TESIS DE LA UNIVERSIDAD DE ZARAGOZA
2022
126

José Luis Pérez Lasierra

Estilos de vida, condicionantes genéticos, y enfermedades asociadas al envejecimiento

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ISSN 2254-7606

Prensas de la Universidad
Universidad Zaragoza

ISSN 2254-7606

Universidad Zaragoza
1542

Tesis Doctoral

ESTILOS DE VIDA, CONDICIONANTES GENÉTICOS, Y ENFERMEDADES ASOCIADAS AL ENVEJECIMIENTO

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UNIVERSIDAD DE ZARAGOZA
Escuela de Doctorado

Programa de Doctorado en Ciencias de la Salud y del Deporte

2022

Tesis Doctoral Internacional *[International Doctoral Thesis]*

Curso Académico 2021-2022

ESTILOS DE VIDA, CONDICIONANTES GENÉTICOS, Y ENFERMEDADES ASOCIADAS AL ENVEJECIMIENTO

LIFESTYLE, GENETICS, AND AGEING RELATED DISEASES

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Estilos de vida, condicionantes genéticos, y enfermedades asociadas al envejecimiento

Lifestyle, genetics, and ageing related diseases

JOSÉ LUIS PÉREZ LASIERRA

A mis padres, Mª del Mar y José Luis

por creer en mi y apoyarme en todo momento.

A mis directores, José Antonio, Belén y Alex

y a mis compañeros del grupo GENUD.

Gracias por vuestra ayuda y apoyo.

Sobre cada montaña, hay un sendero, aunque no se pueda ver desde el valle.

Theodore Roethke

No conquistamos las montañas, sino a nosotros mismos.

Edmund Hillary

Estilos de vida, condicionantes genéticos, y enfermedades asociadas al envejecimiento

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Fdo. Alejandro González de Agüero Lafuente

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Proyectos y contratos de investigación

La Tesis Doctoral que se presenta a continuación, así como varios de los artículos que la conforman, se enmarcan dentro del proyecto Aragon Workers' Health Study (AWHS), fruto del convenio de colaboración entre el Instituto Aragonés de Ciencias de la Salud del Gobierno de Aragón y la Fundación Centro Nacional de Investigaciones Cardiovasculares del Instituto de Salud Carlos III del Ministerio de Ciencia e Innovación, así como entre General Motors España S.L. (GME) y la Comunidad Autónoma de Aragón (BOA Nº 198, 26 de noviembre de 2008).

A su vez, otros de los artículos que componen la presente Tesis Doctoral se han desarrollado gracias a los datos americanos de uso público que proporciona el National Center for Health Statistics (NCHS) a través del estudio National Health and Nutrition Examination Survey (NHANES).

Contratos de investigación

José Luis Pérez Lasierra fue contratado por la Universidad de Zaragoza gracias a las ayudas destinadas a la Formación de Profesorado Universitario del Ministerio de Educación, Cultura y Deporte del Gobierno de España (FPU16/02539).

Estancias de investigación

A lo largo del periodo de realización de la presente Tesis Doctoral el doctorando D. José Luis Pérez Lasierra, realizó dos estancias de investigación. Las características de las estancias de investigación se detallan a continuación:

I. Estancia de investigación internacional en la Universidad Johns Hopkins, Departamento de Epidemiología (Baltimore, Estados Unidos de América). Supervisor: Prof. Eliseo Guallar Castillón. Duración: 2 meses y 19 días (02/09/2019 – 19/11/2019). Temática de la estancia: Estilos de vida y enfermedades relacionadas con el envejecimiento.

II. Estancia de investigación internacional en la Universidad Johns Hopkins, Departamento de Epidemiología (Baltimore, Estados Unidos de América). Supervisor: Prof. Eliseo Guallar Castillón. Duración: 3 meses y 5 días (11/12/2020 – 15/03/2021). Temática de la estancia: Estilos de vida y enfermedades relacionadas con el envejecimiento.

Listado de publicaciones

La presente Tesis Doctoral es un compendio de trabajos científicos previamente publicados o en proceso de publicación. A continuación, se detallan las referencias de cada uno de los artículos que componen este documento:

I. **Perez-Lasierra ,Jose Luis**, Casajús Jose Antonio, Casasnovas José Antonio, Arbones-Mainar Jose Miguel, Lobo Antonio, Lobo Elena, Moreno-Franco Belén, González-Agüero Alejandro. Can Physical Activity Reduce the Risk of Cognitive Decline in Apolipoprotein e4 Carriers? A Systematic Review. Int J Environ Res Public Health. 2021 Jul; 18;(14), 7238. doi: 10.3390/ijerph18147238.

II. **Perez-Lasierra ,Jose Luis**, Laclaustra Martin, Guallar-Castillón Pilar, Casasnovas Jose Antonio, Casajús Jose Antonio, Jarauta Estibaliz, González-Agüero Alejandro, Moreno-Franco Belén. Daily Sitting for Long Periods Increases the Odds for Subclinical Atheroma Plaques. J Clin Med. 2021 Mar; 10;(6), 1229. doi: 10.3390/jcm10061229.

III. **Perez-Lasierra ,Jose Luis**, Casajús Jose Antonio, González-Agüero Alejandro, Moreno-Franco Belén. Association of physical activity levels and prevalence of major degenerative diseases: Evidence from the national health and nutrition examination survey (NHANES) 1999-2018. Exp Gerontol. 2022 Feb; 158, 111656. doi: 10.1016/J.EXGER.2021.111656.

IV. **Perez-Lasierra ,Jose Luis**, Moreno-Franco Belén, González-Agüero Alejandro, Lobo Elena, Casajús Jose Antonio. A cross-sectional analysis of the association between physical activity, depression, and all-cause mortality in Americans over 50 years old. Sci Rep. 2022 Feb 10;12(1):2264. doi: 10.1038/s41598-022-05563-7.

V. **Perez-Lasierra ,Jose Luis**, Casajús Jose Antonio, González-Agüero Alejandro, Arbones-Mainar José Miguel, Casasnovas José Antonio, Laclaustra Martin, Moreno-Franco Belén. Cardiorespiratory fitness decreases the odds for subclinical carotid plaques in apolipoprotein e4 homozygotes. Atherosclerosis. [Sometido].

Listado de abreviaturas

AF	*Actividad física*
AFM	*Actividad física moderada*
AFMV	*Actividad física moderada – vigorosa*
AFV	*Actividad física vigorosa*
ANOVA	*Análisis de varianza de medidas repetidas*
APOE e2	*Apolipoproteína E épsilon 2*
APOE e4	*Apolipoproteína E épsilon 4*
AWHS	*Aragon Workers Health Study*
CEICA	*Comité de ética de la investigación de la Comunidad Autónoma de Aragón*
CF	*Condición física*
DCL	*Deterioro cognitivo leve*
DXA	*Densitometría dual de rayos X*
e2	*Épsilon 2*
e3	*Épsilon 3*
e4	*Épsilon 4*
ECV	*Enfermedades cardiovasculares*
EF	*Ejercicio físico*
ENT	*Enfermedad no transmisible*
FC	*Frecuencia cardiaca*
GIM-c	*Grosor intima media carotídeo*
GME	*General Motors España*
GPAQ	*Global physical activity questionnaire*
HDL-c	*Colesterol de alta densidad*
IMC	*Índice de masa corporal*
LDL-c	*Colesterol de baja densidad*
MET	*Equivalente metabólico*
MoCA	*Montreal cognitive assessment*
N	*Cantidad de muestra*
NCHS	*National Center for Health Statistics*
NDI	*National death index*
NHANES	*National Health and Nutrition Examination Survey*
NOS	*Newcastle-Ottawa scale*
OMS	*Organización Mundial de la Salud*

OR	*Odds ratio*
PHQ-9	*Patient health questionnaire*
PRISMA	*Preferred reporting items for systematic reviews and meta-analyses*
SD	*Desviación estándar*
SE	*Error estándar*
SPSS	*Statistical Package for the Social Sciences*
$VO_{2máx}$	*Consumo máximo de oxígeno*

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Resumen general

Introducción

El envejecimiento poblacional experimentado en las últimas décadas ha producido un incremento en la incidencia y prevalencia de las enfermedades no transmisibles. Este fenómeno ha generado un desajuste entre la esperanza de vida y la esperanza de vida saludable de las personas, produciendo un descenso en la calidad de vida y un aumento de la discapacidad y dependencia en la población. Existe una amplia heterogeneidad entre las características patológicas de estas enfermedades, pero todas surgen de la combinación de diferentes factores genéticos, fisiológicos, ambientales, y comportamentales.

Entre los factores modificables que pueden incidir en el riesgo de desarrollar diferentes enfermedades no transmisibles se encuentran el estilo de vida y la condición física, estando el primero determinado por los niveles de actividad física y sedentarismo, la alimentación, y el consumo de sustancias nocivas, y siendo el segundo muy dependiente del primero, pero estando a su vez modulado por factores genéticos, que no podemos modificar, y que también influyen en el riesgo de desarrollar diferentes enfermedades no transmisibles.

Por tanto, el objetivo general de esta tesis es analizar la importancia del estilo de vida en la probabilidad de presentar diferentes enfermedades no transmisibles asociadas al proceso de envejecimiento.

Metodología

La presente tesis doctoral está compuesta por 5 artículos científicos, siendo uno de ellos una revisión sistemática elaborada a partir de las indicaciones de la guía PRISMA, y siendo los otros 4 estudios originales derivados de dos proyectos diferentes, el AWHS y el NHANES. El estudio de cohortes AWHS cuenta con una muestra de 5678 personas a las que se les han evaluado diferentes determinantes del estilo de vida (actividad física,

sedentarismo, alimentación, etc.), diferentes marcadores de aterosclerosis y salud cardiovascular, y diferentes parámetros bioquímicos. El estudio NHANES proporciona diferentes datos transversales y representativos de la población estadounidense de manera periódica. A partir de ellos podemos obtener la prevalencia de diferentes enfermedades, y el grado de exposición a diferentes factores de riesgo a nivel ambiental y comportamental.

Resultados

La actividad física se asocia de forma positiva a una menor probabilidad de presentar deterioro cognitivo leve en sujetos con un riesgo genético incrementado para ello. A su vez, la actividad física también se asocia a una menor probabilidad de presentar sarcopenia, osteoporosis, osteoartritis, y depresión independientemente de otros factores de riesgo. Aunque diferentes dosis de actividad física parecen estar asociadas a mayores beneficios para cada enfermedad, ninguna de las dosis estudiadas ha resultado tener efectos nocivos o perjudiciales.

El sedentarismo se asocia a una mayor probabilidad de presentar aterosclerosis subclínica en diferentes territorios vasculares de manera independiente de otros factores de riesgo tradicionales y de los niveles de actividad física.

Una mayor condición física cardiorrespiratoria se asocia a una menor probabilidad de presentar aterosclerosis subclínica en las arterias carótidas independientemente de otros factores de riesgo en sujetos con un riesgo genético incrementado para ello.

Conclusión

El estilo de vida juega un papel fundamental en la disminución de la probabilidad de padecer diferentes enfermedades no transmisibles asociadas al proceso de envejecimiento, incluso cuando algunos sujetos presentan una mayor predisposición genética para ello.

General abstract

Introduction

The ageing population experienced in the last decades has produced an increase in the incidence and prevalence of non-communicable diseases. This phenomenon has generated an imbalance between life expectancy and healthy life expectancy, decreasing quality of life, and increasing disability and dependency. There is a wide heterogeneity between the pathological features of these diseases, but all of them are the result of different genetic, physiologic, environmental, and behavioral factors combination.

Among the modifiable factors that can affect the risk of developing different non-communicable diseases are lifestyle and fitness. The first one is determined by physical activity, sedentary behavior, diet, and by consumption of potential harmful substances. On the other hand, fitness is linked and modulated by lifestyle, but also by genetics. Genetic factors also play a role in the probability of developing non-communicable diseases, but in contrast to modifiable factors, genetic features cannot be modified.

Therefore, the general aim of this thesis is to analyze the importance of lifestyle in the probability to present different non-communicable diseases associated to the ageing process.

Methodology

This thesis is composed of 5 scientific papers, the first one is a systematic review that was carried out following the PRISMA guidelines, and the other 4 are original studies from two different projects, the AWHS and the NHANES. The AWHS cohort study was composed of 5678 participants. This project included the assessment of lifestyle factors, such as physical activity, sedentary behavior and diet, several cardiovascular health outcomes and the presence of atherosclerosis, and other biochemical parameters. The NHANES study provides on a regular basis cross-sectional representative data of the

United States population. These data allow us to obtain the prevalence of different diseases, and the exposure of participants to different environmental and behavioral risk factors.

Results

Physical activity is positively associated with lower probability of mild cognitive impairment in subjects with an increased genetic risk. Indeed, physical activity is also associated with lower probability to present sarcopenia, osteoporosis, osteoarthritis, and depression, independently of other risk factors. Although different doses of physical activity seem to be associated with more benefits for presenting each disease, none of the doses analyzed are associated with harmful effects.

Sedentarism is associated with an increased probability to present subclinical atherosclerosis in different vascular territories, independently of other traditional risk factors and physical activity levels.

A higher cardiorespiratory fitness is associated with a lower probability to present subclinical carotid atherosclerosis in persons with an increased genetic risk, independently of other potential risk factors.

Conclusions

Lifestyle plays a key role to decreasing the probability to present different non-communicable diseases associated to the ageing process, even when some persons have an increased genetic risk to develop some of these diseases.

1. Introducción

La presente Tesis Doctoral tiene como objetivo analizar la importancia del estilo de vida en relación con diferentes enfermedades no transmisibles (ENT) comúnmente asociadas al envejecimiento. Por ello, la introducción se estructurará en los siguientes cuatro apartados: 1) el envejecimiento, la esperanza de vida y la esperanza de vida saludable, 2) enfermedades asociadas al envejecimiento y a los últimos años de vida, 3) factores modificables y enfermedades no transmisibles, y 4) condicionantes genéticos: Apolipoproteína E.

1.1 El envejecimiento, la esperanza de vida, y la esperanza de vida saludable

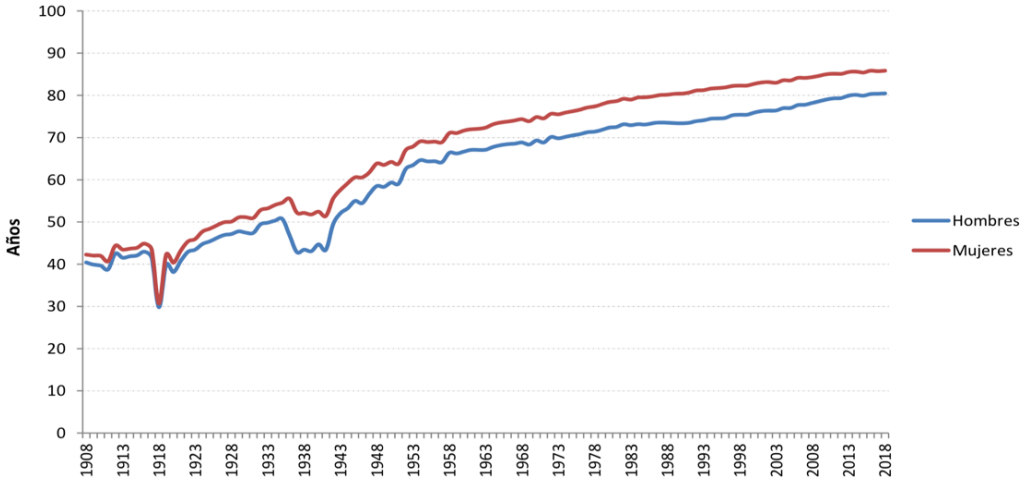
El envejecimiento es un proceso natural dependiente del tiempo y caracterizado por una serie de alteraciones funcionales en el organismo a nivel celular y molecular (Figura 1) (1). La velocidad de envejecimiento es variable entre especies, pero también entre diferentes individuos de la misma especie. Esta heterogeneidad en la velocidad de envejecimiento entre individuos de la misma especie, es producida por diferentes factores genéticos, epigenéticos, ambientales y fortuitos (1).

Figura 1. Principales marcadores del envejecimiento.

Fuente: La figura pertenece al artículo «The hallmarks of aging». (1) Licencia: 5261230806696 (Copyright Clearance Center licence number autorization)

El aumento de la esperanza de vida experimentado en las sociedades desarrolladas en las últimas décadas es un hecho (Figura 2), y sin duda un éxito fruto del desarrollo y mejora de la medicina, la salud pública y los servicios sanitarios (2,3). Concretamente, en el año 2018, los españoles tenían al nacer una esperanza de vida media de 83,2 años (85,9 años las mujeres y 80,5 años los hombres), y los españoles de 65 años una esperanza de vida de 23,5 años para las mujeres y 19,5 años para los hombres (4).

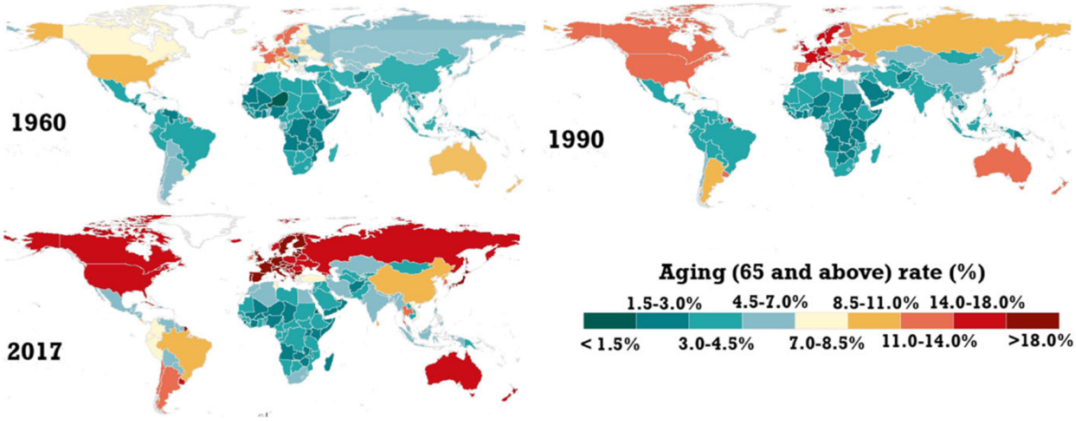
Figura 2. Esperanza de vida al nacer según el sexo desde 1908 hasta 2018.



Fuente: La figura pertenece al informe «Envejecimiento en red nº 25, un perfil de las personas mayores de España (2020)». (4) Licencia: Creative Commons (CC BY-SA).

Sin embargo, aunque pueda parecer que el aumento de la esperanza de vida por si solo supone un éxito sin efectos colaterales adversos, esto no siempre es así. El envejecimiento de la población, que incrementa exponencialmente, es actualmente una nueva realidad demográfica (Figura 3), y una fuente de preocupación para los sistemas sanitarios (5). Aunque el envejecimiento no es un proceso patológico per se, el incremento de la edad está asociado al incremento de la probabilidad de desarrollar diferentes patologías y enfermedades (6). Una vida más larga en muchos casos no es equivalente a una vida acompañada de salud y plenitud funcional. Ya lo advertía el filósofo Giacomo Leopardi a comienzos del siglo pasado, "La vejez es el mal supremo, porque nos priva de todos los placeres, dejándonos solo el apetito por ellos, y trae consigo todos los sufrimientos" (7). Y es que el aumento de la esperanza de vida experimentado en las últimas décadas (2–4), no se corresponde con el aumento de la esperanza de vida saludable (8), definida como el número de años que se puede esperar que una persona viva con completa salud (9).

Figura 3. Distribución de la ratio de envejecimiento global desde 1960 hasta 2017.



Fuente: Adaptación de la figura que pertenece al artículo «Spatiotemporal evolution of global population ageing from 1960 to 2017». (5) Licencia: Creative Commons (CC-BY 4.0)

En España concretamente, en el año 2018, el porcentaje de vida saludable a los 65 años respecto a la esperanza de vida a esa misma edad era del 59,0% para los hombres, y del 48,1% para las mujeres (4). En otros países desarrollados como Estados Unidos, la esperanza de vida para los hombres y mujeres de 65 años de edad es de 17,7 y 20,3 años respectivamente, mientras que la esperanza de vida saludable para esos mismos sujetos es de 12,9 y 14,8 años respectivamente (10). Estos años de diferencia entre la esperanza de vida y la esperanza de vida saludable están marcados en muchas ocasiones por la presencia de multimorbilidad, discapacidad funcional y dependencia, lo que supone una baja calidad de vida para el individuo, y una carga económica elevada para los sistemas de salud comunitarios.

1.2 Enfermedades asociadas al envejecimiento y a los últimos años de vida

Hace décadas, entre las principales causas de muerte se encontraban las enfermedades infecciosas, pero con el avance de la medicina, de los sistemas de salud, la mejora de la higiene, y el desarrollo de los antibióticos, la incidencia de este tipo de enfermedades ha descendido (11,12). Desde hace unos años y gracias a estos avances, se experimentó un cambio de tendencia, y actualmente las ENT ocupan el primer puesto entre las causas de muerte más comunes en los países desarrollados (13), además de ser una de las principales causas de discapacidad funcional y dependencia (14).

Según la Organización Mundial de la Salud (OMS), las ENT, también conocidas como enfermedades crónicas, tienden a ser de larga duración y resultan de la combinación de diferentes factores genéticos, fisiológicos, ambientales y comportamentales (15). Esta definición abarca una gran cantidad de enfermedades, existiendo una amplia heterogeneidad entre todas ellas, pero con la característica común de ser enfermedades no infecciosas.

La aparición de este tipo de enfermedades lleva asociado consigo, en la mayoría de las ocasiones, un descenso de la calidad de vida, un aumento en la probabilidad de desarrollar otro tipo de enfermedades, dando lugar a la aparición de comorbilidad, y un aumento en la probabilidad de fallecer. A su vez, el coste económico asociado a estas patologías es elevado, incrementándose de manera exponencial con la existencia de comorbilidad (16).

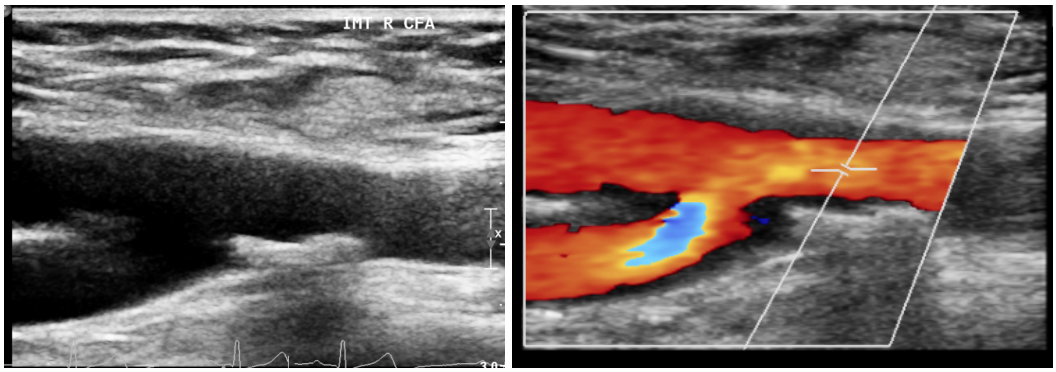
La prevalencia de este tipo de enfermedades es muy alta en los países desarrollados (17). Aunque existen muchas ENT, algunas de las más relevantes son las enfermedades cardiovasculares (ECV), las enfermedades cognitivas y trastornos mentales, las enfermedades asociadas a diferentes cambios en la composición corporal, las enfermedades musculoesqueléticas degenerativas, y diferentes tipos de neoplasias.

1.2.1 Enfermedades cardiovasculares

Las ECV son el resultado de diferentes problemas o anomalías a nivel funcional en el propio miocardio o en el sistema vascular. Actualmente son la causa de muerte dominante a nivel mundial, alcanzando un total de 18,6 millones de muertes al año en 2019 (18), lo que supone aproximadamente la mitad de todas las muertes atribuibles a las ENT (19). Entre las ECV podemos destacar la aterosclerosis, que es la principal causa de discapacidad y mortalidad en un gran número de países (19,20).

La aterosclerosis es un proceso patológico complejo en el que materiales grasos y el colesterol se van depositando y acumulando durante años dentro del lumen de arterias medianas y grandes (19). Estos depósitos se denominan placas, y hacen que la superficie interna de los vasos sanguíneos se vuelva irregular y se estreche su lumen, lo que dificulta el flujo sanguíneo a través del vaso y lo vuelve menos flexible (Figura 4). Eventualmente esas placas se pueden romper desencadenando la formación de un coagulo dentro del vaso, que en caso de progresar puede terminar produciendo un infarto agudo de miocardio o un accidente cerebrovascular (19).

Figura 4. Imagen ecográfica de una placa aterosclerótica en la arteria femoral.



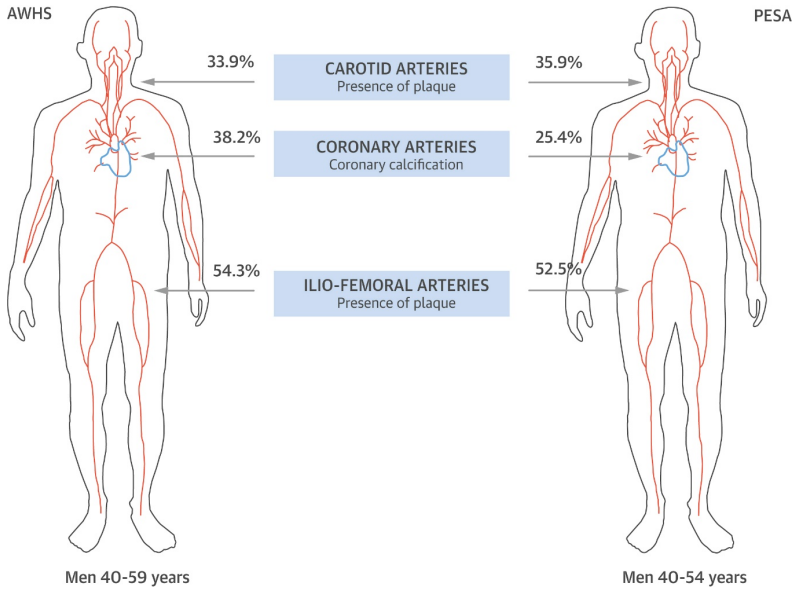
Fuente: Imagen obtenida a través del Estudio Aragon Workers' Health Study (AWHS)

Los primeros cambios funcionales y patológicos derivados de la aterosclerosis suelen aparecer en adultos jóvenes, y lentamente progresan a lo largo de la vida hasta que se manifiestan como ECV clínicas, lo que habitualmente ocurre en la quinta década de vida. En muchas ocasiones estas patologías clínicas suelen derivar en la aparición de diferentes eventos cardiacos, entre los que destacan el infarto agudo de miocardio y los accidentes cerebrovasculares, asociados ambos con una alta tasa de mortalidad y discapacidad (18).

Antes de su manifestación como ECV clínica, se generan los primeros depósitos de materiales grasos y colesterol en determinados territorios vasculares, dando lugar a la aparición de aterosclerosis subclínica, que se presenta de forma asintomática. Entre los territorios que habitualmente se ven afectados primero se encuentran las arterias carótidas, las arterias coronarias, y las arterias ilio-femorales (21,22). Esto es debido a sus características hemodinámicas, aunque la medida en que la aterosclerosis afecta a diferentes territorios es multifactorial (22). La aterosclerosis subclínica presenta una alta prevalencia poblacional (Figura 5), alcanzando una prevalencia para cualquier territorio vascular de entre un 52,7% y un 91,6% en función de la cantidad de factores de riesgo presentes (23).

Por último, cabe destacar que la presencia de ECV fomenta la comorbilidad, ya que aumenta el riesgo de padecer otro tipo de enfermedades, tanto a nivel cardiovascular (24) como a nivel cognitivo (25).

Figura 5 Prevalencia de la aterosclerosis subclínica en diferentes territorios vasculares en participantes de 2 estudios de cohortes diferentes, el AWHS (Aragon Workers' Health Study) y el PESA (Progression of Early Subclinical Atherosclerosis).



Fuente: La figura pertenece al artículo «Femoral and carotid subclinical aterosclerosis association with risk factors and coronary calcium. The AWHS study». (23) Licencia: 5264690941665 (Copyright Clearance Center licence number autorization).

1.2.2 Enfermedades cognitivas y trastornos mentales

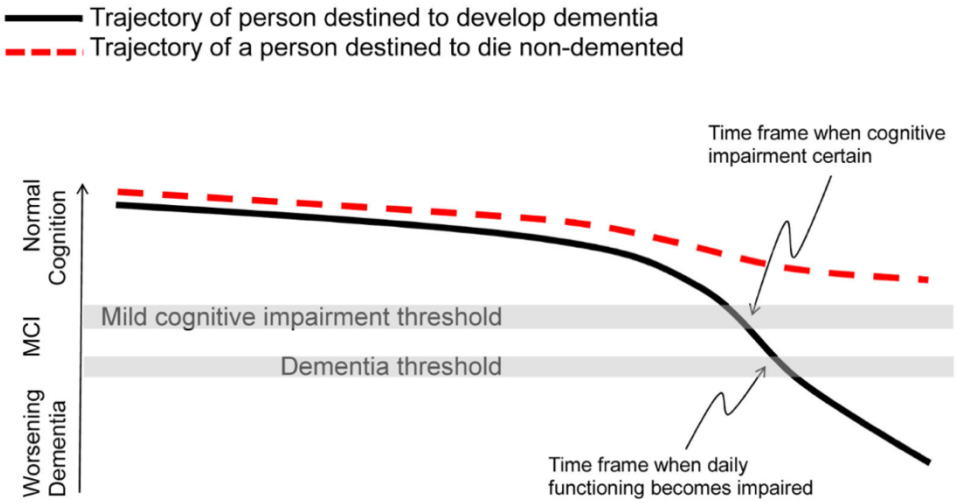
1.2.2.1 Deterioro cognitivo leve

El cerebro, al igual que el resto del cuerpo, cambia a medida que el individuo envejece, produciéndose alteraciones a nivel estructural y funcional (26). El envejecimiento del cerebro y de las neuronas, sumado al aumento de la esperanza de vida, hace que la función cognitiva se vea afectada, incrementando la probabilidad de sufrir pérdidas de memoria, y de experimentar una reducción en el desempeño de tareas cognitivas (26,27).

Por otro lado, y diferenciándolo del descenso en el rendimiento cognitivo habitual debido al envejecimiento (Figura 6), se encuentra el deterioro cognitivo leve (DCL), que se define como la evidencia objetiva de un rendimiento cognitivo más bajo de lo esperado

para la edad y nivel educativo del individuo en uno o más dominios cognitivos, pero sin interferir sustancialmente en las actividades diarias, conservando por tanto la independencia con ayudas o asistencia mínimas (28). Muchos autores han defendido la idea de que el DCL era la antesala de la progresión hacia la demencia, concretamente hacia el Alzheimer (Figura 6) (29).

Figura 6. Trayectorias de la función cognitiva durante el envejecimiento.

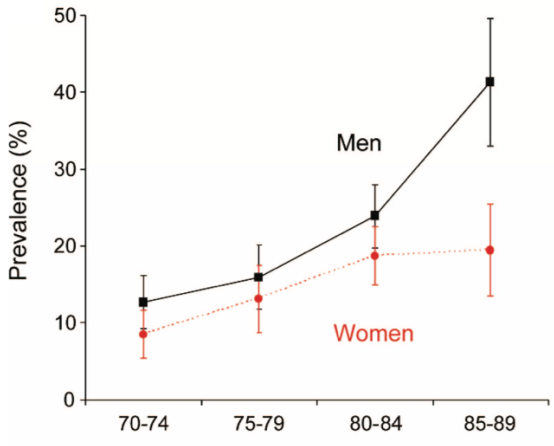


Fuente: La figura pertenece al artículo «Mild cognitive Impairment and mild dementia: a clinical perspective». (28) El eje X representa la edad, y el eje Y el deterioro cognitivo. Los umbrales de deterioro cognitivo leve y demencia se representan mediante franjas horizontales grises. Licencia: 5261261171635 (Copyright Clearance Center licence number autorization)

Sin embargo, algunos estudios muestran como tan solo entre un 5-10% de los pacientes diagnosticados con DCL, finalmente progresan hacia la demencia en un periodo de 4,5 años (30). Teniendo esto en cuenta, otros autores han considerado que el DCL puede ser interpretado como un índice de salud que merece una atención individualizada y al margen de la demencia, ya que el DCL presenta una alta prevalencia en la población mayor

(31), especialmente entre los hombres (Figura 7), y se ha asociado a una mayor probabilidad de discapacidad y a una mayor mortalidad (32–35).

Figura 7. Prevalencia del deterioro cognitivo leve en función de la edad y el sexo.

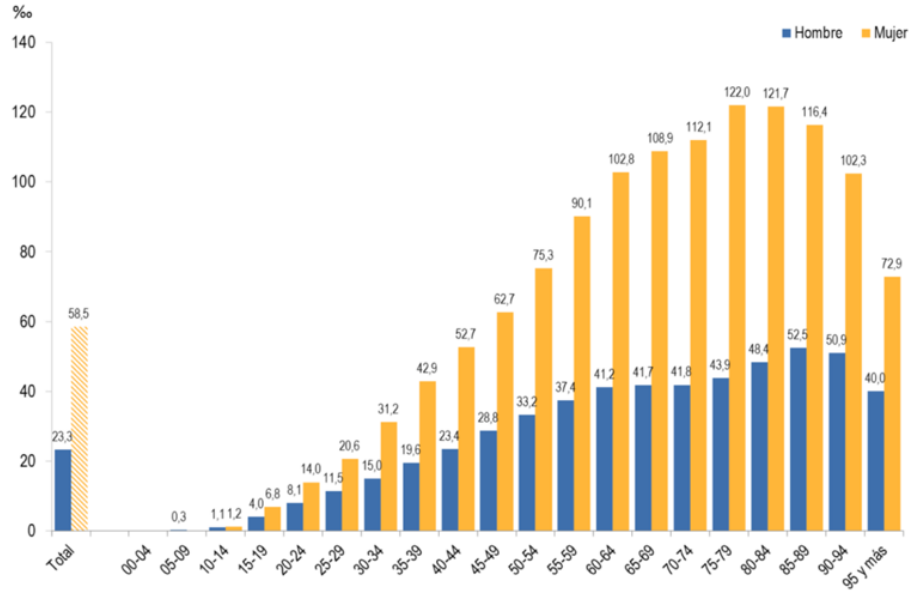


Fuente: La figura pertenece al artículo «Prevalence of mild cognitive Impairment is higher in men. The Mayo Clinic Study of Aging». (31) El eje X representa la edad. Licencia: 5264890651797 (Copyright Clearance Center licence number autorization).

1.2.2.2 Trastorno por depresión

Por otro lado, los trastornos mentales como el trastorno por depresión o el trastorno por ansiedad, han evidenciado un claro aumento en los últimos años, especialmente la ansiedad en los adultos jóvenes (36), y la depresión severa en la población mayor de 65 años (37). Concretamente en España, la prevalencia de depresión es mucho más elevada en mujeres que en hombres, y es más elevada en la población cuanto mayor es su edad, hasta alcanzar niveles máximos en torno a la población de 80 años, a partir de la cual la prevalencia vuelve a disminuir (Figura 8) (38).

Figura 8. Prevalencia de depresión en España en función de la edad y del sexo.



Fuente: La figura pertenece al informe «Salud mental en datos: prevalencia de los problemas de salud y consumo de psicofármacos y fármacos relacionados a partir de registros clínicos de atención primaria (2020)», elaborado a partir de la base de datos clínicos de atención primaria-BDCAP. El eje X representa la edad.

Se estima que la depresión afecta a más de 264 millones de personas, y que es la tercera causa de discapacidad a nivel mundial (39), aumentando además el riego de mortalidad en un 52% (40). Las personas con depresión presentan habitualmente diferentes síntomas emocionales, neurovegetativos y neurocognitivos, entre ellos bajos niveles de ánimo, anhedonia, sentimiento de inutilidad o culpa, fatiga, falta de energía, apetito y sueño disruptivos, y dificultad para pensar o concentrarse (41). Con frecuencia, estos problemas llevan a una disminución de la calidad de vida, discapacidad, ideación suicida, o incluso intentos de suicidio (42,43).

Además, cabe destacar que existe una relación bidireccional entre la depresión y las ECV, haciendo que el riesgo de incidencia de depresión sea mayor para los que presentan alguna ECV, y que el riesgo de desarrollar alguna ECV sea a su vez mayor entre los que presentan depresión (44), aumentando por tanto el riesgo de comorbilidad.

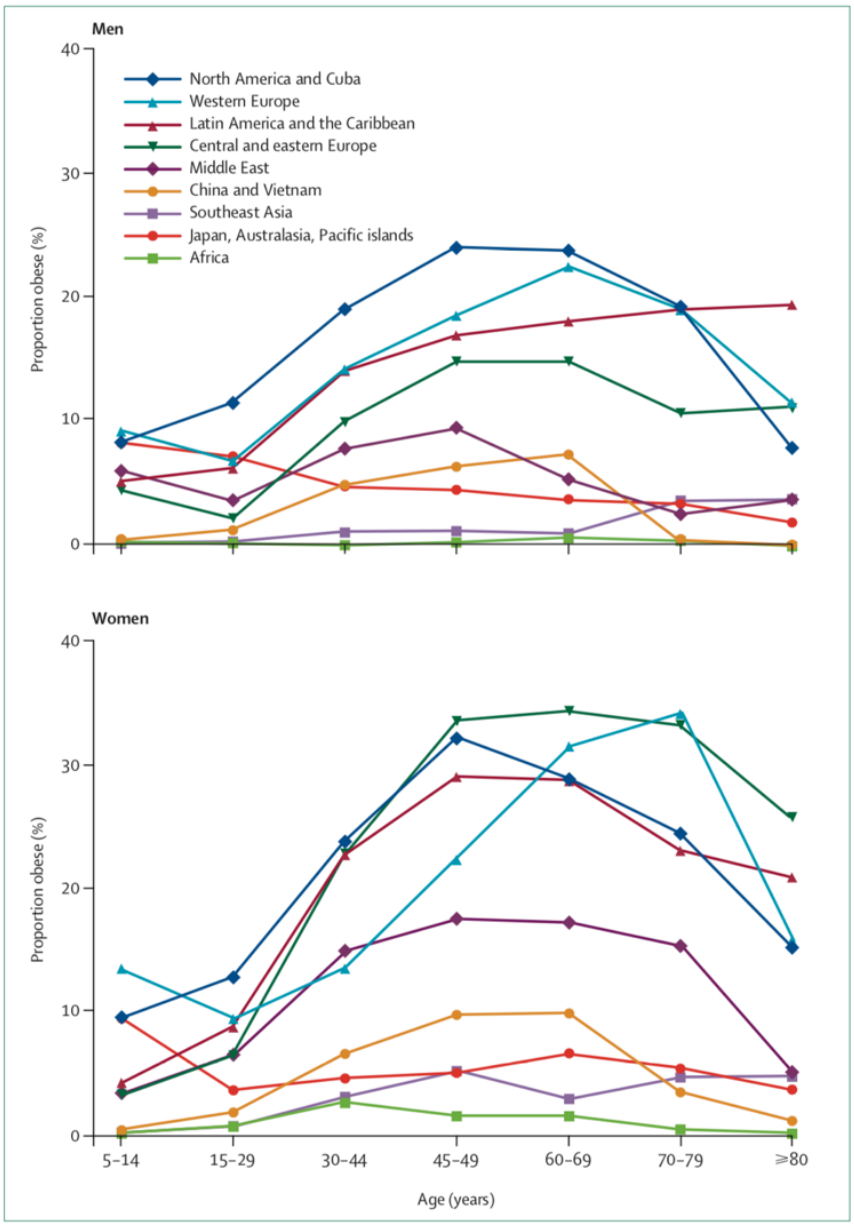
1.2.3 Enfermedades asociadas a cambios en la composición corporal

El envejecimiento está asociado a diferentes cambios en la composición corporal, que incluso pueden llegar a suceder en ausencia de variación del peso (45). Entre los cambios habituales más destacables, encontramos un aumento de la masa grasa, y un descenso de la masa ósea y de la masa muscular. Cuando esos cambios son de una magnitud determinada y tienen una serie de consecuencias concretas, dan lugar a diferentes patologías, entre las que destacamos las siguientes:

- Obesidad
- Osteoporosis
- Sarcopenia

La obesidad, catalogada por la OMS como una epidemia, ha sido tradicionalmente definida como un exceso de grasa corporal que es perjudicial para la salud (46). Habitualmente, se afirma que un individuo presenta obesidad cuando su índice de masa corporal (IMC) es ≥30 kg/m^2 (46). Desde el año 1980, la prevalencia de obesidad se ha duplicado en más de 70 países, y ha continuado creciendo en la mayoría de regiones a nivel mundial (47). A su vez, cabe destacar que existen diferencias en la prevalencia de obesidad en función de diferentes condicionantes, entre los que podemos destacar el sexo, la edad, o el país de procedencia (Figura 9) (48).

Figura 9. Prevalencia de obesidad en función del sexo, edad y región geográfica de procedencia.



Fuente: La figura pertenece al artículo «Obesity», publicado en 2005 en *The Lancet*. (48)

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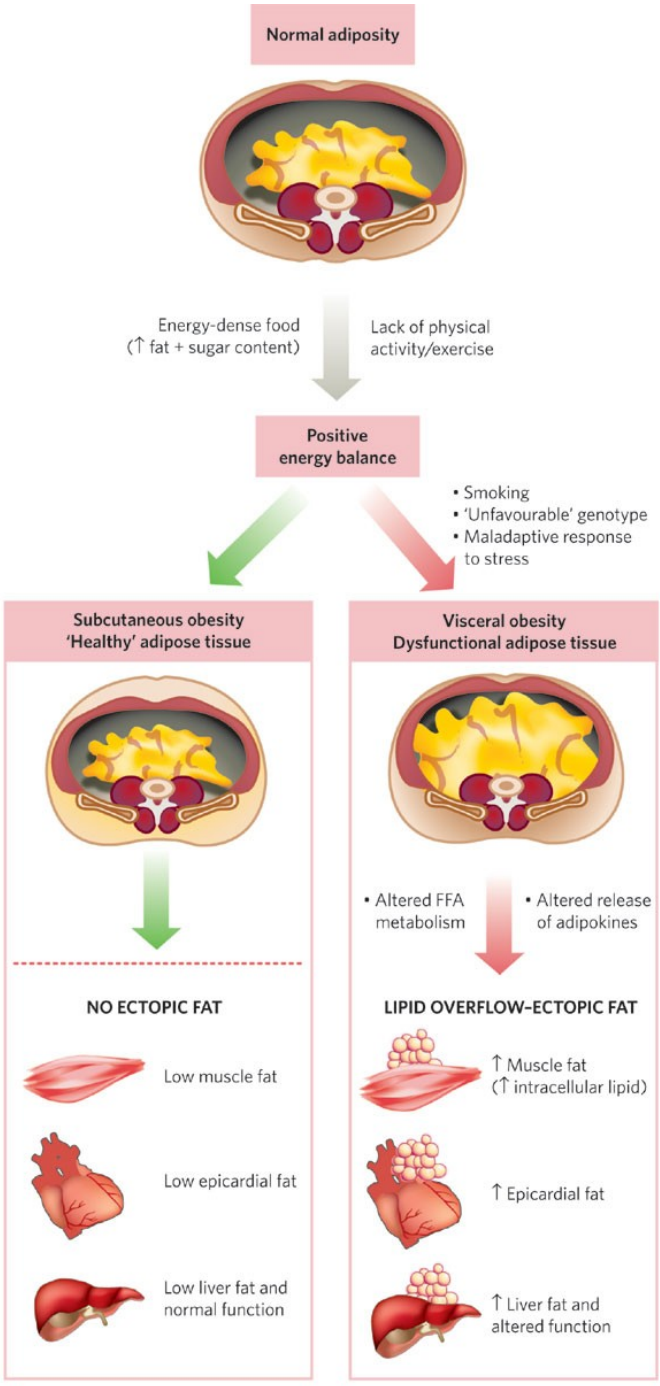
La presencia de obesidad se asocia al desarrollo de otras patologías, destacando entre otras la diabetes, hipercolesterolemia, síndrome metabólico o hipertensión, pero la obesidad también se asocia al aumento de discapacidad y mortalidad, llegándosele a atribuir unas 300.000 muertes al año en Estados Unidos (48). Quizá más importante que el

aumento de la masa grasa en sí, es el lugar en el que se localiza, ya que la acumulación de tejido adiposo en ciertas zonas, como por ejemplo las zonas viscerales, presenta mayores riesgos para la salud que la acumulación de tejido graso en otras zonas (Figura 10) (49,50).

Figura 10. Diferentes zonas de acumulación adiposa y sus consecuencias para la salud.



Fuente: La figura pertenece al artículo «Abdominal obesity and metabolic syndrome». (50)

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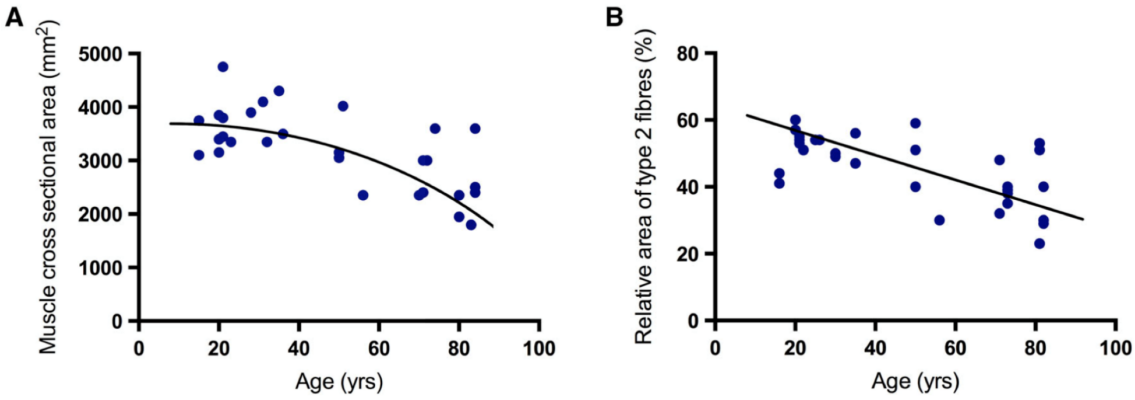
La osteoporosis es una enfermedad esquelético-degenerativa que afecta tanto a la cantidad como a la calidad del hueso, ya que se caracteriza por la disminución de la densidad mineral ósea y del contenido mineral óseo, y por un deterioro en la microarquitectura del tejido óseo, haciéndolo más vulnerable a posibles fracturas por sobrecarga (51). La prevalencia de la osteoporosis es más elevada a medida que envejecemos, y más elevada en mujeres que en hombres, especialmente tras la menopausia (51,52). Aproximadamente un 10% de las mujeres de 60 años padece osteoporosis, llegando a aumentar el porcentaje hasta el 40% a los 80 años e incluso al 66% entre las mujeres de 90 años (52). La mayor complicación de la osteoporosis son las fracturas, que pueden ocurrir en cualquier zona ósea, siendo las más comunes el cuello del fémur y la zona vertebral (52).

La sarcopenia es una enfermedad progresiva caracterizada por la disminución de la masa muscular esquelética y los niveles de fuerza, que provoca un deterioro de la capacidad funcional (53). Varios estudios han demostrado que, debido al envejecimiento, la síntesis proteica y el anabolismo muscular decrecen, provocando una disminución tanto en el número como en el tamaño de las fibras musculares, especialmente en las fibras tipo II, y afectando a su capacidad contráctil (54,55) (Figura 11). La sarcopenia, que afecta habitualmente a la población mayor, alcanza una prevalencia de entre un 1-30% entre la población mayor comunitaria, y de entre un 14-68% entre la población mayor institucionalizada (56).

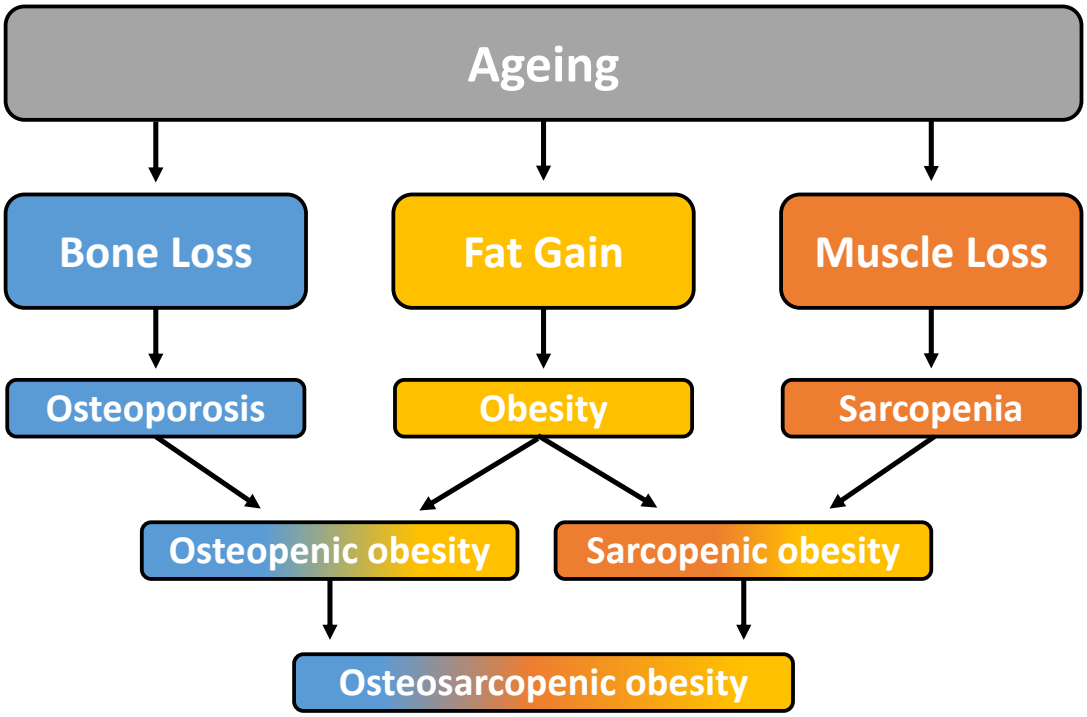
Figura 11. Pérdida de masa muscular y descenso de fibras tipo II a lo largo de la vida.



Fuente: La figura pertenece al artículo «Live strong and prosper: the importance of skeletal muscle strength for healthy ageing». (57) Licencia: Creative Commons (CC-BY 4.0).

El desarrollo de obesidad, osteoporosis y sarcopenia depende de diferentes factores genéticos, ambientales y comportamentales, pero sin duda el envejecimiento aumenta la probabilidad de desarrollar estas patologías (48,52,55). Estas enfermedades están asociadas a diferentes consecuencias negativas para la salud, entre las que podemos destacar la pérdida de capacidad funcional, fragilidad, fracturas, dolor, caídas, e incluso un aumento en la probabilidad de morir (6,51–53,58). A su vez, es común encontrar casos en los que un mismo individuo presenta la coexistencia de dos o más de estas patologías, dando lugar a la comorbilidad. Diferentes autores ya han descrito la coexistencia de estas enfermedades acuñando nuevos términos (Figura 12) y describiendo sus principales consecuencias (58,59).

Figura 12. Nuevos términos para la coexistencia de diferentes patologías asociadas a cambios en la composición corporal.

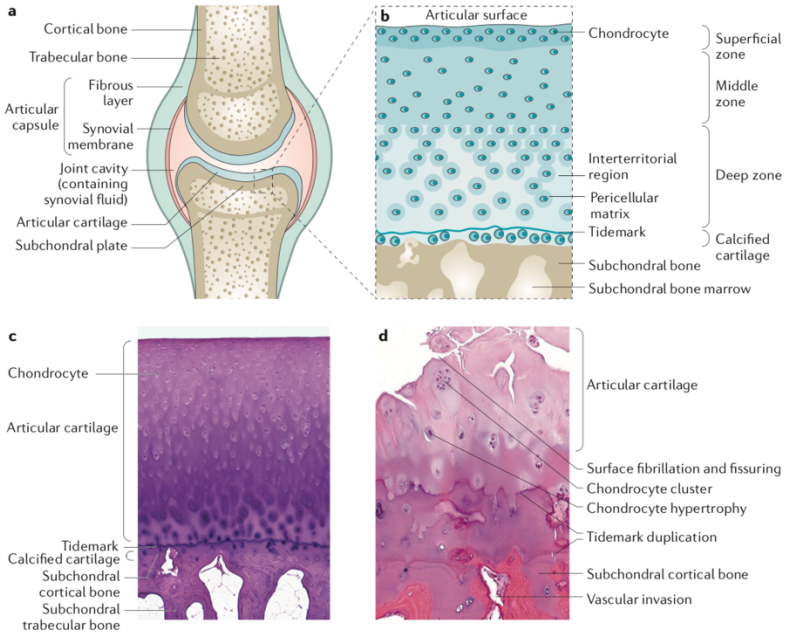


Fuente: Elaboración propia

1.2.4 Enfermedades musculoesqueléticas degenerativas

Entre las enfermedades musculoesqueléticas degenerativas más comunes asociadas al envejecimiento se encuentra la osteoartritis, caracterizada por la presencia de alteraciones en el cartílago articular, el hueso subcondral, los ligamentos, y la cápsula y membrana sinoviales, que finalmente conducen al fallo articular (60). La osteoartritis se desarrolla lentamente, y afecta tanto a articulaciones pequeñas, como a articulaciones grandes, como pueden ser la de la rodilla o la cadera (60,61). Entre los daños estructurales más comunes se encuentra la pérdida de cartílago articular, la presencia de osteofitos, cambios en el hueso subcondral, y alteraciones en los meniscos (Figura 13).

Figura 13. Articulación de la rodilla saludable y con presencia de osteoartritis.



Fuente: La figura pertenece al artículo «Osteoarthritis». (60) (A) Articulación que une dos huesos adyacentes cubiertos por una capa de cartílago articular mediante una capsula de tejido conectivo revestida por una membrana sinovial formada por macrófagos y fibroblastos. (B, C) Corte transversal de la superficie articular esquemático (b) e histológico (c). (D) Corte transversal histológico de una superficie articular que presenta un estado avanzado de osteoartritis. Licencia: 5264711459403 (Copyright Clearance Center licence number autorization).

Esta patología, suele ir acompañada de una mala y reducida funcionalidad articular al realizar incluso las tareas cotidianas más simples y básicas como vestirse o andar, y también suele producir rigidez y dolor articular (60,62). Como consecuencia de todo ello, la osteoartritis se relaciona con una baja calidad de vida, y con una mayor probabilidad de discapacidad funcional y muerte prematura por cualquier tipo de causa (60,62).

La osteoartritis, que es el tipo más común de artritis, alcanza una prevalencia entre la población mayor de 65 años de aproximadamente un 60% para las articulaciones de la mano, un 33% para las de las rodillas, y un 5% para las de las caderas, siendo más común en mujeres que en hombres (60). La patogénesis de la osteoartritis es multifactorial, pero

entre los factores de riesgo más importantes para su desarrollo se encuentran la edad y la obesidad (60,62,63).

1.3 Factores modificables y enfermedades no transmisibles

1.3.1 Estilo de vida

El estilo de vida es una combinación de factores comportamentales que modifican e influyen en la probabilidad de desarrollar la mayoría de las ENT (52,55,60,64–73). Entre los factores más relevantes que determinan el estilo de vida de una persona podemos encontrar la actividad física (AF), el sedentarismo, la alimentación, o el consumo de diferentes tipos de sustancias como el alcohol o el tabaco.

Aproximadamente un 80% de las ENT se pueden prevenir evitando el tabaquismo, manteniendo una dieta saludable y un peso equilibrado, practicando AF, y controlando los niveles de hipertensión, diabetes y colesterol (20).

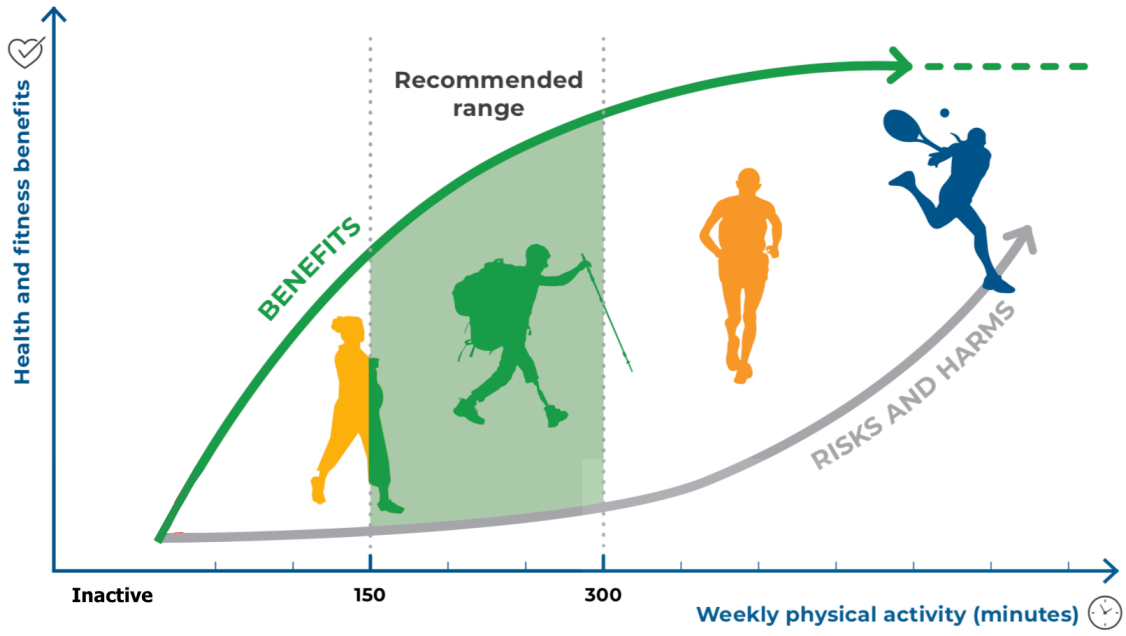
1.3.1.1 Actividad física

La AF es definida como cualquier movimiento producido por los músculos esqueléticos que requiera un consumo de energía (74). El consumo de energía asociado a cada AF dependerá principalmente del tipo de actividad, de su duración, y de su intensidad.

Actualmente, existen una serie de recomendaciones de AF y sedentarismo a nivel mundial establecidas por la OMS en función de la edad y otros condicionantes, a través de las cuales, podemos obtener multitud de beneficios para la salud (75). Concretamente, las recomendaciones mundiales de AF aeróbica establecidas para personas mayores de 18 años, aconsejan realizar a la semana al menos entre 150-300 min de actividad física moderada (AFM), o al menos entre 75-150 min de actividad física vigorosa (AFV), o una combinación equivalente de actividad física moderada—vigorosa (AFMV) (75).

En base al cumplimiento o no de las recomendaciones de AF de la OMS, podemos diferenciar a los sujetos que son activos de los que son inactivos. Cualquier nivel de AF es beneficioso para la salud, pero tal y como indican las recomendaciones, los sujetos activos obtendrán mayores beneficios que los que no alcancen el nivel de AF recomendado, manteniendo además unos riesgos asociados a la práctica de AF mínimos si nos mantenemos en el rango recomendado (Figura 14) (75).

Figura 14. Relación entre los niveles de actividad física y los beneficios para la salud.

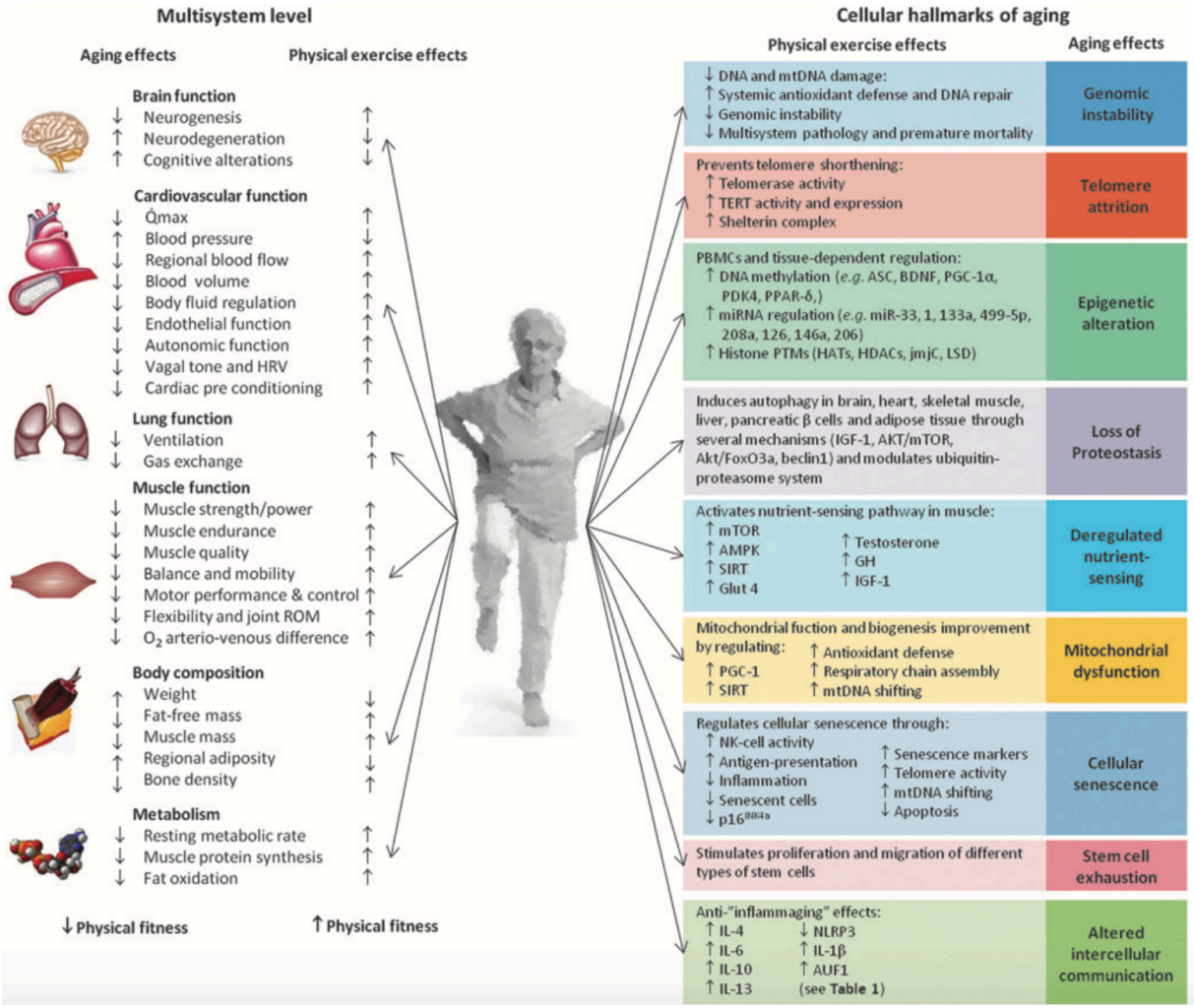


Fuente: Adaptación de la figura que pertenece a la guía «WHO Guidelines on physical activity and sedentary behaviour (2020)». Licencia: Creative Commons (CC BY-NC-SA 3.0 IGO).

Así pues, la AF es un factor clave en el estilo de vida de las personas, ya que se ha demostrado su papel protector frente a las ECV, diferentes enfermedades o trastornos mentales, y varias enfermedades musculoesqueléticas entre otras (65–68). A su vez, existe evidencia de que la práctica de ejercicio físico (EF), definido como la AF planificada, estructurada, y repetitiva que tiene un objetivo concreto (74), es eficaz a la hora de

disminuir los principales marcadores del envejecimiento a nivel celular y molecular (Figura 15) (76), siendo también eficaz en el tratamiento de hasta 26 ENT, entre las que se encuentran la depresión, la ansiedad, la demencia, la obesidad, la diabetes, el síndrome metabólico, la hipertensión, la osteoartritis, la osteoporosis y el cáncer (77).

Figura 15. Resumen de los principales efectos antienvejecimiento del ejercicio físico regular ante los efectos del envejecimiento multisistémicos.



Fuente: La figura pertenece al artículo «Exercise attenuates the major hallmarks of ageing». (76)

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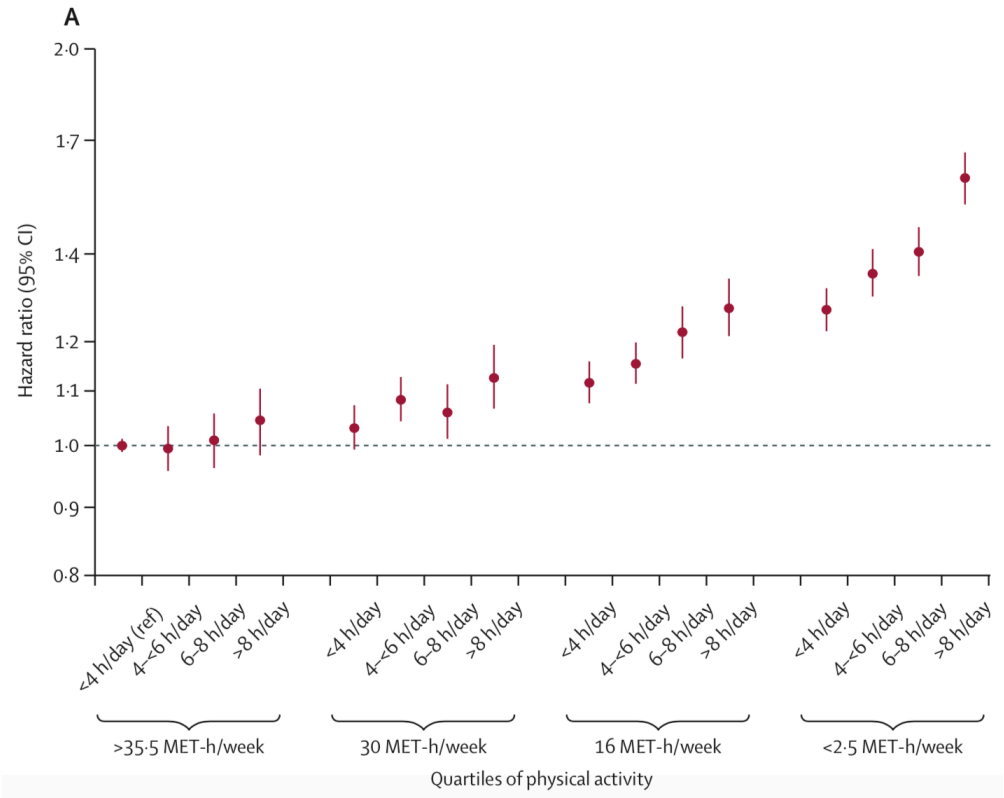
A pesar de la eficacia demostrada de la práctica de AF para mejorar la salud, aproximadamente un 31,1% de los adultos a nivel mundial, y un 36,2% a nivel europeo son inactivos (78,79). Desde hace unos años, la inactividad física se ha catalogado de pandemia, dado que es una de las principales causas de riesgo de muerte a nivel mundial (80).

1.3.1.2 Sedentarismo

La investigación sobre las consecuencias del sedentarismo para la salud ha aumentado exponencialmente en los últimos años, proliferando multitud de definiciones del mismo concepto que han creado cierta confusión (81). Según los últimos acuerdos alcanzados por el Sedentary Behavior Research Network, el comportamiento sedentario se define como cualquier comportamiento durante el día caracterizado por un gasto energético ≤1,5 equivalentes metabólicos (MET), mientras se está sentado, reclinado, o tumbado (81).

Es importante no confundir el ser sedentario, que implica pasar mucho tiempo teniendo un comportamiento sedentario, con el ser inactivo, que implica no cumplir las recomendaciones mundiales de AF de la OMS. En los últimos años, muchos estudios han evidenciado el efecto negativo para la salud que tiene el sedentarismo independientemente de los niveles de AF alcanzados (72), aunque los estudios más recientes relacionados con el sedentarismo, los niveles de AF, y el riesgo de mortalidad por diferentes causas, han evidenciado como el sedentarismo tiene una baja influencia sobre el riesgo mortalidad en sujetos muy activos (Figura 16) (82–84).

Figura 16. Riesgo de mortalidad por cualquier tipo de causa según el nivel de actividad física y las horas que pasamos sentados al día.



Fuente: La gráfica pertenece al artículo «Does physical activity attenuate, or even eliminate, the detrimental association of Sitting time with mortality? A harmonized meta-analysis of data from more than 1 million men and women». (82) Licencia: 5264720833413 (Copyright Clearance Center licence number autorization).

Aunque en algunos casos altos niveles de AF (60-75 min/día de AFM) puedan paliar los efectos negativos que tiene el sedentarismo sobre algunos indicadores de salud (82–84), el sedentarismo se ha convertido sin duda en un factor de riesgo independiente de los niveles de AF para muchas ENT, ya que genera unas adaptaciones fisiológicas negativas propias y diferentes de las que supone la inactividad (85,86).

1.3.1.3 Alimentación

El patrón alimentario, que representa la totalidad de lo que un individuo come y bebe habitualmente, juega un papel fundamental en la salud de las personas, influyendo en la probabilidad de desarrollar o prevenir ciertas ENT (87).

En las últimas décadas, la investigación en el campo de la nutrición se ha centrado en estudiar cómo influyen diferentes patrones alimentarios en la salud, en lugar de estudiar el efecto que tienen ciertos nutrientes o alimentos de manera individual, ya que pueden existir ciertos efectos sinérgicos o antagónicos entre los diferentes alimentos que componen la dieta (88).

Varios de estos patrones se han relacionado con beneficios para la salud (89,90), pero entre ellos, uno de los más estudiados y que mayores beneficios proporciona es el patrón de la dieta mediterránea, que se ha asociado a un descenso de la mortalidad y multimorbilidad que generan las ENT asociadas al envejecimiento (91). Concretamente, la adherencia a la dieta mediterránea se ha asociado con una menor probabilidad de ECV, diabetes, obesidad, síndrome metabólico, algunos tipos de cáncer, depresión, deterioro cognitivo, demencia, e incluso un menor riesgo de fractura ósea (90–94).

1.3.1.4 Consumo de sustancias

El consumo de ciertas sustancias nocivas para la salud como el alcohol o el tabaco, son conductas que podemos modificar, y que sin duda inciden en la probabilidad de desarrollar diferentes tipos de ENT (95–97).

Aunque algunos estudios previos hayan defendido que el consumo de cierta cantidad de algunas bebidas alcohólicas pueda ser beneficioso para prevenir ciertas enfermedades o incluso la mortalidad por cualquier tipo de causa en algunas poblaciones (98), el conjunto de la evidencia actual permite afirmar que desde una visión global de la salud, el consumo de cualquier cantidad de alcohol es perjudicial (96), por lo que el balance

riesgo beneficio del consumo de alcohol indica que la única cantidad de alcohol segura para la salud es cero.

Por otro lado, el consumo de tabaco, independientemente de la forma de consumo, ya sea a través de cigarrillos, cigarrillos electrónicos o pipas de agua (cachimbas), está asociado a una mayor probabilidad de desarrollar diferentes tipos de cáncer, enfermedades y eventos cardiovasculares, enfermedades respiratorias, y en definitiva a un aumento en la probabilidad de morir (97,99).

1.3.2 Condición física

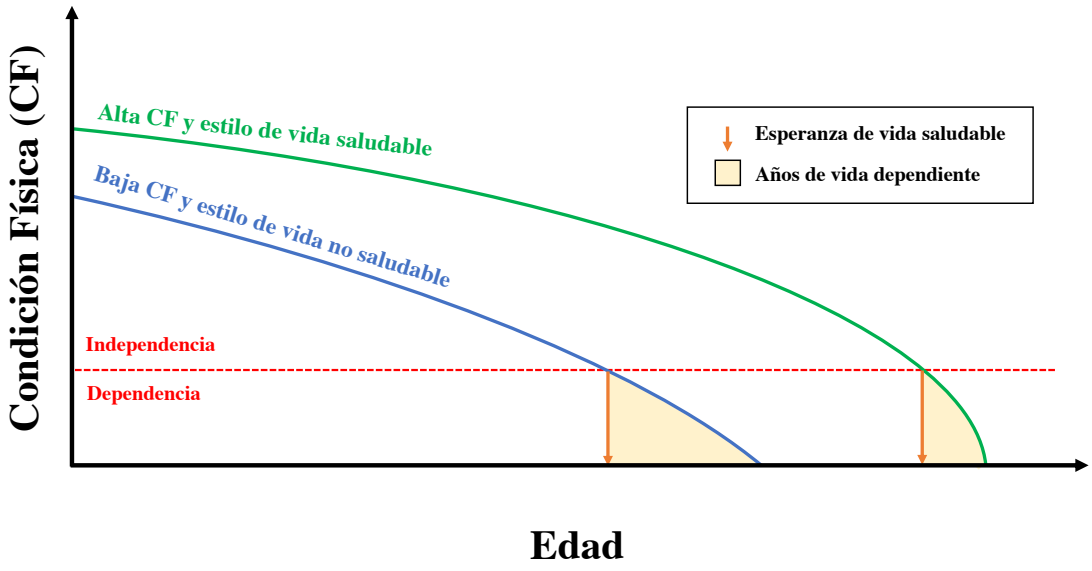
La condición física (CF) se define como la capacidad de llevar a término las actividades de la vida diaria con vigor y diligencia, sin cansancio indebido, y con energía suficiente para disfrutar de las actividades del tiempo libre y para afrontar las emergencias imprevistas que se presenten (74). Teniendo en cuenta esta definición, podemos afirmar que la CF influye en gran medida en la capacidad funcional, grado de dependencia y calidad de vida de las personas.

Alcanzar y mantener una buena CF debe ser un objetivo prioritario a lo largo de la vida, pero aún más durante el envejecimiento. Tener una buena CF nos garantiza una mayor calidad de vida (100), un menor riesgo de desarrollar ENT (101), y en general, un menor riesgo de dependencia, discapacidad y muerte (101–103).

No obstante, a medida que envejecemos, los diferentes componentes involucrados en definir la CF de una persona (resistencia cardiorrespiratoria, fuerza muscular, velocidad, potencia, flexibilidad, equilibrio, composición corporal, etc.) se ven alterados, produciendo un descenso en el nivel de CF (102,104). La magnitud y rapidez de la disminución de CF debida al envejecimiento dependerá de diferentes factores, entre los que se encuentran el nivel máximo de CF alcanzado a lo largo de la vida, y el estilo de vida que adoptemos mientras envejecemos (105) (Figura 17). La CF está directamente asociada a todos los

factores que definen el estilo de vida, y aunque diferentes aspectos genéticos también influyen en tener una mejor o peor CF (106), son los diferentes aspectos modificables (AF, alimentación, y sedentarismo principalmente) los que van a cobrar un mayor peso, siendo además sobre los que podemos incidir.

Figura 17. Cambios en la condición física y sus consecuencias durante el envejecimiento en función del nivel de condición física máximo alcanzado y el estilo de vida adoptado durante el proceso de envejecimiento.



Fuente: Elaboración propia

1.4 Condicionantes genéticos: Apolipoproteína E

Existen diferentes factores inherentes a la persona sobre los que no tenemos control ni posibilidad de variación o modificación, entre los que se encuentran los condicionantes genéticos, que entre otras cosas, pueden predisponer en mayor o menor medida al desarrollo de ENT (107).

Entre los condicionantes genéticos más estudiados en la literatura relacionados con las ENT, se encuentra la apolipoproteína E, que es polimórfica y presenta tres alelos en humanos: épsilon 3 (*e3*), épsilon 2 (*e2*), y épsilon 4 (*e4*). La variante *e3* es el alelo más frecuente (~77,1% en la población general), mientras que las variantes *e2* y *e4* son menos comunes, con unas tasas de ocurrencia aproximadas de 7,8% y 15,1% respectivamente (108). De la combinación de estos tres alelos, surgen seis genotipos diferentes, tres homocigotos (*e2e2*, *e3e3*, y *e4e4*), y tres heterocigotos (*e2e3*, *e2e4*, y *e3e4*).

La apolipoproteína E épsilon 4 (*APOE e4*), se ha asociado a diferentes ECV (109,110), principalmente porque su presencia está asociada con la hipertensión, unos niveles de colesterol de baja densidad (LDL-c) y triglicéridos plasmáticos incrementados, y también con un grosor íntima-media carotídeo (GIM-c) mayor (111–113). Además, la presencia de la *APOE e4* también se ha asociado con varias enfermedades cognitivas, entre ellas el DCL, la demencia, y la enfermedad de Alzheimer (109,114), siendo los homocigotos *e4e4* los que mayor riesgo presentan, lo que sugiere que existe un efecto dosis-respuesta (114,115). Sin embargo, en algunos grupos de población no se dan estas asociaciones (116–118), por lo que se ha sugerido en la literatura que diferentes factores ambientales o comportamentales como la AF, pueden afectar a la expresión de este factor de riesgo genético minimizando sus consecuencias patogénicas en ciertas ENT (119–124).

Por otro lado, la presencia de la apolipoproteína E épsilon 2 (*APOE e2*), se ha asociado a unos niveles más bajos de LDL-c, y por tanto a la protección frente a ciertos

tipos de ECV (111,125). Incluso algunos estudios han evidenciado la asociación entre la presencia de la *APOE e2* y un menor riesgo de Alzheimer proponiendo cual sería el mecanismo neuroprotector implicado (126). Por último, también cabe destacar su asociación a una menor fertilidad, pero a una mayor longevidad (125,127).

2. Hipótesis

Los diferentes componentes que definen el estilo de vida son clave e influyen en la probabilidad de presentar diferentes enfermedades no transmisibles durante el proceso de envejecimiento.

Un estilo de vida saludable y una buena condición física proporcionarán beneficios para la salud incluso en individuos que presenten un riesgo genético incrementado para desarrollar diferentes enfermedades no transmisibles.

2. Hypotheses

Lifestyle is a key factor in the probability to present different non-communicable diseases during the ageing process.

Healthy lifestyle and high fitness have benefits in health outcomes even in persons with increased genetic risk of developing different non-communicable diseases.

3. Objetivos

El *objetivo general* de la presente Tesis Doctoral es analizar la importancia del estilo de vida en la probabilidad de presentar diferentes enfermedades no transmisibles asociadas al proceso de envejecimiento.

Los *objetivos específicos* enmarcados en cada uno de los cinco artículos que componen esta Tesis Doctoral son:

Artículo I. (1) Analizar y sintetizar la evidencia científica relacionada con los niveles de actividad física y el riesgo de deterioro cognitivo leve en portadores sanos del genotipo *APOE e4*.

Artículo I. (2) Revisar la literatura que investiga si el efecto de la actividad física en estos sujetos está directamente asociado a su cantidad e intensidad.

Artículo II. Examinar la asociación entre el tiempo sentado y la presencia de placas de ateroma carotideas y femorales en adultos varones.

Artículo III. Analizar la asociación de diferentes niveles de actividad física con la prevalencia de sarcopenia, osteoporosis y osteoartritis en la población americana no institucionalizada de 50 años o más.

Artículo IV. (1) Analizar la asociación de cumplir con las recomendaciones mundiales de actividad física aeróbica y la depresión en la población americana no institucionalizada de 50 años o más.

Artículo IV. (2) Analizar el riesgo de mortalidad por cualquier tipo de causa que tiene la asociación conjunta de actividad física y depresión en esa población.

Artículo V. (1) Analizar la asociación de diferentes genotipos *APOE* con la presencia de aterosclerosis en las arterias carótidas.

Artículo V. (2) Analizar la asociación del nivel de condición física cardiorrespiratorio y la presencia de aterosclerosis en las arterias carótidas en portadores del genotipo *APOE e4e4*.

3. Aims

The *general aim* of the present Thesis is to analyze the importance of lifestyle in the probability of having different non-communicable diseases associated to the ageing process.

The *specific aims* of each of the five articles that compose this Thesis are:

Manuscript I. (1) To analyze and synthetize the scientific evidence related to physical activity levels and cognitive decline risk in cognitively healthy *APOE e4* subjects.

Manuscript I. (2) To review the literature that investigates whether the effect of physical activity in these subjects is directly associated with amount and intensity.

Manuscript II. To examine the association of sitting time with the presence of carotid and femoral atherosclerosis plaques on male adults.

Manuscript III. To analyze the association of physical activity with the prevalence of sarcopenia, osteoporosis, and osteoarthritis in non-institutionalized Americans over 50 years old.

Manuscript IV. (1) To analyze the association of meeting the aerobic physical activity recommendations and depression in non-institutionalized Americans over 50 years old.

Manuscript IV. (2) To analyze the all-cause mortality risk of the joint association of physical activity and depression in non-institutionalized Americans over 50 years old.

Manuscript V. (1) To analyze the association of *APOE e4* carrier status with carotid atherosclerosis.

Manuscript V. (2) To analyze the association of cardiorespiratory fitness with carotid atherosclerosis in *APOE e4e4* carriers.

4. Material y métodos

En este apartado se detallarán los procedimientos generales utilizados para obtener los resultados que dan lugar a la presente Tesis Doctoral. Esta consta de un artículo de tipo revisión sistemática y de cuatro artículos originales derivados de diferentes proyectos y estudios de investigación. La metodología concreta de cada estudio individual puede consultarse en el manuscrito correspondiente en la sección de *Resultados y Discusión* de este documento.

4.1 Revisión sistemática

La revisión sistemática que forma parte de la presente Tesis Doctoral fue elaborada siguiendo las indicaciones detalladas en la guía PRISMA (*Preferred reporting ítems for systematic reviews and meta-analyses*) (128).

El protocolo de revisión fue establecido de manera previa al comienzo del proceso de revisión de la literatura científica.

4.1.1 Fuentes de datos y estrategia de búsqueda

Para la elaboración del estudio se revisaron sistemáticamente las bases de datos de PubMed, SportDiscus, Cochrane Library, y Web of Science, con el objetivo de recopilar todas las investigaciones de interés publicadas hasta el 13 de abril del 2021.

La estrategia de búsqueda utilizada en las diferentes bases de datos se realizó a través de la combinación de diferentes términos indexados y de texto libre:

- PubMed (términos indexados): “Exercise” [Mesh] AND “Cognitive Dysfunction” [Mesh] AND “Apolipoprotein E4” [Mesh].
- PubMed, SportDiscus, Cochrane Library, y Web of Science (texto libre): (“Physical Activity” OR “Exercise”) AND (“Memory impairment” OR “Age-associated

memory impairment" OR "Late-life forgetfulness" OR "age-related cognitive decline" OR "Mild Cognitive Impairment" OR "Cognitive Decline" OR "Cognitive Dysfunction") AND ("Apolipoprotein E4" OR "Apoe4" OR "Apo E4" OR "Apoe 4" OR "Apoe epsilon 4" OR "Apolipoprotein E-4" OR "Apo E-4" OR "Apo E 4" OR "Apolipoprotein E epsilon4").

4.1.2. Criterios de inclusión y de exclusión

Para elaborar la revisión solo se recopilaron estudios originales, excluyendo todo tipo de informes de simposios, cartas al editor, resúmenes de conferencias, libros, opiniones de expertos y revisiones de cualquier tipo.

Los criterios de inclusión utilizados fueron que los estudios trataran el tema de interés de la revisión, fueran estudios de diseño longitudinal, utilizaran a humanos como participantes, estuvieran publicados en inglés, y que la muestra que utilizaran estuviera formada por personas sin lesiones cerebrales o diagnosticadas con algún tipo de trastorno mental en el momento de comenzar el estudio.

Por otro lado, los estudios fueron excluidos si no proporcionaban información de cómo se había evaluado la función cognitiva o la AF, si evaluaban la AF conjuntamente con el resto de actividades llevadas a cabo en el tiempo libre (incluyendo actividades sedentarias), o si utilizaban como variable resultado la demencia y no el DCL. Los estudios que no evaluaron específicamente el impacto de la AF en el DCL en portadores del genotipo *APOE e4* y aquellos relacionados con la experimentación animal fueron asimismo excluidos.

4.1.3. Evaluación del riesgo de sesgo

Se evaluó el riesgo de sesgo de los estudios incluidos a través de diferentes herramientas en función del diseño que presentaba cada investigación. Se utilizó la

Newcastle-Ottawa Scale (NOS) (129) para evaluar los estudios de cohortes, y la escala PEDro (130) para evaluar los ensayos controlados aleatorizados.

La NOS utiliza un sistema de puntos para evaluar la calidad de los estudios basándose en varios aspectos de tres elementos clave: la selección de los participantes, la comparabilidad de los grupos de estudio, y las variables de interés. La puntuación más alta que puede obtener un estudio es de 9 puntos, y la más baja de 0. Por otro lado, la escala PEDro utiliza once criterios para evaluar la calidad metodológica de los estudios del cero al once basándose en tres aspectos: la validez interna, la interpretabilidad, y la validez externa o aplicabilidad.

En base a las puntuaciones obtenidas por cada estudio, a los evaluados a través de la NOS se los denominó como de calidad baja, moderada y alta para las puntuaciones de 0-3, 4-6 y 7-9 respectivamente, mientras que a los estudios evaluados a través de la escala PEDro, se los denominó de calidad baja, moderada y alta cuando obtuvieron las puntuaciones de 0-4, 5-8, y 9-11 respectivamente.

4.2 Proyecto AWHS y subproyecto APOE

4.2.1 Muestra y diseño del proyecto

El proyecto AWHS es un estudio longitudinal de cohorte que comenzó en febrero de 2009 con el propósito de evaluar las diferentes trayectorias de los factores de riesgo cardiovascular tradicionales y emergentes, y su asociación con las anomalías metabólicas y la aterosclerosis subclínica en la población española de mediana edad y libre de cualquier ECV clínica (131).

La cohorte está compuesta por 5678 trabajadores de una fábrica de automoción ubicada en Figueruelas (Zaragoza), y aunque el proyecto AWHS abarca muchas tomas de diferentes datos a lo largo del tiempo (Figura 18), para el desarrollo de esta Tesis Doctoral,

se han utilizado datos de 2646 participantes de entre 39 y 59 años pertenecientes a la cohorte y que fueron invitados y accedieron entre 2011 y 2014 a completar una serie de exploraciones adicionales relacionadas con la evaluación de la presencia de aterosclerosis subclínica, y cuestionarios sobre estilos de vida.

Figura 18. Cronograma del proyecto AWHS.

ACTIVITY	2009 / 10	2011	2012	2013	2014	2015	2016	2017	2018	2019
Enrollment and informed consent	X	*	*	*	*	*	*	*	*	*
Clinical exam	X	X	X	X	X	X	X	X	X	X
Laboratory analyses	X	X	X	X	X	X	X	X	X	X
Biobanking	X	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3
DNA extraction	X									
Ancillary questionnaires		1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3
Carotid, aortic, iliac and femoral ultrasound and ABI index		1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3	1/3
Calcium coronary scoring		1/3	1/3	1/3				1/3	1/3	1/3
Follow-up for clinical events	X	X	X	X	X	X	X	X	X	X

Fuente: La figura pertenece al artículo «Aragon workers' health Study – design and cohort description». (131) Licencia: Creative Commons (CC BY).

Por último, a finales del año 2017 se elaboró un subproyecto cuyo objetivo era relacionar el estilo de vida y la CF con la salud tanto a nivel cardiovascular como mental en función de la presencia de diferentes genotipos *APOE*. Para ello se localizó a todos los participantes del proyecto AWHS que habían sido identificados como portadores del genotipo *APOE e4e4* en fases previas del proyecto, y se seleccionó de manera aleatoria mediante emparejamiento (*matching*) al mismo número de participantes pertenecientes al

proyecto AWHS identificados como *APOE e3e3*, y *APOE e3e4* en base a su edad, sexo, nivel de estudios y hábitos de tabaquismo. Una vez seleccionados, se les invitó a participar en el subproyecto, que incluía la reevaluación de presencia de aterosclerosis subclínica, la evaluación de la AF y sueño mediante acelerometría, la evaluación de diferentes parámetros de CF, y la evaluación del estilo de vida, diferentes factores comportamentales, y función cognitiva mediante cuestionarios. En este subproyecto se invitó a participar a 120 trabajadores pertenecientes a la cohorte original del AWHS, de los que finalmente aceptaron participar 90.

4.2.2 Comité de ética del proyecto

El proyecto se llevó a cabo siguiendo las Normas Deontológicas reconocidas por la Declaración de Helsinki de 1975 (revisada en la 64 Asamblea General, Fortaleza, Brasil, octubre 2013), las Normas de Buena Práctica Clínica y cumpliendo la legislación y la normativa legal española que regula la investigación clínica en humanos (Real Decreto 223/2004 sobre regulación de ensayos clínicos). El proyecto fue aprobado por el Comité de Ética de Investigación Clínica de Aragón (CEICA), recibiendo el dictamen favorable de dicho Comité (anexo 1).

Todos los participantes fueron correctamente informados de los objetivos del estudio, las pruebas a las que serían sometidos explicando detalladamente las incidencias y posibles complicaciones o efectos secundarios de las mismas, y el manejo que se daría a los datos y resultados obtenidos a través de dichas pruebas. Como requisito indispensable y de manera previa a la participación en el estudio, todos los participantes tuvieron que firmar un consentimiento informado (anexo 2).

4.2.3 Pruebas y valoraciones del proyecto y subproyecto

4.2.3.1 Valoración de la aterosclerosis subclínica

Para valorar la presencia de aterosclerosis subclínica, se utilizó la ultrasonografía. Concretamente se utilizó el sistema de ultrasonidos Philips IU22 (Philips Healthcare, Bothell, WA, USA) para observar la presencia de placas en dos territorios vasculares (femoral y carotídeo) en ambos lados, izquierdo y derecho. Las imágenes por ultrasonidos fueron tomadas mediante sondas lineales bidimensionales de alta frecuencia (Philips Transducer L9-3, Philips Healthcare), utilizando el protocolo del Bioimage Study para las arterias carótidas (132), y un protocolo diseñado específicamente para las arterias femorales (133). La presencia de placa se definió como una estructura focal que sobresale en la luz de la arteria al menos 0,5 mm o ≥50% del grosor de la capa intima-media circundante. Todas las mediciones se analizaron utilizando marcos controlados por electrocardiograma correspondientes a las diástoles terminales (onda R) (134).

4.2.3.2 Valoración de la actividad física y sedentarismo

Para evaluar la AF habitual de los participantes, se utilizaron dos tipos de metodologías diferentes, los cuestionarios y la acelerometría.

El cuestionario utilizado para valorar la AF y comportamiento sedentario de todos los participantes del proyecto AWHS fue la versión española (135) del cuestionario sobre la frecuencia de realización de AF utilizado en el Nurses' Health Study (136) y el Health Professionals' Follow-up Study (137) (anexo 3).

Para calcular el nivel de AF total realizado por cada participante, se asignó un coste metabólico a cada actividad utilizando los MET establecidos en el compendio de actividades físicas propuesto por Ainsworth (138), y se multiplicó por el tiempo medio que el participante indicó haber practicado esa actividad durante el último año. De la suma total de todas las actividades, se obtuvo el nivel de AF de cada participante en MET-h/semana.

Para evaluar el comportamiento sedentario habitual entre semana y de los fines de semana, se utilizaron diferentes preguntas en base a la duración de diferentes conductas sedentarias habituales que implicaran estar sentado o recostado (ver la televisión, estar sentado en el ordenador, estar conduciendo…), pudiendo contestar desde nunca, hasta más de 9 horas/día en diferentes intervalos de horas o fracciones de las mismas en caso de ser la duración inferior a 1 hora.

Por otro lado, en la muestra del subproyecto APOE, se utilizaron los acelerómetros ActiGraph GT3x+ (ActiGraph, Pensacola, FL, USA) para evaluar los niveles de AF de los participantes. Para ello, se pidió a los voluntarios que llevaran el dispositivo en su muñeca no dominante durante 7 días consecutivos. Los acelerómetros se programaron para registrar aceleraciones a 30 Hz con un rango dinámico de ±6 G. Posteriormente, los registros de aceleración fueron descargados y procesados con el software ActiLife v.6.13.4 (ActiGraph, Pensacola, FL, USA). Los registros se dieron como válidos si incluían al menos 10 h/día durante ≥4 días, siendo necesario que al menos 3 días fueran entre semana y 1 día de fin de semana. El tiempo de sedentarismo y de AF a diferentes intensidades se clasificó utilizando los puntos de corte propuestos por Montoye (139): (a) Sedentarismo: ≤2859 counts/min, (b) AF ligera: 2860 – 3940 counts/min, (c) AFM: 3941 – 5612 counts/min, (d) AFV: ≥5613 counts/min, (e) AFMV: ≥3941 counts/min.

4.2.3.3 Valoración de la ingesta alimentaria habitual

La evaluación de la ingesta habitual de alimentos se realizó a través de un cuestionario de frecuencia de consumo de alimentos previamente validado en España (140) (anexo 3). Dicho cuestionario recoge la frecuencia de consumo en el último año de 136 alimentos, incluyendo además preguntas sobre consumo de suplementos y dietas especiales. El cuestionario valora la frecuencia de consumo de cada alimento considerando

nueve frecuencias diferentes, que van desde "nunca o casi nunca", hasta "más de seis veces al día".

4.2.3.4 Valoración del genotipo

Se obtuvieron muestras de sangre de todos los participantes. Posteriormente, se aisló el ADN de las muestras de sangre completas utilizando el kit FlexiGene DNA AGF3000 (Qiagen, Valencia, CA, USA) en una estación de trabajo AutoGenFlex 3000 (Autogen, Holliston, MA, USA), y la identificación del genotipo se realizó en la Unidad de Genética del Parque Científico de Madrid. Las muestras de ADN se colocaron sobre placas de 384 pocillos y se diluyeron en una mezcla de TaqMan Genotyping MasterMix y una mezcla de TaqMan SNP genotyping assays. Las reacciones cuantitativas de PCR se realizaron en un aparato de PCR a Tiempo Real HT7900 (Applied Biosystems) y SDS 2.4 (Applied Biosystems). Se utilizaron muestras con genotipos conocidos como controles positivos y negativos para facilitar la identificación del genotipo, así como blancos, para la amplificación dentro de cada medida.

4.2.3.5 Valoración de la función cognitiva

La función cognitiva fue evaluada a través de un cuestionario a los participantes incluidos en el subproyecto APOE.

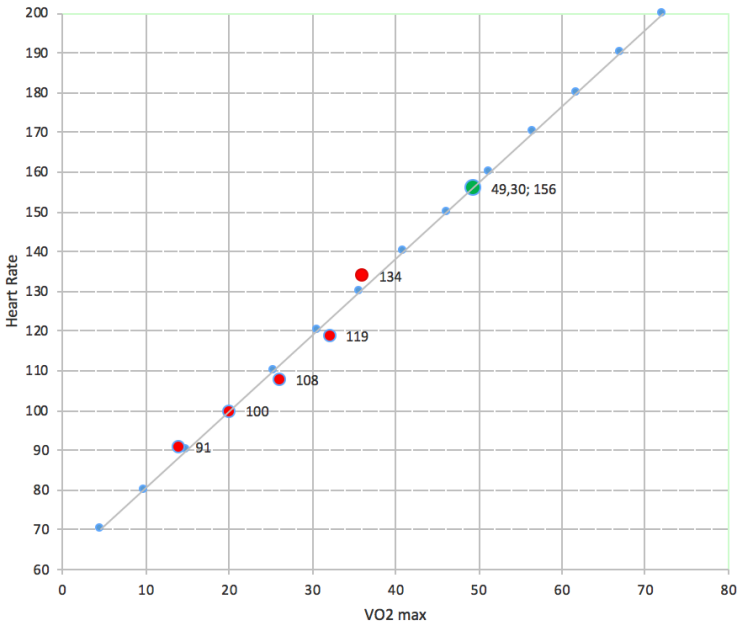
El cuestionario utilizado era una versión ampliada mediante diferentes test cognitivos del Montreal Cognitive Assessment (MoCA). La ampliación del cuestionario original se creó para que la batería de test fuera más precisa a la hora de detectar DCL en sujetos adultos de mediana edad, incluyendo a su vez otros cuestionarios, como el de actividad funcional de Pfeffer (141) (anexo 4).

4.2.3.6 Valoración de la condición física

La CF fue evaluada a través de diferentes pruebas a la muestra que compuso el subproyecto APOE.

Se evaluó la CF cardiorrespiratoria a través del test de banco submáximo Chester Step Test (142). Para ello, los participantes debían subir y bajar de un escalón al ritmo indicado mediante un metrónomo. El test comienza a un ritmo de 15 ciclos/min durante 2 min (primer nivel), y se va incrementando la velocidad 5 ciclos/min cada 2 min hasta el quinto nivel (final del test), o hasta que el participante alcance una frecuencia cardiaca (FC) del 80% de su FC máxima estimada mediante la fórmula (220 – edad), lo que ocurra primero. Al final de cada nivel se registra la FC y la percepción subjetiva de esfuerzo. Finalmente, el consumo máximo de oxígeno (VO_{2max}) se estimó trazando la línea en un gráfico FC/VO_{2max} que mejor se ajustaba a las frecuencias cardiacas registradas durante la prueba, y proyectando una línea hasta la FC máxima estimada, estimando así el consumo de oxígeno correspondiente, que será el VO_{2max} estimado del participante (Figura 19). La estimación del VO_{2max} a través de este método presenta un error estándar de 3,9 mL/kg/min de O_2 frente al VO_{2max} medido durante un test máximo a través de una ergoespirometría con análisis de gases respiración a respiración (142), siendo este el método de referencia para medir el VO_{2max}.

Figura 19. Estimación del consumo máximo de oxígeno a través de los datos de frecuencia cardiaca obtenidos durante el Chester Step Test.



Fuente: Elaboración propia

También se evaluó la fuerza máxima de prensión manual a través de una dinamometría manual máxima utilizando un dinamómetro (TKK 5001, grip A, Takei). Para ello los participantes se encontraban en bipedestación, con el brazo del lado evaluado en ligera abducción (~10º, sin tocar el resto del cuerpo), y el codo y la muñeca en 0º de flexión. Se registraron dos intentos con cada una de las manos dejando un periodo de recuperación adecuado entre intentos, dando por válido finalmente el mejor intento de cada una de las manos.

4.2.3.7 Datos clínicos y sociodemográficos

Entre otras valoraciones, también se llevaron a cabo la medición de la presión arterial y FC en reposo a través de un oscilómetro digital OMRON M10-IT (OMRON Healthcare Co. Ltd., Japón), el peso (báscula SECA 778), la talla para el cálculo del índice de masa corporal (IMC), y el perímetro de cintura (cinta métrica GulicK II 67019). Los participantes aportaron a su vez información acerca de su historia clínica, indicando antecedentes personales y familiares de ECV precoz, toma de medicamentos, tabaquismo

(fumador, no fumador, exfumador) y diagnóstico de hipertensión arterial, hipercolesterolemia y diabetes mellitus.

Las concentraciones de glucosa, triglicéridos, HDL-c y colesterol total se determinaron en condiciones de ayuno >8 h mediante análisis enzimáticos con el equipo ILab 650 de Instrumentation Laboratory. La insulina ultrasensible se determinó mediante inmunoanálisis de quimioluminiscencia con el equipo Access de Beckman Coulter. La hemoglobina glicosilada se determinó mediante intercambio de cationes en columna de fase inversa usando el equipo ADAMS A1c HA-810 de Arkray Factory. Las concentraciones de LDL-c se calcularon usando la fórmula de Friedewald cuando los valores de triglicéridos eran < 400 mg/dL (143).

Los participantes completaron un cuestionario adicional sobre características sociodemográficas que incluía: edad, nivel de estudios (primarios, bachillerato, formación profesional y universitarios), estado civil, situación laboral actual, tipo de puesto de trabajo (manual o de oficina), turno de trabajo (rotatorio mañana-tarde, rotatorio mañana-tarde-noche, central, o noche), y numero de convivientes en la unidad familiar.

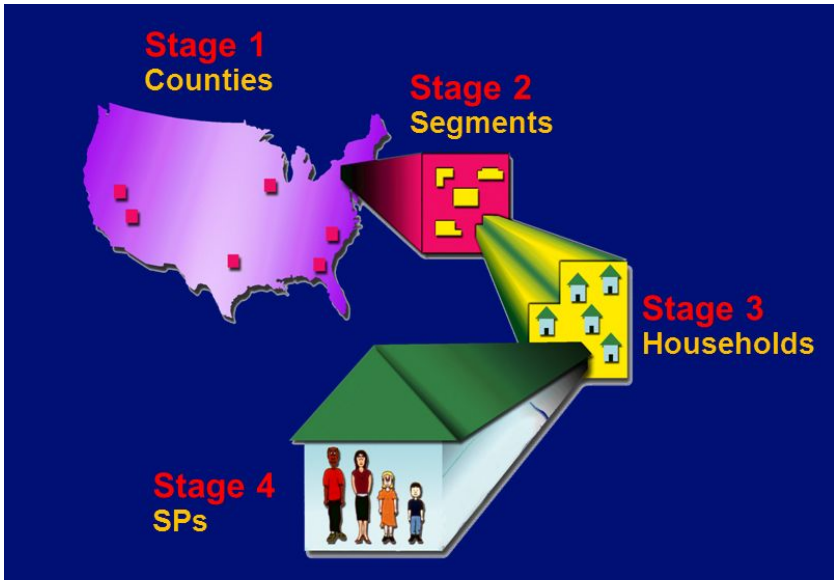
4.3 Estudio NHANES

El NHANES es un estudio llevado a cabo por el NCHS de manera anual con el objetivo de evaluar el estado nutricional y de salud de una muestra representativa de la población no institucionalizada de los Estados Unidos de América.

4.3.1 Muestra y diseño del estudio

Se trata de un estudio de carácter transversal que utiliza un diseño de muestreo complejo, aleatorio, estratificado y de múltiples etapas (Figura 20), que sobremuestrea ciertos subgrupos de población, como ciertas minorías étnicas o adultos mayores, lo que proporciona una excelente validez externa. El estudio selecciona aleatoriamente cada año a unos 7000 residentes de todo el país, y les invita a participar de manera voluntaria y confidencial.

Figura 20. Diseño del proceso de selección de la muestra del estudio NHANES.



Fuente: Figura tomada de la ponencia «NHANES: History, data collection, and future directions», llevada a cabo por la Dr. Kristen Herrick.

El diseño del estudio permite combinar datos de diferentes oleadas para agrupar a un mayor número de participantes, obteniendo así una potencia estadística mayor y unos resultados más fiables. Las instrucciones para combinar diferentes oleadas están disponibles a través de diferentes tutoriales (144).

4.3.2 Comité de ética del estudio

Todos los participantes del estudio han proporcionado un consentimiento informado de manera previa a su participación en el estudio, y el Ethics Review Board del NCHS ha aprobado los procedimientos de las diferentes mediciones llevadas a cabo y de la recogida de datos de las diferentes oleadas, y ha publicado los datos anonimizados en línea para su uso público (145).

4.3.3 Pruebas y valoraciones del estudio

El estudio completo incluye una amplia batería de pruebas, test y cuestionarios en cada una de las oleadas en las que se ha llevado a cabo, por lo que detallar y explicar todas y cada una de ellas va más allá de los objetivos e intereses de la presente Tesis Doctoral. Además, cabe destacar que en cada oleada desde el comienzo del estudio se han ido modificando tanto las pruebas a realizar como la metodología empleada para ello, por lo que para un mayor detalle de la metodología empleada en cada oleada para obtener los diferentes datos y resultados podemos recurrir a la página web del estudio, en la que se indican de manera pormenorizada los procedimientos utilizados (146).

En los siguientes apartados vamos a detallar la metodología empleada para obtener los datos utilizados en los artículos que forman parte de la presente Tesis Doctoral y utilizan datos del estudio NHANES.

4.3.3.1 Datos sociodemográficos

En el estudio se valoran diferentes aspectos sociodemográficos a través de cuestionarios. En ellos se incluyen preguntas para obtener información a nivel personal, familiar, y del entorno doméstico y laboral.

Entre los datos sociodemográficos más relevantes encontramos el sexo, la edad, la etnia, el lugar de nacimiento, la nacionalidad, el dominio del idioma, el estado civil, el nivel educativo, el número de personas de la familia y que habitan en el domicilio, o los ingresos anuales familiares y del domicilio.

4.3.3.2 Valoración de la composición corporal

Para evaluar la composición corporal, se utilizó entre otros métodos la densitometría dual de rayos X (DXA). Se utilizó un densitómetro Hologic QDR-4500 (Bedford, MA) para realizar valoraciones de cuerpo completo, y densitómetros Hologic QDR-4500 (Bedford, MA) y Hologic Discovery (Bedford, MA) para la valoración del cuello femoral y de la columna vertebral. Posteriormente las imágenes obtenidas fueron analizadas utilizando los softwares APEX™ 4.0 y Discovery 12.4 en función de la oleada en la que se realizaron las valoraciones pero, como se ha comprobado, la combinación de datos analizados con ambos softwares es compatible y no altera los resultados (147). Los detalles de los protocolos concretos pueden ser consultados en los manuales del estudio (148).

Mediante estas valoraciones se obtuvieron datos relativos a la cantidad y porcentaje de grasa total y por diferentes áreas corporales, al contenido mineral óseo y densidad mineral ósea total y en diferentes áreas de interés, y a la masa magra total y por diferentes áreas corporales.

4.3.3.3 Valoración de la actividad física

Para evaluar la AF se utilizaron dos cuestionarios diferentes. El primero se utilizó de 1999 a 2006, y el segundo desde 2007 hasta las últimas oleadas llevadas a cabo.

En el cuestionario utilizado entre 1999 – 2006, se evaluaba la AF llevada a cabo en los últimos 30 días, teniendo en cuenta la actividad en el trabajo o doméstica, la actividad para el transporte o viajar, y la actividad realizada en el tiempo libre. Se registró cada una de las actividades que los participantes afirmaron realizar, así como su duración y su frecuencia, y se les asignó un gasto energético en MET utilizando como referencia el compendio de actividades desarrollado y actualizado por Ainsworth en aquel momento (149). Finalmente se calcularon los MET-min/semana de cada actividad multiplicando el valor MET asignado a cada actividad por el número total de minutos a la semana de esa actividad, después, se sumaron todos los MET-min/semana de cada una de las actividades realizadas para obtener el cálculo total de la AF llevada a cabo por cada participante.

El cuestionario utilizado para valorar la AF de los participantes incluidos desde el año 2007 es el Global Physical Activity Questionnaire (GPAQ), creado por la OMS (150). Este cuestionario (anexo 5), analiza la AF habitual realizada durante una semana en tres dominios diferentes (AF en el trabajo o doméstica, AF para el transporte o viajar, y AF realizada en el tiempo libre). El cuestionario también considera durante cuánto tiempo se ha llevado a cabo cada actividad, y a qué intensidad (moderada o vigorosa). Finalmente y siguiendo el protocolo del GPAQ, podemos calcular la AF total llevada a cabo por cada participante en MET-min/semana (151).

4.3.3.4 Valoración de otros datos de salud

Para evaluar o cuantificar otros aspectos que pueden ser relevantes para la salud de los participantes, el estudio NHANES cuenta con una amplia batería de cuestionarios. Entre ellos encontramos uno de condiciones médicas, en el que se pregunta a los participantes

sobre la prevalencia de diferentes enfermedades como el asma, anemia, artritis, cáncer, gota, diferentes eventos cardiacos … y también sobre las consecuencias que hayan podido tener estas enfermedades en el pasado y también actualmente (episodios agudos, visitas o ingresos hospitalarios, tratamientos…).

Entre los cuestionarios utilizados en varias oleadas del estudio, también se incluye el Patient Health Questionnaire-9 (PHQ-9), que fue creado y ha sido ampliamente utilizado para evaluar la depresión (152). Este cuestionario cuenta con nueve ítems que evalúan los síntomas de depresión de las dos últimas semanas (anexo 6). El resultado del test varía entre 0 y 27 puntos, ya que cada una de las nueve preguntas puede ser respondida desde 0 (para nada), hasta 3 (casi todos los días). Los resultados de ≥10 puntos representan la presencia clínica de síntomas de depresión (153). Este es un punto de corte utilizado habitualmente, y que se ha demostrado que maximiza la combinación de sensibilidad y especificidad (154).

Por último, cabe destacar que también se incluyen otros cuestionarios sobre diferentes hábitos de vida como son el consumo de alcohol (anexo 7) o de tabaco (anexo 8).

4.3.4 Registros de mortalidad asociados

Aunque el estudio NHANES es de carácter transversal, existen datos de mortalidad de uso público de la base de datos National Death Index (NDI) vinculados a los participantes del estudio NHANES que participaron entre las oleadas de 1999 – 2014. Estos datos han sido vinculados por el NCHS, y proporcionan información sobre la mortalidad de los participantes desde su fecha de inicio en el estudio NHANES hasta el 31 de diciembre de 2015. En estos datos se registran tanto las defunciones como las causas.

4.4 Análisis estadísticos

Dado que algunos de los análisis estadísticos utilizados en los diferentes artículos que componen la presente Tesis Doctoral son comunes, se ha decidido optar por describir los diferentes análisis utilizados en un mismo apartado. No obstante, algunos de los métodos descritos a continuación solo se han utilizado en uno de los artículos, por ello, para conocer en mayor detalle los análisis estadísticos llevados a cabo en cada estudio, los mismos se pueden consultar en la siguiente sección de *Resultados y discusión*.

Merecen especial mención los artículos III y IV, dado que para llevar a cabo todos los análisis estadísticos se tuvo en cuenta el diseño complejo de la selección de la muestra, por lo que dichos análisis se realizaron considerando e incorporando las variables de estratificación y los pesos combinados apropiados proporcionados por NHANES (144).

En la mayoría de las ocasiones el software utilizado para llevar a cabo los diferentes análisis estadísticos fue el Statistical Package for the Social Sciences (SPSS) (v. 24.0 IBM Corp., Armonk, NY, USA), aunque para realizar algunos análisis o procesos concretos también se utilizó el lenguaje de programación estadística R (v. 3.4.4 y v. 4.0.4, R Foundation for Statistical Computing, Vienna, Austria) utilizando diferentes paquetes. El nivel de significación estadística para todos los test se fijó en $p< 0,05$.

4.4.1 Análisis descriptivos

En los cuatro artículos originales que componen la presente Tesis Doctoral se han llevado a cabo en primer lugar análisis estadísticos descriptivos de las principales variables utilizadas en cada caso. Los análisis descriptivos se han llevado a cabo para toda la muestra, y para diferentes subgrupos creados en cada caso en función de diferentes variables de interés. Las variables continuas se han presentado a través de su media y desviación

estándar (SD), o a través de su media y error estándar (SE), las variables categóricas se han presentado a través de su porcentaje y número de participantes (n).

A su vez, se estudió la normalidad en la distribución de las variables continuas mediante el test de Kolmogorov-Smirnov.

4.4.2 Diferencias entre grupos

Se estudiaron las diferencias entre los diferentes grupos creados a través de test para muestras independientes (test t de Student) cuando se trataba de dos grupos, o a través de test de análisis de las varianzas (ANOVA) con ajuste de Bonferroni para las pruebas post-hoc cuando se trataba de más de dos grupos.

En el caso de las variables categóricas, se estudió la diferencia en la distribución entre grupos a través de tablas de contingencia aplicando el test Chi-cuadrado.

4.4.3 Análisis de regresión

En los cuatro estudios se utilizaron modelos de regresión logística binaria con el objetivo de utilizar los coeficientes para calcular el Odds Ratio (OR) para cada situación determinada, (principalmente presencia o ausencia de determinada enfermedad o condición), en función de la variable de exposición seleccionada. En cada caso se introdujo un ajuste en el análisis en base a las potenciales variables de confusión identificadas.

4.4.4 Análisis de mortalidad

En uno de los estudios (artículo IV) se utilizaron modelos de regresión de riesgos proporcionales de Cox para calcular las razones de riesgo de muerte en cada grupo de interés. Al igual que en los anteriores modelos de regresión, en cada caso se introdujo un ajuste en el análisis en base a las potenciales variables de confusión identificadas. También se utilizaron curvas de supervivencia ajustadas para ilustrar la supervivencia libre de eventos de los diferentes grupos o patrones creados. Con el objetivo de minimizar el sesgo de causalidad inversa, se excluyeron las muertes sucedidas dentro del primer año de

seguimiento. A través del estudio del paralelismo de los gráficos log-log supervivencia y a través de la correlación del tiempo de seguimiento y los residuos de Schoenfeld se comprobó la ausencia de violación de los supuestos de riesgos proporcionales de los modelos creados.

4.4.5 Imputación de datos y análisis de sensibilidad

En uno de los estudios, antes de realizar los análisis, después de considerar la naturaleza aleatoria de los datos faltantes, y después de comprobar que la cantidad de datos faltantes era inferior al 20% por cada variable de las que presentaban datos faltantes, se decidió imputar los datos de los casos con alguna variable faltante para no perder potencia estadística. Para ello se utilizó la estimación de valores por máxima verosimilitud a través del algoritmo EM (Estimación y Maximización). La selección de las variables utilizadas para imputar los datos faltantes se basó en la importancia y relación con la variable a imputar, y en el cálculo de coeficientes de correlación de Pearson para evaluar la relación entre las variables. Después de realizar los diferentes análisis pertinentes con todos los datos (incluyendo los casos con valores imputados), se decidió comprobar la robustez de los resultados obtenidos realizando los mismos análisis tan solo con los casos completos (no incluyendo casos con valores imputados).

5. Resultados y discusión [Results and discussion]

5.1 Artículo I

International Journal of
Environmental Research and Public Health

MDPI

Systematic Review

Can Physical Activity Reduce the Risk of Cognitive Decline in Apolipoprotein e4 Carriers? A Systematic Review

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Citation: Perez-Lasierra, J.L.; Casajús, J.A.; Casasnovas, J.A.; Arbones-Mainar, J.M.; Lobo, A.; Lobo, E.; Moreno-Franco, B.; Gonzalez-Agüero, A. Can Physical Activity Reduce the Risk of Cognitive Decline in Apolipoprotein e4 Carriers? A Systematic Review. *Int. J. Environ. Res. Public Health* **2021**, *18*, 7238. https://doi.org/10.3390/ijerph18147238

Academic Editors: Fernando Sánchez-Santed, Maria Teresa Colomina Fosch, Caridad López Granero and Ingrid Reverte

Received: 21 April 2021
Accepted: 1 July 2021
Published: 6 July 2021

Abstract: Physical activity (PA) reduces the risk of cognitive decline (CD) in the general population. However, little is known about whether the presence of the apolipoprotein E epsilon 4 allele (*APOE e4*) could modify this beneficial effect. The aim of this systematic review was to analyze and synthetize the scientific evidence related to PA levels and CD risk in cognitively healthy *APOE e4* carriers. Four electronic databases were analyzed. Only original articles with longitudinal study design were selected to analyze the relationship between PA and CD in *APOE e4* carriers. Five studies were included in the systematic review. All studies except one stated that PA is a protective factor against CD in *APOE e4* carriers. Moreover, partial support was found for the hypothesis that a greater amount and intensity of PA are more beneficial in CD prevention. The results support the idea that PA is a protective factor against CD in *APOE e4* carriers. Nevertheless, it would be necessary to carry out further studies that would allow these findings to be contrasted.

Keywords: exercise; *APOE e4*; Cognitive Dysfunction; mild cognitive impairment

1. Introduction

Cognitive decline (CD) in older adults is an ever-growing problem because the number of older adults is increasing [1] and because longer life increases the likelihood of loss of memory and decline in the performance of cognitive tasks [2,3]. Cognitive performance is considered an important aspect of healthy aging [4]. There is an increasing interest in this subject, which is now a priority in public health [5], and the European Innovation Partnership on Active and Health Aging [6], has also recognized its relevance. Therefore, the prevention of CD should be an area of increasing interest for research in public health.

Mild cognitive impairment (MCI) is one construct of CD that has received considerable attention in the literature, mainly because it has been widely sustained that MCI progresses to dementia, Alzheimer's disease in particular, in a high proportion of individuals [7], and also because of its high prevalence, even in the community. Several studies have suggested the idea that CD, which includes MCI, may be considered a health index because both

have been associated with an increased mortality rate in the general population [8–10]. Moreover, it has been shown that CD is associated with disability in the general population, even after controlling for dementia [11]. Consequently, the prevention of CD should be a subject of particular attention.

A multitude of risk factors of different typology have been associated with CD [2,12,13]; age is the most important one [3], although cardiovascular diseases are also risk factors [2,12,14]. Other characteristics such as low educational level [12], depression and anxiety [10,15,16], vitamin B12 or D deficiency [12] as well as certain lifestyles that include smoking and physical inactivity [14] may also play a role in CD.

Among the previously documented risk factors of CD, a major genetic risk factor is the presence of the apolipoprotein E epsilon 4 allele (*APOE e4*) [2,17]. The human apolipoprotein E gene is polymorphic, presenting three alleles: epsilon 3 (*e3*), epsilon 2 (*e2*) and epsilon 4 (*e4*). The *e3* variant is the most frequent allele (~77% in the general population), while *e2* and *e4* are less common, with occurrence rates of 7.8% and 15.1%, respectively [18]. *APOE e4* presence is associated with several risk factors and diseases [19–22], including faster CD [2,23]. Homozygotes have the highest risk, followed by heterozygotes, implying that the effect of *APOE e4* on CD is dose-dependent [17,24]. The underlying mechanisms by which *APOE e4* is associated with early CD suggest that *APOE e4* is deficient in beta-amyloid clearance and accelerates beta-amyloid deposition to form amyloid plaques in the brain [20]. The toxic amyloid plaques injure synapses and ultimately cause neurodegeneration and CD [25].

On the other hand, protective factors of CD have also been reported [12,14,26], including intellectual activity and lifestyles aspects such as social interaction and physical activity (PA) [27,28]. PA may act on several risk factors related to CD, and a relationship between PA and neurogenesis, plasticity and higher white matter volume has been shown [29–31]. Several studies demonstrate how cardiovascular diseases and their risk factors are associated with CD and lower performance in multiple cognitive domains [32,33]. However, PA is beneficial in the prevention of cardiovascular diseases and their risk factors [34–36], as well as other known risk factors for CD such as depression or anxiety [35,37]. Therefore, PA may indirectly prevent CD by counteracting the CD risk factors. It has also been reported that PA is beneficial for the prevention of MCI and CD in the general population [12,29,31,38,39]. Recent meta-analyses also suggest that PA reduces the risk of suffering CD [27,40]; however, none of the studies reviewed were differentiated according to the genotyping of the participants based on the presence of *APOE e4*. This is a source of concern, since perhaps the effect of PA in subjects with high genetic risk of CD, *APOE e4* carriers, is different from the effect that PA has in subjects without genetic risk, *APOE e4* non-carriers. In order to create useful strategies for the prevention of severe neurodegenerative diseases, it is crucial to investigate the role that PA may have against neurodegeneration in these subjects with increased genetic risk.

Some studies have analyzed to what extent the amount and intensity of PA influence its potential beneficial effect for the prevention of CD in the general population. Several studies have suggested that higher levels of PA (exercise engaged in $\geq$3 days/week at intensity greater than walking) or vigorous intensity PA is more beneficial [41–44], but discrepant results have also been reported [40], and the question is not settled.

On the basis of the previous reports, the main aim of this systematic review was to analyze and synthetize the scientific evidence related to PA levels and CD risk in cognitively healthy *APOE e4* subjects (without previous CD) and to review the literature that investigates whether the effect of PA in these subjects is directly associated with amount and intensity.

2. Materials and Methods

This work used the model of preferred reporting items for a systematic review and meta-analysis (PRISMA) [45] to ensure accuracy and comprehensiveness. A review protocol was written prior to reviewing the literature.

As all analyses were based on publicly available summary statistics, no ethical approval from an institutional review board or informed patient consent was required.

Four electronic databases were analyzed systematically using different keywords and Boolean operators. The databases analyzed were PubMed, SportDiscus, Cochrane Library and Web of Science (WOS). The strategy included searching by index terms (MeSH) and by free text. The search by index terms used in PubMed was: "Exercise" [Mesh] AND "Cognitive Dysfunction" [Mesh] AND "Apolipoprotein E4" [Mesh]. The search by free text used in PubMed, SportDiscus, Cochrane Library and WOS was: ("Physical Activity" OR "Exercise") AND ("Memory impairment" OR "Age-associated memory impairment" OR "Late-life forgetfulness" OR "age-related cognitive decline" OR "Mild Cognitive Impairment" OR "Cognitive Decline" OR "Cognitive Dysfunction") AND ("Apolipoprotein E4" OR "Apoe4" OR "Apo E4" OR "Apoe 4" OR "Apoe epsilon 4" OR "Apolipoprotein E-4" OR "Apo E-4" OR "Apo E 4" OR "Apolipoprotein E epsilon4").

For the present systematic review, only original articles published up to 13 April 2021 were analyzed, excluding all types of symposium reports, letters to the editor, conference abstracts, books, opinions of experts and reviews of any kind. The inclusion criteria used were that the studies covered the theme of the present review (relationship between the risk of CD (which includes cases of MCI), and PA in participants genotyped with *e4* allele), were longitudinal studies, used humans as participants, were published in English and that the sample used was composed of people without brain injuries or diagnosed mental disorders at baseline.

Studies were excluded if they did not provide information on how the cognitive function or the PA was evaluated, if the PA was evaluated jointly with the rest of the activities carried out in the free time (including sedentary leisure activities) or if they were related to dementia and not specifically to CD. Papers that did not specifically assess the impact of PA on CD in *APOE e4* and those related to animal experimentation were all excluded.

Applying these criteria and using the search strategy described, the process of article selection is illustrated in Figure 1.

Two reviewers (J.L.P.-L. and A.G.A.) carried out the selection process independently based on the criteria previously established. Any discrepancies were resolved by consensus, and in some cases, a third reviewer (J.A.C.) was consulted to resolve disagreements. Starting from the initial search after applying the search criteria and strategies, the articles were first selected based on the title and abstract, identifying key and thematic keywords related or unrelated to our aim, then those duplicated articles were eliminated, and the last screening was performed after a complete and exhaustive reading of the full text, selecting the papers to be included in our systematic review. Finally, after the article selection process, the bibliography of the articles was reviewed in order to identify articles that could meet our inclusion criteria.

Data from the studies, such as the longitudinal follow-up time, the population sample used and its characteristics (average age of the participants in the baseline, sex and genotype), the cognitive function and PA evaluation method, the covariates (confounders) used in the studies, the study design and the main results, were extracted based on a second reading of the full text of the articles included in the systematic review. Moreover, the results of the included studies were grouped according to whether they compared the risk for CD among different PA levels only in *e4* carriers or in *e4* carriers vs. non-carriers. Participants of the studies were considered *APOE e4* carriers if they carried at least one *e4* allele.

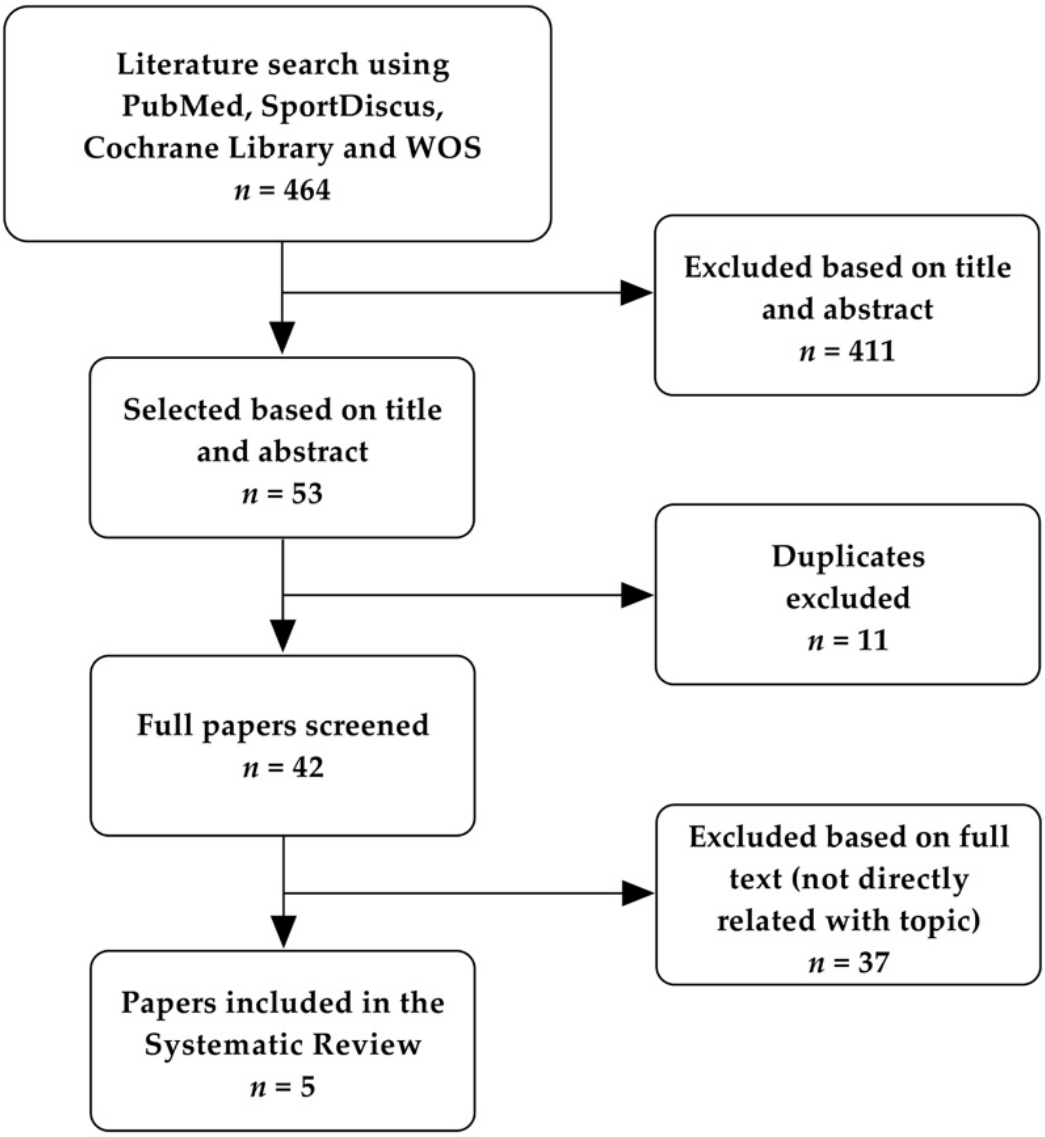

Figure 1. Flow diagram of search strategy in databases (PubMed, SportDiscus, Cochrane Library and Web of Science). Note: WOS—Web of Science.

Quality assessment was performed using the Newcastle–Ottawa Scale (NOS) for cohort studies [46] and the PEDro scale for randomized controlled trials [47]. The NOS for cohort studies uses a "star" rating system to judge quality based on three aspects of the study: (a) selection of participants, (b) comparability of study groups and (c) outcome of interest. The maximum number of stars a study may receive in each of these three categories is 4, 2 and 3, respectively. The highest rating a study can receive is 9 stars. A detailed description of the NOS criteria for assessing quality can be found in the Supplementary Materials.

The PEDro scale consists of 11 criteria that assess the methodological quality of the experimental studies based on three aspects: internal validity (criteria 2–9), interpretability (criteria 10–11) and external validity or applicability (criterion 1).

In the NOS, we assigned scores of 0–3, 4–6 and 7–9 to indicate low, moderate and high-quality studies, respectively, and, in the PEDro scale, we assigned scores of 0–4, 5–8, and 9–11 to indicate low, moderate and high-quality studies, respectively.

3. Results

3.1. Study Selection

As shown in Figure 1, the initial electronic database search yielded 464 hits in total. A total of 411 articles were excluded following screening titles and abstracts, 11 articles were removed for duplicates and 37 articles were excluded following screening of full texts. A total of five articles from the electronic search met the inclusion criteria, and, after the manual search, no articles were added.

3.2. Description of Included Studies

There are several differences in the design and the methodology of the different included studies. Four of them have a prospective cohort design [48–51], and the other is a randomized controlled trial [52]. Between-study differences were observed in the methods to assess the exposure and outcome. Moreover, the range of follow-up time among the studies is quite broad, from 18 months [51] to 10 years [52].

Several studies were carried out on Americans [48,50,52], one on Mexican-Americans [50] and another on American populations of Indian, African, Asian and Hispanic descent [52]. In the latter study, it should be noted that all the participants in the baseline had type II diabetes and overweight or obesity [52]. Another study was conducted on Caucasians [51], and the last one was carried out on Asians [49].

Regarding all the participants of the studies that compared the risk for CD among different PA levels in *e4* carriers vs. non-carriers (Table 1), a total of 7148 participants were included, and 1425 of them were *APOE e4* carriers, 5043 were non-carriers and the remaining 680 were reported as having a missing or unknown genotype (nine study participants from one article [48], and 671 study participants from another [52]). Moreover, the population sample of the studies ranged from 78 participants [51] to 3802 participants [52].

Taking into account all the participants of the studies that compared the risk for CD among different PA levels in *e4* carriers (Table 2), a total of 792 participants were included, but the samples of the studies also varied between 26 participants [51] and 474 participants [48].

3.3. Assessment of Main Variables

To assess PA, different types of questionnaires were used. Two of the studies used questionnaires created ad hoc that were not validated [49,50], while the others used validated questionnaires such as the Minnesota Leisure Time Physical Activity Questionnaire and the 1985 National Health Interview Survey [48], Paffenbarger Physical Activity Questionnaire [52] or the Stanford Brief Activity Survey [51].

To assess cognitive function, all the studies used specific tests or questionnaires, and although there is considerable diversity, in all the studies except one [48], the Mini-Mental State Examination (MMSE) [49,51], or its modified version, the Modified Mini-Mental State Examination (3MSE) [50,52], were used, both of which are employed worldwide [53].

Tables 1 and 2 show the questionnaire used in each of the studies to assess PA, cognitive function and the criteria for establishing CD or MCI based on the cognitive assessment tests used throughout the follow-up. Three of the included studies used MMSE or 3MSE cutoff points to define CD [49,50,52], another used Petersen's criteria for MCI [48] and the remaining study used cutoff points in at least one of the principal outcome indices (DRS-2 and RAVLT) [51]. Tables 1 and 2 display the different criteria used in each study to classify the participants among the different PA levels.

3.4. Risk of Bias Assessment

There was a heterogeneous quality in the methodology of the included studies, since two had high quality [48,50], and the others had moderate quality [49,51,52] (Table 3).

Table 1. Summary of the reviewed articles that compare the risk for CD among different PA levels in *APOE e4* carriers vs. non-carriers.

Study, (Study Design)	Follow-Up, y, Mean, (Range)	Method to Assess PA	Method to Assess Cognitive Function	Adjudication of CD or MCI	Confounders	Study Subgroups	Main Results	Sample				
								n	Female Sex, *n* (%)	Age, y, mean (SD), and/or Range	*APOE e4*, *n*, (*e4e4*, *n*)	No *APOE e4*, *n*
Espeland, M. et al. (intervention)	9.8 (8.4–11.1)	- Paffebarger Physical Activity Questionnaire	- 3MSE - FAQ	3MSE test score fell below prespecified age- and education-specific cutoff points	- Age	- Groups by: - Genotype - Randomization: - Intervention: >175 min PA/week brisk walking - Control: PA, diet and social support	- Non-carriers control group OR = 1.00 (ref. group) - Carriers control group OR = NI - Carriers intervention group OR = 0.84 (95%CI: 0.52, 1.36, $p > 0.05$)	3802	2323 (61)	45–76	724 (57)	2407
Krell, J. et al. (observational)	3.2 (1.9–4.7)	- Minnesota leisure time physical activity questionnaire - 1985 National Health Interview Survey	- Neurological evaluation - STMS - WAIS-R - WMS-R	Petersen criteria for MCI 2004	- Age - Sex - Educational level - Comorbidities - Depression	- Groups by: - Genotype - Intensity of PA: - LPA - MPA - VPA - Moment in life when PA was performed: - Midlife - Late life	Midlife and LPA: - Non-carriers and not LPA: HR = 1.00 (ref. group) - Carriers and not LPA: HR = 1.32 (95%CI: 0.70, 2.50, $p > 0.05$) - Carriers and LPA: HR = 0.97 (95%CI: 0.66, 1.43, $p > 0.05$)	1830	919 (50.2)	78 74–83	474 (NI)	1347
							Midlife and MPA: - Non-carriers and not MPA: HR = 1.00 (ref. group) - Carriers and not MPA: HR = 2.07 (95%CI: 1.32, 3.26, $p < 0.05$) - Carriers and MPA: HR = 1.53 (95%CI: 1.10, 2.15, $p < 0.05$)					
							Midlife and VPA: - Non-carriers and not VPA: HR = 1.00 (ref. group) - Carriers and not VPA: HR = 1.84 (95%CI: 1.42, 2.39, $p < 0.05$) - Carriers and VPA: HR = 1.32 (95%CI: 0.93, 1.89, $p > 0.05$)					
							Late life and LPA: - Non-carriers and not LPA: HR = 1.00 (ref. group) - Carriers and not LPA: HR = 2.03 (95%CI: 1.23, 3.35, $p < 0.05$) - Carriers and LPA: HR = 1.35 (95%CI: 0.95, 1.91, $p > 0.05$)					
							Late life and MPA: - Non-carriers and not MPA: HR = 1.00 (ref. group) - Carriers and not MPA: HR = 1.89 (95%CI: 1.37, 2.61, $p < 0.05$) - Carriers and MPA: HR = 1.43 (95%CI: 1.05, 2.95, $p < 0.05$)					
							Late life and VPA: - Non-carriers and not VPA: HR = 1.00 (ref. group) - Carriers and not VPA: HR = 1.90 (95%CI: 1.51, 2.40, $p < 0.05$) - Carriers and VPA: HR = 1.18 (95%CI: 0.67, 2.07, $p > 0.05$)					

Table 1. *Cont.*

Study, (Study Design)	Follow-Up, y, Mean, (Range)	Method to Assess PA	Method to Assess Cognitive Function	Adjudication of CD or MCI	Confounders	Study Subgroups	Main Results	Sample				
								n	Female Sex, *n* (%)	Age, y, mean (SD), and/or Range	*APOE e4*, *n*, (*e4e4*, *n*)	No *APOE e4*, *n*
Shih, I. et al. (observational)	6.5	- MET-h/week of 18 common activities for older adults (based on the Compendium of Physical activities) (not validated)	- 3MSE - SEVLT (Delayed word recall) - SENAS	Score 3MSE or SEVLT fell less than the 20th percentile/decreased ≥8 in 3MSE or ≥3 points in SEVLT and scores less than 20th percentile at follow-up	- Age - Sex - Educational level - Diabetes - Smoking - History of stroke - Hours standing/walking at work	Groups by: - Genotype - Level of PA: - Low PA: <35MET-h/week - High PA: >35MET-h/week	- No *APOE e4* and High PA: HR = 1.00 (ref. group) - No *APOE e4* and Low PA: HR = 1.39 (95%CI: 0.94, 2.07, $p > 0.05$) - *APOE e4* and High PA: HR = 2.20 (95%CI: 1.29, 3.74, $p < 0.05$) - *APOE e4* and Low PA: HR = 3.44 (95%CI: 1.85, 6.39, $p < 0.05$)	1438	840 (58)	69.7 (6.2)	201 (11)	1237
Woodard, J.L. et al. (observational)	1.5	- Stanford Brief Activity Survey	- MMSE - GDS - MDRS-2 - RAVLT	≥1 SD reduction on at least one of the principal outcomes indices (DRS-2, RAVLT Sum of trials 1–5, RAVLT delayed word recall)	NI	Groups by: - Genotype - Level of PA: - Low: ≤2 d/week of low intensity (does not meet ACSM recom.) - High: ≥3 d/week of moderate to heavy intensity (meets ACSM recom.)	- *APOE e4* Low-PA demonstrated higher probability of decline than No-*APOE e4* Low-PA; *APOE e4* High-PA and No-*APOE e4* High-PA (all $p < 0.05$) - *APOE e4* High-PA probability of CD was not statistically different compared to No-*APOE e4* Low-PA and No-*APOE e4* High-PA ($p > 0.05$)	78	57 (73)	72.6 (5.0)	26 (1)	52

Abbreviations: n = sample size; y = years; NI = Not Informed; STMS = Short Test of Mental Status; WAIS-R = Wechsler Adult Intelligence Scale—Revised; WMS-R = Wechsler Memory Scale—Revised; PA = Physical Activity; LPA = Light Physical Activity; MPA = Moderate Physical Activity; VPA = Vigorous Physical Activity; HR = Hazard Ratio; MET = Metabolic Equivalent; h = hours; d = days; min = minutes; 3MSE = Modified Mini Mental Status Examination; SEVLT = Spanish English Verbal Learning Test; SENAS = Spanish English Neuropsychological Assessment Scale; FAQ = Functional Assessment Questionnaire; OR = Odds Ratio; MMSE = Mini Mental State Examination; GDS = Geriatric Depression Scale; MDRS-2 = Mattis Dementia Rating Scale-2; RAVLT = Rey Auditory Verbal Learning Test; SD = Standard Deviation; ACSM = American College of Sports Medicine; recom. = recommendations.

Table 2. Summary of the reviewed articles that compare the risk for CD among different PA levels in *APOE e4* carriers.

Study, (Study Design)	Follow-Up, y, Mean, (Range)	Method to Assess PA	Method to Assess Cognitive Function	Adjudication of CD or MCI	Confounders	Study Subgroups	Main Results		Sample		
									n, (*e4e4*, *n*)	Female Sex, *n* (%)	Age, y, Mean (SD), and/or Range
Krell, J. et al. (observational)	3.2 (1.9–4.7)	- Minnesota leisure time physical activity questionnaire - 1985 National Health Interview Survey	- Neurological evaluation - STMS - WAIS-R - WMS-R	Petersen criteria for MCI 2004	- Age - Sex - Educational level - Comorbidities - Depression	Groups by: - Intensity of PA: - LPA - MPA - VPA - Moment in life when PA was performed: - Never - In midlife - In late life - Always	LPA	- Never LPA: HR = 1.00 (ref. group) - Only LPA in midlife: HR = 0.80 (95%CI: 0.33, 1.98, $p > 0.05$) - Only LPA in late life: HR = 0.59 (95%CI: 0.19, 1.82, $p > 0.05$) - Always LPA: HR = 0.56 (95%CI: 0.26, 1.21, $p > 0.05$)	474 (NI)	NI	NI
							MPA	- Never MPA: HR = 1.00 (ref. group) - Only MPA in midlife: HR = 0.90 (95%CI: 0.52, 1.55, $p > 0.05$) - Only MPA in late life: HR = 0.96 (95%CI: 0.43, 2.13, $p > 0.05$) - Always MPA: HR = 0.68 (95%CI: 0.40, 1.14, $p > 0.05$)			
							VPA	- Never VPA: HR = 1.00 (ref. group) - Only VPA in midlife: HR = 0.87 (95%CI: 0.57, 1.33, $p > 0.05$) - Only VPA in late life: HR = 1.00 (95%CI: 0.40, 2.49, $p > 0.05$) - -Always VPA: HR = 0.46 (95%CI: 0.22, 0.95, $p < 0.05$)			
Niti, M. et al. (observational)	(1–2)	- Frequency of participation in physical exercise routines; walking; active sports; tai chi (not validated)	- MMSE	Decline ≥1 points in MMSE between baseline and follow-up	- Age - Sex - Educational level - Number of comorbidities - Functional status - Vascular risk factors - Depression - Smoking - Alcohol	Groups by: - At least one PA: - Yes - No	- No-PA OR = 1.00 (ref. group) - Yes-PA OR = 0.34 (95%CI: 0.17, 0.68, $p < 0.05$)		292 (NI)	NI	NI
Woodard, J.L. et al. (observational)	1.5	- Stanford Brief Activity Survey	- MMSE - GDS - MDRS-2 - RAVLT	≥1 SD reduction on at least one of the principal outcomes indices (DRS-2, RAVLT Sum of trials 1–5, RAVLT delayed word recall)	NI	Groups by: - Level of PA: - Low: ≤2 d/week of low intensity (does not meet ACSM recom.) - High: ≥3 d/week of moderate to heavy intensity (meets ACSM recom.)	- Low-PA group demonstrated higher probability of decline than High-PA group ($p < 0.05$)		26 (1)	NI	NI

Abbreviations: n = sample size; y = years; STMS = Short Test of Mental Status; WAIS-R = Wechsler Adult Intelligence Scale; WMS-R = Wechsler Memory Scale; PA = Physical Activity; LPA = Light Physical Activity; MPA = Moderate Physical Activity; VPA = Vigorous Physical Activity; HR = Hazard Ratio; MET = Metabolic Equivalent; h = hours; d = days; min = minutes; 3MSE = Modified Mini Mental Status Examination; SEVLT = Spanish English Verbal Learning Test; FAQ = Functional Assessment Questionnaire; OR = Odds Ratio; MMSE = Mini Mental State Examination; GDS = Geriatric Depression Scale; MDRS-2 = Mattis Dementia Rating Scale-2; RAVLT = Rey Auditory Verbal Learning Test; ACSM = American College of Sports Medicine; recom. = recommendations; NI = Not Informed.

Table 3. Newcastle–Ottawa and PEDro Quality Assessment of the studies included in the systematic review.

Study	Quality Assessment of Cohort Studies with NOS									
	Selection				Comparability		Outcome			NOS QS
	1	2	3	4	5	6	7	8	9	
Krell, J. et al.	*	*		*	*	*	*		*	7
Niti, M. et al.	*	*		*	*	*	*			6
Shih, I. et al.	*	*		*	*	*	*	*	*	8
Woodard, J.L. et al.	*	*		*			*		*	5

Study	Quality Assessment of RCT Studies with PEDro Scale											
	1	2	3	4	5	6	7	8	9	10	11	PEDro QS
Espeland, M. et al.	*	*	*	*			*			*	*	7

Abbreviations: NOS = Newcastle–Ottawa Scale; QS = Quality Score; RCT = Randomized Controlled Trial; * = One point.

3.5. Risk of CD among Different PA Levels

Two tables summarize the results of the included studies. Table 1 compares the studies that analyze the risk in *APOE e4* carriers vs. non-carriers in relation to PA levels, and Table 2 compares the studies that analyze the risk between carriers with different PA levels.

Regarding the results provided in Table 1, Shih et al. stated that those carrying *APOE e4* who accomplished more than 35 MET-hours/week had a 2.20-fold increased hazard ratio (HR) (95%CI: 1.29, 3.74, $p < 0.05$), and those carrying *APOE e4* who performed less than 35 MET-hours/week had a 3.44-fold increased HR (95%CI: 1.85, 6.39, $p < 0.05$) of developing cognitive impairment compared to persons who were not *APOE e4* carriers and who performed more than 35 MET-hours/week [50]. Krell-Roesch et al. stratified their results based on the intensity of PA (light, moderate and vigorous) and also based on the moment in life when PA was performed (midlife or late life). Based on this stratification, no differences in MCI risk were found for light and vigorous PA performed in midlife or late life between *APOE e4* carriers and non-carriers (Table 1). Nevertheless, when compared to *APOE e4* non-carriers who did not perform moderate PA in midlife, *APOE e4* carriers who did not perform moderate PA in midlife had a 2.07-fold increased HR for MCI (95%CI: 1.32, 3.26, $p < 0.05$), while *APOE e4* carriers who performed moderate PA in midlife had a 1.53-fold increased HR for MCI (95%CI: 1.10, 2.15, $p < 0.05$). When compared to *APOE e4* non-carriers who did not perform moderate PA in late life, *APOE e4* carriers who did not perform moderate PA in late life had a 1.89-fold increased HR for MCI (95%CI: 1.37, 2.61, $p < 0.05$), and those *APOE e4* carriers who performed moderate PA in late life had a 1.43-fold increased HR for MCI (95%CI: 1.05, 2.95, $p < 0.05$) [48]. Woodard et al. revealed that the predicted probability of CD for *APOE e4* carriers who reported low levels of PA ($\leq$2 days/week of low intensity) was significantly higher ($p = 0.006$) compared to *APOE e4* non-carriers who reported low or high levels of PA ($\leq$2 days/week of low intensity, or $\geq$3 days/week of moderate to heavy intensity, respectively) [51]. The article by Woodard et al. also shows that the predicted probability of CD for *APOE e4* carriers who reported high levels of PA was not statistically different from that for *APOE e4* non-carriers who reported low or high levels of PA ($p > 0.05$) [51]. Espeland et al. found that *APOE e4* carriers who were involved in the PA intervention group had reduced odds for CD (OR = 0.84, 95%CI: 0.52, 1.36, $p > 0.05$) as compared with non-carriers belonging to the control group [52].

Referring to the results provided in Table 2 that compare the risk of CD among *APOE e4* carriers who showed different PA levels, Woodard et al. revealed that the predicted probability of CD for *APOE e4* carriers who reported low levels of PA ($\leq$2 days/week of low intensity) was significantly higher ($p = 0.006$) compared to carriers who reported high levels of PA ($\geq$3 days/week of moderate to heavy intensity) [51]. Krell-Roesch et al. stratified their results based on the intensity of the PA (light, moderate and vigorous) and also based on the moment in life when PA was performed (never, only in midlife, only in late life, or always in midlife and late life). Based on this stratification, no significant

differences for suffering MCI were found among carriers who performed or who did not perform light or moderate PA in different moments of their lifespan; however, those carriers who had performed vigorous intensity activity in midlife and late life (always) had lower risk of developing MCI in comparison with those who never performed vigorous PA: HR = 0.46 (95%CI: 0.22, 0.95, $p < 0.05$) [48]. Niti et al. reported that participants who performed at least one PA had 0.34 odds for CD (95%CI: 0.17, 0.68, $p < 0.05$) compared with participants who did not perform any PA [49].

4. Discussion

In relation to the aims of this systematic review, the results support the notion that PA is a protective factor against CD in *APOE e4* carriers independently of the methodology used to assess the PA or the criteria to establish CD and PA levels, since this is confirmed by four of the five studies included in this systematic review. It is remarkable in this first review of previous reports in the literature that PA was effective in preventing CD in high-risk individuals such as *APOE e4* carriers. However, there are several issues related to the methodology of included studies that must be taken into account in the interpretation of the results.

As can be seen in Tables 1 and 2, all the studies except one [51] showed results adjusted by different confounders, the most common being age and educational level. However, only two studies carried out a follow-up period >5 years [50,52]. Moreover, the short period of follow-up in some cases, the >20% loss of participants, the self-reported PA and the fact that the results were not adjusted by important confounders such as age, educational level or cardiovascular risk factors could affect the results.

The methodology used to assess PA in the studies had some weaknesses, as the researchers used questionnaires, which are based on self-reported PA and, therefore, may over- or underestimate participants' PA. Some advantages of this type of questionnaire in studies such as those included in the present review are apparent, such as their simplicity, low cost, and ease of administration in large samples in a short period of time [54]. There is wide recognition that the choice of method may be a trade-off between accuracy level and feasibility [55], but when the aim is establishing a dose–response relationship, the use of motion sensors such as accelerometers would be important, although their use in large population studies is less feasible and they are not 100% accurate [54,56,57].

In addition, as can be seen in Tables 1 and 2, the use of different questionnaires to assess PA among studies, and the use of different cutoff points to define the different PA levels, make it difficult to compare among studies. Moreover, the heterogeneity in the method to assess cognitive function and in the definition and criteria used for establishing CD or MCI also complicates the comparison among studies. Nevertheless, the comparability of studies is supported to some extent because these criteria are commonly used in clinical practice to confirm the presence of CD.

Furthermore, most of the included studies only specified if the participants were *APOE e4* carriers, and therefore cases, or were *APOE e4* non-carriers, and therefore controls, but did not specify whether there were any *APOE e2* carriers in control groups. This could be of interest, because, as evidence suggests, *APOE e2* has been associated with a reduced CD risk [58].

Despite these circumstances, all the included studies except one [52] support the idea that PA is a beneficial factor in terms of CD. The absence of significant differences in this study might be due to different reasons: first, the different type of sample used compared to the rest of the included studies, since all the participants had type 2 diabetes and overweight or obesity, and these are independent risk factors for CD [59] and can be improved with PA. Second, the intervention in this study may not be long enough to observe differences. Third, the results were only adjusted by age.

4.1. Physical Activity Dose and Risk of CD

Regarding the results of the studies, this review found that some studies suggest that an increased amount and/or intensity of PA is more effective in reducing the risk of CD for *APOE e4* carriers [48,50,51]. This finding may be explained with previous reports, which suggest that higher levels of PA may be associated with mitigating the increased risk of beta-amyloid deposition in *APOE e4* carriers [60].

However, the sample size in studies such as the one by Woodard et al. was small [51], and PA amount and intensity were only self-reported, such that the precision of the dose–response relationships may be affected. Therefore, although this review finds some support for the hypothesis that an increased amount and intensity of PA is more protective against CD in *APOE e4* carriers, the evidence is limited, and new research may be needed to document the precise amount and intensity of PA to recommend in *APOE e4* carriers.

It could be conceivable that previous studies in the general population, non-stratified by *APOE e4* status, might shed some light on this subject. According to several longitudinal studies, exercise intensity might be more beneficial than duration regarding cognitive function in the general population [41,42]. However, analyzing some studies that reported results related to the amount and intensity of PA adequate for the prevention of CD in the general population, contradictory results were found. While some studies suggest that moderate intensity seems to be sufficient to show a beneficial effect, although higher intensity is more effective [27,61], others stated that light intensity, such as a leisurely walk after dinner, is better than vigorous PA for the prevention of MCI [48].

It could be thought that the optimal dose of PA for *APOE e4* persons to prevent CD should not be very different from that of *APOE e4* non-carriers. However, according to Shih et al., the same PA level in *APOE e4* carriers and non-carriers results in a different risk reduction in CD, obtaining greater benefits for non-carriers [50]. Therefore, it is possible that carriers should perform more PA to reduce the risk to the same extent as non-carriers, but, on the other hand, a study conducted by Schuit et al. reported that, when performing the same PA level in both groups, *APOE e4* carriers obtain greater risk reduction for CD than non-carriers [62]. It is, therefore, apparent that new studies are required to determine the appropriate dose of PA to recommend in preventing CD.

4.2. Strengths and Limitations

The major strength of this review, which used the PRISMA system, is that, to our knowledge, it is the first systematic review focused on studies that stratify by apolipoprotein E genotype in relation to the association of PA and CD. Despite this, there are other limitations that must be considered. First, there is a heterogeneous methodology among the studies to assess the main variables and define the outcome. In the five included studies, a total of six different questionnaires were used to assess the PA, and a total of five different criteria were used to classify the participants in terms of PA levels; further, different criteria to diagnose CD were used in the included studies. Second, as discussed above, there are no studies that assess the PA with an objective method. Third, there are few studies in the literature related to our topic that show results by subgroups based on PA levels and the *APOE e4* status of the participants, and studies such as the one by Woodard et al. were carried out on small samples.

4.3. Future Recommendations

Although there is some evidence indicating that PA can be a protective factor against CD in *APOE e4* persons, future research will be needed in order to corroborate this. It seems that the literature is mainly limited by the failure to present data separately for *APOE e4* carriers and non-carriers, and more studies that stratify in groups according to the genotype and PA levels of the participants should, therefore, be conducted. It is also important to perform studies that evaluate PA objectively by means of motion sensors such as accelerometry, as, despite some limitations, it allows a more precise assessment of the amount and intensity of PA. It would also be interesting to perform intervention studies,

ideally RCTs, in *APOE e4* persons in order to provide data on the amount and intensity of PA that will be optimal for the prevention/slowdown of CD in this type of population. Currently, some clinical trials such as U.S. POINTER, IGNITE and PAAD-2 [63–65] are focused on the role that PA and other lifestyle variables have in cognition among different types of population, including *APOE e4* carriers. The results of these clinical trials could shed some light on this area.

5. Conclusions

The results of the studies included in this systematic review support the idea that PA is a protective factor against CD in individuals of high genetic risk, specifically *APOE e4* carriers. These findings have high clinical and public health significance. Moreover, the results suggest that in this population, a higher dose of PA (amount and/or intensity) might have greater benefits, but it would be necessary to carry out further studies that would allow these findings to be contrasted, since the existing evidence is limited. Further studies should try to establish the optimal dose of PA to effectively and efficiently prevent CD in *APOE e4* carriers.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijerph18147238/s1, Text S1: Quality assessment information. Newcastle–Ottawa Scale (NOS) criteria.

Author Contributions: Conceptualization, J.L.P.-L., J.A.C. (Jose Antonio Casajús), J.A.C. (José Antonio Casasnovas), A.L., E.L., B.M.-F. and A.G.-A.; methodology, J.L.P.-L., J.A.C. (Jose Antonio Casajús), B.M.-F. and A.G.-A.; formal analysis, J.L.P.-L., J.A.C. (Jose Antonio Casajús) and A.G.-A.; investigation, J.L.P.-L., J.A.C. (Jose Antonio Casajús), J.M.A.-M., B.M.-F. and A.G.-A.; writing—original draft preparation, J.L.P.-L. and A.G.-A.; writing—review and editing, J.A.C. (Jose Antonio Casajús), J.A.C. (José Antonio Casasnovas), J.M.A.-M., A.L., E.L. and B.M.-F.; visualization, J.L.P.-L. and A.G.-A.; supervision, J.L.P.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported in part by CIBERCV and grant PI19/00948 from the Instituto de Salud Carlos III (co-supported by the European Regional Development Fund "Investing in your future"). J.L.P.-L. received a Grant FPU 2016 (FPU16/02539) from the Ministerio de Educación Cultura y Deporte (Spain).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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5.2 Artículo II

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Daily Sitting for Long Periods Increases the Odds for Subclinical Atheroma Plaques

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Citation: Perez-Lasierra, J.L.; Laclaustra, M.; Guallar-Castillón, P.; Casasnovas, J.A.; Casajús, J.A.; Jarauta, E.; Gonzalez-Agüero, A.; Moreno-Franco, B. Daily Sitting for Long Periods Increases the Odds for Subclinical Atheroma Plaques. *J. Clin. Med.* **2021**, *10*, 1229. https://doi.org/10.3390/jcm10061229

Academic Editor: Jaakko Tuomilehto

Received: 3 February 2021
Accepted: 12 March 2021
Published: 16 March 2021

Abstract: Sedentarism is a risk factor for cardiovascular disease (CVD), but currently it is not clear how a sedentary behavior such as long sitting time can affect atherosclerosis development. This study examined the relationship between sitting time and the prevalence of carotid and femoral subclinical atherosclerosis. A cross-sectional analysis based on a subsample of 2082 participants belonging to the Aragon Workers' Health Study was carried out. Ultrasonography was used to assess the presence of plaques in carotid and femoral territories; the validated Spanish version of the questionnaire on the frequency of engaging in physical activity used in the Nurses' Health Study and the Health Professionals' was used to assess physical activity and sitting time; and demographic, anthropometric, and clinical data were obtained by trained personnel during the annual medical examination. Participants were categorized into <9 h/day and ≥9 h/day sitting time groups. After adjusting for several confounders, compared with participants that remain seated <9 h/day, those participants who remain seated ≥9 h/day had, respectively, OR = 1.25 (95%CI: 1.01, 1.55, $p < 0.05$) and OR = 1.38 (95%CI: 1.09, 1.74, $p < 0.05$) for carotid and any-territory plaque presence. Remaining seated ≥9 h/day is associated with higher odds for carotid and any-territory plaque presence independently of physical activity levels and other cardiovascular risk factors.

Keywords: sitting time; sedentary behavior; subclinical atherosclerosis; cardiovascular disease

1. Introduction

The first functional and pathological changes of atherosclerosis appear early in the youth and it slowly progresses all through life [1] until it manifests as clinical cardiovascular disease (CVD), which occurs mainly from the fifth decade of life on, creating a remarkable personal and social burden. Acting upon lifestyle and behavior is one of the keystones in CVD prevention and control [1]. Sitting time has been identified in the last decade as a lifestyle factor that increases the prevalence of CVD and its risk factors, independent of physical activity performed [2]. In fact, there is evidence that the sitting time and

physical inactivity affects cardiovascular physiology and metabolism differently, each with a specific biological effect [3–5]. While physical inactivity has been ranked as the fourth global leading cause of death [6], sitting time has been described as an independent major mortality risk factor, responsible for 3.8% of all deaths [7]. Prospective studies evidenced that two hours per day of additional sitting time increases the risk of cardiovascular mortality and cardiovascular events on 5% and 17%, respectively, regardless of the physical activity level [2].

Adopting the sitting position has been associated with changes in carotid artery hemodynamics, and these, in turn, have been hypothesized to lead to atherosclerosis in that territory [8]. Despite evidence on long term effects of sitting time, being able to show early deleterious effects is important in order to reinforce the knowledge on the causal chain, and to provide solid evidence to health promotion campaigns, which should intervene early in life. However, although epidemiological research discovered the association between sitting time and several CVD risk factors such as incident type 2 diabetes [9], inflammatory markers [10,11], and poor lipid profile [12], data about the effect of sitting time on subclinical atherosclerosis are very scarce. Few studies have evaluated the association of sitting time or sedentary time with the presence of plaques in the carotid arteries and they could not demonstrate statistically significant results [13–15]. Thus, relying on a wide sample, meticulously studied for subclinical atherosclerosis, we aim to re-examine the association of sitting time with the presence of carotid and femoral atherosclerosis plaques on male workers from a factory in Spain who belong to the Aragon Workers' Health Study (AWHS) cohort.

2. Materials and Methods

2.1. Study Design and Participants

This cross-sectional analysis was carried out in a subsample of participants belonging to the AWHS [16]. The AWHS is a prospective cohort aiming to investigate the determinants of the development and progression of metabolic abnormalities and subclinical atherosclerosis in 5678 workers of a car-manufacturing factory, free of clinical CVD, and recruited between 2009 and 2012. From 2011 to 2014, 2646 participants between 39 and 59 years of age accepted detailed explorations when they were additionally invited to undergo subclinical atherosclerosis measurements, and to complete diet, behavior, and lifestyle questionnaires. We excluded women (n = 132) and those with missing data on relevant variables (n = 432), so the final sample was composed of 2082 men. The study was approved by the Clinical Research Ethics Committee of Aragon (CEICA). All participants provided written informed consent.

2.2. Subclinical Atherosclerosis Imaging

A Philips IU22 ultrasound system (Philips Healthcare, Bothell, WA, USA) was used to assess the presence of plaques in 2 vascular territories (carotid and femoral) at both sides, right and left, which were considered together as a single site, as both side share similar hemodynamics. Ultrasound images were acquired with linear high-frequency 2-dimensional probes (Philips Transducer L9-3, Philips Healthcare), using the Bioimage Study protocol for the carotid arteries [17] and a specifically designed protocol for the femoral arteries [18]. A plaque was defined as a focal structure that protrudes into the lumen of the carotid artery at least 0.5 mm or $\geq$50% thicker than the surrounding intima-media thickness. All measurements were analyzed using electrocardiogram gated frames corresponding to end-diastole (R-wave) [19]. Presence of subclinical atherosclerosis was defined as the presence of at least 1 plaque in any of the 2 vascular territories.

2.3. Physical Activity and Sedentary Assessment

To estimate sedentary time, we used the question "How many hours do you usually spend sitting or reclining in a typical working day?", with values ranging from "never" to "nine or more than nine hours a day," and that took into account both working and

leisure time in a typical working day. In the analysis, the sample was divided into two groups: those who reported being seated <9 h/day, and those who reported being seated ≥9 h/day. Physical activity was assessed using the validated Spanish version [20] of the questionnaire on the frequency of engaging in physical activity used in the Nurses' Health Study [21] and the Health Professionals' Follow-up Study [22]. To compute the volume of activity performed by each participant, a metabolic cost was assigned to each activity using Ainsworth's compendium for physical activities [23], and multiplied by the time the participant reported practicing that activity. From the sum of all activities, we obtained a value of overall weekly METs-h.

2.4. Demographic, Clinical, and Biochemical Characteristics

Demographic, anthropometric, and clinical data were obtained by trained personnel during the annual medical examination of the manufacturing company. They included age; body mass index (BMI), which was calculated as weight (in kilograms) divided by height (in meters) squared (kg/m^2); waist circumference; and blood pressure. Likewise, biochemical measurements of total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides, and fasting serum glucose concentrations were determined by enzyme analysis using the ILAB 650 analyzer from Instrumentation Laboratory (Bedford, MA, USA). Blood samples were collected in fasting (>8 h) conditions. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula [24] when the triglyceride levels were <400 mg/dL. In all participants, non-HDL-c was calculated by subtracting the HDL-c value from the total cholesterol. We defined arterial hypertension as having systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or self-reported use of antihypertensive medication [25]. Dyslipidemia was defined as having total cholesterol ≥240 mg/dL, LDL-c ≥160 mg/dL, HDL-c <40 mg/dL, or self-reported use of lipid-lowering drugs [26]. Diabetes was defined as fasting plasma ≥126 mg/dL or self-reported treatment with hypoglycemic medication [25]. Smoking habits were categorized as ever smoker (current and former smoker) if the participant reported having smoked in the last year, or having smoked at least 50 cigarettes in his lifetime, and never smoker.

2.5. Statistical Analysis

Descriptive statistics were reported as mean, standard deviation, and percentage. Presence of atherosclerotic plaques in carotid arteries, in femoral arteries, or in any of both territories were fitted separately with logistic regression models depending on sitting time and adjusted for age, BMI, hypertension, dyslipidemia, diabetes, smoking status, and physical activity (METs-h/week). Coefficients were used to calculate odds ratios (OR) for plaque presence of each sitting exposure. In particular, ORs for the presence of plaque for each group of sitting time were calculated using as reference the biggest sample group (≥9 h-sitting/day). A model exploring hourly groups suggested that most differences appeared between the last group and the rest, which focused the analyses on this threshold. *P*-values below 0.05 were considered statistically significant. R statistical software (ver. 3.4.4) was used for the analyses (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Among the 2082 AWHS participants (mean age 50.9; SD 3.9 years), 528 declared spending ≥9 h-sitting/day. They had higher BMI and waist circumference, as well as slightly lower LDL-c than those participants who spent <9 h-sitting/day (Table 1).

Table 1. Baseline characteristics of study participants according to sitting time category.

Variable	Overall	<9 h/day	≥9 h/day	*p*-Value
n	2082	1554	528	
Age, years	50.9 (3.9)	50.8 (3.9)	51.0 (3.9)	0.353
BMI, kg/m^2	27.6 (3.3)	27.5 (3.2)	28.0 (3.4)	0.007
Waist circumference, cm	97.3 (8.9)	96.9 (8.8)	98.4 (8.8)	0.001
Systolic blood pressure, mmHg	125.4 (13.9)	125.4 (14.0)	125.4 (13.7)	0.941
Diastolic blood pressure, mmHg	82.4 (9.4)	82.4 (9.5)	82.4 (9.2)	0.899
Total cholesterol, mg/dL	220.1 (36.4)	220.9 (36.5)	217.7 (36.1)	0.084
HDL-c, mg/dL	53.0 (11.4)	53.2 (11.3)	52.6 (11.5)	0.299
Non-HDL-c, mg/dL	167.1 (35.2)	167.7 (35.2)	165.1 (35.3)	0.146
LDL-c, mg/dL	137.9 (31.4)	138.8 (31.2)	135.4 (31.8)	0.034
Triglycerides, mg/dL	150.1 (97.1)	148.8 (97.1)	153.9 (97.1)	0.303
Glucose, mg/dL	97.7 (17.5)	97.4 (16.5)	98.8 (20.1)	0.092
Hypertension, %	37.5 (781)	37.3 (580)	38.1 (201)	0.760
Dyslipidemia, %	49.2 (1025)	48.8 (758)	50.6 (267)	0.477
Diabetes, %	5.6 (117)	5.1 (79)	7.2 (38)	0.076
Ever smokers, %	77.1 (1606)	76.9 (1195)	77.8 (411)	0.655

BMI: body mass index; HDL-c: High-density lipoprotein cholesterol. Values are mean (SD) or % (number).

Whn studying ORs for plaque development in any of the analyzed territories in each group in a disaggregated variable of total hours sitting time per day, at the detail provided by the collecting instrument (hourly detail), a steep difference was apparent at the 9 h threshold (Figure 1). Although a dose-response curve was not apparent, subsequent analyses shown below confirmed this difference.

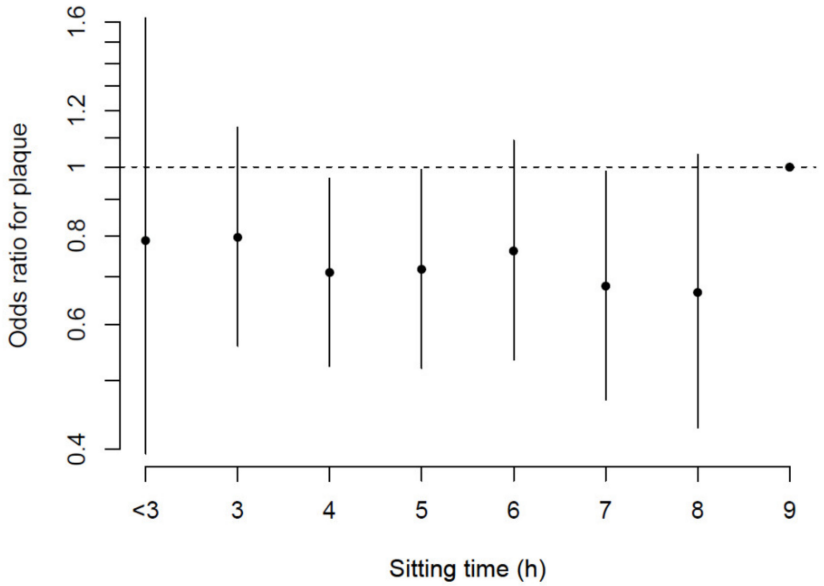

Figure 1. Odds Ratio (95%CI) for the presence of plaque in any of the analyzed territories (carotid and femoral) in terms of total hours of sitting time in the day.

At least one carotid plaque was present in 764 participants (36.7% of the overall sample), 35.4% in the <9 h-sitting/day group and 40.5% of the ≥9 h-sitting/day group. The odds for having a carotid plaque were 1.25 (95%CI: 1.01, 1.55, $p < 0.05$) times higher among those who spent ≥9 h-sitting/day than among the rest, after adjusting for BMI, diabetes, dyslipidemia, hypertension, smoking habit, and physical activity (Table 2).

Table 2. Odds ratio (95%CI) for the presence of plaque in different territories by sitting time category.

Different Models in the Analyzed Territories	Sitting Time		*p*-Value
	<9 h/day	≥9 h/day	
Number with carotid plaque/Total	550/1554	214/528	
Age-adjusted	1.00 (ref)	1.23 (0.99, 1.51)	0.051
Multivariable-adjusted 1	1.00 (ref)	1.24 (1.00, 1.53)	0.047
Multivariable-adjusted 2	1.00 (ref)	1.25 (1.01, 1.55)	0.037
Number with femoral plaque/Total	868/1554	313/528	
Age-adjusted	1.00 (ref)	1.13 (0.92, 1.39)	0.231
Multivariable-adjusted 1	1.00 (ref)	1.14 (0.92, 1.42)	0.237
Multivariable-adjusted 2	1.00 (ref)	1.16 (0.93, 1.45)	0.175
Number with plaque in any territory/Total	1020/1554	380/528	
Age-adjusted	1.00 (ref)	1.33 (1.07, 1.67)	0.010
Multivariable-adjusted 1	1.00 (ref)	1.35 (1.07, 1.71)	0.010
Multivariable-adjusted 2	1.00 (ref)	1.38 (1.09, 1.74)	0.007

Model 1 adjusted for age, body mass index, hypertension, dyslipidemia, diabetes, and smoking status. Model 2 additionally adjusted for physical activity (METs-h/week); (ref): Reference.

Femoral plaques were more frequent as they were present in 1181 participants (56.7% of the overall sample). Although the odds for having a femoral plaque also tended to be higher in the ≥9 h-sitting/day group (Adjusted odds ratio 1.16; 95%CI: 0.93, 1.45, $p = 0.175$), the difference was not statistically significant (Table 2).

Overall, 1400 participants had at least one plaque in any of the territories. A sitting time ≥9 h/day was significantly associated with having at least one plaque (Adjusted odds ratio 1.38; 95%CI: 1.09, 1.74, $p < 0.01$) (Table 2).

4. Discussion

In this cross-sectional analysis conducted in young and middle-aged asymptomatic workers, we provide evidence that a lifestyle that implies ≥9 h-sitting/day is associated with higher odds for the presence of subclinical atherosclerosis in the carotid territory, compared with those who were sitting <9 h/day independent of physical activity levels and the presence of other CVD risk factors.

An association between sitting time and CVD risk factors and mortality has been previously described [2,5,7]. However, the association between sitting time and the presence of subclinical atheroma plaques is under-examined, even when it is known that subclinical atherosclerosis constitutes an intermediate process towards clinical CVD and death. In this way, vascular ultrasound is a non-invasively way to assess atherosclerosis and predict major cardiovascular events. Carotid plaque and intima-media thickness (cIMT) measurements are early markers of subclinical atherosclerosis. However, the former is much more relevant, given that a recent research carried out by Sillesen et al. has concluded that cIMT did not improve the risk prediction of major cardiovascular events significantly while atheroma plaque did [27]. Furthermore, in a meta-analysis published by Inaba et al., the ultrasound assessment of carotid plaque has shown to be more accurate than cIMT in the diagnostic of coronary artery disease [19].

Only two studies have evaluated the presence of carotid artery plaque, and they have been carried out in mostly female populations [13,14]. In a recent European study conducted by Lazaros et al., an upward trend was observed between sitting time and the prevalence of carotid atheroma plaque, the mean cIMT, and the maximum cIMT, although no statistic significance was found [13]. Besides, another study conducted in a small sample ($n = 340$) of a Mexican American population [14] did not find any significant association between total sitting time and the presence of carotid plaque, mean cIMT, or cIMT ≥75%. However, when stratified by levels of physical activity, they found association in one of the strata. Thus, with our results, we finally confirm this previously elusive fact that an association of long sitting time with atherosclerosis exists as early as in the stage of subclinical atherosclerosis.

The independency between the levels of physical activity and sitting time has been debated [28,29]. Several prospective studies suggest that physical activity may not necessarily undo the harms from excessive sitting time [2], but more recent studies on mortality have shown a reduced influence of sitting time at higher amounts of physical activity, and vice-versa, a reduced influence of physical activity among those that spend less time sitting [5,28,29]. All these studies, which performed dose-response analysis, coincided that for above 8 h/day of sitting time there was a substantial increase in the risk, except for those at higher levels of physical activity where this risk did not increase. However, that amount of physical activity is likely to be performed by only a small proportion of the general population, and it is likely to be worthwhile to act independently of physical activity. In the present study, the average level of physical activity performed by our sample was 32.8 METs-h/week [30], below the levels where it has been shown to counteract the influence of sitting time on mortality. Thus, we could indicate that with more detailed methods, we are able to show that there is a relationship between sitting time and the presence of subclinical atherosclerosis, which seems independent from physical activity in the range performed by the general population. Using this intermediate endpoint may have been essential to the demonstration because demonstrating an effect on mortality requires extremely big samples and long follow-ups and it is obscured by the sum of several other influences.

It is remarkable that we found a more intense association in the carotid territory than in the femoral one, while at the age of our sample participants the femoral territory is more affected by atherosclerosis and its presence is more intensely associated with traditional risk factors [31]. The extent to which atherosclerosis affects different sites depends on several factors such as the genetic background, immune status, gender, and oxidative stress, among others [32]. Our results hinted that longer sitting time was associated more with carotid atherosclerosis than with femoral atherosclerosis. Atherosclerosis susceptibility is conditioned by hemodynamic features, which play a major role in the localization of atherosclerosis lesions [32]. Several studies showed that body position affects hemodynamics [8,33,34], and erect posture might play a role in the atherogenesis of leg arteries [33,35], so the sitting position could modify hemodynamics, assumedly protecting leg arteries, and therefore, justify our findings. Another possible explanation is that the greater association of traditional risk factors on femoral arteries atherosclerosis blurs the effect of sitting time on that territory.

As opposed to physical activity, which is agreed to be measured in terms of energy expenditure, researchers have used multiple definitions and methods to assess the sedentary behavior. It is usually measured by accelerometry or questionnaires, and it has been defined in the literature in several ways, for example, the duration of all activities that required an energy expenditure at the level of 1.0–1.5 METs during waking hours [36], the total screen time, the total TV-viewing time, or the total sitting time during working and leisure time in a day or a week [37]. This last definition, and no other sedentary behavior definitions that involve non-fatiguing muscle contractions such as standing position, is associated with a reduced contractile stimulation activity in postural skeletal muscles, causing a decrease of lipoprotein lipase enzyme activity [3,4,38], limiting the uptake of triglycerides and free fatty acid, and also reducing HDL-cholesterol plasma concentration [38] and promoting a proinflammatory state [10].

This study has the strength of a reasonable sample size and the use of high-quality data collection methods to obtain information on subclinical atherosclerosis and other variables. Besides, we believe that missing data did not create selection bias because there were no statistically significant differences in the sitting time distribution between participants excluded and those analyzed. However, several limitations should be acknowledged in our study. First, the cross-sectional analysis does not allow us to establish a causal temporal link between sitting time and the presence of subclinical atheroma plaque, although being subclinical it is unlikely to be responsible of reverse causation. Second, the sample includes only men, and therefore, our results may not be generalizable to women. Third, although

personal interviews to collect data about sitting time and physical activity have been carried out by trained interviewers, the use of self-reported information could be subject to bias.

In conclusion, sitting ≥9 h/day is associated with higher odds for carotid and any territory plaque development independently of physical activity levels and other CVD risk factors.

Author Contributions: Conceptualization, B.M.-F.; methodology, B.M.-F.; formal analysis, M.L.; data curation, J.L.P.-L., M.L. and B.M.-F.; writing—original draft preparation, J.L.P.-L. and B.M.-F.; writing— review and editing, J.L.P.-L., M.L., P.G.-C., J.A.C. (Jose Antonio Casasnovas), J.A.C. (Jose Antonio Casajús), E.J., A.G.-A. and B.M.-F.; supervision, B.M.-F.; funding acquisition, E.J. and B.M.-F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded in part by CIBERCV and grants PI18/01929 and PI19/00948 from the Instituto de Salud Carlos III (co-supported by the European Regional Development Fund 'Investing in your future'). J.L.P.-L. received a Grant FPU 2016 (FPU16/02539) from the Ministerio de Educación, Cultura y Deporte (Spain). M.L.'s research activity is funded by Agencia Aragonesa para la Investigación y el Desarrollo (ARAID).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of CEICA (PI07/09).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not public due to ethical reasons.

Acknowledgments: The authors thank the study participants, doctors and health-care professionals at the Opel factory. The authors also thank the AWHS technical staff.

Conflicts of Interest: The authors declare no conflict of interest.

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5.3 Artículo III

Experimental Gerontology 158 (2022) 111656

Contents lists available at ScienceDirect

Experimental Gerontology

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Association of physical activity levels and prevalence of major degenerative diseases: Evidence from the national health and nutrition examination survey (NHANES) 1999–2018

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ARTICLE INFO

Section editor: Anna-Karin Welmer

Keywords:
Sarcopenia
Osteoporosis
Osteoarthritis
Exercise
Aging

ABSTRACT

Objectives: Degenerative diseases are associated with lower healthy life expectancy and higher mortality. Physical activity (PA) has demonstrated a fundamental role in the prevention and control of several pathologies associated to the aging process. The aim of this study was to analyze the association of PA with the prevalence of sarcopenia, osteoporosis and osteoarthritis in non-institutionalized American population.
Methods: Cross-sectional study carried out in participants aged ≥50 years from the 1999–2018 National Health and Nutrition Examination Survey (NHANES). Sarcopenia was defined using appendicular lean mass adjusted for body mass index (ALM:BMI; men <0.789 kg/m^2, women <0.512 kg/m^2). Osteoporosis was defined as bone mineral density T-score ≤−2.5 of femur neck. Osteoarthritis and PA were self-reported, and total PA was used to classify participants in groups. The Odds Ratios among the different PA levels for each disease were examined.
Results: Performing at least 150 MET-min/week of PA was associated with reduced odds for sarcopenia; performing >1800 MET-min/week was associated with reduced odds for osteoporosis; and performing 150–1800 MET-min/week of PA was associated with reduced odds for osteoarthritis after adjust the results by several confounders.
Conclusions: The benefits of PA in sarcopenia, osteoporosis, and osteoarthritis prevention are evident among Americans aged ≥50 years.

1. Introduction

The aging population, which is increasing exponentially, is currently a new demographic reality and a source of concern for health systems. Life expectancy in developed countries has significantly increased over recent decades (Oeppen and Vaupel, 2002; Kochanek et al., 2019), although this is not always consistent with the healthy life expectancy (GBD 2017 DALYs and HALE Collaborators, 2018), defined as the number of years a person can be expected to live with complete health (WHO, 2009). In the United States the life expectancy for 65 year old men and women is 17.7 and 20.3 years respectively, while the healthy life expectancy for men and women at this age is 12.9 and 14.8 respectively (CDC, 2013).

Major degenerative diseases that impair quality of life are commonly associated with aging, and frequently closely linked with each other. Sarcopenia, is a progressive and generalized disorder defined as the loss of skeletal muscle mass and strength, and therefore functional capacity (Studenski et al., 2014). Osteoporosis, is defined as a skeletal disorder characterized by decreased density of normally mineralized bone and microarchitectural deterioration of bone tissue, making it more

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https://doi.org/10.1016/j.exger.2021.111656
Received 2 September 2021; Received in revised form 1 December 2021; Accepted 7 December 2021
Available online 14 December 2021

vulnerable to fracture due to overload (Glaser and Kaplan, 1997). Moreover, osteoarthritis is the most common joint disorder, characterized by the degeneration of articular cartilage, and also frequently associated with significant functional impairment (Martel-Pelletier et al., 2016). These disorders have been associated with changes in body composition (Martel-Pelletier et al., 2016; Greco et al., 2019), and also with negative health outcomes, including loss of functional capacity, frailty, fractures, falls, pain, and even an increased risk of death (Studenski et al., 2014; Glaser and Kaplan, 1997; Martel-Pelletier et al., 2016; Greco et al., 2019; Batsis et al., 2014; Johnston and Dagar, 2020).

It is known that lifestyle plays a key role in increasing and preserving healthy life expectancy (Mehta and Myrskylä, 2017). In this regard, physical activity (PA) has demonstrated a fundamental role in the improvement of quality of life through the prevention or treatment of degenerative diseases associated with the aging process (Roos and Arden, 2016; Fransen et al., 2015; Beaudart et al., 2017; Pedersen and Saltin, 2015). Moreover, PA is a useful way to reduce the most common negative health outcomes associated with sarcopenia, osteoporosis, and osteoarthritis (Fransen et al., 2015; Beaudart et al., 2017; Pedersen and Saltin, 2015; Varahra et al., 2018).

Prevalence of degenerative diseases among the general population has been analyzed in many studies (Looker et al., 2017; Batsis et al., 2016; Batsis et al., 2015; Vina and Kwoh, 2018), however, few of them have analyze this prevalence among different PA levels in a representative sample of a country. This could be of interest in order to add more information in terms of exercise prescription purposes, or exploring a possible dose-response effect. Therefore, the objective of this study was to analyze the association of PA with the prevalence that these degenerative diseases have in non-institutionalized American population.

2. Material and methods

2.1. Study design and population

The present study was carried out with data from the 1999–2018 National Health and Nutrition Examination Surveys (NHANES), conducted annually by the National Center for Health Statistics (NCHS) with the aim to assess the health and nutritional status of a representative sample of non-institutionalized United States population. The survey uses a multistage, complex, stratified probability sampling design that oversamples minorities and older adults, providing excellent external validity. Only participants aged ≥50 years old were included in the study. We excluded participants with lacked data in any of the analyzed variables, so the final sarcopenia dataset (data from 1999 to 2004) included 4505 participants, the osteoporosis dataset (data from 2007 to 2018) included 7210 participants, and the osteoarthritis dataset (data from 2011 to 2018) included 8820. The selection of the survey periods to analyze each disease is based on the availability of data obtained throughout the same methodology assessment.

All participants provided written informed consent, and The Ethics Review Board of the NCHS approved measurement procedures and data collection and posting of the data online for public use.

2.2. Sarcopenia, osteoporosis, and arthritis definition

Sarcopenia was defined following the Foundation for the National Institutes of Health (FNIH) definition using the appendicular lean mass (ALM) and body mass index ratio (ALM:BMI). ALM was defined as the sum of the lean mass of all limbs expressed in kilograms. To assess the lean mass a dual energy x-ray absorptiometry (DXA) QDR-4500 Hologic scanner (Bedford, MA) was used. The cut points for sarcopenia were <0.789 kg/m^2 and <0.512 kg/m^2 for men and women respectively (Studenski et al., 2014).

Osteoporosis was defined as bone mineral density (BMD) ≤−2.5 standard deviation (SD) (T-score ≤−2.5 SD) at femur neck (Shuhart et al., 2019). BMD of the femur neck was obtained with Hologic QDR-4500 and Hologic Discovery DXA scanners (Bedford, MA). APEX™ version 4.0 and Discovery 12.4 software were used to analyze femur scans in the different period data, but results showed no differences in mean BMD at femur neck between both software's (Looker et al., 2017), details of the DXA examination protocol have been published elsewhere (Centers for Disease Control, 2007). T-scores were calculated as (BMD participant – BMD reference group)/SD reference group. As recommended by the International Society for Clinical Densitometry (ISCD), the reference group for calculation T-scores for the femur neck consisted of 20–29 year-old non-Hispanic white females from NHANES III (Shuhart et al., 2019).

The osteoarthritis diagnosis was self-reported through the following question "Has a doctor or other health professional ever told you that you have arthritis?". For this study, only participants with osteoarthritis were included.

2.3. Assessment of physical activity

Physical activity was assessed by interview using a questionnaire. For the period 1999–2004 PA of the last 30 days was evaluated by means of a questionnaire taking into account PA at work/domestic, in transport/travel, and in leisure time. Each of the activities were awarded an energy expenditure on METs using the criteria established by Ainsworth (Ainsworth et al., 2011). The MET-min per week of each activity was calculated by multiplying the standard MET value of each activity by the total number of minutes per week of each activity, then the total MET-min per week was calculated as the sum of MET-min per week of each activity. This physical activity quantification method was identical to that in previous studies (Steeves et al., 2016; Fowler et al., 2020).

For the period 2007–2018 PA was evaluate through the Global Physical Activity Questionnaire (GPAQ) created by WHO (Armstrong and Bull, 2006). This questionnaire analyze the usual PA performed by participants in a typical week in 3 different fields (PA at work/domestic, PA in transport/travel, and PA in leisure time) as long as it has been carried out in continuous periods of 10 min. The questionnaire also takes into account the intensity at which it has been performed (moderate or vigorous). The total MET-min per week was calculated following the GPAQ protocol (World Health Organization, n.d.).

Finally and based in previous cut-points (Ekelund et al., 2016), the subjects were classified into different groups based on their PA level. The groups were defined as Very Low PA (VLPA) (<150 MET-min/week), Low PA (LPA) (150–960 MET-min/week), Medium PA (MPA) (961–1800 MET-min/week) and High PA (HPA) (>1800 MET-min/week).

2.4. Assessment of additional covariates

Age, sex, race/ethnicity, annual household income, educational level, smoking status, alcohol consumption, and BMI were assessed. Age was classified in periods of 10 years from the aged of 50 years (50–59; 60–69; 70–79; ≥80). Race/ethnicity variable difference among Mexican American, Other Hispanic, Non-Hispanic Withe, Non-Hispanic Black, and Other (including Multi-Racial). Annual household income variable classifies the participants in four groups (0–19,999 $; 20,000–44,999 $; 45,000–74,999 $ and ≥75,000 $). Educational level groups were less 9th grade, 9th–12th grade with no diploma, high school graduate or equivalent, college or Associate's degree, and college graduate or above. Finally, participants were classified by alcohol consumption in the last 12 months (0 drinks/day, <2 drinks/day, and ≥2 drinks/day), smoking status (never smoking, former smoker, and smokers), and obesity status (obesity was defined as BMI ≥30 kg/m^2).

2.5. Statistical analysis

According to the NHANES analytical guidelines, all data was downloaded, merged, and analyzed incorporating appropriated

Table 1
Baseline characteristics of study participants according to levels of PA[a].

	Overall	VLPA	LPA	MPA	HPA	*P* value for interaction
Sarcopenia dataset						
N, %	100 (4505)	36.9 (1924)	31.1 (1330)	15.2 (579)	16.8 (672)	<0.001
Sarcopenia, %	16.9 (1021.4)	24.6 (563.6)	14.8 (272.4)	11.2 (96.4)	9.1 (89)	<0.001
Age, %						
50–59	46.0 (1317)	41.1 (498)	46.7 (403)	53.0 (198)	49.4 (218)	<0.001
60–69	26.7 (1463)	26.5 (617)	27.9 (440)	24.3 (183)	27.2 (223)	
70–79	19.0 (1066)	20.4 (463)	18.3 (306)	16.9 (132)	19.0 (165)	
>80	8.3 (659)	12.0 (346)	7.2 (181)	5.8 (66)	4.4 (66)	
Male, %	51.3 (2517)	44.4 (992)	53.3 (746)	53.1 (338)	61.1 (441)	<0.001
ALM, kg	21.28 (0.11)	20.42 (0.18)	21.51 (0.21)	21.68 (0.33)	22.35 (0.21)	
Race/Ethnicity, %						
Mexican American	3.4 (830)	4.5 (426)	3.3 (237)	1.9 (375)	2.5 (92)	<0.001
Other Hispanic	4.2 (155)	5.7 (84)	3.5 (37)	2.2 (12)	3.8 (22)	
Non-Hispanic Withe	81.7 (2721)	75.3 (993)	83.0 (847)	87.9 (406)	87.4 (475)	
Non-Hispanic Black	7.7 (695)	10.9 (378)	6.6 (171)	5.4 (72)	4.8 (74)	
Other	3.1 (104)	3.5 (43)	3.6 (38)	2.7 (14)	1.5 (9)	
Obese, %	32.7 (1461)	37.8 (684)	32.9 (433)	29.2 (176)	24.2 (168)	<0.001
Annual Household income, %						
0–19,999 $	20.2 (1292)	29.9 (715)	16.8 (343)	12.9 (113)	11.6 (121)	<0.001
20,000–44,999 $	32.4 (1569)	37.5 (719)	31.3 (463)	26.9 (181)	28.1 (206)	
45,000–74,999 $	22.9 (856)	18.2 (285)	25.8 (273)	27.0 (137)	24.3 (161)	
≥75,000 $	24.6 (788)	14.4 (205)	26.2 (251)	33.1 (148)	36.1 (184)	
Educational level, %						
Less 9th Grade	8.7 (873)	13.8 (516)	7.7 (227)	4.4 (63)	3.1 (67)	<0.001
9th–12th Grade No diploma	13.1 (699)	19.7 (397)	9.6 (160)	9.5 (71)	8.4 (71)	
High School Graduate	26.0 (1033)	28.2 (435)	25.5 (308)	25.2 (141)	22.9 (149)	
College or AA degree	27.3 (1037)	24.5 (371)	30.4 (347)	27.5 (145)	27.4 (174)	
College Graduate or above	24.9 (863)	13.8 (205)	26.8 (288)	33.4 (159)	38.3 (211)	
Alcohol consumers, %						
0 drinks/day	30.8 (1573)	40.7 (827)	27.8 (414)	24.8 (169)	20.0 (163)	<0.001
<2 drinks/day	61.5 (2613)	52.1 (969)	65.1 (831)	66.7 (366)	70.5 (447)	
≥2 drinks/day	7.7 (319)	7.1 (128)	7.1 (85)	8.5 (44)	9.5 (62)	
Smoking status, %						
Never	37.9 (1730)	34.9 (700)	40.0 (535)	42.2 (246)	36.7 (249)	<0.001
Former	43.5 (1995)	41.2 (814)	42.8 (580)	44.8 (272)	48.9 (329)	
Smoker	18.6 (780)	23.9 (410)	17.2 (215)	12.9 (61)	14.4 (94)	
Osteoporosis dataset						
N, %	100 (7210)	28.5 (2393)	22.9 (1631)	11.7 (816)	36.9 (2370)	<0.001
Osteoporosis, %	4.9 (372)	8.0 (188)	4.9 (82)	4.4 (36)	2.7 (66)	<0.001
Age, %						
50–59	43.7 (2427)	34.5 (625)	42.1 (530)	40.7 (252)	52.8 (1020)	<0.001
60–69	31.9 (2549)	32.7 (846)	32.4 (583)	33.8 (307)	30.4 (813)	
70–79	16.9 (1480)	20.2 (554)	17.7 (350)	19.0 (182)	13.3 (394)	
>80	7.5 (754)	12.7 (368)	7.9 (168)	6.5 (75)	3.5 (143)	
Male, %	51.6 (4039)	42.8 (1179)	44.5 (826)	52.1 (450)	62.6 (1584)	<0.001
BMC, g/cm^2	4.11 (0.02)	3.98 (0.02)	4.00 (0.03)	4.10 (0.05)	4.29 (0.03)	
Race/Ethnicity, %						
Mexican American	4.7 (912)	5.6 (332)	4.2 (173)	3.7 (90)	4.7 (317)	0.002
Other Hispanic	3.7 (663)	3.9 (216)	3.6 (149)	3.3 (69)	3.8 (229)	
Non-Hispanic Withe	77.6 (3652)	74.1 (1165)	78.1 (826)	78.3 (428)	79.8 (1233)	
Non-Hispanic Black	8.8 (1461)	11.0 (547)	8.9 (340)	7.7 (147)	7.3 (427)	
Other	5.2 (522)	5.3 (133)	5.2 (143)	7.0 (82)	4.4 (164)	
Obese, %	37.2 (2634)	44.7 (976)	33.3 (558)	36.7 (281)	33.9 (819)	<0.001
Annual Household income, %						
0–19,999 $	12.9 (1540)	17.1 (612)	13.6 (350)	10.3 (151)	10.0 (427)	<0.001
20,000–44,999 $	28.2 (2424)	32.5 (861)	27.7 (528)	28.3 (276)	25.2 (759)	
45,000–74,999 $	20.8 (1387)	20.4 (431)	19.4 (309)	20.3 (154)	22.1 (493)	
≥75,000 $	38.1 (1859)	30.0 (489)	39.3 (444)	41.1 (235)	42.7 (691)	
Educational level, %						
Less 9th Grade	4.6 (785)	7.5 (342)	4.2 (160)	2.9 (70)	3.2 (213)	<0.001
9th–12th Grade No diploma	9.8 (1002)	14.3 (441)	7.9 (190)	5.9 (72)	8.8 (299)	
High School Graduate	25.2 (1722)	27.0 (583)	22.7 (379)	22.9 (189)	25.9 (571)	
College or AA degree	29.2 (1994)	29.9 (627)	29.2 (460)	27.3 (225)	29.4 (682)	
College Graduate or above	31.1 (1707)	21.3 (400)	36.0 (442)	40.9 (260)	32.7 (605)	
Alcohol consumers, %						
0 drinks/day	24.4 (2191)	32.8 (901)	21.5 (444)	20.2 (216)	21.0 (630)	<0.001
<2 drinks/day	67.6 (4476)	61.2 (1353)	71.7 (1075)	73.0 (543)	68.4 (1505)	
≥2 drinks/day	8.1 (543)	6.1 (139)	7.0 (112)	6.9 (57)	10.6 (235)	
Smoking status, %						
Never	47.2 (3173)	43.0 (1005)	51.0 (755)	52.0 (382)	46.5 (1031)	<0.001
Former	36.8 (2750)	38.1 (908)	36.0 (614)	36.9 (322)	36.2 (906)	
Smoker	16.0 (1287)	18.9 (480)	13.0 (262)	11.2 (112)	17.3 (433)	
Osteoarthritis dataset						

(*continued on next page*)

Table 1 (*continued*)

	Overall	VLPA	LPA	MPA	HPA	*P* value for interaction
N, %	100 (8820)	28.2 (2898)	22.4 (1978)	12.1 (1002)	37.3 (2942)	<0.001
Osteoarthritis, %	29.1 (2212)	35.1 (871)	27.0 (460)	25.3 (225)	26.9 (656)	<0.001
Age, %						
50–59	44.1 (3161)	35.7 (825)	41.5 (694)	45.3 (344)	51.7 (1298)	<0.001
60–69	31.6 (2986)	31.5 (969)	33.4 (686)	29.8 (355)	31.0 (976)	
70–79	16.7 (1737)	20.3 (655)	16.8 (387)	18.2 (209)	13.5 (486)	
>80	7.6 (936)	12.5 (449)	8.3 (211)	6.7 (94)	3.7 (182)	
Male, %	50.4 (4894)	43.2 (1437)	44.4 (998)	47.2 (532)	60.4 (1927)	<0.001
Race/Ethnicity, %						
Mexican American	4.4 (1081)	5.3 (399)	3.7 (200)	3.0 (97)	4.5 (385)	<0.001
Other Hispanic	3.6 (872)	4.2 (297)	3.5 (189)	3.1 (96)	3.5 (290)	
Non-Hispanic Withe	78.4 (4327)	75.4 (1383)	77.9 (949)	79.9 (510)	80.6 (1485)	
Non-Hispanic Black	8.0 (1750)	9.4 (603)	8.6 (412)	7.3 (190)	6.8 (545)	
Other	5.6 (790)	5.7 (216)	6.3 (228)	6.8 (109)	4.6 (237)	
Obese, %	37.9 (3321)	47.8 (1265)	35.9 (717)	37.3 (356)	31.9 (983)	<0.001
Annual Household income, %						
0–19,999 $	12.0 (1810)	16.0 (721)	12.4 (396)	9.9 (179)	9.3 (514)	<0.001
20,000–44,999 $	27.3 (2922)	33.9 (1052)	26.0 (639)	26.7 (324)	23.3 (907)	
45,000–74,999 $	21.0 (1686)	19.7 (512)	20.9 (378)	18.5 (180)	22.7 (616)	
≥75,000 $	39.8 (2402)	30.4 (613)	40.6 (565)	44.9 (319)	44.7 (905)	
Educational level, %						
Less 9th Grade	4.2 (912)	6.9 (395)	3.4 (177)	2.7 (81)	3.0 (259)	<0.001
9th–12th Grade No diploma	8.8 (1140)	13.7 (511)	6.8 (203)	5.9 (97)	7.1 (329)	
High School Graduate	23.1 (2000)	26.0 (705)	21.1 (432)	19.5 (198)	23.3 (665)	
College or AA degree	29.7 (2479)	29.5 (763)	30.9 (579)	26.9 (278)	29.9 (859)	
College Graduate or above	34.3 (2289)	24.0 (524)	37.8 (587)	44.9 (348)	36.6 (830)	
Alcohol consumers, %						
0 drinks/day	22.8 (2584)	31.9 (1080)	20.9 (538)	17.2 (241)	18.9 (725)	<0.001
<2 drinks/day	68.7 (5579)	62.0 (1657)	71.5 (1302)	76.2 (702)	69.6 (1918)	
≥2 drinks/day	8.5 (657)	6.1 (161)	7.6 (138)	6.5 (59)	11.6 (299)	
Smoking status, %						
Never	47.4 (3990)	44.0 (1248)	49.7 (935)	55.6 (499)	45.9 (1308)	<0.001
Former	36.9 (3272)	38.0 (1089)	37.0 (730)	35.8 (381)	36.3 (1072)	
Smoker	15.7 (1558)	18.0 (561)	13.3 (313)	8.6 (122)	17.8 (562)	

VLPA: Very Light Physical Activity (<150 MET-min/week); LPA: Light Physical Activity (150–960 MET-min/week); MPA: Medium Physical Activity (961–1800 MET-min/week); HPA: High Physical Activity (>1800 MET-min/week); Other: Other race including Multi-Racial; AA degree: Associate's degree.

[a] Data are expressed as weighted percentages and unweighted number of participants for categorical variables, and as weighted mean (standard error) for continuous variables.

combined weights, primary sampling unit, and strata provided by NHANES (National center for health statistics, n.d.). Categorical variables were expressed as frequency (%), and continuous variables are presented as mean and standard error (SE). Descriptive analyses were carried out for the overall samples and divided by PA level groups. Weighted logistic regressions models were also carried out for each degenerative disease analyzed according to the PA levels of the participants to examine the adjusted odds ratios (OR). Regression models were adjusted for the following variables: age-adjusted, adjusted by age, sex, race/ethnicity, annual household income, and educational level (Model 1), and additionally adjusted by smoking status, alcohol consumption, and obesity (Model 2). A two-sided *p*-value of 0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical software ver. 24.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Prevalence of major degenerative diseases in Americans

The prevalence of sarcopenia, osteoporosis, and osteoarthritis in ≥50 years old non-institutionalized Americans were 16.9%, 4.9%, and 29.1% respectively. The prevalence of sarcopenia, osteoporosis and osteoarthritis in VLPA, LPA, MPA and HPA groups were 24.6%, 14.8%, 11.2% and 9.1% respectively for sarcopenia; 8.0%, 4.9%, 4.4% and 2.7% respectively for osteoporosis; and 35.1%, 27.0%, 25.3% and 26.9% respectively for osteoarthritis (Table 1).

The prevalence for each degenerative disease according to sex and PA level group can be seen in the Supplementary Table 1, and according to sex and race/ethnicity in the Supplementary Table 2. The prevalence presented in both supplementary tables is also representative of non-institutionalized US-population.

3.2. Association of physical activity levels and degenerative diseases

Data from 4505 participants were used to study the association between sarcopenia and PA levels. The baseline characteristics of participants according to PA level group and overall can be seen in Table 1. The weighted odds for having sarcopenia were 0.60 (95%CI: 0.47, 0.76, $p < 0.05$) for LPA group, 0.49 (95%CI: 0.35, 0.69, $p < 0.05$) for MPA group, and 0.39 (95%CI: 0.29, 0.52, $p < 0.05$) for the HPA group compared to VLPA group after adjusting the results by confounders included in regression model 2 (Fig. 1 and Table 2).

Data from 7210 participants were used to study the association between osteoporosis and PA levels. Table 1 shown the baseline characteristics of participants according to PA level group and overall. As can be seen in Table 2 and Fig. 1, the weighted odds for having osteoporosis were not significant for LPA and MPA group compared with VLPA, 0.68 (95%CI: 0.46, 1.01, $p > 0.05$) for LPA group, and 0.71 (95%CI: 0.43, 1.18, $p > 0.05$) for MPA group. However, the HPA had 0.57 (95%CI: 0.42, 0.76, $p < 0.05$) lower odds for having osteoporosis than VLPA after adjusting the results by confounders included in regression model 2.

Data from 8820 participants were used to study the association between osteoarthritis and PA levels. The baseline characteristics of participants according to PA level group and overall can be seen in Table 1. The weighted odds for having osteoarthritis were 0.75 (95%CI: 0.62, 0.92, $p < 0.05$) for LPA group, 0.73 (95%CI: 0.55, 0.95, $p < 0.05$) for MPA group, and 0.97 (95%CI: 0.81, 1.16, $p > 0.05$) for the HPA group compared to VLPA group after adjusting the results by confounders included in regression model 2 (Fig. 1 and Table 2).

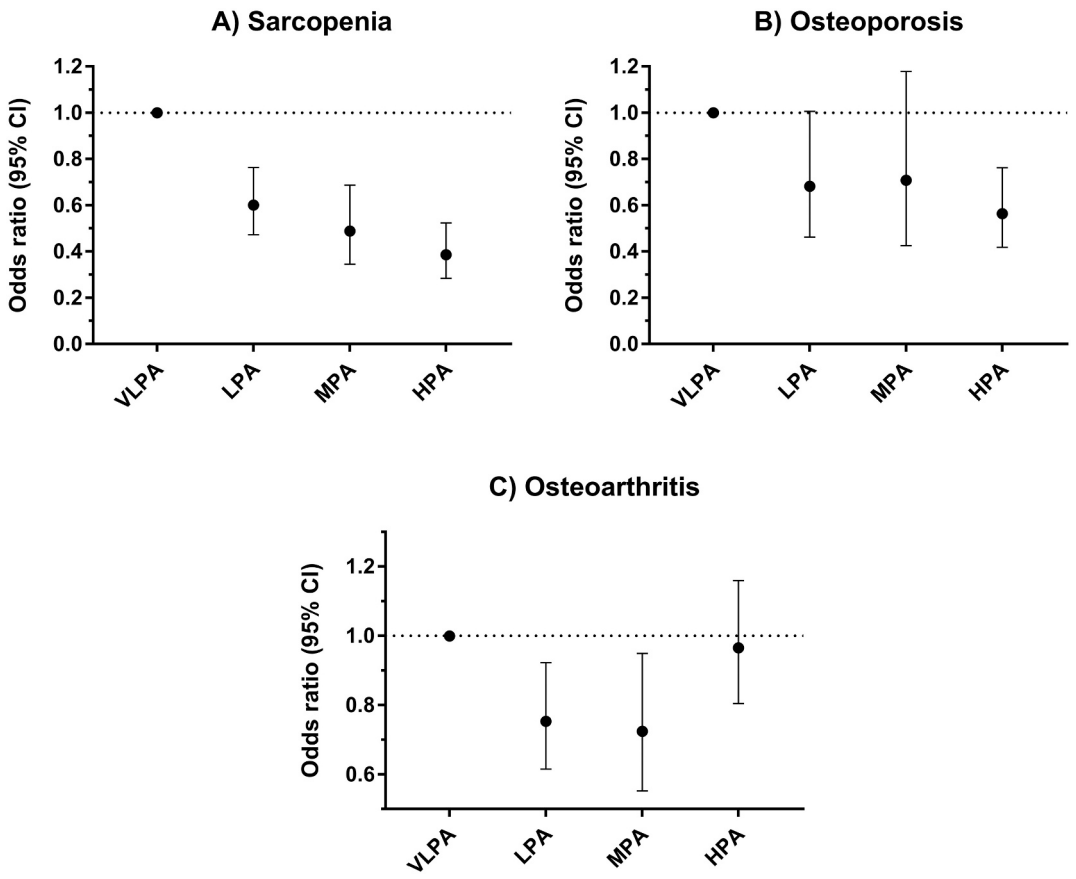

Fig. 1. Odds Ratio (95%CI) for major degenerative diseases according to physical activity level group. Data are representative of non-institutionalized American population.
A) Odds ratio for Sarcopenia B) Odds ratio for Osteoporosis C) Odds ratio for Osteoarthritis.
VLPA: Very Light Physical Activity (<150 MET-min/week); LPA: Light Physical Activity (150–960 MET-min/week); MPA: Medium Physical Activity (961–1800 MET-min/week); HPA: High Physical Activity (>1800 MET-min/week).
All odds ratio were adjusted by age, sex, race/ethnicity, annual household income, educational level, smoking status, alcohol consumption and obesity.

4. Discussion

In this study, we demonstrate that American adults who perform at least 150 MET-min/week of PA presented reduced odds for sarcopenia, those who perform 150–1800 MET-min/week had reduced odds for osteoarthritis, and those who perform more than 1800 MET-min/week had reduced odds for osteoporosis. It seems to be indicating that performing around 1800 MET-min/week (i.e. 64 min/day of moderate PA) is an effective method to prevent some diseases that affect quality of life in a large part of the population over 50 years.

The results observed in our study about the association between sarcopenia and PA levels are in accordance with the main literature findings in this field collected in a recent meta-analysis, which proves that PA protects against sarcopenia and reduces the odds of acquiring it in later life OR = 0.45 (95%CI: 0.37, 0.55, $p < 0.05$) (Steffl et al., 2017). Several previous investigations have focus on if people performed or not PA, but few have stratified their results by PA levels based in frequencies of activity per week/month or METs (Ryu et al., 2013; Beavers et al., 2009; Murphy et al., 2014). The present study shows a possible dose-response relationship, results that agree with a study in Koreans conducted by Ryu et al., which manifested that high levels of PA (≥3000 MET-min/week) are better than moderate (>600 MET-min/week) or low (<600 MET-min/week) levels of PA to prevent sarcopenia in men, although not significant results were reported in women (Ryu et al., 2013). On the other hand, according with reports by Beavers et al., and Murphy et al., PA protects against sarcopenia and reduces the odds of acquiring it in later life, but it is not clear if a dose-response relationship exists (Beavers et al., 2009; Murphy et al., 2014). Previous studies suggest that among others, mitochondrial dysfunction is critical on the sarcopenia pathogenesis (Yoo et al., 2018). Overall, PA appears to ameliorate the mitochondrial-related problems associated with sarcopenia, and consequently, PA has a direct impact on muscle mass quality and quantity, increasing muscle strength and muscle mass in older adults (Yoo et al., 2018; Fragala et al., 2015).

In our study, only the most active group (>1800 MET-min/week) is protected against osteoporosis. These results are similar to those obtained by several previous studies, which demonstrates that only the more active participants were protected against osteoporosis and fracture risk (Kim et al., 2019; Morseth et al., 2012). Kim et al., showed that compared to no activity group, the odds for having osteoporosis were 0.40 (95%CI: 0.16, 0.99, $p < 0.05$) and 0.35 (95%CI: 0.14, 0.90, $p < 0.05$) times lower among those men who performed moderate and vigorous PA respectively, but no differences were found between those walking (low level of PA) and no activity group (Kim et al., 2019). Morseth et al., concluded that compared with those who did not perform PA, only the most active men and women, (those who performed either light or hard PA at least 3 h/week), had respectively 40% (HR = 0.60, 95%CI: 0.41, 0.90) and 26% (HR = 0.74, 95%CI: 0.58, 0.94) reduced fracture risk in the hip (Morseth et al., 2012). On the other hand, some studies reported no differences in the protective effect among different PA levels (Mackey et al., 2011). These literature discrepancies could be explained through differences in the sample (race, sex, age…), differences in the methodology used to assess the PA, and mainly through differences in the type of PA that people usually performed, because of

Table 2
Odds ratio (95%CI) for degenerative diseases according to physical activity levels. Data are representative of non-institutionalized American population.

	Physical activity levels (MET-min/week)			
	VLPA (<150)	LPA (150–960)	MPA (961–1800)	HPA (>1800)
Sarcopenia				
Age-adjusted	1.00 (ref)[abc]	0.57 (0.45, 0.71)	0.43 (0.31, 0.60)	0.33 (0.25, 0.45)
Multivariable-adjusted 1	1.00 (ref)[abc]	0.58 (0.46, 0.73)	0.46 (0.34, 0.63)	0.34 (0.25, 0.46)
Multivariable-adjusted 2	1.00 (ref)[abc]	0.60 (0.47, 0.76)	0.49 (0.35, 0.69)	0.39 (0.29, 0.52)
Osteoporosis				
Age-adjusted	1.00 (ref)[abc]	0.68 (0.48, 0.96)	0.62 (0.39, 0.99)	0.46 (0.34, 0.62)
Multivariable-adjusted 1	1.00 (ref)[c]	0.70 (0.48, 1.01)	0.71 (0.43, 1.17)	0.60 (0.44, 0.81)
Multivariable-adjusted 2	1.00 (ref)[c]	0.68 (0.46, 1.01)	0.71 (0.43, 1.18)	0.57 (0.42, 0.76)
Osteoarthritis				
Age-adjusted	1.00 (ref)[abc]	0.72 (0.60, 0.87)	0.68 (0.52, 0.88)	0.79 (0.67, 0.94)
Multivariable-adjusted 1	1.00 (ref)[ab]	0.69 (0.57, 0.84)	0.66 (0.50, 0.86)	0.86 (0.72, 1.03)
Multivariable-adjusted 2	1.00 (ref)[ab]	0.75 (0.62, 0.92)	0.73 (0.55, 0.95)	0.97 (0.81, 1.16)

VLPA: Very Low Physical Activity; LPA: Low Physical Activity; MPA: Medium Physical Activity; HPA: High Physical Activity.
Model 1 Adjusted by age, sex, race/ethnicity, annual household income and educational level.
Model 2 Additionally adjusted by smoking status, alcohol consumption and obesity.
[a] Significative differences between Very Low and Low physical activity levels.
[b] Significative differences between Very Low and Medium physical activity levels.
[c] Significative differences between Very Low and High physical activity levels.

the potential preventive effect of PA against osteoporosis depends of the biomechanical impact of the activity (Gomez-Bruton et al., 2017). Maybe one possible reason to explain why in our study only the most active group is protected against osteoporosis, is that persons who belong to this group probably perform more impact loading activities other than walking, putting under high mechanical load the bone tissue than the experienced in daily activities (mainly walking). As a previous theory suppose, the mechanical loads induce stimuli in the bone tissue, resulting in bone mass preservation at sites of mechanical stress (Turner, 2006).

The results provided in our study related to the association of PA and osteoarthritis show a beneficial effect of low and medium level of PA (150–1800 MET-min/week), but this association is not present in the most active group (>1800 MET-min/week). Other authors however, demonstrated a protective effect of vigorous intensity PA on knee osteoarthritis (Racunica et al., 2007), but intensity is not the same as dose (combination of PA intensity and amount). Vigorous activities (such as running or jumping) usually implies a contact stress between the adjacent cartilages surfaces of the joint in the range of 4–9 Nm^2, but at least a 25 Nm^2 contact stress is needed to cause an acute disruption in articular cartilage (Vuori, 2001). However, chronic or repetitive stresses less than 25 Nm^2 may cause articular damage or degeneration (Vuori, 2001). This fact suggests that the main problem is in the amount and not in the intensity of PA, so taking into account the results of our study, low to medium levels of PA seems to be more beneficial than nothing or too much PA in this case.

The three major degenerative diseases analyzed in this study that affect a large part of the population over 50 years old, are not only related with functional disability and so with an impairment in the quality of life (Martel-Pelletier et al., 2016; Greco et al., 2019). In addition, several studies related these diseases with mortality (Martel-Pelletier et al., 2016; Batsis et al., 2014; Johnston and Dagar, 2020). Moreover, the economic burden in the United States due to these diseases are $18.5 billion for sarcopenia, $17–25 billion for osteoporosis, and $193.9 billion for osteoarthritis (Janssen et al., 2004; Burge et al., 2007; Zhao et al., 2019). Therefore, PA can improve both, life expectancy and quality of life. Furthermore, the beneficial effect associated to PA would have an expected impact on the national economic burden that these diseases have.

This study has the strength of the excellent external validity provided by the complex sample design of NHANES, the use of multiple survey periods for each disease, and the use of high-quality data collection methods to assess body composition variables. However, several limitations should be acknowledged in our study. First, the cross-sectional analysis does not allow us to establish a causal temporal link between PA level and the major degenerative diseases analyzed. Second, PA was analyzed taking into account the amount and the intensity, but without taking into account the type of activity performed, and as mention above, this could be a relevant aspect in some degenerative diseases. Third, although data collection about osteoarthritis and PA have been carried out by trained interviewers, the use of self-reported information could be subject to bias. Fourth, only non-institutionalized adults are included in this analysis, so the results can only be applied to this population.

5. Conclusions

The results of the study sustain that PA is associated with lower odds for sarcopenia, osteoporosis and osteoarthritis, three of the major degenerative diseases that affect a large part of the population over 50 years in the United States. Although different doses of PA seem to be more beneficial to prevent each disease, none of the doses analyzed are associated with harmful effects. Overall, PA is effective to prevent these diseases, but the most beneficial dose will depend on conditions of each individual. This implies that public health strategies should focus on PA promotion.

Funding

JLPL received a Grant FPU 2016 (FPU16/02539) from the Ministerio de Educación Cultura y Deporte (Spain).

CRediT authorship contribution statement

Name	Location	Role	Contribution
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2021.111656.

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5.4 Artículo IV

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A cross-sectional analysis of the association between physical activity, depression, and all-cause mortality in Americans over 50 years old

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Depression is estimated to be the second leading cause of disability in the United States and is associated with a 52% increased risk of death. Lifestyle components may have an important role in depression pathogenesis. The aims of this study were to analyze the association of meeting the physical activity (PA) recommendation guidelines and depression, and to analyze the all-cause mortality risk of the joint association of PA and depression. This cross-sectional study included 7201 participants from the 2007–2014 National Health and Nutrition Examination Survey aged ≥ 50 years and linked to National Death Index records through December 31, 2015. Depression was defined as a score ≥ 10 using the Patient Health Questionnaire (PHQ-9). PA was self-reported, and total PA was used to classify participants as more active (≥ 600 MET-min/week) or less active (< 600 MET-min/week). The odds ratios for depression were examined according to be more active or less active. The hazard ratios (HR) for the association of PA level and depression status with all-cause mortality were examined. Being more active was associated with reduced odds for depression. Compared with less active participants with depression, those who were more active and having depression had HR 0.45 (95% CI 0.22, 0.91, p = 0.026) for all-cause mortality. Being more active is associated with lower odds for depression and seems to be a protective factor against the increased all-cause mortality risk due to depression.

Depression is a common mental disorder affecting more than 264 million people worldwide[1]. In the United States, it is estimated to be the second cause of disability[2], with an increasing trend in non-institutionalized population, mainly in older people[3].

People suffering from depression usually show different neurovegetative, neurocognitive, and emotional symptoms[4]. Among them are low levels of mood, anhedonia, feelings of worthlessness or guilt, fatigue or loss of energy, disruptive appetite, sleep disturbance, and difficulty to think or concentrate[4], which frequently lead to decreased quality of life, disability, suicide ideation, or even suicide attempts[5,6].

Lifestyle components may have an important role in depression pathogenesis. Unhealthy behaviors such as sedentarism, physical inactivity, poor diet, or substance abuse have been associated with a significantly higher risk of depression[7,8], contrary to the protective effect of a healthy lifestyle[8]. Physical activity (PA), defined as any bodily movement produced by skeletal muscles that requires energy expenditure[9], is a cornerstone in the primary prevention of chronic diseases, including depression[8,10,11]. Moreover, evidence also suggests that PA is a recognized strategy in secondary prevention[12] and that PA plays a significant role concerning late-life depression

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consequences, such as suicide[13]. Biological theories about the antidepressant mechanisms of PA are mainly based on the improvement of neuroplasticity, and in the reduction of inflammation and oxidative stress, while psychosocial theories are based on the improvement of self-esteem, social support, and self-efficacy[14].

Although the benefits of PA against depression are well documented[8,10,11], few studies have analyzed whether compliance with PA recommendations[9] is enough to obtain a preventive effect against depression[10]. Furthermore, those studies are mainly focused on young or middle-aged women, health care workers, or college students[10], and only two studies are focused on older people[15,16]. On the contrary, the protective effect of complying with PA recommendations against all-cause mortality in the general population is widely known[9,17]. Evidence also suggests that depression is associated, by itself, with a higher mortality risk, reaching a 52% increased risk of death[18]. Despite the evidence of these associations with mortality, to our knowledge, no study has analyzed the all-cause mortality risk of the joint association of PA and depression in older adults. Therefore, the purpose of this study was to analyze the association of meeting the PA recommendations and depression and to analyze the all-cause mortality risk of the joint association of PA and depression in non-institutionalized, older American adults.

Methods

Study design and population. The National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics (NCHS), is an annual national cross-sectional survey of a representative sample of non-institutionalized United States population. The survey uses a stratified, multistage sample design to randomly select approximately 7000 residents across the country each year. Participation in the survey is confidential and voluntary. Public-use linked mortality files from the National Death Index (NDI) are available for continuous NHANES 1999–2014, providing mortality data from the date of survey participation through December 31, 2015.

The present study used data from 4 cross-sectional NHANES waves conducted from 2007 to 2014 and their linked mortality files. Details about linkage of NHANES data with NDI records have been published elsewhere[19]. For this analysis, sample was reduced to participants ≥ 50 years old who were followed up for mortality outcomes ≥ 12 months after the enrollment in the study to minimize bias from reverse causation (n = 10,908). Participants with missing data on PA (n = 2523), depression (n = 811), and other covariables (n = 373) were excluded, so the final sample included 7201 participants.

All participants provided written informed consent, and all methods were carried out in accordance with relevant guidelines and regulations. The Ethics Review Board of the NCHS approved measurement procedures, data collection, and posting of the data online for public use.

Definition and assessment of depression. Depression was assessed by means of the Patient Health Questionnaire-9 (PHQ-9), a widely-used self-report depression screener that consists of 9 items to assess depressive symptoms over the last 2 weeks[20]. The PHQ-9 score can range from 0 to 27, since each of the 9 items can be scored from 0 (not at all) to 3 (nearly every day)[20]. Scores ≥ 10 represent clinically significant depressive symptoms[21], so for this study, depression has been defined as score ≥ 10 in the PHQ-9. This is a common cut-point that has been used in previous studies[22] and maximized combined sensitivity and specificity[23].

Assessment of physical activity. PA was assessed by interview using the Global Physical Activity Questionnaire (GPAQ) created by the World Health Organization (WHO)[24]. This questionnaire analyzes the usual PA performed in a typical week in 3 different domains (PA at work/domestic, PA in transport/travel, and PA in leisure time), as long as it has been carried out in continuous periods of 10 min. The questionnaire also considers the intensity at which it has been performed (moderate or vigorous). The total metabolic equivalent per minute per week (MET-min/week) was calculated following the GPAQ protocol[25].

Based on PA recommendation guidelines by the WHO[9], the subjects were classified into two different groups. Those who performed at least 150 min of moderate to vigorous PA (≥ 600 MET-min/week) and met the PA recommendations for adults compose the more-active group, and those who performed less than 150 min of moderate to vigorous PA (< 600 MET-min/week) and thus did not meet the recommendations, composed the less-active group.

Mortality. Survival time was counted from the date of survey participation to the date of death or the end of the study follow-up period (December, 31, 2015), whichever came first. In this study all-cause mortality was used as the main outcome for mortality, classifying participants as alive or deceased.

Assessment of additional covariates. Demographic, lifestyle, anthropometric, and health data were obtained and used to adjust the results of regression models. The selection of these specific variables was based on their possible confounding role in the associations analyzed[6,26].

Demographics included age (50–59; 60–69; 70–79; ≥ 80), sex, race/ethnicity (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other (including Multi-Racial)), annual household income (0–19,999 USD; 20,000–44,999 USD; 45,000–74,999 USD, and ≥ 75,000 USD), and educational level (less than 9th grade; 9th–12th grade, no diploma; high school graduate; college or Associate´s degree; and college graduate or above).

Lifestyle risk factors included alcohol consumption in the last 12 months classified as 0 drinks/day, < 2 drinks/day, and ≥ 2 drinks/day, and smoking status was defined as never smoked, former smoker, and smoker.

Anthropometric included body mass index (BMI), calculated as weight in kilograms divided by height in meters squared and classified in < 25.0 kg/m^2, 25.0–29.9 kg/m^2, and ≥ 30.0 kg/m^2. Self-reported medical diagnosis of hypertension, dyslipidemia, or type 1 and 2 diabetes, or self-reported use of antihypertensive medication,

lipid-lowering drugs, or hypoglycemic medication, were used to classify participants as having arterial hypertension, dyslipidemia, and diabetes, respectively.

Statistical analysis. According to the NHANES analytical guidelines, all data were downloaded, merged, and analyzed, incorporating appropriated combined weights, primary sampling unit, and strata provided by NHANES[27]. Moreover, public-use linked mortality files from NDI were merged with NHANES data following the appropriate guidelines[19].

Categorical variables were expressed as frequency (%), and continuous variables were presented as mean and standard error (SE). Descriptive analyses were carried out for the overall samples and divided by PA groups. Logistic regressions models according to the PA group of the participants were conducted to examine the adjusted odds ratios (OR) for depression. The first model was unadjusted, and the second only age-adjusted. Model A was adjusted by age, sex, race/ethnicity, annual household income, and educational level. Model B was additionally adjusted by smoking status, alcohol consumption, BMI, arterial hypertension, dyslipidemia, and diabetes.

Cox proportional hazards regression models were performed to examine hazard ratios (HRs) and 95% CIs for the association between PA level and depression status with all-cause mortality. Furthermore, adjusted survival curves were plotted. When the PA level and depression status joint association with all-cause mortality was analyzed, the less-active (< 600MET-min/week) and with-depression (PHQ-9 ≥ 10) subgroup was considered the reference group when hazard ratios for the three other subgroups were calculated. In this case, the model was adjusted for potential confounders, including age at baseline, sex, race/ethnicity, annual household income, educational level, smoking status, alcohol consumption, BMI, arterial hypertension, dyslipidemia, and diabetes. The proportional hazards assumption was not violated as examined by log–log survival plots and correlations of follow-up time and Schoenfeld residuals from the adjusted Cox models[28].

A two-sided p-value of 0.05 was considered statistically significant. Statistical analysis was performed using SPSS statistical software (ver. 24.0 IBM Corp., Armonk, NY, USA) and R statistical software (ver. 4.0.4).

Results

The overall prevalence of depression in ≥ 50-years-old non-institutionalized Americans was 7.8%. The prevalence among those who met and did not meet the PA recommendations for adults was 5.3% and 11.0%, respectively (Table 1). The more-active group had a lower prevalence of obesity, hypertension, dyslipidemia, and diabetes, as well as higher educational level and annual household income than the less-active group (Table 1).

The likelihood of having depression was lower for those participants in the more-active group compared to those in the less-active group. The weighted odds for having depression after adjusting the results by age, sex, race/ethnicity, annual household income, educational level, alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes were 0.57 (95% CI 0.44, 0.72, $p < 0.001$) for the more-active group compared to the less-active group (Table 2). Additionally, Supplementary Table 1 includes tests of the weighted odds for having depression among three PA levels subgroups: < 600 MET-min/week, 600–1200 MET-min/week, and > 1200 MET-min/week.

In addition, if the total PA was divided according to the different domains analyzed (PA at work/domestic, PA in leisure time, and PA in transport/travel), only those who performed ≥ 600 MET-min/week of leisure-time PA had significantly lower odds for having depression compared to those who performed < 600 MET-min/week of leisure-time PA (OR 0.47, 95% CI 0.32, 0.67, $p < 0.001$) (Supplementary Table 2).

During a median 54.0 months (interquartile range 12–108 months) of follow-up, 655 deaths occurred among 7201 individuals in the study. The percentage of deaths among those who met and did not meet the PA recommendations for adults were 4.1% and 9.4%, respectively (Table 1). Moreover, the percentage of deaths in participants with and without depression were 9.4% and 6.1%, respectively.

When studying HRs for all-cause mortality, those with depression had a 1.55-fold increased HR of death (95% CI 1.18, 2.03, $p = 0.002$) compared to those without depression (Table 3 and Fig. 1a). Moreover, those who performed < 600 MET-min/week had a 1.73-fold increased HR of death (95% CI 1.45, 2.07, $p < 0.001$) compared to those who performed ≥ 600 MET-min/week (Table 4 and Fig. 1b). These HRs were adjusted by age, sex, race/ethnicity, annual household income, educational level, alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes.

When the joint association of depression and PA was analyzed in relation to the risk of all-cause mortality, those who were more active without depression had the lowest risk of death compared to those who were less active and with depression, HR 0.38 (95% CI 0.28, 0.52, $p < 0.001$). Those who were less active without depression, and those who were more active with depression, also had a lower risk of death compared to those who were less active and with depression, 0.63 HR (95% CI 0.46, 0.85, $p = 0.003$), and 0.45 HR (95% CI 0.22, 0.91, $p = 0.026$), respectively (Fig. 2). These HRs were adjusted by age, sex, race/ethnicity, annual household income, educational level, alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes.

Discussion

This study provided evidence that performing at least 150 min/week of moderate to vigorous PA was associated with reduced odds for depression among an American population aged 50 and older. Furthermore, among those with depression, performing 150 min/week of moderate to vigorous PA was associated with a 55.1% reduced risk of all-cause mortality compared to those who performed less PA.

The beneficial effect of PA in depression prevention has been analyzed in-depth through specific systematic reviews, concluding that PA may prevent depression[10,11,29]. Nevertheless, only a few studies were focused on older people[15,16,30–33], and in addition, only two of those studies conducted in European and Asian populations analyzed the relationship between depression and PA assessed as a dose (combined amount and intensity)[15,16]. However,

	Overall	Less active	More active	p value for interaction
N, %	100 (7201)	43.1 (3439)	56.9 (3762)	**< 0.001**
Depression, %	7.8 (676)	11.0 (437)	5.3 (239)	**< 0.001**
PHQ-9 Score, points	2.92 (0.07)	3.62 (0.11)	2.38 (0.08)	**< 0.001**
Deaths, %	6.4 (655)	9.4 (428)	4.1 (227)	**< 0.001**
Age, %				
50–59	43.5 (2403)	37.4 (972)	48.1 (1431)	**< 0.001**
60–69	32.2 (2542)	32.0 (1201)	32.4 (1341)	
70–79	16.5 (1501)	19.5 (809)	14.2 (692)	
> 80	7.8 (755)	11.1 (457)	5.4 (298)	
Male, %	49.7 (3829)	41.7 (1601)	55.8 (2228)	**< 0.001**
Race/ethnicity, %				
Mexican American	3.8 (765)	4.3 (386)	3.4 (379)	**< 0.001**
Other Hispanic	3.2 (660)	3.6 (323)	2.8 (337)	
Non-Hispanic White	80.1 (3769)	77.6 (1727)	82.0 (2042)	
Non-Hispanic Black	9.0 (1581)	10.7 (823)	7.7 (758)	
Other	3.9 (426)	3.7 (180)	4.0 (246)	
Annual household income, %				
0–19,999 USD	13.6 (1592)	17.7 (887)	10.5 (705)	**< 0.001**
20,000–44,999 USD	28.2 (2405)	31.4 (1225)	25.8 (1180)	
45,000–74,999 USD	21.4 (1376)	19.9 (607)	22.6 (769)	
≥ 75,000 USD	36.7 (1828)	31.0 (720)	41.1 (1108)	
Educational level, %				
Less than 9th Grade	4.9 (755)	7.0 (442)	3.4 (313)	**< 0.001**
9th–12th Grade, No diploma	10.3 (1038)	12.9 (596)	8.4 (442)	
High School Graduate	22.4 (1656)	24.0 (820)	21.2 (836)	
College or AA Degree	30.0 (2009)	29.7 (903)	30.2 (1106)	
College Graduate or Above	32.3 (1743)	26.4 (678)	36.7 (1065)	
Alcohol consumers, %				
0 drinks/day	24.1 (2213)	28.9 (1232)	20.5 (981)	**< 0.001**
< 2 drinks/day	67.8 (4490)	64.8 (2022)	70.1 (2468)	
≥ 2 drinks/ day	8.1 (498)	6.3 (185)	9.4 (313)	
Smoking status, %				
Never	45.1 (3102)	43.6 (1431)	46.3 (1671)	**0.046**
Former	38.6 (2845)	38.5 (1367)	38.7 (1478)	
Smoker	16.2 (1254)	17.9 (641)	15.0 (613)	
BMI, %				
< 25	25.3 (1750)	21.5 (745)	28.1 (1005)	**< 0.001**
25–29.9	35.6 (2533)	31.3 (1110)	38.9 (1423)	
≥ 30	39.1 (2918)	47.1 (1584)	33.0 (1334)	
Arterial hypertension, %	51.1 (4056)	57.5 (2136)	46.2 (1920)	**< 0.001**
Dyslipidemia, %	56.1 (4067)	58.7 (2015)	54.1 (2052)	**0.003**
Diabetes, %	16.8 (1573)	21.9 (912)	12.8 (661)	**< 0.001**

Table 1. Baseline characteristics of study participants according to level of physical activity. Data are expressed as weighted percentages and unweighted number of participants for categorical variables, and as weighted mean (standard error) for continuous variables. Less active: performed < 600 MET-min/week; More active: performed ≥ 600 MET-min/week; Other: other race, including Multi-Racial; AA degree: Associate's degree. Significant values are in bold.

controversial findings were reported by these two studies. Mc Dowell et al. conducted a study with more than 7800 participants and supported an association between meeting PA guidelines and lower odds of depression after adjusting the results by age, sex, and BMI (OR 0.56, 95% CI 0.47, 0.66)[16]. On the other hand, Wang et al. did not find an association between meeting PA guidelines (performing 600–2249 MET-min/week of PA) and lower odds of depression (OR 1.02, 95% CI 0.78, 1.33), but found an association between performing more than 2250 MET-min/week of PA and higher odds of depression (OR 1.22, 95% CI 1.01, 1.47)[15]. As Wang et al. discussed, the association of very high levels of PA with a higher risk of depression may be due to the purpose of PA, since higher frequency, longer duration, and larger volume of heavy-labor work may indicate lower household income,

	Physical activity level (MET-min/week)		
	Less active (< 600)	More active (≥ 600)	*p*-value
Unadjusted	1.00 (ref)[a]	0.46 (0.36, 0.57)	< 0.001
Age-adjusted	1.00 (ref)[a]	0.41 (0.33, 0.52)	< 0.001
Multivariable-adjusted Model A	1.00 (ref)[a]	0.52 (0.41, 0.65)	< 0.001
Multivariable-adjusted Model B	1.00 (ref)[a]	0.57 (0.44, 0.72)	< 0.001

Table 2. Odds ratio (95% CI) for depression according to physical activity levels. Data are representative of non-institutionalized American population. Model A is adjusted by age, sex, race/ethnicity, annual household income, and educational level. Model B is additionally adjusted by alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes. [a]Significant differences between Less-active and More-active groups.

	Depression status		
	Without depression	With depression	*p*-value
Unadjusted	1.00 (ref)[a]	1.72 (1.28, 2.30)	0.001
Age-adjusted	1.00 (ref)[a]	2.35 (1.75, 3.16)	< 0.001
Multivariable-adjusted Model A	1.00 (ref)[a]	1.88 (1.40, 2.51)	< 0.001
Multivariable-adjusted Model B	1.00 (ref)[a]	1.55 (1.18, 2.03)	0.002

Table 3. Hazard ratio (95% CI) for all-cause mortality according to depression status. Data are representative of non-institutionalized American population. Model A is adjusted by age, sex, race/ethnicity, annual household income, and educational level. Model B is additionally adjusted by alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes. With depression: scored ≥ 10 in PHQ-9; Without depression: scored < 10 in PHQ-9. [a]Significant differences between without-depression and with-depression groups.

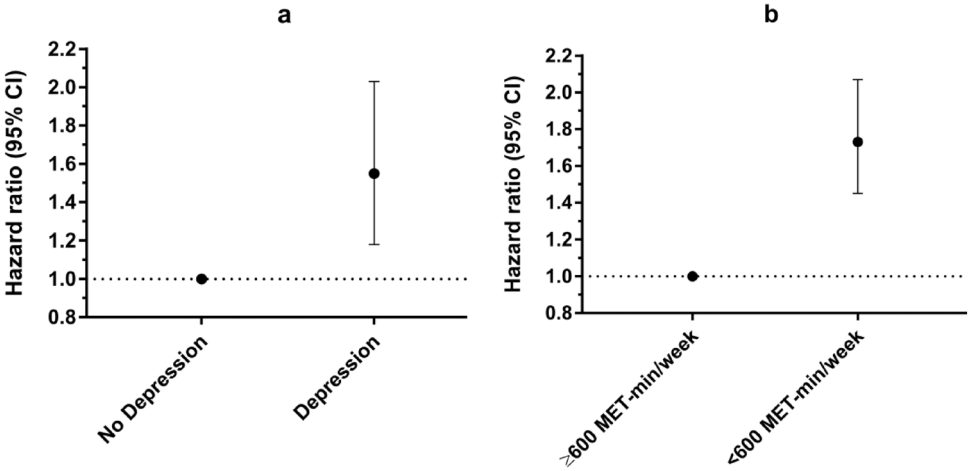

Figure 1. Hazard ratio (95% CI) for all-cause mortality according to (**a**) depression status, and (**b**) physical activity level. Data are representative of non-institutionalized American population. All hazard ratios were adjusted by age, sex, race/ethnicity, annual household income, educational level, alcohol consumption, smoking status, body mass index, arterial hypertension, dyslipidemia, and diabetes.

which leads to increasing the risk of depression[15]. The findings of our study are in line with those of Mc Dowell, reinforcing the idea that meeting PA guidelines can help prevent depression among persons older than 50 years.

As we mentioned above, increasing evidence shows a beneficial effect of PA in depression prevention[10,11,29]. However, the causality and direction of this association have been discussed in the literature, suggesting that PA may protect against depression, and/or depression may result in decreased PA. This could be a source of concern in ascertaining the role of PA in depression prevention. Nevertheless, a meta-analysis of prospective studies, and other recent study using bidirectional mendelian randomization provide evidence to establish a causal relationship between PA and a reduced risk for depression[11,34].

Previous studies have analyzed the association between meeting the PA recommendations in adults and all-cause mortality, establishing that those meeting the recommendations have a 40% decreased risk of death[9,17].

	Physical activity level (MET-min/week)		
	More active (≥ 600)	Less active (< 600)	*p*-value
Unadjusted	1.00 (ref)[a]	2.52 (2.07, 3.06)	< 0.001
Age-adjusted	1.00 (ref)[a]	1.91 (1.58, 2.30)	< 0.001
Multivariable-adjusted Model A	1.00 (ref)[a]	1.85 (1.53, 2.24)	< 0.001
Multivariable-adjusted Model B	1.00 (ref)[a]	1.73 (1.45, 2.07)	< 0.001

Table 4. Hazard ratio (95% CI) for all-cause mortality according to physical activity level. Data are representative of non-institutionalized American population. Model A is adjusted by age, sex, race/ethnicity, annual household income, and educational level. Model B is additionally adjusted by alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes. [a]Significant differences between Less-active and More-active groups.

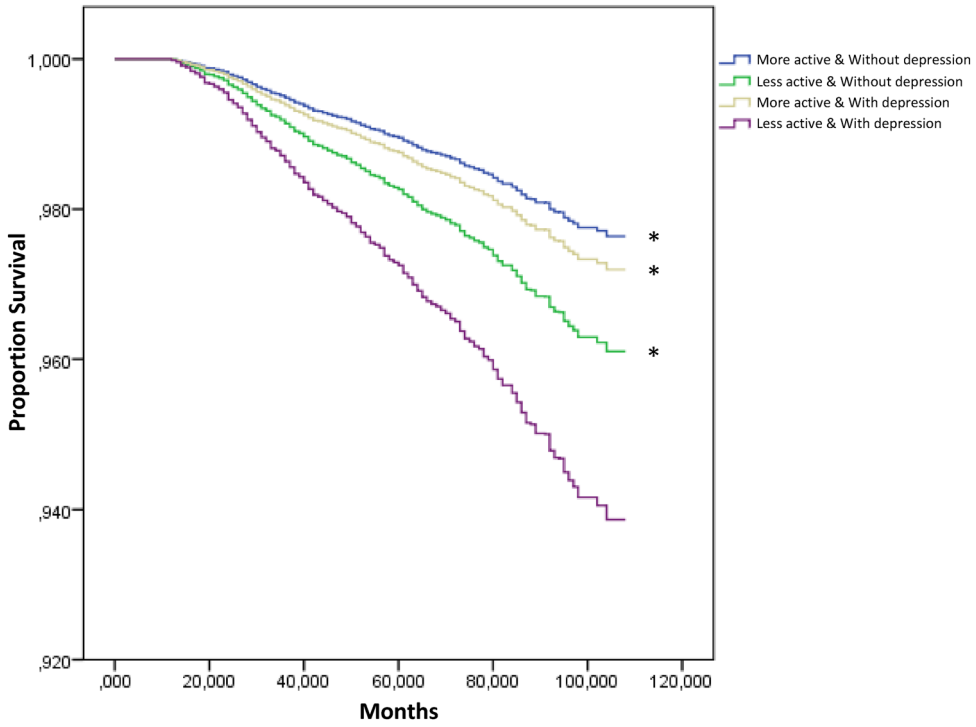

Figure 2. Survival curves for all-cause mortality according to physical activity group and depression status. Data are representative of non-institutionalized American population. Less active: Performed < 600 MET-min/week; More active: Performed ≥ 600 MET-min/week; With depression: Scored ≥ 10 in the PHQ-9; Without depression: Scored < 10 in the PHQ-9. Curves are adjusted by age, sex, race/ethnicity, annual household income, educational level, alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes. *Significant difference with Less active & with depression group.

Other previous studies also have ascertained a positive association between depression and all-cause mortality[18]. The results of our study are consistent with those previous studies, establishing that 150 min of moderate to vigorous PA can be a protective factor against all-cause mortality and that having depression is associated with a higher all-cause mortality risk. However, the present study also analyzes the combined effects of meeting the PA recommendations and depression status with the risk of all-cause mortality among persons older than 50 years. As could be expected, those without depression in the more-active group had the lowest HR for all-cause mortality compared with those with depression in the less-active group. Interestingly, those with depression in the more-active group had a lesser HR than those without depression in the less-active group, compared with the reference group (with depression and less active). This fact reveals that PA could counteract the higher mortality risk due to depression.

Analyzing the influence of PA as a dose (combined amount and intensity) in relation to depression and all-cause mortality, and not only as frequency, as in other studies[22,31,32], is essential. However, it could be interesting to analyze whether the type of PA influences the relationship of PA's preventive role against depression and whether the combined effect of PA and depression status on all-cause mortality is PA-type dependent. Other studies have elucidated the relationship between PA and mortality, finding that it is dependent on the type of PA[17]. Therefore, differentiating at least between endurance and resistance activities in PA quantification may determine if any type

of PA is more protective than others against depression. One previous study has shown that regular flexibility, and no other type of exercise, such as muscular strength or walking, was independently related to depression prevention[22]. However, as mentioned above, in this study, the assessment of PA only as frequency may not show the real role of each PA type in depression prevention[22]. Maybe future studies could shed some light on this issue.

To the best of our knowledge, this is the first study that analyzed the joint association between PA and depression with all-cause mortality in a representative sample of the American population aged 50 and older. However, several limitations should be acknowledged in our study. First, regarding the association of PA and depression, the cross-sectional analysis does not allow us to establish a causal, temporal link. Second, although data collection about PA has been carried out by trained interviewers, the use of self-reported information could be subject to bias[35]. Third, depression status was only assessed once (at baseline), and it was not possible to consider the course of depression. Fourth, to increase the statistical power, only two subgroups of total PA were used to test in combination with depression status, its joint association with mortality. Fifth, only non-institutionalized adults were included in this analysis, so the results can only be applied to this population.

Conclusions

In summary, performing 150 min/week of moderate to vigorous PA is associated with reduced odds for depression and seems to be a preventive factor against the increased all-cause mortality risk due to depression. From a population health perspective, promoting moderate to vigorous PA for at least 150 min/week among Americans aged over 50 years with depression may be an important health-promotion strategy that can reduce the increased all-cause mortality risk associated with depression.

Received: 7 October 2021; Accepted: 11 January 2022
Published online: 10 February 2022

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Acknowledgements

This research was funded by the Aragon Regional Government Fund as reference group (Grupo DGA B12), by fondos FEDER "otra manera de hacer Europa", and by Grant PI19/00948 from the Instituto de Salud Carlos III. J.L.P.L. received a Grant FPU 2016 (FPU16/02539) from the Ministerio de Educación, Cultura y Deporte (Spain).

Author contributions

J.L.P.L. designed and conceptualized the study, analyzed and interpreted the data, and drafted the manuscript for intellectual content. B.M.F. analyzed and interpreted the data and drafted the manuscript for intellectual content. A.G.A. and E.L. interpreted the data and drafted the manuscript for intellectual content. J.A.C. designed and conceptualized the study, interpreted the data, and drafted the manuscript for intellectual content. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-05563-7.

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5.5 Artículo V, actualmente sometido en Atherosclerosis

Cardiorespiratory fitness decreases the odds for subclinical carotid plaques in apolipoprotein *e4* homozygotes

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ABSTRACT

Background and aims: Atherosclerosis is a chronic arterial disease and a major cause of death. Some studies suggest that being apolipoprotein e4 (*APOE e4*) carrier increases the risk of atherosclerosis, and others, that cardiorespiratory fitness (CRF) could play a key role in atherosclerotic prevention. Our aim is to analyze the association of *APOE e4* with carotid atherosclerosis, and the association of CRF with atherosclerosis in *APOE e4* carriers.

Methods: A cross-sectional analysis based on a subsample of 90 participants belonging to the Aragon Workers' Health Study was carried out. Ultrasonography was used to assess the presence of plaques in carotid territory; the submaximal Chester Step Test was used to assess CRF; and behavioral, demographic, anthropometric, and clinical data were obtained by trained personnel during the annual medical examination. *APOE e4e4* participants were categorized into Low-CRF (VO_{2max} <35 mL/kg/min) and High-CRF (VO_{2max} ≥35 mL/kg/min) groups.

Results: After adjusting for several confounders, compared with *APOE e3e3*, those participants genotyped as *APOE e3e4* and *APOE e4e4* had, respectively OR= 1.60 (95%CI: 0.45, 5.71) and OR= 4.29 (95%CI: 1.16, 15.91) for carotid atherosclerosis. Compared to Low-CRF *APOE e4e4* carriers, the odds for having a carotid plaque were 0.09 (95%CI: 0.008, 0.98) times lower among High-CRF *APOE e4e4* carriers.

Conclusions: *APOE e4e4* genotype was associated with higher carotid atherosclerosis, but CRF is a modifiable factor that may be targeted by *APOE e4e4* in order to decrease their increased atherosclerotic risk due to their genetic condition.

Keywords: Fitness; atherosclerosis; cardiovascular disease; carotid arteries; APOE e4; genetics.

1. INTRODUCTION

Atherosclerosis is a chronic arterial disease and a major cause of death. The first functional and pathological changes appear in early- to mid-life and slowly progress through time [1] conditioned by several modifiable behaviors [2,3]. However, other potential non-modifiable risk factors could play an important role in this progression.

Apolipoprotein E epsilon 4 allele (*APOE e4*) is one of the most investigated genetic factor related to cognitive function and also, with cardiovascular disease (CVD) [4–6], probably due to its association with elevated levels of low density lipoprotein cholesterol (LDL-c) and plasma triglycerides. Although the presence of *APOE e4* has been related to increased carotid intima media thickness (cIMT) [7,8], its association with the presence of peripheral atherosclerosis continues to be the subject of debate. In this context, while some studies have reported no associations between *APOE e4* and the presence of atheroma plaques [9–12], others have established that *APOE e4* could be a genetic risk factor, especially in men [13–16].

Cardiorespiratory fitness (CRF) reflects the integrated ability to transport oxygen from the atmosphere to the mitochondria to perform physical work, and is a strong and independent marker of risk for adverse health outcomes, among them all-cause mortality [17]. Moreover, the recent literature suggest that CRF level may be involved in the appearance and development of subclinical atherosclerosis [18,19]. Although several studies have reported an inverse association between CRF and cIMT [20,21], and between CRF and artery calcification [22], only few studies have investigated the association between CRF and subclinical atherosclerosis [18,20], and to our knowledge, no study has investigated this last association in *APOE e4* carriers.

Therefore, the aims of this study were firstly to analyze the association of *APOE e4* carrier status with carotid atherosclerosis, and secondly to analyze the association of CRF with carotid atherosclerosis in *APOE e4* carriers.

2. MATERIALS AND METHODS

2.1. Study participants

This cross-sectional analysis was carried out in a subsample of participants belonging to the Aragon Workers' Health Study (AWHS) [23]. From the total AWHS genotyped sample (n= 5322), those genotyped as *APOE e4e4* (n= 46) were invited to undergo CRF, PA, and subclinical atherosclerosis measurements, and to complete diet and behavior questionnaires. Some of these potential participants refused to participate (n= 16), so a total of 30 participants genotyped as *APOE e4e4* were assessed and randomly matched by age, sex, educational level and smoking habits with 30 participants genotyped as *APOE e3e4* and 30 participants genotyped as *APOE e3e3* who were invited to undergo the measurements and complete the questionnaires. Therefore, the final sample for this analysis was composed of 90 participants. The study was approved by the Clinical Research Ethics Committee of Aragon (CEICA). All participants provided written informed consent.

2.2. Carotid ultrasound

A Philips IU22 ultrasound system (Philips Healthcare, Bothell, WA, USA) was used to assess the presence of plaques in carotid territory at both sides, right and left, which were considered together as a single site, as both side share similar hemodynamics. Ultrasound images were acquired with linear high-frequency 2-dimesional probes (Philips Transducer L9-3, Philips Healthcare), using the Bioimage Study protocol [24]. A plaque was defined as a focal structure that protrudes into the lumen of the carotid artery at least 0.5 mm or ≥50% thicker than the surrounding intima media thickness. All measurements were analyzed using electrocardiogram gated frames corresponding to end-diastole (R-wave) [25]. Presence of subclinical atherosclerosis was defined as the presence of at least 1 plaque in the carotid territory.

2.3. Physical activity

Physical activity (PA) assessment was performed using accelerometers ActiGraph GT3X+ (ActiGraph, Pensacola, FL, USA). Participants were required to wear the device on their non-dominant wrist for 7 consecutive days. Accelerometers were initialized to record accelerations at 30 Hz with a dynamic range of ±6 G. The acceleration records were downloaded and processed with the ActiLife v.6.13.4 software (ActiGraph,

Pensacola, FL, USA). Time in sedentarism (SED) and PA intensities was classified using previously proposed thresholds by Montoye [26] in the non-dominant wrist: (a) SED: ≤2859 counts/min, (b) light PA (LPA): 2860 – 3940 counts/min, (c) moderate PA (MPA): 3941 – 5612 counts/min, (d) vigorous PA (VPA): ≥5613 counts/min, (e) moderate to vigorous PA (MVPA): ≥3941 counts/min. Participants records were valid when they included at least 10 h/day during ≥4 days, requiring at least 3 weekdays and 1 weekend day.

2.4. Cardiorespiratory fitness assessment

CRF assessment was performed through a submaximal multistep test, the Chester Step Test (CST) [27]. The CST required participants to step up and down a single step to a metronome beat (on a prerecorded audio beat). Stepping started at 15 steps/min for 2 min (first level) and increasing 5 steps/min each 2 min until the five level (end of the test) or until participants reached a heart rate of 80% of the predicted maximum (220 - age), whichever came first [27]. At the end of each level heart rate and rating of perceived exertion were recorded. Maximum oxygen uptake (VO_{2max}) was predicted by plotting the recorded heart rates on a graphical datasheet, where a visual line of best-fit is drawn between data points, projecting the line up to predicted maximum heart rate and then estimating the matching oxygen uptake value. Among the 30 *e4e4* carriers, two CRF groups were created using the median as cut-off, the Low-CRF group (VO_{2max} ≤35 mL/kg/min) and the High-CRF group (VO_{2max} >35 mL/kg/min).

2.5. APOE genotyping

DNA isolation from whole blood was performed by using the FlexiGene DNA AGF3000 kit (Qiagen, Valencia, CA, USA) on an AutoGenFlex 3000 workstation (Autogen, Holliston, MA, USA) and genotyping was carried out in the Genetics Unit-Parque Científico de Madrid (Madrid, Spain). DNA samples were spotted onto 384 plates using a Beckman BioMek 2000 automated liquid handler (Beckman High Wycombe, UK) and diluted in a mix consisting in TaqMan Genotyping MasterMix (Applied Biosystems, Foster City, California) and a mixture of pre-made TaqMan SNP genotyping assays; C_3084793_20 (rs429358) and C_904973_10 (rs7412) (Applied Biosystems). qPCR reactions were made in a HT7900 Fast Real-Time PCR System (Applied Biosystems) and SDS 2.4 software (Applied Biosystems) used for genotype calling. Samples with known

genotypes as well as negative amplification blanks were included within each run to serve as positive/negative controls and assist genotyping.

2.6. Mediterranean diet adherence

Diet was assessed by a 136 items semi-quantitative food frequency questionnaire (FFQ) previously validated in Spain [28]. The Alternate Mediterranean Dietary Index (aMED) score was calculated based on a scale including nine components: whole grains, vegetables (excluding potatoes), fruits (juices included), legumes, nuts, fish, ratio of monounsaturated fats to saturated fats, red and processed meats, and alcohol. The total aMED score range from 0 to 9, with higher scores reflecting higher Mediterranean diet adherence.

2.7. Sociodemographic, clinical and biological data

Study participants reported age, sex, and educational level. Clinical data included weight, height, body mass index (BMI), blood pressure, medical history, and the current use of medication. Laboratory measurements were performed on blood samples collected in fasting conditions (>8 h). Total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides, and fasting serum glucose concentrations were determined by enzyme analysis using the ILAB 650 analyzer from Instrumentation Laboratory (Bedford, MA, USA). Non-HDL-c was calculated by subtracting the HDL-c value from the total cholesterol. LDL-c was calculated using the Friedewald formula [29] when the triglyceride levels were <400 mg/dL. We defined arterial hypertension as having systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or self-reported use of antihypertensive medication [30]. Dyslipidemia was defined as having total cholesterol ≥240 mg/dL, LDL-c ≥160 mg/dL, HDL-c <40 mg/dL, or self-reported use of lipid-lowering drugs [31]. Diabetes was defined as fasting plasma ≥126 mg/dL or self-reported treatment with hypoglycemic medication [30]. Smoking habits were categorized as current smoker if the participant reported having smoked in the last year, former smoker if the participant having smoked at least 50 cigarettes in his lifetime, and never smoker.

2.8. Statistical analyses

The continuous variables will be presented as mean and standard deviation (SD), and the categorical variables as percentage and number. Presence of atherosclerotic plaque in carotid arteries was fitted with logistic regression models depending on genotype and

adjusted for BMI, hypertension, dyslipidemia, diabetes, MVPA, VO_{2max}, and aMED score. Odds ratios (ORs) for the presence of carotid plaques for each genotype group were calculated using as reference the *APOE e3e3* genotype. Also ORs for the presence of carotid plaques for each CRF group were calculated in *APOE e4e4* carriers using as reference the Low-CRF group. A two-sided p-value lower than 0.05 was considered statistically significant. Before conducting the analyses, and after considering the missing at random or missing completely at random nature, participants with missing data in VO_{2max} (n=13) or MVPA (n=8) (Supplementary Table 1) were imputed using expectation maximization algorithm [32]. The variables used to impute missing data were the genotype, age, sex, educational level, smoking status, BMI, VO_{2max}, and a compendium of accelerometry variables related to physical activity and sedentary behavior. Statistical analysis was performed using SPSS statistical software ver. 24.0 (IBM Corp, Armonk, NY, USA).

3. RESULTS

The sample included 90 men (mean age 60.0; SD 4.5 years). The 30 *APOE e4e4* participants had higher systolic blood pressure than *APOE e3e3* participants, as well as higher adherence to Mediterranean diet than *APOE e3e4* participants (Table 1).

3.1. Odds ratios for carotid atherosclerosis by genotype

At least one carotid plaque was present in 48 participants (53.3% of the overall sample), 36.7% of the *APOE e3e3* group, 60.0% of the *APOE e3e4* group, and 63.3% of the *APOE e4e4* group (Table 1). The odds for having a carotid plaque were 4.29 times higher (95%CI: 1.16, 15.91, $p<0.05$) among those genotyped as *APOE e4e4* than those genotyped as *APOE e3e3* after adjusting for BMI, hypertension, dyslipidemia, diabetes, MVPA, VO_{2max}, and aMED score (Table 2).

Although the odds for having a carotid plaque also tended to be higher in the *APOE e3e4* participants than in the *APOE e3e3* participants (Adjusted ORs 1.60; 95%CI: 0.45, 5.71, $p=0.46$), the difference was not statistically significant (Table 2).

3.2. Odds ratios for carotid atherosclerosis by CRF in *APOE e4e4* participants

Among the 15 *APOE e4e4* participants who were in the Low-CRF group, 12 presented at least one carotid plaque, and among the 15 *APOE e4e4* participants who were in the High-

CRF group, at least one carotid plaque was present in 7 participants (Table 3). The odds for having a carotid plaque were 0.09 times lower (95%CI: 0.008, 0.98, p<0.05) among those *APOE e4e4* who present High-CRF (VO_{2max} >35 mL/kg/min) than those *APOE e4e4* who present Low-CRF (VO_{2max} ≤35 mL/kg/min) after adjusting for age, BMI, hypertension, dyslipidemia, diabetes, smoking status, MVPA, and aMED score (Table 3).

In order to test the robustness of the results related to CRF as a potential protective factor against carotid plaques in *APOE e4e4* carriers, a sensitivity analysis was conducted with the *APOE e4e4* participants with no imputed VO_{2max} (n=26) (Table 4). After createing new CRF groups using the median VO_{2max} of these 26 participants as cut-off (median VO_{2max} 34.3 mL/kg/min), ORs for carotid plaque were studied. The odds for having a carotid plaque were 0.003 times lower (95%CI: 0.0001, 0.89, p<0.05) among those *APOE e4e4* who present High-CRF (VO_{2max} >34.3 mL/kg/min) than those *APOE e4e4* who present Low-CRF (VO_{2max} ≤34.3 mL/kg/min) after adjusting for age, BMI, hypertension, dyslipidemia, diabetes, smoking status, MVPA, and aMED score (Table 4).

4. DISCUSSION

This cross-sectional analysis of middle-aged men sustains that being *APOE e4* homozygote is associated with higher odds for the presence of carotid plaque compared with the *APOE e3* homozygotes independently of other CVD and lifestyle risk factors. Besides, in *APOE e4* homozygotes, a higher CRF was associated with lower odds for carotid atherosclerosis plaques compared with a lower CRF.

Our results about the association between the *APOE* genotype and carotid plaques are in accordance with previous studies [13,15,16], but only two of them reported results for *APOE e4e4* and *APOE e3e4* carriers separately, and not for the overall *APOE e4* carriers (*APOE e4e4* and *APOE e3e4* together) [13,16]. The results of our study are similar to those observed by Debette et al., which proves that *APOE e4* homozygotes present higher odds for carotid plaque than *APOE e3* homozygotes (Adjusted ORs 2.12; 95%CI: 1.27, 3.53, p=0.004), and that despite the odds for carotid plaque in *APOE e3e4* carriers in relation to *APOE e3* homozygotes tend to be higher, the association is not statistically significant (ORs 1.08; 95%CI: 0.93, 1.25, p=0.33) [13]. Also, in the same line, Beilby et al. reported a linear trend with increasing risk of carotid plaque per unit change in

genotype among men with *APOE e3e4* and *APOE e4e4* in relation with those *APOE e3e3* men (ORs 1.72; 95%CI: 1.05, 2.80, p=0.03) [16].

On the other hand, as mentioned above, other studies reported no associations between *APOE e4* and atherosclerotic carotid plaques after adjusting by major confounders [9–12]. As previous studies supports, adjusting the results by important confounders such as smoking or hypertension seems to be crucial [33]. Yet, most of these studies have not adjusted their results by relevant and usually overlooked factors such as PA, CRF, or adherence to some specific dietary patterns. This omission could be relevant for the reproducibility of the results [19,34]. Also, although the total sample included in these studies seems to be reasonable, the number of participants genotyped as *APOE e4e4* is very scarce. For example, only 10 participants among the total sample (n=544) were *APOE e4e4* carriers in the Djoussé et al. study [12]. Likewise, most of these studies are mainly limited to present data separately for *APOE e3e4* and *APOE e4e4* carriers [9,10,12], and as support this study and the conducted by Debette et al., only the *APOE e4e4* polymorphism is mainly associated with higher odds for carotid plaque [13].

The health benefits of a high CRF in the general population are well described in the literature [17,35]. However, the role of CRF in atherosclerosis development is under-examined. Only two studies have focused on the association of CRF with carotid plaques, finding an inversely association [18,20]. Although higher CRF seems to be a protective factor against atherosclerosis in the general population [18,20], no study has investigated this potential effect in subjects with increased genetic risk of atherosclerosis, *APOE e4e4* carriers. To the best of our knowledge, this is the first study that has investigated the association of CRF and carotid plaques specifically in *APOE e4e4* carriers, finding that higher CRF is associated with decreased odds for atherosclerosis. Bearing in mind that atherosclerosis represents an intermediate step towards CVD and death, this finding has high clinical and public health significance.

This study was of modest sample size but benefited from significant inclusion of a significative amount of *APOE e4e4* carriers and other matched participants by randomization. Besides, the study has the strength of a high-quality data collection methods to obtain information on subclinical atherosclerosis and other variables. However, several limitations should be acknowledged in our study. First, the cross-

sectional design does not allow us to establish causal temporal links. Second, the sample includes only Southern European men, and therefore, these results may not be generalizable to women and other race or ethnic groups of population. Third, CRF assessment was performed using a previously validated but indirect technique, therefore VO_{2max} was estimated and not directly measured.

In conclusion, the *APOE e4e4* but not the *APOE e3e4* genotype was associated with higher carotid atherosclerosis prevalence in men independently of traditional and other atherosclerotic risk factors, supporting the previous observations that had elucidated this association. On the other hand, CRF is a modifiable physiological attribute that may be targeted by *APOE e4e4* carriers in order to decrease their increased atherosclerosis risk due to their genetic condition.

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Financial support:

JLPL received a grant FPU 2016 (FPU16/02539) from the Ministerio de Educación Cultura y Deporte (Spain).

Author contributions:

Conceptualization, JLPL and BMF; methodology, JLPL, JAC, AGA, JMAM, JAC, ML, and BMF; formal analysis, JLPL; data curation; JLPL, JMAM, ML, and BMF; writing-original draft preparation, JLPL; writing-review and editing; JAC, AGA, JMAM, JAC, ML, and BMF.

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Table 1 Baseline characteristics of study participants according to *Apolipoprotein E* genotype.

	Overall	***APOE e3e3***	***APOE e3e4***	***APOE e4e4***	**p-value**
N	90	30	30	30	
Age, years	60.0 (4.5)	60.4 (3.6)	60.0 (3.8)	59.7 (5.8)	0.842
BMI, kg/m^2	27.4 (3.0)	27.4 (2.9)	27.6 (3.5)	27.3 (5.8)	0.934
Total cholesterol, mg/dL	219.6 (40.4)	214.6 (43.1)	220.5 (43.9)	223.7 (34.6)	0.690
HDL-c, mg/dL	50.4 (10.1)	51.7 (10.3)	49.8 (11.4)	49.6 (8.7)	0.694
Non-HDL-c, mg/dL	169.7 (37.1)	162.9 (39.2)	170.1 (39.7)	176.1 (32.3)	0.405
LDL-c, mg/dL	142.2 (34.3)	138.2 (34.8)	141.4 (34.8)	147.6 (34.0)	0.596
Triglycerides, mg/dL	140.8 (79.5)	124.2 (53.6)	145.6 (62.4)	152.7 (110.4)	0.369
Glucose, mg/dL	104.4 (22.6)	100.3 (21.0)	102.7 (14.4)	110.1 (29.4)	0.229
SBP, mmHg	137.1 (16.8)	133.5 (14.6)	133.8 (16.2)	143.9 (18.0)	**0.024**
DBP, mmHg	85.3 (8.9)	84.4 (7.9)	84.7 (8.4)	87.0 (10.3)	0.459
VO_2max, mL/kg/min	38.8 (7.9)	38.9 (7.8)	40.7 (8.2)	36.8 (7.7)	0.172
MVPA time, min/day	109.6 (81.2)	103.3 (69.3)	112.0 (87.4)	113.5 (87.9)	0.872
Hypertension, %	67.8 [61]	63.3 [19]	70.0 [21]	70.0 [21]	0.816
Dyslipidemia, %	70.0 [63]	63.3 [19]	66.7 [20]	80.0 [24]	0.329
Diabetes, %	12.2 [11]	13.3 [4]	6.7 [2]	16.7 [5]	0.484
Carotid plaque, %	53.3 [48]	36.7 [11]	60.0 [18]	63.3 [19]	0.079
aMED score, points	4.12 (1.8)	4.07 (1.5)	3.50 (2.1)	4.80 (1.6)	**0.019**

BMI: body mass index; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; MVPA: moderate to vigorous physical activity; aMED score: alternate Mediterranean dietary index. Values are mean (SD) or % [number].

Table 2 Odds ratio (95%CI) for the presence of plaque in carotid territory by *Apolipoprotein E* genotype.

	Genotype		
	APOE e3e3	***APOE e3e4***	***APOE e4e4***
Number with carotid plaque/Total	11/30	18/30	19/30
Unadjusted	1.00 (ref)	2.59 (0.91, 7.34)	2.98 (1.04, 8.53)
Model 1	1.00 (ref)	3.05 (1.01, 9.28)	3.38 (1.08, 10.53)
Model 2	1.00 (ref)	3.08 (1.01, 9.34)	3.42 (1.09, 10.67)
Model 3	1.00 (ref)	3.11 (1.02, 9.49)	3.30 (1.05, 10.34)
Model 4	1.00 (ref)	3.12 (1.02, 9.53)	3.34 (1.06, 10.49)
Model 5	1.00 (ref)	1.60 (0.45, 5.71)	4.29 (1.16, 15.91)

Model 1: Adjusted for body mass index, hypertension, dyslipidemia, and diabetes.

Model 2: Model 1 additionally adjusted for MVPA (min/day).

Model 3: Model 1 additionally adjusted for VO_2max (mL/kg/min).

Model 4: Model 1 additionally adjusted for MVPA (min/day) and VO_2max (mL/kg/min).

Model 5: Model 4 additionally adjusted for aMED score (0-9)

Table 3 Odds ratio (95%CI) for the presence of plaque in carotid territory by cardiorespiratory fitness group in *Apolipoprotein e4e4* carriers (n=30).

	Cardiorespiratory Fitness (CRF)	
	Low-CRF	**High-CRF**
Number with carotid plaque/Total	12/15	7/15
Unadjusted	1.00 (ref)	0.22 (0.04, 1.11)
Model 1	1.00 (ref)	0.13 (0.16, 1.10)
Model 2	1.00 (ref)	0.09 (0.008, 0.98)

Low-CRF: VO_2max ≤ 35 mL/kg/min; High-CRF: VO_2max > 35 mL/kg/min.

Model 1: Adjusted for age, body mass index, hypertension, dyslipidemia, diabetes and smoking status (current smoker, no-smoker, and former smoker).

Model 2: Additionally adjusted for MVPA (min/day) and aMED score (0-9).

Table 4 Odds ratio (95%CI) for the presence of plaque in carotid territory by cardiorespiratory fitness group in *Apolipoprotein e4e4* carriers with no imputed data in VO_2max (n=26).

	Cardiorespiratory Fitness (CRF)	
	Low-CRF	**High-CRF**
Number with carotid plaque/Total	10/13	7/13
Unadjusted	1.00 (ref)	0.35 (0.07, 1.90)
Model 1	1.00 (ref)	0.16 (0.01, 2.61)
Model 2	1.00 (ref)	0.003 (0.0001, 0.89)

Low-CRF: VO_2max ≤ 34.3 mL/kg/min; High-CRF: VO_2max > 34.3 mL/kg/min.

Model 1: Adjusted for age, body mass index, hypertension, dyslipidemia, diabetes, and smoking status (Current smoker, no-smoker, and former smoker).

Model 2: Additionally adjusted for MVPA (min/day) and aMED score (0-9).

6. Discusión global

El estilo de vida y en definitiva todos los componentes que lo modulan y definen, parecen jugar un papel clave y de vital importancia en la disminución de la probabilidad de padecer diferentes ENT asociadas al proceso de envejecimiento, incluso cuando en algunos casos los sujetos presentan una mayor predisposición genética para ello.

La AF es uno de los pilares fundamentales del estilo de vida y que sin duda, como hemos podido comprobar en los diferentes manuscritos que componen esta Tesis Doctoral y en la literatura científica, influye en la salud de las personas (65–68,75). Realizar AF se asocia con una menor probabilidad de padecer ECV, enfermedades degenerativas, y también enfermedades y trastornos mentales, así como riesgo de muerte.

Ante la abrumadora evidencia científica a favor de la AF, uno de los siguientes retos es determinar la dosis adecuada, entendida como la combinación de frecuencia, duración e intensidad, para mejorar la salud. Esta es una pregunta que los investigadores están tratando de abordar, y al hacerlo es cuando comienzan a surgir las discrepancias en la literatura científica (155,156). Muchos organismos a nivel nacional e internacional han establecido unas pautas en relación a la AF que la población debería hacer para mejorar su salud (75,157,158). Entre ellas, se encuentra la OMS, que elabora y actualiza periódicamente una serie de recomendaciones mundiales de AF en base a la literatura científica de alta calidad disponible relacionada con la AF y diferentes enfermedades (75). Las últimas recomendaciones de la OMS defienden que, aunque cualquier cantidad de AF es beneficiosa, más es mejor, a pesar de asumir a su vez mayores riesgos, aunque existe una cantidad óptima en la que los beneficios obtenidos superan ampliamente los riesgos asumidos. No obstante, esto son recomendaciones generalistas, enfocadas a mejorar la salud en su más amplia definición en un gran público en el que encontramos una amplia heterogeneidad. Por ello, al centrarnos en diferentes subgrupos, casos individuales y

situaciones determinadas en las que se tienen en cuenta otros factores, ya sean genéticos, ambientales o comportamentales, y su interrelación, podría darse el caso de que la cantidad recomendada de AF fuera insuficiente para obtener la mejora esperada, como sucede en diferentes estudios en los que los portadores del genotipo *APOE e4* no obtienen el mismo beneficio de la AF que los no portadores ante el riesgo de DCL o demencia (122,159). Otro buen ejemplo de ello son dos estudios elaborados por Ekelund et al., en los que se relaciona el riesgo de muerte por varias ENT con los niveles combinados de sedentarismo y AF, haciendo evidente que si pasamos mucho tiempo sentados, necesitaremos realizar más AF para obtener el mismo beneficio que una persona que no pasa tanto tiempo sentada (82,84). Por otro lado, también podría darse el caso contrario, en el que una alta cantidad de AF pudiera tener efectos adversos para determinados marcadores de salud, como han evidenciado algunos estudios que han investigado la prevalencia de aterosclerosis según diferentes niveles de AF (160).

A pesar de que la búsqueda de la dosis de AF idónea en función de diferentes factores sea un buen objetivo de investigación y un campo en desarrollo, quizá sea más importante conseguir que la población simplemente haga AF, ya que a pesar de sus ya demostrados amplios beneficios para la salud, un alto porcentaje de la población mundial sigue siendo inactiva (78,79). Sin duda focalizar esfuerzos en fomentar la transferencia de los resultados de las investigaciones a través de diferentes estrategias en función del público objetivo es un aspecto prioritario (161). Por un lado porque así conseguiríamos mejorar la salud de las personas, pero a su vez, también conseguiríamos reducir la carga económica que suponen las ENT a las arcas públicas, cuyo coste se sitúa en Estados Unidos entre 17 y 25 billones de dólares en el caso de la osteoporosis (162), o en 193,9 billones de dólares en el caso de la osteoartritis (163), llegando a alcanzar en su conjunto un gasto del 7,8% del producto interior bruto entre los países miembros de la Unión Europea (164).

En lo relativo al sedentarismo, las recomendaciones para los adultos de los diferentes organismos, se limitan a recomendar reducirlo y sustituirlo siempre que sea posible por cualquier tipo de AF, incluso de baja intensidad (75,157). Cada vez parece más evidente que cuanto menos sedentario se sea, menos riesgos para la salud asumiremos (75,165). Sin embargo, quizá sería interesante ir más allá, y analizar si además de por ejemplo la cantidad de tiempo sentado que uno pasa al día, influye en qué estamos invirtiendo ese tiempo (trabajar, desplazarnos, lectura, tiempo de televisión o pantalla…). Ya hay estudios que han evidenciado cómo el tiempo que pasamos sentados viendo la televisión puede aumentar en mayor medida el riesgo para la salud que el tiempo que estamos sentados realizando otra tarea (82,166). Esto es debido en muchas ocasiones a que el tiempo de televisión habitualmente va acompañado de otras conductas poco saludables (ingesta de alimentos poco saludables, consumo de alcohol…), pero podría darse el caso de que el tiempo sentado invertido en una actividad determinada se asociara a peores consecuencias para la salud por el simple hecho de realizar esa actividad. Sin duda es un tema poco estudiado y que podría merecer especial atención.

Por otro lado, algunos estudios han evidenciado como además del tiempo total de sedentarismo, también es importante analizar si el tiempo total es el sumatorio de muchos comportamientos sedentarios de corta duración interrumpidos por periodos activos, o por el contrario es el sumatorio de pocos comportamientos sedentarios pero mantenidos durante un largo periodo de tiempo (167).

Sin duda en la actualidad la investigación sobre cómo los estilos de vida influyen en el desarrollo de ENT es de vital importancia, y por ello este campo se encuentra en pleno desarrollo. Serán las futuras investigaciones las que vayan aportando nuevo conocimiento al respecto, a través del cual sin duda conseguiremos mejorar la salud y calidad de vida de las personas.

6.1 Limitaciones y fortalezas

A continuación, se van a exponer las diferentes limitaciones encontradas y asumidas, así como las principales fortalezas de los diferentes proyectos y manuscritos que componen esta Tesis Doctoral.

6.1.1 Limitaciones y fortalezas de la revisión sistemática

Quizá la principal limitación de la revisión sistemática fue la baja cantidad de estudios que se encontraron en la literatura relacionados con nuestro objetivo de investigación en los que se aportaran resultados para diferentes subgrupos creados en función del nivel de AF y del genotipo *APOE*.

Por otro lado, cabe destacar la amplia heterogeneidad metodológica existente entre los estudios incluidos a la hora de evaluar la AF. Aunque todos ellos utilizan cuestionarios, en cada estudio se utiliza uno diferente, estableciendo además distintos puntos de corte para crear los grupos por niveles de AF. Esto hace que las comparaciones entre estudios sean más complicadas. A su vez, los diferentes estudios también presentan cierta heterogeneidad a la hora de establecer el criterio diagnóstico del DCL, pero la comparación entre estudios en este caso es más factible dado que son criterios utilizados habitualmente en la práctica clínica para confirmar la presencia de DCL.

También es importante tener en cuenta que la mayoría de los estudios incluidos en la revisión solo especifican si los participantes son portadores del genotipo *APOE e4* o no, pero no especifican si alguno de los portadores del alelo *e4*, es también portador del alelo *e2*, (genotipo *APOE e2e4*). Este aspecto puede ser importante, dado que como sugiere la evidencia científica, ser portador del genotipo *APOE e2* está asociado entre otras cosas con un menor riesgo de DCL (168).

A pesar de estas limitaciones, la revisión sistemática también presenta varias fortalezas, entre ellas el uso de una metodología adecuada basada en las recomendaciones de la guía PRISMA y en la evaluación del sesgo de los estudios incluidos a través de diferentes herramientas adecuadas a sus diferentes diseños metodológicos. También cabe destacar que, a nuestro juicio, y basados en nuestro conocimiento actual, esta es la primera revisión sistemática que combina y compara los resultados de los diferentes estudios publicados que evalúan el riesgo de DCL estratificando sus resultados en función de diferentes subgrupos muestrales creados según el nivel de AF y el genotipo *APOE*.

6.1.2 Limitaciones y fortalezas del proyecto AWHS

El proyecto AWHS también presenta algunas limitaciones, siendo necesario tenerlas en cuenta a la hora de interpretar los diferentes resultados obtenidos a través del mismo. Por otro lado, también presenta varias fortalezas que sitúan al proyecto como un referente en su campo de investigación.

6.1.2.1. Limitaciones

Como se ha indicado en anteriores apartados, el proyecto AWHS surgió con el objetivo de evaluar las diferentes trayectorias de los factores de riesgo cardiovascular tradicionales y emergentes, y su asociación con las anomalías metabólicas y la aterosclerosis subclínica en la población española de mediana edad y libre de cualquier ECV clínica. Sin embargo, la muestra del proyecto se ha compuesto de 5678 trabajadores de una fábrica de automoción, en la que la mayoría de ellos son hombres y de raza caucásica, por lo que en muchos casos, como sucede en los dos manuscritos derivados de este proyecto que forman parte de la presente Tesis Doctoral, la muestra utilizada estaba compuesta tan solo por hombres de raza caucásica. Sin duda este hecho limita la validez externa de los resultados de investigación, haciendo que tan solo sean aplicables a la población de sexo masculino y de raza caucásica.

A su vez, también cabe destacar que algunas de las mediciones y variables registradas en el proyecto fueron autorreportadas por los participantes y que, aunque las entrevistas a través de las cuales fueron obtenidos esos datos fueron efectuadas por personal investigador entrenado para ello, el uso de información autorreportada puede estar sujeto a sesgos debido a la subjetividad y al grado de colaboración que cada participante presenta. Las variables evaluadas a través de esta metodología y que se utilizan en alguno o varios estudios que forman parte de esta Tesis Doctoral son el nivel educativo, la AF y el sedentarismo evaluados desde la primera oleada, el patrón alimentario, y el consumo de diferentes sustancias como el tabaco o el alcohol.

Por otro lado, la evaluación de la CF cardiorrespiratoria se realizó mediante una estimación a través de un test submáximo, que a pesar de estar validado, su uso implica asumir ciertos errores que serían menores en el caso de utilizar otro tipo de metodología. Sin duda una ergoespirometría sería el método más adecuado para valorar este componente de la CF, pero esto aumentaría el tiempo necesario para evaluar a los participantes, y además encarecería el coste de evaluación.

6.1.2.2. Fortalezas

Como una de las principales fortalezas del proyecto podemos encontrar el tamaño muestral, además de la alta calidad metodológica utilizada para evaluar muchas de las variables del estudio. Entre ellas la presencia de aterosclerosis subclínica, o las diferentes variables derivadas de los análisis bioquímicos. A su vez, también cabe destacar la incorporación de la acelerometría en la segunda oleada del proyecto como método adicional y objetivo de evaluación de la AF y sedentarismo.

6.1.3 Limitaciones y fortalezas del estudio NHANES

El estudio NHANES presenta ciertas limitaciones, que tanto los investigadores que utilizan sus datos para generar nuevo conocimiento como los lectores deben tener en cuenta. Por otro lado, un estudio de tal magnitud cuenta con diferentes fortalezas que aseguran una alta calidad.

6.1.3.1 Limitaciones

Una de las principales limitaciones del estudio NHANES es que en muchas de las variables que se registran se utilizan para ello cuestionarios de autorreporte. Este método de evaluación se utiliza en gran cantidad de variables, entre ellas varias de las que se utilizan en manuscritos que componen esta Tesis Doctoral, como la AF, el consumo de alcohol y tabaco, presencia de diferentes enfermedades (hipertensión, hipercolesterolemia, diabetes y osteoartritis), o diferentes variables de corte sociodemográfico, como el nivel educativo o los ingresos anuales del domicilio.

Otra de las limitaciones del estudio es que en cada oleada se selecciona a una nueva muestra representativa de la población de manera aleatoria, por lo que no se realiza un seguimiento de los sujetos que ya han sido evaluados en anteriores fases del estudio. Esto por un lado permite que en cada evaluación se pueda focalizar la atención en determinados subgrupos poblaciones de interés sobremuestreándolos (minorías étnicas, población mayor, etc.), pero a su vez al no hacerse un seguimiento de los mismos sujetos a lo largo del tiempo, no se puede establecer causalidad entre las posibles causas y efectos de interés.

Por último, cabe destacar que en el estudio sólo se incluye población no institucionalizada, por lo que los resultados obtenidos a partir de cualquier estudio que utilice datos del NHANES solo pueden ser extrapolados a la población no institucionalizada.

6.1.3.2 Fortalezas

La principal fortaleza del estudio la encontramos en su diseño muestral, que asegura una buena validez externa gracias a su diseño de muestreo complejo, aleatorio, estratificado y de múltiples etapas. A su vez, cabe destacar la posibilidad de combinar los datos de diferentes oleadas del estudio, que permite aumentar la muestra con la que trabajar, y asegura una buena potencia estadística.

También es destacable la alta calidad metodológica utilizada para obtener algunas de las variables del estudio. En el caso de la composición corporal, el uso del DXA asegura unos errores mínimos en la cuantificación de diferentes variables, siendo además el método de referencia para cuantificar tanto la densidad como la cantidad mineral ósea.

El hecho de que los datos derivados del estudio NHANES se puedan combinar con otras bases de datos como la del NDI que proporciona datos de mortalidad también es de gran interés científico, y sin duda una fortaleza añadida del diseño del estudio.

7. Conclusiones

- **Artículo 1.** La revisión sistemática muestra que la actividad física es un factor protector contra el deterioro cognitivo leve en sujetos portadores del genotipo *APOE e4*, y que por tanto presentan un alto riesgo genético para dicha enfermedad. Estos hallazgos tienen una alta relevancia clínica y de salud pública. Además, los resultados parecen indicar que en portadores del genotipo *APOE e4*, una dosis mayor de actividad física (cantidad y/o intensidad), podría reportar mayores beneficios, pero son necesarios futuros estudios que contrasten estos resultados, ya que la evidencia disponible es limitada.
- **Artículo 2.** Permanecer sentado durante 9 horas o más al día está asociado a una mayor probabilidad de presentar placas de ateroma en las arterias carótidas o en cualquier territorio vascular independientemente del nivel de actividad física y de otros factores de riesgo cardiovascular.
- **Artículo 3.** La actividad física está asociada con una menor probabilidad de presentar sarcopenia, osteoporosis y osteoartritis, tres de las principales enfermedades degenerativas que afectan a gran parte de la población mayor de 50 años. Aunque diferentes dosis de actividad física parecen ser más beneficiosas para cada enfermedad, ninguna de las dosis analizadas se asocia con efectos nocivos. En general, la actividad física parece efectiva para prevenir estas enfermedades, pero la dosis más beneficiosa dependerá de las condiciones de cada individuo.
- **Artículo 4.** Realizar al menos 150 minutos/semana de actividad física moderada – vigorosa (≥600 MET-min/semana) está asociado con una menor probabilidad de presentar depresión, y además parece que es un factor protector frente al aumento

del riesgo de mortalidad por cualquier tipo de causa debido a la depresión. Desde una perspectiva de salud poblacional, promover la actividad física moderada – vigorosa durante al menos 150 minutos/semana entre la población mayor de 50 años con depresión puede ser una estrategia importante para reducir el riesgo de muerte.

- **Artículo 5.** El genotipo *APOE e4e4* pero no el *APOE e3e4* está asociado con una mayor presencia de placas de ateroma en las arterias carótidas en hombres independientemente de otros factores de riesgo como la actividad física, la condición física y la adherencia a determinados patrones alimentarios. Por otro lado, una mayor condición física cardiorrespiratoria está asociada a una menor probabilidad de presentar placas de ateroma en las arterias carótidas entre los portadores del genotipo *APOE e4e4*. Esto supone que la condición física es un factor fisiológico modificable a través del que los portadores del genotipo *APOE e4e4* pueden disminuir su riesgo incrementado de aterosclerosis debido a su condición genética.

7. Conclusions

- **Manuscript I.** The systematic review support the idea that physical activity is a protective factor against cognitive decline in individuals of high genetic risk, specifically *APOE e4* carriers. These findings have high clinical and public health significance. Moreover, the results suggest that in this population, a higher dose of physical activity (amount and/or intensity) might have greater benefits, but it would be necessary to carry out further studies that would allow these findings to be contrasted, since the existing evidence is limited.
- **Manuscript II.** Remain seated for 9 hours/day or more, is associated with higher odds for carotid and any vascular territory plaque development independently of physical activity levels and other cardiovascular risk factors.
- **Manuscript III.** Physical activity is associated with lower odds for sarcopenia, osteoporosis and osteoarthritis, three of the major degenerative diseases that affect a large part of the population over 50 years old. Although different doses of physical activity seem to be more beneficial to prevent each disease, none of the doses analysed are associated with harmful effects. Overall, physical activity is effective to prevent these diseases, but the most beneficial dose will depend on conditions of each individual.
- **Manuscript IV.** Performing at least 150 min/week of moderate to vigorous physical activity (≥600 MET-min/week) is associated with reduced odds for depression and seems to be a preventive factor against the increased all-cause mortality risk due to depression. From a population health perspective, promoting moderate to vigorous physical activity for at least 150 min/week among individuals

aged over 50 years old with depression may be an important strategy to reduce the mortality risk.

- **Manuscript V.** The *APOE e4e4* but not the *APOE e3e4* genotype was associated with higher carotid atherosclerosis in men independently of atherosclerosis risk factors such as physical activity, fitness and adherence to some dietary patterns. On the other hand, a higher cardiorespiratory fitness is associated with lower odds for carotid plaques among the *APOE e4e4* carriers. This implies that cardiorespiratory fitness is a modifiable physiological attribute that *APOE e4e4* carriers may modify in order to decrease their increased atherosclerosis risk due to their genetic condition.

8. Futuras líneas de investigación

Las futuras líneas de investigación derivadas de los estudios que componen esta Tesis Doctoral se pueden dividir en varios apartados: a) estudiar la asociación de diferentes estilos de vida con otras ENT, b) estudiar los efectos dosis-respuesta de diferentes estímulos combinados (AF y sedentarismo) ante las diferentes ENT analizadas u otras nuevas, c) desarrollar propuestas de intervención mediante EF en sujetos con un alto riesgo genético de desarrollar diferentes ENT.

Fomentar y generar conocimiento sobre la investigación y el desarrollo del posible rol protector de diferentes niveles de AF frente a diferentes ENT no es un campo de investigación novedoso o innovador, pero sin duda todavía quedan aspectos por explorar, o que han sido poco explorados. Aún más evidente es el caso del sedentarismo, cuyo papel en la fisiopatología de las ENT quizá haya sido menos estudiado por el momento. Un campo emergente y en el que en los últimos años la comunidad científica ha mostrado especial interés es en investigar si una misma cantidad absoluta de AF o sedentarismo, tiene las mismas consecuencias ante diferentes marcadores de salud si se lleva a cabo de manera ininterrumpida o en diferentes periodos temporales interrumpidos por otros comportamientos. Este sin duda es un aspecto muy interesante, ya que se tiene una perspectiva más veraz del comportamiento real de las personas.

Un paso más allá se situaría la investigación que combina diferentes interacciones entre los componentes del estilo de vida ante diferentes marcadores de salud, teniendo en cuenta los diferentes patrones en función de la dosis-respuesta. Es cierto que ya existen investigaciones al respecto que por ejemplo han estudiado cómo influye la combinación de diferentes cantidades de sedentarismo y AF en el riesgo de muerte, pero sin duda es un

campo en desarrollo en el que debemos profundizar utilizando como variable resultado el desarrollo de diferentes ENT. Ampliar el conocimiento científico en este campo es clave, ya que a través de él, podremos ser más efectivos en el campo de la prevención primaria y por tanto en mejorar la salud de las personas.

Por último, sería interesante ampliar el conocimiento científico en base a la influencia de diferentes programas de ejercicio en el riesgo de desarrollar ENT en personas que presenten un riesgo incrementado para ello por alguna condición genética. En el caso del genotipo *APOE e4*, es necesario ampliar los conocimientos sobre la influencia de los diferentes componentes del estilo de vida en enfermedades mentales y cardiovasculares.

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Apéndice

Características de las revistas [Journal characteristics]

Factor de impacto y clasificación de cada revista en el "*ISI Web of Knowledge – Journal Citation Reports"* dentro de sus áreas correspondientes.

[*Impact factor and ranking of each journal in "ISI Web of Knowledge – Journal Citation Reports" within their subject categories.]*

Artículos publicados [Published manuscripts]

Artículo *[Manuscript]*	Revista *[Journal]*	Factor de impacto *[Impact factor]*
I	International Journal of Environmental Research and Public Health JCR 2020 (Public, environmental & occupational health - Social Sciences Citation Index): 41/176 – Q1 JCR 2020 (Public, environmental & occupational health - Science Citation Index Expanded): 68/203 – Q2 JCR 2020 (Environmental sciences): 118/274 – Q2	**3,390**
II	Journal of Clinical Medicine JCR 2020 (Medicine, General & Internal - Social Citation Index Expanded): 39/167 – Q1	**4,241**
III	Experimental Gerontology JCR 2020 (Geriatrics & Gerontology - Science Citation Index Expanded): 23/53 – Q2	**4,032**
IV	Scientific Reports JCR 2020 (Multidisciplinary Sciences - Science Citation Index Expanded): 17/72 – Q1	**4,380**

Artículos sometidos [Submitted manuscripts]

Artículo *[Manuscript]*	Revista *[Journal]*	Factor de impacto *[Impact factor]*
V	Atherosclerosis JCR 2020 (Peripheral Vascular Disease - Science Citation Index Expanded): 14/65 – Q1 JCR 2020 (Cardiac & Cardiovascular Systems - Science Citation Index Expanded): 43/142 – Q2	**5,162**

Contribución del doctorando en el proyecto AWHS

El proyecto de cohorte prospectivo AWHS está compuesto por diferentes evaluaciones generales de diferentes aspectos a toda la cohorte, y evaluaciones más específicas sobre determinados parámetros a diferentes submuestras. El doctorando ha podido participar en la planificación de diferentes pruebas a realizar, la recogida de datos, la confección de las bases de datos, y el análisis y explotación de datos nuevos y de evaluaciones anteriores.

El doctorando ha sido el encargado de llevar a cabo en una submuestra las evaluaciones de la condición física a través de diferentes test máximos y submáximos, la evaluación de la actividad física, sedentarismo y sueño a través de la acelerometría, y la evaluación de diferentes hábitos alimentarios y comportamentales, así como la función cognitiva a través de diferentes cuestionarios.

Una vez obtenidos los datos de la nueva submuestra, y con datos existentes previamente todavía no explotados, el doctorando confeccionó las bases de datos y verificó la ausencia de errores de manera previa al comienzo de los diferentes análisis llevados a cabo. A partir de dichos análisis, se han elaborado diferentes artículos que componen parte de la presente Tesis Doctoral.

Agradecimientos

El contenido de este documento es un buen resumen de los logros de más de cuatro años de investigación y formación desde que comenzaron mis andanzas como doctorando en el grupo de investigación GENUD y en el proyecto AWHS. En este documento aparece reflejado el producto final, pero el proceso para llegar hasta aquí y que en muchos casos pasa desapercibido es lo que realmente considero mi Tesis Doctoral. Quedaría muy bien decir que todo ha sido fácil desde el principio, pero no nos vamos a engañar, no todo ha sido un camino de rosas. No me cabe la menor duda de que sin mis directores de tesis y sin mis compañeros y amigos jamás habría logrado llegar tan lejos, por ello, no tengo más que palabras de agradecimiento hacia ellos.

Para ***José Antonio***, porque desde que fue mi profesor en 3º de carrera noté como despertaba en mi el interés por la investigación, por poner en duda mis conocimientos y los nuevos aprendizajes. Recuerdo una clase en la que nos vendiste tan bien el doctorado "Son 4 años en los que os lo pasáis muy bien, tenéis un sueldo decente mientras os formáis, viajáis y acabáis con un buen currículum", que al terminar tenía claro que yo tenía que intentar tirar por allí, y que quería que fuera contigo. En la llegada a buen puerto de todo esto tu tienes gran parte de culpa, y es que no se me ocurre un director mejor para mi tesis. Creo que eres un referente para todos en cuanto a dedicación, éxito, y ética de trabajo. Espero poder parecerme a ti algún día.

A ***Belén***, por ser mi guía durante este periodo desde el día en que nos conocimos, no podré agradecerte lo suficiente tu inestimable ayuda y apoyo. Creo que has conseguido guiarme y que no perdiera la esperanza en muchas ocasiones, al final te he ido haciendo caso, y creo que gracias a ti el resultado no habría podido ser mejor. Eres un ejemplo de trabajo duro y dedicación.

A ***Alex***, por ayudarme y guiarme especialmente en los comienzos del proceso cuando todo me venía de nuevas. Creo que he aprendido mucho de ti, y seguro que lo voy a seguir haciendo, porque sin duda aún me queda mucho por delante y considero que tu eres un gran referente y un gran profesional del que aprender. Espero que me puedas seguir mandando muchos "Todo OK José Luis".

Los directores son una parte esencial de este proceso, y no siento más que agradecimiento y admiración hacia ellos, pero el equipo y las personas de las que te rodeas en tu día a día también son importantes, por ello quiero agradecer al grupo GENUD al completo su ayuda y apoyo durante estos años, en especial a los miembros de la rama Exercise, que han sido una segunda familia durante estos años:

A ***Nuria***, ***German*** y ***Alba***, he tenido la suerte de aprender de vosotros como alumno, y de contar con vosotros también como doctorando, porque aunque no sois mis directores de tesis, siempre que habéis podido y os lo he pedido me habéis ayudado. Creo que sois referentes y un pilar fundamental del grupo GENUD.

A ***Matute***, por ayudarme en todo lo posible desde que lo conocí cuando estaba con mi TFM. También por hacerme ver el funcionamiento de las cosas, entre ellas las grandes mentiras y verdades de GENUD, una salida en raquetas fraguada durante 4 años es un buen ejemplo de que algunas mentiras se convierten en verdades.

A ***Bruton***, por estar siempre disponible para echarme una mano con lo que necesitara, y por esas salidas en bici que a lo que te despistas se convierten en cronos encubiertas hasta la siguiente señal. Siempre tendré alguna buena ruta que recomendarte en agradecimiento por muchos peros y condiciones que me pongas.

A ***Gabriel***, porque has sido un guía en el camino, y creo que no hay nadie más dispuesto a echar una mano que tu, siempre allí para cualquier trámite, duda o problema. Tampoco me olvido de tus chistes, y es que esos momentos de risas son la mejor desconexión en el despacho.

A ***Borja***, porque desde que te conocí y hasta hoy, me has parecido el ejemplo perfecto de sacrificio y trabajo duro. Considero que tu éxito te lo has ganado a pulso, y sirves de ejemplo para los que venimos por detrás.

A ***Jorge***, porque siempre estás disponible para cualquier conflicto matemático que se presente, en la tesis y en el día a día. Tampoco me olvido de las noches de juegos de mesa, que aunque no han sido tantas como me habría gustado, me han ayudado a desconectar.

A ***Navarrete***, porque hemos compartido parte del camino juntos, en el despacho y en las aulas. No me cabe duda de que te espera un gran futuro, y que tus alumnos tendrán suerte de que seas su profesor, espero que sigamos trabajando juntos aunque te conviertas en Rector.

A ***Ana***, la nutri del equipo, por su ayuda, compañerismo y amistad dentro y fuera del trabajo. Aunque seas la nutri infiltrada, eres la que más entrena de todos, cuento contigo para seguir haciendo equipo a relevos cortos para no dejar ganar a Bruton ni a las chapas.

A ***Adrián***, porque hemos ido a la par desde que empezamos, y tener un compañero y amigo en el camino como tú ha sido un autentico lujo. Creo que no podría haber tenido más suerte con el compañero cuando empecé con todo esto. Nos parecemos en muchas cosas, incluso algunos dicen que demasiado y nos llegan a confundir, pero siempre he admirado tu capacidad, compatibilizar medicina con el doctorado, y tener tiempo para los amigos, entrenar, y mantener una sonrisa y la alegría, no está al alcance de cualquiera.

Espero que podamos seguir compartiendo el camino, lleguemos a trabajar juntos, y tengamos que seguir rellenando más papelotes a la par.

A ***Cris***, por la alegría que transmite, y por su ayuda, compañerismo y confianza, creo que tu trabajo duro y dedicación te permitirán hacer lo que te propongas. Siempre recordaré las tardes de exergames que nos hemos pegado, quién nos iba a decir en 1º de carrera que estaríamos hoy aquí?

A ***Lorena***, porque desde que llegaste has sido un apoyo más dentro del equipo, en el despacho y en las clases. No me cabe duda de que alcanzaras las metas que te propongas.

A ***Ángel Iván***, por estar siempre dispuesto a ayudar con lo que sea. Un tío currante donde los haya, y un ejemplo de compromiso y dedicación.

A ***Jorge Subías***, que desde que llegó viene pisando fuerte y con ganas de hacer cosas. Necesitábamos un oscense más para el equipo, y el fichaje no pudo ser mejor.

A ***Irina***, por la alegría y dedicación que transmite al equipo. Creo que se ha integrado a la perfección, y aporta mucho al equipo.

A ***Dani***, última incorporación del equipo, del que seguro podemos esperar grandes cosas. Mucho ánimo, al final todo el esfuerzo merece la pena.

Tampoco me podía olvidar de la otra gran parte del equipo que ha permitido que todo este trabajo saliera adelante, los que componen y son el proyecto AWHS. En especial quería agradecer a ***José Antonio Casasnovas*** su inestimable apoyo y ayuda inmediata siempre que ha hecho falta. Gracias a él pude vivir la experiencia de viajar a Baltimore a continuar mi formación investigadora. Aprovecho para agradecer la acogida de ***Eliseo***, ***Elena*** y ***Carol*** en Baltimore, que a pesar de sus muy apretadas agendas, me dedicaron todo el tiempo que pudieron a nivel laboral y personal, haciendo que me sintiera como uno más.

Nada de esto habría sido posible sin el trabajo de ***Eli***, ***Raquel***, ***Rosa*** y ***Martín*** entre muchos otros trabajadores involucrados en el AWHS, por ello os doy las gracias. Tampoco

nada sería posible sin la desinteresada y altruista colaboración de los participantes, que voluntariamente nos dedican su tiempo para que juntos podamos avanzar en el conocimiento científico y en definitiva en la búsqueda de una vida mejor.

También considero que merecen una especial mención ***Antonio Lobo*** y ***Elena Lobo***, que han dedicado parte de su tiempo en ayudarme cuando algún tema sobrepasaba mis conocimientos, y en enseñarme y ayudarme a entender parte de su trabajo.

Aunque el apoyo dentro del mundillo researcher y las personas de las que te rodeas son aspectos importantes, el apoyo de los tuyos lo es más, familiares y amigos que lo son desde que tengo uso de razón, y que han supuesto un apoyo monumental en los buenos momentos y en los baches y contratiempos. Por ello, les quiero dar las gracias, y quiero que sepan que parte de todo esto también es gracias a ellos.

A ***mis padres***, porque me han dado todo lo que tengo, y han influido en todo lo bueno que soy, nunca tendré páginas suficientes para agradecer todo lo que me han ayudado. Desde pequeño me han querido y educado, inculcándome los valores del esfuerzo y dedicación. Que esta tesis haya salido adelante, y en definitiva yo haya llegado hasta aquí es gracias a vosotros.

A ***mi hermana***, porque aunque entre nosotros lo disimulamos muy bien, en el fondo nos entendemos y nos llevamos muy bien. Gracias por la visita a Baltimore durante la estancia, fue la semana más entretenida y ociosa en 3 meses, pero sobre todo gracias por mantenerme ágil aquí arriba todos estos años, siempre que tengamos alguna frase celebre de alguna película/serie en la mente y nos riamos, nos acordaremos el uno del otro.

A ***Yaya***, que nos ha cuidado, mimado y educado a todos desde que tengo memoria y hasta ahora, a sus 93 años. Ella no ha necesitado investigar para darse cuenta de que el ejercicio es salud, y para ella, el huerto y el jadico han sido su mejor medicina.

A mis tíos ***Luis***, ***Maribel***, ***Raquel*** y ***Jesús***, primas ***Raquel***, ***Patricia*** e ***Isabel***, y primos ***Jesús*** y ***Jorge***, por su cariño y apoyo desde siempre. Recuerdo con nostalgia los momentos y celebraciones en familia que han pasado, y espero con ilusión los que están por llegar, y esto es así gracias a todos vosotros. Las cenas y comidas de Navidad, los cumpleaños, las comidas de San Lorenzo, las tardes de verano en Arguis, los años de juegos en casa de Yaya, las vacaciones en el Marius, las sesiones de monopoly maratonianas… Sin duda los mejores momentos son en familia, y aunque no seamos muchos, a mi no me podría haber tocado una mejor. Tampoco me olvido de ***Elías*** y ***Diego***, porque no sé quién se lo pasa mejor en los paseos en carretillo por el huerto, si ellos o yo.

A ***Emilio*** y ***Magdalena***, que me han acogido como a uno más en la familia, y a los que yo sin duda considero parte de la mía.

A mis amigos y amigas de Ainsa, con los que durante todos estos años no he vivido más que buenos momentos y alegrías. Aunque cada vez sea más complicado, espero que pronto nos podamos juntar todos de nuevo, porque los años del IES Sobrarbe han sido de los mejores que recuerdo, y sin duda es gracias a vosotros.

Especialmente quiero agradecer a ***Joaquín*** y ***Fes*** su amistad, porque en estos últimos años hemos vivido infinidad de momentos y aventuras. Desde aquella primera incursión en el mundo de la montaña en el 2014 en la que tiendas de campaña con águilas fosforitas sitiaron Goriz, no han dejado de sucederse escapadas montañeras, en las que todo queda apartado, solo amigos y montaña. Creo que nunca olvidaremos momentos como el STA1 con César, o frases como "*No te cases en la primera cita*", y como esa miles de anécdotas más sobre el hielo, la nieve o la roca. He encontrado mi vía de escape, parece que los problemas y preocupaciones desaparecen a cota 2000m cuando nos juntamos, por ello os doy las gracias y espero que sigamos escapando a las montañas, a esquiar, escalar, correr, andar… lo que sea, pero con vosotros.

A mis amigos de la carrera, con los que compartí los primeros aprendizajes en lo que ahora me apasiona, y con los que hemos pasado horas discutiendo sobre entrenamiento y ejercicio físico. Los años de carrera no habrían sido lo mismo sin vosotros, y aunque ahora sea complicado coincidir, por escrito queda que a la siguiente Finkbrau invito yo.

Por último, pero como siempre no por ello menos importante, sino más bien todo lo contrario, quiero dar las gracias a ***Cristina***, mi novia, porque puede que sea la persona que mejor sabe lo que ha costado llegar hasta aquí. Me ha acompañado y ha celebrado conmigo las pequeñas victorias, pero también ha sabido apoyarme y ayudarme en los momentos de flaqueza, desmotivación y frustración. Has aguantado y escuchado todas mis historietas y problemas esperando a que me desahogara con un abrazo y un "todo irá bien" como respuesta. No soy una persona demasiado sentimental, pero no se me ocurre una mejor compañera de vida, y echando la vista atrás y aún sabiendo lo duro que ha sido vivir a más de 700 km, creo que todo ha merecido la pena.

Material suplementario incluido en los artículos de la tesis doctoral

MATERIAL SUPLEMENTARIO ARTÍCULO 1

International Journal of
Environmental Research and Public Health

MDPI

Review

Can Physical Activity Reduce the Risk of Cognitive Decline in Apolipoprotein e4 Carriers? A Systematic Review

Supplementary Text S1: Quality assessment information. Newcastle-Ottawa Scale (NOS) criteria

In section (a), the representativeness of the exposed cohort, the selection of the non-exposed cohort, the ascertainment of exposure, and the demonstration that outcome of interest was not present at start of the study were evaluated and scored or not with a star.

* Representativeness of the exposed cohort:

a) truly representative of the average PA in the community →Star (1)

b) somewhat representative of the average PA in the community →Star (1)

c) selected group of users eg nurses, volunteers →No star (0)

d) no description of the derivation of the cohort →No star (0)

* Selection of the non-exposed cohort:

a) drawn from the same community as the exposed cohort →Star (1)

b) drawn from a different source →No star (0)

c) no description of the derivation of the non-exposed cohort →No star (0)

* Ascertainment of exposure:

a) secure record (eg surgical records) →Star (1)

b) structured interview →Star (1)

c) written self-report →No star (0)

d) no description →No star (0)

* Demonstration that outcome of interest was not present at start of study: 24

a) yes →Star (1)

b) no →No star (0)

In section (b), the NOS for cohort studies evaluate the variables of control that studies use to adjust their results. A star was scored for the studies that adjusted their results by the confounder age, and with another star if they also adjusted for the confounder education level or years of education received. Age was selected as the most relevant factor, supported by conclusions in several studies [1], and as second relevant factor authors selected education based on the results of a systematic review [2].

* Comparability of cohorts on the basis of the design or analysis

a) study controls for age →Star (1)

b) study controls for education level / years of education received →Star (1)

In section (c) the assessment of the outcome was taken into account, the follow-up period, and the loss of participants throughout the follow-up. The second item scored with a star in those studies in which the follow-up from the baseline was ≥5 years, and the third item scored with a star when the loss of participants in the study between the baseline and the follow-up was £20%. We believe that participant losses >20% may compromise the results of the studies, and in view of clinical experience, we also believe that 5 years can be a reasonable period of time for follow-up; moreover, other similar studies use this time-frame [3], and we think that the use of similar criteria is an advantage for comparative studies.

* Assessment of outcome:

a) independent blind assessment →Star (1)

b) record linkage →Star (1)

c) self-report →No star (0)

d) no description →No star (0)

* Was follow-up ≥5 years?:

a) yes →Star (1)

b) no →No star (0)

* Adequacy of follow-up of cohorts:

a) complete follow-up − all subjects accounted for →Star (1)

b) subjects lost £20% →Star (1)

c) follow up rate <80% and no description of those lost →No star (0)

d) no statement →No star (0)

References

1 Lipnicki DM, Makkar SR, Crawford JD, Thalamuthu A, Kochan NA, Lima-costa MF, et al. Determinants of cognitive performance and decline in 20 diverse ethno-regional groups: A COSMIC collaboration cohort study. *PLoS Med*. **2019**, *16*, 1–27.

2 Luck T, Luppa M, Briel S, Riedel-Heller SG. Incidence of Mild Cognitive Impairment: A Systematic Review. *Dement Geriatr Cogn Disord*. **2010**, *29*, 164–175.

3 Blondell SJ, Hammersley-Mather R, Veerman JL. Does physical activity prevent cognitive decline and dementia?: A systematic review and meta-analysis of longitudinal studies. *BMC Public Health*. **2014**, *14*, 1–12.

MATERIAL SUPLEMENTARIO ARTÍCULO 3

Supplementary Table 1 Prevalence of the different diseases in ≥50 years old Americans analyzed according to sex and physical activity level[a].

	Males					Females				
	OA	**VLPA**	**LPA**	**MPA**	**HPA**	**OA**	**VLPA**	**LPA**	**MPA**	**HPA**
Sarcopenia	19.2%	28.6%	17.0%	13.6%	12.1%	14.5%	21.3%	12.3%	8.5%	4.3%
Osteoporosis	2.0%	3.9%	2.1%	1.4%	1.1%	8.1%	11.2%	7.1%	7.8%	5.5%
Osteoarthritis	21.3%	25.2%	15.9%	21.7%	21.5%	36.9%	42.7%	35.8%	28.6%	35.1%

[a] *Data are weighted and represent non-institutionalized American population.*
OA: Overall; VLPA: Very Light Physical Activity (<150 MET-min/week); LPA: Light Physical Activity (150-960 MET-min/week); MPA: Medium Physical Activity (961-1800 MET-min/week); HPA: High Physical Activity (>1800 MET-min/week).

Supplementary Table 2 Prevalence of the different diseases in ≥50 years old Americans analyzed according to sex and race/ethnicity[a].

	Males					Females				
	MA	**OH**	**NHW**	**NHB**	**OR**	**MA**	**OH**	**NHW**	**NHB**	**OR**
Sarcopenia	32.5%	26.3%	19.2%	4.9%	30.2%	35.6%	21.5%	14.2%	4.8%	16.6%
Osteoporosis	1.8%	1.5%	2.1%	0.7%	2.9%	4.6%	7.2%	8.8%	2.1%	12.2%
Osteoarthritis	10.2%	11.3%	23.8%	12.5%	17.6%	21.9%	22.6%	39.4%	26.5%	34.4%

[a] *Data are weighted and represent non-institutionalized American population.*
MA: Mexican American; OH: Other Hispanic; NHW: Non-Hispanic White; NHB: Non-Hispanic Black; OR: Other race including Multi-Racial.

MATERIAL SUPLEMENTARIO ARTÍCULO 4

	Physical activity level (MET-min/week)		
	Low (<600)	**Medium (600 - 1200)**	**High (>1200)**
N	43.1 %	14.5 %	42.4 %
Unadjusted	1.00 (ref)	0.54 (0.38, 0.78)*	0.43 (0.33, 0.55)*
Age-adjusted	1.00 (ref)	0.52 (0.36, 0.75)*	0.17 (0.14, 0.21)*
Multivariable-adjusted Model A	1.00 (ref)	0.59 (0.41, 0.84)*	0.49 (0.38, 0.63)*
Multivariable-adjusted Model B	1.00 (ref)	0.66 (0.46, 0.95)*	0.53 (0.41, 0.70)*

Supplementary Table 1: Odds ratio (95% CI) for depression according to physical activity levels. Data are representative of non-institutionalized American population. Model A is adjusted by age, sex, race/ethnicity, annual household income, and educational level. Model B is additionally adjusted by alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes. ***** Significant differences with Low group ($p<0.05$).

	Physical activity (MET-min/week)		*p*-Value
	Less active (<600)	More active (≥600)	
Physical activity at work/domestic			
N	69.5 %	30.5 %	
Unadjusted	1.00 (ref)	0.80 (0.59, 1.07)	0.125
Age-adjusted	1.00 (ref)	0.73 (0.55, 0.99) *	0.041
Multivariable-adjusted Model A	1.00 (ref)	0.78 (0.58, 1.06)	0.108
Multivariable-adjusted Model B	1.00 (ref)	0.82 (0.60, 1.11)	0.196
Physical activity in leisure time			
N	67.8 %	32.2 %	
Unadjusted	1.00 (ref)	0.31 (0.22, 0.44) *	<0.001
Age-adjusted	1.00 (ref)	0.30 (0.21, 0.42) *	<0.001
Multivariable-adjusted Model A	1.00 (ref)	0.42 (0.30, 0.60) *	<0.001
Multivariable-adjusted Model B	1.00 (ref)	0.47 (0.32, 0.67) *	<0.001
Physical activity in transport/travel			
N	88.7 %	11.3 %	
Unadjusted	1.00 (ref)	0.77 (0.52, 1.15)	0.198
Age-adjusted	1.00 (ref)	0.73 (0.49, 1.09)	0.124
Multivariable-adjusted Model A	1.00 (ref)	0.71 (0.48, 1.04)	0.078
Multivariable-adjusted Model B	1.00 (ref)	0.79 (0.53, 1.16)	0.225

Supplementary Table 2: Odds ratio (95% CI) for depression according to physical activity performed in different domains (work/domestic, leisure time, transport/travel). Data are representative of non-institutionalized American population. Model A is adjusted by age, sex, race/ethnicity, annual household income, and educational level. Model B is additionally adjusted by alcohol consumption, smoking status, BMI, arterial hypertension, dyslipidemia, and diabetes. * Significant differences between Less-active and More-active groups.

MATERIAL SUPLEMENTARIO ARTÍCULO 5

Supplementary Table 1 Number of participants with missing and imputed data by variable and by genotype.

Imputed variables	**Overall**	***APOE e3e3***	***APOE e3e4***	***APOE e4e4***
VO_2max, %	14.4 [13]	10.0 [3]	20.0 [6]	13.3 [4]
MVPA, %	8.9 [8]	10.0 [3]	6.7 [2]	10.0 [3]

Values are % [number].

Anexos

Anexo 1. Informe Dictamen del Comité de Ética de Investigación

GOBIERNO DE ARAGON
Departamento de Salud y Consumo

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 3
50009 Zaragoza

Dña. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

Que el proyecto de investigación titulado "**Aragón Workers Health Study**"

Investigador Principal: **Dr. Jose Antonio Casasnovas Lenguas.**

Ha sido evaluado por este Comité y tras la revisión de la documentación aportada, este CEIC resuelve **AUTORIZAR** la realización del estudio, según consta en el acta 09/2007, de 16 de mayo de 2007, y en este sentido se informa al Investigador Principal del proyecto.

Lo que firmo en Zaragoza, a 23 de mayo de 2007.

DIPUTACIÓN GENERAL DE ARAGÓN
DEPARTAMENTO DE SALUD Y CONSUMO
COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA

María González Hinjos

DNV

GOBIERNO DE ARAGON
Departamento de Salud y Consumo

COMITÉ ÉTICO DE INVESTIGACIÓN
CLÍNICA DE ARAGÓN (CEICA)
Avda. Gómez Laguna, 25 planta 3
50009 Zaragoza

LISTA DE MIEMBROS DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA DE ARAGÓN

Dra. María González Hinjos, Secretaria del Comité Ético de Investigación Clínica de Aragón,

CERTIFICA

Que en la reunión de este Comité celebrada el día 16 de abril de 2007 estuvieron presentes las siguientes personas:

- Carlos Aibar Remón; Médico. Servicio de Medicina Preventiva y Salud Pública. Hospital Clínico Universitario Lozano Blesa. Profesional Sanitario experto en epidemiología clínica.
- Marina Heredia Ríos; Representante de las Organizaciones de Consumidores y Usuarios.
- Gabriel Hernández Delgado; Médico. Servicio de Radiología. Hospital Universitario Miguel Servet. Representante de Comisión de Investigación.
- Angela Idoipe Tomás; Farmacéutica. Servicio de Farmacia. Hospital Universitario Miguel Servet. Farmacéutica de Hospital.
- María Jesús Lallana Álvarez. Farmacéutica de Atención Primaria de Zaragoza Sector III.
- Cesar Loris Pablo; Médico. Servicio de Pediatría. Hospital Universitario Miguel Servet. Presidente del Comité. Representante de Comisión de Investigación.
- Jesús Magdalena Belío; Médico. Centro de Salud de Azuara. Médico con labor asistencial y representante del Comité de Ética Asistencial del Área de Atención Primaria II y V.
- Javier Perfecto Ejarque; Médico. Centro de Salud Arrabal. Médico con labor asistencial.
- Susana Torrente Gari; Jurista. Centro de Estudios Sociales. Licenciada en Derecho ajena a la profesión sanitaria.
- María González Hinjos; Farmacéutica. Secretaria del Comité Ético de Ensayos Clínicos.

Lo que firmo en Zaragoza, a 23 de mayo de 2007

DIPUTACIÓN GENERAL DE ARAGÓN

Firmado: María González Hinjos

Anexo 2. Consentimiento informado

Instituto Aragonés de Ciencias de la Salud
Programa de Investigación Cardiovascular de Aragón.
Gobierno de Aragón
Centro Nacional de Investigaciones Cardiovasculares.
Instituto de Salud Carlos III. Ministerio de Sanidad Consumo.
Gobierno de España

HOJA DE INFORMACIÓN PARA EL TRABAJADOR

Naturaleza del proyecto

Aragón, al igual que otras sociedades desarrolladas, está sufriendo una epidemia de sobrepeso y de obesidad, que conlleva un riesgo mayor para desarrollar diabetes, síndrome metabólico, enfermedad cardiovascular, cáncer, enfermedades hepáticas, respiratorias y músculo-esqueléticas. Sin embargo no afecta por igual a todas las personas, algunas están más predispuestas que otras. Sabemos que los factores genéticos y ambientales (como el estilo de vida) pueden influir en el desarrollo futuro de la enfermedad.

El Aragón Workers Health Study es el estudio de salud de trabajadores de Aragón, el **objetivo** de este estudio es conocer mejor cómo se desarrolla la enfermedad cardiovascular, la obesidad, la diabetes, la hipertensión. Saber cuales son los hábitos de vida y los factores genéticos que nos protegen y los que hacen que progrese la enfermedad y nos lleven a sufrir angina de pecho, infarto de miocardio, claudicación de extremidades, infarto o hemorragia cerebral, etc.

Nuestro propósito es lograr una prevención cardiovascular más eficaz, centrándola sobre los trabajadores más predispuestos o con mayor riesgo de sufrir la enfermedad.

Este estudio está promovido conjuntamente por el departamento de Salud y Consumo del **Gobierno de Aragón** y por la fundación Centro Nacional de Investigaciones Cardiovasculares. Los investigadores responsables de este estudio pertenecen al Instituto Aragonés de Ciencias de la Salud (I+CS), a la Universidad de Zaragoza, al Centro Nacional de investigaciones cardiovasculares (CNIC) y al Servicio Aragonés de la Salud (SALUD).

En qué consiste la participación

Solicitamos su consentimiento informado para que usted nos autorice a:

- **Acceder a los datos clínicos** que se han recogido en su reconocimiento médico de su empresa, de forma totalmente anónima y exclusivamente con fines de investigación.

- **Utilizar los restos de sangre**, suero , plasma y orina que son desechados tras el análisis de sangre de su reconocimiento médico para realizar en esos restos los análisis bioquímicos y genéticos que nos permitan encontrar marcadores que nos indiquen de forma temprana su riesgo de enfermedad cardiovascular.

- **Realizar distintas técnicas de imagen indoloras**, no invasivas y sin riesgos a trabajadores más predispuestos a enfermar, para poder detectarles lesiones iniciales de enfermedad aterosclerótica en sus arterias, y poder así aplicarle la mejor prevención cardiovascular posible.

Qué hacen los investigadores con las muestras de sangre

Las muestras de sangre recogida se procesarán en el laboratorio de General Motors de Figueruelas.

Se separará y guardará en pequeños contenedores parte de suero, plasma, orina y sangre. De las células sanguíneas se extraerá el material genético (el DNA) necesario.

Estas muestras se congelarán y guardarán en un banco de muestras por si en un futuro surgen nuevas líneas de investigación. Este material podrá ser compartido con otros grupos de investigación, procedimiento que siempre se hará bajo las normas de seguridad y confidencialidad necesarias y con autorización previa del comité científico del estudio, la dirección del Instituto Aragonés de Ciencias de la Salud (I+CS) y el Centro Nacional de Enfermedades Cardiovasculares (CNIC) que además serán los responsables de la custodia de dichas muestras.

En todos los aspectos referidos a la conservación y destrucción de las muestras recogidas y almacenadas, aseguramos el cumplimiento de la ley 14/2007 de investigación biomédica.

Qué hacen los investigadores con los datos que recogen

Los datos se guardan en ficheros, en bases de datos informatizadas. Estos ficheros identifican a cada participante con un código, por tanto no contienen ni su nombre ni otro dato que permita identificarle a ningún miembro del equipo investigador. Por lo tanto la comunicación de resultados tendrá que ser a través de su médico de empresa, que es quien únicamente conoce ese código.

Finalmente los resultados derivados de estos análisis se publican en revistas científicas. Estos datos no se utilizarán para otra finalidad que no sea la descrita y para su uso se seguirá siempre: su voluntad, la normativa vigente respecto a protección de datos de carácter personal y en general a la ética en la investigación científica.

Los resultados de los análisis de sangre, tanto de los habituales, como de los especiales y de DNA, así como los resultados de las exploraciones de imagen, serán entregados directamente al Servicio de Prevención de Riesgos Laborales de General Motors Figueruelas, para que sea su médico de empresa el que le transmita la información confidencial a cada trabajador.

Beneficios y riesgos de participar en el estudio

El beneficio del estudio para la sociedad es **profundizar en el conocimiento de la enfermedad cardiovascular** y así intentar encontrar herramientas (marcadores) para detectar la enfermedad antes de que provoque síntomas, para mejorar su prevención y tratamiento,

detectando a las personas que tienen más riesgo de padecer enfermedad cardiovascular: infarto de miocardio, angina, infarto cerebral etc.

A corto plazo no se prevé que los resultados obtenidos del estudio puedan beneficiar directamente al individuo participante, aunque esperamos obtener estas herramientas diagnósticas útiles a partir de los tres años de iniciado el estudio. Por ello, es probable que usted pueda salir beneficiado de unas novedosas e incruentas técnicas de imagen para el diagnóstico de enfermedad que aún no ha producido síntomas y un conocimiento más profundo sobre sus metabolismo, hábitos de vida y, por tanto, sobre su riesgo cardiovascular.

Los riesgos para los participantes del estudio son inexistentes.

Garantía de participación voluntaria

Si a pesar de todo decide no participar, su atención por el personal médico de la factoría General Motors España Figueruelas no se verá afectada en nada, Además, en el caso que usted acepte participar, ha de saber que se puede retirar **en cualquier momento sin tener que dar explicaciones,** simplemente diciéndoselo a un miembro del equipo médico de General Motors. Su muestra será retirada del banco de almacenamiento y sus datos clínicos eliminados.

En el caso de que se jubilara de forma ordinaria o debido a una incapacidad podría continuar en el estudio, asumiendo completamente el equipo de investigación todos los procedimientos que se realizaran.

Muchas gracias por su tiempo y atención.

EL EQUIPO MEDICO INVESTIGADOR

HOJA DE CONSENTIMIENTO INFORMADO

PROYECTO: Estudio de la salud de los trabajadores de Aragón

Yo, ...

(nombre y apellidos)

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido suficiente información sobre el estudio.

He hablado con: ....
.....

(nombre y apellidos de la persona que ha explicado este consentimiento investigador)

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

1) cuando quiera
2) sin tener que dar explicaciones
3) sin que esto repercuta en mis cuidados médicos

Presto libremente mi conformidad para participar en el estudio.

Doy mi conformidad para participar en este estudio cuyos procedimientos y objetivos se me han explicado.

He recibido una copia firmada de este Consentimiento Informado.

Fecha:

Firma del participante :

He explicado la naturaleza y el propósito del estudio al paciente mencionado

Fecha:

Firma del Investigador:

Deseo dejar de participar en este estudio desde la fecha indicada en adelante, aunque el equipo investigador podrá utilizar los datos clínicos y las muestras que hasta ahora habían recogido.

Fecha:

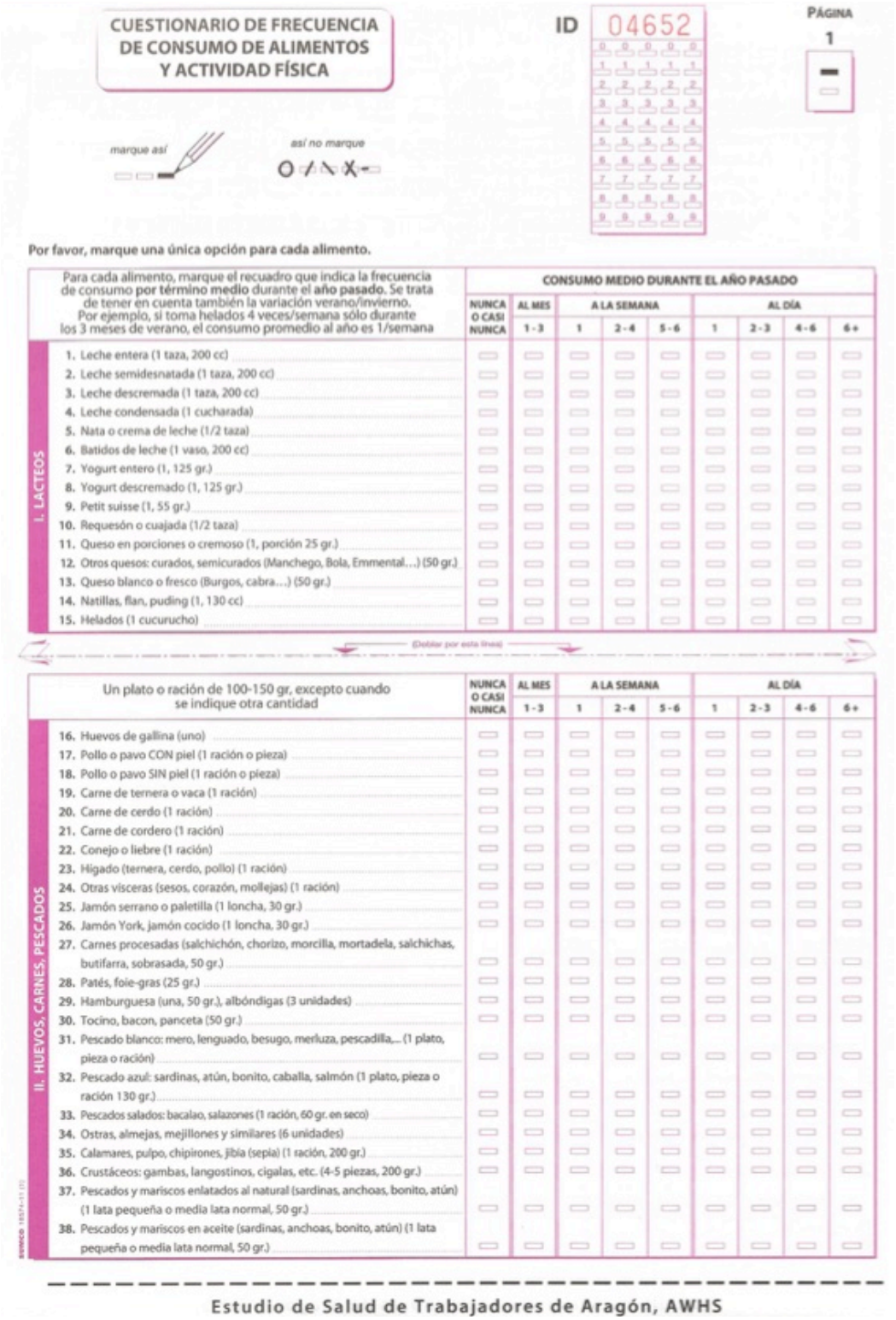
Firma del participante:

Deseo dejar de participar en este estudio y, por lo tanto, deberán destruirse las muestras biológicas almacenadas, así como los datos clínicos aportados para este estudio hasta el momento.

Fecha:

Firma del participante:

Anexo 3. Cuestionario utilizado para valorar la frecuencia de consumo de alimentos, actividad física y sedentarismo



CUESTIONARIO DE FRECUENCIA DE CONSUMO DE ALIMENTOS Y ACTIVIDAD FÍSICA

ID 04652

PÁGINA 1

marque así

así no marque

Por favor, marque una única opción para cada alimento.

Para cada alimento, marque el recuadro que indica la frecuencia de consumo **por término medio** durante el **año pasado**. Se trata de tener en cuenta también la variación verano/invierno. Por ejemplo, si toma helados 4 veces/semana sólo durante los 3 meses de verano, el consumo promedio al año es 1/semana	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
I. LÁCTEOS									
1. Leche entera (1 taza, 200 cc)									
2. Leche semidesnatada (1 taza, 200 cc)									
3. Leche descremada (1 taza, 200 cc)									
4. Leche condensada (1 cucharada)									
5. Nata o crema de leche (1/2 taza)									
6. Batidos de leche (1 vaso, 200 cc)									
7. Yogurt entero (1, 125 gr.)									
8. Yogurt descremado (1, 125 gr.)									
9. Petit suisse (1, 55 gr.)									
10. Requesón o cuajada (1/2 taza)									
11. Queso en porciones o cremoso (1, porción 25 gr.)									
12. Otros quesos: curados, semicurados (Manchego, Bola, Emmental...) (50 gr.)									
13. Queso blanco o fresco (Burgos, cabra...) (50 gr.)									
14. Natillas, flan, puding (1, 130 cc)									
15. Helados (1 cucurucho)									

(Doblar por esta línea)

Un plato o ración de 100-150 gr, excepto cuando se indique otra cantidad	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
II. HUEVOS, CARNES, PESCADOS									
16. Huevos de gallina (uno)									
17. Pollo o pavo CON piel (1 ración o pieza)									
18. Pollo o pavo SIN piel (1 ración o pieza)									
19. Carne de ternera o vaca (1 ración)									
20. Carne de cerdo (1 ración)									
21. Carne de cordero (1 ración)									
22. Conejo o liebre (1 ración)									
23. Hígado (ternera, cerdo, pollo) (1 ración)									
24. Otras vísceras (sesos, corazón, mollejas) (1 ración)									
25. Jamón serrano o paletilla (1 loncha, 30 gr.)									
26. Jamón York, jamón cocido (1 loncha, 30 gr.)									
27. Carnes procesadas (salchichón, chorizo, morcilla, mortadela, salchichas, butifarra, sobrasada, 50 gr.)									
28. Patés, foie-gras (25 gr.)									
29. Hamburguesa (una, 50 gr.), albóndigas (3 unidades)									
30. Tocino, bacon, panceta (50 gr.)									
31. Pescado blanco: mero, lenguado, besugo, merluza, pescadilla,... (1 plato, pieza o ración)									
32. Pescado azul: sardinas, atún, bonito, caballa, salmón (1 plato, pieza o ración 130 gr.)									
33. Pescados salados: bacalao, salazones (1 ración, 60 gr. en seco)									
34. Ostras, almejas, mejillones y similares (6 unidades)									
35. Calamares, pulpo, chipirones, jibia (sepia) (1 ración, 200 gr.)									
36. Crustáceos: gambas, langostinos, cigalas, etc. (4-5 piezas, 200 gr.)									
37. Pescados y mariscos enlatados al natural (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									
38. Pescados y mariscos en aceite (sardinas, anchoas, bonito, atún) (1 lata pequeña o media lata normal, 50 gr.)									

Estudio de Salud de Trabajadores de Aragón, AWHS

Por favor, marque una única opción para cada alimento.

III. VERDURAS Y HORTALIZAS

Un plato o ración de 200 grs, excepto cuando se indique	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
39. Acelgas, espinacas	☐	☐	☐	☐	☐	☐	☐	☐	☐
40. Col, coliflor, brócoles	☐	☐	☐	☐	☐	☐	☐	☐	☐
41. Lechuga, endivias, escarola (100 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
42. Tomate crudo (1, 150 gr)	☐	☐	☐	☐	☐	☐	☐	☐	☐
43. Zanahoria, calabaza (100 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
44. Judías verdes	☐	☐	☐	☐	☐	☐	☐	☐	☐
45. Berenjenas, calabacines, pepinos	☐	☐	☐	☐	☐	☐	☐	☐	☐
46. Pimientos (150 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
47. Espárragos	☐	☐	☐	☐	☐	☐	☐	☐	☐
48. Gazpacho andaluz (1 vaso, 200 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
49. Otras verduras (alcachofa, puerro, cardo, apio)	☐	☐	☐	☐	☐	☐	☐	☐	☐
50. Cebolla (media unidad, 50 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
51. Ajo (1 diente)	☐	☐	☐	☐	☐	☐	☐	☐	☐
52. Perejil, tomillo, laurel, orégano, etc. (una pizca)	☐	☐	☐	☐	☐	☐	☐	☐	☐
53. Patatas fritas comerciales (1 bolsa, 50 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
54. Patatas fritas caseras (1 ración, 150 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
55. Patatas asadas o cocidas	☐	☐	☐	☐	☐	☐	☐	☐	☐
56. Setas, níscalos, champiñones	☐	☐	☐	☐	☐	☐	☐	☐	☐

IV. FRUTAS

Una pieza o ración	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
57. Naranja (una), pomelo (uno), o mandarinas (dos)	☐	☐	☐	☐	☐	☐	☐	☐	☐
58. Plátano (uno)	☐	☐	☐	☐	☐	☐	☐	☐	☐
59. Manzana o pera (una)	☐	☐	☐	☐	☐	☐	☐	☐	☐
60. Fresas/fresones (6 unidades, 1 plato postre)	☐	☐	☐	☐	☐	☐	☐	☐	☐
61. Cerezas, picotas, ciruelas (1 plato de postre)	☐	☐	☐	☐	☐	☐	☐	☐	☐
62. Melocotón, albaricoque, nectarina (una pieza)	☐	☐	☐	☐	☐	☐	☐	☐	☐
63. Sandía (1 tajada, 200-250 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
64. Melón (1 tajada, 200-250 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
65. Kiwi (1 unidad, 100 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
66. Uvas (un racimo, 1 plato postre)	☐	☐	☐	☐	☐	☐	☐	☐	☐
67. Aceitunas (10 unidades)	☐	☐	☐	☐	☐	☐	☐	☐	☐
68. Frutas en almíbar o en su jugo (2 unidades)	☐	☐	☐	☐	☐	☐	☐	☐	☐
69. Dátiles, higos secos, uvas-pasas, ciruelas-pasas (50 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
70. Almendras, cacahuetes, avellanas, pistachos, piñones (30 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
71. Nueces (30 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐

72. ¿Cuántos días a la semana toma fruta como postre?		0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐

V. LEGUMBRES Y CEREALES

Un plato o ración	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
73. Lentejas (1 plato, 150 gr. cocidas)	☐	☐	☐	☐	☐	☐	☐	☐	☐
74. Alubias (pintas, blancas o negras) (1 plato, 150 gr. cocidas)	☐	☐	☐	☐	☐	☐	☐	☐	☐
75. Garbanzos (1 plato, 150 gr. cocidos)	☐	☐	☐	☐	☐	☐	☐	☐	☐
76. Guisantes, habas (1 plato, 150 gr. cocidas)	☐	☐	☐	☐	☐	☐	☐	☐	☐
77. Pan blanco, pan de molde (3 rodajas, 75 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
78. Pan negro o integral (3 rodajas, 75 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
79. Cereales desayuno (30 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
80. Cereales integrales: muesli, copos avena, all-bran (30 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐
81. Arroz blanco (60 gr. en crudo)	☐	☐	☐	☐	☐	☐	☐	☐	☐
82. Pasta: fideos, macarrones, espaguetis, otras (60 gr. en crudo)	☐	☐	☐	☐	☐	☐	☐	☐	☐
83. Pizza (1 ración, 200 gr.)	☐	☐	☐	☐	☐	☐	☐	☐	☐

SIVIMED 18574-11 (Rev. 1)

CUESTIONARIO DE FRECUENCIA DE CONSUMO DE ALIMENTOS Y ACTIVIDAD FÍSICA

ID

Repita el número de la 1ª hoja y vuelva a marcarlo

PÁGINA 3

Por favor, marque una única opción para cada alimento.

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
Una cucharada o porción individual. Para freír, untar, mojar en el pan, aliñar o para ensaladas, utiliza en total:	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
VI. ACEITES Y GRASAS									
84. Aceite de oliva (una cucharada sopera)									
85. Aceite de oliva virgen (una cucharada sopera)									
86. Aceite de oliva de orujo (una cucharada sopera)									
87. Aceite de maíz (una cucharada sopera)									
88. Aceite de girasol (una cucharada sopera)									
89. Aceite de soja (una cucharada sopera)									
90. Mezcla de los anteriores (una cucharada sopera)									
91. Margarina (porción individual, 12 gr.)									
92. Mantequilla (porción individual, 12 gr.)									
93. Manteca de cerdo (10 gr.)									
94. Marca de aceite de oliva que usa habitualmente:									

No marque aquí

CONSUMO MEDIO DURANTE EL AÑO PASADO	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
VII. BOLLERÍA Y PASTELERÍA									
95. Galletas tipo María (4-6 unidades, 50 gr.)									
96. Galletas integrales o de fibra (4-6 unidades, 50 gr.)									
97. Galletas con chocolate (4 unidades, 50 gr.)									
98. Repostería y bizcochos hechos en casa (50 gr.)									
99. Croissant, ensaimada, pastas de té u otra bollería industrial comercial... (uno, 50 gr.)									
100. Donuts (uno)									
101. Magdalenas (1-2 unidades)									
102. Pasteles (uno, 50 gr.)									
103. Churros, porras y similares (1 ración, 100 gr.)									
104. Chocolates y bombones (30 gr.)									
105. Cacao en polvo-cacaos solubles (1 cucharada de postre)									
106. Turrón (1/8 de barra, 40 gr.)									
107. Mantecados, mazapán (90 gr.)									

CONSUMO MEDIO DURANTE EL AÑO PASADO	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1-3	1	2-4	5-6	1	2-3	4-6	6+
VIII. MISCELÁNEA									
108. Croquetas, empanadillas, precocinados (una ración)									
109. Sopas y cremas de sobre (1 plato)									
110. Mostaza (una cucharadita de postre)									
111. Mayonesa comercial (1 cucharada sopera = 20 gr.)									
112. Salsa de tomate frito, ketchup (1 cucharadita)									
113. Picante: tabasco, pimienta, pimentón (una pizca)									
114. Sal (una pizca)									
115. Mermeladas (1 cucharadita)									
116. Azúcar (1 cucharadita)									
117. Miel (1 cucharadita)									
118. Snacks distintos de patatas fritas: gusanitos, palomitas, maíz, etc. (1 bolsa, 50 gr.)									
119. Otros alimentos de frecuente consumo:									
119.1									
119.2									
119.3									

119. Otros alimentos de frecuente consumo

119.1 (No marque aquí) 119.2 (No marque aquí) 119.3 (No marque aquí)

IX. BEBIDAS

Por favor, marque una única opción para cada alimento.

	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
120. Bebidas carbonatadas con azúcar: bebidas con cola, limonadas, tónicas, etc. (1 botellín, 200 cc)									
121. Bebidas carbonatadas bajas en calorías, bebidas light (1 botellín, 200 cc)									
122. Zumo de naranja natural (1 vaso, 200 cc)									
123. Zumos naturales de otras frutas (1 vaso, 200 cc)									
124. Zumos de frutas en botella o enlatados (200 cc)									
125. Café descafeinado (1 taza, 50 cc)									
126. Café (1 taza, 50 cc)									
127. Té (1 taza, 50 cc)									
128. Vaso de vino rosado (100 cc)									
129. Vaso de vino tinto (100 cc)									
130. Vaso de vino blanco (100 cc)									
131. Cerveza (1 jarra, 330 cc)									
132. Licores, anís o anisetes... (1 copa, 50 cc)									
133. Destilados: whisky, vodka, ginebra, coñac (1 copa, 50 cc)									

Habitualmente, ¿qué hace con la grasa de la carne? 1 La como ☐ 2 Se la quito ☐

	SÍ	NO		SÍ	NO
¿Procura tomar mucha fibra?	☐	☐	¿Evita el consumo de mantequilla?	☐	☐
¿Procura tomar mucha fruta?	☐	☐	¿Procura reducir el consumo de grasa?	☐	☐
¿Procura tomar mucha verdura?	☐	☐	¿Procura reducir el consumo de carne?	☐	☐
¿Procura tomar mucho pescado?	☐	☐	¿Limita la sal en las comidas?	☐	☐
¿Suele comer entre comidas (picotear)?	☐	☐	¿Le añade azúcar a algunas bebidas?	☐	☐
¿Sigue una dieta especial?	☐	☐	¿Procura reducir el consumo de dulces?	☐	☐

Si ha contestado SÍ, señale el tipo de dieta: .....

No debe marcar esta zona sombreada

Si durante el año pasado tomó vitaminas y/o minerales (incluyendo calcio) o productos dietéticos especiales (salvado, aceite de onagra, leche con ácidos grasos omega-3, flavonoides, etc.), por favor indique la marca y la frecuencia con que los tomó:

Marcas de los suplementos de vitaminas o minerales o de los productos dietéticos	CONSUMO MEDIO DURANTE EL AÑO PASADO								
	NUNCA O CASI NUNCA	AL MES	A LA SEMANA			AL DÍA			
		1 - 3	1	2 - 4	5 - 6	1	2 - 3	4 - 6	6+
134.									
134.1									
134.2									

134 (No marque aquí) 134.1 (No marque aquí) 134.2 (No marque aquí)

SUMCO 10374-11 (Ref. 2)

PÁGINA 5

ID

Repita el número de la 1ª hoja y vuelva a marcarlo

135. Cuando hace ejercicio o deporte siguiendo su modo típico de hacerlo, ¿cuál cree que es su grado de intensidad en el esfuerzo?. Puntúelo de 0 (el mínimo posible) a 10 (el máximo).

☐ Nunca hago deporte 0 1 2 3 4 5 6 7 8 9 10

136. Habitualmente, ¿cuánto tiempo anda al día?

☐ < 10 minutos ☐ 10 - 20 minutos ☐ 21 - 30 minutos
☐ 1/2 - 1 hora ☐ 1 - 2 horas ☐ > 2 horas

137. Su paso habitual al andar por la calle es...

☐ Lento ☐ Normal, medio ☐ Rápido ☐ Muy rápido

138. ¿Cuántos pisos sube al día por escaleras en total?

☐ 2 ó menos ☐ 3 - 4 ☐ 5 - 9 ☐ 10 - 14 ☐ 15 ó más

marque así

139. Por término medio, ¿cuántos kilómetros hace al año en coche, ya sea conduciendo usted o conduciendo otro?

☐ < 1.000 ☐ 1.000 - 10.000 ☐ 10.001 - 20.000 ☐ 20.001 - 50.000 ☐ > 50.000

así no marque

¿Y en moto?

☐ Ninguno ☐ < 1.000 ☐ 1.000 - 5.000 ☐ 5.001 - 10.000 ☐ > 10.000

140. ¿Hace ejercicio?

☐ No (responda solo a la pregunta **142**) ☐ Sí (conteste a las preguntas **141** y **142**)

141. ¿Cuánto tiempo por término medio dedicó a las siguientes actividades en el último año?
(por favor rellene la frecuencia media durante la semana y los meses al año que practica la actividad)

	FRECUENCIA MEDIA DURANTE LA SEMANA									MESES AL AÑO		
	NUNCA	MINUTOS / SEMANA			HORAS / SEMANA							
		1 - 4	5 - 19	20 - 59	1 - 1,5	2 - 3	4 - 6	7 - 10	≥ 11	< 3	3 - 6	> 6
Andar o pasear fuera de casa (incluye golf)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Correr o hacer jogging despacio	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Correr más competitivo y rápido (atletismo, etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pasear en bicicleta	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bicicleta estática	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nadar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tenis, frontón, squash, otros de raqueta o pala	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fútbol, futbito	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Otros de equipo (baloncesto, balonmano...)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Baile, danza, aerobic	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Excursiones al monte, escalada	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gimnasia	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cuidado del jardín y/o piscina, bricolaje, etc.	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Esquí, patinaje	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Judo, karate u otras artes marciales	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vela	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Otras actividades físicas-deporte no mencionadas	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

142. Tiempo por término medio en las siguientes actividades en el último año. Distinga y conteste ENTRE SEMANA y FIN DE SEMANA

TIEMPO AL DÍA	DÍA TÍPICO DE TRABAJO ENTRE SEMANA				DÍA TÍPICO DE FIN DE SEMANA			
	NUNCA	< 30 MIN.	30 - 59 MIN.	HORAS / DÍA	NUNCA	< 30 MIN.	30 - 59 MIN.	HORAS / DÍA
Ver televisión-vídeo	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Sentado ante pantalla ordenador	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Conduciendo	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Estar sentado (en total)	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Dormir por las noches	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Dormir la siesta	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Tomando el sol (verano)	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Tomando el sol (invierno)	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Salir con los amigos	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
De pie en el trabajo	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Tareas domésticas	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+
Actividad en el trabajo más intensa que estar de pie	☐	☐	☐	1 2 3 4 5 6 7 8 9+	☐	☐	☐	1 2 3 4 5 6 7 8 9+

SUMCO 18574-11 (2)

Muchas gracias por su colaboración

Estudio de Salud de Trabajadores de Aragón, AWHS

Anexo 4. Cuestionario utilizado para valorar la función cognitiva en el subproyecto APOE

Cuaderno de Recogida de Datos (CRD)

Proyecto ANTORCHA

Entrevistador: ……..

Participante: ……..

Número de participante en el estudio |A|P|O|E|__|__|__|

Fecha y hora de la entrevista |__|__|-|__|__|-|__|__|__|__| |__|__|:|__|__|

día mes año

RECORDAR QUE AL ENTREVISTADO HAY QUE PREGUNTARLE LA EDAD Y EL AÑO DE NACIMIENTO AL PRINCIPIO DE LA ENTREVISTA…

(1) *¿Puede decirme que edad tiene?* **__ /1**

(2) *¿Me puede decir en qué año nació?* **__ /1**

Fijándonos en la información recogida en los aparatados anteriores, vemos que ha tenido algún problema de salud. Ahora le voy a preguntar sobre su memoria:

(3) *¿Conserva bien la memoria?* **__ /1**

(4) *¿Ha tenido algún problema con su memoria?* **__ /1**

(5) *En caso afirmativo, ¿supone un problema para Vd.?* **__ /1**

(6) **MoCA** TMT Parte A Y Parte B…… **__ /1**

TMT Parte A (tiempo en segundos) **_____**
TMT Parte B (tiempo en segundos) **_____**

Ambas partes del TRAIL MAKING TEST constan de 25 círculos distribuidos en una hoja de papel. En la Parte A, los círculos están numerados del 1 al 25, y el paciente debe dibujar líneas para conectar los números en orden ascendente. En la Parte B, los círculos incluyen números (1 - 13) y letras (A - L); como en la Parte A, el paciente tiene que conectar los círculos en un patrón ascendente, pero alternando números y letras (es decir, 1-A-2-B-3-C, etc.).

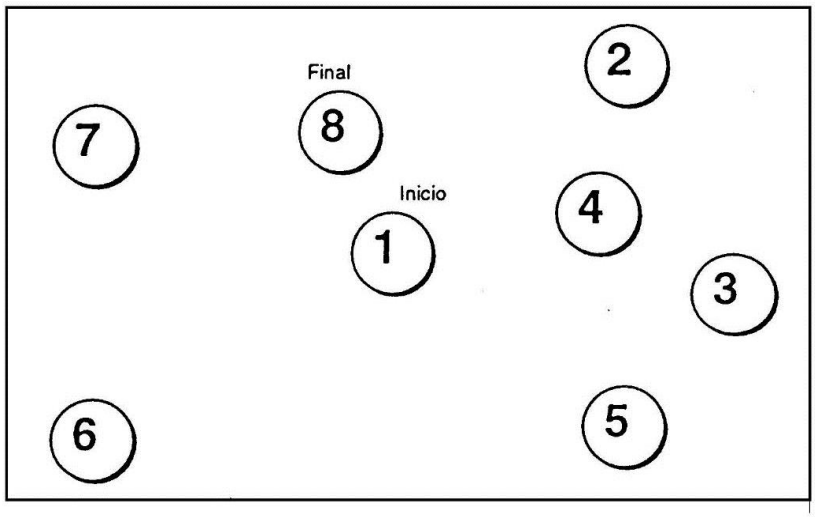
PARTE A: *"Tiene que unir los números lo más rápido posible en orden ascendente, sin levantar el lápiz del papel. Por ejemplo 1, 2, 3…* [Lo hago yo] *Continúe usted…* [Se le da el lápiz al participante]*"*

Nos aseguramos que lo ha entendido, y preparamos el cronómetro. Damos la vuelta a la hoja y le indicamos el inicio en el 1 y el final en el 25, después tapamos la hoja, hasta que empecemos a cronometrar. Si el paciente comete un error, señalarlo y permitir que él lo corrija. Los errores afectan a la puntuación solo porque incrementa el tiempo de finalización de la tarea. No es necesario continuar con la prueba si el paciente no ha completado ambas partes después de que hayan transcurrido cinco minutos.

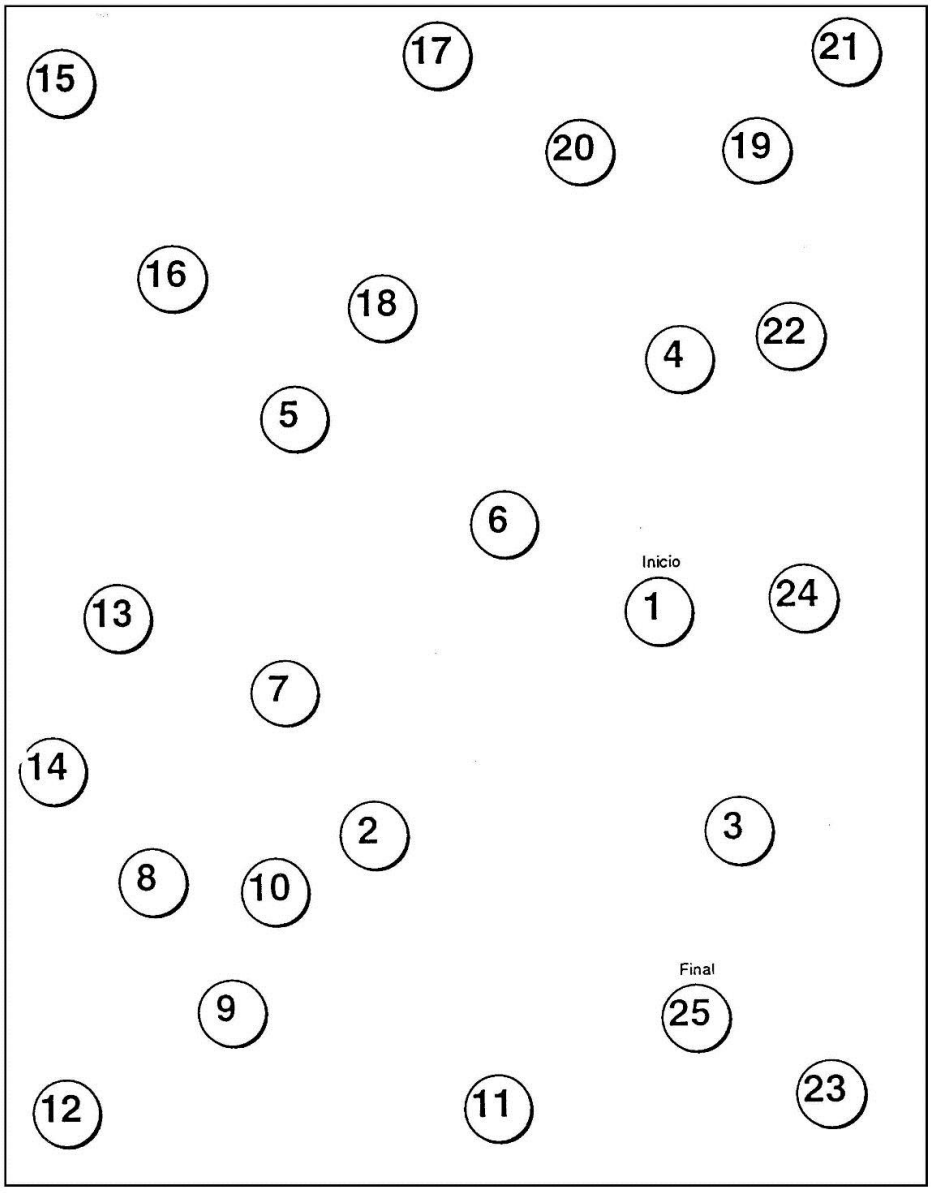
PARTE B: *"Ahora tiene que unir números y letras. ¿Conoce el abecedario? Por ejemplo: 1A, 2B, 3C…* [Lo hago yo] *Continúe usted…* [Se le da el lápiz al participante]*"*

TRAIL MAKING TEST A

EJEMPLO



TEST


15
17
21
20
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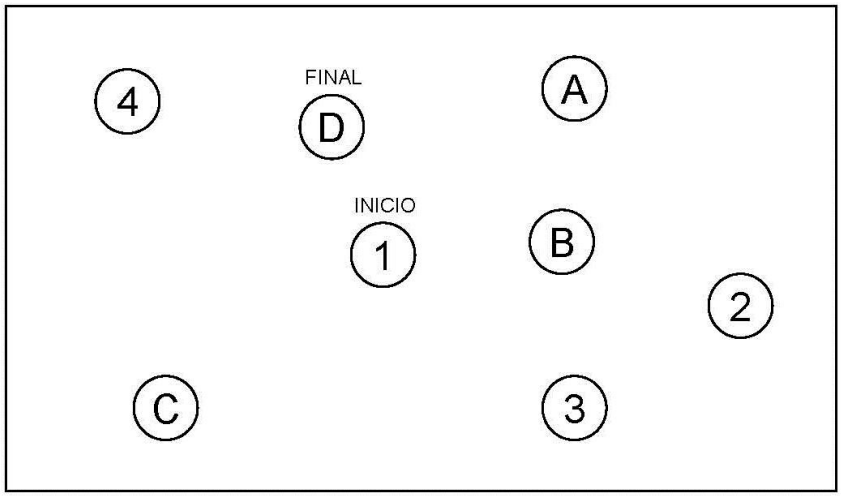
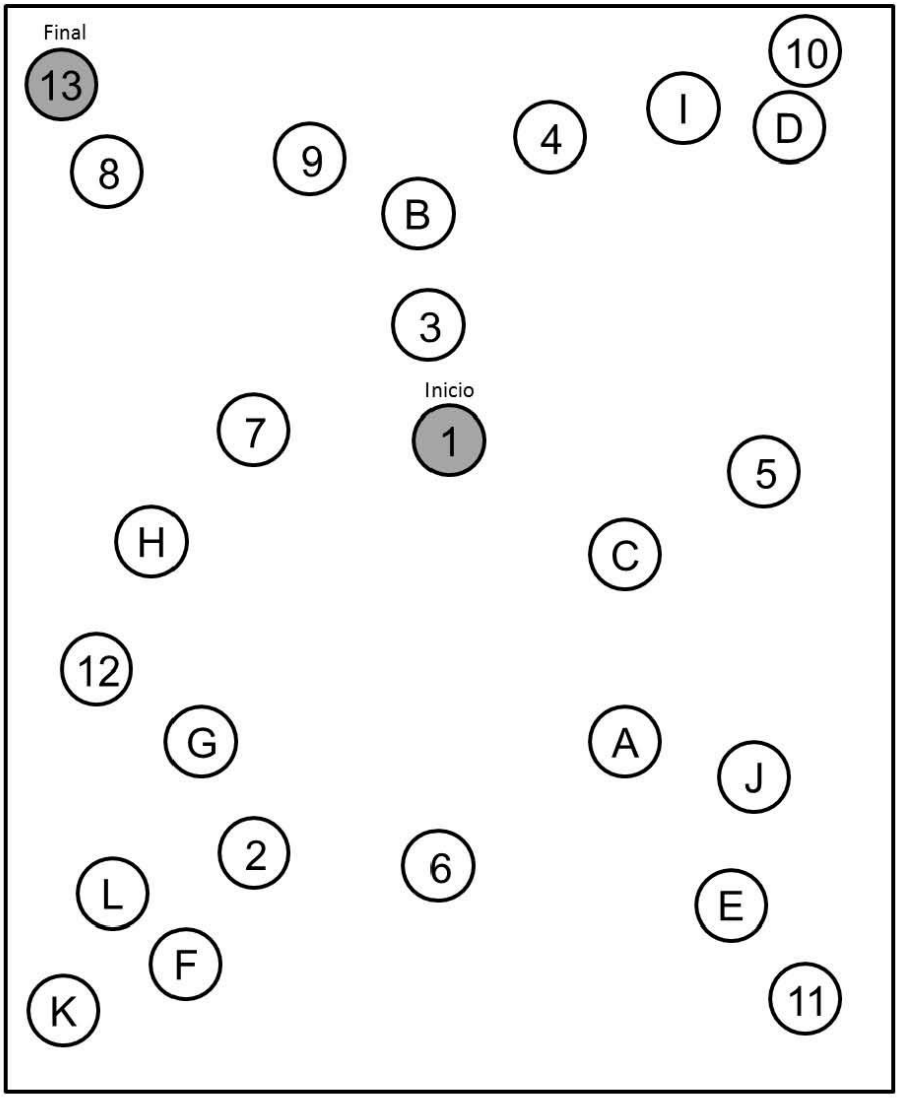
16
18
22
4
5
6
Inicio
1
24
13
7
14
2
3
8
10
Final
9
25
12
11
23

TRAIL MAKING TEST B

EJEMPLO

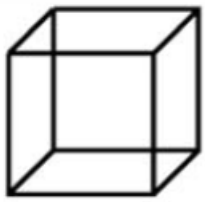


TEST



(7) Copiar el cubo…… **__ /1**

"Me gustaría que copie este dibujo de la manera más precisa posible"



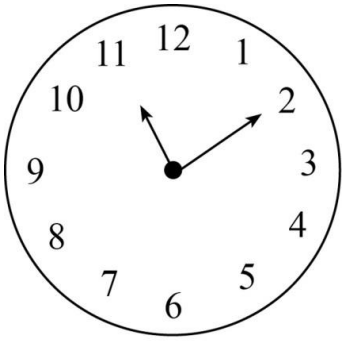
(8) Dibujar un reloj (Once y diez)
Contorno…… **__ /1**
Números…… **__ /1**
Agujas…… **__ /1**

Puntuación:

Test Reloj a la orden	__ / 10
Test Reloj a la copia	__ / 10

"Me gustaría que dibujara un reloj redondo y grande en esta hoja, colocando en él todos sus números y cuyas manecillas marquen las once y diez. En caso de que cometa algún error, aquí tiene una goma de borrar para que pueda rectificarlo. Esta prueba no tiene tiempo límite, por lo que le pedimos que la haga con tranquilidad" (a la orden)

"Copie este dibujo" (a la copia)



CONTORNO

Puntos	Resultados
2	Dibujo normal. Esfera circular u ovalada con pequeñas distorsiones por temblor
1	Incompleto o con alguna distorsión significativa. Esfera muy asimétrica
0	Ausencia de dibujo totalmente distorsionado

AGUJAS

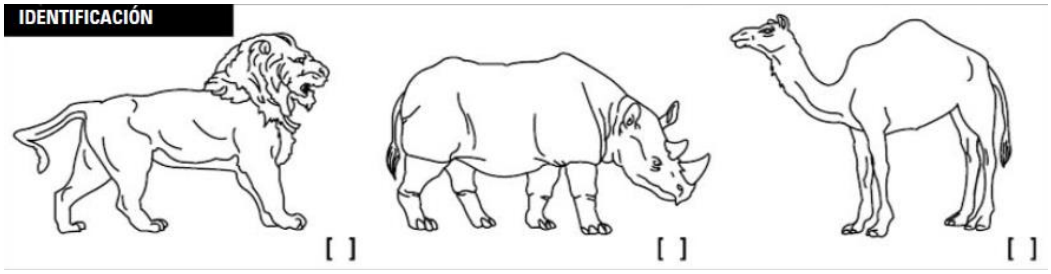
Puntos	Resultados
4	Las manecillas están en posición correcta y con las proporciones adecuadas de tamaño (la de la hora más corta)
3,5	Las manecillas en posición correcta pero ambas de igual tamaño
3	Pequeños errores de localización de las manecillas (situar una de las agujas en el espacio destinado al número anterior o posterior)
3	Aguja de los minutos más corta que la de la hora, con pauta horaria correcta
2	Gran distorsión en la localización de las manecillas (incluso si marcan las once y diez), cuando los números presentan errores significativos en la localización espacial
2	Cuando las manecillas no se juntan en el punto central y marcan la hora correcta
1	Cuando las manecillas no se juntan en el punto central y marcan una hora incorrecta
1	Presencia de una sola manecilla o un esbozo de las dos
0	Ausencia de manecillas o perseveración en el dibujo de las mismas
0	Efecto en forma de "rueda de carro"

NÚMEROS

Puntos	Resultados
4	Todos los números presentes y en el orden correcto. Sólo "pequeños errores" en la localización espacial en menos de 4 números (por ejemplo, colocar el nº 8 en el espacio del nº 9)
3,5	Cuando los "pequeños errores" en la localización se dan 4 o más números
3	Todos presentes con error significativo en la localización espacial (por ejemplo, colocar el nº 3 en el espacio del nº 6)
3	Número con algún desorden de frecuencia
2	Omisión o adición de algún nº , pero sin grandes distorsiones en los números restantes
2	Número con algún desorden de frecuencia (4 o más números)
2	Los doce números colocados en sentido antihorario (rotación inversa)
2	Todos los números presentes, pero con gran distorsión (número fuera de reloj o dibujados en media esfera, etc.)
2	Presencia de los 12 números en una línea vertical, horizontal u oblicua (alineación numérica)
1	Ausencia o exceso de números con gran distorsión espacial
1	Alineación numérica con falta o exceso de números
1	Rotación inversa con falta o exceso de números
0	Ausencia o escasa representación de números (menos de 6 números dibujados)

(9) Identificación……. **__ /3**

"Nombre cada uno de los animales de izquierda a derecha"



(10) Memoria

1 er intento: "Ésta es una prueba de memoria. Le voy a leer una lista de palabras que debe recordar. Escuche con atención y, cuando yo termine, me gustaría que me diga todas las palabras que pueda recordar, en el orden que desee".

	ROSTRO	SEDA	IGLESIA	CLAVEL	ROJO	**Sin puntos**
1er intento						
2º intento						

(11) Atención (**dígitos WAIS**) …… **__ /2**

"Voy a leer algunos números. Escuche con cuidado y cuando yo haya terminado, diga los mismos números en el mismo orden".

DÍGITOS (ORDEN DIRECTO)

TERMINACIÓN: Puntuación de 0 en los dos intentos de cualquier elemento.

PUNTUACIÓN: En cada intento 0 a 1 punto en cada respuesta

ELEMENTO			**PUNT. INTENTO**		**PUNTUACIÓN ELEMENTO**		
1	1	**1-7**	0	1	0	1	2
	2	**6-3**	0	1	0	1	2
2	1	**5-8-2**	0	1	0	1	2
	2	**6-9-4**	0	1	0	1	2
3	1	**6-4-3-9**	0	1	0	1	2
	2	**7-2-8-6**	0	1	0	1	2
4	1	**2-1-8-5-4**	0	1	0	1	2
	2	**7-5-8-3-6**	0	1	0	1	2
5	1	**6-1-9-4-7-3**	0	1	0	1	2
	2	**3-9-2-4-8-7**	0	1	0	1	2
6	1	**5-9-1-7-4-2-8**	0	1	0	1	2
	2	**4-1-7-9-3-8-6**	0	1	0	1	2
7	1	**5-8-1-9-2-6-4-7**	0	1	0	1	2
	2	**3-8-2-9-5-1-7-4**	0	1	0	1	2
8	1	**2-7-5-8-6-2-5-8-4**	0	1	0	1	2
	2	**7-1-3-9-4-2-5-6-8**	0	1	0	1	2
Puntuación total (máximo 16)							

"Ahora voy a decir otros números. Pero en esta ocasión cuando me detenga, quiero que los repita en orden inverso, al revés. Por ejemplo: si yo digo 7-1-9, ¿qué diría usted?". Si responde correctamente (9-1-7) diga: *Correcto*

DÍGITOS (ORDEN INVERSO)
TERMINACIÓN: Puntuación de 0 en los dos intentos de cualquier elemento.
PUNTUACIÓN: En cada intento 0 a 1 punto en cada respuesta

ELEMENTO			PUNT. INTENTO		PUNTUACIÓN ELEMENTO		
1	1	**2-4**	0	1	0	1	2
	2	**5-7**	0	1	0	1	2
2	1	**7-4-2**	0	1	0	1	2
	2	**4-1-5**	0	1	0	1	2
3	1	**3-2-7-9**	0	1	0	1	2
	2	**4-9-6-8**	0	1	0	1	2
4	1	**1-5-2-8-6**	0	1	0	1	2
	2	**6-1-8-4-3**	0	1	0	1	2
5	1	**5-3-9-4-1-8**	0	1	0	1	2
	2	**7-2-4-8-5-6**	0	1	0	1	2
6	1	**8-1-2-9-3-6-5**	0	1	0	1	2
	2	**4-7-3-9-1-2-8**	0	1	0	1	2
7	1	**9-4-3-7-6-2-5-8**	0	1	0	1	2
	2	**7-2-8-1-9-6-5-3**	0	1	0	1	2
Puntuación total (máximo 14)							

***<u>AVISO</u>: en este momento hay que volver a leer la misma lista de palabras y marcar en la CASILLA DEL ÍTEM 10 como 2º INTENTO, todas las palabras que recuerde.**

2º intento: "Ahora le voy a leer la misma lista de palabras que le leí antes. Intente acordarse del mayor número posible de palabras, incluyendo las que repitió en la primera ronda.
Preste atención, ya que más adelante le preguntaré por estas mismas palabras"

(12) Concentración ……. **__ /1**

"Voy a leerle una serie de letras. Cada vez que diga la letra A, dé un golpecito con la mano. Cuando diga una letra que no sea la A, no dé ningún golpecito".
[No se asignan puntos si ≥ 2 errores]
Puntuación: No se asigna ningún punto si se comete más de un error (ej., la persona da el golpecito con una letra equivocada o no da el golpecito con la letra 'A').

F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

(13) Restar de 7 en 7 empezando desde 100.

"Ahora me gustaría que calcule 100 menos 7, y así sucesivamente: continúe restando 7 a la cifra de su respuesta anterior, hasta que le pida que pare".

[ ] 93 **[ ] 86** **[ ] 79** **[ ] 72** **[ ] 65** **__ /3**

Puntuación: 4 o 5 correctas: **3 puntos** / 2 o 3 correctas: **2 puntos**/1 correcta: **1 punto**/ 0 correctas: **0 puntos**
Si el entrevistado comete un error en la resta y da una cifra errónea, pero resta 7 correctamente de dicha cifra errónea, se asignan puntos, por ejemplo, 100 - 7 = 92 - 85 - 78 -71 –64. "92" es incorrecto, pero todos los números siguientes son correctos. Dado que se trata de 4 respuestas correctas, la puntuación es de tres puntos.

(14) Lenguaje: repetición de frases.
"Ahora le voy a leer una frase y me gustaría que la repitiera a continuación: 'El gato se esconde bajo el sofá cuando los perros entran en la sala'". Acto seguido, el examinador dice: *"Ahora le voy a leer una segunda frase y usted la va a repetir a continuación: 'Espero que él le entregue el mensaje una vez que ella se lo pida'".*

Puntuación: Se asigna un punto por cada frase repetida correctamente. La repetición debe ser exacta. El examinador debe prestar atención a los errores de omisión, sustitución o adición.

Repetir:
El gato se esconde bajo el sofá cuando los perros entran en la sala [ ]
Espero que él le entregue el mensaje una vez que ella se lo pida [ ] **__ /2**

(15) Fluidez verbal.

Fluencia fonética (P-A-S)

"Quiero saber cuántas palabras conoce que comiencen por la letra P (o A o S) en 1 minuto. Puede comenzar por ejemplo con PATATA (o Alto o SAL), procurando no repetirse. Si dice el singular (patata) no val el plural (patatas) o si nombra el femenino no vale el masculino. También debe procurar evitar los nombres propios (Paco, París, etc.) y las palabras derivadas (patata, patatero…). Empiece cuando le diga. Tiempo!"

Puntuación: Se asigna un punto si el sujeto dice 11 palabras o más en un minuto. **__ /1**

Puntuación total:

P	
A	
S	

P:	**Patata**	**Total:**
A:	**Alto**	**Total:**
S:	**Sal**	**Total:**

Fluencia SEMÁNTICA (CATEGORÍAS) FAS

"Quiero saber cuántas palabras conoce que se refieran a animales en 1 minuto. Puede comenzar por ejemplo con GATO, procurando no repetirse. Si dice el singular no vale el plural o si nombra el femenino, no vale el masculino. Empiece cuando quiera."

Puntuación total:

Animales	

ANIMALES:	**Gato**	**Total:**

(16) Similitudes **(SEMEJANZAS WAIS-III)** **__ /2**

Se empieza la prueba por el ítem 1 hasta el 10, consecutivamente. La puntuación máxima de los ítems 1 al 5 es de 1 punto, mientras que la de los ítems 6 al 10 es de 2 puntos. En caso de que el sujeto cometa 4 errores consecutivos (0 puntos), se finaliza la prueba.

«Ahora voy a leerle 2 palabras y usted tendrá que decirme en qué se parecen». En cada elemento, se hace la pregunta insertando las palabras correspondientes: *« ¿En qué se parecen naranja y pera?».* Si la respuesta dada por el sujeto es ambigua o va seguida de una (P) en la lista de ejemplos de respuesta, se le insiste: *«Dígame algo más»,* o bien *« ¿A qué se refiere?».* Si el sujeto da varias respuestas correctas, se puntúa la mejor. Si da algunas incorrectas y otras correctas, preguntar: *«Entonces, ¿cuál es su respuesta?».*

	Elemento	Respuesta	Puntuación		
1	Naranja-Pera		0	1	
2	Chaqueta-Pantalón		0	1	
3	Perro-León		0	1	
4	Calcetines-Zapatos		0	1	
5	Tenedor- Cuchara		0	1	
6	Mesa-Silla		0	1	2
7	TREN-BICICLETA		0	1	2
8	RELOJ-REGLA		0	1	2
9	Ojo-Oído		0	1	2
10	Aire-Agua		0	1	2
		Puntación directa			

(17) Recuerdo diferido

"Antes le leí una serie de palabras y le pedí que las recordase. Dígame ahora todas las palabras de las que se acuerde". El examinador marca las palabras que el paciente recuerde sin necesidad de pistas, por medio de una cruz (x) en el espacio reservado a dicho efecto.

Puntuación: Se asigna un punto por cada una de las palabras **recordadas espontáneamente, sin pistas.**

ROSTRO	SEDA	IGLESIA	CLAVEL	ROJO	
[ ]	[ ]	[ ]	[ ]	[ ]	__ /5

(18) Orientación

"Dígame en qué día estamos hoy". Si el paciente ofrece una respuesta incompleta, el examinador dice: *"Dígame el año, el mes, el día del mes (fecha) y el día de la semana"*. A continuación, el examinador pregunta*: "Dígame cómo se llama el lugar donde estamos ahora y en qué localidad nos encontramos".*

Puntuación: Se asigna un punto por cada una de las respuestas correctas. El paciente debe decir la fecha exacta y el lugar exacto (hospital, clínica, oficina, etc.). No se asigna ningún punto si el paciente se equivoca por un día en el día del mes y de la semana.

[ ] Día del mes (fecha) **[ ] Mes** **[ ] Año** **[ ] Día de la semana** **[ ] Lugar** **[ ] Localidad** **__ /6**

(19) Puntuación total del MoCA: sumar todas las casillas sombreadas. La puntuación máxima es 30.

Normal ≥ 26/30

TOTAL	**___ /30**
Añadir 1 punto si tiene ≤ 12 años de estudios	

(20) Problemas de memoria objetivos: si la puntuación del MoCA está por debajo del punto de corte, (menos de 26 puntos) puntuará 1. Si no, 0. (Rodear con un círculo)

0 /1

(21) Problemas de memoria, corroborados por un informante: esposa, hijo, amigo, vecino, cuidador, compañero de trabajo… [No: 0 / Sí: 1] (Rodear con un círculo)

0 /1

Si el entrevistado no viene o identifica un informador fiable*, pasar al apartado de juicios. [No: 0 / Sí: 1]

Apartado de juicios [No: 0 / Sí: 1]

- En opinión del entrevistador, el entrevistado sufre problemas de memoria y son un problema para llevar a cabo su vida con normalidad.
- El actual estado cognoscitivo del entrevistado data desde el nacimiento o se debe a patología anterior (ej.: retraso mental moderado o grave) y no es debida a enfermedad mental de inicio en los últimos años (ACV, TCE…).

*Informador fiable: persona que mantiene contacto habitual con el entrevistado de al menos 2 horas a la semana.

(22) Realización de tareas habituales: el entrevistado, en el estado cognoscitivo actual, no puede realizar por sí mismo tareas cotidianas o necesitaría ayuda (ej.: labores de casa, gestiones comunes y cuidado de la familia -hijos, nietos…- en el caso de que su situación laboral actual sea **no activo laboralmente**). (**Personas activas** laboralmente, puede realizar su trabajo en el puesto habitual, sin problemas).

0 / 1/ 2

NOTA:
0: NO TIENE PROBLEMA→ El entrevistado puntúa 0 si la puntuación FAQ es 0
1: NO TIENE PROBLEMA, AUNQUE NECESITARÍA AYUDA → El entrevistado puntúa 1 si la puntuación en FAQ es < 6
2: TIENE PROBLEMAS, DEPENDIENTE → El entrevistado puntúa 2 si la puntuación en FAQ es ≥ 6, o alguna de las actividades de la vida diaria (AVD) se ha puntuado con 3 puntos.

Puntuación en AVD's (FAQ de Pfeffer-) valorar si es necesario obtener la puntuación en todos, o solo en aquellos que estén jubilados…

Cuestionario de ACTIVIDAD FUNCIONAL de PFEFFER (FAQ)

Pfeffer et al. (1982)

Informador (relación con el paciente):
Nombre: Varón [ ] Mujer [ ]
Fecha: F. nacimiento: Edad:
Estudios/Profesión: N. Hª:
Observaciones:

Puntuar cada ítem del modo siguiente:

0 ***Normal; o nunca lo hizo pero podría hacerlo solo/a***
1 ***Con dificultad pero se maneja solo; o nunca lo hizo y si tuviera que hacerlo ahora tendría dificultad***
2 ***Necesita ayuda (pero lo hace)***
3 ***Dependiente (no puede realizarlo)***

1. ¿Maneja su propio dinero ?	**3**	**2**	**1**	**0**
2. ¿Puede hacer solo/a la compra (alimentos, ropa, cosas de la casa)?	**3**	**2**	**1**	**0**
3. ¿Puede preparase solo/a el café o el té y luego apagar el fuego?	**3**	**2**	**1**	**0**
4. ¿Puede hacerse solo/a la comida?	**3**	**2**	**1**	**0**
5. ¿Está al corriente de las noticias de su vecindario, de su comunidad?	**3**	**2**	**1**	**0**
6. ¿Puede prestar atención, entender y discutir las noticias de la radio y los programas de TV, libros, revistas?	**3**	**2**	**1**	**0**
7. ¿Recuerda si queda con alguien, las fiestas familiares (cumpleaños, aniversarios), los días festivos?	**3**	**2**	**1**	**0**
8. ¿Es capaz de manejar su propia medicación?	**3**	**2**	**1**	**0**
9. ¿Es capaz de viajar solo/a fuera de su barrio y volver a casa?	**3**	**2**	**1**	**0**
10. ¿Saluda apropiadamente a sus amistades?	**3**	**2**	**1**	**0**
11. ¿Puede salir a la calle solo/a sin peligro?	**3**	**2**	**1**	**0**
PUNTUACIÓN TOTAL				

Una puntuación por debajo de 6 indica normalidad (no dependencia)
Una puntuación de 6 o más indica alteración funcional

Anexo 5. Cuestionario NHANES que incluye el GPAQ

PHYSICAL ACTIVITY AND PHYSICAL FITNESS – PAQ
Target Group: SPs 2+

BOX 1
CHECK ITEM PAQ.700: IF SP AGE 2-11, GO TO PAQ706. IF SP AGE <2 OR SP 12-15, GO TO NEXT SECTION. IF SP AGE 16+, CONTINUE.

PAQ.605 Next I am going to ask you about the time {you spend/SP spends} doing different types of physical activity in a typical week.

Think first about the time {you spend/he spends/she spends} doing work. Think of work as the things that {you have/he has/she has} to do such as paid or unpaid work, household chores, and yard work.

Does {your/SP's} work involve **vigorous**-intensity activity that causes **large increases** in breathing or heart rate like carrying or lifting heavy loads, digging or construction work for **at least 10 minutes continuously**?

YES	1	
NO	2	(PAQ.620)
REFUSED	7	(PAQ.620)
DON'T KNOW	9	(PAQ.620)

PAQ.610 In a typical week, on how many days {do you/does SP} do **vigorous**-intensity activities as part of {your/his/her} work?

PROBE IF NEEDED: Vigorous-intensity activity causes large increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

INTERVIEWER: REMEMBER, WE ARE ONLY ASKING ABOUT WORK AND CHORES IN THIS QUESTION.

HARD EDIT: 1-7.
ERROR MESSAGE: THE NUMBER OF DAYS SHOULD BE BETWEEN 1 AND 7.

|___|___|
ENTER NUMBER OF DAYS

REFUSED	77	(PAQ.620)
DON'T KNOW	99	(PAQ.620)

PAQ.615 Q/U How much time {do you/does SP} spend doing **vigorous**-intensity activities at work on a typical day?

PROBE IF NEEDED: Think about a typical day when {you do/he does/she does} vigorous-intensity activities during {your/his/her} work.

PROBE IF NEEDED: Vigorous-intensity activity causes large increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

INTERVIEWER: REMEMBER, WE ARE ONLY ASKING ABOUT WORK AND CHORES.

SOFT EDIT: >4 HOURS.
ERROR MESSAGE: INTERVIEWER, YOU HAVE RECORDED THAT THE SP SPENDS MORE THAN 4 HOURS DOING VIGOROUS-INTENSITY ACTIVITIES AT WORK ON A TYPICAL DAY. PLEASE CONFIRM WITH SP THAT OVER 4 HOURS IS CORRECT.

HARD EDIT: ≥24 HOURS.
HARD EDIT: <10 MINUTES.
ERROR MESSAGE: THE TIME SHOULD BE 10 MINUTES OR MORE, BUT LESS THAN 24 HOURS.

|___|___|___|
ENTER NUMBER OF MINUTES OR HOURS

REFUSED	7777	(PAQ.620)
DON'T KNOW	9999	(PAQ.620)

ENTER UNIT

MINUTES	1
HOURS	2

PAQ.620 Does {your/SP's} work involve **moderate**-intensity activity that causes **small increases** in breathing or heart rate such as brisk walking or carrying light loads for **at least 10 minutes continuously**?

YES	1	
NO	2	(PAQ.635)
REFUSED	7	(PAQ.635)
DON'T KNOW	9	(PAQ.635)

PAQ.625 In a typical week, on how many days {do you/does SP} do **moderate**-intensity activities as part of {your/his/her} work?

PROBE IF NEEDED: Moderate-intensity activity causes small increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

INTERVIEWER: REMEMBER, WE ARE ONLY ASKING ABOUT WORK AND CHORES.

HARD EDIT: 1-7.
ERROR MESSAGE: THE NUMBER OF DAYS SHOULD BE BETWEEN 1 AND 7.

|___|___|
ENTER NUMBER OF DAYS

REFUSED	77	(PAQ.635)
DON'T KNOW	99	(PAQ.635)

PAQ.630 Q/U How much time {do you/does SP} spend doing **moderate**-intensity activities at work on a typical day?

PROBE IF NEEDED: Think about a typical day when {you do/he does/she does} moderate-intensity activities during {your/his/her} work.

PROBE IF NEEDED: Moderate-intensity activity causes small increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

INTERVIEWER: REMEMBER, WE ARE ONLY ASKING ABOUT WORK AND CHORES.

SOFT EDIT: >4 HOURS.
ERROR MESSAGE: INTERVIEWER, YOU HAVE RECORDED THAT THE SP SPENDS MORE THAN 4 HOURS DOING MODERATE-INTENSITY ACTIVITIES AT WORK ON A TYPICAL DAY. PLEASE CONFIRM WITH SP THAT OVER 4 HOURS IS CORRECT.

HARD EDIT: ≥24 HOURS.
HARD EDIT: <10 MINUTES.
ERROR MESSAGE: THE TIME SHOULD BE 10 MINUTES OR MORE, BUT LESS THAN 24 HOURS.

|___|___|___|
ENTER NUMBER OF MINUTES OR HOURS

REFUSED	7777	(PAQ.635)
DON'T KNOW	9999	(PAQ.635)

|___|
ENTER UNIT

MINUTES	1
HOURS	2

PAQ.635 The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way {you travel/SP travels} to and from places. For example to work, for shopping, to school.

In a typical week {do you/does SP} walk or use a bicycle for **at least 10 minutes continuously** to get to and from places?

YES	1	
NO	2	(PAQ.650)
REFUSED	7	(PAQ.650)
DON'T KNOW	9	(PAQ.650)

PAQ.640 In a typical week, on how many days {do you/does SP} walk or bicycle for **at least 10 minutes continuously** to get to and from places?

HARD EDIT: 1-7.
ERROR MESSAGE: THE NUMBER OF DAYS SHOULD BE BETWEEN 1 AND 7.

|___|___|
ENTER NUMBER OF DAYS

REFUSED	77(PAQ.650)
DON'T KNOW	99(PAQ.650)

PAQ.645 Q/U How much time {do you/does SP} spend walking or bicycling for travel on a typical day?

PROBE IF NEEDED: Think about a typical day when {you walk or bicycle/SP walks or bicycles} for travel.

SOFT EDIT: >4 HOURS.
ERROR MESSAGE: INTERVIEWER, YOU HAVE RECORDED THAT THE SP SPENDS MORE THAN 4 HOURS WALKING OR BICYCLING TO GET TO AND FROM PLACES ON A TYPICAL DAY. PLEASE CONFIRM WITH SP THAT OVER 4 HOURS IS CORRECT.

HARD EDIT: ≥24 HOURS.
HARD EDIT: <10 MINUTES.
ERROR MESSAGE: THE TIME SHOULD BE 10 MINUTES OR MORE, BUT LESS THAN 24 HOURS.

|___|___|___|
ENTER NUMBER OF MINUTES OR HOURS

REFUSED	7777	(PAQ.650)
DON'T KNOW	9999	(PAQ.650)

|___|
ENTER UNIT

MINUTES	1
HOURS	2

PAQ.650 The next questions exclude the work and transportation activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities.

In a typical week {do you/does SP} do any **vigorous**-intensity sports, fitness, or recreational activities that cause **large increases** in breathing or heart rate like running or basketball for **at least 10 minutes continuously**?

YES	1	
NO	2	(PAQ.665)
REFUSED	7	(PAQ.665)
DON'T KNOW	9	(PAQ.665)

PAQ.655 In a typical week, on how many days {do you/does SP} do **vigorous**-intensity sports, fitness or recreational activities?

PROBE IF NEEDED: Vigorous-intensity activity causes large increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

HARD EDIT: 1-7.
ERROR MESSAGE: THE NUMBER OF DAYS SHOULD BE BETWEEN 1 AND 7.

|___|___|
ENTER NUMBER OF DAYS

REFUSED	77	(PAQ.665)
DON'T KNOW	99	(PAQ.665)

PAQ.660 Q/U How much time {do you/does SP} spend doing **vigorous**–intensity sports, fitness or recreational activities on a typical day?

PROBE IF NEEDED: Think about a typical day when {you do/SP does} vigorous-intensity sports, fitness or recreational activities.

SOFT EDIT: >4 HOURS.
ERROR MESSAGE: INTERVIEWER, YOU HAVE RECORDED THAT THE SP SPENDS MORE THAN 4 HOURS DOING VIGOROUS-INTENSITY RECREATIONAL ACTIVITIES ON A TYPICAL DAY. PLEASE CONFIRM WITH SP THAT OVER 4 HOURS IS CORRECT.

HARD EDIT: ≥24 HOURS.
HARD EDIT: <10 MINUTES.
ERROR MESSAGE: THE TIME SHOULD BE 10 MINUTES OR MORE, BUT LESS THAN 24 HOURS.

|___|___|___|
ENTER NUMBER OF MINUTES OR HOURS

REFUSED	7777	(PAQ.665)
DON'T KNOW	9999	(PAQ.665)

|___|
ENTER UNIT

MINUTES	1
HOURS	2

PAQ.665 In a typical week {do you/does SP} do any **moderate**-intensity sports, fitness, or recreational activities that cause a **small increase** in breathing or heart rate such as brisk walking, bicycling, swimming, or golf for **at least 10 minutes continuously**?

YES	1	
NO	2	(PAQ.680)
REFUSED	7	(PAQ.680)
DON'T KNOW	9	(PAQ.680)

PAQ.670 In a typical week, on how many days {do you/does SP} do **moderate**-intensity sports, fitness or recreational activities?

PROBE IF NEEDED: Moderate-intensity sports, fitness or recreational activities cause small increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

HARD EDIT: 1-7.
ERROR MESSAGE: THE NUMBER OF DAYS SHOULD BE BETWEEN 1 AND 7.

|___|___|
ENTER NUMBER OF DAYS

REFUSED	77	(PAQ.680)
DON'T KNOW	99	(PAQ.680)

PAQ.675 Q/U How much time {do you/does SP} spend doing **moderate**-intensity sports, fitness or recreational activities on a typical day?

PROBE IF NEEDED: Think about a typical day when {you do/SP does} moderate-intensity sports, fitness or recreational activities.

PROBE IF NEEDED: Moderate-intensity sports, fitness or recreational activities cause small increases in breathing or heart rate and is done for at **least 10 minutes continuously**.

SOFT EDIT: >4 HOURS.
ERROR MESSAGE: INTERVIEWER, YOU HAVE RECORDED THAT THE SP SPENDS MORE THAN 4 HOURS DOING MODERATE-INTENSITY RECREATIONAL ACTIVITIES ON A TYPICAL DAY. PLEASE CONFIRM WITH SP THAT OVER 4 HOURS IS CORRECT.

HARD EDIT: ≥24 HOURS.
HARD EDIT: <10 MINUTES.
ERROR MESSAGE: THE TIME SHOULD BE 10 MINUTES OR MORE, BUT LESS THAN 24 HOURS.

|___|___|___|
ENTER NUMBER OF MINUTES OR HOURS

REFUSED	7777	(PAQ.680)
DON'T KNOW	9999	(PAQ.680)

|___|
ENTER UNIT

MINUTES	1
HOURS	2

PAQ.680 Q/U The following question is about sitting at work, at home, getting to and from places, or with friends, including time spent sitting at a desk, traveling in a car or bus, reading, playing cards, watching television, or using a computer. Do not include time spent sleeping.

How much time {do you/does SP} usually spend sitting on a typical day?

|___|___|___|
ENTER NUMBER OF MINUTES OR HOURS

REFUSED	7777	(BOX 2)
DON'T KNOW	9999	(BOX 2)

|___|
ENTER UNIT

MINUTES	1
HOURS	2

SOFT EDIT: 18 HOURS OR MORE AND LESS THAN 3 HOURS.
ERROR MESSAGE: PLEASE VERIFY TIMES OF 18 HOURS OR MORE OR LESS THAN 3 HOURS.

HARD EDIT: 24 HOURS OR MORE.
ERROR MESSAGE: THE TIME SHOULD BE LESS THAN 24 HOURS.

BOX 2

CHECK ITEM PAQ.720:
IF SP AGE 16+, GO TO PAQ.710.

PAQ.706 Now I'd like to ask you some questions about {your/SP's} activities.

During the **past 7 days**, on how many days {were you/was SP} physically active for a total of **at least 60 minutes per day**? Add up all the time {you/he/she} spent in any kind of physical activity that increased {your/his/her} heart rate and made {you/him/her} breathe hard some of the time.

0 days	0
1 day	1
2 days	2
3 days	3
4 days	4
5 days	5
6 days	6
7 days	7
REFUSED	77
DON'T KNOW	99

PAQ.710 Now I will ask you first about TV watching and then about computer use.

Over the past 30 days, on average how many **hours per day** did {you/SP} sit and watch TV or videos? Would you say . . .

less than 1 hour,	0
1 hour,	1
2 hours,	2
3 hours,	3
4 hours,	4
5 hours or more, or	5
{You do/SP does} not watch TV or videos	8
REFUSED	77
DON'T KNOW	99

CAPI INSTRUCTION:
SOFT EDIT: IF SP AGE => 16 AND THE TIME PAQ.710 > THE TIME IN PAQ.680.
ERROR MESSAGE: PLEASE VERIFY PAQ.710 TIME (TV WATCHING) SHOULD NOT BE MORE THAN PAQ.680 (TIME SITTING).

PAQ.715 Over the past 30 days, on average how many **hours per day** did {you/SP} use a computer or play computer games outside of work or school? Include Playstation, Nintendo DS, or other portable video games. Would you say . . .

less than 1 hour,	0
1 hour,	1
2 hours,	2
3 hours,	3
4 hours,	4
5 hours or more, or	5
{You do/SP does} not use a computer outside of work or school	8
REFUSED	77
DON'T KNOW	99

HELP SCREEN:
If the SP watches T.V. or video at the same time as working on the computer, count this time as watching T.V. or video.

BOX 2b

CHECK ITEM PAQ.718:
IF 3-11, CONTINUE.
ELSE, GO TO END OF SECTION.

PAQ.722 For the next questions, think about the sports, lessons, or physical activities {you/SP} may have done during the **past 7 days**? {Please do not include things {you/he/she} did during the school day like PE or gym class.}

Did {you/SP} do any physical activities during the **past 7 days?**

YES	1	
NO	2	(BOX 3)
REFUSED	7	(BOX 3)
DON'T KNOW	9	(BOX 3)

CAPI INSTRUCTION: IF SP AGE IS 3-4 YEARS OLD, DO NOT DISPLAY {Please do not include things {you/he/she} did during the school day like PE or gym class.}

PAQ.724 OS

What physical activities did {you/SP} do during the **past 7 days**? Don't include activities {you/SP} did during gym or PE.
[PROBE: Did {you/he/she} do any other physical activities?}

CODE ALL THAT APPLY

AEROBICS/WEIGHT TRAINING/GYM/ EXERCISE	1
BASEBALL/SOFTBALL/CATCH/PITCHING	2
BASKETBALL	3
BIKE RIDING/DIRT BIKING/MOUNTAIN BIKING	4
CHEERLEADING	5
DANCE	6
FIELD HOCKEY/STREET HOCKEY/ ROLLER HOCKEY	7
FOOTBALL	8
FRISBEE/ULTIMATE FRISBEE	29
GOLF	9
GYMNASTICS/TUMBLING	10
HIKING	11
ICE HOCKEY	12
ICE SKATING	13
JUMPING ROPE	14
LACROSSE	15
MARTIAL ARTS (KARATE/TAE KWON DO/ JUDO, ETC.)	16
PLAYING GAMES (PROBE: WERE YOU PHYSICALLY ACTIVE? IF NO, DON'T COUNT)	17
BACKYARD/PLAYGROUND GAMES AND ACTIVITIES	30
ROLLER BLADING/ROLLER SKATING	18
RUNNING/JOGGING	19
SCOOTER RIDING (PROBE: DOES IT HAVE A MOTOR? IF YES, DON'T COUNT)	20
SKATEBOARDING	21
SOCCER	22
SWIMMING	23
TENNIS	24
TRACK & FIELD	25
TRAMPOLINE	31
VOLLEYBALL	26
WALKING	27
WRESTLING	28
OTHER (SPECIFY)_____	91
REFUSED	77
DON'T KNOW	99

BOX 3

CHECK ITEM PAQ.726:
IF SP AGE 3-4, GO TO END OF SECTION.
IF SP AGE 5-11, CONTINUE.

PAQ.731 During the **past 7 days**, on how many days did {you/SP} play **active** video games such as Wii Sports, Wii Fit, Xbox 360, Xbox Kinect, Playstation 3, or Dance, Dance Revolution?

0 days	0	(PAQ.755)
1 day	1	
2 days	2	
3 days	3	
4 days	4	
5 days	5	
6 days	6	
7 days	7	
REFUSED	77	
DON'T KNOW	99	

PAQ.733 On average, for how long did {you/SP} play these **active** video games?

_____
Q/U
|___|___|___|
ENTER NUMBER (OF MINUTES OR HOURS)

REFUSED	7777	(PAQ.755)
DON'T KNOW	9999	(PAQ.755)

ENTER UNIT

MINUTES	1
HOURS	2

SOFT EDIT: IF THE HOURS EXCEED 4 SAY UNUSUAL.
SOFT EDIT: IF THE MINUTES ARE LESS THAN 10 CONFIRM THAT IT IS MINUTES NOT HOURS.

PAQ.755 The following are activities that may be done before, during, or after school **other than** during {PE or gym class/recess}. If {you are/SP is} not currently in school, think about {your/his/her} activities when {you were/he was/she was} **last in school**.} {Do you/Does SP} participate in school sports or physical activity clubs?

CAPI INSTRUCTION: IF SP AGE 5-11, DISPLAY {recess}

YES	1	
NO	2	(PAQ.762)
REFUSED	7	(PAQ.762)
DON'T KNOW	9	(PAQ.762)

PAQ.759 OS In what school **sports** or **physical activity** clubs {do you/does SP} participate?

CODE ALL THAT APPLY

HAND CARD PAQ1

BASEBALL/SOFTBALL	1	
BASKETBALL	2	
BOCCE BALL	3	
CHEERLEADING	4	
DANCE	17	
FOOTBALL	5	
FRISBEE/ULTIMATE FRISBEE	18	
GOLF	6	
GYMNASTICS	7	
HOCKEY	8	
LACROSSE	9	
RUNNING	19	
SOCCER	10	
SWIMMING/DIVING	11	
TENNIS	12	
TRACK AND FIELD	13	
TRAMPOLINE	20	
VOLLEYBALL	14	
WRESTLING	15	
OTHER (SPECIFY)_____	16	
REFUSED	77	
DON'T KNOW	99	

PAQ.762 {Do you/Does SP} have recess during school days?

YES	1	
NO	2	(PAQ.750)
REFUSED	7	(PAQ.750)
DON'T KNOW	9	(PAQ.750)

PAQ.764 How often {do you/does SP} have recess?

1 day a week	1
2 days a week	2
3 days a week	3
4 days a week, or	4
Every day	5
REFUSED	7
DON'T KNOW	9

PAQ.766 On average, how long is the recess period?

LESS THAN 10 MINUTES	1
10-15 MINUTES	2
16-30 MINUTES	3
MORE THAN 30 MINUTES	4
REFUSED	7
DON'T KNOW	9

PAQ.750 I am going to read a statement and I want you to let me know if you strongly agree, agree, neither agree nor disagree, disagree or strongly disagree with the statement. {I enjoy participating in PE or gym class.}

CAPI INSTRUCTION: IF SP AGE 5-11, DISPLAY { {SP} enjoys participating in recess}

HAND CARD PAQ2

STRONGLY AGREE	1	
AGREE	2	
NEITHER AGREE NOR DISAGREE	3	
DISAGREE	4	
STRONGLY DISAGREE	5	
REFUSED	7	
DON'T KNOW	9	

PAQ.770 In the past year, did {you/SP} receive a Physical Fitness Test award, such as a President's Challenge or Fitnessgram award?

YES	1	
NO	2	(END OF SECTION)
REFUSED	7	(END OF SECTION)
DON'T KNOW	9	(END OF SECTION)

PAQ.772 OS What Physical Fitness Test award did {you/SP} receive?

PROBE IF NEEDED: Examples of physical fitness test awards are the FITNESSGRAM and the PRESIDENT'S CHALLENGE. CODE ALL THAT APPLY.

Fitnessgram	1	
President's Challenge	2	
OTHER (SPECIFY)_____	3	
REFUSED	7	
DON'T KNOW	9	

Anexo 6. Cuestionario PHQ-9 para evaluar la depresión

DEPRESSION SCREEN – DPQ
Target Group: SPs 12+

BOX 1

CHECK ITEM DPQ.001:
- IF INTERVIEW DONE ONLY WITH SURVEY PARTICIPANT (CODED '1' IN RIQ.005), CONTINUE.
- OTHERWISE, GO TO NEXT SECTION.

DPQ.010 Over the **last 2 weeks**, how often have you been bothered by the following problems:

little interest or pleasure in doing things? Would you say . . .

HANDCARD DPQ1

Not at all,	0
several days,	1
more than half the days, or	2
nearly every day?	3
REFUSED	7
DON'T KNOW	9

DPQ.020 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

feeling down, depressed, or hopeless?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.030 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

trouble falling or staying asleep, or sleeping too much?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.040 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

feeling tired or having little energy?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.050 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

poor appetite or overeating?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.060 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

feeling bad about yourself – or that you are a failure or have let yourself or your family down?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.070 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

trouble concentrating on things, such as reading the newspaper or watching TV?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.080 [Over the **last 2 weeks**, how often have you been bothered by the following problems:]

moving or speaking so slowly that other people could have noticed? Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual?

HANDCARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

DPQ.090 Over the last 2 weeks, how often have you been bothered by the following problem:

Thoughts that you would be better off dead or of hurting yourself in some way?

HAND CARD DPQ1

NOT AT ALL	0
SEVERAL DAYS	1
MORE THAN HALF THE DAYS	2
NEARLY EVERY DAY	3
REFUSED	7
DON'T KNOW	9

BOX 2

CHECK ITEM DPQ.095:
- IF RESPONSE TO ANY OF QUESTIONS DPQ.010 – DPQ.090 = 1, 2, OR 3, GO TO DPQ.100.
- OTHERWISE, GO TO NEXT SECTION.

DPQ.100 How **difficult** have these problems made it for you to do your work, take care of things at home, or get along with people?

Not at all difficult,	0
Somewhat difficult,	1
Very difficult,	2
Extremely difficult?	3
REFUSED	7
DON'T KNOW	9

Anexo 7. Cuestionario NHANES para evaluar el consumo de alcohol

ALCOHOL USE – ALQ
Target Group: SPs 18+ (CAPI)

ALQ.101 The next questions are about drinking alcoholic beverages. Included are liquor (such as whiskey or gin), beer, wine, wine coolers, and any other type of alcoholic beverage.

In **any one year**, {have you/has SP} had at least 12 drinks of any type of alcoholic beverage? By a drink, I mean a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

HAND CARD ALQ1

YES	1	(ALQ.120)
NO	2	
REFUSED	7	
DON'T KNOW	9	

ALQ.110 In {your/SP's} **entire life**, {have you/has he/has she} had at least 12 drinks of any type of alcoholic beverage?

YES	1	
NO	2	(END OF SECTION)
REFUSED	7	(END OF SECTION)
DON'T KNOW	9	(END OF SECTION)

ALQ.120 Q/U In the **past 12 months**, how often did {you/SP} drink any type of alcoholic beverage?

PROBE: How many days per week, per month, or per year did {you/SP} drink?

ENTER '0' FOR NEVER.

|___|___|___|
ENTER QUANTITY

REFUSED	777
DON'T KNOW	999

ENTER UNIT

WEEK	1
MONTH	2
YEAR	3
REFUSED	7
DON'T KNOW	9

BOX 1

CHECK ITEM ALQ.125:
IF SP DIDN'T DRINK (CODED '0') IN ALQ.120, GO TO ALQ.151.
OTHERWISE, CONTINUE WITH ALQ.130.

ALQ.130 In the **past 12 months**, on those days that {you/SP} drank alcoholic beverages, on the **average**, how many drinks did {you/he/she} have? (By a drink, I mean a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.)

HAND CARD ALQ1

IF LESS THAN 1 DRINK, ENTER '1'.
IF 95 DRINKS OR MORE, ENTER '95'.

CAPI INSTRUCTION:
SOFT EDIT: IF RESPONSE >=20, THEN DISPLAY "YOU SAID ON THE DAYS THAT YOU DRINK YOU HAVE ON **AVERAGE** {DISPLAY QUANTITY} DRINKS, IS THAT CORRECT?"

HARD EDIT: If ALQ.101 = 2 or 9, ALQ.130 must be less than 12.
Error Message: "Number of drinks per day cannot be greater than number of drinks in any one year."

|___|___|___|
ENTER # OF DRINKS

REFUSED 777
DON'T KNOW 999

ALQ.141 Q/U In the **past 12 months**, on how many **days** did {you/SP} have {DISPLAY NUMBER} or more drinks of any alcoholic beverage?
PROBE: How many days per week, per month, or per year did {you/SP} have {DISPLAY NUMBER} or more drinks in a single day?

ENTER '0' FOR NONE.

CAPI INSTRUCTION:
IF SP = MALE, DISPLAY = 5
IF SP = FEMALE, DISPLAY = 4

HARD EDIT: If ALQ.101 = 2 or 9, ALQ.141 must be less than 3 times per year.
Error Message: "Number of drinks must be less than 3 if SP never had more than 12 drinks per year."

|___|___|___|
ENTER QUANTITY

REFUSED 777
DON'T KNOW 999

ENTER UNIT

WEEK..... 1
MONTH 2
YEAR..... 3
REFUSED 7
DON'T KNOW 9

ALQ.151 Was there ever a time or times in {your/SP's} life when {you/he/she} **drank {DISPLAY NUMBER} or more drinks** of any kind of alcoholic beverage **almost every day**?

CAPI INSTRUCTION:
IF SP = MALE, DISPLAY = 5
IF SP = FEMALE, DISPLAY = 4

YES	1
NO	2
REFUSED	7
DON'T KNOW	9

BOX 2

CHECK ITEM ALQ.154:
IF ALQ.141 IS CODED '0' OR IF ALQ.120 IS CODED '0', GO TO END OF SECTION.
OTHERWISE, CONTINUE WITH ALQ.160.

ALQ.160 During the **past 30 days**, how many times did {you/SP} **drink {DISPLAY NUMBER} or more drinks** of any kind of alcohol in about **two hours**?

ENTER '0' FOR NEVER.

CAPI INSTRUCTION:
IF SP = MALE, DISPLAY = 5
IF SP = FEMALE, DISPLAY = 4

SOFT EDIT: IF RESPONSE IS > 60 TIMES, THEN DISPLAY "YOU SAID THAT IN THE **PAST 30 DAYS**, YOU HAD **{DISPLAY NUMBER} OR MORE** DRINKS OF ANY KIND OF ALCOHOL IN ABOUT **TWO HOURS**, (DISPLAY QUANTITY) TIMES. IS THAT CORRECT"?

|___|___|
ENTER QUANTITY

REFUSED	777
DON'T KNOW	999

Anexo 8. Cuestionario NHANES para evaluar el consumo de tabaco

TOBACCO – SMQ
Target Group: SPs 12+ (CAPI)

BOX 1

CHECK ITEM SMQ.859:
IF SP AGED 12-17, GO TO SMQ.860.
OTHERWISE, CONTINUE.

SMQ.681 The following questions ask about use of tobacco products in the past **5 days**.

During the past **5 days**, including today, did {you/he/she} smoke cigarettes, pipes, cigars, little cigars or cigarillos, water pipes, hookahs, or e-cigarettes?

YES	1	
NO	2	(SMQ.851)
REFUSED	7	(SMQ.851)
DON'T KNOW	9	(SMQ.851)

SMQ.692 Which of these products did {you/he/she} smoke?

(CHECK ALL THAT APPLY)

Cigarettes	1	
Pipes	2	
Cigars, or little cigars, or cigarillos	3	
Water pipes or Hookahs	4	
E-cigarettes	5	
REFUSED	77	(SMQ.851)
DON'T KNOW	99	(SMQ.851)

BOX 2

CHECK ITEM SMQ.701:
IF 'CIGARETTES' (CODE 1) IN SMQ.692, GO TO SMQ.710.
IF 'PIPES' (CODE 2) IN SMQ.692, GO TO SMQ.740.
IF 'CIGARS' (CODE 3) IN SMQ.692, GO TO SMQ.771.
IF 'WATER PIPES OR HOOKAHS' (CODE 4) IN SMQ.692, GO TO SMQ.845.
IF 'E-CIGARETTE' (CODE 5) IN SMQ.692, GO TO SMQ.849.

SMQ.710 During the past **5 days,** including today, on how many days did {you/he/she} smoke cigarettes?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED	7
DON'T KNOW	9

SMQ.720 During the past **5 days**, including today, on the days {you/he/she} smoked, how many cigarettes did {you/he/she} smoke each day?

IF R SAYS 95 OR MORE CIGARETTES PER DAY, ENTER 95.

HARD EDIT: RANGE 1 – 95.

|___|___|
ENTER NUMBER OF CIGARETTES

REFUSED 777
DON'T KNOW 999

SMQ.725 When did {you/he/she} smoke {your/his/her} last cigarette? Was it . . .

today,..... 1
yesterday, or..... 2
3 to 5 days ago?..... 3
REFUSED 7
DON'T KNOW 9

BOX 3

CHECK ITEM SMQ.731:
IF 'PIPES' (CODE 2) IN SMQ.692, GO TO SMQ.740.
IF 'CIGARS' (CODE 3) IN SMQ.692, GO TO SMQ.771.
IF 'WATER PIPES OR HOOKAHS' (CODE 4) IN SMQ.692, GO TO SMQ.845.
IF 'E-CIGARETTE' (CODE 5) IN SMQ.692, GO TO SMQ.849.
OTHERWISE, GO TO SMQ.851.

SMQ.740 During the past **5 days,** including today, on how many days did {you/he/she} smoke a pipe?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED 7
DON'T KNOW 9

BOX 4

CHECK ITEM SMQ.761:
IF 'CIGARS' (CODE 3) IN SMQ.692, GO TO SMQ.771.
IF 'WATER PIPES OR HOOKAH IN SMQ.692, GO TO SMQ.845.
IF 'E-CIGARETTE' (CODE 5) IN SMQ.692, GO TO SMQ.849.
OTHERWISE, GO TO SMQ.851.

SMQ.771 During the past **5 days,** including today, on how many days did {you/he/she} smoke cigars, or little cigars or cigarillos?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED 7
DON'T KNOW 9

BOX 5

CHECK ITEM SMQ.791:
IF 'WATER PIPE' (CODE 4) IN SMQ.692, GO TO 845.
IF 'E-CIGARETTE' (CODE 5) IN SMQ.692, GO TO 849.
OTHERWISE, GO TO SMQ.851.

SMQ.845 During the past **5 days,** including today, on how many days did {you/he/she} smoke tobacco in a water pipe or Hookah?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED 7
DON'T KNOW 9

BOX 6

CHECK ITEM SMQ.847:
IF 'E-CIGARETTE' (CODE 5) IN SMQ.692, GO TO 849.
OTHERWISE, GO TO SMQ.851.

SMQ.849 During the past **5 days,** including today, on how many days did {you/he/she} smoke an e-cigarette?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED 7
DON'T KNOW 9

SMQ.851 Smokeless tobacco products are placed in the mouth or nose and include chewing tobacco, snuff, snus, or dissolvables.

During the past **5 days**, including today, did {you/he/she} use any smokeless tobacco?

(Please do not include nicotine replacement products like patches, gum, lozenge, or spray which are considered products to help {you/him/her} stop smoking.)

YES	1	
NO	2	(SMQ.863)
REFUSED	7	(SMQ.863)
DON'T KNOW	9	(SMQ.863)

SMQ.853 Which of these products did {you/he/she} use?

(CHECK ALL THAT APPLY)

Chewing tobacco	1	
Snuff	2	
Snus	3	
Dissolvables	4	
REFUSED	7	(SMQ.863)
DON'T KNOW	9	(SMQ.863)

BOX 7

CHECK ITEM SMQ.855:
- IF 'CHEWING' (CODE 1) IN SMQ.853, GO TO SMQ.800.
- IF 'SNUFF' (CODE 2) IN SMQ.853, GO TO SMQ.817.
- IF 'SNUS' (CODE 3) IN SMQ.853, GO TO SMQ.857.
- IF 'DISSOLVABLES' (CODE 4) IN SMQ.853, GO TO SMQ.861.

SMQ.800 During the past **5 days,** including today, on how many days did {you/he/she} use chewing tobacco, such as Redman, Levi Garrett or Beechnut?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED	7
DON'T KNOW	9

BOX 8

CHECK ITEM SMQ.818:
IF 'SNUFF' (CODE 2) IN SMQ.853, GO TO SMQ.817.
IF 'SNUS' (CODE 3) IN SMQ.853, GO TO SMQ.857.
IF DISSOLVABLES (CODE 4) IN SMQ.853, GO TO SMQ.861.
OTHERWISE, GO TO SMQ.863.

SMQ.817 During the past **5 days,** including today, on how many days did {you/he/she} use snuff, such as Skoal, Skoal Bandits, or Copenhagen?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED 7
DON'T KNOW 9

BOX 9

CHECK ITEM SMQ.821:
IF 'SNUS' (CODE 3) IN SMQ.853, GO TO SMQ.857.
IF DISSOLVABLES (CODE 4) IN SMQ.853, GO TO SMQ.861.
OTHERWISE, GO TO SMQ.863.

SMQ.857 During the past **5 days,** including today, on how many days did {you/he/she} use snus?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED 7
DON'T KNOW 9

BOX 10

CHECK ITEM SMQ.859:
IF DISSOLVABLES (CODE 4), CONTINUE.
OTHERWISE, GO TO SMQ.863.

SMQ.861 During the past **5 days,** including today, on how many days did {you/he/she} use dissolvables such as strips or orbs?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED	7	
DON'T KNOW	9	

SMQ.863 During the past **5 days**, including today, did {you/he/she} use any nicotine replacement therapy products such as nicotine patches, gum, lozenges, inhalers, or nasal sprays?

YES	1	
NO	2	(END OF SECTION)
REFUSED	7	(END OF SECTION)
DON'T KNOW	9	(END OF SECTION)

SMQ.830 During the past **5 days,** including today, on how many days did {you/he/she} use any nicotine replacement therapy products such as nicotine patches, gum, lozenges, inhalers, or nasal sprays?

HARD EDIT: RANGE 1 – 5.

|___|
ENTER NUMBER OF DAYS

REFUSED	7	
DON'T KNOW	9	

SMQ.840 When did {you/he/she} last use a nicotine replacement therapy product? Was it . . .

today,	1	(END OF SECTION)
yesterday, or	2	(END OF SECTION)
3 to 5 days ago?	3	(END OF SECTION)
REFUSED	7	(END OF SECTION)
DON'T KNOW	9	(END OF SECTION)

SMQ.860 The next questions are about {your/his/her} exposure to other people's tobacco smoke.

During the last 7 days, did {you/SP} spend time in a **restaurant?**

YES	1	
NO	2	(SMQ.870)
REFUSED	7	(SMQ.870)
DON'T KNOW	9	(SMQ.870)

SMQ.862 While {you were/SP was} in a **restaurant**, did someone else smoke cigarettes or other tobacco products indoors?

YES	1	
NO	2	
REFUSED	7	
DON'T KNOW	9	

SMQ.870 During the last 7 days, did {you/SP} ride in a **car or motor vehicle?**

YES	1	
NO	2	(SMQ.874)
REFUSED	7	(SMQ.874)
DON'T KNOW	9	(SMQ.874)

SMQ.872 While {you were/SP was} riding in **a car or motor vehicle,** did someone else smoke cigarettes or other tobacco products?

YES	1	
NO	2	
REFUSED	7	
DON'T KNOW	9	

SMQ.874 During the last 7 days, did {you/SP} spend time in **a home other than {your/his/her} own?**

YES	1	
NO	2	(SMQ.878)
REFUSED	7	(SMQ.878)
DON'T KNOW	9	(SMQ.878)

SMQ.876 While {you were/SP was} in **a home other than {your/his/her} own,** did someone else smoke cigarettes or other tobacco products indoors?

YES	1	
NO	2	
REFUSED	7	
DON'T KNOW	9	

SMQ.878 During the last 7 days, {were you/was SP} in **any other indoor area?**

YES	1	
NO	2	(END OF SECTION)
REFUSED	7	(END OF SECTION)
DON'T KNOW	9	(END OF SECTION)

SMQ.880 While {you were/SP was} in the **other indoor** area, did someone else smoke cigarettes or other tobacco products?

YES	1
NO	2
REFUSED	7
DON'T KNOW	9