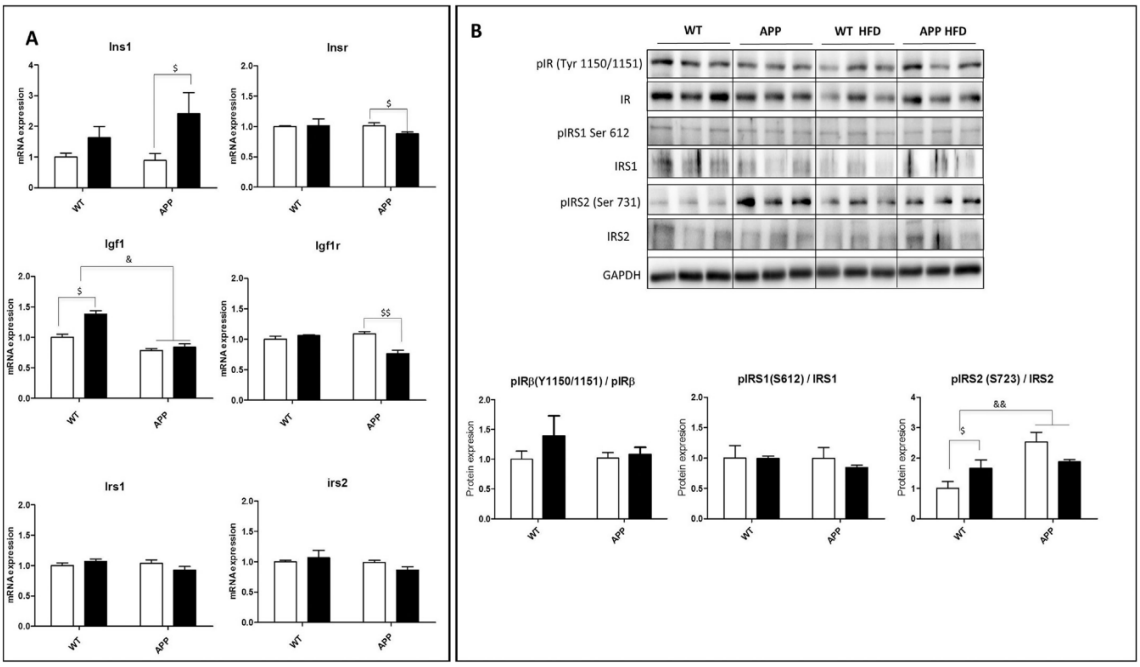


Fig. 3. (A) mRNA expression profile (n = 4–6 independent samples per group, with 3 technical replicates per sample) and (B) representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of molecules related to proximal insulin signaling in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. Mouse pSer^{612}-IRS1 and pSer^{723}-IRS2 correspond to human pSer^{616}-IRS1 and pSer^{731}-IRS2 (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$; $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

desensitization of proximal insulin signaling. We did not detect differences in IRS1 phosphorylation, however, our data demonstrated a significant increase in pSer^{723}-IRS2 in WH vs. WC ($P = 0.039$) and in APP/PS1 vs. WT (2-way ANOVA, $P = 0.0078$) mice (Fig. 3b), indicating a potential role of IRS2 in response to both the hypercaloric diet treatment and in AD-like phenotype.

3.4. Effects of HFD on signaling kinases and tau phosphorylation

Having determined that the proximal insulin signaling is likely perturbed in the hippocampi of HFD-exposed and APP/PS1 transgenic animals, we turned our attention to the signaling kinases implicated both in insulin signaling and senile plaque formation. Apart from their diverse cellular roles, these kinases (except for CDK5) have also been shown to phosphorylate IRS proteins directly, thus modulating the activity of these adaptor molecules. We measured protein levels of pSer^{473}-Akt, pTyr^{216}-Gsk3β, pThr^{183}/pTyr^{185}-JNK1, pThr^{202}/pTyr^{204}-ERK1/2, pTyr^{15}-CDK5 and its activator molecule p35 in the hippocampal extracts of 6-month-old animals. All of the above mentioned phosphorylations result in the activation of the respective kinases. Interestingly, we have observed significant differences in the activation state in all of the kinases tested when compared to at least one of the groups (Fig. 4). For example, ERK1/2 and CDK5/p35 were overactivated in WH vs. WC group only, while JNK1 activity was enhanced in WH vs. WC and TH vs. TC hippocampi. Basal GSK3β activation was higher in the TC vs. WC groups, with the HFD resulting in additional activation in WH group only. In contrast, Akt activity was inhibited in all of the groups when compared to WC (WH vs. WC; TC vs WC; TH vs. TC, as well as in APP/PS1 vs. WT – 2-way ANOVA, $P = 0.0085$). As kinase-mediated Tau hyperphosporylation is one of the principal diagnostic criteria of AD, we have also measured the phosphorylation state of the Tau protein at Ser^{404}. HFD treatment resulted in a significant increase in pSer^{404}-Tau in the WH vs. WC group, an increase which was comparable to the levels observed in the hippocampus of the APP/PS1 animals (Fig. 4). Collectively, our data suggest that the HFD treatment shares some of the features of the distal insulin signaling abnormalities observed in AD-like model.

3.5. Amyloid degrading enzymes (ADE) and HFD

Peripheral hyperinsulinemia and insulin resistance disrupts insulin transport into the CNS, resulting in the reduction of brain insulin levels. Under the normal circumstances, excess insulin is removed by Insulin Degrading Enzyme (IDE), increased expression of which forms a part of a negative feedback loop triggered by insulin itself. Thus, low local insulin levels will result in the reduced expression of IDE. As expected, we have observed a significant reduction in the protein levels of IDE in the hippocampi of both WH and TH mice (Fig. 5). However, IDE may also participate in β-amyloid clearance, and the reduction in its levels may potentially exacerbate APP/PS1 phenotype. Neprilysin is another well-known ADE that had been implicated in Aβ degradation. Interestingly, we detected a significant upregulation of neprilysin levels in TH vs. TC group (Fig. 5), suggesting a possible compensatory mechanism to counteract elevated amyloid load in HFD-treated APP/PS1 mice.

3.6. Mitochondrial metabolism is altered in the hippocampi of HFD-fed WT and APP/PS1 mice

So far, we have mainly focused on the insulin route and have not discussed the implications of altered insulin signaling on mitochondrial homeostasis. As mitochondria are the principal organelles involved in cell metabolism, we measured hippocampal mRNA and protein expression levels of a number of molecules associated with mitochondrial energy status and biogenesis. We did not detect changes in the mRNA expression of α- catalytic subunits of one of the major kinases activated in response to ATP depletion – AMPK (*Prkaa1* and *Prkaa2* levels were not affected in any of the groups). However, we observed a marginal but significant reduction in response to HFD in a key transcriptional

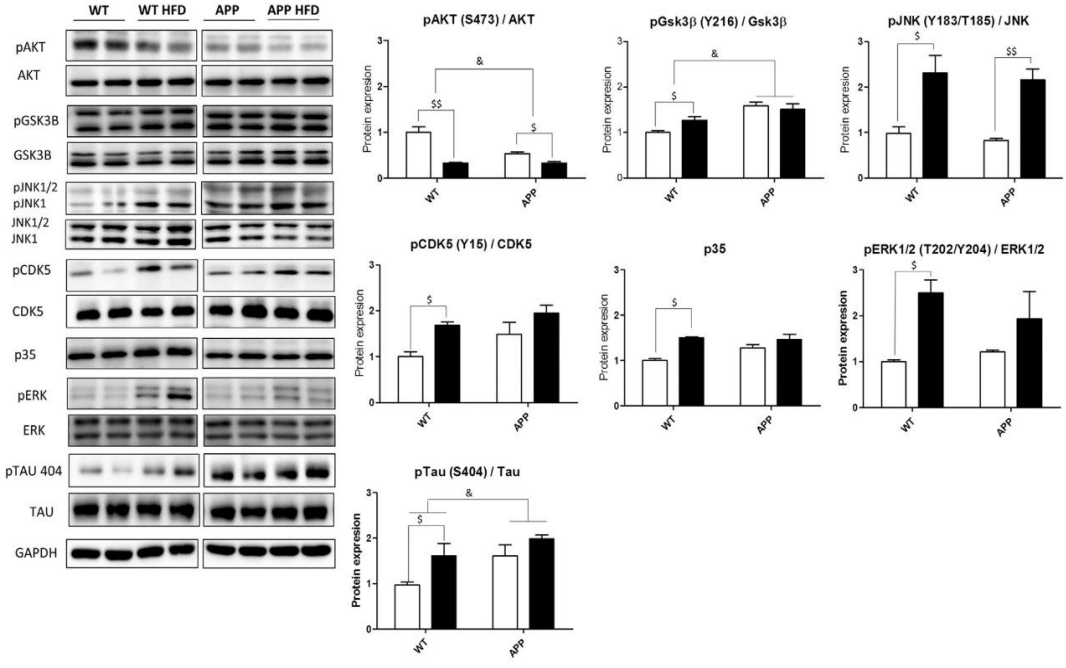


Fig. 4. Representative GAPDH-normalized immunoblot images and quantification ($n = 4–6$ independent samples per group) of molecules related to distal insulin signaling in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$; $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$); open bar: chow, closed bar: high-fat diet.

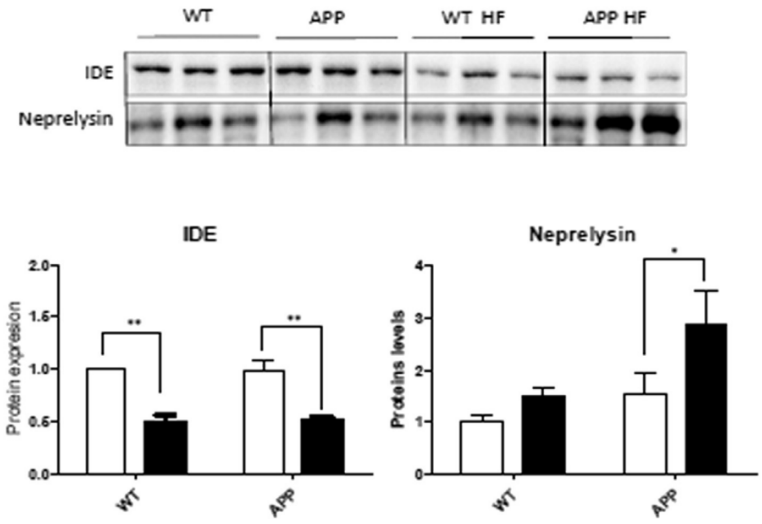


Fig. 5. Representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of enzymes implicated in β-amyloid degradation in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with one-way ANOVA with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

co-regulator of mitochondrial biogenesis PGC-1α, which is partly regulated by AMPK (*ppargc1a* was reduced in APP/PS1 vs. WT groups; 2-way ANOVA, P = 0.0144; PGC-1α was reduced in WH vs. WC and TH vs. TC, P = 0.0044 and P = 0.0022, respectively) (Fig. 6A, B).

We have also determined the expression levels of PGC-1α co-regulated transcription factors, including peroxisome proliferator-activated receptors α (PPAR-α) and γ (PPAR-γ), mRNA and protein levels of which remained unchanged (Fig. 6A, B). Differences in the mRNA expression of the nuclear respiratory factors 1 (*nrf1*) and 2 (*nrf2*) between the groups were very small, even though statistically significant, and were comparable to the changes observed in estrogen-related receptor α (*esrra*) and mitochondrial transcription factor A (*tfam*) transcripts (Fig. 6A). At the protein level, we detected a significant reduction of TFAM in WH vs. WC and TH vs. TC groups (Fig. 6B).

In addition, we evaluated mitochondrial function impairment via immunoblotting analysis of OXPHOS complexes. Our results demonstrated significant reduction in OXPHOS I, II, III and IV in the hippocampi of 6-months-old WH vs. WC mice. All of the OXPHOS complexes were down regulated in the basal state in TC vs. WC animals, and were not further reduced in response to HFD treatment (Fig. 7). Taken together, our data indicate significant perturbations in cellular energy metabolism in the brains of HFD-treated and APP/PS1 mice.

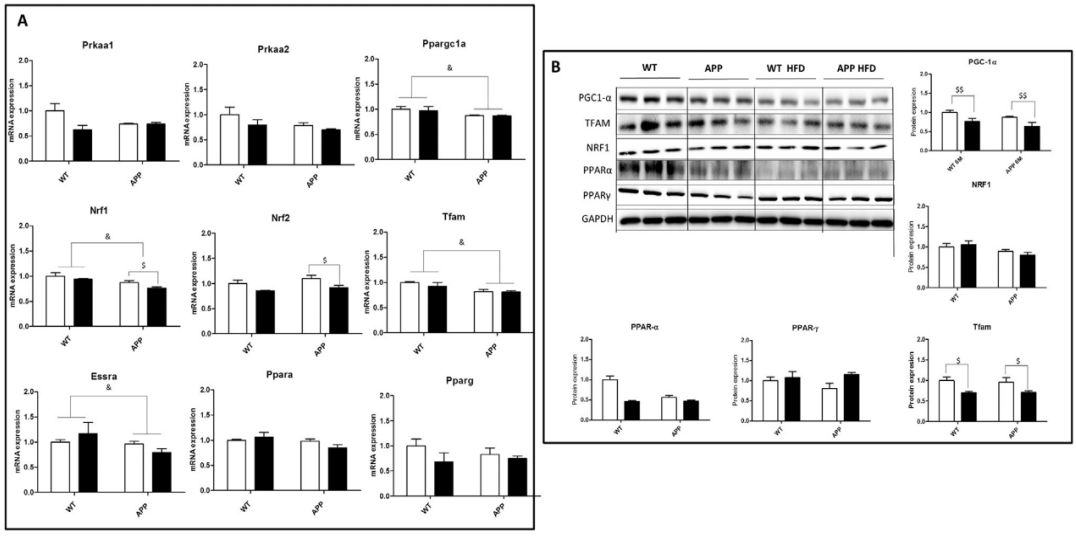


Fig. 6. (A) mRNA expression profile (n = 4–6 independent samples per group, with 3 technical replicates per sample) and (B) representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of molecules related to distal insulin signaling and mitochondrial homeostasis in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$; regular 2-way ANOVA, where & denotes $p < 0.05$); open bar: chow, closed bar: high-fat diet.

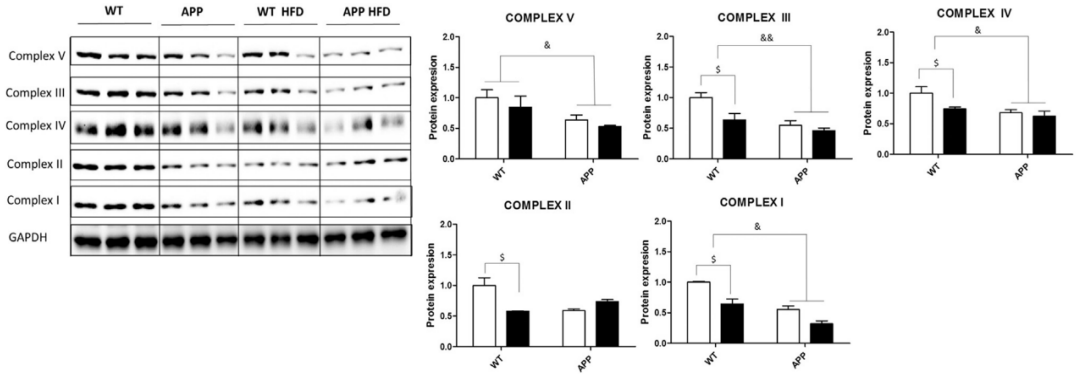


Fig. 7. Representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of mitochondrial OXPHOS complexes in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

4. Discussion

The original "amyloid cascade" hypothesis postulated over 20 years ago in a seminal *Science* paper by Hardy and Higgins [10] has undoubtedly led to major breakthroughs in understanding the pathophysiology of AD. At its core, the hypothesis defined the cascade as a sequence of events starting from abnormal β-amyloid peptide processing leading to β-amyloid deposition, tau phosphorylation and neurofibrillary tangle formation, all of which ultimately result in cellular dysfunction and neuronal death [11]. Since then, significant research efforts on the part of academic labs and the pharmaceutical industry have largely focused on the amyloid cascade as the principal target for AD treatment. Unfortunately, none of the molecules that reached Phase II and III clinical trials targeting this pathway have proven to be more effective than placebo at treating AD (reviewed in [36]). Mounting criticism of the amyloid cascade hypothesis has recently prompted John Hardy himself to address the issues plaguing AD drug development [37]. The general failure of the amyloidocentric approach in pharmaceutical development strongly suggests that the β-amyloid is unlikely to be solely responsible for AD progression.

It has been long suspected that mitochondria play a significant role in neurodegenerative diseases [38–40]. By 2004, Swerdlow & Khan [41] proposed a comprehensive model which takes into account mitochondrial alterations occurring during the course of AD. The "mitochondrial cascade" hypothesis suggests that the cause and effect relationship in AD are reversed when pitted against an "amyloid cascade" hypothesis. The theory implies that the damage to the mitochondria occurs prior to the β-amyloid accumulation. This view has recently been expanded [42–44] and is supported by a number of studies (reviewed in [45]) which classify AD as a primarily metabolic disorder. According to this view, dementia develops as a result of the diminished ability of the brain to efficiently utilize the available glucose, leading to reduced neuronal plasticity which affects cerebral capacity to form and retain memories.

In the present paper, we present results that reinforce the hypothesis that AD may be viewed as a metabolic disorder, with the disease neuropathology at least partially related to insulin signaling failure and energy depletion in hippocampal neurons. Our data notwithstanding, before we continue the discussion regarding the implications of insulin signaling and mitochondrial regulation on hippocampal memory, we would like to address some of the discrepancies in peripheral metabolic parameters in our results compared to previously published studies. Ramos-Rodriguez and colleagues recently reported that a similar HFD treatment did not result in an increase in basal fasting blood glucose levels, nor in plasma triglycerides, but instead increased plasma cholesterol in 6 months-old APP/PS1 animals [46]. Our data clearly demonstrate elevated basal blood glucose in TH group, an increase in total blood triglyceride levels in WH vs. WC and in TH vs. TC groups, and no changes in cholesterol levels. Regarding blood glucose measurements, our methodology is very similar with 16 hours-long fast applied in both studies. However, the glucose values approximating 60 mg/dl detected by Ramos-Rodriguez *et al*, suggest that the measurements were taken at room temperature, which may explain the observed differences in the basal glucose levels in WC animals (~80 mg/dl in our study), as our tests were conducted at 28-29C [32]. As for the differences in cholesterol and triglycerides levels, these can be easily explained by the different diets used (60% Kcal obtained mainly from animal fat (lard) in Ramos-Rodriguez *et al*, vs. 45% Kcal mainly from hydrogenated coconut oil (vegetable fat) in our study). Interestingly, increases in plasma insulin levels in mice treated with either diet were comparable. Taken together, our data indicate that metabolic syndrome is indeed present in our model.

The connection between AD, obesity and type-2 diabetes has been known by epidemiologists for some time now. In fact, as early as 2004, in a study carried out at the Mayo clinic, investigators reported either impaired fasting glucose or diabetes in 81% of human subjects suffering from AD [47]. However, it is only recently that we are beginning to understand the pathophysiological processes linking peripheral metabolic abnormalities and neuronal dysfunction. Defective insulin signaling and mitochondrial redox imbalances appear to play a major role in the development of both the metabolic syndrome and AD. Crucially, it has been suggested that the insulin-dependent signaling in the brain may potentially be regulated via the pathways independent of its glucoregulatory functions in the periphery [48–50]. In the present study, we compared molecular pathways involved in memory loss in mice as a result of a HFD treatment, with animals which lose memory as a consequence of an APP/PS1 genotype. It was reported previously that the continuous exposure of juvenile (3-weeks-old) mice to a HFD causes relational memory loss (hippocampus-dependent) in a significant number of animals by 5 months of age [51]. We have chosen a similar experimental approach, but have focused on 6-months-old animals, with our data indicating that virtually all HFD-fed WT mice demonstrate pronounced memory loss at this age, as measured by NOR test. Importantly, 6-months-old APP/PS1 control animals present with similar memory deficits when compared to WH group. This has permitted us to make direct comparisons between the treated and untreated WT and APP/PS1 animals. One of the intriguing findings in our study is that the HFD-induced memory loss appears to be independent of the levels of cerebral β-amyloid peptide in mice not predisposed to abnormal βA processing (even though we detected a significant increase in insoluble βA_{1-42} levels in cortical homogenates in TH vs. TC group).

In our experiments, HFD treatment produces metabolic syndrome which includes peripheral hyperinsulinemia, peripheral insulin resistance, peripheral and central hyperglycemia and dyslipidemia among others. So, what are the components of metabolic syndrome that result in memory loss in HFD-fed WT animals? Assuming that the insulin is involved, we need to consider that one of the key differences in brain insulin signaling between AD and diet-induced obesity is the availability/quantity of insulin hormone in the brain. Diet-induced obesity results in the disruption of the Blood-Brain-Barrier (BBB), which limits receptor-mediated insulin transport to the CNS. Therefore, somewhat counterintuitively, peripheral hyperinsulinemia actually provokes insulin deficiency in the brain [52–56]. On the other hand, in AD, cerebral insulin resistance has been widely implicated in disease pathogenesis (reviewed in [48]). While the distinction between insulin deficiency and insulin resistance may seem trivial to some authors in the neuroscience field (to the point that a large number of studies use both terms interchangeably), it may have a significant impact on potential treatment strategies. For example, in an insulin-resistant (at the CNS level) non-obese AD patient, it may actually be beneficial to choose an oral anti-diabetic drug treatment as a means for improving AD symptoms. Conversely, the same treatment in an obese patient may not have the desired effects due to the inability of the extra insulin to cross BBB, in which case the intranasal administration may provide better outcomes. Regardless of the root cause, if we consider insulin signaling perturbations to be one of the contributors to memory loss and AD-like symptoms in our model, then both the proximal and distal insulin signaling pathways should be altered in the hippocampal region. Proximal insulin signaling consists of an insulin-IR/IGF-1R-IRS axis, whereby the binding of the insulin to the extracellular alpha subunit of the IR or IGF-1R initiates a series of Tyr autophosphorylations in the beta subunit, disinhibiting intracellular Tyr kinase activity towards IRS, thereby allowing these adaptor molecules to interact with a large number of targets. Once activated, IRS1 and 2 are further regulated via a highly complex mechanism involving multiple Ser/Thr kinases, which can phosphorylate the tail regions of IRS molecules at over 50 Ser/Thr residues. Unlike Tyr phosphorylation by the IR, which activates IRS, modifications at Ser/Thr residues are capable of both promoting and inhibiting downstream IRS-mediated signaling [57]. As previously mentioned, cerebral insulin resistance has been implicated in AD pathogenesis. Such a resistance may stem from defects in IR itself [58], or may be mediated via negative regulation of the IRS. Our data suggest that the IR is functional in the hippocampi of 6 months-old HFD-treated WT and APP/PS1 mice. Furthermore, we have also detected a significant increase in *igf1* transcripts in WH vs. WC animals, possibly in response to cerebral insulin deficiency. We then considered the possibility that the downregulation of the IRS pathway, via inhibitory Ser/Thr phosphorylation, may attenuate downstream insulin signaling. As it is nearly impossible to determine the phosphorylation state of all of the 50+ Ser/Thr residues of the IRS molecules within the scope of a single paper, we have performed immunoblotting analysis of mouse pSer^{612}-IRS1 and pSer^{723}-IRS2 (corresponding to human pSer^{616}-IRS1 and pSer^{731}-IRS2). Elevated levels of pSer^{616}-IRS1 were previously detected in neurons of AD patients and were associated with insulin resistance [59], and an increase in pSer^{723}-IRS2 protein levels was observed in dorsal root ganglia neurons of diabetic mice [60]. As indicated in our findings, there were no differences in pSer^{612}-IRS1 for any groups. However, we detected a significant increase in hippocampal pSer^{723}-IRS2 in WH, TC and TH mice. While our analysis is by no means exhaustive, it does support the hypothesis that the hippocampus-dependent memory loss is, at least in part, dependent on insulin signaling deficiencies.

Ser/Thr phosphorylation of the IRS molecules is mediated by both insulin-dependent (autologous) and insulin-independent (heterologous) kinases. Autologous regulation is thought to be the predominant form of downstream insulin-mediated signaling under physiological conditions. In a disease state, however, preferential activation of the heterologous pathway may contribute to the underlying pathology. Cellular stress and/or proinflammatory phenotype may cause inappropriate Ser/Thr modifications of IRS, which may result in the "hijacking" of the normal physiological route. In the current study, we measured hippocampal expression of IRS-regulating Ser/Thr kinases, which are also known to play a role in neurodegenerative diseases. These include autologous (Akt, ERK 1/2), heterologous capable of phosphorylating IRS in the basal cellular state (AMPK, GSK3β), as well as heterologous kinases activated in response to cellular stress and sympathetic activation (JNK1) [57].

4.1. Insulin-IRS-Akt-GSK-3β pathway

Apart from the involvement of insulin, insulin-related molecules and their receptors in the cognitive loss observed in HFD-fed animals, we would like to further discuss key pathways and molecules related to insulin signaling. This pathway is one of the major regulators of distal insulin signaling. Activated IRS recruits PI3K, thus activating downstream signaling cascade involving 3-phosphoinositide-dependent protein kinase-1 (PDK1), Akt and GSK-3β. In the context of glucose regulation, GSK-3β promotes glycogen synthesis, but it is also one of the principal kinases responsible for Tau phosphorylation [61]. Successful activation of Akt requires not only phosphorylation at Thr308 by PDK1, but also additional "priming" at Ser473 residue by mammalian target of rapamycin (mTOR) complex [62]. The activity of GSK-3β is, in turn, negatively regulated by phosphorylation at Ser9 residue by activated Akt, as well as positively regulated by autophosphorylation at Tyr216 [63]. Our data demonstrate both HFD- and transgene-dependent decrease in hippocampal pSer^{473}-Akt levels, accompanied by an increase in activated pTyr^{216}-GSK-3β protein levels in 6-months-old mice. Furthermore, we have also observed an upregulation in pSer^{404}-Tau, which suggests abnormalities in IRS-Akt-GSK-3β signaling. These results are in line with previously published postmortem examinations of the brains of human patients suffering from AD and diabetes [64].

4.2. AMPK, PPAR and ERK

ERK has been implicated as a key molecule involved in hippocampal memory consolidation. While directly stimulated by insulin, it may also be partially activated via IRS-independent mechanisms [57], thus offering a potentially attractive modulatory target for the pharmaceutical intervention. ERK acts via ERK/CREB/CBP pathway, activation of which ultimately results in the transcription of genes required for neuronal plasticity and long-term potentiation (LTP), in particular. In the brain, ERK was shown to be positively regulated by AMPK [65], a key energy sensor in the peripheral tissues, the role of which in the CNS remains controversial [66]. In addition, activated ERK was shown to be recruited (thus activating) to PPARγ, a nuclear receptor and a transcription factor which activates a number of genes related to insulin sensitivity and cognition [67]. We did not detect any changes in the α subunit of the AMPK, nor in PPARα and γ levels, but we did observe an increase in pThr^{202}/pTyr^{204}-ERK1/2 in response to HFD treatment. Considering that ERK expression is positively correlated with memory consolidation our results may seem contradictory, however this is not the case. Feld *et al* [68] have recently demonstrated that a minimum threshold of ERK expression is required to maintain memory function, and that the aberrant overexpression of ERK protein leads to memory impairment in a similar manner as ERK deficiency.

4.3. JNK1

This stress activated pro-inflammatory and pro-apoptotic MAPK has been previously linked to neurodegeneration and to AD, albeit the existing evidence for its importance is rather strenuous (reviewed in [69]). There is much more support for the involvement of JNK1 in diet-induced obesity and the peripheral insulin resistance (reviewed

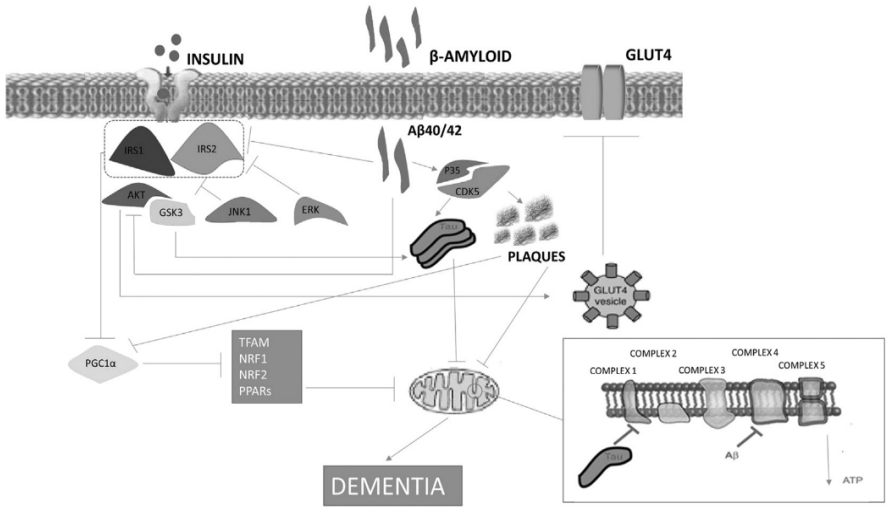


Fig. 8. A proposed mechanism whereby insulin signaling and mitochondrial dysregulation contribute to hippocampal phenotype in HFD-fed and AD-like mice. Increased circulating triglyceride levels in response to HFD treatment initially provoke cerebral insulin deficiency, which results in the downregulation of the canonical insulin signaling pathway in the hippocampus. At the same time, the free fatty acid-mediated increased metabolic stress subverts autologous IR-IRS-Ser/Thr kinase axis signaling, thus favoring heterologous regulation. This route, once activated, initiates a series of self-propagating events which ultimately lead to insulin resistance in a manner similar to that observed in response to elevated β-amyloid levels. As functional IR signaling in the hippocampus is, at least partially, regulating neuronal glucose entry [74], the inhibition of this pathway may result in the reduced supply of readily available energy to the mitochondria, affecting neuroplasticity. Mitochondrial OXPHOS metabolism deficiencies may thus be explained by two possible mechanisms: (a) as a direct result of reduced glucose availability, and (b) as a consequence of decreased mitochondrial biogenesis (PGC1) and/or disruptions to mitochondrial function due to hyperphosphorylated Tau protein and increased β-amyloid levels.

in [70]). The fact that we detected a significant increase in $pThr^{183}$/$pTyr^{185}$-JNK1 in the hippocampi of HFD-fed mice, irrespective of the β-amyloid load, is curious for two reasons. One: it suggests that hippocampal insulin signaling may in fact be strikingly similar to the peripheral phenotype. Two: very high levels of β amyloid, as observed in 6-months-old APP/PS1 control animals, are not sufficient to trigger elevated JNK1 expression.

4.4. CDK5

Cyclin-dependent kinase 5 (co-activated by p35) does not phosphorylate IRS directly, however increased activity of this kinase (and GSK-3β) contributes to Tau hyperphoshorylation, resulting in neurofibrillary tangle formation (reviewed in [71]). We have detected a significant upregulation of both the CDK5 and GSK-3β kinases in the hippocampi of HFD-fed WT mice. These data correlate well with the observed increase in $pSer^{404}$-Tau.

4.5. PGC-1α

Our data demonstrate a significant decrease in insulin signaling and impaired glucose homeostasis in the hippocampus. As hippocampal neurons require copious amounts of energy in order to be able to form and retain memories, such deficiencies will likely have an effect on mitochondrial energy metabolism. Therefore, in the latter part of this study, we have focused on the pathways related to mitochondrial biogenesis and OXPHOS pathway.

The peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a transcriptional co-activator which, apart from other functions, plays a critical role in mitochondrial biogenesis. PGC-1α was previously shown to regulate the expression, either directly or indirectly, of the nuclear respiratory factors 1 (NRF1) and 2 (NRF 2), estrogen-related receptor α (ESRRa) and mitochondrial transcription factor A (TFAM) (reviewed in [72]). While we have observed a significant reduction in PGC-1α protein levels in response to HFD treatment, and various statistically significant changes in the above mentioned molecules, most of the differences were rather modest in numerical values, and may or may not represent the behavior of individual targets under similar experimental conditions. Taken together, however, our data suggest clear impairments in mitochondrial function. Regarding the expression of OXPHOS proteins, we detected significant decreases in mitochondrial OXPHOS metabolism in both the HFD-fed WT mice and in APP/PS1 animals. This finding is especially significant as OXPHOS deregulation has been previously linked to both the βA and Tau pathologies in AD brains (reviewed by [73]).

In conclusion, we have demonstrated some parallels between the hippocampus-dependent HFD-induced memory loss vs. the memory loss occurring in a mouse model of Alzheimer disease. Our results indicate that the brain β-amyloid levels seem not to be the primary cause of the HFD-induced memory perturbations. It appears that the reductions in brain insulin signaling and the resulting mitochondrial dysfunction are among the key culprits leading to cognitive decline in early-stage AD-like rodent models. A summarized view of our hypothesis is provided in Fig. 8.

Disclosure statement

The authors declare no competing financial interests.

Conflict of interest

All authors don't have any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations. All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

Acknowledgments

This study was funded by grant 2009/SGR00853 from the Generalitat de Catalunya (autonomous government of Catalonia), by grants SAF2011-23631, SAF2012-39852-C02-01 and SAF2012-30708 from the Spanish Ministerio de Ciencia e Innovación. Grant 0177594 from the CONACYT (Mexico). Project "Prometeo" from SENESCYT (Government of Ecuador).

References

[1] W.B. Traill, M. Mazzocchi, B. Shankar, D. Hallam, Importance of government policies and other influences in transforming global diets, Nutr. Rev. 72 (2014) 591–604, http://dx.doi.org/10.1111/nure.12134.
[2] E. a Finkelstein, D.S. Brown, L. a Wrage, B.T. Allaire, T.J. Hoerger, Individual and aggregate years-of-life-lost associated with overweight and obesity, Obesity 18 (2010) 333–339, http://dx.doi.org/10.1038/oby.2009.253.
[3] a E. Field, E.H. Coakley, a Must, J.L. Spadano, N. Laird, W.H. Dietz, et al., Impact of overweight on the risk of developing common chronic diseases during a 10-year period, Arch. Intern. Med. 161 (2001) 1581–1586.
[4] M. Kivipelto, T. Ngandu, L. Fratiglioni, M. Viitanen, I. Kåreholt, B. Winblad, et al., Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease, Arch. Neurol. 62 (2005) 1556–1560, http://dx.doi.org/10.1001/archneur.62.10.1556.
[5] M.A. Beydoun, H.A. Beydoun, Y. Wang, Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis, Obes. Rev. 9 (2008) 204–218, http://dx.doi.org/10.1111/j.1467-789X.2008.00473.x.
[6] A.L. Fitzpatrick, L.H. Kuller, O.L. Lopez, P. Diehr, E.S. O'Meara, W.T. Longstreth, et al., Midlife and late-life obesity and the risk of dementia: cardiovascular health study, Arch. Neurol. 66 (2009) 336–342, http://dx.doi.org/10.1001/archneurol.2008.582.
[7] B. Nepal, L.J. Brown, K.J. Anstey, Rising midlife obesity will worsen future prevalence of dementia, PLoS One 9 (2014) e99305, http://dx.doi.org/10.1371/journal.pone.0099305.
[8] D.E. Barnes, K. Yaffe, The projected effect of risk factor reduction on Alzheimer's disease prevalence, Lancet Neurol. 10 (2011) 819–828, http://dx.doi.org/10.1016/S1474-4422(11)70072-2.
[9] S. Norton, F.E. Matthews, D.E. Barnes, K. Yaffe, C. Brayne, Potential for primary prevention of Alzheimer's disease: an analysis of population-based data, Lancet Neurol. 13 (2014) 819–828, http://dx.doi.org/10.1016/S1474-4422(11)70072-2.
[10] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, Science 256 (1992) 184–185.
[11] J. Hardy, D. Allsop, Amyloid deposition as the central event in the aetiology of Alzheimer's disease, Trends Pharmacol. Sci. 12 (1991) 383–388.
[12] S.T. Ferreira, W.L. Klein, The Aβ oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease, Neurobiol. Learn. Mem. 96 (2011) 529–543, http://dx.doi.org/10.1016/j.nlm.2011.08.003.
[13] E. Steen, B.M. Terry, E.J. Rivera, J.L. Cannon, T.R. Neely, R. Tavares, et al., Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? J. Alzheimers Dis. 7 (2005) 63–80.
[14] X. Zhu, G. Perry, M. a Smith, Insulin signaling, diabetes mellitus and risk of Alzheimer disease, J. Alzheimers Dis. 7 (2005) 81–84.
[15] S.M. de la Monte, J.R. Wands, Alzheimer's disease is type 3 diabetes – evidence reviewed, J. Diabetes Sci. Technol. 2 (2008) 1101–1113, http://dx.doi.org/10.1177/193229680800200619.
[16] M. Hokama, S. Oka, J. Leon, T. Ninomiya, H. Honda, K. Sasaki, et al., Altered expression of diabetes-related genes in Alzheimer's disease brains: the hisayama study, Cereb. Cortex 24 (2014) 2476–2488, http://dx.doi.org/10.1093/cercor/bht101.
[17] G.V.W. Johnson, Tau phosphorylation and proteolysis: insights and perspectives, J. Alzheimers Dis. 9 (2006) 243–250.
[18] B. Kim, C. Backus, S. Oh, J.M. Hayes, E.L. Feldman, Increased tau phosphorylation and cleavage in mouse models of type 1 and type 2 diabetes, Endocrinology 150 (2009) 5294–5301, http://dx.doi.org/10.1210/en.2009-0695.
[19] Y. Li, K.B. Duffy, M.A. Ottinger, B. Ray, J.A. Bailey, H.W. Holloway, et al., GLP-1 receptor stimulation reduces amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease, J. Alzheimers Dis. 19 (2010) 1205–1219, http://dx.doi.org/10.3233/JAD-2010-1314.
[20] M.F. White, IRS2 integrates insulin/IGF1 signalling with metabolism, neurodegeneration and longevity, Diabetes Obes. Metab. 16 (Suppl. 1) (2014) 4–15, http://dx.doi.org/10.1111/dom.12347.
[21] S.M. Gold, I. Dziobek, V. Sweat, a. Tirsi, K. Rogers, H. Bruehl, et al., Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes, Diabetologia 50 (2007) 711–719, http://dx.doi.org/10.1007/s00125-007-0602-7.
[22] H. Bruehl, O.T. Wolf, V. Sweat, A. Tirsi, S. Richardson, A. Convit, Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus, Brain Res. 1280 (2009) 186–194, http://dx.doi.org/10.1016/j.brainres.2009.05.032.
[23] X. Wang, W. Zheng, J.-W. Xie, T. Wang, S.-L. Wang, W.-P. Teng, et al., Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model, Mol. Neurodegener. 5 (2010) 46, http://dx.doi.org/10.1186/1750-1326-5-46.
[24] R. Ravona-Springer, E. Moshier, J. Schmeidler, J. Godbold, J. Akrivos, M. Rapp, et al., Changes in glycemic control are associated with changes in cognition in non-diabetic elderly, J. Alzheimers Dis. 30 (2012) 299–309, http://dx.doi.org/10.3233/JAD-2012-120106.
[25] S. Hoyer, Glucose metabolism and insulin receptor signal transduction in Alzheimer disease, Eur. J. Pharmacol. 490 (2004) 115–125, http://dx.doi.org/10.1016/j.ejphar.2004.02.049.
[26] G.D. Femminella, P. Edison, Evaluation of neuroprotective effect of glucagon-like peptide 1 analogs using neuroimaging, Alzheimers Dement. 10 (2014) S55–S61, http://dx.doi.org/10.1016/j.jalz.2013.12.012.
[27] E.C. McNay, A.K. Recknagel, Reprint of: "Brain insulin signaling: A key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes,", Neurobiol. Learn. Mem. 96 (2011) 517–528, http://dx.doi.org/10.1016/j.nlm.2011.11.001.
[28] C. Hölscher, Drugs developed for treatment of diabetes show protective effects in Alzheimer ' s and Parkinson ' s diseases, Acta Phys. Sin. 66 (2014) 497–510, http://dx.doi.org/10.13294/j.aps.2014.0059.
[29] G. Castro, M.F.C. Areias, L. Weissmann, P.G.F. Quaresma, C.K. Katashima, M.J. a Saad, et al., Diet-induced obesity induces endoplasmic reticulum stress and insulin resistance in the amygdala of rats, FEBS Open Bio 3 (2013) 443–449, http://dx.doi.org/10.1016/j.fob.2013.09.002.
[30] H. Oh, S. Boghossian, D. a York, M. Park-York, The effect of high fat diet and saturated fatty acids on insulin signaling in the amygdala and hypothalamus of rats. Brain Res. 1537 (2013) 191–200, http://dx.doi.org/10.1016/j.brainres.2013.09.025.
[31] S.E. Arnold, I. Lucki, B.R. Brookshire, G.C. Carlson, C. a Browne, H. Kazi, et al., High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice, Neurobiol. Dis. 67 (2014) 79–87, http://dx.doi.org/10.1016/j.nbd.2014.03.011.
[32] J.E. Ayala, V.T. Samuel, G.J. Morton, S. Obici, C.M. Croniger, G.I. Shulman, et al., Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice, Dis. Model. Mech. 3 (2010) 525–534, http://dx.doi.org/10.1242/dmm.006239.
[33] I. Pedrós, D. Petrov, M. Allgaier, F. Sureda, E. Barroso, C. Beas-Zarate, et al., Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease, Biochim. Biophys. Acta 1842 (2014) 1556–1566, http://dx.doi.org/10.1016/j.bbadis.2014.05.025.
[34] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987) 156–159, http://dx.doi.org/10.1006/abio.1987.9999.
[35] D. Porquet, C. Griñán-Ferré, I. Ferrer, A. Camins, C. Sanfeliu, J. Del Valle, et al., Neuroprotective role of trans-resveratrol in a murine model of familial Alzheimer's disease, J. Alzheimers Dis. 42 (2014) 1209–1220, http://dx.doi.org/10.3233/JAD-140444.
[36] L.S. Schneider, F. Mangialasche, N. Andreasen, H. Feldman, E. Giacobini, R. Jones, et al., Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014, J. Intern. Med. 275 (2014) 251–283, http://dx.doi.org/10.1111/joim.12191.
[37] E. Karran, J. Hardy, A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease, Ann. Neurol. 76 (2014) 185–205, http://dx.doi.org/10.1002/ana.24188.
[38] A.H. Schapira, Oxidative stress and mitochondrial dysfunction in neurodegeneration, Curr. Opin. Neurol. 9 (1996) 260–264.
[39] J.B. Schulz, R.T. Matthews, T. Klockgether, J. Dichgans, M.F. Beal, The role of mitochondrial dysfunction and neuronal nitric oxide in animal models of neurodegenerative diseases, Mol. Cell. Biochem. 174 (1997) 193–197.
[40] M.P. Mattson, Mother's legacy: mitochondrial DNA mutations and Alzheimer's disease, Trends Neurosci. 20 (1997) 373–375.
[41] R.H. Swerdlow, S.M. Khan, A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease, Med. Hypotheses 63 (2004) 8–20, http://dx.doi.org/10.1016/j.mehy.2003.12.045.
[42] R.H. Swerdlow, J.M. Burns, S.M. Khan, The Alzheimer's disease mitochondrial cascade hypothesis, J. Alzheimers Dis. 20 (Suppl. 2) (2010) S265–S279, http://dx.doi.org/10.3233/JAD-2010-100339.
[43] R.H. Swerdlow, Mitochondria and cell bioenergetics: increasingly recognized components and a possible etiologic cause of Alzheimer's disease, Antioxid. Redox Signal. 16 (2012) 1434–1455, http://dx.doi.org/10.1089/ars.2011.4149.
[44] J.E. Selfridge, E. Lezi, J. Lu, R.H. Swerdlow, Role of mitochondrial homeostasis and dynamics in Alzheimer's disease, Neurobiol. Dis. 51 (2013) 3–12, http://dx.doi.org/10.1016/j.nbd.2011.12.057.
[45] L. a Demetrius, J. Driver, Alzheimer's as a metabolic disease, Biogerontology 14 (2013) 641–649, http://dx.doi.org/10.1007/s10522-013-9479-7.
[46] J.J. Ramos-Rodriguez, O. Ortiz-Barajas, C. Gamero-Carrasco, P.R. de la Rosa, C. Infante-Garcia, N. Zopeque-Garcia, et al., Prediabetes-induced vascular alterations exacerbate central pathology in APPswe/PS1dE9 mice, Psychoneuroendocrinology 48 (2014) 123–135, http://dx.doi.org/10.1016/j.psyneuen.2014.06.005.
[47] J. Janson, T. Laedtke, J.E. Parisi, P. O'Brien, R.C. Petersen, P.C. Butler, Increased risk of type 2 diabetes in Alzheimer disease, Diabetes 53 (2004) 474–481.
[48] F.G. De Felice, Alzheimer's disease and insulin resistance: translating basic science into clinical applications, J. Clin. Invest. 123 (2013) 531–539, http://dx.doi.org/10.1172/JCI64595.
[49] F.G. De Felice, S.T. Ferreira, Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease, Diabetes 63 (2014) 2262–2272, http://dx.doi.org/10.2337/db13-1954.
[50] S. Takeda, N. Sato, H. Rakugi, R. Morishita, Molecular mechanisms linking diabetes mellitus and Alzheimer disease: beta-amyloid peptide, insulin signaling, and

neuronal function, Mol. BioSyst. 7 (2011) 1822–1827, http://dx.doi.org/10.1039/c0mb00302f.
[51] C. Boitard, N. Etchamendy, J. Sauvant, A. Aubert, S. Tronel, A. Marighetto, et al., Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice, Hippocampus 22 (2012) 2095–2100, http://dx.doi.org/10.1002/hipo.22032.
[52] K.J. Kaiyala, R.L. Prigeon, S.E. Kahn, S.C. Woods, M.W. Schwartz, Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs, Diabetes 49 (2000) 1525–1533.
[53] D.P. Begg, J.D. Mul, M. Liu, B.M. Reedy, D. a D'Alessio, R.J. Seeley, et al., Reversal of diet-induced obesity increases insulin transport into cerebrospinal fluid and restores sensitivity to the anorexic action of central insulin in male rats, Endocrinology 154 (2013) 1047–1054, http://dx.doi.org/10.1210/en.2012-1929.
[54] M. Heni, P. Schöpfer, A. Peter, T. Sartorius, A. Fritsche, M. Synofzik, et al., Evidence for altered transport of insulin across the blood-brain barrier in insulin-resistant humans, Acta Diabetol. 51 (2014) 679–681, http://dx.doi.org/10.1007/s00592-013-0546-y.
[55] S.E. Kanoski, T.L. Davidson, Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity, Physiol. Behav. 103 (2011) 59–68, http://dx.doi.org/10.1016/j.physbeh.2010.12.003.
[56] W. a Banks, J.B. Owen, M. a Erickson, Insulin in the brain: there and back again, Pharmacol. Ther. 136 (2012) 82–93, http://dx.doi.org/10.1016/j.pharmthera.2012.07.006.
[57] K.D. Copps, M.F. White, Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2, Diabetologia 55 (2012) 2565–2582, http://dx.doi.org/10.1007/s00125-012-2644-8.
[58] J.E. Pessin, A.R. Saltiel, Signaling pathways in insulin action: molecular targets of insulin resistance, J. Clin. Invest. 106 (2000) 165–169, http://dx.doi.org/10.1172/JCI10582.
[59] A.M. Moloney, R.J. Griffin, S. Timmons, R. O'Connor, R. Ravid, C. O'Neill, Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling, Neurobiol. Aging 31 (2010) 224–243, http://dx.doi.org/10.1016/j.neurobiolaging.2008.04.002.
[60] C.W. Grote, J.K. Morris, J.M. Ryals, P.C. Geiger, D.E. Wright, Insulin receptor substrate 2 expression and involvement in neuronal insulin resistance in diabetic neuropathy, Exp. Diabetes Res. 2011 (2011) 212571, http://dx.doi.org/10.1155/2011/212571.
[61] A. Takashima, GSK-3 is essential in the pathogenesis of Alzheimer's disease, J. Alzheimers Dis. 9 (2006) 309–317.
[62] D.D. Sarbassov, D. a Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (2005) 1098–1101, http://dx.doi.org/10.1126/science.1106148.
[63] A. Cole, S. Frame, P. Cohen, Further evidence that the tyrosine phosphorylation of glycogen synthase kinase-3 (GSK3) in mammalian cells is an autophosphorylation event, Biochem. J. 377 (2004) 249–255, http://dx.doi.org/10.1042/BJ20031259.
[64] Y. Liu, F. Liu, I. Grundke-Iqbal, K. Iqbal, C.-X. Gong, Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes, J. Pathol. 225 (2011) 54–62, http://dx.doi.org/10.1002/path.2912.
[65] K.T. Dineley, J.B. Jahrling, L. Denner, Insulin resistance in Alzheimer's disease, Neurobiol. Dis. 72 (2014) 92–103, http://dx.doi.org/10.1016/j.nbd.2014.09.001.
[66] G.V. Ronnett, S. Ramamurthy, A.M. Kleman, L.E. Landree, S. Aja, AMPK in the brain: its roles in energy balance and neuroprotection, J. Neurochem. 109 (2009) 17–23, http://dx.doi.org/10.1111/j.1471-4159.2009.05916.x (Suppl.).
[67] J.B. Jahrling, C.M. Hernandez, L. Denner, K.T. Dineley, PPARγ recruitment to active ERK during memory consolidation is required for Alzheimer's disease-related cognitive enhancement, J. Neurosci. 34 (2014) 4054–4063, http://dx.doi.org/10.1523/JNEUROSCI.4024-13.2014.
[68] M. Feld, M.C. Krawczyk, M. Sol Fustiñana, M.G. Blake, C.M. Baratti, A. Romano, et al., Decrease of ERK/MAPK overactivation in prefrontal cortex reverses early memory deficit in a mouse model of Alzheimer's disease, J. Alzheimers Dis. 40 (2014) 69–82, http://dx.doi.org/10.3233/JAD-131076.
[69] L. Chami, F. Checler, BACE1 is at the crossroad of a toxic vicious cycle involving cellular stress and β-amyloid production in Alzheimer's disease, Mol. Neurodegener. 7 (2012) 52, http://dx.doi.org/10.1186/1750-1326-7-52.
[70] G. Sabio, R.J. Davis, cJun NH2-terminal kinase 1 (JNK1): roles in metabolic regulation of insulin resistance, Trends Biochem. Sci. 35 (2010) 490–496, http://dx.doi.org/10.1016/j.tibs.2010.04.004.
[71] G.S. Desai, C. Zheng, T. Geetha, S.T. Mathews, B.D. White, K.W. Huggins, et al., The pancreas-brain axis: insight into disrupted mechanisms associating type 2 diabetes and Alzheimer's disease, J. Alzheimers Dis. 42 (2014) 347–356, http://dx.doi.org/10.3233/JAD-140018.
[72] S. Austin, J. St-Pierre, PGC1α and mitochondrial metabolism–emerging concepts and relevance in ageing and neurodegenerative disorders, J. Cell Sci. 125 (2012) 4963–4971, http://dx.doi.org/10.1242/jcs.113662.
[73] A. Eckert, K.L. Schulz, V. Rhein, J. Götz, Convergence of amyloid-beta and tau pathologies on mitochondria in vivo, Mol. Neurobiol. 41 (2010) 107–114, http://dx.doi.org/10.1007/s12035-010-8109-5.
[74] C. a Grillo, G.G. Piroli, R.M. Hendry, L.P. Reagan, Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent, Brain Res. 1296 (2009) 35–45, http://dx.doi.org/10.1016/j.brainres.2009.08.005.

CAPÍTULO V. DISCUSIÓN

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

CAPÍTULO V. DISCUSIÓN

La mayoría de las investigaciones sobre la EA se realiza mediante modelos transgénicos de ratón caracterizados por mutaciones que producen un aumento en los niveles de péptidos Aβ, en comparación con los animales control. La función de estos modelos animales es conseguir reproducir los síntomas y las lesiones responsables de la EA. Por lo tanto, constituyen una herramienta decisiva para el conocimiento de las enfermedades neurodegenerativas y permiten el diseño de nuevas estrategias terapéuticas.

Existen modelos animales basados en mutaciones en los genes que regulan el metabolismo de la proteína precursora del amiloide (APP), de la presenilina (PS1/2) o de ambos (APP/PS1). Otros están basados en alteraciones de la proteína tau (tau-JNPL3) e incluso existen triple transgénicos PS/APP/tau. También se dispone de cepas con alteración de la expresión de la neprilisina, una de las principales enzimas que participan en la degradación de Aβ, así como de APOE. Una revisión detallada sobre las características diferenciales de los modelos de ratón de la enfermedad se puede encontrar en la publicación de Bilkei-Gorzo (Bilkei-Gorzo 2014).

En la cepa de ratones transgénicos APP/PS1 se combinan dos mutaciones en los genes *app* y *ps1,* alterando la producción y el procesamiento de la proteína APP. Este hecho provoca un aumento de los niveles de Aβ42 (Kurt et al. 2001; Radde et al. 2006; Bilkei-Gorzo 2014). Dichos animales presentan alteraciones patológicas, como la amiloidogénesis, a las 6-8 semanas de edad y presentan placas en la corteza cingulada y en el hipocampo en el 4-5 mes. También se caracterizan por activación de la glía, alteraciones sinápticas y déficits en el aprendizaje y en la memoria (Holcomb et al. 1998; Savonenko et al. 2005; Garcia-Alloza et al. 2006). El presente trabajo de investigación ha utilizado el ratón APPswe / PS1dE9 (APP / PS1) con la mutación Sueca (K594M/N595L).

Como ampliamente descrito en la introducción, la EA es una patología compleja de etiología todavía desconocida. Aparte del componente genético y de la vejez, se han identificado otros factores de riesgo metabólicos y ambientales que contribuyen a la EA.

Por una parte se encuentran los factores metabólicos, como la obesidad, la hipertensión, la hiperlipidemia y la resistencia a la insulina. Por otra parte, se pueden identificar factores ambientales como el tabaquismo, la depresión y las lesiones cerebrales (Baumgart et al. 2015) .

Varios estudios epidemiológicos correlacionan la EA con comorbilidades metabólicas, aunque los mecanismos moleculares implicados sigan todavía desconocidos. La correlación observada entre las alteraciones en la vía de la

señalización de la insulina y la EA ha sido tal que se ha llegado a denominar la patología como "diabetes tipo 3" (Steen et al. 2005). Según esa hipótesis, no sólo se daría una continua alteración de la señalización de la insulina a nivel periférico, sino también a nivel central. Esa alteración en la señalización de la insulina podría comprometer y alterar la captación de glucosa por parte de las células neuronales, afectando a la producción eficiente de energía en el cerebro a partir de la glucosa (Yao et al. 2011).

En la presente tesis doctoral se presentan tres trabajos. El primero ha tenido como objetivo establecer si la señalización de insulina a nivel periférico y central se ve afectada en el modelo murino de EAf representado por los ratones APP/PS1, antes y después de la formación de las placas amiloides. El segundo trabajo consistió en evaluar los efectos de adipocinas implicadas en el control de la ingesta de alimentos en el hipocampo. Por último, en el tercer trabajo, evaluamos el impacto de la dieta grasa en el proceso de amiloidogénesis tanto en los ratones salvajes como en los transgénicos.

A continuación se realiza una discusión en detalle de los resultados obtenidos siguiendo el siguiente esquema:

1. **Caracterización de modelo de EAf**
   - 1.1. Alteraciones metabólicas periféricas
   - 1.2. Alteraciones en el SNS (hipocampo y corteza)
2. **Efectos de la dieta HFAT en ratones salvajes y APP/PS1 de 6 meses de edad**
   - 2.1. Alteraciones periféricas que produce la dieta HFAT.
   - 2.2. Alteraciones que produce la dieta HFAT en el SNS (hipocampo y corteza).

1. Caracterización de modelo de EAf.

1.1 Alteraciones metabólicas periféricas

Se evaluaron las alteraciones metabólicas en los ratones transgénicos a la edad de 3 y 6 meses, con el fin de detectar si el proceso de amiloidogénesis podía estar relacionado con alteraciones en el metabolismo de la glucosa.

Por lo que se refiere a los niveles de insulina en sangre a los tres meses de edad, no se observaron diferencias significativas entre las dos cepas; un ligero incremento se observó en la curva de tolerancia a la glucosa (IP-GTT) pero las diferencias entre las AUC no resultaron significativas. Tampoco se observaron alteraciones en los niveles de triglicéridos y de colesterol, por lo que los datos

sugieren que el fenotipo metabólico periférico no se ve afectado a esta edad. Estos datos corroboran los de otros grupos de investigación, que muestran que los ratones APP/PS1 entre 5 y 7 semanas de edad no muestran alteraciones en el metabolismo de la glucosa y de la insulina (Jiménez-Palomares et al. 2012).

En cambio, a los 6 meses, los ratones APP/PS1 presentaban diferencias significativas en el metabolismo energético. Concretamente se observaba una disminución de la capacidad de metabolizar la glucosa y una disminuida sensibilidad a la insulina. Estos datos sugieren que la síntesis de Aβ42 podría inducir a un estado pre-diabético antes de que se formen las placas seniles.

Para explicar este fenómeno hay dos posibles hipótesis: en la primera, el fragmento Aβ sintetizado en el cerebro migra a la periferia a través del líquido cefalorraquídeo y del plasma, para producir los efectos metabólicos mencionados (DeMattos et al. 2002); en la segunda, la síntesis de Aβ se produce a nivel periférico, en concreto por parte de adipocitos (Roher et al. 2009; Y.-H. Lee et al. 2008). Un reciente estudio indicaría que la primera de las hipótesis sería la correcta, ya que el Aβ provocaría resistencia hepática a la insulina mediante una desregulación de la vía de JAK2/STAT3/SOCS1 (Y. Zhang et al. 2012).

La resistencia hepática a la insulina promueve la lipogénesis y también produce un incremento del estrés oxidativo y de la disfunción mitocondrial. Además la lipogéneis produce un incremento de ceramidas, lípidos tóxicos que se relacionan con la EA (de la Monte 2012).

Por lo que se refiere al metabolismo de los lípidos, recientemente se ha publicado que existe una relación entre el colesterol y la amiloidosis cerebral (Reed et al. 2014), sin embargo en nuestro modelo no encontramos diferencias en los niveles de colesterol entre las distintas cepas. En cambio, los niveles de triglicéridos aumentaron en el ratón APP/PS1 a la edad de 6 meses, por lo que parece de que sí existirían cambios en la lipogénesis.

1.2 Alteraciones en el SNC (hipocampo y corteza)

Amiloidogénesis y proteína tau

Para demostrar las alteraciones en la actividad transcripcional inducida en los animales transgénicos, se analizó la actividad transcripcional del gen APP y se encontraron niveles elevados de ARNm de APP en el hipocampo de ratones transgénicos a 3 y 6 meses. Por otro lado, se observaron niveles elevados de Aβ42 insoluble en ratones de 3 meses, aunque no hubiera presencia de placas seniles. En los ratones de 6 meses se detectaron niveles elevados de Aβ40 soluble e insoluble, así como niveles incrementados de Aβ42 soluble e insoluble; microscópicamente se observaron placas seniles.

Estos datos contrastan con otros trabajos publicados en este modelo de ratón, en los que se detectan niveles elevados de Aβ40 pero no de Aβ42 a la edad de 3 meses, mientras que a los 6 meses se detectan ambos péptidos solubles e insolubles (Aso et al. 2012).

Estos datos sugieren que, a la edad de 3 meses, los mecanismos endógenos de defensa que protegen contra la agregación amiloide serían capaces de neutralizar el exceso de péptidos amiloides mientras en edades más avanzadas los animales pierden esa capacidad protectora.

En cuanto a la hiperfosforilación de la proteína tau, en nuestro estudio hemos observado diferentes residuos fosforilados en ratones transgénicos de 3 meses, precisamente los residuos de serina 199 y 404 y de treonina 205. Estos datos indican que las alteraciones de tau pueden comenzar a manifestarse a los 3 meses de edad. A los 6 meses se mantienen las fosforilaciones y se reporta una fosforilación más en el residuo de serina 396. Como comentado en la introducción, las cinasas implicadas y los residuos susceptibles a la hiperfosforilación de tau son múltiples (Martin et al. 2013).

La determinación de las cinasas responsables de estas fosforilaciones ha sido uno de nuestros objetivos. Por ello, se determinaron diferentes cinasas mediante la técnica de Western-blot, entre ellas AMPK, ERK, JNK1, AKT, GSK3β, y CDK5/P35, cuyo resultado ha sido el siguiente:

Lo niveles de ERK no se vieron modificados, aunque la cinasa esté implicada en la consolidación de la memoria en el hipocampo. ERK está regulada por AMPK (Dineley, Jahrling, et al. Denner 2014), actúa a través de la vía ERK/CREB/CBP y permite transcribir genes necesarios para la plasticidad neuronal y la potenciación a largo plazo (LTP).

La cinasa JNK1 está implicada en la diabetes tipo II y la obesidad (Sabio & Davis 2010). En nuestro modelo aparece un aumento de su expresión génica en los ratones sometidos a dieta HFAT (Petrov et al. 2015) aunque no se observan cambios entre los ratones transgénicos y los controles. Estos datos sugieren que JNK1 no es responsable de la fosforilación de tau en el modelo de ratón APP/PS1.

Otros estudios indican CDK5 como la principal cinasa responsable de la fosforilación de tau, además de otras cinasas cuales AKT y GSK3β (Sundaram et al. 2012; Shukla, Skuntz, and Pant 2012). De acuerdo con ello, nuestros resultados muestran que los niveles de CDK5/P35, AKT y GSK3β resultan más elevados en el ratón transgénico respecto al control (Pedrós et al. 2014; Petrov et al. 2015).

La activación de CDK5/P35 está estrechamente relacionada con alterados niveles de glucosa y con los defectos en la vía de la insulina (Ubeda et al. 2004). Por lo

tanto, varios estudios han sugerido que la EA y la DM2 pueden compartir una vía común de la patología: la hiperactivación de CDK5 (Sadowski et al. 2004; Vignini et al. 2013).

Vía de señalización de la insulina en el hipocampo

Algunos estudios publicados recientemente sugieren una estrecha relación entre la diabetes y el proceso de amiloidosis cerebral (Sato and Morishita 2013; Hokama et al. 2013; Ramos-Rodriguez et al. 2014).

La presencia de depósitos de sustancia amiloide en los islotes pancreáticos es una característica fisiopatológica en la DM2, que se ha descrito en más del 90% de las necropsias de los pacientes afectos de dicha enfermedad. Estos depósitos están formados por la pro IAPP (pro-Islet *amyloid polypeptide)* que es un precursor de la amilina. La amilina es una proteína de 37 aminoácidos, sintetizada y secretada por las células beta pancreáticas (Haataja et al. 2008).

Parece que el proceso patológico de amiloidogénesis que sufre el cerebro en la EA y el páncreas en la DM2 están ligados y comparten alteraciones moleculares comunes en el procesamiento de péptidos implicando la vía de señalización de la insulina (De Felice 2013; Jiménez-Palomares et al. 2012; Haataja et al. 2008; Hokama et al. 2014).

Por ejemplo, se ha descrito que el uso de estreptozotocina, un compuesto químico que induce diabetes, provoca aumento de los niveles de Aβ y formación de placas neuríticas, además de déficits en la memoria espacial (Wang et al. 2010).

Según la información publicada, la resistencia periférica a la insulina actuaría como factor de riesgo para el desarrollo de la EA, aunque está descrito que este fenómeno puede producirse únicamente a nivel local, en el cerebro, (concretamente en el hipocampo y en menor medida en la corteza del cerebelo) sin que necesariamente se produzca un cuadro de DM2 (Talbot et al. 2012).

Las principales proteínas implicadas en la resistencia a insulina en el cerebro son el RI, el receptor del factor de crecimiento insulínico (IGFR) y los substratos del receptor de insulina 1 y la 2 (IRS1 y IRS2). En nuestro modelo transgénico se han podido observar fenómenos que indicarían un proceso de resistencia sistémica a la insulina.

En primer lugar, observamos una reducción de los niveles de mRNA del RI en los ratones de tres meses de edad (Pedrós et al. 2014); en ratones de 6 meses observamos un incremento de la fosforilación inactivadora de IRS2 en serina 723 (Petrov et al. 2015). Estos datos indican que la resistencia a insulina es un proceso

que se inicia antes de la formación de placas y se va agravando a medida que avanza la amiloidogénesis.

En efecto, se ha descrito que el aumento en los niveles de Aβ42 en el cerebro de ratones de APP/PS1 a los 15 meses de edad está relacionado con un bloqueo de la vía de señalización de insulina en el cerebro (Chua et al. 2012). Es de señalar que la mayoría de los estudios publicados se centran en etapas muy tardías de la enfermedad, cuando las placas de Aβ están completamente desarrolladas (Jucker & Walker 2013).

Proceso de aprendizaje y memoria

El proceso de aprendizaje y memoria en el hipocampo está regulado por múltiples procesos y factores de crecimiento. Entre las moléculas implicadas está el neuropéptido Y (NPY) (Ramos et al. 2006; Rangani et al. 2012). Varios estudios han demostrado que los niveles de NPY están reducidos en los pacientes con EA (Sperk et al. 2007). También se ha comprobado que en plasma y en el líquido cefalorraquídeo existe una reducción de NPY en pacientes con EA (Minthon et al. 1996). En nuestro modelo de ratón observamos una reducción del mRNA en el hipocampo de ratón transgénico a los 3 meses en comparación con el control.

Además, se observó una reducción significativa en la expresión de los genes relacionados con la memoria, *Arc* y *Fos* que están regulados por la señalización de insulina (Guillod-Maximin et al. 2004; Kremerskothen et al. 2002).

Mediante el test de conducta que evalúa la memoria hipocampal pudimos observar un déficit significativo en los ratones transgénicos de 6 meses de edad. Estos resultados coinciden con otros estudios referentes a la pérdida de memoria hipocampal, que indican que a los 6 meses esta cepa desarrolla deterioro cognitivo (Aso et al. 2012).

Vía de señalización de la leptina en el hipocampo

En el hipocampo, la leptina se ha implicado en los procesos de aprendizaje y memoria, en la neuroprotección y en la plasticidad sináptica (Irving & Harvey 2014). Recientemente se han descrito alteraciones en la vía de señalización de leptina en pacientes humanos con EA (Bonda et al. 2014). En dicho estudio, los autores detectaron un aumento significativo en los niveles de leptina tanto en el líquido cefalorraquídeo (LCR) como en el hipocampo de pacientes con EA. Curiosamente, un aumento en los niveles circulantes de la hormona se acompañó a una reducción en los niveles de ARNm de receptor de leptina haciendo alusión a la posibilidad de resistencia a la leptina (Bonda et al. 2014).

En nuestro modelo de Alzheimer se detectó una reducción del receptor de leptina a los 6 meses, tanto en la expresión de su mRNA como en el contenido proteico.

Paralelamente, se estudió otra adipocina llamada prolactina que activa la misma ruta y parece tener efectos neuroprotectores en las neuronas hipocampales (Warren et al. 2012). Ambas moléculas comparten receptores del tipo citocina, lo cual sugiere la posibilidad de que tengan efectos sinérgicos entre ellas. Los efectos protectores de la prolactina se han puesto de manifiesto en un modelo murino de epilepsia (Morales 2011); parece que esta adipocina tenga una función relevante en el control de la ingesta de alimentos en humanos (Garfield et al. 2012; Lacquaniti et al. 2013). Otro estudio relaciona la prolactina con el proceso de la neurogénesis y la mielinización de las neuronas en el hipocampo (Mak & Weiss 2010).

Nuestros resultados en ratones de 3 meses muestran que se produce una reducción del receptor de prolactina, tanto en la expresión del mRNA como en los niveles de proteína, y una desregulación de Stat5b dependiente del receptor de prolactina.

Tanto leptina como prolactina comparten la vía de señalización JAK/STAT/SOCS que implica diferentes factores de transcripción citoplasmáticos de la familia STAT. En los ratones APP/PS1 de 3 meses de edad se ha detectado una reducción de la subunidad de del gen STAT5b, junto con una reducción de SOCS1, SOCS2 y SOCS3. Esto podría estar indicando que hay una desregulación de la vía en una fase inicial de la amiloidogénesis.

Ácidos grasos y colesterol

El colesterol es especialmente abundante en las estructuras mielinizadas del cerebro y del sistema nervioso central. La concentración de colesterol en el SNC es la mayor de todos los tejidos corporales (23mg/g). Así el SNC representa solo el 2,1 % del peso corporal pero contiene el 23% de todo el colesterol presente en el cuerpo. (Dietschy & Turley 2004).

La homeostasis del colesterol en el sistema nervioso central es independiente de la que se realiza en el resto del cuerpo. Por lo tanto, en el cerebro todo el colesterol se sintetiza de forma endógena. El colesterol no se puede degradar en el cerebro ya que las altas concentraciones de colesterol libre son tóxicas para las neuronas y, por lo tanto, se secreta a la circulación sistémica. Cerca del 60% se secreta en forma del metabolito conocido como 24S-hidroxi-colesterol (24OHC), y el 40% restante a través de vías aún desconocidas, que pueden implicar la

apolipoproteina E (ApoE) y el transportador *ATP-Binding Cassette transporter* (ABCA1) (Vanmierlo et al. 2010).

La colesterol 24S-hidroxilasa (CYP46A1) es una enzima que convierte el colesterol en 24OHC, uno de los principales metabolitos del colesterol en el cerebro, capaz de atravesar la barrera hematoencefálica (Dietschy & Turley 2004). Una disminución en los niveles 24OHC en el cerebro puede ser, por tanto, un marcador indirecto de niveles elevados de colesterol.

En nuestro estudio, hemos detectado una reducción significativa en el ARNm de cyp46a1 en el hipocampo a 3 meses de edad en los ratones transgénicos, en comparación con los controles. Nuestros resultados están en concordancia con estudios que han evaluado los niveles en plasma de pacientes con EA, e identifican una disminución de los niveles de 24OHC (Heverin et al. 2004; Ali et al. 2013).

En conjunto, nuestros resultados sugieren que las alteraciones en las vías del metabolismo de colesterol del cerebro pueden estar asociados con los primeros signos de EA como en un modelo de ratón APP/PS1.

Metabolismo y biogénesis mitocondrial

La fisiopatología de la EA se desarrolla cuando las mitocondrias pierden su capacidad funcional, e incrementan la apoptosis neuronal, la deposición de Aβ y ovillos neurofibrilares.

El metabolismo mitocondrial del hipocampo está alterado en los ratones transgénicos APP/PS1 a los 3 meses de edad como pone de manifiesto la reducción significativa de los complejos OXPHOS, es decir en la fosforilación oxidativa de los complejos I, II, II y IV. Por otra parte, hemos detectado cambios significativos en el contenido de las moléculas que participan en la biogénesis mitocondrial incluyendo PGC-1α, NRF1 y NRF2.

PGC1-α es un miembro de la familia de co-activadores transcripcionales que desempeña un papel central en la regulación del metabolismo de la energía celular. Estimula la biogénesis mitocondrial y participa en la regulación del metabolismo de carbohidratos y lípidos. Por tanto, participa directamente en los trastornos metabólicos como la obesidad y la diabetes. Sin embargo, su papel en el SNC es menos claro. Se ha publicado que PGC-1α podría ser un biomarcador potencial de la enfermedad EA. En efecto, se ha observado una reducción de la actividad transcripcional y del contenido de PGC-1α en los cerebros *post mortem* de pacientes con EA (Shirwany & Zou 2014; Kim et al. 2007; Qin et al. 2009; Galluzzi et al. 2009)

PGC1α, además de regular directamente la biogénesis mitocondrial activando OXPHOS, también es un regulador transcripcional clave de la IDE (Hoover et al. 2010; Shukla et al. 2012; L. Zhang et al. 2012) y el metabolismo oxidativo de PGC1α interviene en la eliminación de insulina (Austin & St-Pierre 2012).

No detectamos diferencias de expresión en IDE, y la razón podría encontrarse en que los cambios de IDE mitocondrial serían enmascarados por la IDE citosólica (Shukla et al. 2012).

Proteínas sinápticas

La pérdida de conexiones sinápticas puede ocurrir como resultado de la acumulación de oligómeros Aβ, en fases anteriores a la formación de las placas seniles. Hemos medido los niveles de SYP y PSD95, en los ratones APP/PS1, que son marcadores sinápticos. Los resultados muestran que no se vieron afectados en el ratón transgénico. Estos resultados indican que, a estas edades todavía no se han producido alteraciones importantes en la neurotransmisión.

2. Efectos de la dieta HFAT en ratones salvajes y APP/PS1 a 6 meses

2.1 Dieta HFAT y alteraciones periféricas

El presente trabajo trata de considerar la enfermedad de Alzheimer como un desorden metabólico, en el cual los trastornos cognitivos están relacionados con alteraciones en la vía de señalización de la insulina y del metabolismo energético a nivel periférico y central.

El objetivo de esta parte de la tesis doctoral fue evaluar los cambios metabólicos que produce una dieta HFAT en el modelo murino APP/PS1. En particular, se quiso determinar si la dieta grasa acelera el desarrollo de la enfermedad en nuestro modelo.

En nuestro trabajo observamos que la dieta grasa es capaz de inducir pérdida de memoria en ratones de 6 meses de edad y sugiere que sea debido al incremento de la resistencia a la insulina a nivel periférico.

La dieta control suministrada a los ratones tenía un 18% de Kcal provenientes de la grasa; mientras que la dieta HFAT aportó un 45%. Los ratones sometidos a HFAT presentan obesidad inducida por la dieta, con diferencias en el peso corporal que empiezan a ser detectadas alrededor de los 2 meses de edad. Al final del tratamiento (5 meses), se registró un aumento de peso de aproximadamente el 50%.

Las características clásicas del síndrome metabólico están presentes tanto en los ratones control como en los ratones transgénicos tratados con la dieta HFAT. Es decir detectamos obesidad, dislipidemia, hiperinsulinemia periférica e intolerancia a la glucosa e insulina.

Por tanto, la dieta causó el efecto esperado y, por ello, se estudiaron en detalle las diferencias entre las dos cepas. En los ratones control los niveles de glucosa basal en sangre se mantuvieron normales mientras que en los transgénicos se observó un incremento muy significativo. No obstante los niveles de insulina en plasma se incrementaron en los dos tipos de ratón. Este dato nos indicaría que la glucosa no se está metabolizando adecuadamente en el ratón transgénico. Para comprobar está hipótesis se realizaron las curvas de glucosa e insulina, se observó que el ratón salvaje HFAT y el APP/PS1 control compartían un patrón de eficiencia similar en la metabolización de glucosa, mientras que el ratón APP/PS1 alimentado con dieta HFAT emporaba su rendimiento de forma significativa.

Así pues, parece que una elevada secreción cerebral del péptido Aβ es suficiente para producir cambios en el metabolismo periférico de los hidratos de carbono que son comparables a los que se observan con la administración crónica de una dieta grasa.

Es importante recordar que no se conoce todavía si el Aβ es responsable por si solo del fenotipo metabólico del ratón APP/PS1. Algunos estudios correlacionan los niveles de Aβ42 en plasma con el incremento de índice de masa corporal en pacientes sanos (Balakrishnan et al. 2005). La insulina en plasma en condiciones normales es degradada por la IDE enzima que también degrada Aβ (Deprez-Poulain et al. 2015). Los dos substratos podrían producir una inhibición competitiva de la enzima IDE. Esto podría teóricamente llevar a un aumento de los niveles plasmáticos de insulina y producir intolerancia a la glucosa.

2.2 Dieta HFAT y alteraciones en el sistema nervioso central

Existe una relación bien establecida entre la obesidad humana y el deterioro cognitivo (Beydoun et al. 2008; Lee 2011). La obesidad y dietas hipercaloricas alteran funciones cerebrales específicas relacionadas con la memoria contextual y espacial en el hipocampo pueden ser particularmente vulnerables (Greenwood & Winocur 2005; Valladolid-Acebes et al. 2012; Yamada-Goto et al. 2012; Kosari et al. 2012). Curiosamente, se ha demostrado que los animales obesos cuya dieta se revierte de nuevo a una dieta estándar, recuperan la funcionalidad de la memoria (Sobesky et al. 2014).

En nuestros resultados la dieta grasa produce pérdida de memoria en ratones de 6 meses de edad. Según una prueba conductual, asociada a evaluar la pérdida de

memoria, los ratones APP/PS1 y los ratones salvajes alimentados con dieta grasa, pierden la memoria. Confirmando el papel de la dieta hipercalórica en el proceso del déficit cognitivo. Es importante remarcar que se empezó a administrar la dieta en el momento del destete, cuando aún los procesos de neurogénesis están en curso. Estudios recientes han comprobado que el tipo de dieta condiciona el desarrollo neuronal (Boitard et al. 2012).

Los mecanismos moleculares a través de los cuales una dieta rica en grasa es capaz de empeorar los procesos cognitivos en modelos animales no están del todo explicados.

Amiloidogénesis y tau

Los resultados de la presente tesis doctoral muestran que los niveles de péptidos Aβ en la corteza de ratones salvajes con y sin dieta grasa no fueron significativos. Al contrario, observamos un incremento significativo de los niveles de Aβ42 insoluble en el córtex de ratones transgénicos alimentados con dieta grasa, respecto a los ratones transgénicos sometidos a la dieta control.

Este resultado podría sugerir que la dieta grasa contribuye a la formación de placas amiloides en animales transgénicos. Lamentablemente, el número y el tamaño de las placas en los animales APP/PS1 no resultaron modificadas en respuesta al tratamiento HFAT, dejando abierta la cuestión de si una dieta hipercalórica contribuye de forma aditiva al desarrollo de la patología de Alzheimer en ratones transgénicos.

Por otro lado, la proteína tau, fosforilada en serina 404, resultó incrementada en ratones de tipo salvaje sometidos a una dieta rica en grasa. Otros autores han publicado que la obesidad acelera la fosforilación de tau en ratones predispuestos al desarrollo de déficits cognitivos, acelerando la progresión de la enfermedad (Gendron et al. 2013).

En otro estudio publicado con ratones 3xTgAD (modelo murino de Alzheimer), no se encuentra incrementada la fosforilación de tau S202 después de administrar una dieta HFAT (Knight et al. 2014). Otros autores en cambio sí observan variaciones en tau en serina 214, tau 422 y tau 404. (Leboucher et al. 2013). Esta diversidad de resultados podría ser debida a la complejidad de evaluar de forma individual las fosforilaciones de la proteína tau. Es importante recordar que dicha proteína puede fosforilarse en 85 residuos distintos, y 28 son específicos de la EA (Martin et al. 2013).

En los ratones salvajes alimentados con dieta hipercalórica, observamos un incremento de CDK5/p35, una disminución de la vía de señalización AKT/GSK3β y un incremento notable de las cinasas JNK1 y ERK. La desregulación de las vías

AKT/GSK3β y CDK5/P35 ya estaba presente en los ratones APP/PS1 alimentados con dieta control. Por lo tanto, la administración de la dieta HFAT tendría un impacto en la patología a nivel central ya que también se produce una alteración en la actividad de las cinasas ERK y JNK1.

Estas cinasas pueden explicar el aumento observado en la fosforilación de tau en S404 en ratones de tipo salvaje alimentados con dieta HFAT.

Vía de señalización de la insulina en el hipocampo

Los resultados de la presente tesis doctoral muestran que el tratamiento con la dieta HFAT provocó cambios en la señalización de la vía de la insulina en el hipocampo en comparación con la dieta normal, tanto en los ratones salvajes como en los transgénicos.

La insulina/Igf es reconocida por sus receptores mediante la subunidad alfa. Una vez formado el complejo se inicia una serie de autofosforilaciones en los residuos Tyr de la subunidad beta, que a su vez fosforila IRS1/2, permitiendo así que estas proteínas adaptadoras puedan interactuar con un gran número de moléculas.

La activación de IRS1 y 2 se realiza mediante un mecanismo que involucra múltiples cinasas que fosforilan IRS en más de 50 residuos, (Copps & White 2012). Mediante la activación de IRS se activan diferentes rutas como PI3K/AKT/GSK3β; AMPK/PGC1α; que regulan el correcto metabolismo energético.

En el presente trabajo, se evaluaron los niveles de mRNA de preproinsulina (INS1), del IR, IRS1, IRS2, IGF1 e IGFR. La dieta HFAT produjo un incremento significativo en la INS1, lo que puede significar dos cosas: A) No llegaría suficiente insulina a las células del cerebro, lo que podría dar lugar a los mecanismos compensatorios que activan la expresión endógena de insulina directamente en el hipocampo; B) La obesidad inducida por dieta produciría resistencia a la insulina en el hipocampo.

Otro hallazgo muy interesante es el comportamiento de IGF1 y su receptor IGF1R. La expresión del mRNA de IGF1 se incrementó en ratones salvajes alimentados con HFAT y fueron ligeramente reducidos en ratones transgénicos, mientras que la expresión del mRNA de IGF1R disminuyó en los ratones transgénicos expuestos a la dieta hipercalórica. Es de destacar que en muestras de cerebros *post-mortem* de pacientes con EA se han descrito niveles reducidos de IGFR (Freude et al. 2009).

La reducción de la expresión de IGF1 se ha correlacionado con el deterioro cognitivo en la EA (Kimoto et al. 2015). Así pues, nuestros datos están de acuerdo con estas observaciones. La razón por la cual se produce un aumento de

expresión en ratones de tipo salvaje tratados con HFAT es menos evidente, pero puede implicar mecanismos compensatorios similares a los descritos anteriormente para la insulina.

También se evaluaron los niveles proteicos de IRS2 mediante Western blot. Los análisis realizados en extractos hipocampales mostraron que IRS2 se encontraba inactiva tanto en ratones transgénicos como por la administración de la dieta hipercalórica.

El IRS2 modula el comportamiento alimentario y reproductor; los ratones IRS-2-/- (knockout para el IRS-2) dan como resultado ratones hiperfágicos y obesos como consecuencia de la defectuosa señal de la insulina (Masaki et al. 2004). Estudios con imágenes cerebrales de humanos indican que la incorrecta utilización de glucosa en el cerebro precede a los déficits cognitivos (de la Monte 2012b). En este contexto, tanto la insulina, como IRS1 y IRS2 podrían modular la plasticidad sináptica del hipocampo y la consolidación de la memoria (M. de la Monte 2012; Leal et al. 2013; de la Monte 2012b; de la Monte & Wands 2008).

Metabolismo y biogénesis mitocondrial

Como se ha comentado anteriormente, la funcionalidad de la mitocondria tiene un papel fundamental en el proceso de neurodegeneración. La hipótesis de la cascada mitocondrial fue propuesta por Swerdlow & Khan. Los autores proponen que el daño mitocondrial en la patología de Alzheimer es anterior a la acumulación del β-amiloide (Swerdlow & Khan 2004b). Conociendo que una dieta rica en grasa puede alterar la funcionalidad mitocondrial (Godoy J.A. et al, 2014), en nuestro trabajo hemos querido evaluar en qué medida una dieta hipercalórica puede afectar a la actividad mitocondria a nivel del sistema nervioso central, y de esta forma contribuir al desarrollo de la patología de Alzheimer.

En este sentido los resultados de este trabajo han permitido observar que la obesidad inducida por la dieta grasa en los ratones salvajes ha producido una reducción significativa de todos los complejos OXPHOS. Cuando se evaluaron los ratones transgénicos con y sin dieta HFAT no se observaron diferencias entre los diferentes complejos OXPHOS, probablemente porque la alteración mitocondrial ya era demasiado grave. Aun así, estos datos demuestran que una dieta rica en grasa es suficiente por sí sola, para provocar una disfunción mitocondrial. Por lo tanto la cadena de transporte de electrones se ve alterada de forma similar entre la obesidad/DM2 y la EA. Por otro lado, el daño mitocondrial en condiciones basales en los ratones transgénicos es suficientemente grave y el síndrome metabólico no lo empeora.

También se evaluaron otros marcadores de la funcionalidad mitocondrial, como los reguladores de la biogénesis mitocondrial: los factores de transcripción nuclear factor respiratorio 1 y 2 (NRF-1 y NRF-2), el factor de transcripción mitocondrial A (Tfam) y el coactivador PGC-1α.

Se observó que la dieta HFAT reducía los niveles de estos marcadores tanto en ratones salvajes como en los transgénicos. En conjunto, nuestros datos demuestran claramente que el síndrome metabólico afecta significativamente a la funcionalidad y a la biogénesis mitocondrial del hipocampo en ratones de tipo salvaje. En el grupo transgénico, las deficiencias en estos procesos ya están presentes incluso antes de la administración de la dieta grasa.

Mecanismos de eliminación de los agregados y depósitos amiloides

El mecanismo principal para la eliminar el fragmento Aβ se produce por degradación *in situ* mediante proteasas específicas. Entre estas proteasas, las que tienen mayor relevancia fisiológica en el cerebro son la neprilisina (NEP), la enzima degradadora de insulina (IDE) y la enzima convertidora de endotelina (ECE) (Iwata et al. 2001; Qiu et al. 1998).

En un estado de resistencia periférica a la insulina, el transporte de insulina a nivel del sistema nervioso central está alterado y, en consecuencia, se observa una fuerte reducción de los niveles de insulina en el cerebro. En condiciones basales, el exceso de insulina circulante es eliminado por la IDE. La disminución de los niveles de insulina en el hipocampo, producida por un estado de resistencia a la insulina, provoca una reducción en la actividad de IDE.

De acuerdo con ello, en el presente trabajo observamos una reducción significativa en la expresión de IDE en el hipocampo de los ratones alimentados con dieta grasa, tanto en el grupo salvaje como en el grupo transgénico. Estos datos confirman que la administración de la dieta rica en grasa altera la tolerancia a la insulina no solo a nivel periférico, sino también en el cerebro. Por otro lado, IDE está también involucrada en un mecanismo de defensa que implica la degradación del péptido Aβ. Por lo tanto, la reducción en los niveles de IDE que se producen en consecuencia al estado de resistencia a la insulina podría contribuir a exacerbar el fenotipo transgénico. En estas circunstancias, para compensar la falta de una de las proteasas responsables de la degradación del péptido β-amiloide, parece que haya un aumento de neprilisina, otra enzima degradadora de péptidos de Aβ.

3. Resumen general

El proceso de amiloidogénesis y las alteraciones de la vía de señalización de insulina en estados iniciales de la enfermedad afecta a moléculas relevantes de la biogénesis mitocondrial como son AMPK, PGC1α, OXPHOS y NRF1.

La activación del complejo de P35/CDK5 participa en la resistencia a insulina periférica y en nuestro modelo participa en la hiperfosforilación de tau antes de la formación de placas. Identificar los procesos antes y después de la formación de placas ha sido uno de nuestros objetivos (figura 19).

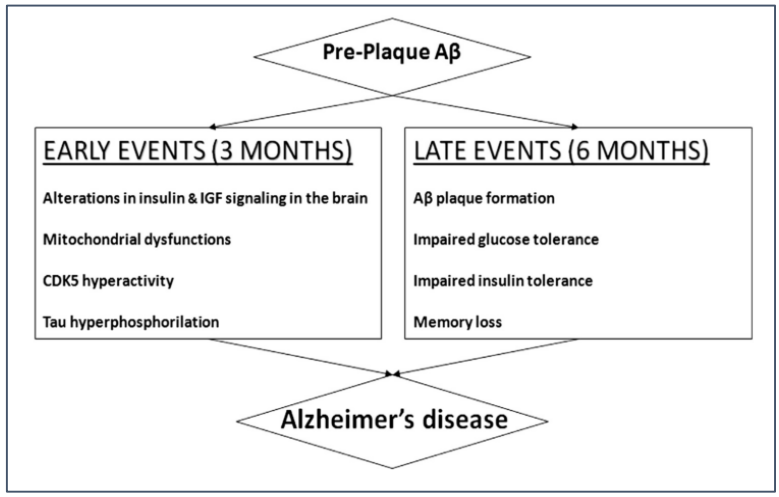


Figura 19. **Resumen de los procesos que conducen a la EA antes y después de la formación de placas** (Pedrós et al. 2014).

La dieta rica en grasa produce obesidad, resistencia a insulina (a nivel periférico y central) y provoca pérdida de memoria en ratones control y transgénicos.

La dieta HFAT y los niveles elevados de Aβ, provocan una activación de las cinasas JNK1 y ERK, de forma que inhiben la vía de señalización de la insulina en el hipocampo mediante el bloqueo de IRS.

La insulina es responsable de la activación de moléculas clave en el metabolismo energético como PGC1α. Estas funciones quedan alteradas y provocan una reducción en la fosforilación oxidativa y del ATP generado. En los ratones transgénicos los niveles de Aβ40 y Aβ42, las placas seniles y los niveles elevados de tau incrementan el daño mitocondrial, contribuyendo al desarrollo de trastornos cognitivos (Figura 20)

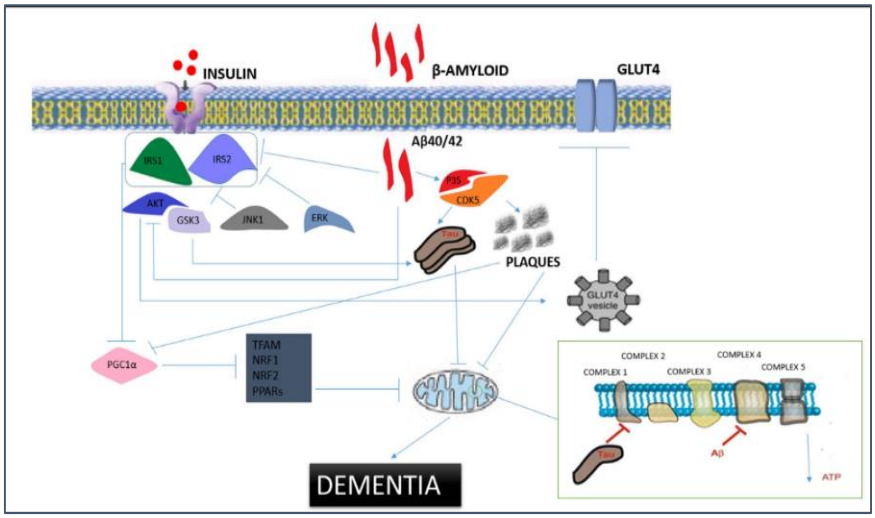


Figura 20. **Mecanismo propuesto que relaciona la vía de señalización de insulina y la desregulación mitocondrial en el hipocampo de ratones APP/PS1 y salvajes alimentados con una dieta grasa** (Petrov et al. 2015).

CAPÍTULO VI. CONCLUSIONES

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

CAPÍTULO VI. CONCLUSIONES

A partir del trabajo experimental desarrollado en esta Tesis Doctoral, puede concluirse:

I. Si bien no se han hallado depósitos de placas de péptido ß-amiloide en ratones transgénicos APP/PS1 a los tres meses de edad, sí se ha hallado un aumento en la actividad transcripcional del gen app en el hipocampo. Igualmente, se ha observado la presencia de péptido amiloide ß42 soluble en los extractos corticales.

II. Se ha observado la implicación de genes relacionados con la biogénesis mitocondrial en fases tempranas de la amilodogénesis, con una disminución en la expresión de la proteína PGC1-α en el hipocampo de ratones APP/PS1 a los tres meses de edad, así como de la expresión de NRF1.

III. Las alteraciones en la fosforilación oxidativa mitocondrial (OXPHOS) en ratones APP/PS1 tienen lugar ya a los tres meses de edad, y se mantienen a los 6 meses.

IV. El ratón APP/PS1 presenta una mayor fosforilación de la proteína tau a partir de los 3 meses de edad, fenómeno que se intensifica a los 6 meses, probablemente relacionado con una mayor expresión de la cinasa CDK5.

V. Los ratones APP/PS1 presentan una reducción de la actividad de la proteína IRS2 y cambios en la expresión de genes involucrados en la señalización de la insulina y de IGF, que podrían estar involucrados en alteraciones en la tolerancia a la glucosa y la insulina.

VI. Los ratones APP/PS1 de seis meses de edad presentan una disminución en la expresión génica del receptor de la leptina, de Neuropéptido Y y de la hormona corticótropa, respecto a los controles, así como la expresión de proteínas de la cascada de activación de la prolactina (SOCS1 y 2). Por todo ello, parece que en este modelo experimental estarían afectadas vías de señalización de adipocinas.

VII. La alimentación con dieta HFAT provoca un incremento en las concentraciones plasmáticas de insulina, triglicéridos e intolerancia a la glucosa e insulina tanto en ratones APP/PS1 como en salvajes, pero sólo los ratones transgénicos presentan hiperglucemia basal.

VIII. La dieta HFAT contribuye a elevar las concentraciones de péptidos ß-amiloides en el cerebro, altera la expresión de genes relacionados con la señalización de insulina en el hipocampo, así como incrementa el déficit cognitivo.

IX. La dieta HFAT, en ratones salvajes, incrementa la fosforilación de JNK1, CDK5/P35 y ERK. En ratones APP/PS1 los niveles son por si elevados y solo incrementa la JNK1. Estas cinasas son responsables de la fosforilación de tau S404.

X. El metabolismo mitocondrial se halla alterado en el hipocampo de ratones salvajes alimentados con dieta HFAT y en los APP/PS1 con dieta control y HFAT. En concreto, se afectan todas las proteínas estudiadas, desde el complejo I al V.

PUBLICACIONES ADICIONALES

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

REVIEW

Neuroprotective and anti-ageing role of leptin

Jaume Folch[2], Ignacio Pedrós[2], Iván Patraca[2], Francesc Sureda[2], Fèlix Junyent[1,2], Carlos Beas-Zarate[4], Ester Verdaguer[3], Mercè Pallàs[1], Carme Auladell[3] and Antoni Camins[1]

[1]Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Institut de Biomedicina (IBUB), Centros de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Universitat de Barcelona, Nucli Universitari de Pedralbes, 08028 Barcelona, Spain

[2]Unitats de Bioquímica i Farmacologia, Facultat de Medicina i Ciències de la Salut, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat Rovira i Virgili, C./St. Llorenç 21, 43201 Reus, Tarragona, Spain

[3]Departament de Biologia Cel·lular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

[4]División de Neurociencias, Departamento de Biología Celular y Molecular, C.U.C.B.A, Instituto Mexicano del Seguro Social (IMSS), Centro de Investigación Biomédica de Occidente (CIBO), Universidad de Guadalajara, Sierra Mojada 800, Col. Independencia, Guadalajara, Jalisco 44340, Mexico

(Correspondence should be addressed to A Camins; Email: camins@ub.edu)

Abstract

Leptin (Lep), an adipose-derived hormone, exerts very important functions in the body mainly on energy storage and availability. The physiological effects of Lep controlling the body weight and suppressing appetite are mediated by the long form of Lep receptor in the hypothalamus. Lep receptor activates several downstream molecules involved in key pathways related to cell survival such as STAT3, PI3K, MAPK, AMPK, CDK5 and GSK3β. Collectively, these pathways act in a coordinated manner and form a network that is fully involved in Lep physiological response. Although the major interest in Lep is related to its role in the regulation of energy balance, and since resistance to Lep affects is the primary risk factor for obesity, the interest on their effects on brain cognition and neuroprotection is increasing. Thus, Lep and Lep mimetic compounds now await and deserve systematic exploration as the orchestrator of protective responses in the nervous system. Moreover, Lep might promote the activation of a cognitive process that may retard or even partially reverse selected aspects of Alzheimer's disease or ageing memory loss.

Journal of Molecular Endocrinology (2012) **49,** R149–R156

Introduction

It is well documented that hormones play a vital role in the regulation of numerous biochemical pathways throughout the body (Fernandez & Torres-Alemán 2012). In recent years it has been demonstrated that a range of hormones can be found within the CNS jointly with its receptors and this is evidence of an existence of physiological effects in the brain under regulation of hormonal signalling (Scott *et al.* 2009). Although a wide number of hormonal systems can be potential candidates to study the effects on the brain, the largest bodies of work concentrate mainly around leptin (Lep) and insulin due to their modulation on hippocampal function (Plum *et al.* 2005, Signore *et al.* 2008). Likewise, there are other potential targets regulated by Lep such as brain-derived neurotrophic factor (BDNF), which is a neurotrophin also involved in hypothalamic food intake regulation and represents a potential target for developing new anti-obesity therapies. Furthermore, cytokines such as interleukin 6 and other hormones also regulate the physiological actions of Lep (Sadagurski *et al.* 2010, Rosas-Vargas *et al.* 2011).

Alterations in lipid metabolism have been related to neurodegenerative disorders, in particular Alzheimer's disease (AD; Lieb *et al.* 2009, Tezapsidis *et al.* 2009). Interestingly, clinical studies have also shown that patients with diabetes mellitus have an increased risk of suffering from AD, which reveals the link between hormonal diseases and neurodegenerative processes (Salminen *et al.* 2011, Li *et al.* 2012). Furthermore, the association between obesity and altered signalling mechanisms of insulin implies a greater susceptibility to neurodegenerative processes (Fig. 1).

However, controversy exists in determining the specific relationships between obesity and Alzheimer's disease (AD). Although some authors consider that obesity in middle age is due to a risk of dementia, others questioned the existence of a relationship between the two processes, particularly in humans (Doruk *et al.* 2010, Arab *et al.* 2011). On the other hand, it is well known that both insulin resistance, associated with an

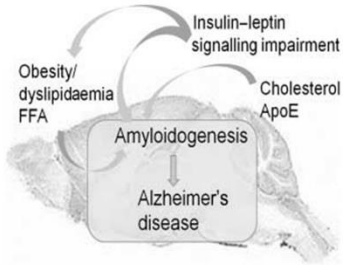


Figure 1 Factors involved in the amyloidogenesis: free fatty acids (FFA), cholesterol, lipoproteins (Apo lipoprotein E), obesity and dislipidaemia status and impairment of insulin and leptin signalling (adapted from Merlo S, Spampinato S, Canonico PL, Copani A & Sortino MA 2010 Alzheimer's disease: brain expression of a metabolic disorder? *Trends in Endocrinology & Metabolism* **21** 537–544).

increase in plasmatic levels of fatty acids, and high VLDL content contribute to amyloidogenesis (Arab *et al.* 2011).

Probably, the main characteristic and important problem to solve in AD is memory loss. It is well known that the hippocampus has been recognized to play a fundamental role in the process of learning and memory (Bonda *et al.* 2011). Thus, hormonal modulation of hippocampal neuronal function by insulin and Lep has great interest and numerous implications (Bjørbaek & Kahn 2004, Harvey *et al.* 2006, Harvey 2007, Carro 2009).

The aim of the present review is to discuss the potential beneficial effects of the hormone Lep in neurological disorders basically in two aspects: its neuroprotective role and beneficial effects on memory (Tezapsidis *et al.* 2009).

Physiological functions of Lep in the CNS

The Lep gene (*OB* (*LEP*)) encodes a polypeptide of 16 kDa. The primary sequence and the crystallographic data suggest that Lep adopts a helical three-dimensional structure, which is reminiscent of some cytokines such as interleukin 2 (Elmquist *et al.* 1998, Elias *et al.* 2000).

The physiological functions of Lep are known to be mediated through the binding to Lep receptor (Ob-R), and elicit an array of subsequent intracellular signalling cascades (Guo *et al.* 2008). For instance, previous studies demonstrated that mice with a deficiency in Lep develop morbid obesity and diabetes and plasma levels of Lep are highly correlated with the amount of body fat (Thio *et al.* 2006, Coccurello *et al.* 2009, Doruk *et al.* 2010).

Within the CNS, Lep receptors are located in the hypothalamus, where they are known to be involved in the control of energy homeostasis. Besides the regulation of body energy homeostasis and neuroendocrine functions in the hypothalamus, Lep may have more widespread actions in the brain. Examples of Lep functions in the brain are the modulation of the excitability of hippocampal neurons by activating potassium channels. Another interesting point is that, since Lep receptor-deficient mice have impaired spatial learning ability, it was suggested that Lep signalling may influence neuronal excitability and synaptic plasticity (Oomura *et al.* 2006).

The regulation of appetite and energy expenditure by Lep takes place by inhibiting serotonin synthesis and releasing it in brainstem neurons. Therefore, the brain regulation of serotonin is an important biological and medical function of Lep. This effect is due to the localization of Lep receptors, which are found in brainstem serotonergic neurons. Besides, the presence of Ob-Rb mRNA expression has been demonstrated in the substantia nigra. Neurochemical effects of Lep on dopaminergic neurons also include the increase of tyrosine hydroxylase content and the regulation of dopamine transporter activity (Scott *et al.* 2009). Therefore, Lep is able to modulate the mesolimbic dopaminergic system (Bjørbaek & Kahn 2004, Scott *et al.* 2009).

On the other hand, Lep receptors have been shown to be expressed in neuronal cell cultures of hippocampal and glial cells (Marwarha *et al.* 2012). As Lep is a modulator of hippocampal function, the study of the effects of Lep in this brain area on glutamate receptors, especially *N*-methyl-D-aspartate (NMDA) and AMPA, is therefore of particular interest, because they are potential modulators of not only learning and memory processes but also with regard to CNS-driven diseases such as epilepsy (Moult & Harvey 2008, 2011, Morley & Banks 2010). These roles are interesting, because it has been demonstrated that Lep exerts an important role in protecting from kainate excitoxicity and modulating synaptic plasticity and dendritic morphology (Shanley *et al.* 2002).

Lep receptor signalling in neurons

From the Lep receptor gene a total of six different isoforms are synthesized. All are membrane proteins with the exception of the soluble isoform, Ob-Re. The Ob-R form is the longest isoform and is solely responsible for signalling induced by ligand binding. The best-described signalling pathway used by Lep involves the coordinated activation of JAK2/STAT3. The binding of Lep, or an agonist, to its receptor stimulates the activation of JAK2, which in turn phosphorylates tyrosine residues in the intracellular domain of the Lep receptor. STAT3 is a transcriptional factor that, upon phosphorylation, dimerizes and is transported to the nucleus, where it controls

the transcription of target genes (Guo *et al.* 2008, Signore *et al.* 2008). This favours the activation of different molecular mechanisms critical for Lep effects on body weight, control the route of phosphatidylinositol 3 kinase (PI3K), the kinase AKT, MAPK and protein kinase activated by 5′-AMP (Shanley *et al.* 2002). The signalling mechanism of the Lep receptor is negatively regulated by the molecule suppressor of cytokine signalling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B). In particular, SOCS3 is a member of cytokine-inducible signalling inhibitors, which inhibits LepR signalling by blocking JAK2 activity (Hubschle *et al.* 2001, Avraham *et al.* 2010; Fig. 2).

More recently, it has been suggested that Cdk5/p35 modulate signalling triggered by Lep (He *et al.* 2009). Thus p35, a co-activator of p35, favours Lep signalling manifested by STAT3 and SOCS3 activation. Moreover, cdk5 was co-localized with Lep receptor in the same cells in the hypothalamus (He *et al.* 2009).

Another downstream target of Lep signalling is peroxisome proliferator-activated receptor γ (PPARγ (PPARG)). Lep is involved in the regulation of PPARγ levels both *in vivo* in peripheral tissues and in neuronal cultures of human neuroblastoma SH-SY5Y (Greco *et al.* 2009*a*,*b*, 2011).

Moreover, Lep modulates activation of endogenous cellular energy sensors in neurons such as AMP and NAD^+, AMPK and sirtuin 1 (SIRT1; Avraham *et al.* 2010). It is well known that AMPK is an important enzyme involved in the regulation of cellular metabolic activity (Marwarha *et al.* 2010, Greco *et al.* 2011). Among them, AMPK regulates the cellular uptake of glucose, the β-oxidation of fatty acids and the biogenesis of glucose transporter 4 (GLUT4) and mitochondria (Greco *et al.* 2009*a*,*b*, 2011, Salminen *et al.* 2011). Thus, through changes in hypothalamic AMPK activity, Lep could regulate human metabolism. Furthermore, downstream targets of AMPK, such as the mammalian target of rapamycin pathway, are important not only in food intake regulation but also in the control of the ageing process and autophagia (Greco *et al.* 2011).

Lep as a neuroprotective agent

Different studies conducted in *in vitro* models show that Lep is neuroprotective not only in dopaminergic cells but also in other cell types and brain areas (Dicou *et al.* 2001, Tezapsidis *et al.* 2009, Oury and Karsentry 2011). These data suggest the potential application of Lep in the treatment of neurological disorders such as AD and Parkinson's disease (PD; discussed below).

Role of Lep in AD

AD has been well characterized as a multifactorial neurodegenerative process characterized by progressive neuronal loss, gliosis and accumulation of two markers of disease: senile plaques (aggregates of β-amyloid peptides) and neurofibrillary tangles (hyperphosphorylated tau protein) (Greco *et al.* 2008, 2009*a*). The formation of β-amyloid aggregates is the result of an abnormal amyloid precursor protein cleavage by β- and γ-secretases. It seems that the presence of free fatty acids, cholesterol, lipoprotein and APOE

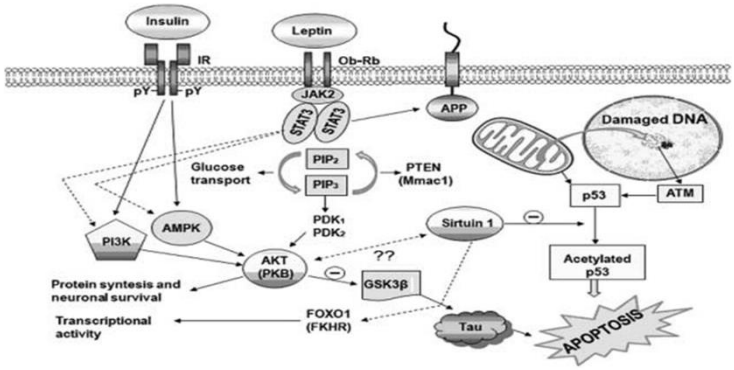


Figure 2 Proposed anti-apoptotic mechanisms of leptin. The leptin signalling pathway can contribute to reduce the neuronal apoptosis through, hypothetically, the activation of sirtuin 1 activity that, in turn, would reduce the stabilization of p53 by an acetylation process. Currently it is unknown how SIRT1 regulates AKT (??). Activation of p53 is promoted by mitochondrial metabolism impairment and the concomitant generation of reactive oxygen species. The oxidative species damage DNA and activate ataxia telangiectasia mutated (ATM) protein, which also acts due to the activation of p53.

promotes amyloidogenesis, whereas Lep facilitates their elimination. Intervention in one or more of these factors could slow or limit the process of amyloidogenesis. In the context of the β-amyloid hypothesis for AD, Lep may interfere with the pathogenesis of AD in different ways by a) inhibiting the amyloidogenic process (Greco *et al.* 2008); b) decreasing the activity of glycogen synthase kinase-3β (GSK3β) and, thus, reducing the levels of tau protein phosphorylation (Greco *et al.* 2009*b*) and c) improving the cognitive function (Tezapsidis *et al.* 2009).

Firstly, it appears that Lep may reduce amyloidogenesis, decreasing the activity of the enzyme responsible for the breakdown of the β site APP (BACE) in neurons and, thus, decreasing the amount of protein β-amyloid formed (Marwarha *et al.* 2010). It has been suggested that this effect may be indirect, and is related to the lipolytic activity of Lep. Similarly, Lep may also enhance the elimination of β-amyloid protein through the effect of APOE-dependent uptake. It has been demonstrated that Lep signalling is probably related to changes in *Apoe* gene expression and Lep would exert this effect on removal of β-amyloid aggregates with the same intensity. These observations have been confirmed in *in vitro* and *in vivo* experiments using a transgenic mice model for AD in which mice have been administered Lep chronically (Greco *et al.* 2010, Marwarha *et al.* 2010). It was shown that the treatment of a murine model of AD (TgCRND8) with Lep significantly improves all parameters of experimental AD such as decrease in β-amyloid and phospho-tau levels and cognitive function improvement (Tezapsidis *et al.* 2009).

Another mechanism involved in the neuroprotective effects of Lep may be the activation of AMPK and SIRT1, because these enzymes constitute potential targets associated with AD (Fig. 2; Greco *et al.* 2011). Low levels of Lep contribute to an insufficient stimulation of AMPK which, in turn, favours an increase in β-amyloid levels and phosphorylated tau. SIRT1 activation exerts beneficial effects in AD probably by upregulation of α-secretase production (Bonda *et al.* 2011). Moreover, additional neuroprotective effects in AD of SIRT1 may also be mediated, at different levels, through the acetylation state (and thus the localization and activity) of basic transcription factors like *p53* (*TP53*), *NF-κB*, *FOXO* and *KU70* (*XRCC6*), and this might trigger pro-survival pathways in the neuronal cell (Camins *et al.* 2010).

Published data suggest that Lep regulates tau phosphorylation through a pathway involving both AMPK and GSK3β, leading the enzyme to this inactive form by the activation of serine-9 phosphorylation (Greco *et al.* 2008, 2009*a*,*b*). Thus, Lep, which ameliorates both amyloid β- and τ-related pathological pathways, holds promise as a therapeutic for AD (Fig. 3).

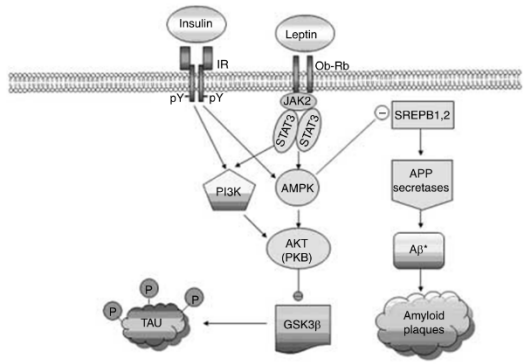


Figure 3 Insulin and leptin signalling. Leptin inhibits the hyperphosphorylation of tau protein through PI3K and AKT activation that, in turn, promotes the formation of inactive form Ser-9 GSK3β. Leptin also inhibits GSK3β activity acting on AMPK, and reducing the activity of transcription factors SREPB1,2 and the formation of amyloid deposits (Aβ*).

Effects of Lep on memory

Recent evidence indicates that Lep plays an important role in modulating hippocampal synaptic plasticity and affecting glutamate receptor trafficking, mainly NMDA and AMPA receptors (Moult *et al.* 2010).

The regulation of NMDA by Lep is important because receptor-dependent long-term potentiation (LTP) induced in the hippocampal CA1 region has been implicated in spatial learning and memory. It is well established that the synaptic activation of NMDA receptors is associated with a postsynaptic rise in intracellular Ca^{2+}, which is crucial for the induction of LTP in hippocampal CA1 synapses. Treatment of hippocampal neurons with Lep stimulates CaMKII phosphorylation and facilitates the development of LTP. It has been shown that neonatal Lep administration in rodents is able to increase the expression of the NR1 subunit of NMDA receptors in the hippocampus (Walker *et al.* 2007). Subsequently, this treatment increases the density of hippocampal synapses. This process is dependent on the synaptic activation of NR2A-containing NMDA receptors. The role of Lep in regulating dendritic morphogenesis has important implications in hippocampal synaptic plasticity and neuronal development (Moult *et al.* 2010, O'Malley *et al.* 2007). These effects of Lep are not limited to hippocampal neurons, as Lep receptors are expressed in cerebellar neurons and treatment of these neurons with Lep facilitated NR2B NMDA receptor-mediated calcium influx. Therefore, these effects could explain, in part, the beneficial effects of Lep on memory.

Lep promotes an increase in the synaptic expression of GLUR2-lacking AMPA receptors in adult hippocampal slices resulting in a persistent increase in the efficacy of excitatory synaptic transmission (Moult & Harvey 2011). AMPA receptors are permeable to Ca^{2+}, which allows the activation of specific intracellular signalling pathways required for synaptic efficacy.

Role of Lep in PD

Several reports show that weight loss is a common characteristic in patients with PD (Fiszer *et al.* 2010). The relationship between serum body weight with Lep in PD patients has been studied (Loreflät *et al.* 2009, Aziz *et al.* 2011). However, a decreased body fat mass is probably the better parameter to be correlated with lower Lep levels in PD patients (Novakova *et al.* 2011).

It was reported that Lep exerts a cytoprotective effect against the mitochondrial neurotoxin MPP^+, an experimental model of PD (Lu *et al.* 2006). In these studies two neuroprotective pathways are mainly proposed. The first one suggests that the activation by Lep of PI3K/AKT favours the survival of SH-SY5Y neuroblastoma cells (Lu *et al.* 2006, Ho *et al.* 2010). Another study suggests that Lep neuroprotective effects are mediated through the expression of mitochondrial uncoupling protein 2 (UCP2). Thus, Lep favours an increase of UCP2 that restores ATP levels *in vitro* and *in vivo* and preserves energy supply, as has been observed in the neuroblastoma SH-SY5Y cell line. These data are interesting as they show the association between Lep and the increase in mitochondrial efficiency (Ho *et al.* 2010).

6-hydroxydopamine (6-OHDA) is another well known neurotoxin. In the MN9D dopaminergic cell line, subjected to the toxic action of 6-OHDA, the administration of Lep reverses cell loss (Weng *et al.* 2007). The neuroprotective effect is related to the modulation of the route of mitogenic extracellular kinase and ERK. Lep treatment has shown significant protective effects by rescuing dopaminergic neurons from 6-OHDA toxicity *in vivo* due to pCREB increased BDNF levels. Lep-induced increase of BDNF levels may be the main potential mechanism that mediates neuroprotection and gives support to the application of Lep as a neuroprotective drug in experimental PD models.

Lep and epilepsy

The interest in Lep as anti-epileptogenic therapy has emerged after the observation that the ketogenic diet, an effective anticonvulsant therapy used to treat intractable epilepsy, elevates serum Lep levels in rodents (Thio *et al.* 2006). Thus, current data suggest that Lep will be an endogenous anticonvulsant (Shanley *et al.* 2002). This hypothesis is based on the observation that mice deficient in Lep receptors (*ob/ob* mice) are more susceptible to seizures induced by pentylenetetrazol (PTZ), an experimental proconvulsant agent used as experimental model of epilepsy (Erbayat-Altay *et al.* 2008). Moreover, it was evidenciated that the *ob/ob* mice are more susceptible to PTZ-induced generalized seizures and cell death than wild-type mice. These data suggest that elevated blood Lep levels may decrease neuronal excitability and also provide an anticonvulsant effect. Likewise, Lep treatment also significantly diminished seizure activity induced by other chemical models, such as i.c. injections of 4-aminopyridine (voltage-gated potassium channel inhibitor), and i.p. injections of PTZ (a non-competitive GABA antagonist) in mice (Xu *et al.* 2008).

Furthermore, in Lep-deficient *ob/ob* mice model, Lep was able to protect hippocampal neurons against kainate excitotoxicity, another experimental model of epilepsia. This experimental model favours seizure activity by activation of glutamate receptors (Guo *et al.* 2008).

How Lep exerts this anticonvulsant effect in this model is unknown. However, several hypotheses have been proposed. The anticonvulsant effect of Lep may result from NMDA receptor modulation or via activation of large conductance calcium-activated potassium channels (Walker *et al.* 2007). Calcium-activated potassium channels are important in determining the excitability of hippocampal neurons and may contribute to aberrant firing such as during seizure activity (Moult & Harvey 2011).

The inhibition of AMPAR-mediated synaptic transmission constitutes another potential mechanism involved in Lep anti-epileptic properties. This effect on AMPA synaptic responses is mediated by binding to its receptor and activating the JAK2/PI3K pathway.

In addition, previous studies demonstrated that Lep has an *in vitro* neuroprotective effect against NMDA receptor-induced excitotoxicity (a receptor implicated in kainate-induced hippocampal cell death) and oxidative stress favours neuronal damage (Dicou *et al.* 2001). The mechanisms involved in these neuroprotective mechanisms may be the induction of Bcl-xL and Mn-SOD, through a STAT3-dependent manner. Mn-SOD is a mitochondrial antioxidant enzyme, whereas Bcl-xL stabilizes mitochondrial membranes, and both proteins favour mitochondrial protection mediated by Lep. This hormone has also shown neuroprotective effects against death induced by trophic factor withdrawal, a model relevant to natural developmental cell death.

Neuroprotective effects of Lep in models of ischaemia

In addition to the beneficial effects on the potential treatment of neurodegenerative diseases, Lep exerts a neuroprotective role in rodent models of cerebral ischaemia. In these studies, it has been demonstrated that Lep neuroprotective mechanisms involve ERK1/2, AKT, NF-κβ transcription and STAT3 signalling pathways (Weng *et al.* 2007, Zhang & Chen 2008, Guo *et al.* 2008), which are all downstream signalling events of Lep receptor activation. With respect to the transcription factor NF-κB, activation is typical of neuroprotective molecules and is associated with the induction of the Bcl-xL gene, an anti-apoptotic protein which is member of the BCL-2 family (valerio *et al.* 2009). Therefore, the anti-apoptotic properties of Lep in ischaemia could be explained by modification of the Bcl-xL/Bax ratio towards an anti-apoptotic state. Likewise, the neuroprotective properties of Lep could be also explained by the activation of ERK1/2 that can phosphorylate Bad at Ser-112 and, thus, prevent its apoptotic activity. The nuclear translocation of p65 and p50, which then form a complex with c-Rel that is also involved in cell survival, is another neuroprotective effect of Lep.

Conclusions and future perspectives

It has been demonstrated that Lep plays an important role in neuroprotection and cognitive improvement against some experimental neuropathological conditions such as ischaemia, AD, PD and epilepsy. Evidence suggests that Lep, through binding to its receptor, modulates key pathways namely CDK5, AMPK, GSK3β, STAT3 and others involved in neuroprotection (Tezapsidis *et al.* 2009). In addition, Lep modulates glutamate receptors and improves cognition (Moult & Harvey 2011). The regulation or modulation of the mitochondria function is another area of interest in the neuroprotective functions of this hormone. AMPK, and the PPARγ coactivator (PGC)/PPAR are pathways activated by Lep which supports mitochondrial function. Lep-dependent mitochondrial metabolic activation and regulation may exert a trophic and protective effect that contributes to the restoration of energetic status in neurons altered in neurological disorders. The molecular mechanisms driving these mitochondrial changes induced by Lep should be investigated in depth, because the regulation of BCL-2 family of proteins is a key factor involved in apoptotic neuronal cell death.

Finally, the development of peptides designed as potential agonists of Lep could be relevant for the treatment of diseases associated with the Lep receptor. In reference to this, an area of pharmacological interest is the treatment of rheumatoid arthritis, where antagonists of Lep receptor probably could be of therapeutic use (Otvos *et al.* 2008, 2011).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

This work was funded by grant 2009/SGR00853 from the Generalitat de Catalunya (autonomous government of Catalonia), by grants BFU2010-19119/BFI, SAF2011-23631 and SAF2009-13093 from the Spanish Ministerio de Ciencia e Innovación, grant PS09/01789 from the Instituto de Salud Carlos III and grant 610RT0405 from Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo (CYTED) and SEP-CONACYT Grant 000177594 (CBZ).

References

Arab L, Sadeghi R, Walker DG, Lue LF & Sabbagh MN 2011 Consequences of aberrant insulin regulation in the brain: can treating diabetes be effective for Alzheimer's disease. *Current Neuropharmacology* **9** 693–705. (doi:10.2174/157015911798376334)

Avraham Y, Davidi N, Porat M, Chernoguz D, Magen I, Vorobeiv L, Berry EM & Leker RR 2010 Leptin reduces infarct size in association with enhanced expression of CB2, TRPV1, SIRT-1 and leptin receptor. *Current Neurovascular Research* **7** 136–143. (doi:10.2174/156720210791184943)

Aziz NA, Pijl H, Frölich M, Roelfsema F & Roos RA 2011 Leptin, adiponectin, and resistin secretion and diurnal rhythmicity are unaltered in Parkinson's disease. *Movement Disorders* **26** 760–761. (doi:10.1002/mds.23463)

Bonda DJ, Lee HG, Camins A, Pallàs M, Casadesus G, Smith MA & Zhu X 2011 The sirtuin pathway in ageing and Alzheimer disease: mechanistic and therapeutic considerations. *Lancet Neurology* **10** 275–279. (doi:10.1016/S1474-4422(11)70013-8)

Bjørbaek C & Kahn BB 2004 Leptin signaling in the central nervous system and the periphery. *Recent Progress in Hormone Research* **59** 305–331. (doi:10.1210/rp.59.1.305)

Camins A, Sureda FX, Junyent F, Verdaguer E, Folch J, Pelegri C, Vilaplana J, Beas-Zarate C & Pallàs M 2010 Sirtuin activators: designing molecules to extend life span. *Biochimica et Biophysica Acta* **1799** 740–749. (doi:10.1016/j.bbagrm.2010.06.005)

Carro EM 2009 Therapeutic approaches of leptin in Alzheimer's disease. *Recent Patents on CNS Drug Discovery* **4** 200–208. (doi:10.2174/157488909789104848)

Coccurello R, Brina D, Caprioli A, Conti R, Ghirardi O, Schepis F & Moles A 2009 30 Days of continuous olanzapine infusion determines energy imbalance, glucose intolerance, insulin resistance, and dyslipidemia in mice. *Journal of Clinical Psychopharmacology* **29** 576–583. (doi:10.1097/JCP.0b013e3181bfe13e)

Dicou E, Attoub S & Gressens P 2001 Neuroprotective effects of leptin *in vivo* and *in vitro*. *Neuroreport* **12** 3947–3951. (doi:10.1097/00001756-200112210-00019)

Doruk H, Naharci MI, Bozoglu E, Isik AT & Kilic S 2010 The relationship between body mass index and incidental mild cognitive impairment, Alzheimer's disease and vascular dementia in elderly. *Journal of Nutrition, Health & Aging* **14** 834–838. (doi:10.1007/s12603-010-0113-y)

Elias CF, Kelly JF, Lee CE, Ahima RS, Drucker DJ, Saper CB & Elmquist JK 2000 Chemical characterization of leptin-activated neurons in the rat brain. *Journal of Comparative Neurology* **423** 261–281. (doi:10.1002/1096-9861(20000724)423:2<261::AID-CNE6>3.0.CO;2-6)

Elmquist JK, Bjørbæk C, Ahima RS, Flier JS & Saper CB 1998 Distributions of leptin receptor mRNA isoforms in the rat brain. *Journal of Comparative Neurology* **395** 535–547. (doi:10.1002/(SICI)1096-9861(19980615)395:4<535::AID-CNE9>3.0.CO;2-2)

Erbayat-Altay E, Yamada KA, Wong M & Thio LL 2008 Increased severity of pentylenetetrazol induced seizures in leptin deficient ob/ob mice. *Neuroscience Letters* **433** 82–86. (doi:10.1016/j.neulet.2007.12.051)

Fernandez AM & Torres-Alemán I 2012 The many faces of insulin-like peptide signalling in the brain. *Nature Reviews. Neuroscience* **13** 225–239. (doi:10.1038/nrn3209)

Fiszer U, Michałowska M, Baranowska B, Wolińska-Witort E, Jeske W, Jethon M, Piaścik-Gromada M & Marcinowska-Suchowierska E 2010 Leptin and ghrelin concentrations and weight loss in Parkinson's disease. *Acta Neurologica Scandinavica* **121** 230–236. (doi:10.1111/j.1600-0404.2009.01185.x)

Greco SJ, Sarkar S, Johnston JM, Zhu X, Su B, Casadesus G, Ashford JW, Smith MA & Tezapsidis N 2008 Leptin reduces Alzheimer's disease-related tau phosphorylation in neuronal cells. *Biochemical and Biophysical Research Communications* **376** 536–541. (doi:10.1016/j.bbrc.2008.09.026)

Greco SJ, Sarkar S, Johnston JM & Tezapsidis N 2009*a* Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. *Biochemical and Biophysical Research Communications* **380** 98–104. (doi:10.1016/j.bbrc.2009.01.041)

Greco SJ, Sarkar S, Casadesus G, Zhu X, Smith MA, Ashford JW, Johnston JM & Tezapsidis N 2009*b* Leptin inhibits glycogen synthase kinase-3β to prevent tau phosphorylation in neuronal cells. *Neuroscience Letters* **455** 191–194. (doi:10.1016/j.neulet.2009.03.066)

Greco SJ, Bryan KJ, Sarkar S, Zhu X, Smith MA, Ashford JW, Johnston JM, Tezapsidis N & Casadesus G 2010 Leptin reduces pathology and improves memory in a transgenic mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease* **19** 1155–1167.

Greco SJ, Hamzelou A, Johnston JM, Smith MA, Ashford JW & Tezapsidis N 2011 Leptin boosts cellular metabolism by activating AMPK and the sirtuins to reduce tau phosphorylation and β-amyloid in neurons. *Biochemical and Biophysical Research Communications* **414** 170–174. (doi:10.1016/j.bbrc.2011.09.050)

Guo Z, Jiang H, Xu X, Duan W & Mattson MP 2008 Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. *Journal of Biological Chemistry* **283** 1754–1763. (doi:10.1074/jbc.M703753200)

Harvey J 2007 Leptin regulation of neuronal excitability and cognitive function. *Current Opinion in Pharmacology* **7** 643–647. (doi:10.1016/j.coph.2007.10.006)

Harvey J, Solovyova N & Irving A 2006 Leptin and its role in hippocampal synaptic plasticity. *Progress in Lipid Research* **45** 369–378. (doi:10.1016/j.plipres.2006.03.001)

He Y, Kastin AJ, Hsuchou H & Pan W 2009 The Cdk5/p35 kinases modulate leptin-induced STAT3 signaling. *Journal of Molecular Neuroscience* **39** 49–58. (doi:10.1007/s12031-008-9174-3)

Ho PW, Liu HF, Ho JW, Zhang WY, Chu AC, Kwok KH, Ge X, Chan KH, Ramsden DB & Ho SL 2010 Mitochondrial uncoupling protein-2 (UCP2) mediates leptin protection against MPP+ toxicity in neuronal cells. *Neurotoxicity Research* **17** 332–343. (doi:10.1007/s12640-009-9109-y)

Hubschle T, Thom E, Watson A, Roth J, Klaus S & Meyerhof W 2001 Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. *Journal of Neuroscience* **21** 2413–2424.

Li J, Deng J, Sheng W & Zuo Z 2012 Metformin attenuates Alzheimer's disease-like neuropathology in obese, leptin-resistant mice. *Pharmacology, Biochemistry, and Behavior* **101** 564–574. (doi:10.1016/j.pbb.2012.03.002)

Lieb W, Beiser AS, Vasan RS, Tan ZS, Au R, Harris TB, Roubenoff R, Auerbach S, DeCarli C, Wolf PA *et al.* 2009 Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. *Journal of the American Medical Association* **302** 2565–2572. (doi:10.1001/jama.2009.1836)

Lorefält B, Toss G & Granérus AK 2009 Weight loss, body fat mass, and leptin in Parkinson's disease. *Movement Disorders* **24** 885–890. (doi:10.1002/mds.22466)

Lu J, Park CS, Lee SK, Shin DW & Kang JH 2006 Leptin inhibits 1-methyl-4-phenylpyridinium-induced cell death in SH-SY5Y cells. *Neuroscience Letters* **407** 240–243. (doi:10.1016/j.neulet.2006.08.053)

Marwarha G, Dasari B, Prabhakara JP, Schommer J & Ghribi O 2010 β-Amyloid regulates leptin expression and tau phosphorylation through the mTORC1 signaling pathway. *Journal of Neurochemistry* **115** 373–384. (doi:10.1111/j.1471-4159.2010.06929.x)

Marwarha G, Dasari B & Ghribi O 2012 Endoplasmic reticulum stress-induced CHOP activation mediates the down-regulation of leptin in human neuroblastoma SH-SY5Y cells treated with the oxysterol 27-hydroxycholesterol. *Cellular Signalling* **24** 484–492. (doi:10.1016/j.cellsig.2011.09.029)

Morley JE & Banks WA 2010 Lipids and cognition. *Journal of Alzheimer's Disease* **20** 737–747.

Moult PR & Harvey J 2008 Hormonal regulation of hippocampal dendritic morphology and synaptic plasticity. *Cell Adhesion & Migration* **2** 269–275. (doi:10.4161/cam.2.4.6354)

Moult PR, Cross A, Santos SD, Carvalho AL, Lindsay Y, Connolly CN, Irving AJ, Leslie NR & Harvey J 2010 Leptin regulates AMPA receptor trafficking via PTEN inhibition. *Journal of Neuroscience* **30** 4088–4101.

Moult PR & Harvey J 2011 NMDA receptor subunit composition determines the polarity of leptin-induced synaptic plasticity. *Neuropharmacology* **61** 924–936. (doi:10.1016/j.neuropharm.2011.06.021)

Novakova L, Haluzik M, Jech R, Urgosik D, Ruzicka F & Ruzicka E 2011 Hormonal regulators of food intake and weight gain in Parkinson's disease after subthalamic nucleus stimulation. *Neuroendocrinology Letters* **32** 437–441.

O'Malley D, MacDonald N, Mizielinska S, Connolly CN, Irving AJ & Harvey J 2007 Leptin promotes rapid dynamic changes in hippocampal dendritic morphology. *Molecular and Cellular Neurosciences* **35** 559–572. (doi:10.1016/j.mcn.2007.05.001)

Oomura Y, Hori N, Shiraishi T, Fukunaga K, Takeda H, Tsuji M, Matsumiya T, Ishibashi M, Aou S, Li XL *et al.* 2006 Leptin facilitates learning and memory performance and enhances hippocampal CA1 long-term potentiation and CaMK II phosphorylation in rats. *Peptides* **27** 2738–2749. (doi:10.1016/j.peptides.2006.07.001)

Otvos L Jr, Terrasi M, Cascio S, Cassone M, Abbadessa G, De Pascali F, Scolaro L, Knappe D, Stawikowski M, Cudic P *et al.* 2008 Development of a pharmacologically improved peptide agonist of the leptin receptor. *Biochimica et Biophysica Acta* **1783** 1745–1754. (doi:10.1016/j.bbamcr.2008.05.007)

Otvos L Jr, Shao WH, Vanniasinghe AS, Amon MA, Holub MC, Kovalszky I, Wade JD, Doll M, Cohen PL, Manolios N *et al.* 2011 Toward understanding the role of leptin and leptin receptor antagonism in preclinical models of rheumatoid arthritis. *Peptides* **32** 1567–1574. (doi:10.1016/j.peptides.2011.06.015)

Oury F & Karsenty G 2011 Towards a serotonin-dependent leptin roadmap in the brain. *Trends in Endocrinology and Metabolism* **22** 382–387. (doi:10.1016/j.tem.2011.04.006)

Plum L, Schubert M & Brüning JC 2005 The role of insulin receptor signaling in the brain. *Trends in Endocrinology and Metabolism* **16** 59–65. (doi:10.1016/j.tem.2005.01.008)

Rosas-Vargas H, Martínez-Ezquerro JD & Bienvenu T 2011 Brain-derived neurotrophic factor, food intake regulation, and obesity. *Archives of Medical Research* **42** 482–494. (doi:10.1016/j.arcmed.2011.09.005)

Salminen A, Kaarniranta K, Haapasalo A, Soininen H & Hiltunen M 2011 AMP-activated protein kinase: a potential player in Alzheimer's disease. *Journal of Neurochemistry* **118** 460–474. (doi:10.1111/j.1471-4159.2011.07331.x)

Sadagurski M, Norquay L, Farhang J, D'Aquino K, Copps K & White MF 2010 Human IL6 enhances leptin action in mice. *Diabetologia* **53** 525–535. (doi:10.1007/s00125-009-1580-8)

Scott MM, Lachey JL, Sternson SM, Lee CE, Elias CF, Friedman JM & Elmquist JK 2009 Leptin targets in the mouse brain. *Journal of Comparative Neurology* **514** 518–532. (doi:10.1002/cne.22025)

Shanley LJ, O'Malley D, Irving AJ, Ashford ML & Harvey J 2002 Leptin inhibits epileptiform-like activity in rat hippocampal neurones via PI 3-kinase-driven activation of BK channels. *Journal of Physiology* **545** 933–944. (doi:10.1113/jphysiol.2002.029488)

Signore AP, Zhang F, Weng Z, Gao Y & Chen J 2008 Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials. *Journal of Neurochemistry* **106** 1977–1990. (doi:10.1111/j.1471-4159.2008.05457.x)

Tezapsidis N, Johnston JM, Smith MA, Ashford JW, Casadesus G, Robakis NK, Wolozin B, Perry G, Zhu X, Greco SJ *et al.* 2009 Leptin: a novel therapeutic strategy for Alzheimer's disease. *Journal of Alzheimer's Disease* **16** 731–740.

Thio LL, Erbayat-Altay E, Rensing N & Yamada KA 2006 Leptin contributes to slower weight gain in juvenile rodents on a ketogenic diet. *Pediatric Research* **60** 413–417. (doi:10.1203/01.pdr.0000238244.54610.27)

Valerio A, Dossena M, Bertolotti P, Boroni F, Sarnico I, Faraco G, Chiarugi A, Frontini A, Giordano A, Liou HC *et al.* 2009 Leptin is induced in the ischemic cerebral cortex and exerts neuroprotection through NF-κB/c-Rel-dependent transcription. *Stroke* **40** 610–617. (doi:10.1161/STROKEAHA.108.528588)

Walker CD, Long H, Williams S & Richard D 2007 Long-lasting effects of elevated neonatal leptin on rat hippocampal function, synaptic proteins and NMDA receptor subunits. *Journal of Neuroscience Research* **85** 816–828. (doi:10.1002/jnr.21173)

Weng Z, Signore AP, Gao Y, Wang S, Zhang F, Hastings T, Yin XM & Chen J 2007 Leptin protects against 6-hydroxydopamine-induced dopaminergic cell death via mitogen activated protein kinase signaling. *Journal of Biological Chemistry* **282** 34479–34491. (doi:10.1074/jbc.M705426200)

Xu L, Rensing N, Yang XF, Zhang HX, Thio LL, Rothman SM, Weisenfeld AE, Wong M & Yamada KA 2008 Leptin inhibits 4-aminopyridine- and pentylenetetrazole-induced seizures and AMPAR-mediated synaptic transmission in rodents. *Journal of Clinical Investigation* **118** 272–280. (doi:10.1172/JCI33009)

Zhang F & Chen J 2008 Leptin protects hippocampal CA1 neurons against ischemic injury. *Journal of Neurochemistry* **107** 578–587. (doi:10.1111/j.1471-4159.2008.05645.x)

Received in final form 5 September 2012
Accepted 11 September 2012
Made available online as an Accepted Preprint
11 September 2012

Life Sciences xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Life Sciences

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

Minireview

The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary hormone prolactin

Jaume Folch [a,c], Iván Patraca [a,c], Nohora Martínez [a,c], Ignacio Pedrós [a,b], Dmitry Petrov [b,c], Miren Ettcheto [b,c], Sonia Abad [b,c], Miguel Marin [f], Carlos Beas-Zarate [d,e], Antoni Camins [b,c,f,*]

[a] Unitats de Bioquímica i Farmacologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C./ St. Llorenç 21, 43201 Reus, Tarragona, Spain
[b] Unitat de Farmacologia i Farmacognòsia Facultat de Farmàcia, Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain
[c] Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos Tercero, Madrid, Spain
[d] Departamento de Biología Celular y Molecular, C.U.C.B.A., Universidad de Guadalajara and División de Neurociencias, Centro de Investigación Biomédica de Occidente (CIBO), Mexico
[e] Instituto Mexicano del Seguro Social (IMSS), Sierra Mojada 800, Col. Independencia, Guadalajara, Jalisco 44340, Mexico
[f] Centro de Biotecnología, Universidad Nacional de Loja, Av. Pío Jaramillo Alvarado y Reinaldo Espinosa, La Argelia, Loja, Ecuador

ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form 5 May 2015
Accepted 11 May 2015
Available online xxxx

Keywords:
Leptin
Amylin
Ghrelin
Prolactin
Insulin receptor
High-fat diet
Obesity
Hippocampus
Alzheimer's disease
PGC-1α
mTOR

ABSTRACT

Leptin (Lep) is emerging as a pivotal molecule involved in both the early events and the terminal phases of Alzheimer's disease (AD). In the canonical pathway, Lep acts as an anorexigenic factor via its effects on hypothalamic nucleus. However, additional functions of Lep in the hippocampus and cortex have been unravelled in recent years. Early events in the sporadic form of AD likely involve cellular level alterations which can have an effect on food intake and metabolism. Thus, AD can be conceivably interpreted as a multiorgan pathology that not only results in a dramatic neuronal loss in brain areas such as the hippocampus and the cortex (ultimately leading to a significant cognitive impairment) but as a disease which also affects body-weight homeostasis. According to this view, body-weight control disruptions are to be expected in both the early- and late-stage AD, concomitant with changes in serum Lep content, alterations in Lep transport across the blood–brain barrier (BBB) and Lep receptor-related signalling abnormalities. Lep is a member of the adipokine family of molecules, while the Lep receptor belongs to the class I cytokine receptors. Since cellular response to adipokine signalling can be either potentiated or diminished as a result of specific ligand–receptor interactions, Lep interactions with other members of the adipokine family including amylin, ghrelin and hormones such as prolactin require further investigation. In this review, we provide a general perspective on the functions of Lep in the brain, with a particular focus on the sporadic AD.

Contents

* Corresponding author at: Unitat de Farmacologia i Farmacognosia, Facultat de Farmàcia Universitat de Barcelona, Avda/Diagonal 643, E-08028 Barcelona, Spain.
E-mail address: camins@ub.edu (A. Camins).

http://dx.doi.org/10.1016/j.lfs.2015.05.002
0024-3205/

1. Introduction

Alzheimer's disease is one of the most common causes of senile dementia and it is estimated that by 2050, the number of cases will rise to 110 million [1,2]. The vast majority of patients suffer from the sporadic AD, with only a small subset of the population presenting with the familial form as a result of mutations in amyloid precursor protein (APP) Presenilin 1 or Presenilin 2 genes [3,4].

AD progression is associated with the formation of senile β-amyloid (Aβ) plaques and accumulations of hyperphosphorylated Tau proteins called neurofibrillary tangles in the brain [5,6]. Clinically, AD is characterized by a progressive loss of cognitive abilities as a result of severe hippocampal neurodegeneration, with the biggest impact on memory and learning faculties [7,8]. Aβ peptides are generated by a specific proteolytic cleavage of the APP. In this amyloidogenic pathway, the β- and γ-Secretases cleave APP at the N- and C-termini of the Aβ, respectively. The relationship between the aberrant APP processing and Aβ generation caused the formulation of the widely accepted "amyloid cascade hypothesis". It states that mutations in APP (or other genes) lead to an increase in Aβ which, when accumulated, leads to disease [9,10].

Apart from the Aβ itself, a host of other factors contributing to AD pathology have been identified: oxidative stress and ROS generation, alterations in glucose metabolism, deregulation of Ca^{2+} signalling, deregulation of glial cell activity, alterations in nutritional behaviour, metabolic syndrome and obesity, hypertension and type 2 diabetes mellitus (T2DM) [11–19]. Thus, it is difficult to point out a single pathogenic mechanism leading to the onset and progression of this devastating disease. It has been suspected for many years that AD may in fact be a multifactorial metabolic disorder influenced by several risk factors such as hypertension, diabetes, hypercholesterolaemia, neuroinflammation and hypoxia, among others [13–15].

In the next sections we discuss some of the metabolic aspects of AD, with a special emphasis on adipokines in general, and leptin in particular.

2. Lifestyle and AD

As a means to slow down the onset of disease symptoms, researchers have turned their attention to the relationship between lifestyle and AD [20]. Lifestyle-related diseases are potentially preventable, and their incidence can be decreased with adequate changes in diet, amount of physical activity and modification of the environment. The common feature at the core of most lifestyle diseases is obesity, and accumulating evidence indicates that obesity is an independent risk factor for developing AD. Obesity is a pandemic and a serious global health concern. Obesity is also a risk factor for multiple conditions and contributes to multi-morbidities, resulting in increased healthcare costs and millions of deaths each year [21]. Obesity has been associated with changes in brain structure, cognitive deficits, dementia and AD. In agreement with this, high-fat diet (HFD)-induced obesity also causes a variety of health disorders including cognitive decline in experimental animal models [22]. There is a well-established link between human obesity and cognitive decline [23]. Specific brain functions related to the hippocampus may be particularly vulnerable as evidenced in a large number of studies in rodents linking high-caloric diets with decreased contextual and spatial memory [24–29]. Significantly, it has been demonstrated that obese animals whose diet regimen is reversed from HFD back to standard chow, recover memory function [22].

3. Obesity, type 2 diabetes mellitus and leptin resistance

As mentioned above, obesity significantly increases cognitive decline and AD risk, supporting the notion that AD is a degenerative metabolic disease in which brain glucose uptake and utilization are impaired [30]. Thus, several early biomarkers rely on the definition of AD as a "Cognitive Metabolic Syndrome" or "Type 3 Diabetes" [31]. Biological plausibility for this relationship has been framed within the "Metabolic cognitive syndrome" concept. Even more, it has been proposed that Aβ accumulation can be considered a consequence rather than the real etiologic basis for the disease. A growing body of epidemiological evidence suggests that metabolic syndrome and its components (impaired glucose tolerance, abdominal or central obesity, hypertension, hypertriglyceridemia, and reduced high-density lipoprotein cholesterol) may be important in the development of age-related cognitive decline, mild cognitive impairment, vascular dementia, and AD [32]. Furthermore, results from the "Hysayama Study" indicate that altered expression of genes related to diabetes mellitus in AD brains is a result of AD pathology, which may thereby be exacerbated by peripheral insulin resistance or diabetes mellitus [33].

In fact, adults with newly diagnosed prediabetes, or type 2 diabetes mellitus (T2DM), show insulin resistance associated with reductions in regional cerebral glucose metabolism and subtle cognitive impairments [34]. Furthermore, most obese individuals show increased food intake despite high circulating Lep levels [35]. These findings imply a state of Lep resistance that causes a reduced responsiveness to Lep anorexigenic effect, with a concomitant loss of appetite- and weight gain-suppressing effects [36,37].

4. Leptin functions in the hypothalamus

The peripheral actions of metabolic hormones are well documented. However, the functions of these pleiotropic hormones are not restricted to the periphery, with growing evidence suggesting that both Lep and insulin can readily cross the BBB, producing widespread central effects in brain areas like the hypothalamus. In the periphery, the fat mass participates in the regulation of glucose and insulin metabolism through the release of hormones in a bidirectional feedback loop, a mechanism called the "Adipoinsular axis" (Fig. 1) [38,39]. This axis links adipose tissue and pancreatic β-cells via leptin and insulin, respectively. As insulin directly stimulates Lep release by adipose tissue, Lep feeds back to reduce both insulin secretion and insulin gene expression in β-cells by modulation of K^{+}_{ATP} channels and activation of cyclic nucleotide phosphodiesterase 3B and subsequent suppression of cAMP levels [40,41]. The suppressive effect of Lep on insulin production is not only mediated by direct actions via Lep receptors (LepR) on β-cells, but also by the autonomic nervous system (ANS). Lep-dependent ANS regulation of body weight is largely achieved via a negative afferent loop involving the hypothalamus [42,43].

It has been shown that hypercaloric diets (HCD) used in a majority of diet-induced obesity studies, typically induce glucose metabolism abnormalities and insulin resistance (including diabetes mellitus) and persistent hyperleptinaemia [44]. In addition, the consumption of Western diets, rich in sugar and saturated fat, stimulates an inflammatory response in the hypothalamus, a contributing factor to the development of central Lep resistance and obesity [36]. In the hypothalamus, specialized groups of neurons are sensitive to signals derived from several organs related to food intake or starvation. Hypothalamic pro-opiomelanocortin (POMC) and neuropeptide Y–Agouti-related peptide (NPY–AgRP) neurons produce anorexigenic and orexigenic neuropeptides and neurotransmitters, and express the long signalling form of the leptin receptor (LRb). The anorexigenic properties and the regulatory functions of Lep in the control of energy and glucose homeostasis are largely dependent on the POMC and NPY–AgRP circuits in the arcuate nucleus of the hypothalamus. POMC and NPY neurons are considered as major Lep effector sites, with the food-intake regulation directly dependent on the LRb/STAT3 (activator of transcription 3) interaction [45,46].

Recent evidence suggests that sirtuin-1 (SIRT1) activation and expression are essential for leptin-induced anorexigenic effects in the hypothalamic POMC neurons [47]. Moreover, results in Lep deficient ob/ob mouse model show a lack of SIRT1 activation in the hypothalamus in response to caloric restriction, compared to age-matched controls [48].

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

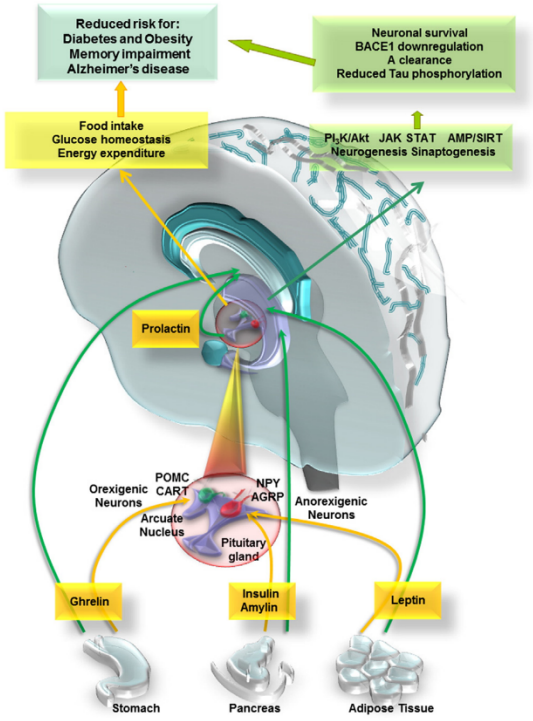


Fig. 1. Leptin, amylin and insulin decrease appetite by inhibiting neurons that produce the molecules NPY and AgRP, while stimulating melanocortin-producing neurons in the arcuate-nucleus region of the hypothalamus, near the third ventricle of the brain. NPY and AgRP stimulate eating, and melanocortins inhibit eating, via other neurons. Activation of NPY/AgRP-expressing neurons inhibits melanocortin-producing neurons. The gastric hormone ghrelin stimulates appetite by activating the NPY/AgRP-expressing neurons. In the hypothalamus, leptin regulates food intake, glucose homeostasis, and energy expenditure. The hippocampus integrates signals from Lep ghrelin, insulin, amylin and prolactin, and contributes to decrease risk of memory impairment related to obesity and T2DM, which are all risk factors for Alzheimer's disease.
Adapted from Marwarha and Ghribi [72], and from Schwartz and Morton [132].

The complexity of AD pathology is illustrated by the fact that apart from clear links between AD and obesity, weight loss is another prominent early feature of AD that often precedes cognitive decline and clinical diagnosis. In fact, pathologically low Lep levels and disruptions in orexigenic NPY hypothalamic neuron signalling were described in a mouse model overexpressing Aβ, suggesting that Lep regulation may be involved [49].

A large body of work has identified inflammatory signalling in the hypothalamus as an essential mediator of the sickness response: anorexia, cachexia, fever, inactivity, lethargy, anhedonia and adipsia are all triggered by systemic inflammatory stimuli and promote negative energy balance [50].

5. Leptin, amylin and ghrelin

Alzheimer's disease and T2DM share many common features including the deposition of amyloidogenic proteins — Aβ and amylin (islet amyloid polypeptide), respectively [51]. The hormone amylin, discovered in 1987, is the second β-cell hormone described. It is co-secreted with insulin by the pancreatic β-cells in response to nutrient stimuli, in the context of the "Adipoinsular axis" (Fig. 1). Dysregulation of the axis may contribute to obesity and the development of hyperinsulinaemia associated with diabetes [39,52]. In addition, impaired β-cell secretion of amylin in response to a meal had been described in T2DM patients [53,54]. Recent evidence suggests that both Aβ and amylin may act via the amylin receptor, although the precise mechanisms for this interaction at a cellular level are unknown.

One of the roles of amylin is to slow gastric emptying, thereby delaying the delivery of nutrients to the circulation [55]. A second effect is to decrease food intake, and a third effect is to reduce postprandial hyperglucagonemia, thereby inhibiting hepatic glucose release. Despite the beneficial effects of amylin in the CNS, it has also been shown to induce neurotoxicity in embryonic rat hippocampal primary cultures in vitro [56]. This effect may contribute to the prominent neurite degeneration in AD [57]. Furthermore, centrally administered amylin resulted in impaired memory retention (on a footshock avoidance conditioning in a T maze) in mice given strong training [58].

Besides the involvement of Lep and amylin in energy regulation, an additional adipokine ghrelin may also be implicated in weight loss observed in AD patients. Leptin and insulin stimulate anorexigenic POMC neurons, whereas ghrelin stimulates orexigenic NPY–AgRP neurons. Ghrelin is an endogenous ligand for GH secretagogue receptor type 1α (GHSR1α). It is a multifunctional hormone produced and released mainly from the stomach, which assists in the promotion of sensations of hunger [59]. Ghrelin has been associated with the progression of obesity and metabolic syndrome, but has been also linked to neuromodulation, neuroprotection, memory and learning processes [60]. Ghrelin receptors are prominently expressed in different regions of the brain, including the arcuate nucleus, ventromedial nuclei, in CA2 and CA3 regions of the hippocampus, in the *substantia nigra*, in the ventral tegmental area, the dentate gyrus of the hippocampal formation, and the dorsal and median raphe nuclei [61,62].

It has been shown that intracerebroventricular injections of ghrelin increased memory retention in rats [63–65]. In fact, ghrelin administration rescued memory deficits and prevented neuronal and synaptic degeneration in the hippocampi of mice injected with Aβ [66]. Furthermore, ghrelin also decreased the levels of hyperphosphorylated Tau via the PI3K/Akt–GSK pathway, in hippocampal neurons [67]. These studies suggest that ghrelin plays a vital role not only in metabolic control, but also in regulating cognitive function and memory capacity. Therefore, abnormal ghrelin signalling could be one of the causes and/or consequences of AD symptomatology [64,65,68,69].

Thus, it can be concluded that there is an association between obesity, T2DM and AD, where altered mechanisms of insulin, Lep, ghrelin and amylin signalling lead to a greater susceptibility to neurodegenerative processes. Taken together, these results suggest that peripheral metabolic peptides, in particular Lep and ghrelin, might be considered as potential preventive strategies for ameliorating the hypothalamic alterations in AD [70].

6. Leptin functions in the hippocampus

As mentioned earlier, circulating Lep is transported across the BBB into the brain, where it regulates food intake, glucose homeostasis, and energy expenditure mainly via the hypothalamic circuits. Loss of Lep signalling may increase the likelihood of developing atherosclerosis, obesity and T2DM, all independent risk factors for AD. However, functional Lep receptors (LepR or ObRb) also have been reported to be expressed in the hippocampus and other cortical regions of the brain. The physiological significance of this observation has been explored during recent years. Then, besides the described canonical functions of insulin, Lep, and ghrelin in the control of metabolism, each of these hormones has been implicated in the control of neuronal survival, astrocyte activity and neuroprotection in extrahypothalamic regions, in particular the hippocampus.

It is well known that the hippocampus is an area which is severely affected during the course of AD. The cognitive deficits associated with T2DM have been linked to impaired central insulin modulation in the hippocampus, which is a critical region for memory processing [71].

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

Furthermore, epidemiological studies have demonstrated that higher circulating Lep levels are associated with lower risk of dementia including AD, whereas lower circulating levels of Lep have been reported in patients with AD [72]. It has been demonstrated that chronic Lep administration has led to memory improvements in the CRND8 transgenic mouse model of AD [73,74]. The hippocampus expresses high levels of both insulin and Lep receptors, as well as key components of their associated signalling cascades. Recent studies indicate that both hormones are potential cognitive enhancers [75]. Indeed, it has been demonstrated that both Lep and insulin markedly influence key cellular events that underlie hippocampal learning and memory, including activity-dependent synaptic plasticity and the trafficking of glutamate receptors to and away from the hippocampal synapses [74,75]. Furthermore, the induction of apoptosis among hippocampal cells can be related to impairments in either Lep or insulin function. Thus, it appears that Lep confers protection against hippocampal-dependent AD neuropathology.

An important question is whether leptin deficiency plays a role in the causation of AD and/or its progression. In fact, Lep deficiency in AD contributes to a neuronal imbalance in handling energy requirements, leading to higher Aβ and phosphorylated Tau. Additionally, Lep modulates AD pathological pathways in vitro through a mechanism involving the energy sensor, AMP-activated protein kinase (AMPK) [74]. Lep activates the PI_3K/Akt, JAK STAT, and AMPK/SIRT pathways, promoting neuronal survival, reducing Aβ production and increasing its clearance, and reducing Tau hyperphosphorylation [72,76].

We can conclude that Lep promotes neurogenesis and synaptogenesis, thus facilitating learning and memory processes in the hippocampus (Fig. 1). Nevertheless, the ability of Lep to regulate hippocampal synaptic function markedly declines with age, and these changes have been linked to neurodegenerative disorders such as AD [77]. In fact, it has been described that individuals with early AD, or mild cognitive impairment, show low plasma Lep levels [78]. Accumulating data suggest that AD patients may benefit from Lep replacement therapy, and it may constitute a very significant application of Lep [79]. Then, Lep deficiency in AD can be restored by replenishing low Lep levels, and this may also be a legitimate strategy for therapy [74].

7. Leptin signalling

As discussed before, several studies have shown that AD and T2DM may share common pathways to pathology. In fact, Lep and insulin signalling pathways may act synergistically with the insulin receptor leading to the development of neurodegenerative processes [80]. Early alterations in glucose, insulin signalling and energy metabolism pathways have been demonstrated in an APPswe/PS1dE9 mouse model of familial AD. The observed changes are complex and related to impairment in insulin and adipokine receptors, all along with alterations in cholesterol and fatty acid metabolism [81].

Leptin and insulin receptors are widely expressed in the central nervous system. The human ObRb is a member of the superfamily of cytokine receptor class I (gp130). The best-described signalling pathway activated in response to receptor activation involves the coordinated functions of JAK2/STAT3 (Fig. 2). STAT3 is a transcription factor that, upon phosphorylation, dimerizes and is translocated to the nucleus, where it controls the transcription of target genes. ObRb is negatively regulated by the suppressor of cytokine signalling 3 (SOCS3) and protein tyrosine phosphatase 1β (PTP1β). Key downstream effectors of ObRb include AMP-activated protein kinase (AMPK), PGC-1α (involved in mitochondrial biogenesis), as PPAR, as well as aspartyl protease β-site AβPP-cleaving enzyme (BACE1). Among the targets of the receptor we can also find kinases involved in Tau phosphorylation and microtubule stability, including the mammalian target of rapamycin (mTOR).

Leptin is involved in the activity of kinases like mTOR, which could perhaps explain the significance of Lep resistance to both AD and T2DM. As Orr and colleagues propose, since mTOR hyperactivity is common to both diabetes and AD, mTOR signalling could be considered a molecular link between these two age-related diseases [82]. Leptin signalling also induces the activation of the ubiquitous and broad spectrum PI3K/Akt/mTOR pathway [83]. Indeed, it has been shown that Lep binding to its long-form receptor can activate four major signal transduction pathways: JAK/STAT pathway; ERK pathway; PI3K/Akt/mTOR pathway as well as the AMPK/SIRT1 signal transduction pathways [72,76].

The mammalian mTOR plays a key role in maintaining energy homeostasis in the brain and other tissue types [82,84]. As an energy sensor, mTOR regulates numerous cellular pathways including protein translation, cell growth and proliferation. In fact, mTOR mediates the synthesis and aggregation of Tau, resulting in compromised microtubule stability [85]. mTOR has been shown to modulate insulin signalling in a context of high nutrient exposure. mTOR directly phosphorylates the insulin receptor leading to its internalization; this, in turn, results in a decrease of mTOR signalling [82,86]. However, through the same mechanisms, chronic mTOR hyperactivity leads to insulin resistance, a key feature of T2DM [87].

AMPK is a cellular energy sensor highly conserved among eukaryotes. AMPK regulates the activities of a number of key metabolic enzymes through phosphorylation [88]. It protects cells from stresses that cause ATP depletion by switching off ATP-consuming biosynthetic pathways. Furthermore, previous data suggest that AMPK can also phosphorylate substrates like Tau proteins, thereby favouring their aggregation. Phosphorylated Tau becomes soluble and causes microtubule disassembly. In extreme cases, including in AD, hyperphosphorylation of Tau leads to the formation of neurofibrillary tangles.

AMPK can phosphorylate and directly activate PGC-1α that, in turn, controls major metabolic functions through the co-activation of PPARγ and other transcription factors [89]. PGC-1α is a PPAR transcriptional coactivator, and elevated levels of PGC1α change the composition of peroxisomes, so that they might exhibit decreased insulin degradation and purine metabolism. PPARs are ligand-activated transcription factors of the nuclear receptor superfamily. The levels of PPARs have been reported to decline with age [90]. PPARγ is highly expressed in adipose tissue and is a major regulator of insulin and glucose metabolism. It can be therefore suggested that the link between energy metabolism and the amyloid cascade hypothesis stems from the fact that PPARγ regulates the transcription of BACE1, a key enzyme involved in Aβ generation. The enzyme BACE1 catalyses the rate-limiting step in Aβ production, a peptide at the nexus of neurodegenerative cascade in AD. Leptin has been shown to reduce Aβ production by decreasing BACE1 activity and expression levels [72,76]. Leptin increases the expression and activity of SIRT1, which results in decreased NF-κβ-mediated transcription of BACE1.

Furthermore, since PGC-1α appears to decrease Aβ generation, therapeutic modulation of PGC-1α could have real potential as a treatment for AD. Thus, Lep emerges as a general activity modulator of AMPK, PGC-1α and the molecules downstream of them: PPARγ and BACE1.

8. Leptin and inflammation

Chronic inflammatory processes are common in obesity, cancer and neurodegenerative diseases like AD. Although the BBB restricts access of immune cells and mediators from the blood, innate immune activation can occur throughout the brain in response to both local and systemic inflammatory stimuli [91]. The apoE4 genotype, lifestyle-induced obesity and dyslipidaemia could be considered as systemic inflammatory stimuli, whereas Aβ and Tau protein accumulation are considered local stimuli. Chronic inflammation is known to cause Lep resistance that, as previously discussed, is a status related to T2DM and AD [92].

It has been shown that both the accumulation of Aβ and apoE4 genotype result in a transient enhancement of Lep signalling that might lead to Lep resistance over time [93]. The mechanisms by which Aβ and apoE4 affect LepR expression are unknown. Both molecules have been

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

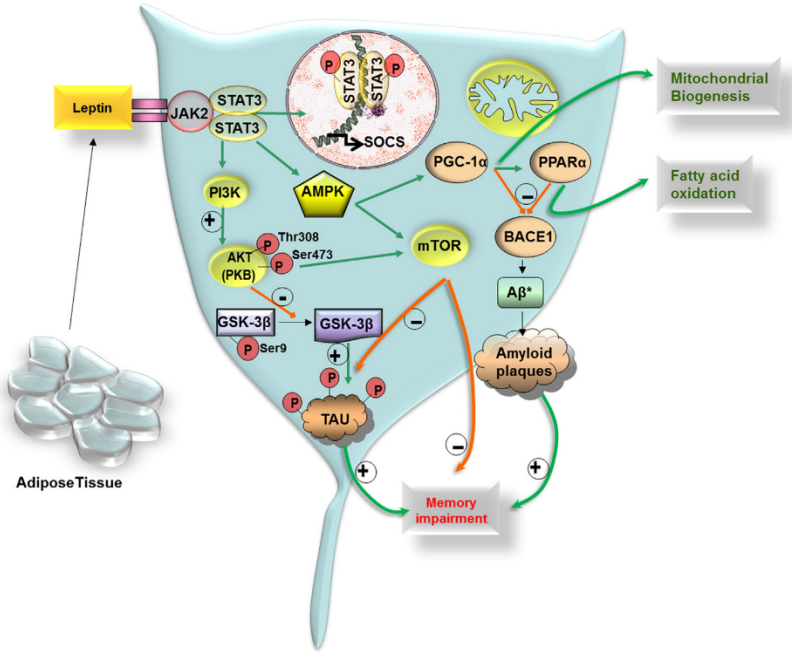


Fig. 2. Leptin signalling. In the hippocampus, Lep activates the PI_3K/Akt, JAK STAT, and AMK/SIRT pathways, thus promoting neuronal survival, BACE1 down-regulation, reducing Aβ production and increasing its clearance, and reducing Tau phosphorylation. Leptin activation of Lep receptor induces STAT-3 phosphorylation activating the transcription of genes from SOCS family, among others. Leptin inhibits the hyperphosphorylation of Tau protein through PI3K and AKT activation that, in turn, promotes the formation of inactive form ser-9 GSK3-β. Leptin also inhibits GSK3-β activity acting on AMPK, and reducing the formation of Aβ deposits. Leptin activates PGC-1α that, in turn, causes the inhibition of BACE1 and Aβ accumulation. PGC-1α activation induces mitochondrial biogenesis and induces PPARα activity and fatty acid oxidation.
Adapted from Greco et al. [74].

shown to cause inflammation, and LepR upregulation has been demonstrated in response to proinflammatory stimuli, such as treatments with LPS and TNFα [94]. In fact, transgenic animal models overexpressing human forms of Aβ or human apoE4, showed enhanced inflammatory reactions in the brain, including TNFα generation and gliosis [95,96]. Thus, it may be speculated that the initial up-regulation of LepR could result from the pro-inflammatory effects of Aβ or apoE4.

Current evidence indicates that hypothalamic proinflammatory phenotype could play a key role in central Lep and insulin resistance. Recent studies have shown that diet-induced obesity (DIO), or HFD-induced obesity, accelerates AD progression. Hypothalamic inflammation, induced by HFD feeding, favours increases in body weight [58]. It has been shown that DIO increases amyloid deposition in amyloidogenic transgenic mice [97]. In WT rats, DIO causes Tau phosphorylation, increases glial fibrillary acidic protein (GFAP) expression and astroglial activation in the hippocampus and impairs cognitive function [98–100]. Interestingly, these changes were associated with enhanced astrocytic LepR expression and mild microgliosis, but not Aβ accumulation. The involvement of microglia and astrocytes in the onset and progress of neurodegenaration in AD is increasingly recognized [101]. Glial cells maintain brain plasticity and protect the brain to ensure functional recovery from injuries. Dysfunction of glial cells may promote neurodegeneration and, eventually, the retraction of neuronal synapses, which leads to cognitive deficits [100,101].

The hippocampus may be particularly vulnerable to the negative consequences of HFD, and it is suspected that the HFD may 'prime' the proinflammatory response in this area, a process which is potentially regulated by fatty acid signalling [22]. Several studies have attempted to correlate hypothalamic expression of specific cytokines (IL-1β, TNFα, IL-6 and IL-10) to either DIO or to intraventricular administration of saturated fatty acids [102,103]. Results show that saturated fatty acids can stimulate microglia, leading to upregulation of NF-kβ and pro-inflammatory cytokine expression [103–104].

Proinflammatory cytokines such as Interleukin-1 (IL-1), IL-4, IL-6, IL-10, IL-12, and IL-18, IFN-γ, TNFα, TGF-β, and several chemokines known to cause glial activation, have been suggested as potential AD biomarkers. These and other related pro- and anti-inflammatory molecules are rapidly overexpressed by glia in adult rodent hippocampus in various models of limbic seizures [104–106]. In addition, it has been demonstrated that in the hippocampus, neuronal injury occurs only when the cytokines are induced in glia, and cytokine synthesis precedes the appearance of degenerating neurons [106]. In this context, neuronal injury is more pronounced when both IL-6 and TNF-α are produced in addition to IL-1β.

Adipokines and circulating food-intake-controlling metabolites are capable of reducing glial activation in models of excitoxicity. It has been demonstrated that Lep was able to protect hippocampal neurons against kainate excitotoxicity in an experimental model of epilepsia in Lep deficient ob/ob mice [107]. Likewise, ghrelin prevented kainate-induced activation of microglia and astrocytes, and inhibited the expression of proinflammatory mediator TNFα, IL-1β, and cyclooxygenase-2 [104,105]. The inhibitory effect of ghrelin appears to be associated with the reduction in matrix metalloproteinase-3 expression in damaged hippocampal neurons [104]. Furthermore, recent findings demonstrate that desacyl-ghrelin, hexarelin and EP-80317 ligands display relevant anticonvulsive properties in models of limbic seizures [108].

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

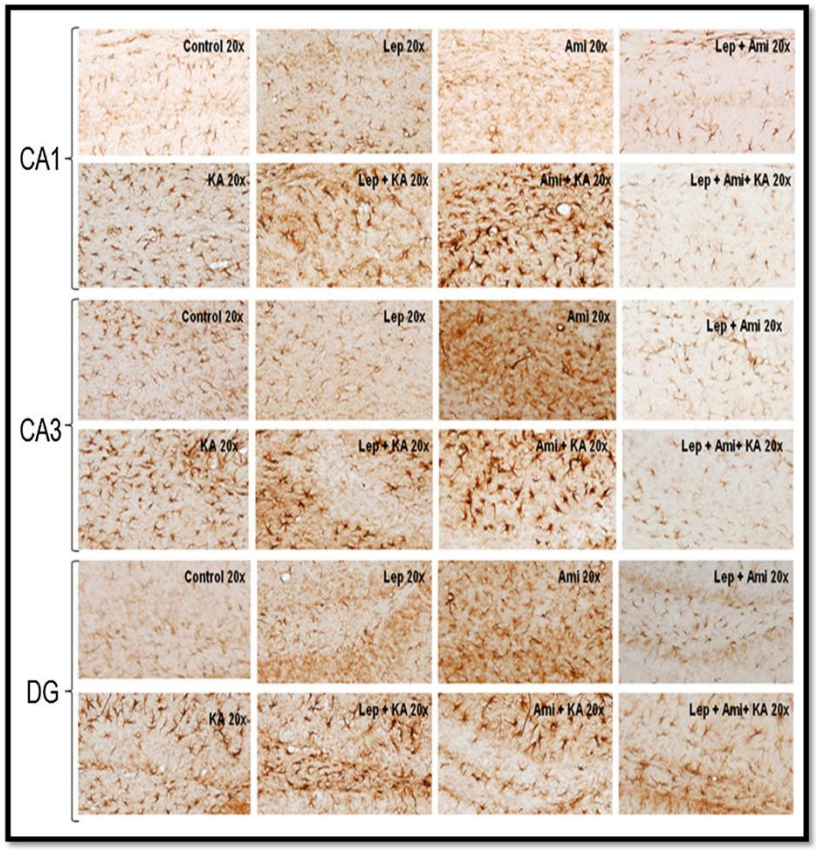


Fig. 3. Effect of Lep and amylin administration on hippocampal gliosis. As it is shown, treatment with KA 24.5 mg/kg, caused a reactive process of gliosis, as revealed from the immunohistochemistry of GFAP. The gliosis was especially intense in both CA1 and DG regions, when compared with the controls. Whereas treatment with amylin and Lep alone did not produce significant differences when compared with the controls, the combined exposure to amylin and Lep was able to decrease the process of reactive gliosis in CA1 and CA2 areas.

Lactation is a natural model for neuroprotection because it effectively prevents acute and chronic cell damage of the hippocampus that can be induced by excitotoxicity. It is becoming apparent that neuroendocrine hormones including oxytocin, progesterone and prolactin (PRL), apart from their roles in lactation, may also have neuroprotective effects on hippocampal neurons [109].

Among the pituitary hormones, PRL is the most versatile in the spectrum and number of functions it regulates. PRL modulates virtually every aspect of vertebrate physiology, including osmoregulation, growth, metabolism, development, reproduction, parental behaviour, and immune function [110]. In mammals, this hormone may act in cooperation with Lep to transfer information to the brain about the caloric state of the animal [111].

It has been shown that PRL prevents the damaging effects of excitotoxicity in the dorsal hippocampus [112]. Trophic actions of PRL in the CNS include mediating development and maturation of dopaminergic tuberoinfundibular neurons [113]. It also regulates neurogenesis and brain cell proliferation [114]. Prolactin also shows mitogenic actions on astroglia and protects hippocampal neurogenesis in the dentate gyrus of chronically stressed mice [115,116]. Prolactin is also involved in immune regulation [117]. In the brain, prolactin receptors (Prl-Rs), which belong to the class I cytokine receptor superfamily, have been detected in the cortex, hypothalamus and hippocampus, in both astrocytes and glial cells [77]. Neuroprotective properties of PRL have also been demonstrated in a kainic acid-induced rat model of epilepsy, where the administration of PRL to ovariectomised rats significantly reduced seizures and neurotoxicity in the hippocampus [118]. This is noteworthy, as recent data suggest that PRL may be a possible marker for obesity in humans [77].

Finally, recently published results from our group show that PRL gene is down-regulated at early stages of amyloidogenesis, in an APPswe/PS1dE9 double transgenic murine model of AD [80,81]. We observed a significant down-regulation of the Prl-Rs and PRL genes in the hippocampus of 3 month-old APPswe/PS1dE9 mice, when compared to a wild-type control group. Thus, our results indicate early perturbations in this particular biological route, at early stages of the neurodegenerative process, when both cognitive impairments and Aβ deposits have yet to develop.

It has been shown that Lep is a powerful stimulator of in vitro PRL release and that its actions occur in part through stimulation of ERK1/2 [119]. This observation, once again, demonstrates the existence of a complex net of interactions among different adipokines, and related hormones like PRL, that should be extensively analysed to gain a better understanding of AD aetiology.

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

9. Synergic effects among adipokines

Besides the compelling evidence concerning the effects of adipokines Lep, amylin and ghrelin, and pituitary-derived hormones like PRL in the brain, there is a relative lack of knowledge in relation to either the synergistic or antagonistic effects of their combinations.

The first evidence of effects from combined actions of these molecules comes from the fact that the high circulating levels of PRL (or placental lactogens) during pregnancy, may directly interfere with Lep receptor signalling, possibly predisposing to Lep resistance [120]. Furthermore, pregnancy is characterized by marked changes in the control of energy balance [121,122]. In addition, Lep is a very potent in vitro secretagogue of PRL release in ectotherms [119]. However, the physiological significance of Lep actions in stimulating PRL release, including any similar effects on PRL secretion in vivo, remains uncertain.

Another point of interest is the synergic effects of amylin and Lep in reducing body weight and adiposity. Unfortunately, treatment of DIO animals and obese humans with Lep alone, did not demonstrate the dramatic weight-reducing benefits seen in ob/ob mice. As De Paoli stated in a recent review, the possibility to potentiate or enable the weight-reducing properties of Lep has led to significant efforts to combine Lep with other interventions or agents known to induce weight loss in order to overcome leptin resistance [123]. It seems that either a resistance to the weight-reducing actions of Lep exists in DIO mice and obese humans, or that Lep is not actively functioning to reduce body weight in the obese state. These observations led to the conclusion that, in the absence of endogenous amylin, Lep signalling is likely diminished. Results from a diet-induced leptin-resistant obese rat model, show that pre-treatment with amylin, for 1 week, restored Lep-induced neuronal activation of phosphorylated STAT3 signalling, within the ventromedial hypothalamus [124–126]. Furthermore, it has been shown that amylin augmented Lep signalling/receptor binding [126]. More recent data show that amylin-induced microglial IL-6 production is the likely mechanism by which amylin treatment interacts with Lep signalling to increase its anorexigenic effects in the hippocampus [127]. Taken together, these data establish a novel role for Lep and amylin in the processes of mouse hippocampal neurogenesis, and provide new insights into the mechanisms of neurogenic regulation [123].

It has been demonstrated that Lep and amylin, alone and in combination, activate signal transducer STAT3, AMPK, Akt, and extracellular signal-regulated kinase signalling pathways in human primary adipocytes, human peripheral blood mononuclear cells, and ex vivo in human adipose tissue from male versus female subjects [moon]. However, the authors conclude that in humans Lep and amylin activate overlapping intracellular signalling pathways and have additive, but not synergistic, effects.

Unpublished results from our laboratory suggest synergic effects of the combination of amylin and Lep in reducing kainate-induced neurotoxicity in mouse hippocampus (Fig. 3). In our experiments, a single treatment with KA 24.5 mg/kg caused a reactive gliosis at 72 h, clearly evident with GFAP immunohistochemistry. The gliosis was intense in both CA1 and DG regions, when compared with the respective controls, and treatment with amylin and Lep alone did not result in significant decreases in reactive gliosis. The combined exposure to amylin and Lep, however, produced a significant reduction in gliosis in CA1 and CA2 areas. Further experiments demonstrated that these effects were specifically related to GSK-3β and Akt activities. In fact, it has been shown that in vitro exposure of HEK293 cells to Aβ1–42 and human amylin increased cytosolic cAMP and $Ca^{(2+)}$ levels and triggered multiple pathways involving the signal transduction mediators protein kinase A, MAPK, Akt, and c-Fos [126,127].

The effects of amylin and Lep on AMPK activity are particularly remarkable. AMP-activated protein kinase, a master regulator of cellular energy homeostasis and a central player in glucose and lipid metabolism, is potentially implicated in the pathogenesis of AD [62]. AMPK activity decreases in AD brain, indicating decreased mitochondrial biogenesis and function. The roles of AMPK in the pathogenesis of AD include Aβ generation and Tau phosphorylation at Thr-231 and Ser-396/404, and an inhibition of mTOR signalling pathway, thus facilitating autophagy and promoting lysosomal degradation of Aβ. Then, AMPK emerges as a potential key target in the AD treatment and highlights the potential usefulness of amylin and Lep as neuroprotective agents in the context of AD. In agreement with that, recent translational research findings point to a potential therapeutic approach that incorporates amylin (a beta-cell hormone) and Lep agonism, restoring or enhancing Lep sensitivity [126–132].

10. Concluding remarks

The present review emphasizes the role of adipokines and hormones related to adipoinsular axis in the development of AD. Leptin and amylin modulate the activity of key molecules involved in energy regulation, including AMPK, which plays a role in mitochondrial function, neurogenesis and cognition. Similarly, a number of studies suggest a central regulatory role for ghrelin not only in metabolism, but also in modulation of cognitive functions and memory capacity. In addition, these adipokines can regulate the inflammatory response mediated by the glia cells, a very useful property considering that inflammation plays a large role in AD neuropathology. Both ghrelin and Lep have demonstrable capacity to reduce seizures caused by exposure to kainic acid, and also limit the extent of glial inflammatory response. A comprehensive understanding of Lep functions at the cellular level, including elucidating potential interactions with other members of the adipocytokine family, is required for the development of novel pharmacotherapies targeting sporadic AD. This knowledge can contribute to the long-term goal of both AD prevention and treatment.

As a general conclusion, adipokines in general, and Lep in particular, are promising AD targets.

Conflict of interest statement

The authors declare no competing financial interests.

Abbreviations

Aβ	β-amyloid
AD	Alzheimer's disease
AMPK	AMP-activated protein kinase
ANS	the autonomic nervous system
BACE1	PPAR and aspartyl protease β-site AβPP-cleaving enzyme
BBB	brain blood barrier
CSF	cerebrospinal fluid
DIO	diet-induced obesity
GHSR1α	GH secretagogue receptor type 1α
HCD	high calorie diet
HFD	high-fat diet
IGF-1	insulin-like growth factor-1
JAK	janus tyrosine kinase
LepR (or ObRb)	Lep receptors
LRb	long signalling form of the Lep receptor
MAPK	mitogen-activated protein kinase
NPY–AgRP	neuropeptide Y–Agouti-related peptide
ObRb	leptin receptor
PI3k	phosphoinositide 3-kinase
POMC	pro-opiomelanocortin
PRL	prolactin
Prl-Rs	prolactin receptors
PTP1β	protein tyrosine phosphatase 1β
SIRT1	sirtuin-1
STAT3	signal transducers and activators of transcription
SOCS3	suppressor of cytokine signalling 3
T2DM	type 2 diabetes mellitus

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

Acknowledgements

This study was funded by grant 2009/SGR00853 from the Generalitat de Catalunya (autonomous government of Catalonia), by grant SAF2011-23631 from the Spanish Ministerio de Ciencia e Innovación, Grant 0177594 from the CONACYT (Mexico), and Project "Prometeo" from SENESCYT (Government of Ecuador). We also would like to express our gratitude to Ms. Ana Nieto for revising this manuscript.

References

[1] H. Hampel, D. Prvulovic, S. Teipel, F. Jessen, C. Luckhaus, L. Frolich, M.W. Riepe, R. Dodel, T. Leyhe, L. Bertram, W. Hoffmann, F. Faltraco, German Task Force on Alzheimer's Disease. The future of Alzheimer's disease: the next 10 years, Prog. Neurobiol. 95 (2011) 718–728, http://dx.doi.org/10.1016/j.pneurobio.2011.11.008.
[2] A. Wimo, L. Jonsson, J. Bond, M. Prince, B. Winblad, Alzheimer Disease International, The worldwide economic impact of dementia 2010, Alzheimer's Dement, J. Alzheimer's Assoc 9 (2013) 1–11, http://dx.doi.org/10.1016/j.jalz.2012.11.006.
[3] C. Qiu, L. Fratiglioni, Epidemiology of dementia, in: P. McNamara (Ed.), Dementia Treatments and Developments, ABC-CLIO, California 2011, pp. 1–33.
[4] C. Reitz, C. Brayne, R. Mayeux, Epidemiology of Alzheimer disease, Nat. Rev. Neurol. 7 (2011) 137–152, http://dx.doi.org/10.1038/nrneurol.2011.2.
[5] A. Erol, An integrated and unifying hypothesis for the metabolic basis of sporadic Alzheimer's disease, J. Alzheimers Dis. 13 (2008) 241–253.
[6] S.W. Pimplikar, Reassessing the amyloid cascade hypothesis of Alzheimer's disease, Int. J. Biochem. Cell Biol. 41 (2009) 1261–1268, http://dx.doi.org/10.1016/j.biocel.2008.12.015.
[7] C. Ballard, S. Gauthier, A. Corbett, C. Brayne, D. Aarsland, E. Jones, Alzheimer's disease, Lancet 377 (2011) 1019–1031, http://dx.doi.org/10.1016/S0140-6736(10)61349-9.
[8] R.J. Castellani, R.K. Rolston, M.A. Smith, Alzheimer disease, Dis. Mon. 56 (2010) 484–546.
[9] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, Science 256 (1992) 184–185, http://dx.doi.org/10.1126/science.1566067.
[10] J.A. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356, http://dx.doi.org/10.1126/science.1072994.
[11] N. Sato, R. Morishita, Plasma β-amyloid: a possible missing link between Alzheimer disease and diabetes, Diabetes 62 (2013) 1005–1006, http://dx.doi.org/10.2337/db12-1549.
[12] W. Zhang, M. Bai, Y. Xi, J. Hao, Multiple inflammatory pathways are involved in the development and progression of cognitive deficits in APPswe/PS1dE9 mice, Neurobiol. Aging 33 (2012) 2661–2677, http://dx.doi.org/10.1016/j.neurobiolaging.2011.12.023.
[13] M. Garcia-Alloza, E.M. Robbins, S.X. Zhang-Nunes, S.M. Purcell, Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease, Neurobiol. Dis. 24 (2009) 516–524, http://dx.doi.org/10.1016/j.nbd.2006.08.017.
[14] Y. Urano, S. Ochiai, N. Noguchi, Suppression of amyloid-β production by 24S-hydroxycholesterol via inhibition of intracellular amyloid precursor protein trafficking, FASEB J. 27 (2013) 4305–4315, http://dx.doi.org/10.1096/fj.13-231456.
[15] B. Reed, S. Villeneuve, W. Mack, C. Decarli, H.C. Chui, W. Jagust, Associations between serum cholesterol levels and cerebral amyloidosis, JAMA Neurol 71 (2014) 195–200.
[16] E. Barbero-Camps, A. Fernández, L. Martínez, J.C. Fernández-Checa, A. Colell, APP/PS1 mice overexpressing SREBP-2 exhibit combined Aβ accumulation and tau pathology underlying Alzheimer's disease, Hum. Mol. Genet. 22 (2013) 3460–3476, http://dx.doi.org/10.1093/hmg/ddt201.
[17] A.S. Buchman, R.S. Wilson, J.L. Bienias, R.C. Shah, D.A. Evans, D.A. Bennett, Change in body mass index and risk of incident Alzheimer disease, Neurology 65 (2005) 892–897, http://dx.doi.org/10.1212/01.wnl.0000176061.33817.90.
[18] L. Haataja, T. Gurlo, C.J. Huang, P.C. Butler, Islet amyloid in type 2 diabetes, and the toxic oligomer hypothesis, Endocr. Rev. 29 (2008) 303–316, http://dx.doi.org/10.1210/er.2007-0037.
[19] M. Hiltunen, V.K. Khandelwal, N. Yaluri, T. Tiilikainen, M. Tusa, H. Koivisto, M. Krzisch, S. Vepsäläinen, P. Mäkinen, S. Kemppainen, P. Miettinen, A. Haapasalo, H. Soininen, M. Laakso, H. Tanila, Contribution of genetic and dietary insulin resistance to Alzheimer phenotype in APP/PS1 transgenic mice, J. Cell. Mol. Med. 16 (2012) 1206–1222, http://dx.doi.org/10.1111/j.1582-4934.2011.01384.x.
[20] S. Koga, A. Kojima, S. Kuwabara, Y. Yoshiyama, Immunohistochemical analysis of tau phosphorylation and astroglial activation with enhanced leptin receptor expression in diet-induced obesity mouse hippocampus, Neurosci. Lett. 571 (2014) 11–16, http://dx.doi.org/10.1016/j.neulet.2014.04.028.
[21] I.A. Arnoldussen, A.J. Kiliaan, D.R. Gustafson, Obesity and dementia: adipokines interact with the brain, Eur. Neuropsychopharmacol. 24 (2014) 1982–1999, http://dx.doi.org/10.1016/j.euroneuro.2014.03.002.
[22] J.L. Sobesky, R.M. Barrientos, H.S. De May, B.M. Thompson, M.D. Weber, L.R. Watkins, S.F. Maier, High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism, Brain Behav. Immun. 42 (2014) 22–32, http://dx.doi.org/10.1016/j.bbi.2014.06.017.
[23] K.S. Sellbom, J. Gunstad, Cognitive function and decline in obesity, J. Alzheimers Dis. 30 (Suppl. 2) (2012) S89–S95.
[24] S.E. Kanoski, T.L. Davidson, Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity, Physiol. Behav. 103 (2011) 59–68, http://dx.doi.org/10.1016/j.physbeh.2010.12.003.
[25] G. Winocur, C.E. Greenwood, Studies of the effects of high fat diets on cognitive function in a rat model, Neurobiol. Aging 26 (Suppl. 1) (2005) 46–49, http://dx.doi.org/10.1016/j.neurobiolaging.2005.09.003.
[26] I. Valladolid-Acebes, P. Stucchi, V. Cano, M.S. Fernandez-Alfonso, B. Merino, M. Gil-Ortega, A.A. Fole, L. Morales, M. Ruiz-Gayo, N. Del Olmo, High-fat diets impair spatial learning in the radial-arm maze in mice, Neurobiol. Learn. Mem. 95 (2011) 80–85, http://dx.doi.org/10.1016/j.nlm.2010.11.007.
[27] S. Kosari, E. Badoer, J.C. Nguyen, A.S. Killcross, T.A. Jenkins, Effect of western and high fat diets on memory and cholinergic measures in the rat, Behav. Brain Res. 235 (2012) 98–103, http://dx.doi.org/10.1016/j.bbr.2012.07.017.
[28] A.P. Ross, E.C. Bruggeman, A.W. Kasumu, J.G. Mielke, M.B. Parent, Nonalcoholic fatty liver disease impairs hippocampal-dependent memory in male rats, Physiol. Behav. 106 (2012) 133–141, http://dx.doi.org/10.1016/j.physbeh.2012.01.008.
[29] N. Yamada-Goto, G. Katsuura, Y. Ochi, K. Ebihara, T. Kusakabe, K. Hosoda, K. Nakao, Impairment of fear-conditioning responses and changes of brain neurotrophic factors in diet-induced obese mice, J. Neuroendocrinol. 24 (2012) 1120–1125, http://dx.doi.org/10.1111/j.1365-2826.2012.02327.x.
[30] S. Hoyer, Glucose metabolism and insulin receptor signal transduction in Alzheimer disease, Eur. J. Pharmacol. 490 (2004) 115–125, http://dx.doi.org/10.1016/j.ejphar.2004.02.049.
[31] S. Merlo, S. Spampinato, P.L. Canonico, A. Copani, M.A. Sortino, Alzheimer's disease: brain expression of a metabolic disorder? Trends Endocrinol. Metab. 21 (2010) 537–544, http://dx.doi.org/10.1016/j.tem.2010.05.005.
[32] V. Frisardi, V. Solfrizzi, D. Seripa, C. Capurso, A. Santamato, D. Sancarlo, G. Vendemiale, A. Pilotto, F. Panza, Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease, Ageing Res. Rev. 9 (2010) 399–417, http://dx.doi.org/10.1016/j.arr.2010.04.007.
[33] M. Hokama, S. Oka, J. Leon, T. Ninomiya, H. Honda, K. Sasaki, T. Iwaki, T. Ohara, T. Sasaki, F.M. Laferla, Y. Kiyohara, Y. Nakabeppu, Altered expression of diabetes-related genes in Alzheimer's disease brains: the Hisayama Study, Cereb. Cortex 24 (2014) 2476–2488, http://dx.doi.org/10.1093/cercor/bht101.
[34] L.D. Baker, D. Cross, S. Minoshima, D. Belongia, G. Stennis, S. Watson, Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes, Arch. Neurol. 68 (2011) 51–57, http://dx.doi.org/10.1001/archneurol.2010.225.
[35] R.C. Frederich, A. Hamann, S. Anderson, B. Löllmann, B.B. Lowell, J.S. Flier, Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action, Nat. Med. 1 (1995) 1311–1314, http://dx.doi.org/10.1038/nm1295-1311.
[36] K.C. de Git, R.A. Adan, Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation, Obes. Rev., 2015, http://dx.doi.org/10.1111/obr.12243.
[37] L. Zhou, C. Yuan, J. Zhang, R. Yu, M. Huang, I.M. Adcock, X. Yao, Circulating leptin concentrations in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis, Respiration 86 (2013) 512–522, http://dx.doi.org/10.1159/000354191.
[38] G.H. Ballantyne, A. Gumbs, I.M. Modlin, Changes in insulin resistance following bariatric surgery and the adipoinsular axis: role of the adipocytokines, leptin, adiponectin and resistin, Obes. Surg. 15 (2005) 692–699, http://dx.doi.org/10.1381/0960892053923789.
[39] T.J. Kieffer, J.F. Habener, The adipoinsular axis: effects of leptin on pancreatic beta-cells, Am. J. Physiol. Endocrinol. Metab. 278 (2000) E1–E14.
[40] J.C. Henquin, H.P. Meissner, Opposite effects of tolbutamide and diazoxide on 86Rb+ fluxes and membrane potential in pancreatic B cells, Biochem. Pharmacol. 31 (1982) 1407–1415, http://dx.doi.org/10.1016/0006-2952(82)90036-3.
[41] R. Alemzadeh, K.M. Tushaus, Modulation of adipoinsular axis in prediabetic zucker diabetic fatty rats by diazoxide, Endocrinology 145 (2004) 5476–5484, http://dx.doi.org/10.1210/en.2003-1523.
[42] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432, http://dx.doi.org/10.1038/372425a0.
[43] L. Gautron, J.K. Elmquist, Sixteen years and counting: an update on leptin in energy balance, J. Clin. Invest. 121 (2011) 2087–2093, http://dx.doi.org/10.1172/JCI45888.
[44] S. Koga, A. Kojima, C. Ishikawa, S. Kuwabara, K. Arai, Y. Yoshiyama, Effects of diet-induced obesity and voluntary exercise in a tauopathy mouse model: implications of persistent hyperleptinemia and enhanced astrocytic leptin receptor expression, Neurobiol. Dis. 71 (2014) 180–192, http://dx.doi.org/10.1016/j.nbd.2014.08.015.
[45] J.M. Zigman, J.K. Elmquist, Minireview: from anorexia to obesity—the yin and yang of body weight control, Endocrinology 144 (2003) 3749–3756.
[46] S.H. Bates, W.H. Stearns, T.A. Dundon, M. Schubert, A.W. Tso, Y. Wang, A.S. Banks, H.J. Lavery, A.K. Haq, E. Maratos-Flier, B.G. Neel, M.W. Schwartz Jr., M.G. Myers, STAT3 signalling is required for leptin regulation of energy balance but not reproduction, Nature 421 (2003) 856–859, http://dx.doi.org/10.1038/nature01388.
[47] G. Ramadori, T. Fujikawa, M. Fukuda, J. Anderson, D.A. Morgan, R. Mostoslavsky, R.C. Stuart, M. Perello, C.R. Vianna, E.A. Nillni, K. Rahmouni, R. Coppari, SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity, Cell Metab. 12 (2010) 78–87, http://dx.doi.org/10.1016/j.cmet.2010.05.010.
[48] G. Ramadori, C.E. Lee, A.L. Bookout, S. Lee, K.W. Williams, J. Anderson, J.K. Elmquist, R. Coppari, Brain SIRT1: anatomical distribution and regulation by energy availability, J. Neurosci. 28 (2008) 9989–9996, http://dx.doi.org/10.1523/JNEUROSCI.3257-08.2008.

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

[49] M. Ishii, G. Wang, G. Racchumi, J.P. Dyke, C. Iadecola, Transgenic mice overexpressing amyloid precursor protein exhibit early metabolic deficits and a pathologically low leptin state associated with hypothalamic dysfunction in arcuate neuropeptide Y neurons, J. Neurosci. 34 (2014) 9096–9106, http://dx.doi.org/10.1523/JNEUROSCI.0872-14.2014.
[50] J.P. Thaler, S.J. Choi, M.W. Schwartz, B.E. Wisse, Hypothalamic inflammation and energy homeostasis: resolving the paradox, Front. Neuroendocrinol. 31 (2010) 79–84, http://dx.doi.org/10.1016/j.yfrne.2009.10.002.
[51] W. Fu, A. Ruangkittisakul, D. MacTavish, J.Y. Shi, K. Ballanyi, J.H. Jhamandas, Amyloid β (Aβ) peptide directly activates amylin-3 receptor subtype by triggering multiple intracellular signaling pathways, J. Biol. Chem. 287 (2012) 18820–18830, http://dx.doi.org/10.1074/jbc.M111.331181.
[52] S. Söderberg, B. Ahrén, M. Eliasson, B. Dinesen, T. Olsson T. The association between leptin and proinsulin is lost with central obesity, J. Intern. Med. 252 (2002) 140–148, http://dx.doi.org/10.1046/j.1365-2796.2002.01018.x.
[53] E. Hartter, T. Svoboda, B. Ludvik, M. Schuller, B. Lell, E. Kuenburg, M. Brunnbauer, W. Woloszczuk, R. Prager. Basal and stimulated plasma levelsof pancreatic amylin indicate itsco-secretion with insulin in humans, Diabetologia, 34 (1991) 52–54. http://dx.doi.org/10.1007/BF00404025.
[54] J.E. Koda, M. Fineman, T.J. Rink, G.E. Dailey, D.B. Muchmore, L.G. Linarelli, Amylin concentrations and glucose control, Lancet 339 (1992) 1179–1180, http://dx.doi.org/10.1016/0140-6736(92)90785-2.
[55] A.A. Young, B. Gedulin, W. Vine, A. Percy, T.J. Rink, Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin, Diabetologia 38 (1995) 642–648, http://dx.doi.org/10.1007/BF00401833.
[56] T. Riediger, M. Rauch, H. Schmid, Actions of amylin on subfornical organ neurons and on drinking behaviour in rats, Am. J. Physiol. 276 (1999) R514–R521.
[57] P.C. May, L.N. Boggs, K.S. Fuson, Neurotoxicity of human amylin in rat primary hippocampal cultures: similarity to Alzheimer's disease amyloid-beta neurotoxicity, J. Neurochem. 61 (1993) 2330–2333, http://dx.doi.org/10.1111/j.1471-4159.1993.tb07480.x.
[58] J.E. Morley, J.F. Flood, S.A. Farr, H.J. Perry, F.E. Kaiser, P.E. Morley, Effects of amylin on appetite regulation and memory, Can. J. Physiol. Pharmacol. 73 (1995) 1042–1046, http://dx.doi.org/10.1139/y95-147.
[59] C. De Vriese, C. Delporte, Influence of ghrelin on food intake and energy homeostasis, Curr. Opin. Clin. Nutr. Metab. Care 10 (2007) 615–659, http://dx.doi.org/10.1097/MCO.0b013e32829fb37c.
[60] M.D. Gahete, A. Rubio, J. Córdoba-Chacón, F. Gracia-Navarro, R.D. Kineman, J. Avila, R.M. Luque, J.P. Casta-o, Expression of the ghrelin and neurotensin systems is altered in the temporal lobe of Alzheimer's disease patients, J. Alzheimers Dis. 22 (2010) 819–828.
[61] X.M. Guan, H. Yu, O.C. Palyha, K.K. McKee, S.D. Feighner, D.J. Sirinathsinghji, R.G. Smith, L.H. Van der Ploeg, A.D.J. Howard, Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues, Brain Res. Mol. Brain Res. 48 (1997) 23–29, http://dx.doi.org/10.1016/S0169-328X(97)00071-5.
[62] M. Nakazato, N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, S. Matsukura, A role for ghrelin in the central regulation of feeding, Nature 409 (2001) 194–198, http://dx.doi.org/10.1038/35051587.
[63] V.P. Carlini, M.E. Monzon, M.M. Varas, A.B. Cragnolini, H.B. Schioth, T.N. Scimonelli, S.R. Barioglio, Ghrelin increases anxiety-like behavior and memory retention in rats, Biochem Biophys Res Commun 299 (2002) 739–743, http://dx.doi.org/10.1016/S0006-291X(02)02740-7.
[64] H. Cai, W.N. Cong, J.I. WN, S. Ji, S. Rothman, S. Maudsley, B. Martin, Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders, Curr. Alzheimer Res. 9 (2012) 5–17, http://dx.doi.org/10.2174/156720512799015064.
[65] Z. Cai, L.J. Yan, K. Li, S.H. Quazi, B. Zhao, Roles of AMP-activated protein kinase in Alzheimer's disease, Neuromolecular Med. 14 (2012) 1–14, http://dx.doi.org/10.1007/s12017-012-8173-2.
[66] M. Moon, J.G. Choi, D.W. Nam, H.S. Hong, Y.J. Choi, M.S. Oh, I. Mook-Jung, Ghrelin ameliorates cognitive dysfunction and neurodegeneration in intrahippocampal amyloid-b1-42 oligomer-injected mice, J. Alzheimers Dis. 23 (2011) 147–159.
[67] Y. Chen, C.P. Cao, C.R. Li, W. Wang, D. Zhang, L.L. Han, X.Q. Zhang, A. Kim, S. Kim, G.L. Liu, Ghrelin modulates insulin sensitivity and tau phosphorylation in high glucose-induced hippocampal neurons, Biol. Pharm. Bull. 33 (2010) 1165–1169, http://dx.doi.org/10.1248/bpb.33.1165.
[68] V. Giordano, G. Peluso, M. Iannuccelli, P. Benatti, M. Calvani, Systemic and brain metabolic dysfunction as a new paradigm for approaching Alzheimer's dementia, Neurochem. Res. 32 (2007) 555–567, http://dx.doi.org/10.1007/s11064-006-9125-8.
[69] W.N. Cong, E. Golden, N. Pantaleo, C.M. White, S. Maudsley, B. Martin, Ghrelin receptor signaling: a promising therapeutic target for metabolic syndrome and cognitive dysfunction, CNS Neurol. Disord. Drug Targets 9 (2010) 557–563, http://dx.doi.org/10.2174/187152710793361513.
[70] S. Gomes, I. Martins, A.C. Fonseca, C.R. Oliveira, R. Resende, C.M. Pereira, Protective effect of leptin and ghrelin against toxicity induced by amyloid-β oligomers in a hypothalamic cell line, J. Neuroendocrinol. 26 (2014) 176–185, http://dx.doi.org/10.1111/jne.12138.
[71] E.C. McNay, A.K. Recknagel, Brain insulin signaling: a key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes, Neurobiol. Learn. Mem. 96 (2011) 432–442, http://dx.doi.org/10.1016/j.nlm.2011.08.005.
[72] G. Marwarha, O. Ghribi, Leptin signaling and Alzheimer's disease, Am J Neurodegener Dis 1 (2012) 245–265.
[73] S.J. Greco, K.J. Bryan, S. Sarkar, X. Zhu, M.A. Smith, J.W. Ashford, J.M. Johnston, N. Tezapsidis, G. Casadesus, Leptin reduces pathology and improves memory in a transgenic mouse model of Alzheimer's disease, J. Alzheimers Dis. 19 (2010) 1155–1167.
[74] S.J. Greco, A. Hamzelou, J.M. Johnston, M.A. Smith, J.W. Ashford, N. Tezapsidis, Leptin boosts cellular metabolism by activating AMPK and the sirtuins to reduce tau phosphorylation and β-amyloid in neurons, Biochem. Biophys. Res. Commun. 14 (2011) 170–174, http://dx.doi.org/10.1016/j.bbrc.2011.09.050.
[75] G. McGregor, Y. Malekizadeh, J. Harvey, Minireview: food for thought: regulation of synaptic function by metabolic hormones, Mol. Endocrinol. 29 (2015) 3–13, http://dx.doi.org/10.1210/me.2014-1328.
[76] G. Marwarha, S. Raza, C. Meiers, O. Ghribi, Leptin attenuates BACE1 expression and amyloid-β genesis via the activation of SIRT1 signaling pathway, Biochim. Biophys. Acta 1842 (2014) 1587–1595, http://dx.doi.org/10.1016/j.bbadis.2014.05.015.
[77] A.J. Irving, J. Harvey, Leptin regulation of hippocampal synaptic function in health and disease, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 369 (2013) 2013–2055, http://dx.doi.org/10.1098/rstb.2013.0155.
[78] J.M. Johnston, W.T. Hu, D.W. Fardo, S.J. Greco, G. Perry, T.J. Montine, J.Q. Trojanowski, L.M. Shaw, J.W. Ashford, N. Tezapsidis, Low plasma leptin in cognitively impaired ADNI subjects: gender differences and diagnostic and therapeutic potential, Curr. Alzheimer Res. 1 (2) (2014) 165–174.
[79] J.M. Johnston, S.J. Greco, A. Hamzelou, J.W. Ashford, N. Tezapsidis, Repositioning leptin as a therapy for Alzheimer's disease, Therapy 8 (5) (2011) 481–490.
[80] J. Folch, I. Pedrós, I. Patraca, F. Sureda, F. Junyent, C. Beas-Zarate, E. Verdaguer, M. Pallàs, C. Auladell, A. Camins, Neuroprotective and anti-ageing role of leptin, J. Mol. Endocrinol. 49 (2012) R149–R156, http://dx.doi.org/10.1530/JME-12-0151.
[81] I. Pedrós, D. Petrov, M. Allgaier, F. Sureda, E. Barroso, C. Beas-Zarate, C. Auladell, M. Pallàs, M. Vázquez-Carrera, G. Casadesús, J. Folch, A. Camins, Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease, Biochim. Biophys. Acta 1842 (2014) 1556–1566, http://dx.doi.org/10.1016/j.bbadis.2014.05.025.
[82] M.E. Orr, A. Salinas, R.A. Buffenstein, S. Oddo, Mammalian target of rapamycin hyperactivity mediates the detrimental effects of a high sucrose diet on Alzheimer's disease pathology, Neurobiol. Aging 35 (2014) 1233–1242, http://dx.doi.org/10.1016/j.neurobiolaging.2013.12.006.
[83] K. Hegyi, K. Fülöp, K. Kovács, S. Tóth, A. Falus, Leptin-induced signal transduction pathways, Cell Biol. Int. 28 (2004) 159–169, http://dx.doi.org/10.1016/j.cellbi.2003.12.003.
[84] M. Mannaa, S. Kramer, M. Boschmann, M. Gollasch, mTOR and regulation of energy homeostasis in humans, J. Mol. Med. 91 (2013) 1167–1175.
[85] Z. Tang, E. Bereczki, H. Zhang, S. Wang, C. Li, X. Ji, R.M. Branca, J. Lehtiö, Z. Guan, P. Filipcik, S. Xu, B. Winblad, J.J. Pei, Mammalian target of rapamycin (mTor) mediates tau protein dyshomeostasis: implication for Alzheimer disease, J. Biol. Chem. 31 (288) (2013) 15556–155570.
[86] S. Wullschleger, R. Loewith, M.N. Hall, TOR signaling in growth and metabolism, Cell 124 (2006) 471–484, http://dx.doi.org/10.1016/j.cell.2006.01.016.
[87] A.K. Saha, X.J. Xu, T.W. Balon, A. Brandon, E.W. Kraegen, N.B. Ruderman, Insulin resistance due to nutrient excess: is it a consequence of AMPK downregulation? Cell Cycle 10 (2011) 3447–3451, http://dx.doi.org/10.4161/cc.10.20.17886.
[88] N.A. Shirwany, M.H. Zou, AMPK: a cellular metabolic and redox sensor. A minireview, Front Biosci (Landmark Ed), 19 (2014) 447–474. 10.2741/4218.
[89] L. Katsouri, C. Parr, N. Bogdanovic, M. Willem, M. Sastre, PPARγ co-activator-1α (PGC-1α) reduces amyloid-β generation through a PPARγ-dependent mechanism, J. Alzheimers Dis. 25 (2011) 151–162.
[90] A. Louis, A. Bartke, M.M. Masternak, Effects of growth hormone and thyroxine replacement therapy on insulin signaling in Ames dwarf mice, J. Gerontol. A Biol. Sci. Med. Sci. 65 (2010) 344–352, http://dx.doi.org/10.1093/gerona/glq018.
[91] N. Laflamme, S. Rivest, Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components, FASEB J. 15 (2001) 155–163, http://dx.doi.org/10.1096/fj.00-0339com.
[92] S.S. Martin, A. Qasim, M.P. Reilly, Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease, J. Am. Coll. Cardiol. 52 (2008) 1201–1210, http://dx.doi.org/10.1016/j.jacc.2008.05.060.
[93] S. Maioli, M. Lodeiro, P. Merino-Serrais, F. Falahati, W. Khan, E. Puerta, A. Codita, R. Rimondini, M.J. Ramirez, A. Simmons, F. Gil-Bea, E. Westman, A. Cedazo-Minguez, The Alzheimer's disease neuroimaging initiative. Alterations in brain leptin signalling in spite of unchanged CSF leptin levels in Alzheimer's disease, Aging Cell 14 (2015) 122–129, http://dx.doi.org/10.1111/acel.12281.
[94] H. Hsuchou, W. Pan, M.J. Barnes, A.J. Kastin, Leptin receptor mRNA in rat brain astrocytes, Peptides 30 (2009) 2275–2280, http://dx.doi.org/10.1016/j.peptides.2009.08.023.
[95] G. Munch, J. Apelt, J. Rosemarie, E. Kientsch, P. Stahl, H.J. Luth, R. Schliebs, Advanced glycation endproducts and pro-inflammatory cytokines in transgènic Tg2576 mice with amyloid plaque pathology, J. Neurochem. 86 (2003) 283–289, http://dx.doi.org/10.1046/j.1471-4159.2003.01837.x.
[96] Y. Zhu, E. Nwabuisi-Heath, S.B. Dumanis, L.M. Tai, C. Yu, G.W. Rebeck, M.J. LaDu, APOE genotype alters glial activation and loss of synaptic markers in mice, Glia 60 (2012) 559–569, http://dx.doi.org/10.1002/glia.22289.
[97] E. Fuente-Martín, C. García-Cáceres, M. Granado, M.L. de Ceballos, M.A. Sánchez-Garrido, B. Sarman, Z.W. Liu, M.O. Dietrich, M. Tena-Sempere, P. Argente-Arizón, F. Díaz, J. Argente, T.L. Horvath, J.A. Chowen, Leptin regulates glutamate and glucose transporters in hypothalamic astrocytes, J. Clin. Invest. 122 (2012) 3900–3913, http://dx.doi.org/10.1172/JCI64102.
[98] B.T. Jeon, E.A. Jeong, H.J. Shin, Y. Lee, D.H. Lee, H.J. Kim, S.S. Kang, G.J. Cho, W.S. Choi, G.S. Roh, Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet, Diabetes 61 (2012) 1444–1454, http://dx.doi.org/10.2337/db11-1498.

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

[99] P. Agostinho, R.A. Cunha, C. Oliveira, Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's Disease, Curr. Pharm. Des. 16 (2010) 2766–2778, http://dx.doi.org/10.2174/138161210793176572.
[100] D. Farfara, V. Lifshitz, D. Frenkel, Neuroprotective and neurotoxic properties of glial cells in the pathogenesis of Alzheimer's disease, J. Cell. Mol. Med. 12 (2008) 762–780, http://dx.doi.org/10.1111/j.1582-4934.2008.00314.x.
[101] G. Azizi, A. Mirshafiey, The potential role of proinflammatory and antiinflammatory cytokines in Alzheimer disease pathogenesis, Immunopharmacol. Immunotoxicol. 34 (2012) 881–895, http://dx.doi.org/10.3109/08923973.2012.705292.
[102] C.T. De Souza, E.P. Araujo, S. Bordin, R. Ashimine, R.L. Zollner, A.C. Boschero, M.J. Saad, L.A. Velloso, Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus, Endocrinology 146 (10) (2005) 4192–4199, http://dx.doi.org/10.1210/en.2004-1520.
[103] M. Milanski, G. Degasperi, A. Coope, J. Morari, R. Denis, D.E. Cintra, D.M. Tsukumo, G. Anhe, M.E. Amaral, H.K. Takahashi, R. Curi, H.C. Oliveira, J.B. Carvalheira, S. Bordin, M.J. Saad, L.A. Velloso, Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity, J. Neurosci. 29 (2009) 359–370, http://dx.doi.org/10.1523/JNEUROSCI.2760-08.2009.
[104] J. Lee, E. Lim, Y. Kim, E. Li, S. Park, Ghrelin attenuates kainic acid-induced neuronal cell death in the mouse hippocampus, J. Endocrinol. 205 (2010) 263–270, http://dx.doi.org/10.1677/JOE-10-0040.
[105] M.G. De Simoni, C. Perego, T. Ravizza, D. Moneta, M. Conti, F. Marchesi, A. De Luigi, S. Garattini, A. Vezzani, Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus, Eur. J. Neurosci. 12 (2000) 2623–2633, http://dx.doi.org/10.1046/j.1460-9568.2000.00140.x.
[106] T. Ravizza, M. Rizzi, C. Perego, C. Richichi, J. Velísková, S.L. Moshé, M.G. De Simoni, A. Vezzani, Inflammatory response and glia activation in developing rat hippocampus after status epilepticus, Epilepsia 46 (2005) 113–117, http://dx.doi.org/10.1111/j.1528-1167.2005.01006.x.
[107] E. Erbayat-Altay, A.J. Fessler, M. Gallagher, H.P. Attarian, F. Dehdashti, V.J. Vahle, J. Ojemann, J.L. Dowling, F.G. Gilliam, Correlation of severity of FDG-PET hypometabolism and interictal regional delta slowing in temporal lobe epilepsy, Epilepsia 46 (2005) 573–576, http://dx.doi.org/10.1111/j.0013-9580.2005.08204.x.
[108] G. Biagini, A. Torsello, C. Marinelli, F. Gualtieri, R. Vezzali, S. Coco, E. Bresciani, V. Locatelli, Beneficial effects of desacyl-ghrelin, hexarelin and EP-80317 in models of status epilepticus, Eur. J. Pharmacol. 670 (2011) 130–136, http://dx.doi.org/10.1016/j.ejphar.2011.08.020.
[109] M.W. Warren, L.S. Hynan, M.F. Weiner, Lipids and adipokines as risk factors for Alzheimer's disease, J. Alzheimers Dis. 29 (2012) 151–157.
[110] M.E. Freeman, S. Kanyicska, A. Lerant, Prolactin: structure, function, and regulation of secretion, Physiol. Rev. 80 (2000) 1523–1631.
[111] V. Popovic, L.H. Duntas, Brain somatic cross-talk: ghrelin, leptin and ultimate challengers of obesity, Nutr. Neurosci. 8 (2005) 1–5, http://dx.doi.org/10.1080/10284150400027107.
[112] D. Tejadilla, M. Cerbón, T. Morales, Prolactin reduces the damaging effects of excitotoxicity in the dorsal hippocampus of the female rat independently of ovarian hormones, Neuroscience 169 (2010) 1178–1185, http://dx.doi.org/10.1016/j.neuroscience.2010.05.074.
[113] C.J. Phelps, M.I. Romero, D.L. Hurley, Prolactin replacement must be continuous and initiated prior to 21 d of age to maintain hypothalamic dopaminergic neurons in hypopituitary mice, Endocrine 20 (2003) 139–148, http://dx.doi.org/10.1385/ENDO:20:1-2:139.
[114] M. Furuta, R.S. Bridges, Gestation-induced cell proliferation in the rat brain, Brain Res. Dev. Brain Res. 156 (2005) 61–66, http://dx.doi.org/10.1016/j.devbrainres.2005.01.008.
[115] D. Mangoura, C. Pelletiere, S. Leung, N. Sakellaridis, D.X. Wang, Prolactin concurrently activates src-PLD and JAK/Stat signaling pathways to induce proliferation while promoting differentiation in embryonic astrocytes, Int. J. Dev. Neurosci. 18 (2000) 693–704, http://dx.doi.org/10.1016/S0736-5748(00)00031-9.
[116] L. Torner, S. Karg, A. Blume, M. Kandasamy, H.G. Kuhn, J. Winkler, L. Aigner, I.D. Neumann, Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate, J. Neurosci. 29 (2009) (1826–1833) http://dx.doi.org/10.1523/JNEUROSCI.3178-08.2009.
[117] V. Goffin, N. Binart, P. Touraine, P.A. Kelly, Prolactin: the new biology of an old hormone, Annu Ver Physiol 64 (2002) 47–67, http://dx.doi.org/10.1146/annurev.physiol.64.081501.131049.
[118] T. Morales, Recent findings on neuroprotection against excitotoxicity in the hippocampus of female rats, J. Neuroendocrinol. 23 (2011) 994–1001, http://dx.doi.org/10.1111/j.1365-2826.2011.02141.x.
[119] C.K. Tipsmark, C.N. Strom, S.T. Bailey, R.J. Borski, Leptin stimulates pituitary prolactin release through an extracellular signal-regulated kinase-dependent pathway, J. Endocrinol. 196 (2008) 275–281, http://dx.doi.org/10.1677/JOE-07-0540.
[120] V.S. Nagaishi, L. Cardinali, T.T. Zampieri, I.C. Furigo, M. Metzger, J. Donato Jr., Possible crosstalk between leptin and prolactin during pregnancy, Neuroscience 259 (2014) 71–83, http://dx.doi.org/10.1016/j.neuroscience.2013.11.050.
[121] R.A. Augustine, D.R. Grattan, Induction of central leptin resistance in hyperphagic pseudopregnant rats by chronic prolactin infusion, Endocrinology 149 (2008) 1049–1055, http://dx.doi.org/10.1210/en.2007-1018.
[122] S.R. Ladyman, R.A. Augustine, D.R. Grattan, Hormone interactions regulating energy balance during pregnancy, J. Neuroendocrinol. 22 (2010) 805–817, http://dx.doi.org/10.1111/j.1365-2826.2010.02017.x.
[123] A.M. De Paoli, 20 years of leptin: leptin in common obesity and associated disorders of metabolism, J. Endocrinol. 223 (2014) 71–81, http://dx.doi.org/10.1530/JOE-14-0258.
[124] J.D. Roth, B.L. Roland, R.L. Cole, J.L. Trevaskis, C. Weyer, J.E. Koda, C.M. Anderson, D.G. Parkes, A.D. Baron, Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 7257–7262, http://dx.doi.org/10.1073/pnas.0706473105.
[125] J.L. Trevaskis, V.F. Turek, C. Wittmer, P.S. Griffin, J.K. Wilson, J.M. Reynolds, Y. Zhao, C.M. Mack, D.G. Parkes, J.D. Roth, Enhanced amylin-mediated body weight loss in estradiol-deficient diet-induced obese rats, Endocrinology 151 (2010) 5657–5668, http://dx.doi.org/10.1210/en.2010-0590.
[126] V.F. Turek, J.L. Trevaskis, B.E. Levin, A.A. Dunn-Meynell, B. Irani, G. Gu, C. Wittmer, P.S. Griffin, C. Vu, D.G. Parkes, J.D. Roth, Mechanisms of amylin/leptin synergy in rodent models, Endocrinology 151 (2010) 143–152, http://dx.doi.org/10.1210/en.2009-0546.
[127] C. Le Foll, M.D. Johnson, A. Dunn-Meynell, C.N. Boyle, T.A. Lutz, B.E. Levin, Amylin-induced central IL-6 production enhances ventromedial hypothalamicleptin signalling, Diabetes (2014 Nov 19) pii: DB_140645.
[128] S. Jager, C. Handschin, J. St-Pierre, S.M. Spiegelman, AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12017–12022, http://dx.doi.org/10.1073/pnas.0705070104.
[129] F.Y. Ma, G.M. Anderson, T.D. Gunn, V. Goffin, D.R. Grattan, S.J. Bunn, Prolactin specifically activates signal transducer and activator of transcription 5b in neuroendocrine dopaminergic neurons, Endocrinology 146 (2005) 5112–5119, http://dx.doi.org/10.1210/en.2005-0770.
[130] J.A. Ríos, P. Cisternas, M. Arrese, S. Barja, N.C. Inestrosa, Is Alzheimer's disease related to metabolic syndrome? A Wnt signaling conundrum, Prog. Neurobiol. 121 (2014) 125–146, http://dx.doi.org/10.1016/j.pneurobio.2014.07.004.
[131] M. Sadowski, J. Pankiewicz, H. Scholtzova, Y. Ji, D. Quartermain, C.H. Jensen, Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory, J. Neuropathol. Exp. Neurol. 63 (2004) 418–428.
[132] M.W. Schwartz, G.J. Morton, Obesity: keeping hunger at bay, Nature 418 (2002) 595–597, http://dx.doi.org/10.1038/418595a.

Please cite this article as: J. Folch, et al., The role of leptin in the sporadic form of Alzheimer's disease. Interactions with the adipokines amylin, ghrelin and the pituitary..., Life Sci (2015), http://dx.doi.org/10.1016/j.lfs.2015.05.002

[Frontiers in Bioscience, Landmark, 21, 8-19, January 1, 2016]

Molecular links between early energy metabolism alterations and Alzheimer´s disease

Ignacio Pedros[1,2], Ivan Patraca[1], Nohora Martinez[1], Dmitry Petrov[3], Francesc X. Sureda[2], Carme Auladell[4], Carlos Beas-Zarate[5], Jaume Folch[1]

[1]Unitats de Bioquimica i, [2]Farmacologia, Facultat de Medicina i Ciencies de la Salut, Centro de Investigacion Biomedica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat Rovira i Virgili, C./St. Llorenç 21 43201 Reus (Tarragona), Spain, [3]Unitat de Farmacologia i Farmacognosia Facultat de Farmacia, Institut de Biomedicina (IBUB), Centros de Investigacion Biomedica en Red de Enfermedades Neurodegenerativas (CIBERNED), Universitat de Barcelona, Barcelona, Spain, [4]Departament de Biologia Cellular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain, [5]Departamento de Biologia Celular y Molecular, C.U.C.B.A., Universidad de Guadalajara and Division de Neurociencias, Centro de Investigacion Biomedica de Occidente (CIBO), Instituto Mexicano del Seguro Social (IMSS), Sierra Mojada 800, Col. Independencia, Guadalajara, Jalisco 44340, Mexico

TABLE OF CONTENTS

1. ABSTRACT

Recent studies suggest that the neurobiology of Alzheimer's disease (AD) pathology could not be explained solely by an increase in β-amyloid levels. In fact, success with potential therapeutic drugs that inhibit the generation of beta amyloid has been low. Therefore, due to therapeutic failure in recent years, the scientists are looking for alternative hypotheses to explain the causes of the disease and the cognitive loss. Accordingly, alternative hypothesis propose a link between AD and peripheral metabolic alteration. Then, we review in depth changes related to insulin signalling and energy metabolism in the context of the APPSwe/PS1dE9 (APP/PS1) mice model of AD. We show an integrated view of the changes that occur in the early stages of the amyloidogenic process in the APP/PS1 double transgenic mice model. These early changes affect several key metabolic processes related to glucose uptake and insulin signalling, cellular energy homeostasis, mitochondrial biogenesis and increased Tau phosphorylation by kinase molecules like mTOR and Cdk5.

2. INTRODUCTION

Alzheimer's disease, in the more common sporadic form (SAD), is one of the most common causes of senile dementia and the numbers of new cases of the disease are increasing exponentially. The AD progression is associated with the formation of senile β-amyloid (Aβ) plaques and cognitive decline. In the early 1980s, the biochemical characterization of senile plaques, in patients with Down's syndrome and AD, led to the identification of Aβ peptide as a major component. The Aβ is a product of the Aβ protein precursor (APP), and the relationship between APP and Aβ caused the formulation of the amyloid cascade hypothesis. Then, mutations in APP (or other genes) lead to an increase in Aβ and to disease (1,2).

The majority of AD research is carried out using animal models that have increased Aβ levels compared to controls, and while Aβ pathology is mimicked in these models, many other factors associated with AD pathology are not. The APP/PS1 double transgenic mouse is a genetically modified mouse model that has been generated to try to mimic human AD pathology. In the APP/PS1 line, two strategies are combined to reach elevated Aβ levels: overexpression of the mutant human amyloid precursor protein encoding gene, together with the mutant presenilin-1 gene, which additionally impairs amyloid protein processing leading to elevated Aβ42 levels (3-5).

Despite the existence of several mice models for AD, the early onset of pathological changes as the

cerebral amyloidosis present at 6–8 weeks old mice, allow to consider APP/PS1 mice a good model to study the familial form of AD. A detailed review concerning differential characteristics of the AD mice strains can be found in Bilkei-Gorzo (5). Among them, Tg2576, APP23, APP/PS1 and the triple transgenic 3xtg AD mice strains express the so called Swedish mutation. It consists in a 695-amino acid isoform of human Alzheimer Aβ precursor protein containing the substitution of Lys670 by Asn and Met671 by Leu. Whereas APP/PS1 mice is a good model to study the early onset of pathological changes, the Tg2576, APP23, and 3xtg strains express a late onset form of the disease. Loss of both noradrenergic and cholinergic neurons is unique to double transgenic APP/PS1 and 3xtg mice. By contrast, none of these models show massive neuronal loss in cortex and hippocampus. The APP/PS1 strain show amyloid plaques formation along with Tau protein hyperphosphorylation (3). Only in 3xtg mice strain neurofibrillary tangles can be observed (6).

It has been demonstrated that APP/PS1 mice show increased insoluble β-amyloid production accompanied by brain plaque pathology and early memory loss, becoming evident at the age of 6 months (7-9). Recent data demonstrated that cognitive decline occur early before amyloid plaque deposition in APP/PS1 mice and, then, in this experimental model soluble β-amyloid peptide should be involved in early cognitive impairment. Acutely administered soluble Aβ oligomers have recently been reported to induce impairments in memory function (10,11) possibly by disturbing acetylcholinesterase (ACh) or NMDA receptors signalling systems (12,13). In fact, several studies have demonstrated impaired function of ACh and NMDA receptors signalling systems in multiple transgenic mouse models of Alzheimer's disease like APP/PS1 (11,12). Since PS1/APP mice aggressively generate Aβ (14), excessive concentrations of soluble Aβ oligomers may lead to the observed memory deficits by functionally disrupting the ACh and NMDA receptors signalling pathways. Thus APP/PS1 mice are commonly used in AD research for behavioural tests and studying the molecular mechanisms in plaque formation and thus AD progression (8,9,15).

3. METABOLIC SYNDROME, ADIPOKINES AND AD

Despite the genetic and cell biological evidence that supports the amyloid hypothesis, it is becoming clear that AD aetiology is complex and that Aβ alone is unable to account for all aspects of AD (16,17). For many years, it has been suspected that AD is a generalized metabolic disorder, but little evidence has emerged to confirm this suspicion. Published data have suggested metabolic syndrome as an independent risk factor for AD. Decades of fruitless search for effective therapies have led to the suggestion that the treatment usually starts too late in the course of the disease to be able to modify it, and can only be detected when pathology is already advanced (18).

There is evidence of a relationship between adipokines and AD. The adipokines, are cytokines secreted by adipose tissue. Among them, leptin, adiponectin, tumour necrosis factor (TNF)-alpha, interleukins (IL-6), and also molecules like Pituitary-derived prolactin (PRL), a well-known regulator of the lactating mammary gland, recently shown to be produced by human adipose tissue (19). Adipokines have come to be recognized for their contribution to the mechanisms by which obesity and related metabolic disorders influence diseases like cancer or AD. It has been observed that AD patients display increased circulating levels of anorexigenic adipokines, related to gender, that may contribute to the metabolic changes observed in AD patients (20).

Among the adipokine genes associated to AD, we can find the obese gene (ob) which is responsible of the synthesis of the adipostatic hormone leptin (Lep). Leptin is a hormone secreted by adipose tissue that acts to suppress appetite and regulate energy expenditure. In humans, recent studies have suggested an association between higher Lep levels and a reduced incidence of dementia and AD (21). In rodents, Lep modulates the production and clearance of Aβ (22). Mice with Lep receptor disruption show impaired long-term potentiation, synaptic plasticity and spatial learning, whereas treatment with Lep increases Aβ and tau clearance as well as amelioration of AD-like pathology (23-25). More recently it has been demonstrated that leptin resistance in the hippocampus may play a role in the characteristic changes associated with AD (26). In this study, whereas leptin mRNA was decreased in hippocampus, increased leptin was found and, then, suggesting a discontinuity in the leptin signalling pathway. The lack of leptin signalling within degenerating neurons may represent a novel neuronal leptin resistance in Alzheimer disease.

Similar to the ligand, the prolactin receptor (PRLR) has also been shown to be a member of the larger class of receptors, known as the class 1 cytokine receptor superfamily. Prolactin is secreted by the pituitary, decidua, and lymphoid cells, has been shown to have a regulatory role in reproduction, immune function, and cell growth in mammals. Elevated levels PRL, oxytocin, progesterone and glucocorticoids are characteristics of lactation and the pronounced fluctuation of these hormones occurring in this phase may play a role protecting the hippocampus. Indeed, it has been shown that PRL administration to ovariectomized rats significantly diminishes the deleterious effects of kainic acid (KA) in the dorsal hippocampus and reduces the progression of KA-induced seizures (27). Thus,

lactation is a natural model for neuroprotection because it effectively prevents acute and chronic cell damage of the hippocampus induced by excitotoxicity. Furthermore, it has been shown that PRLR affects energy balance and metabolic adaptation in rodents *via* effects on brown adipose tissue differentiation and function (28). In fact, recent findings show that circulating prolactin improves glucose homeostasis by increasing insulin action and secretion (29). It has been demonstrated that PRL loss resulted in learning and memory deficits in the PRL null mice, as indicated by significant deficits in the standard behavioural tests requiring input from the hippocampus (30).

Despite molecules like PRL have not been clearly associated to AD, it seems clear the presence of PRLR in several brain areas like cortex, hypothalamus and hippocampus, and identified in both astrocytes and glial cells (31). Then, changes downstream prolactin receptor involve key molecules related to fatty acid oxidation, mitochondrial biogenesis, inflammation and memory processes. Among them, we can point out the PPARγ coactivator-1α (PGC-1α) a molecular link between metabolic syndrome, Aβ generation and AD.

3.1. Energy metabolism and AD

Besides the role of adipokines *per se*, it has also been shown that alterations in energy metabolism also promote the development of AD. Mitochondrial structural and functional perturbations in AD have been recognized for some time, and led Swerdlow and Khan to propose the mitochondrial cascade hypothesis (32). This hypothesis proposes that inherited mutations in mtDNA determine the basal functional ability of mitochondria and their ability to respond to and recover from stress signalling. The histopathology of AD develops when the mitochondria loss their functions below a certain point, and includes neuronal apoptosis, β-amyloid deposition, and neurofibrillary tangles (33).

Mitochondrial biogenesis is the process by which cells generate new mitochondria and, if necessary, increase mitochondrial mass. PGC-1α is a member of a family of transcription co-activators that plays a central role in the regulation of cellular energy metabolism and stimulates mitochondrial biogenesis (34). PGC-1α participates in the regulation of both carbohydrate and lipid metabolism (35). Although the role of PGC-1α in peripheral disorders such as obesity and diabetes is well known, the role in neurons is currently a great interest because is a key regulator of energy metabolism (34). In addition, PGC-1α is also involved in the regulation of genes that protects neuronal cells from oxidative stress such as mitochondrial superoxide dismutase. Finally, PGC-1α coordinates mitochondrial biogenesis in at least some tissues such as muscle, heart, liver, and pancreas via co-activation of various transcription factors (33,34).

It has been recently shown that PGC-1α mRNA and protein levels are reduced in AD subject brains (36,37). As Selfridge and colleagues suggested (33), even if PGC-1α changes represent a consequence as opposed to cause of AD pathology, PGC-1α remains an attractive target for therapeutic intervention. Whether mitochondrial mass changes in AD, it is reasonable to postulate that increasing mitochondrial mass may alleviate bioenergetics-related stress in the AD brain.

PGC-1α is regulated by several metabolism-responsive elements like AMPK, which is activated by elevated AMP/ATP ratios. AMPK is a cellular energy sensor conserved in all eukaryotic cells. AMPK regulates the activities of a number of key metabolic enzymes through phosphorylation (38). It protects cells from stresses that cause ATP depletion by switching off ATP-consuming biosynthetic pathways. AMPK can phosphorylate and directly activate PGC-1α (39).

Furthermore, previous data suggest that AMPK, besides the important cellular functions such as cellular energy sensor, can also phosphorylate substrates like Tau protein and, thus, could favour its aggregation. Its phosphorylation makes it soluble and causes microtubule disassembly. In extreme situations as in AD, hyperphosphorylation of Tau leads to the formation of neurofibrillary tangles. It is well established that neurons are elongated cells. To maintain neuronal function they need efficient delivery of cellular organelles (such as mitochondria, endoplasmic reticulum, lysosomes, proteins, and lipids from soma to axons, dendrites and synapses. Hoover *et al.* (40) investigated the localization of abnormal Tau in dendritic spines using rTgP301L tau mice. They found that early Tau-related deficits develop not from the loss of synapses or neurons, but rather as a result of synaptic abnormalities caused by the accumulation of hyperphosphorylated Tau within intact dendritic spines.

PPARs are ligand-activated transcription factors of the nuclear receptors superfamily. The levels of PPARs have been reported to decline with age (41). PPARγ is highly expressed in adipose tissue and is a major regulator of insulin and glucose metabolism. PGC-1α is a PPAR transcriptional co-activator, and elevated levels of PGC1α change the composition of peroxisomes, so that they might exhibit decreased insulin degradation and purine metabolism. Then, it can be suggested that the link between energy metabolism and the amyloid cascade hypothesis can rely in the fact that PPARγ regulates the transcription of β-secretase (BACE1), a key enzyme involved in Aβ generation. In turn PGC-1α controls major metabolic functions through the co-activation of PPARγ and other transcription factors (42). In conclusion, since PGC-1α appears to decrease Aβ generation, therapeutic modulation of PGC-1α could have real potential as a treatment for AD.

3.2. Cholesterol, fatty acids and AD

Several strategies have proved to be effective in slowing down the pathological process or in improving the health status of the APP/PS1 mice (5) and, among them, can be pointed out the caloric restriction (43). From the 1930s it has been reported that caloric restriction (CR) mitigates neurological damage and, furthermore, rats submitted to CR live almost twice as long as non-restricted rats. Since that time, findings from a diverse range of species support the view that CR exerts beneficial effects on health and longevity, and is also able to reduce amyloid accumulation in middle-aged APP/PS1 mice. Then, excessive consumption of calories, particularly fat, opposes healthy brain aging though mechanisms that remain to be elucidated (43).

Hyperlipidemia, hypercholesterolemia, and obesity are all associated with increased accumulation of amyloid in AD and mouse models that form AD-type amyloid plaques. The brain is rich in cholesterol and substantial *in vitro* and animal evidence indicates that cholesterol levels in the brain affect the synthesis, clearance, and toxicity of Aβ (44). Then, elevated cerebral Aβ levels can be associated with cholesterol fractions in a pattern analogous to that found in coronary artery disease. In fact, a large amount of evidence suggests a pathogenic link between cholesterol homeostasis dysregulation and AD, where altered cholesterol metabolism and hypercholesterolemia appear to play fundamental roles in amyloid plaque formation and tau hyperphosphorylation (45). Experiments carried out with the use of low density lipoprotein receptor (LDLR)-deficient mice link hypercholesterolemia with cognitive dysfunction, potentially mediated by increased neuroinflammation and APP processing (46). Furthermore, it has been demonstrated, using an Aβ25-35-injected AD-like pathological mouse model, that hypercholesterolemia accelerated Aβ accumulation and tau pathology, which was accompanied by microglial activation and subsequent aggravation of memory impairment (47).

By contrast, it is unknown if a specific fatty-acid composition influences the development of AD, and published results are controversial. For instance, an study based on the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort show that serum levels of saturated FAs were inversely associated with risk of AD, in sharp contrast to experimental studies (48). By contrast, research carried out in the APP/PS1 mice model show that AD increases susceptibility to body weight gain induced by short-term high-fat diet (HFD) feeding, and to the associated glucose intolerance and insulin resistance (49).

Nevertheless, protective effects of omega-3 fatty acids have been hypothesized (50-52). This can be supported on epidemiologic results and on the evidence that decreased levels of omega-3 fatty acids have been observed in brain tissue of people with AD, specifically in areas that mediate learning and memory. Thus, these observations reinforce an innovative approach that focuses on the protective action exerted by molecules naturally occurring in food and, at higher content, in dietary supplements (52,53).

Then, recognition of the correlation between AD and dyslipemia could be an important step forward for our understanding of AD pathogenesis and, possibly, for the development of new therapeutic strategies. However, the underlying mechanisms remain unknown.

3.3. Alzheimer's disease or "brain diabetes"

It has been described that obesity and diabetes significantly increase cognitive decline and AD risk, supporting the notion that molecular mechanisms of cellular energy homeostasis are linked to AD pathogenesis. Furthermore, biological plausibility for this relationship has been framed within the *metabolic cognitive syndrome* concept. Thus, several early biomarkers have been proposed and many of them rely on the definition of AD as a "Cognitive Metabolic Syndrome" or "Diabetes 3" (54). Then, AD would be a degenerative metabolic disease in which brain glucose uptake and utilization are impaired. Furthermore, a growing body of epidemiological evidence suggested that metabolic syndrome and its components (impaired glucose tolerance, abdominal or central obesity, hypertension, hypertriglyceridemia, and reduced high-density lipoprotein cholesterol) may be important in the development of age-related cognitive decline, mild cognitive impairment, vascular dementia, and AD (55). In fact, results from hippocampal gene expression studies in normal mice, show several aging-dependent up-regulated processes and, among them, lipid catabolism, proteolysis, cholesterol transport, and myelinogenesis (56,57). Additionally, a consistent observation is that persons with AD, despite unchanged eating habits, begin to lose weight several years prior to the onset of clinical symptoms, suggesting the link between adipose tissue metabolism and AD (25,58,59).

Epidemiological, clinical, and basic studies have shown a relationship between AD and Type 2 Diabetes Mellitus (T2DM), and that the main physiological link between both conditions is peripheral and central insulin signalling impairment (60). T2DM triggers a condition of "diabetic encephalopathy" characterized by electrophysiological, structural and neurochemical changes leading to cognitive impairments (61). In fact, results from the so called "Hisayama Study" indicate that altered expression of genes related to diabetes mellitus in AD brains is a result of AD pathology, which may thereby be exacerbated by peripheral insulin resistance or diabetes mellitus (62). These cognitive deficits associated to T2DM have been argued to be due in large part to an impaired central insulin modulation in

the hippocampus, which is a critical region for memory processing (63). In fact, adults with newly diagnosed pre-diabetes or T2DM show insulin resistance associated with reductions in regional cerebral glucose metabolism and subtle cognitive impairments (64). Interestingly, the insulin signalling overlaps with pathways that regulate both synaptic plasticity and memory processes (63). Therefore, insulin has effects on memory storage and synaptic physiology (65,66).

Published results indicate that there is a close link between insulin deficient diabetes and cerebral amyloidosis in the pathogenesis of AD (67,70). Despite the active research on this field in recent years, the molecular mechanisms involved in the pathophysiology observed in both diseases remain unclear. It has been shown that β-amyloid peptide and phosphorylated tau accumulation also occur in T2DM rat models that exhibit neurite degeneration and neuronal loss (71). These changes appear to be associated with insulin resistance and hypercholesterolemia, and emphasize the role of energy metabolism control in the etiopathology of the AD. Results from Chua and colleagues have demonstrated an alteration in brain insulin proteins in APP/PS1 females, and the alteration of this pathway is responsible of the increase in brain β42 level in APP/PS1 mice (72). Thus, authors suggest that the brain insulin signalling impairment is involved in the amyloid accumulation in female APP/PS1 mice. Sadowski and colleagues demonstrated a correlation between the hippocampal levels of amyloid plaques and glucose utilization at 22 months of age (73).

It has been suggested from human brain imaging studies that impaired glucose utilization in AD precedes the onset of cognitive deficits and, thus, it will be the cause of AD. Therefore, brain glucose metabolism defects are strongly associated with memory impairment in AD brain. In agreement with that, early markers related to insulin function, like circulating insulin-like growth factor I (IGF-I) have been recently proposed (18). Furthermore, it has been shown that insulin tolerance tests revealed significant hyperglycaemia in mice overexpressing mutant amyloid precursor protein and presenilin-1 (APdE9), either by cross-breeding them with pancreatic insulin like growth factor 2 (IGF-2) overexpressing mice, or by feeding them with high-fat diet (74). In fact, it has been shown that local and systemic levels of IGF1 are altered in such CNS diseases as Alzheimer. IGF1 has emerged as a crucial factor in the CNS; it is involved in normal cognitive function and successful aging, in addition to development. In this context, insulin binds to the insulin receptor and insulin receptor substrates 1 and 2 (IRS-1 and IRS-2), and is involved in the modulation of hippocampal synaptic plasticity and memory consolidation (75).

Then, it can be concluded that the association between obesity and altered signalling mechanisms of insulin implies a greater susceptibility to neurodegenerative processes.

3.4. The "missing link" between T2DM and AD

Several studies have shown that AD and T2DM may share another common pathways to pathology, both kinases involved in Tau phosphorylation and microtubule stability: the mammalian target of rapamycin (mTOR) and the cyclin dependent kinase 5 (Cdk5). The kinase mTOR plays a key role in maintaining energy homeostasis in the brain and other tissue types (76,77). As an energy sensor, mTOR regulates numerous cellular pathways including protein translation, cell growth and proliferation. In fact, mTor mediates the synthesis and aggregation of Tau, resulting in compromised microtubule stability (78). Furthermore, the authors describe that changes of mTOR activity cause fluctuation of the level of a battery of Tau kinases such as protein kinase A, v-Akt murine thymoma viral oncogene homolog-1, glycogen synthase kinase 3β, cyclin-dependent kinase 5, and Tau protein phosphatase 2A. In addition, compelling evidence indicated that the sequential molecular events such as the synthesis and phosphorylation of Tau can be regulated through p70 S6 kinase, the well characterized immediate downstream target of mTOR. A common pattern observed in both post-mortem AD brains and drug-oriented *in vitro* and *in vivo* models, is an aberrant accumulation of mTOR. Recently, rapamycin has been shown to be neuroprotective in models for Alzheimer's disease in an autophagy-dependent manner. Caccamo and colleagues (79) and Spilman and colleagues (80) showed that rapamycin rescued cognitive deficits by suppressing extracellular Aβ deposition and intracellular Tau accumulation (81). In fact, treatment with rapamycin has proved to reduce Aβ42 levels and to improve cognitive function through inhibition of mTOR signalling in two independent mouse models of AD (77,79,80). Finally, it has been shown that rapamycin exerts neuroprotection via a novel mechanism that involves presynaptic activation (82) and rapamycin-treated hippocampal neurons are resistant to the synaptotoxic effect induced by Aβ oligomers, suggesting that enhancers of presynaptic activity can be therapeutic agents for Alzheimer's disease.

It has been proposed that mTOR modulate insulin signalling in times of high nutrient exposure. mTOR directly phosphorylates the insulin receptor leading to its internalization; this, in turn, results in a decrease of mTOR signalling (83, 77). However, through the same mechanisms, chronic mTOR hyperactivity leads to insulin resistance, a key feature of T2DM (84). Then, as Orr and colleagues propose (77), since mTOR hyperactivity is common to both diabetes and AD, mTOR signalling could be considered a molecular link between these two age-related diseases.

In addition to mTOR, the hyper-activation of Cdk5/p25 can be related to AD and T2DM (85). Cdk5

Energy metabolism in the APP/PS1 mice model

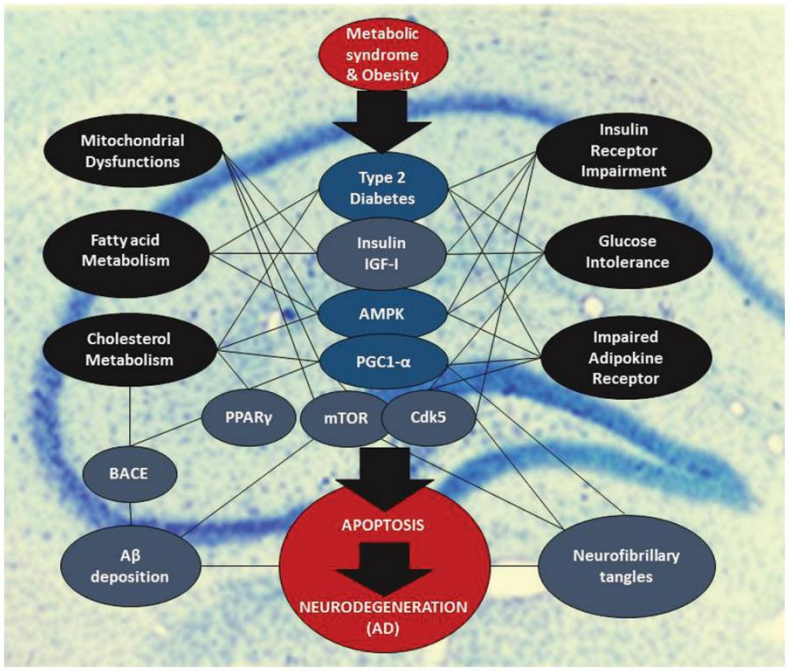


Figure 1. The image shows a complex grid of interactions resulting from the correlations that can be found among metabolic processes and key molecules involved in AD. Results from animal models of AD, like APPswe/PS1dE9, show early down-regulation of glucose and insulin signalling pathways and energy metabolism. The observed changes are complex and are related to insulin and adipokine receptors signalling impairment, all along with alterations in cholesterol and fatty acids metabolism. All together cause changes that affect the activity of key molecules like AMPK and PGC-1α, involved in mitochondrial biogenesis, PPAR and BACE activity regulation and Aβ deposition. Since Tau expression is regulated by insulin/IGF-I, and by AMPK, changes in neurofibrillary tangles can be related to energy impairment. Finally, an increased activity of mTOR, Cdk5 and p35 could be responsible of increased Tau phosphorylation and neurofibrillary tangles formation.

is an atypical cyclin-dependent kinase localized in the brain, and its activity is dependent upon binding to p35/p39. In addition, while cdk5 has important physiological functions related to brain development, the breakdown of cdk5/p35 into cdk5/p25 increases its kinase activity and neurotoxicity. Interestingly, in recent years increased cdk5/p25 expression has been demonstrated in the brains of patients with Alzheimer's and Parkinson's diseases. Experimental studies performed in neuronal cell cultures indicate that cdk5/p25 plays a prominent role in apoptosis. In fact, The Cdk5-p25 forms a more stable and hyperactive complex, causing aberrant phosphorylation of cytoskeletal components like Tau and neurofilaments, and induces cell death. It has been shown that cells treated with high glucose concentrations exhibit an induction of p25, the p35-derived truncated fragment which hyper activates Cdk5 in neurons. Cdk5/p35 has been implicated in cytoskeletal protein phosphorylation in normal brain and in many human neurodegenerative disorders (86). Significant increases in Cdk5 activity and the localization of Cdk5 in neurodegenerative lesions have been demonstrated in several diseases, including AD (87). Studies illustrate that p35 regulates the subcellular distribution of Cdk5 and cytoskeletal proteins in neurons and that Cdk5 has a hierarchical role in regulating the phosphorylation and function of cytoskeletal proteins. All these data supports the hypothesis that cdk5/p25 acts as a master regulator of neuronal cell death. In addition, cdk5/p25 might also interact with other pathways such as GSK-3β and c-JUN kinase.

Recent studies have identified P5, a truncated 24-aa peptide derived from the Cdk5 activator p35, later modified as TFP5, so as to penetrate the blood-brain barrier after intraperitoneal injections in AD model mice (84). Since this treatment inhibited abnormal Cdk5 hyperactivity and significantly rescued AD pathology in these mice, the authors suggest that TFP5 peptide may be a novel candidate for type 2 diabetes therapy.

4. CONCLUDING REMARKS

In summary, the reviewed results show early down-regulation of glucose and insulin signalling pathways

and energy metabolism in an APPswe/PS1dE9 model of Alzheimer disease (Figure 1). These changes affect the activity of key molecules like AMPK and PGC-1α, involved in mitochondrial biogenesis. It reinforces the hypothesis that the preceding events in the amyloidogenesis are related with both insulin signalling and energy metabolism impairment. Then, initial hypothesis of insoluble Aβ fibrils as main responsible of AD is currently changing because Aβ soluble oligomers truly may be the responsible of a synapse failure, neuronal dysfunction and also cognitive deficits. Likewise, experimental data in APP transgenic animal models reinforce this hypothesis because it was demonstrated that cognitive impairment in AD occurs early before amyloid plaque deposition. Since Tau expression is regulated by insulin/IGF-I, and by AMPK, changes in neurofibrillary tangles can be related to energy impairment. Finally, an increased activity of mTOR, Cdk5 and p35 could be responsible of increased Tau phosphorylation and neurofibrillary tangles formation in the APP/PS1 mice model.

5. ACKNOWLEDGMENTS

We are also grateful to the Language Advisory Service of the University of Barcelona for revising this manuscript. This study was funded by grant 2009/SGR00853 from the *Generalitat de Catalunya* (autonomous government of Catalonia), by grants BFU2007-63209/BFI, BFU2010-19119/BFI and SAF2009-13093 from the Spanish *Ministerio de Ciencia e Innovacion*, grant PI080400 and PS09/01789 from the Instituto de Salud Carlos III.

6. REFERENCES

1. J Hardy, DJ Selkoe. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–6 (2002)
DOI: 10.1126/science.1072994
2. JA Hardy, GA Higgins. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185 (1992)
DOI: 10.1126/science.1566067
3. MA Kurt, DC Davies, M Kidd, K Duff, SC Rolph, KH Jennings, DR Howlett. Neurodegenerative changes associated with beta-amyloid deposition in the brains of mice carrying mutant amyloid precursor protein and mutant presenilin-1 transgenes. *Exp Neurol* 171, 59–71 (2001)
DOI: 10.1006/exnr.2001.7717
4. R Radde, T Bolmont, SA Kaeser, J Coomaraswamy, D Lindau, L Stoltze, ME Calhoun, F Jäggi, H Wolburg, S Gengler, C Haass, B Ghetti, C Czech, C Hölscher, PM Mathews, M Jucker. Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep* 7, 940–6 (2006)
DOI: 10.1038/sj.embor.7400784
5. A Bilkei-Gorzo. Genetic mouse models of brain ageing and Alzheimer's disease. *Pharmacol Ther* 142, 244–57 (2014)
DOI: 10.1016/j.pharmthera.2013.12.009
6. S Oddo, A Caccamo, JD Shepherd, MP Murphy, TE Golde, R Kayed, R Metherate, MP Mattson, Y Akbari, FM Laferla. Triple-Transgenic Model of Alzheimer's Disease with Plaques and Tangles : Intracellular A beta and Synaptic Dysfunction. *Neuron* 39, 409–421 (2003)
DOI: 10.1016/S0896-6273(03)00434-3
7. JL Jankowsky, HH Slunt, V Gonzales, NA Jenkins, NG Copeland, DR Borchelt. APP processing and amyloid deposition in mice haplo-insufficient for presenilin 1. *Neurobiol Aging* 25, 885–92 (2004)
DOI: 10.1016/j.neurobiolaging.2003.09.008
8. W Zhang, M Bai, Y Xi, J Hao, L Liu, N Mao, C Su, J Miao, Z Li. Early memory deficits precede plaque deposition in APPswe/PS1dE9 mice: involvement of oxidative stress and cholinergic dysfunction. *Free Radic Biol Med* 52, 1443–52 (2012a)
DOI: 10.1016/j.freeradbiomed.2012.01.023
9. W Zhang, M Bai, Y Xi, J Hao, Z Zhang, C Su, G Lei, J Miao, Z Li. Multiple inflammatory pathways are involved in the development and progression of cognitive deficits in APPswe/PS1dE9 mice. *Neurobiol Aging* 33, 2661–77 (2012b)
DOI: 10.1016/j.neurobiolaging.2011.12.023
10. S Lesné, MT Koh, L Kotilinek, R Kayed, CG Glabe, A Yang, M Gallagher, KH Ashe. A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 440, 352–7 (2006)
DOI: 10.1038/nature04533
11. A Nagakura, Y Shitaka, J Yarimizu, N Matsuoka. Characterization of cognitive deficits in a transgenic mouse model of Alzheimer's disease and effects of donepezil and memantine. *Eur J Pharmacol* 703, 53–61 (2013)
DOI: 10.1016/j.ejphar.2012.12.023

12. I Dewachter, RK Filipkowski, C Priller, L Ris, J Neyton, S Croes, D Terwel, M Gysemans, H Devijver, P Borghgraef, E Godaux, L Kaczmarek, J Herms, F Van Leuven. Deregulation of NMDA-receptor function and down-stream signaling in APP(V717I) transgenic mice. *Neurobiol Aging* 30, 241–56 (2009)
DOI: 10.1016/j.neurobiolaging.2007.06.011

13. Q Liu, Y Huang, F Xue, A Simard, J DeChon, G Li, J Zhang, L Lucero, M Wang, M Sierks, G Hu, Y Chang, RJ Lukas, J Wu. A novel nicotinic acetylcholine receptor subtype in basal forebrain cholinergic neurons with high sensitivity to amyloid peptides. *J Neurosci* 29, 918–29 (2009)
DOI: 10.1523/JNEUROSCI.3952-08.2009

14. K Noda-Saita, A Yoneyama, Y Shitaka, Y Hirai, K Terai, J Wu, T Takeda, K Hyodo, N Osakabe, T Yamaguchi, M Okada. Quantitative analysis of amyloid plaques in a mouse model of Alzheimer's disease by phase-contrast X-ray computed tomography. *Neuroscience* 138, 1205–13 (2006)
DOI: 10.1016/j.neuroscience.2005.12.036

15. M Oksman, H Iivonen, E Hogyes, Z Amtul, B Penke, I Leenders, L Broersen, D Lütjohann, T Hartmann, H Tanila. Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice. *Neurobiol Dis* 23, 563–72 (2006)
DOI: 10.1016/j.nbd.2006.04.013

16. SW Pimplikar. Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *Int J Biochem Cell Biol* 41, 1261–8 (2009)
DOI: 10.1016/j.biocel.2008.12.015

17. SW Pimplikar, R Nixon, NK Robakis, J Shen, LH Tsai. Amyloid-independent mechanisms in Alzheimer's disease pathogenesis. *J Neurosci* 30, 14946–54 (2010)
DOI: 10.1523/JNEUROSCI.4305-10.2010

18. A Trueba-Sáiz, C Cavada, AM Fernandez, T Leon, D González, J Fortea Ormaechea, A Lleó, T Del Ser, A Nuñez, I Torres-Aleman. Loss of serum IGF-I input to the brain as an early biomarker of disease onset in Alzheimer mice. *Transl Psychiatry* 3, e330 (2013)
DOI: 10.1038/tp.2013.102

19. T Brandebourg, E Hugo, N Ben-Jonathan. Adipocyte prolactin: regulation of release and putative functions. *Diabetes Obes Metab* 9, 464–76 (2007)
DOI: 10.1111/j.1463-1326.2006.00671.x

20. AD Intebi, L Garau, I Brusco, M Pagano, RC Gaillard, E Spinedi. Alzheimer's disease patients display gender dimorphism in circulating anorectic adipokines. *Neuroimmunomodulation* 10, 351–358 (2002)
DOI: 10.1159/000071476

21. W Lieb, Beiser, ZS Tan, TB Harris, C Decarli, PA Wolf. Association of Plasma Leptin Levels and MRI Measures of Brain Aging. *JAMA* 302, 2565–2572 (2014)
DOI: 10.1001/jama.2009.1836

22. G Marwarha, B Dasari, JRP Prasanthi, J Schommer, O Ghribi. Leptin reduces the accumulation of Abeta and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *J Alzheimers Dis* 19, 1007–19 (2010)

23. J Harvey, N Solovyova, A Irving. Leptin and its role in hippocampal synaptic plasticity. *Prog Lipid Res* 45, 369–78 (2006)
DOI: 10.1016/j.plipres.2006.03.001

24. SJ Greco, KJ Bryan, S Sarkar, X Zhu, M Smith, JW Ashford, J Johnston, N Tezapsidis, G Casadesus. Leptin reduces pathology and improves memory in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis* 19, 1155–67 (2010)

25. MW Warren, LS Hynan, MF Weiner, T Texas. Lipids and Adipokines as Risk Factors for Alzheimer's Disease. *J Alzheimers Dis* 29, 151–157 (2012)

26. DJ Bonda, JG Stone, SL Torres, SL Siedlak, G Perry, R Kryscio, G Jicha, G Casadesus, M Smith, X Zhu, HG Lee. Dysregulation of leptin signaling in Alzheimer disease: evidence for neuronal leptin resistance. *J Neurochem* 128, 162–72 (2014)
DOI: 10.1111/jnc.12380

27. T Morales. Recent findings on neuroprotection against excitotoxicity in the hippocampus of female rats. *J Neuroendocrinol* 23, 994–1001 (2011)
DOI: 10.1111/j.1365-2826.2011.02141.x

28. J Auffret, S Viengchareun, N Carré, RGP Denis, C Magnan, PY Marie, A Muscat, B Fève, M Lombès, N Binart. Beige differentiation of adipose depots in mice lacking prolactin

receptor protects against high-fat-diet-induced obesity. *FASEB J* 26, 3728–37 (2012)
DOI: 10.1096/fj.12-204958

29. S Park, S Kang, HW Lee, BS Ko. Central prolactin modulates insulin sensitivity and insulin secretion in diabetic rats. *Neuroendocrinology* 95, 332–343 (2012)
DOI: 10.1159/000336501

30. TL Walker, J Vukovic, MM Koudijs, DG Blackmore, EW Mackay, AM Sykes, RW Overall, AS Hamlin, PF Bartlett. Prolactin stimulates precursor cells in the adult mouse hippocampus. *PLoS One* 7, e44371 (2012)
DOI: 10.1371/journal.pone.0044371

31. C Bole-Feysot, V Goffin, M Edery, N Binart, PA Kelly. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev* 19, 225–68 (1998)
DOI: 10.1210/edrv.19.3.0334

32. RH Swerdlow, SM Khan. A “mitochondrial cascade hypothesis” for sporadic Alzheimer’s disease. *Med Hypotheses* 63, 8–20 (2004)
DOI: 10.1016/j.mehy.2003.12.045

33. JE Selfridge, J Lu, RH Swerdlow. Role of mitochondrial homeostasis and dynamics in Alzheimer’s disease. *Neurobiol Dis* 51, 3–12 (2013)
DOI: 10.1016/j.nbd.2011.12.057

34. BN Finck, DP Kelly. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* 116, 615–622 (2006)
DOI: 10.1172/JCI27794

35. H Liang, WF Ward. PGC-1alpha: a key regulator of energy metabolism. *Adv Physiol Educ* 30, 145–51 (2006)
DOI: 10.1152/advan.00052.2006

36. B Sheng, X Wang, B Su, H Lee, G Casadesus, G Perry, X Zhu. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer’s disease. *J Neurochem* 120, 419–29 (2012)
DOI: 10.1111/j.1471-4159.2011.07581.x

37. RH Swerdlow. Mitochondria and Cell Bioenergetics: Increasingly Recognized Components and a Possible Etiologic Cause of Alzheimer’s Disease. *Antioxid Redox Signal* 16, 1434–1455 (2012)
DOI: 10.1089/ars.2011.4149

38. NA Shirwany, MH Zou. AMPK: A cellular metabolic and redox sensor. A minireview. *Front Biosci (Landmark Ed)* 19, 447–74 (2014)
DOI: 10.2741/4218

39. S Jäger, C Handschin, J St-Pierre, BM Spiegelman. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A* 104, 12017–22 (2007)
DOI: 10.1073/pnas.0705070104

40. BR Hoover, MN Reed, J Su, RD Penrod, L Kotilinek, MK Grant, R Pitstick, GA Carlson, LM Lanier, LL Yuan, KH Ashe, D Liao. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron* 68, 1067–81 (2010)
DOI: 10.1016/j.neuron.2010.11.030

41. A Louis, A Bartke, MM Masternak. Effects of growth hormone and thyroxine replacement therapy on insulin signaling in Ames dwarf mice. *J Gerontol A Biol Sci Med Sci* 65, 344–52 (2010)
DOI: 10.1093/gerona/glq018

42. L Katsouri, C Parr, N Bogdanovic, M Willem, M Sastre. PPARγ co-activator-1α (PGC-1α) reduces amyloid-β generation through a PPARγ-dependent mechanism. *J Alzheimers Dis* 25, 151–62 (2011)

43. PR Mouton, ME Chachich, C Quigley, E Spangler, DK Ingram. Caloric restriction attenuates amyloid deposition in middle-aged dtg APP/PS1 mice. *Neurosci Lett* 464, 184–7 (2009)
DOI: 10.1016/j.neulet.2009.08.038

44. B Reed, S Villeneuve, W Mack, C DeCarli, HC Chui, W Jagust. Associations between serum cholesterol levels and cerebral amyloidosis. *JAMA Neurol* 71, 195–200 (2014)
DOI: 10.1001/jamaneurol.2013.5390

45. P Gamba, G Testa, B Sottero, S Gargiulo, G Poli, G Leonarduzzi. The link between altered cholesterol metabolism and Alzheimer’s disease. *Ann N Y Acad Sci* 1259, 54–64 (2012)
DOI: 10.1111/j.1749-6632.2012.06513.x

46. L Thirumangalakudi, A Prakasam, R Zhang, H Bimonte-Nelson, K Sambamurti, MS Kindy, NR Bhat. High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of

working memory in mice. *J Neurochem* 106, 475–85 (2008)
DOI: 10.1111/j.1471-4159.2008.05415.x

47. SH Park, JH Kim, KH Choi, YJ Jang, SS Bae, BT Choi, HK Shin. Hypercholesterolemia accelerates amyloid β-induced cognitive deficits. *Int J Mol Med* 31, 577–82 (2013)
48. E Rönnemaa, B Zethelius, B Vessby, L Lannfelt, L Byberg, L Kilander. Serum fatty-acid composition and the risk of Alzheimer's disease: a longitudinal population-based study. *Eur J Clin Nutr* 66, 885–90 (2012)
DOI: 10.1038/ejcn.2012.63
49. N Mody, A Agouni, GD McIlroy, B Platt, M Delibegovic. Susceptibility to diet-induced obesity and glucose intolerance in the APP (SWE)/PSEN1 (A246E) mouse model of Alzheimer's disease is associated with increased brain levels of protein tyrosine phosphatase 1B (PTP1B) and retinol-binding protein 4 (RBP4), and basal phosphorylation of S6 ribosomal protein. *Diabetologia* 54, 2143–51 (2011)
DOI: 10.1007/s00125-011-2160-2
50. M Söderberg, C Edlund, K Kristensson, G Dallner. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. *Lipids* 26, 421–425 (1991)
DOI: 10.1007/BF02536067
51. PA Dacks, DW Shineman, HM Fillit. Current evidence for the clinical use of long-chain polyunsaturated n-3 fatty acids to prevent age-related cognitive decline and Alzheimer's disease. *J Nutr Health Aging* 17, 240–51 (2013)
DOI: 10.1007/s12603-012-0431-3
52. G Vitiello, S Marino, S Di, AMD Ursi, GD Errico. Omega-3 fatty acids regulate the interaction of the Alzheimer's aβ(25-35) peptide with lipid membranes. *Langmuir* 29, 14239–14245 (2013)
DOI: 10.1021/la403416b
53. JA Luchsinger, JM Noble, N Scarmeas. Diet and Alzheimer's disease. *Curr Neurol Neurosci* Rep 7, 366–372 (2007)
DOI: 10.1007/s11910-007-0057-8
54. S Merlo, S Spampinato, PL Canonico, A Copani, MA Sortino. Alzheimer's disease: brain expression of a metabolic disorder? *Trends Endocrinol Metab* 21, 537–44 (2010)
DOI: 10.1016/j.tem.2010.05.005
55. V Frisardi, V Solfrizzi, D Seripa, C Capurso, A Santamato, D Sancarlo, G Vendemiale, A Pilotto, F Panza. Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. *Ageing Res Rev* 9, 399–417 (2010)
DOI: 10.1016/j.arr.2010.04.007
56. EM Blalock, KC Chen, K Sharrow, JP Herman, NM Porter, TC Foster, PW Landfield. Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. *J Neurosci* 23, 3807–19 (2003)
57. EM Blalock, KC Chen, AJ Stromberg, CM Norris, I Kadish, SD Kraner, NM Porter, PW Landfield. Harnessing the power of gene microarrays for the study of brain aging and Alzheimer's disease: statistical reliability and functional correlation. *Ageing Res Rev* 4, 481–512 (2005)
DOI: 10.1016/j.arr.2005.06.006
58. AS Buchman, RS Wilson, JL Bienias, RC Shah, DA Evans, DA Bennett. Change in body mass index and risk of incident Alzheimer disease. *Neurology* 65, 892–897 (2005)
DOI: 10.1212/01.wnl.0000176061.33817.90
59. BB Cronk, DK Johnson, JM Burns. Body mass index and cognitive decline in mild cognitive impairment. Alzheimer *Dis Assoc Disord* 24, 126–30 (2009)
DOI: 10.1097/WAD.0b013e3181a6bf3f
60. S Hoyer. Glucose metabolism and insulin receptor signal transduction in Alzheimer disease. *Eur J Pharmacol* 490, 115–25 (2004)
DOI: 10.1016/j.ejphar.2004.02.049
61. A Sima. A. Encephalopathies: the emerging diabetic complications. *Acta Diabetol* 47, 279–93 (2010)
DOI: 10.1007/s00592-010-0218-0
62. M Hokama, S Oka, J Leon, T Ninomiya, H Honda, K Sasaki, T Iwaki, T Ohara, Sasaki, FM Laferla, Y Kiyohara, Y Nakabeppu. Altered Expression of Diabetes-Related Genes in Alzheimer's Disease Brains: The Hisayama Study. *Cereb Cortex* 24, 2476-88 (2013)
DOI: 10.1.093/cercor/bht101 (2013)
63. EC McNay, AK Recknagel. Brain insulin signaling: a key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. *Neurobiol*

Learn Mem 96, 432–42 (2011)
DOI: 10.1016/j.nlm.2011.08.005

64. LD Baker, DJ Cross, S Minoshima, D Belongia, GS Watson, S Craft. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol* 68, 51–7 (2011)
DOI: 10.1001/archneurol.2010.225

65. EC McNay, CT Ong, RJ McCrimmon, J Cresswell, JS Bogan, RS Sherwin. Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. *Neurobiol Learn Mem* 93, 546–53 (2010)
DOI: 10.1016/j.nlm.2010.02.002

66. DA Costello, M Claret, H Al-Qassab, F Plattner, EE Irvine, AI Choudhury, KP Giese, DJ Withers, P Pedarzani. Brain deletion of insulin receptor substrate 2 disrupts hippocampal synaptic plasticity and metaplasticity. *PLoS One* 7, e31124 (2012)
DOI: 10.1371/journal.pone.0031124

67. SM Gold, I Dziobek, V Sweat, A Tirsi, K Rogers, H Bruehl, W Tsui, S Richardson, E Javier, E., A Convit. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia* 50, 711–9 (2007)
DOI: 10.1007/s00125-007-0602-7

68. H Bruehl, OT Wolf, V Sweat, A Tirsi, S Richardson, A Convit. Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus. *Brain Res* 1280, 186–94 (2009)
DOI: 10.1016/j.brainres.2009.05.032

69. X Wang, W Zheng, JW Xie, T Wang, SL Wang, WP Teng, ZY Wang. Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model. *Mol Neurodegener* 5, 46 (2010)
DOI: 10.1186/1750-1326-5-46

70. R Ravona-Springer, E Moshier, J Schmeidler, J Godbold, J Akrivos, M Rapp, HT Grossman, M Wysocki, JM Silverman, V Haroutunian, MS Beeri. Changes in glycemic control are associated with changes in cognition in non-diabetic elderly. *J Alzheimers Dis* 30, 299–309 (2012)

71. X Li, F Guo, Q Zhang, T Huo, L Liu, H Wei, L, L Xiong, Q Wang. Electroacupuncture decreases cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice. *BMC Complement Altern Med* 14, 37 (2014)
DOI: 10.1186/1472-6882-14-37

72. LM Chua, ML Lim, PR Chong, ZP Hu, NS Cheung, BS Wong. Impaired neuronal insulin signaling precedes Aβ42 accumulation in female AβPPsw/PS1ΔE9 mice. *J Alzheimers Dis* 29, 783–91 (2012)

73. M Sadowski, J Pankiewicz, H Scholtzova, Y Ji, D Quartermain, CH Jensen, K Duff, RA Nixon, RJ Gruen, T Wisniewski. Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory impairment in APP/PS1 mice. *J Neuropathol Exp Neurol* 63, 418–428 (2004)

74. M Hiltunen, VKM Khandelwal, N Yaluri, T Tiilikainen, M Tusa, H Koivisto, M Krzisch, S Vepsäläinen, P Mäkinen, S Kemppainen, P Miettinen, A Haapasalo, H Soininen, M Laakso, H Tanila. Contribution of genetic and dietary insulin resistance to Alzheimer phenotype in APP/PS1 transgenic mice. *J Cell Mol Med* 16, 1206–22 (2012)
DOI: 10.1111/j.1582-4934.2011.01384.x

75. K Talbot, H Wang, H Kazi, L Han, KP Bakshi, A Stucky, RL Fuino, KR Kawaguchi, AJ Samoyedny, RS Wilson, Z Arvanitakis, JA Schneider, BA Wolf, DA Bennett, JQ Trojanowski, SE Arnold. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest* 122, 1316–1338 (2012)
DOI: 10.1172/JCI59903

76. M Mannaa, S Krämer, M Boschmann, M Gollasch. mTOR and regulation of energy homeostasis in humans. *J Mol Med* (Berl) 91, 1167–75 (2013)
DOI: 10.1007/s00109-013-1057-6

77. ME Orr, A Salinas, R Buffenstein, S Oddo. Mammalian target of rapamycin hyperactivity mediates the detrimental effects of a high sucrose diet on Alzheimer's disease pathology. *Neurobiol Aging* 35, 1233–42 (2014)
DOI: 10.1016/j.neurobiolaging.2013.12.006

78. Z Tang, E Bereczki, H Zhang, S Wang, C Li, X Ji, RM Branca, J Lehtio, Z Guan, P Filipcik, S Xu, B Winblad, JJ Pei. Mammalian

Target of Rapamycin (mTor) Mediates Tau Protein Dyshomeostasis: Implication for Alzheimer Disease. *J Biol Chem* 288, 15556–15570 (2013)
DOI: 10.1074/jbc.M112.435123

79. A Caccamo, S Majumder, A Richardson, R Strong, S Oddo. Molecular interplay between mammalian target of rapamycin (mTOR), amyloid-beta, and Tau: effects on cognitive impairments. *J Biol Chem* 285, 13107–20 (2010)
DOI: 10.1074/jbc.M110.100420

80. P Spilman, N Podlutskaya, M Hart, J Debnath, O Gorostiza, D Bredesen, A Richardson, R Strong, V Galvan. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. *PLoS One* 5, e9979 (2010)
DOI: 10.1371/journal.pone.0009979

81. Y Sun, X Ji, X Mao, L Xie, J Jia, V Galvan, DA Greenberg, K Jin. Differential activation of mTOR complex 1 signaling in human brain with mild to severe Alzheimer's disease. *J Alzheimers Dis* 38, 437–44 (2014)

82. AE Ramírez, CR Pacheco, LG Aguayo, CM Opazo. Rapamycin protects against Aβ-induced synaptotoxicity by increasing presynaptic activity in hippocampal neurons. *Biochim Biophys Acta* 1842, 1495-501 (2014)
DOI: 10.1016/j.bbadis.2014.04.019

83. S Wullschleger, R Loewith, MN Hall. TOR signaling in growth and metabolism. *Cell* 124, 471–84 (2006)
DOI: 10.1016/j.cell.2006.01.016

84. AK Saha, XJ Xu, TW Balon, A Brandon, EW Kraegen, NB Ruderman. Insulin resistance due to nutrient excess: is it a consequence of AMPK downregulation? *Cell Cycle* 10, 3447–3451 (2011)
DOI: 10.4161/cc.10.20.17886

85. BK Binukumar, V Shukla, ND Amin, P Reddy, S Skuntz, P Grant, HC Pant. Topographic regulation of neuronal intermediate filaments by phosphorylation, role of peptidyl-prolyl isomerase 1: significance in neurodegeneration. *Histochem Cell Biol* 140, 23–32 (2013)
DOI: 10.1007/s00418-013-1108-7

86. JL Hallows, K Chen, RA DePinho, I Vincent. Decreased cyclin-dependent kinase 5 (cdk5) activity is accompanied by redistribution of cdk5 and cytoskeletal proteins and increased cytoskeletal protein phosphorylation in p35 null mice. *J Neurosci* 23, 10633–44 (2003)

87. M Takahashi, E Iseki, K Kosaka. Cdk5 and munc-18/p67 co-localization in early stage neurofibrillary tangles-bearing neurons in Alzheimer type dementia brains. *J Neurol Sci* 172, 63–69 (2000)
DOI: 10.1016/S0022-510X(99)00291-9

Abbreviations: AD: Alzheimer's disease; APP/PS1: APPSwe/PS1dE9 mice; SAD: Alzheimer's sporadic form; Aβ: β-amyloid; APP: Aβ protein precursor; Ach: acetylcholinesterase; PRL: Pituitary-derived prolactin; ob: obese gene; Lep: leptin; PRLR: prolactin receptor; PGC-1α: PPARγ coactivator-1α; BACE1: β-secretase; CR: caloric restriction; LDLR: low density lipoprotein receptor; ULSAM: Uppsala Longitudinal Study of Adult Men; HFD: short-term high-fat diet; T2DM: Type 2 Diabetes Mellitus; APdE9: overexpressing mutant amyloid precursor protein and presenilin-1 mice; IGF-2: insulin like growth factor 2; IRS-1/2: insulin receptor substrates 1 and 2; mTOR: mammalian target of rapamycin; Cdk5: cyclin dependent kinase 5

Key Words: APP/PS1, Insulin Receptor, Hippocampus, Alzheimer Disease, Leptin, Prolactin, PGC-1α, mTOR, Cdk5, Review

Send correspondence to: Jaume Folch, Unitat de Bioquimica i Biologia Molecular, Facultat de Medicina i Ciencies de la Salut, Universitat Rovira i Virgili de Tarragona, Spain, C. Sant Llorenc 21. 43201-Reus, Spain, Tel: 34-977759376, Fax: 34-977759322, E-mail: jaume.folch@urv.cat

BIBLIOGRAFÍA

UNIVERSITAT ROVIRA I VIRGILI
ALTERACIONES METABÓLICAS EN EL PROCESO DE AMILOIDOGÉNESIS EN EL HIPOCAMPO DE RATONES APP/PS1
Ignacio Pedrós Martín

A

Abdul-Rahman, O. et al., 2012. Altered gene expression profiles in the hippocampus and prefrontal cortex of type 2 diabetic rats. *BMC genomics*, 13, p.81. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3299604&tool=pmcentrez&rendertype=abstract [Accessed February 14, 2016].

Accardi, G. et al., 2012. Can Alzheimer disease be a form of type 3 diabetes? *Rejuvenation research*, 15(2), pp.217–21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22533436 [Accessed July 9, 2014].

Adeghate, E., Donáth, T. & Adem, A., 2013. Alzheimer disease and diabetes mellitus: do they have anything in common? *Current Alzheimer research*, 10(6), pp.609–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23627758 [Accessed August 19, 2015].

Aido, B.T.C.S., 2013. Review Metabolism of amyloid O peptide and pathogenesis of Alzheimer ' s disease. , 89(7).

Akiyama, H. et al., Inflammation and Alzheimer's disease. *Neurobiology of aging*, 21(3), pp.383–421. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3887148&tool=pmcentrez&rendertype=abstract [Accessed April 6, 2015].

Alexander, G.E. et al., 2002. Longitudinal PET Evaluation of Cerebral Metabolic Decline in Dementia: A Potential Outcome Measure in Alzheimer's Disease Treatment Studies. *The American journal of psychiatry*, 159(5), pp.738–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11986126 [Accessed December 3, 2015].

Ali, Z. et al., 2013. On the regulatory role of side-chain hydroxylated oxysterols in the brain. Lessons from CYP27A1 transgenic and Cyp27a1(-/-) mice. *Journal of lipid research*, 54(4), pp.1033–43. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3605980&tool=pmcentrez&rendertype=abstract [Accessed November 30, 2015].

Alle, H., Roth, A. & Geiger, J.R.P., 2009. Energy-Efficient Action Potentials in Hippocampal Mossy Fibers. *Science*, 325(5946), pp.1405–1408. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19745156 [Accessed October 12, 2015].

Alzheimer, A. et al., 1995. An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clinical anatomy (New York, N.Y.)*, 8(6), pp.429–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8713166 [Accessed March 10, 2015].

Amitani, M. et al., 2013. The role of leptin in the control of insulin-glucose axis. *Frontiers in Neuroscience*, 7(7 APR), pp.1–12.

Andersson, U. & Scarpulla, R.C., 2001. Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. *Molecular and cellular biology*, 21(11), pp.3738–49. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=87014&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Anstey, K.J. et al., 2011. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, 12(5), pp.e426–37.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/21348917 [Accessed September 28, 2015].

Antunes, M. & Biala, G., 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognitive processing*, 13(2), pp.93–110. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3332351&tool=pmcentrez&rendertype=abstract [Accessed March 8, 2015].

Aso, E. et al., 2012. Amyloid generation and dysfunctional immunoproteasome activation with disease progression in animal model of familial Alzheimer's disease. *Brain pathology (Zurich, Switzerland)*, 22(5), pp.636–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22188425 [Accessed April 29, 2014].

Attwell, D. & Laughlin, S.B., 2001. An energy budget for signaling in the grey matter of the brain. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 21(10), pp.1133–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11598490 [Accessed September 5, 2015].

Austin, S. & St-Pierre, J., 2012. PGC1α and mitochondrial metabolism--emerging concepts and relevance in ageing and neurodegenerative disorders. *Journal of cell science*, 125(Pt 21), pp.4963–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23277535 [Accessed July 9, 2014].

B

Babcock, A.A. et al., 2015. Cytokine-producing microglia have an altered beta-amyloid load in aged APP/PS1 Tg mice. *Brain, Behavior, and Immunity*, 48, pp.86–101. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25774009 [Accessed October 7, 2015].

Bado, A. et al., 1998. The stomach is a source of leptin. *Nature*, 394(6695), pp.790–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9723619 [Accessed November 21, 2015].

Balakrishnan, K. et al., 2005. Plasma Abeta42 correlates positively with increased body fat in healthy individuals. *Journal of Alzheimer's disease : JAD*, 8(3), pp.269–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16340084 [Accessed November 30, 2015].

Barnes, J. et al., 2009. A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiology of aging*, 30(11), pp.1711–23. Available at: http://www.sciencedirect.com/science/article/pii/S0197458008000262 [Accessed December 3, 2015].

Bates, S.H. & Myers, M.G., 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. *Trends in endocrinology and metabolism: TEM*, 14(10), pp.447–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14643059 [Accessed February 15, 2016].

Baumgart, M. et al., 2015. Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 11(6), pp.1–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26045020.

Becker, J.T., 2010. Neuroimagen en la enfermedad de Alzheimer: nuevas perspectivas. *Rev*

Neurol, 50(Suppl 5).

Bekris, L.M. et al., 2010. Genetics of Alzheimer disease. *Journal of geriatric psychiatry and neurology*, 23(4), pp.213–27. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3044597&tool=pmcentrez&rendertype=abstract [Accessed July 23, 2015].

Bélanger, M., Allaman, I. & Magistretti, P.J., 2011. Brain energy metabolism: Focus on Astrocyte-neuron metabolic cooperation. *Cell Metabolism*, 14(6), pp.724–738.

Benomar, Y. et al., 2005. Cross down-regulation of leptin and insulin receptor expression and signalling in a human neuronal cell line. *The Biochemical journal*, 388(Pt 3), pp.929–39. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1183474&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Beydoun, M.A., Beydoun, H.A. & Wang, Y., 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity*, 9(3), pp.204–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18331422 [Accessed September 28, 2015].

Bilkei-Gorzo, A., 2014. Genetic mouse models of brain ageing and Alzheimer's disease. *Pharmacology & therapeutics*, 142(2), pp.244–57. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24362083 [Accessed August 31, 2015].

Bingham, E.M. et al., 2002. The Role of Insulin in Human Brain Glucose Metabolism: An 18Fluoro-Deoxyglucose Positron Emission Tomography Study. *Diabetes*, 51(12), pp.3384–3390. Available at: http://diabetes.diabetesjournals.org/content/51/12/3384.abstract [Accessed September 25, 2015].

Boitard, C. et al., 2012. Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. *Hippocampus*, 22(11), pp.2095–100. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22593080 [Accessed July 9, 2014].

Bonda, D.J. et al., 2014. Dysregulation of leptin signaling in Alzheimer disease: evidence for neuronal leptin resistance. *Journal of neurochemistry*, 128(1), pp.162–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23895348 [Accessed July 9, 2014].

Bossy-Wetzel, E., Schwarzenbacher, R. & Lipton, S.A., 2004. Molecular pathways to neurodegeneration. *Nature Medicine*, 10(7), pp.S2–S9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15272266 [Accessed October 13, 2015].

de Bruijn, R.F.A.G. & Ikram, M.A., 2014. Cardiovascular risk factors and future risk of Alzheimer's disease. *BMC medicine*, 12(1), p.130. Available at: http://www.biomedcentral.com/1741-7015/12/130 [Accessed November 16, 2015].

Brunden, K.R., Trojanowski, J.Q. & Lee, V.M.-Y., 2009. Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. *Nature Reviews Drug Discovery*, 8(10), pp.783–793. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2787232&tool=pmcentrez&rendertype=abstract [Accessed November 2, 2015].

Bubber, P. et al., 2005. Mitochondrial abnormalities in Alzheimer brain: Mechanistic implications. *Annals of Neurology*, 57(5), pp.695–703. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15852400 [Accessed October 9, 2015].

Buerger, K. et al., 2006. CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease. *Brain : a journal of neurology*, 129(Pt 11), pp.3035–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17012293 [Accessed December 3, 2015].

Burwell, R.D., Witter, M.P. & Amaral, D.G., 1995. Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus*, 5(5), pp.390–408. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8773253 [Accessed November 13, 2015].

C

Cahill, G.F., 2006. Fuel metabolism in starvation. *Annual review of nutrition*, 26, pp.1–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16848698 [Accessed October 17, 2015].

Candeias, E. et al., 2012. The impairment of insulin signaling in Alzheimer's disease. *IUBMB life*, 64(12), pp.951–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23129399 [Accessed July 9, 2014].

Cao, X. & Südhof, T.C., 2001. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science (New York, N.Y.)*, 293(5527), pp.115–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11441186 [Accessed December 4, 2015].

Cárdenas, A.M. et al., 2012. Role of tau protein in neuronal damage in Alzheimer's disease and Down syndrome. *Archives of medical research*, 43(8), pp.645–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23142525 [Accessed July 9, 2014].

Castellani, R. et al., 2002. Role of mitochondrial dysfunction in Alzheimer's disease. *Journal of neuroscience research*, 70(3), pp.357–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12391597 [Accessed September 3, 2015].

Caviston, J.P. & Holzbaur, E.L.F., 2006. Microtubule motors at the intersection of trafficking and transport. *Trends in Cell Biology*, 16(10), pp.530–537. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16938456 [Accessed October 5, 2015].

Chadwick, E.K. et al., 2011. Continuous neuronal ensemble control of simulated arm reaching by a human with tetraplegia. *Journal of neural engineering*, 8(3), p.034003. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3608269&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Cho, D.-H. et al., 2009. S-Nitrosylation of Drp1 Mediates -Amyloid-Related Mitochondrial Fission and Neuronal Injury. *Science*, 324(5923), pp.102–105. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2823371&tool=pmcentrez&rendertype=abstract [Accessed October 9, 2015].

Chua, L.-M. et al., 2012. Impaired neuronal insulin signaling precedes Aβ42 accumulation in female AβPPsw/PS1ΔE9 mice. *Journal of Alzheimer's disease : JAD*, 29(4), pp.783–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22337827 [Accessed July 9, 2014].

Citron, M. et al., 1997. Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nature medicine*, 3(1), pp.67–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8986743 [Accessed October 7, 2015].

Clark, C.M. et al., 2012. Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-β plaques: a prospective cohort study. *The Lancet. Neurology*, 11(8), pp.669–78. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22749065 [Accessed November 30, 2015].

Clark, I. et al., 2012. Tumor necrosis factor-induced cerebral insulin resistance in Alzheimer's disease links numerous treatment rationales. *Pharmacological reviews*, 64(4), pp.1004–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22966039.

Clarke, D.D. & Sokoloff, L., 1999. Circulation and Energy Metabolism of the Brain. Available at: http://www.ncbi.nlm.nih.gov/books/NBK20413/ [Accessed December 4, 2015].

Cochran, J.N., Hall, A.M. & Roberson, E.D., 2014. The dendritic hypothesis for Alzheimer's disease pathophysiology. *Brain research bulletin*, 103, pp.18–28. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3989444&tool=pmcentrez&rendertype=abstract [Accessed February 13, 2016].

Cooper, C. et al., 2015. Modifiable Predictors of Dementia in Mild Cognitive Impairment: A Systematic Review and Meta-Analysis. *American Journal of Psychiatry*, 172(4), pp.323–334. Available at: http://ajp.psychiatryonline.org/doi/10.1176/appi.ajp.2014.14070878 [Accessed October 12, 2015].

Copps, K.D. & White, M.F., 2012. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia*, 55(10), pp.2565–82.

Corral-Debrinski, M. et al., 1992. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nature genetics*, 2(4), pp.324–9. Available at: http://www.nature.com/ng/journal/v2/n4/pdf/ng1292-324.pdf [Accessed October 9, 2015].

Cota, D. et al., 2008. The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 28(28), pp.7202–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2597379&tool=pmcentrez&rendertype=abstract [Accessed February 15, 2016].

Craft, S., 2012. Alzheimer disease: Insulin resistance and AD--extending the translational path. *Nature reviews. Neurology*, 8(7), pp.360–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22710630 [Accessed July 9, 2014].

Cramer, P.E. et al., 2012. ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models. *Science (New York, N.Y.)*, 335(6075), pp.1503–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3651582&tool=pmcentrez&rendertype=abstract [Accessed December 3, 2015].

Cuadrado-Tejedor, M. et al., 2012. Chronic mild stress accelerates the onset and progression of the Alzheimer's disease phenotype in Tg2576 mice. *Journal of Alzheimer's disease : JAD*, 28(3), pp.567–78. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/22045482 [Accessed December 4, 2015].

D

Davies, P. & Maloney, A.J., 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet (London, England)*, 2(8000), p.1403. Available at: http://www.ncbi.nlm.nih.gov/pubmed/63862 [Accessed January 31, 2016].

Davis, S. & Laroche, S., 2006. Mitogen-activated protein kinase/extracellular regulated kinase signalling and memory stabilization: a review. *Genes, brain, and behavior*, 5 Suppl 2, pp.61–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16681801 [Accessed December 4, 2015].

de la Monte, S.M., 2012a. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. *Current Alzheimer research*, 9(1), pp.35–66. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3349985&tool=pmcentrez&rendertype=abstract [Accessed July 24, 2014].

de la Monte, S.M., 2012b. Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's disease. *Drugs*, 72(1), pp.49–66. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4550303&tool=pmcentrez&rendertype=abstract [Accessed October 6, 2015].

de la Monte, S.M. & Wands, J.R., 2008. Alzheimer's disease is type 3 diabetes-evidence reviewed. *Journal of diabetes science and technology*, 2(6), pp.1101–13. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2769828&tool=pmcentrez&rendertype=abstract [Accessed September 16, 2015].

de la Monte, S.M. & Wands, J.R., 2005. Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 7(1), pp.45–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15750214 [Accessed February 13, 2016].

de la Monte, Suzanne M Wands, J.R., 2008. Alzheimer ' s Disease Is Type 3 Diabetes — Evidence Reviewed. *Journal of Diabetes Science and Technology*, 2(6), pp.1101–1113.

de la Torre, J.C. & Mussivand, T., 1993. Can disturbed brain microcirculation cause Alzheimer's disease? *Neurological research*, 15(3), pp.146–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8103579 [Accessed February 13, 2016].

DeMattos, R.B. et al., 2002. Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. *Journal of neurochemistry*, 81(2), pp.229–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12064470 [Accessed November 30, 2015].

Deprez-Poulain, R. et al., 2015. Catalytic site inhibition of insulin-degrading enzyme by a small molecule induces glucose intolerance in mice. *Nature communications*, 6, p.8250. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4580987&tool=pmcentrez&rendertype=abstract [Accessed December 3, 2015].

Desikan, R.S. et al., 2009. Automated MRI measures identify individuals with mild cognitive

impairment and Alzheimer's disease. *Brain : a journal of neurology*, 132(Pt 8), pp.2048–57. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2714061&tool=pmcentrez&rendertype=abstract [Accessed December 3, 2015].

Diaz Brinton, R. & Yamazaki, R.S., 1998. Advances and challenges in the prevention and treatment of Alzheimer's disease. *Pharmaceutical research*, 15(3), pp.386–98. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9563067 [Accessed October 2, 2015].

Dickerson, B.C. et al., 2009. The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cerebral cortex (New York, N.Y. : 1991)*, 19(3), pp.497–510. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2638813&tool=pmcentrez&rendertype=abstract [Accessed November 30, 2015].

Dietschy, J.M. & Turley, S.D., 2004. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *Journal of lipid research*, 45(8), pp.1375–1397.

DiMauro, S. & Schon, E.A., 2003. Mitochondrial respiratory-chain diseases. *The New England journal of medicine*, 348(26), pp.2656–68. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12826641 [Accessed May 15, 2015].

Dineley, K.T., Jahrling, J.B. & Denner, L., 2014. Insulin resistance in Alzheimer's disease. *Neurobiology of disease*, 72 Pt A, pp.92–103. Available at: http://www.sciencedirect.com/science/article/pii/S0969996114002642 [Accessed July 16, 2015].

Duara, R. et al., 1996. Alzheimer's disease: interaction of apolipoprotein E genotype, family history of dementia, gender, education, ethnicity, and age of onset. *Neurology*, 46(6), pp.1575–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8649551 [Accessed August 26, 2015].

E

Engvall, E., Jonsson, K. & Perlmann, P., 1971. Enzyme-linked immunosorbent assay. II. Quantitative assay of protein antigen, immunoglobulin G, by means of enzyme-labelled antigen and antibody-coated tubes. *Biochimica et biophysica acta*, 251(3), pp.427–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11452886 [Accessed October 7, 2015].

Eskelinen, M.H. et al., 2008. Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study. *International journal of geriatric psychiatry*, 23(7), pp.741–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18188871 [Accessed October 11, 2015].

Fagan, A.M. et al., 2007. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Archives of neurology*, 64(3), pp.343–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17210801 [Accessed December 3, 2015].

De Felice, F.G., 2013. Alzheimer's disease and insulin resistance: translating basic science into clinical applications. *Journal of Clinical Investigation*, 123(2), pp.531–539.

Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3561831&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

F

Fernández, M. & Castro, J., 2008. Marcadores genéticos en la enfermedad de Alzheimer : genes patógenos y de susceptibilidad ma or ci nt. *Revista Alzheimer*, 38(tabla 1), pp.4–13. Available at: http://www.revistaalzheimer.com/PDF/0177.pdf.

Ferri, C.P. et al., 2005. Global prevalence of dementia: a Delphi consensus study. *Lancet*, 366(9503), pp.2112–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2850264&tool=pmcentrez&rendertype=abstract.

Florent-Béchard, S. et al., 2009. The essential role of lipids in Alzheimer's disease. *Biochimie*, 91(6), pp.804–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19303044 [Accessed January 7, 2016].

Formiga, F. & Pérez-Maraver, M., 2014. Diabetes mellitus tipo 3. ¿El renacer de la insulina inhalada? *Endocrinología y Nutrición*, 61(4), pp.173–175. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1575092214000746.

Fortea, J. et al., 2011. Cognitively preserved subjects with transitional cerebrospinal fluid ß-amyloid 1-42 values have thicker cortex in Alzheimer's disease vulnerable areas. *Biological psychiatry*, 70(2), pp.183–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21514924 [Accessed December 3, 2015].

Francis, H.M. & Stevenson, R.J., 2011. Higher reported saturated fat and refined sugar intake is associated with reduced hippocampal-dependent memory and sensitivity to interoceptive signals. *Behavioral neuroscience*, 125(6), pp.943–55. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22023100 [Accessed October 11, 2015].

Freiherr, J. et al., 2013. Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. *CNS drugs*, 27(7), pp.505–14. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3709085&tool=pmcentrez&rendertype=abstract [Accessed October 21, 2015].

Freude, S., Schilbach, K. & Schubert, M., 2009. The role of IGF-1 receptor and insulin receptor signaling for the pathogenesis of Alzheimer's disease: from model organisms to human disease. *Current Alzheimer research*, 6(3), pp.213–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19519303 [Accessed December 6, 2015].

Frühbeck, G., 2006. Intracellular signalling pathways activated by leptin. *The Biochemical journal*, 393(Pt 1), pp.7–20. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1383660&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

G

Galluzzi, L., Blomgren, K. & Kroemer, G., 2009. Mitochondrial membrane permeabilization in neuronal injury. *Nature reviews. Neuroscience*, 10(7), pp.481–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19543220 [Accessed September 6, 2015].

Gammelsaeter, R. et al., 2011. A role for glutamate transporters in the regulation of insulin secretion. *PloS one*, 6(8), p.e22960. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0022960 [Accessed December 4, 2015].

Gao, Q. et al., 2004. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proceedings of the National Academy of Sciences of the United States of America*, 101(13), pp.4661–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=384803&tool=pmcentrez&rendertype=abstract [Accessed August 31, 2015].

Gao, S. et al., 1998. The relationships between age, sex, and the incidence of dementia and Alzheimer disease: a meta-analysis. *Archives of general psychiatry*, 55(9), pp.809–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9736007 [Accessed October 2, 2015].

Garcia-Alloza, M. et al., 2006. Characterization of amyloid deposition in the APPswe/PS1dE9 mouse model of Alzheimer disease. *Neurobiology of Disease*, 24(3), pp.516–524. Available at: http://www.sciencedirect.com/science/article/pii/S0969996106002075 [Accessed October 7, 2015].

Garfield, A.S. et al., 2012. Neurochemical characterization of body weight-regulating leptin receptor neurons in the nucleus of the solitary tract. *Endocrinology*, 153(10), pp.4600–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3507354&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Gasparini, L. et al., 2001. Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 21(8), pp.2561–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11306609 [Accessed December 4, 2015].

Gelling, R.W. et al., 2006. Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. *Cell Metabolism*, 3(1), pp.67–73. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1550413105003499.

Gendron, T.F., Zhang, Y.-J. & Petrucelli, L., 2013. Does obesity-induced τ phosphorylation tip the scale toward dementia? *Diabetes*, 62(5), pp.1365–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3636650&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Graeber, M.B. et al., 1998. Histopathology and APOE genotype of the first Alzheimer disease patient, Auguste D. *Neurogenetics*, 1(3), pp.223–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10737127 [Accessed December 3, 2015].

Greenwood, C.E. & Winocur, G., 2005. High-fat diets, insulin resistance and declining cognitive function. *Neurobiology of aging*, 26 Suppl 1, pp.42–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16257476 [Accessed November 20, 2015].

Guerchet, M., Prina, M. & Prince, M., 2013. Policy Brief for Heads of Government: The Global Impact of Dementia 2013–2050. *Policy Brief for Heads of Government: The Global Impact of Dementia 2013–2050 Published by Alzheimer's Disease International (ADI), London. December 2013*, pp.1–8. Available at:

http://www.alz.co.uk/research/G8-policy-brief.

Guerra, J., Arjona, L. & Díaz, J., 2009. Título:"Demencias y enfermedad de Alzheimer un recorrido por la historia." *Medigraphic.Com*, 9(1). Available at: http://www.medigraphic.com/pdfs/geroinfo/ger-2014/ger141b.pdf.

Guillod-Maximin, E. et al., 2004. Acute intracarotid glucose injection towards the brain induces specific c-fos activation in hypothalamic nuclei: involvement of astrocytes in cerebral glucose-sensing in rats. *Journal of neuroendocrinology*, 16(5), pp.464–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15117340 [Accessed December 3, 2015].

Gustafson, D. et al., 2003. An 18-year follow-up of overweight and risk of Alzheimer disease. *Archives of internal medicine*, 163(13), pp.1524–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12860573 [Accessed October 2, 2015].

Guzmán, M., Sánchez, C. & Galve-Roperh, I., 2001. Control of the cell survival/death decision by cannabinoids. *Journal of molecular medicine (Berlin, Germany)*, 78(11), pp.613–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11269508 [Accessed December 4, 2015].

H

Haass, C., 2004. Take five--BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *The EMBO journal*, 23(3), pp.483–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1271800&tool=pmcentrez&rendertype=abstract [Accessed November 24, 2015].

Haataja, L. et al., 2008. Islet Amyloid in Type 2 Diabetes, and the Toxic Oligomer Hypothesis. *Endocrine Reviews*, 29(3), pp.303–316. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2528855&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

van Hall, G. et al., 2009. Blood lactate is an important energy source for the human brain. *Journal of Cerebral Blood Flow & Metabolism*, 29(6), pp.1121–1129. Available at: http://dx.doi.org/10.1038/jcbfm.2009.35 [Accessed October 22, 2015].

Hardy, J. & Allsop, D., 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in pharmacological sciences*, 12(10), pp.383–8.

Hardy, J. & Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)*, 297(5580), pp.353–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12130773 [Accessed July 10, 2014].

Hardy, J.A. & Higgins, G.A., 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science (New York, N.Y.)*, 256(5054), pp.184–5.

Hay, N. & Sonenberg, N., 2004. Upstream and downstream of mTOR. *Genes & development*, 18(16), pp.1926–45. Available at: http://genesdev.cshlp.org/content/18/16/1926.full [Accessed July 11, 2014].

Head, E. et al., 2012. Aging and down syndrome. *Current Gerontology and Geriatrics Research*, 2012.

Hegyi, K. et al., 2004. Leptin-induced signal transduction pathways. *Cell biology*

international, 28(3), pp.159–69. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14984741 [Accessed December 4, 2015].

van der Heide, L.P. et al., 2005. Insulin modulates hippocampal activity-dependent synaptic plasticity in a N-methyl-d-aspartate receptor and phosphatidyl-inositol-3-kinase-dependent manner. *Journal of neurochemistry*, 94(4), pp.1158–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16092951 [Accessed December 4, 2015].

Herms, J. et al., 2004. Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. *The EMBO journal*, 23(20), pp.4106–15. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=524337&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Heverin, M., Bogdanovic, N. & Lütjohann, D., 2004. Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. *Journal of lipid …*, 45(1), pp.186–193. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14523054 [Accessed March 31, 2014].

Hoffman, L.B. et al., 2009. Less Alzheimer disease neuropathology in medicated hypertensive than nonhypertensive persons. *Neurology*, 72(20), pp.1720–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2683736&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Hokama, M. et al., 2013. Altered Expression of Diabetes-Related Genes in Alzheimer's Disease Brains: The Hisayama Study. *Cerebral cortex (New York, N.Y. : 1991)*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23595620 [Accessed July 9, 2014].

Hokama, M. et al., 2014. Altered expression of diabetes-related genes in Alzheimer's disease brains: the Hisayama study. *Cerebral cortex (New York, N.Y. : 1991)*, 24(9), pp.2476–88. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4128707&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Holcomb, L. et al., 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature medicine*, 4(1), pp.97–100. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9427614 [Accessed January 6, 2016].

Holmquist, G., 1975. Hoechst 33258 fluorescent staining of Drosophila chromosomes. *Chromosoma*, 49(4), pp.333–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/48449 [Accessed October 7, 2015].

Hong, M. & Lee, V.M., 1997. Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. *The Journal of biological chemistry*, 272(31), pp.19547–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9235959 [Accessed December 4, 2015].

Hoover, B.R. et al., 2010. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron*, 68(6), pp.1067–81. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3026458&tool=pmcentrez&rendertype=abstract [Accessed March 20, 2015].

Hua, X. et al., 2008. Tensor-based morphometry as a neuroimaging biomarker for Alzheimer's disease: an MRI study of 676 AD, MCI, and normal subjects. *NeuroImage*, 43(3), pp.458–69. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3197851&tool=pmcentrez&rendertype=abstract [Accessed August 26, 2015].

Huang, H.-J. et al., 2011. Long-term social isolation exacerbates the impairment of spatial working memory in APP/PS1 transgenic mice. *Brain research*, 1371, pp.150–60. Available at: http://www.researchgate.net/publication/49642700_Long-term_social_isolation_exacerbates_the_impairment_of_spatial_working_memory_in_APPPS1_transgenic_mice [Accessed October 7, 2015].

Hyman, B.T. et al., 2012. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 8(1), pp.1–13. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3266529&tool=pmcentrez&rendertype=abstract [Accessed August 6, 2015].

I

Ikonomovic, M.D. et al., 2008. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain : a journal of neurology*, 131(Pt 6), pp.1630–45. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2408940&tool=pmcentrez&rendertype=abstract [Accessed November 17, 2015].

Irving, A.J. & Harvey, J., 2014. Leptin regulation of hippocampal synaptic function in health and disease. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 369(1633), p.20130155. Available at: http://rstb.royalsocietypublishing.org/content/369/1633/20130155 [Accessed November 30, 2015].

Iwata, N. et al., 2001. Metabolic regulation of brain Abeta by neprilysin. *Science (New York, N.Y.)*, 292(5521), pp.1550–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11375493 [Accessed November 28, 2015].

J

Jack, C.R. et al., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet neurology*, 9(1), pp.119–28. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2819840&tool=pmcentrez&rendertype=abstract [Accessed July 17, 2014].

Jack, C.R. et al., 2011. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 7(3), pp.257–62. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3096735/ [Accessed September 28, 2015].

Janson, J. et al., 2004. Increased Risk of Type 2 Diabetes in Alzheimer Disease. *Diabetes*, 53(2), pp.474–481.

Jiang, J. et al., 2013. Inhibition of the prostaglandin receptor EP2 following status

epilepticus reduces delayed mortality and brain inflammation. *Proceedings of the National Academy of Sciences of the United States of America*, 110(9), pp.3591–6. Available at: http://www.pnas.org/content/110/9/3591.abstract.

Jiménez-Palomares, M. et al., 2012. Increased Aβ production prompts the onset of glucose intolerance and insulin resistance. *American journal of physiology. Endocrinology and metabolism*, 302(11), pp.E1373–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22414803 [Accessed October 5, 2015].

Jucker, M. & Walker, L.C., 2013. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465), pp.45–51. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3963807&tool=pmcentrez&rendertype=abstract [Accessed July 13, 2014].

K

Kalaria, R.N. et al., 2008. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *The Lancet. Neurology*, 7(9), pp.812–26. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2860610&tool=pmcentrez&rendertype=abstract [Accessed June 4, 2015].

Kanoski, S.E. et al., 2011. Hippocampal leptin signaling reduces food intake and modulates food-related memory processing. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 36(9), pp.1859–70. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3154104&tool=pmcentrez&rendertype=abstract [Accessed August 31, 2015].

Karran, E., Mercken, M. & Strooper, B. De, 2011. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature Reviews Drug Discovery*, 10(9), pp.698–712. Available at: http://www.nature.com/doifinder/10.1038/nrd3505.

Katsouri, L. et al., 2011. PPARγ co-activator-1α (PGC-1α) reduces amyloid-β generation through a PPARγ-dependent mechanism. *Journal of Alzheimer's disease : JAD*, 25(1), pp.151–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21358044 [Accessed July 9, 2014].

Ke, Y.D. et al., 2009. Experimental diabetes mellitus exacerbates tau pathology in a transgenic mouse model of Alzheimer's disease. *PloS one*, 4(11), p.e7917. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0007917 [Accessed February 14, 2016].

Kiliaan, A.J., Arnoldussen, I.A.C. & Gustafson, D.R., 2014. Adipokines: a link between obesity and dementia? *The Lancet. Neurology*, 13(9), pp.913–23. Available at: http://www.thelancet.com/article/S1474442214700857/fulltext [Accessed November 26, 2015].

Kim, D. et al., 2007. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *The EMBO Journal*, 26(13), pp.3169–3179. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1914106&tool=pmcentrez&rendertype=abstract [Accessed October 6, 2015].

Kim, E.J. et al., 2005. Glucose metabolism in early onset versus late onset Alzheimer's disease: an SPM analysis of 120 patients. *Brain : a journal of neurology*, 128(Pt 8), pp.1790–801. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15888536 [Accessed December 3, 2015].

Kimoto, A. et al., 2015. Serum insulin-like growth factor-I and amyloid beta protein in Alzheimer's disease: relationship with cognitive function. *Psychogeriatrics : the official journal of the Japanese Psychogeriatric Society*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26439951 [Accessed January 6, 2016].

Kivipelto, M. et al., 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Archives of neurology*, 62(10), pp.1556–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16216938 [Accessed September 15, 2015].

Klunk, W.E. et al., 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Annals of neurology*, 55(3), pp.306–19. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14991808 [Accessed November 10, 2015].

Knight, E.M. et al., 2014. High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. *Neurobiology of aging*, 35(8), pp.1821–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24630364 [Accessed July 9, 2014].

Knott, A.B. et al., 2008. Mitochondrial fragmentation in neurodegeneration. *Nature Reviews Neuroscience*, 9(7), pp.505–518. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2711514&tool=pmcentrez&rendertype=abstract [Accessed October 8, 2015].

Könner, A.C., Klöckener, T. & Brüning, J.C., 2009. Control of energy homeostasis by insulin and leptin: targeting the arcuate nucleus and beyond. *Physiology & behavior*, 97(5), pp.632–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19351541 [Accessed December 4, 2015].

Koopman, K. et al., 2009. Improved discrimination of autopsy-confirmed Alzheimer's disease (AD) from non-AD dementias using CSF P-tau(181P). *Neurochemistry international*, 55(4), pp.214–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19524111 [Accessed December 3, 2015].

Korc, M., 2003. Diabetes Mellitus in the era of proteomics. *Molecular & Cellular Proteomics*, pp.399–404. Available at: http://www.mcponline.org/cgi/doi/10.1074/mcp.R300005-MCP200.

Kosari, S. et al., 2012. Effect of western and high fat diets on memory and cholinergic measures in the rat. *Behavioural brain research*, 235(1), pp.98–103. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22820146 [Accessed July 9, 2014].

Kremerskothen, J. et al., 2002. Insulin-induced expression of the activity-regulated cytoskeleton-associated gene (ARC) in human neuroblastoma cells requires p21(ras), mitogen-activated protein kinase/extracellular regulated kinase and src tyrosine kinases but is protein kinase C-indepe. *Neuroscience letters*, 321(3), pp.153–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11880195 [Accessed December 3, 2015].

Kroner, Z., 2009. The relationship between Alzheimer's disease and diabetes: Type 3 diabetes? *Alternative medicine review : a journal of clinical therapeutic*, 14(4),

pp.373–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20030463 [Accessed August 13, 2015].

Kurt, M.A. et al., 2001. Neurodegenerative changes associated with beta-amyloid deposition in the brains of mice carrying mutant amyloid precursor protein and mutant presenilin-1 transgenes. *Experimental neurology*, 171(1), pp.59–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11520121 [Accessed October 5, 2015].

L

Lacquaniti, A. et al., 2013. Prolactin in obese children : a bridge between inflammation and metabolic-endocrine dysfunction. , pp.1–8.

LaFerla, F.M., Green, K.N. & Oddo, S., 2007. Intracellular amyloid-beta in Alzheimer's disease. *Nature reviews. Neuroscience*, 8(7), pp.499–509. Available at: http://dx.doi.org/10.1038/nrn2168 [Accessed July 11, 2014].

Leal, M.C. et al., 2013. Transcriptional regulation of insulin-degrading enzyme modulates mitochondrial amyloid β (Aβ) peptide catabolism and functionality. *The Journal of biological chemistry*, 288(18), pp.12920–31. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3642335&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Leboucher, A. et al., 2013. Detrimental effects of diet-induced obesity on τ pathology are independent of insulin resistance in τ transgenic mice. *Diabetes*, 62(5), pp.1681–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3636620&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Lee, E.B., 2011. Obesity, leptin, and Alzheimer's disease. *Annals of the New York Academy of Sciences*, 1243, pp.15–29. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3564488&tool=pmcentrez&rendertype=abstract [Accessed August 26, 2015].

Lee, G.H. et al., 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature*, 379(6566), pp.632–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8628397 [Accessed December 4, 2015].

Lee, J.W. et al., 2008. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. *Journal of neuroinflammation*, 5, p.37. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2556656&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Lee, Y.-H. et al., 2008. Amyloid precursor protein expression is upregulated in adipocytes in obesity. *Obesity (Silver Spring, Md.)*, 16(7), pp.1493–500. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18483477 [Accessed November 30, 2015].

Leone, T.C. et al., 2005. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS biology*, 3(4), p.e101. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1064854&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Lovestone, S. & McLoughlin, D.M., 2002. Protein aggregates and dementia: is there a

common toxicity? *Journal of neurology, neurosurgery, and psychiatry*, 72(2), pp.152–61. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1737746&tool=pmcentrez&rendertype=abstract [Accessed December 3, 2015].

Lu, F.-P., Lin, K.-P. & Kuo, H.-K., 2009. Diabetes and the Risk of Multi-System Aging Phenotypes: A Systematic Review and Meta-Analysis C. Zhang, ed. *PLoS ONE*, 4(1), p.e4144. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0004144 [Accessed October 8, 2015].

Luchtman, D.W. & Song, C., 2013. Cognitive enhancement by omega-3 fatty acids from child-hood to old age: findings from animal and clinical studies. *Neuropharmacology*, 64, pp.550–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22841917 [Accessed May 28, 2015].

M

M. de la Monte, S., 2012. Brain Insulin Resistance and Deficiency as Therapeutic Targets in Alzheimers Disease. *Current Alzheimer Research*, 9(1), pp.35–66. Available at: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1567-2050&volume=9&issue=1&spage=35.

Mahley, R.W., Weisgraber, K.H. & Huang, Y., 2006. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 103(15), pp.5644–51. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1414631&tool=pmcentrez&rendertype=abstract [Accessed August 1, 2015].

Mak, G.K. & Weiss, S., 2010. Paternal recognition of adult offspring mediated by newly generated CNS neurons. *Nature neuroscience*, 13(6), pp.753–8. Available at: http://www.nature.com/neuro/journal/v13/n6/fig_tab/nn.2550_F4.html [Accessed November 30, 2015].

Mancuso, M. et al., 2009. Is there a primary role of the mitochondrial genome in Alzheimer's disease? *Journal of bioenergetics and biomembranes*, 41(5), pp.411–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19798559 [Accessed February 13, 2016].

Markesbery, W.R., 1997. Oxidative stress hypothesis in Alzheimer's disease. *Free radical biology & medicine*, 23(1), pp.134–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9165306 [Accessed February 13, 2016].

Martin, L. et al., 2013. Tau protein kinases: involvement in Alzheimer's disease. *Ageing research reviews*, 12(1), pp.289–309. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22742992 [Accessed July 9, 2014].

Martin Prince, A. et al., 2014. World Alzheimer Report 2014 Dementia and Risk Reduction an Analysis of Protective and Modifiable Factors Supported. , p.102.

Martin Prince, A. et al., 2015. World Alzheimer Report 2015 The Global Impact of Dementia An AnAlysIs of prevAlence, IncIDence, cosT AnD TrenDs.

Masaki, T. et al., 2004. Obesity in insulin receptor substrate-2-deficient mice: disrupted control of arcuate nucleus neuropeptides. *Obesity research*, 12(5), pp.878–85.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/15166310 [Accessed November 28, 2015].

Mattson, M.P., 2004. Pathways towards and away from Alzheimer's disease. *Nature*, 430(7000), pp.631–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3091392&tool=pmcentrez&rendertype=abstract [Accessed May 17, 2015].

Mayeda, E.R., Whitmer, R. a. & Yaffe, K., 2015. Diabetes and Cognition. *Clinics in Geriatric Medicine*, 31(1), pp.101–115. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0749069014000913.

Mecocci, P., MacGarvey, U. & Beal, M.F., 1994. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Annals of neurology*, 36(5), pp.747–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7979220 [Accessed January 28, 2015].

Meng, X.-F. et al., 2014. Midlife Vascular Risk Factors and the Risk of Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Journal of Alzheimer's disease : JAD*, 42, pp.1295–1310. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25024338.

Metcalfe, M.J. & Figueiredo-Pereira, M.E., Relationship between tau pathology and neuroinflammation in Alzheimer's disease. *The Mount Sinai journal of medicine, New York*, 77(1), pp.50–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2904237&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Minkeviciene, R. et al., 2008. Age-related decrease in stimulated glutamate release and vesicular glutamate transporters in APP/PS1 transgenic and wild-type mice. *Journal of neurochemistry*, 105(3), pp.584–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18042177 [Accessed August 31, 2015].

Minthon, L., Edvinsson, L. & Gustafson, L., 1996. Correlation between clinical characteristics and cerebrospinal fluid neuropeptide Y levels in dementia of the Alzheimer type and frontotemporal dementia. *Alzheimer disease and associated disorders*, 10(4), pp.197–203. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8939279 [Accessed November 27, 2015].

Montine, T.J. et al., 2014. Recommendations of the Alzheimer's Disease-Related Dementias Conference. *Neurology*, 83(9), pp.851–860. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4155046&tool=pmcentrez&rendertype=abstract [Accessed November 25, 2015].

Morales, T., 2011. Recent findings on neuroprotection against excitotoxicity in the hippocampus of female rats. *Journal of neuroendocrinology*, 23(11), pp.994–1001. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21507086 [Accessed November 27, 2015].

Morioka, T. et al., 2007. Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. *The Journal of clinical investigation*, 117(10), pp.2860–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1994606&tool=pmcentrez&rendertype=abstract [Accessed August 19, 2015].

Morris, M.C. & Tangney, C.C., 2014. Dietary fat composition and dementia risk. *Neurobiology of aging*, 35 Suppl 2, pp.S59–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24970568 [Accessed August 29, 2014].

Morton, G.J. et al., 2005. Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. *Cell metabolism*, 2(6), pp.411–20. Available at: http://www.sciencedirect.com/science/article/pii/S1550413105003360 [Accessed December 4, 2015].

Morton, G.J. & Schwartz, M.W., 2011. Leptin and the central nervous system control of glucose metabolism. *Physiological reviews*, 91(2), pp.389–411. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3379883&tool=pmcentrez&rendertype=abstract [Accessed June 29, 2015].

Münzberg, H. & Myers, M.G., 2005. Molecular and anatomical determinants of central leptin resistance. *Nature neuroscience*, 8(5), pp.566–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15856064 [Accessed December 4, 2015].

Myers, M.G., Cowley, M.A. & Münzberg, H., 2008. Mechanisms of leptin action and leptin resistance. *Annual review of physiology*, 70, pp.537–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17937601 [Accessed November 16, 2014].

N

Nakamura, T. et al., 2010. S-nitrosylation of Drp1 links excessive mitochondrial fission to neuronal injury in neurodegeneration. *Mitochondrion*, 10(5), pp.573–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2918703&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Newman, A.B. et al., 2005. Dementia and Alzheimer's disease incidence in relationship to cardiovascular disease in the Cardiovascular Health Study cohort. *Journal of the American Geriatrics Society*, 53(7), pp.1101–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16108925 [Accessed December 4, 2015].

Nicolas, C.S. et al., 2013. The role of JAK-STAT signaling within the CNS. *JAK-STAT*, 2(1), p.e22925. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3670265&tool=pmcentrez&rendertype=abstract [Accessed August 31, 2015].

Nieuwenhuis-Mark, R.E., 2009. Diagnosing Alzheimer's dementia in Down syndrome: problems and possible solutions. *Research in developmental disabilities*, 30(5), pp.827–38. Available at: http://www.sciencedirect.com/science/article/pii/S0891422209000110 [Accessed June 4, 2015].

Niswender, K.D., Baskin, D.G. & Schwartz, M.W., 2004. Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends in endocrinology and metabolism: TEM*, 15(8), pp.362–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15380807 [Accessed December 4, 2015].

Nyaradi, A. et al., 2014. Prospective associations between dietary patterns and cognitive performance during adolescence. *Journal of Child Psychology and Psychiatry*, 55(9), pp.1017–1024. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24673485 [Accessed October 11, 2015].

O

Olney, J.W., Wozniak, D.F. & Farber, N.B., 1997. Excitotoxic neurodegeneration in Alzheimer disease. New hypothesis and new therapeutic strategies. *Archives of neurology*, 54(10), pp.1234–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9341569 [Accessed February 13, 2016].

Orr, M.E. et al., 2014. Mammalian target of rapamycin hyperactivity mediates the detrimental effects of a high sucrose diet on Alzheimer's disease pathology. *Neurobiology of aging*, 35(6), pp.1233–42. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3973159&tool=pmcentrez&rendertype=abstract [Accessed February 15, 2016].

Ossenkoppele, R. et al., 2012. Longitudinal imaging of Alzheimer pathology using [11C]PIB, [18F]FDDNP and [18F]FDG PET. *European journal of nuclear medicine and molecular imaging*, 39(6), pp.990–1000. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22441582 [Accessed December 3, 2015].

P

Paolo, G. & Kim, T.-W., 2011. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nature reviews. Neuroscience*, 12(5), pp.284–96. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3321383&tool=pmcentrez&rendertype=abstract [Accessed November 19, 2015].

Pasquier, F. et al., 2006. Diabetes mellitus and dementia. *Diabetes & metabolism*, 32(5 Pt 1), pp.403–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17110895 [Accessed August 14, 2014].

Payami, H. et al., 1996. Gender difference in apolipoprotein E-associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. *American journal of human genetics*, 58(4), pp.803–11. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1914663&tool=pmcentrez&rendertype=abstract [Accessed October 2, 2015].

Pedros, I. et al., 2016. Molecular links between early energy metabolism alterations and Alzheimer ´ s disease. , pp.8–19.

Pedrós, I. et al., 2014. Early alterations in energy metabolism in the hippocampus of APPswe/PS1dE9 mouse model of Alzheimer's disease. *Biochimica et biophysica acta*, 1842(9), pp.1556–1566. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24887203 [Accessed July 9, 2014].

Peters, A. et al., 2007. Causes of obesity: looking beyond the hypothalamus. *Progress in neurobiology*, 81(2), pp.61–88. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17270337 [Accessed December 4, 2015].

Petrov, D. et al., 2015. High-fat diet-induced deregulation of hippocampal insulin signaling and mitochondrial homeostasis deficiences contribute to Alzheimer disease pathology in rodents. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1852(9), pp.1687–1699. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0925443915001477.

Pickrell, A.M., Fukui, H. & Moraes, C.T., 2009. The role of cytochrome c oxidase deficiency in ROS and amyloid plaque formation. *Journal of bioenergetics and biomembranes*,

41(5), pp.453–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19795195 [Accessed July 9, 2014].

Plattner, F. et al., 2014. Memory enhancement by targeting Cdk5 regulation of NR2B. *Neuron*, 81(5), pp.1070–83. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24607229 [Accessed July 9, 2014].

Pletnikova, O. et al., 2015. Alzheimer Lesions in the Autopsied Brains of People 30 to 50 Years of Age. *Cognitive and behavioral neurology : official journal of the Society for Behavioral and Cognitive Neurology*, 28(3), pp.144–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26413742 [Accessed October 23, 2015].

Pollard, H.B., Arispe, N. & Rojas, E., 1995. Ion channel hypothesis for Alzheimer amyloid peptide neurotoxicity. *Cellular and molecular neurobiology*, 15(5), pp.513–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8719038 [Accessed February 13, 2016].

Popp, J. et al., 2013. Cerebral and extracerebral cholesterol metabolism and CSF markers of Alzheimer's disease. *Biochemical pharmacology*, 86(1), pp.37–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23291240 [Accessed December 3, 2015].

Postina, R. et al., 2004. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. *The Journal of clinical investigation*, 113(10), pp.1456–64. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=406531&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Prince, M., Prina, M. & Guerchet, M., 2013. World Alzheimer Report 2013 Journey of Caring: An Analysis of Long-Term Care for Dementia. *Alzheimer's Disease International*, pp.1–92.

Profenno, L.A., Porsteinsson, A.P. & Faraone, S. V, 2010. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. *Biological psychiatry*, 67(6), pp.505–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19358976 [Accessed May 14, 2015].

Q

Qin, W. et al., 2009. PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia. *Archives of neurology*, 66(3), pp.352–61. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3052997&tool=pmcentrez&rendertype=abstract [Accessed August 31, 2015].

Qiu, C., Kivipelto, M. & von Strauss, E., 2009. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues in clinical neuroscience*, 11(2), pp.111–28. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3181909&tool=pmcentrez&rendertype=abstract [Accessed January 7, 2015].

Qiu, W.Q. et al., 1998. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *The Journal of biological chemistry*, 273(49), pp.32730–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9830016 [Accessed September 23, 2015].

Querfurth, H.W. & Laferla, F.M., 2010. Alzheimer's Disease. , 9, pp.329–344.

R

Radde, R. et al., 2006. Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO reports*, 7(9), pp.940–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1559665&tool=pmcentrez&rendertype=abstract [Accessed August 31, 2015].

Radzimanowski, J. et al., 2008. Structure of the intracellular domain of the amyloid precursor protein in complex with Fe65-PTB2. *EMBO reports*, 9(11), pp.1134–40. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2581855&tool=pmcentrez&rendertype=abstract [Accessed December 4, 2015].

Raji, C.A. et al., 2010. Brain structure and obesity. *Human brain mapping*, 31(3), pp.353–64. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2826530&tool=pmcentrez&rendertype=abstract [Accessed July 13, 2015].

Ramos, B. et al., 2006. Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1xAPP transgenic model of Alzheimer's disease. *Neurobiology of aging*, 27(11), pp.1658–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16271420 [Accessed November 27, 2015].

Ramos-Rodriguez, J.J. et al., 2013. Differential central pathology and cognitive impairment in pre-diabetic and diabetic mice. *Psychoneuroendocrinology*, 38(11), pp.2462–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23790682 [Accessed October 5, 2015].

Ramos-Rodriguez, J.J. et al., 2014. Prediabetes-induced vascular alterations exacerbate central pathology in APPswe/PS1dE9 mice. *Psychoneuroendocrinology*, 48C, pp.123–135. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24998414 [Accessed July 10, 2014].

Rangani, R.J. et al., 2012. Nicotine evoked improvement in learning and memory is mediated through NPY Y1 receptors in rat model of Alzheimer's disease. *Peptides*, 33(2), pp.317–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22266216 [Accessed November 27, 2015].

Reed, B. et al., 2014. Associations between serum cholesterol levels and cerebral amyloidosis. *JAMA neurology*, 71(2), pp.195–200. Available at: http://archneur.jamanetwork.com/article.aspx?articleid=1791528&resultClick=24 [Accessed November 29, 2015].

Reiner, P. et al., 2012. Sulcal span in Azheimer's disease, amnestic mild cognitive impairment, and healthy controls. *Journal of Alzheimer's disease : JAD*, 29(3), pp.605–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22297645 [Accessed October 27, 2015].

Review, N. & Selection, S., 2013. Cholesterol Level and Statin Use in Alzheimer Disease. , 68(11), pp.1385–1392.

Ridha, B.H. et al., 2006. Tracking atrophy progression in familial Alzheimer's disease: a serial MRI study. *The Lancet Neurology*, 5(10), pp.828–834. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16987729 [Accessed December 3, 2015].

Roher, A.E. et al., 2009. Amyloid beta peptides in human plasma and tissues and their

significance for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 5(1), pp.18–29. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2663406&tool=pmcentrez&rendertype=abstract [Accessed November 10, 2015].

Rotz, R.C. et al., 2004. The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. *Journal of cell science*, 117(Pt 19), pp.4435–48. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15331662 [Accessed November 17, 2015].

S

Sabio, G. & Davis, R.J., 2010. cJun NH2-terminal kinase 1 (JNK1): roles in metabolic regulation of insulin resistance. *Trends in biochemical sciences*, 35(9), pp.490–6. Available at: http://www.cell.com/article/S0968000410000733/fulltext [Accessed October 8, 2015].

Sadowski, M. et al., 2004. Amyloid-beta deposition is associated with decreased hippocampal glucose metabolism and spatial memory impairment in APP/PS1 mice. *Journal of neuropathology and experimental neurology*, 63(5), pp.418–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15198121.

Salkovic-Petrisic, M. & Hoyer, S., 2007. Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. *Journal of neural transmission. Supplementum*, (72), pp.217–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17982898 [Accessed December 4, 2015].

Sapolsky, R.M., 2000. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biological psychiatry*, 48(8), pp.755–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11063972 [Accessed December 4, 2015].

Sato, N. & Morishita, R., 2013. Roles of vascular and metabolic components in cognitive dysfunction of Alzheimer disease: short- and long-term modification by non-genetic risk factors. *Frontiers in Aging Neuroscience*, 5, p.64. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3817366&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Saunders, A.M. et al., 1993. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*, 43(8), pp.1467–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8350998 [Accessed September 2, 2015].

Savonenko, A. et al., 2005. Episodic-like memory deficits in the APPswe/PS1dE9 mouse model of Alzheimer's disease: relationships to beta-amyloid deposition and neurotransmitter abnormalities. *Neurobiology of disease*, 18(3), pp.602–17. Available at: http://www.sciencedirect.com/science/article/pii/S0969996104002669 [Accessed September 15, 2015].

Schechter, R. & Abboud, M., 2001. Neuronal synthesized insulin roles on neural differentiation within fetal rat neuron cell cultures. *Brain research. Developmental brain research*, 127(1), pp.41–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11287063 [Accessed December 4, 2015].

Schipper, H.M., 2004. Brain iron deposition and the free radical-mitochondrial theory of

ageing. *Ageing Research Reviews*, 3(3), pp.265–301. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15231237 [Accessed October 9, 2015].

Schneider, L.S. et al., 1996. Effects of estrogen replacement therapy on response to tacrine in patients with Alzheimer's disease. *Neurology*, 46(6), pp.1580–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8649552 [Accessed October 2, 2015].

Schwartz, M.W. et al., 2000. Central nervous system control of food intake. *Nature*, 404(6778), pp.661–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10766253 [Accessed March 4, 2015].

Seubert, P. et al., 1992. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature*, 359(6393), pp.325–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1406936 [Accessed December 4, 2015].

Shanley, L.J., Irving, a J. & Harvey, J., 2001. Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 21(24), p.RC186. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11734601.

Sheng, B. et al., 2012. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. *Journal of neurochemistry*, 120(3), pp.419–29. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3253532&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Shimomura, I. et al., 1999. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature*, 401(6748), pp.73–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10485707 [Accessed December 4, 2015].

Shirwany, N.A. & Zou, M.-H., 2014. AMPK: a cellular metabolic and redox sensor. A minireview. *Frontiers in bioscience (Landmark edition)*, 19, pp.447–74. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4101001&tool=pmcentrez&rendertype=abstract [Accessed October 6, 2015].

Shukla, V., Skuntz, S. & Pant, H.C., 2012. Deregulated Cdk5 activity is involved in inducing Alzheimer's disease. *Archives of medical research*, 43(8), pp.655–62. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3532552&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Snyder, E.M. et al., 2005. Regulation of NMDA receptor trafficking by amyloid-beta. *Nature neuroscience*, 8(8), pp.1051–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16025111 [Accessed January 28, 2016].

Snyder, J.S. et al., 2011. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature*, 476(7361), pp.458–61. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3162077&tool=pmcentrez&rendertype=abstract [Accessed July 10, 2014].

Sobesky, J.L. et al., 2014. High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism. *Brain, behavior, and immunity*, 42, pp.22–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24998196 [Accessed January 6, 2016].

Solano, D.C. et al., 2000. Insulin regulates soluble amyloid precursor protein release via phosphatidyl inositol 3 kinase-dependent pathway. *FASEB journal : official*

publication of the Federation of American Societies for Experimental Biology, 14(7), pp.1015–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10783157 [Accessed December 4, 2015].

Solfrizzi, V. et al., 2011. Metabolic syndrome, mild cognitive impairment, and progression to dementia. The Italian Longitudinal Study on Aging. *Neurobiology of aging*, 32(11), pp.1932–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20045217 [Accessed October 11, 2015].

Sperk, G., Hamilton, T. & Colmers, W.F., 2007. Neuropeptide Y in the dentate gyrus. *Progress in brain research*, 163, pp.285–97. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17765725 [Accessed November 27, 2015].

Sperling, R. a. et al., 2011. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia*, 7(3), pp.280–292. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1552526011000999.

Srinivasa, R.N. et al., 2015. Cardiovascular Risk Factors Associated with Smaller Brain Volumes in Regions Identified as Early Predictors of Cognitive Decline. *Radiology*, p.142488. Available at: http://pubs.rsna.org/doi/abs/10.1148/radiol.2015142488 [Accessed September 8, 2015].

Steen, E. et al., 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? *Journal of Alzheimer's disease : JAD*, 7(1), pp.63–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15750215 [Accessed August 19, 2015].

St-Pierre, J. et al., 2006. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell*, 127(2), pp.397–408. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17055439 [Accessed July 9, 2014].

Stratmann, M. et al., 2014. Insular and Hippocampal Gray Matter Volume Reductions in Patients with Major Depressive Disorder B. Draganski, ed. *PLoS ONE*, 9(7), p.e102692. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4106847&tool=pmcentrez&rendertype=abstract [Accessed October 22, 2015].

Strooper, B., 2007. Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease. *EMBO reports*, 8(2), pp.141–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1796779&tool=pmcentrez&rendertype=abstract [Accessed November 18, 2015].

Strozyk, D. et al., 2003. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*, 60(4), pp.652–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12601108 [Accessed December 3, 2015].

Studies, P., 2013. Cholesterol Level and Statin Use in Alzheimer Disease. , 68(10), pp.1239–1244.

Sundaram, J.R. et al., 2012. Cdk5/p25-induced cytosolic PLA2-mediated lysophosphatidylcholine production regulates neuroinflammation and triggers neurodegeneration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 32(3), pp.1020–34. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/22262900 [Accessed July 9, 2014].

Swanson, D., Block, R. & Mousa, S.A., 2012. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition (Bethesda, Md.)*, 3(1), pp.1–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3262608&tool=pmcentrez&rendertype=abstract [Accessed August 4, 2015].

Swerdlow, R.H., Burns, J.M. & Khan, S.M., 2010. The Alzheimer's disease mitochondrial cascade hypothesis. *Journal of Alzheimer's disease : JAD*, 20 Suppl 2, pp.S265–79. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2883665&tool=pmcentrez&rendertype=abstract [Accessed September 2, 2015].

Swerdlow, R.H. & Khan, S.M., 2004a. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Medical hypotheses*, 63(1), pp.8–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15193340 [Accessed January 7, 2016].

Swerdlow, R.H. & Khan, S.M., 2004b. A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Medical hypotheses*, 63(1), pp.8–20.

T

Talbot, K. et al., 2012. Demonstrated brain insulin resistance in Alzheimer ' s disease patients is associated with IGF-1 resistance , IRS-1 dysregulation , and cognitive decline. , 122(4).

TERRY, R.D. & PENA, C., 1965. Experimental production of neurofibrillary degeneration 2. electron microscopy, phosphatase histochemistry and electron probe analysis. *journal of neuropathology and experimental neurology*, 24, pp.200–10. available at: http://www.ncbi.nlm.nih.gov/pubmed/14280497 [Accessed February 13, 2016].

Tolppanen, A.-M. et al., 2014. Midlife and late-life body mass index and late-life dementia: results from a prospective population-based cohort. *Journal of Alzheimer's disease : JAD*, 38(1), pp.201–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23948937 [Accessed October 10, 2015].

U

Ubeda, M., Kemp, D.M. & Habener, J.F., 2004. Glucose-induced expression of the cyclin-dependent protein kinase 5 activator p35 involved in Alzheimer's disease regulates insulin gene transcription in pancreatic beta-cells. *Endocrinology*, 145(6), pp.3023–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14976144 [Accessed July 9, 2014].

V

Valladolid-Acebes, I. et al., 2012. High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. *American journal of physiology. Endocrinology and metabolism*, 302(4), pp.E396–402. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22114023 [Accessed January 6, 2016].

Vanacore, N., 2013. Neurodegenerative causes of death among retired National Football League players. *Neurology*, 80(13), pp.1266–7. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/23530154 [Accessed November 30, 2015].

Vanmierlo, T. et al., 2010. Alterations in brain cholesterol metabolism in the APPSLxPS1mut mouse, a model for Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 19(1), pp.117–27. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20061631 [Accessed July 9, 2014].

Velasco, G. et al., 2005. Cannabinoids and ceramide: Two lipids acting hand-by-hand. *Life Sciences*, 77(14), pp.1723–1731. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0024320505005035.

Vignini, A. et al., 2013. Alzheimer's disease and diabetes: new insights and unifying therapies. *Current diabetes reviews*, 9(3), pp.218–27. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23363296 [Accessed October 5, 2015].

W

Wang, S. et al., 2001. [Changes and relations of leptin, growth hormone and insulin during puberty in obese and non-obese children]. *Wei sheng yan jiu = Journal of hygiene research*, 30(4), pp.219–20, back cover. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12561520 [Accessed August 20, 2015].

Wang, X. et al., 2010. Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model. *Molecular neurodegeneration*, 5(1), p.46. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2987993&tool=pmcentrez&rendertype=abstract [Accessed July 9, 2014].

Warren, M.W., Hynan, L.S. & Weiner, M.F., 2012. Lipids and adipokines as risk factors for Alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 29(1), pp.151–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3732377&tool=pmcentrez&rendertype=abstract [Accessed August 31, 2015].

Watson, G.S. et al., 2005. Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry*, 13(11), pp.950–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16286438 [Accessed December 4, 2015].

Webster, S.J., Bachstetter, A.D. & Van Eldik, L.J., 2013. Comprehensive behavioral characterization of an APP/PS-1 double knock-in mouse model of Alzheimer's disease. *Alzheimer's research & therapy*, 5(3), p.28. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3706792&tool=pmcentrez&rendertype=abstract [Accessed October 7, 2015].

Weiner, M.W. et al., 2012. The Alzheimer's Disease Neuroimaging Initiative: a review of papers published since its inception. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 8(1 Suppl), pp.S1–68. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3329969&tool=pmcentrez&rendertype=abstract [Accessed December 1, 2015].

Wiesner, G. et al., 1999. Leptin is released from the human brain: influence of adiposity and gender. *The Journal of clinical endocrinology and metabolism*, 84(7), pp.2270–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10404789 [Accessed

December 4, 2015].

Willette, A.A. et al., 2015. Association of Insulin Resistance With Cerebral Glucose Uptake in Late Middle-Aged Adults at Risk for Alzheimer Disease. *JAMA neurology*, 72(9), pp.1013–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26214150 [Accessed August 5, 2015].

X

Xia, Z. et al., 1995. Opposing Effects of ERK and JNK-p38 MAP Kinases on Apoptosis. *Science*, 270(5240), pp.1326–1331. Available at: http://www.sciencemag.org/content/270/5240/1326 [Accessed December 4, 2015].

Y

Yamada-Goto, N. et al., 2012. Impairment of fear-conditioning responses and changes of brain neurotrophic factors in diet-induced obese mice. *Journal of neuroendocrinology*, 24(8), pp.1120–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22487415 [Accessed January 6, 2016].

Yan, S. Du & Stern, D.M., 2005. Mitochondrial dysfunction and Alzheimer's disease: role of amyloid-β peptide alcohol dehydrogenase (ABAD). *International Journal of Experimental Pathology*, 86(3), pp.161–171. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2517415&tool=pmcentrez&rendertype=abstract [Accessed October 9, 2015].

Yao, J. et al., 2011. Inhibition of Amyloid- (A) Peptide-Binding Alcohol Dehydrogenase-A Interaction Reduces A Accumulation and Improves Mitochondrial Function in a Mouse Model of Alzheimer's Disease. *Journal of Neuroscience*, 31(6), pp.2313–2320. Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4717-10.2011.

Yao, J. et al., 2009. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 106(34), pp.14670–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2732886&tool=pmcentrez&rendertype=abstract.

Z

Zhang, F. et al., 1997. Crystal structure of the obese protein leptin-E100. *Nature*, 387(6629), pp.206–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9144295 [Accessed October 16, 2015].

Zhang, L., Ding, Q. & Wang, Z., 2012. Nuclear Respiratory Factor 1 Mediates the Transcription Initiation of Insulin-Degrading Enzyme in a TATA Box-Binding Protein-Independent Manner F. G. Hamel, ed. *PLoS ONE*, 7(8), p.e42035. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3411688&tool=pmcentrez&rendertype=abstract [Accessed October 5, 2015].

Zhang, Y. et al., 2012. Amyloid-β induces hepatic insulin resistance by activating JAK2/STAT3/SOCS-1 signaling pathway. *Diabetes*, 61(6), pp.1434–43. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3357286&tool=pmce

ntrez&rendertype=abstract [Accessed July 9, 2014].

Zhang, Y. et al., 2013. Amyloid-β induces hepatic insulin resistance in vivo via JAK2. *Diabetes*, 62(4), pp.1159–66. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3609589&tool=pmcentrez&rendertype=abstract [Accessed February 8, 2016].

Zhang, Y. et al., 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505), pp.425–32. Available at: http://dx.doi.org/10.1038/372425a0 [Accessed January 7, 2015].

Zhao, H. et al., 2013. Hyperphosphorylation of tau protein by calpain regulation in retina of Alzheimer's disease transgenic mouse. *Neuroscience letters*, 551, pp.12–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23810804 [Accessed July 9, 2014].

Zhao, L. et al., 2004. Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for Alzheimer's disease intervention. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 24(49), pp.11120–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15590928 [Accessed December 4, 2015].

Zhao, Z. et al., 2007. Insulin degrading enzyme activity selectively decreases in the hippocampal formation of cases at high risk to develop Alzheimer's disease. *Neurobiology of aging*, 28(6), pp.824–30. Available at: http://www.neurobiologyofaging.org/article/S0197458006001412/fulltext [Accessed August 17, 2015].