TESIS DE LA UNIVERSIDAD DE ZARAGOZA
2022
128

Miguel Máximo Seral Cortés

Dieta mediterránea, comportamientos sedentarios y factores genéticos como determinantes de la obesidad y el síndrome metabólico en adolescentes Europeos

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ISSN 2254-7606

Prensas de la Universidad
Universidad Zaragoza

ISSN 2254-7606

Universidad Zaragoza
1542

Tesis Doctoral

DIETA MEDITERRÁNEA, COMPORTAMIENTOS SEDENTARIOS Y FACTORES GENÉTICOS COMO DETERMINANTES DE LA OBESIDAD Y EL SÍNDROME METABÓLICO EN ADOLESCENTES EUROPEOS

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UNIVERSIDAD DE ZARAGOZA
Escuela de Doctorado

Programa de Doctorado en Ciencias de la Salud y del Deporte

2022

PORTADA

Dieta Mediterránea, comportamientos sedentarios y factores genéticos como determinantes de la obesidad y el síndrome metabólico en adolescentes Europeos.

Mediterranean diet, sedentary behaviors and genetics as determinants of obesity and metabolic syndrome in European adolescents.

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Tesis Doctoral Internacional

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2022

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Miguel Máximo Seral Cortés

Zaragoza, Junio de 2022

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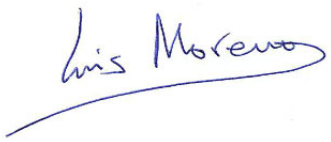

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Fdo.: Idoia Labayen Goñi

En Pamplona, a 9 de Mayo de 2022

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LISTA DE PUBLICACIONES

LIST OF PUBLICATIONS

La presente Tesis Doctoral es un compendio de trabajos científicos previamente publicados. Los artículos que constituyen la presente tesis se detallan a continuación:

I. Seral-Cortes M, Larruy-Garcia A, De Miguel-Etayo P, Labayen I, Moreno LA. Mediterranean Diet and Genetic Determinants of obesity and Metabolic Syndrome in European children and Adolescents. Genes 2022;13(3),420.

II. Seral-Cortes M, Sabroso-Lasa S, De Miguel-Etayo P, Gonzalez-Gross M, Gesteiro E, Molina-Hidalgo C, et al. Development of a Genetic Risk Score to predict the risk of overweight and obesity in European adolescents from the HELENA study. Scientific reports. 2021;11(1).

III. Seral-Cortes M, Sabroso-Lasa S, De Miguel-Etayo P, Gonzalez-Gross M, Gesteiro E, Molina-Hidalgo C, et al. Interaction Effect of the Mediterranean Diet and an Obesity Genetic Risk Score on Adiposity and Metabolic Syndrome in Adolescents: The HELENA Study. Nutrients. 2020;12(12).

IV. Seral-Cortes M, Sabroso-Lasa S, Bailo-Aysa A, Gonzalez-Gross M, Molnár D, Censi L, et al. Mediterranean Diet, Screen-Time-Based Sedentary Behavior and Their Interaction Effect on Adiposity in European Adolescents: The HELENA Study. Nutrients. 2021;13(2)

PROYECTO DE INVESTIGACIÓN

RESEARCH PROJECT

La presente tesis incluye tres artículos basados en datos obtenidos en el proyecto de investigación:

Estudio *HELENA* *(Healthy Lifestyle in Europe by Nutrition in Adolescence).*

Proyecto financiado por la Comisión Europea: *European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034).* Análisis adicionales fueron apoyados por el Ministerio de Economía y Competitividad Español *(Grants RYC-2010-05957 y RYC-2011-09011)*, el Instituto de Salud Carlos III y Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn).

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LISTA DE ABREVIATURAS

LIST OF ABBREVIATIONS

CASTELLANO

% GC	Porcentaje Grasa Corporal
AAP	Asociación Americana de Pediatría
AF	Actividad Física
CC	Circunferencia de Cintura
CEICA	Comité Ético de Investigación Clínica de Aragón
CT	Colesterol Total
DM	Dieta Mediterránea
DMT2	Diabetes Mellitus Tipo 2
ECA	Ensayo Controlado Aleatorizado
ECV	Enfermedad Cardiovascular
IMC	Índice Masa Corporal
IMG	Índice Masa Grasa
KG	Kilógramos
MTA	Media de Tensión Arterial
OMS	Organización Mundial de la Salud
SM	Síndrome Metabólico
TAD	Tensión Arterial Diastólica
TAS	Tensión Arterial Sistólica
TG	Triglicéridos

ENGLISH

% BF	Body Fat Percentage
AAP	American Association of Pediatrics
AUC	Area Under Curve
COVID19	Coronavirus Disease 2019
DASH	Dietary Approaches to Stop Hypertension
FAS	Family Affluence Scale
FFQ	Food Frequency Questionnaire
GLM	Generalized Linear Model
GRS	Genetic Risk Score
GWAS	Genome Wide Association Study
HDL	High Density Lipoprotein
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
HELENA-DIAT	HELENA Dietary Assessment Tool
HOMA	Homeostatic Model Assessment
IDF	International Diabetes Federation
IOTF	International Obesity Task Force
KG	Kilograms
LDL	Low Density Lipoprotein
LRM	Linear Regression Model
MAF	Minimum Allele Frequency
MD	Mediterranean Diet
MDS	Mediterranean Diet Score
MetS	Metabolic Syndrome

MSM	Multiple Source Method
NCBI	National Center for Biotechnology Information
NCEP-ATPIII	National Cholesterol Education Program Adult Treatment Panel III
PA	Physical Activity
PRS	Polygenic Risk Score
PUFA	Polyunsaturated Fatty Acid
ROC	Receiver Operating Characteristic
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SNP	Single Nucleotide Polymorphism
TG	Triglycerides
TC	Total Cholesterol
T2DM	Type 2 Diabetes Mellitus
uGRS	Unweighted Genetic Risk Score
wGRS	Weighted Genetic Risk Score
WHO	World Health Organization
YANA-C	Young Adolescents Nutrition Assessment on Computer

GENES

ADAM17	A Disintegrin And Metalloprotease 17
AMPD1	Adenosine Monophosphate Deaminase 1
ANGPTL4	Angiopoietin Protein-Like 4
CD36	Cluster of Differentiation 36
CETP	Cholesteryl ester transfer protein
CNTF	Ciliary Neurotrophic Factor

DRD2	Dopamine Receptor D2
FASN	Fatty Acid Synthase
FTO	Fat mass and Obesity-associated
IGF1	Insulin like Growth Factor 1
IL-6	Interleukin 6
KCTD10	Potassium Channel Tetramerization Domain Containing 10.
LPA	Lipoprotein A
LXRβ	Liver X Receptor β
MC4R	Melanocortin 4 Receptor
MMAB	Metabolism Of Cobalamin Associated B
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
PLIN	Perilipin 1
PPARγ	Peroxisome Proliferator Activated Receptor γ
PTPN1	Protein Tyrosine Phosphatase Non-Receptor Type 1
THRA	Thyroid Hormone Receptor Alpha
TNFα	Tumour Necrosis Factor

RESUMEN

La obesidad infanto-juvenil es uno de los problemas de salud pública más serios a nivel mundial, con implicaciones negativas sobre la salud tanto a nivel físico como psicológico desde edades tempranas. Junto con la obesidad, la prevalencia del síndrome metabólico (SM) ha sufrido en los últimos años un incremento alarmante entre los jóvenes. Tanto la obesidad como el SM son enfermedades complejas y multifactoriales, resultantes de la interacción entre los factores genéticos y ambientales. Durante las últimas dos décadas, nuevos estudios genéticos a gran escala (*genome wide association studies* (*GWAS*)) han ido desvelando nuevas variantes de ciertos genes relacionados con el riesgo de obesidad en adultos. El estudio de la influencia de los polimorfismos de un solo nucleótido (*single nucleotide polymorphism (SNP)),* ya sea de manera individual o combinada (formando un índice de riesgo genético (*genetic risk score (GRS)),* han sido considerados como herramientas de gran utilidad para estimar la predisposición genética a la obesidad o al SM en diferentes grupos de edad. Sin embargo, la mayoría de los mencionados *GRSs* han sido identificados en población adulta. Entre los factores ambientales y de estilo de vida, una baja adherencia a la dieta Mediterránea (DM) se ha asociado con el riesgo de obesidad y enfermedades cardiometabólicas. Así, los beneficios para la salud que conlleva la adherencia a la DM son ampliamente conocidos, tanto en edades tempranas como en la edad adulta. Sin embargo, los efectos sobre la salud que proporciona la DM se pueden ver influenciados por las características genéticas individuales. Numerosos estudios previos, desarrollados en población adulta, han valorado el efecto modulador de la adherencia a la DM sobre el riesgo genético de desarrollo de obesidad. Sin embargo, una revisión sistemática de la literatura muestra que hay muy poca evidencia de esta interacción genes x DM sobre la obesidad y el SM en población infanto-juvenil Europea. Por otro lado, la adherencia a la DM también se ha

asociado previamente con otros factores de estilo de vida, como el tiempo de uso de pantallas, que desempeña un papel fundamental en el desarrollo de obesidad en adolescentes Europeos. Teniendo en cuenta la elevada prevalencia de enfermedades crónicas desde edades tempranas de la vida y la importancia de la promoción de estilos de vida saludables, en la presente Tesis Doctoral se han planteado los siguientes objetivos:

1) Describir los posibles efectos de la interacción genes x DM que podrían modular el riesgo de desarrollo de obesidad y de SM en jóvenes Europeos.

2) Desarrollar un *GRS* para predecir el riesgo de sobrepeso y obesidad en adolescentes Europeos.

3) Valorar la posible interacción entre la adherencia a la DM y el *GRS* de obesidad y sus efectos sobre la adiposidad y SM en adolecentes Europeos.

4) Valorar si el tiempo sedentario tiene un efecto modulador en la asociación entre la DM y los marcadores de adiposidad.

Para lograr estos objetivos, se consideraron los resultados obtenidos en el estudio *HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence)*, un estudio transversal multicéntrico, que incluyó 10 países Europeos. El tamaño de la muestra del estudio *HELENA* fue de 3.528 adolescentes, de los cuales aproximadamente un tercio del total, 1.172 adolescentes, fueron seleccionados aleatoriamente para extraer una muestra de sangre y de este modo obtener información sobre los parámetros séricos de interés. Para la realización de los artículos originales dentro del estudio HELENA, se consideraron diversos tamaños de muestra en función de la disponibilidad de información completa para estudiar las variables de interés. En el desarrollo del Artículo II, se incluyeron 1.069 adolescentes con datos sobre el perfil genético y adiposidad; en el

Artículo III, un total de 605 adolescentes con información dietética, genética, adiposidad y perfil bioquímico, incluyendo variables relacionadas con el SM, fueron considerados; finalmente, en el Artículo IV se incluyeron 2.047 adolescentes con datos sobre dieta, tiempo sedentario de pantalla y adiposidad.

Dentro el escaso número de artículos que valoran la interacción genes x DM en jóvenes Europeos, se encontró un estudio, el cual mostró que el efecto de los beneficios de adherirse a la DM, en relación con valores de adiposidad y SM, sólo se hizo patente en individuos con un riesgo genético concreto, obteniendo de este modo, un mayor aprovechamiento de la DM cuando el riesgo genético a obesidad era menor. Además, el efecto del riesgo genético sobre el aprovechamiento de la DM fue más pronunciado en chicas adolescentes.

Con el objetivo de valorar la predisposición genética al sobrepeso y la obesidad, se desarrolló un *GRS* formado por 21 *SNPs,* que fueron asociados al sobrepeso y obesidad (p<0.05). Para valorar su capacidad predictiva, se evaluó el área bajo la curva *(area under curve (AUC)).* El formato ponderado del *GRS (weighted genetic risk score (wGRS)),* obtuvo un valor de 0.734, mientras que en el formato no ponderado *(unweighted genetic risk score (uGRS)),* el *AUC* fue de 0.723; los *GRSs* fueron validados internamente. Además, en el estudio de interacción genes x DM, se observó que una mayor adherencia a la DM fue asociada a niveles más bajos de adiposidad y SM en ambos sexos (p<0.05). El mayor aprovechamiento de los beneficios de la DM se observaba cuando la puntuación del *uGRS* era menor (menor número de alelos de riesgo), habiendo diferencias entre ambos sexos (el efecto de interacción fue mayor en chicas que en chicos adolescentes) (p<0.05).

En los análisis que incluyeron las variables de composición corporal (IMC, CC y IMG) y DM, se observó inicialmente una asociación inversa entre éstas en cuanto a IMC (ambos sexos) e IMG (sólo chicas adolescentes) (p<0.05). Además, en el análisis de interacción tiempo sedentario de pantalla x DM, se observó que una mayor adherencia a la DM se asociaba con niveles más bajos de índice de masa corporal (IMC), circunferencia de cintura (CC) e índice de masa grasa (IMG), cuando la exposición al tiempo de pantalla en chicas adolescentes era menor (p<0.05). Este efecto no fue observado en chicos adolescentes.

Los resultados recogidos en los artículos originales presentan una serie de limitaciones y fortalezas inherentes al diseño del estudio HELENA. Las principales limitaciones de los resultados incluidos en la presente Tesis Doctoral fueron: 1) Debido a la naturaleza transversal del estudio *HELENA*, no se puede determinar una relación causa-efecto. 2) En el diseño inicial del estudio *HELENA*, únicamente se seleccionaron lugares específicos del cromosoma que en aquel momento eran considerados como de riesgo. 3) Aunque el modelo para desarrollar los *GRSs* se validó internamente, los resultados obtenidos deben validarse en poblaciones infanto-juveniles más grandes, valorando la incidencia de obesidad, y así comprobar la fiabilidad de estos *GRSs* específicos de obesidad en otras poblaciones de etnia similar. 4) En el desarrollo del *GRS* de obesidad, se encontraron *SNPs* más comunes a *GRSs* no Europeos que a *GRSs* Europeos. Este hallazgo podría deberse a la mayor cantidad de *GRSs* desarrollados en otras etnias, en comparación con la cantidad de *GRSs* realizadas en la población Europea. 5) No se dispone de datos sobre el parentesco o el origen étnico de los participantes estudiados. 6) La baja adherencia a la DM registrada en términos generales y, en particular, en los adolescentes del estudio *HELENA* incluidos en los análisis de la presente Tesis Doctoral, hace necesario que los resultados mostrados se deban interpretar

cuidadosamente. Por otro lado, las principales fortalezas de este estudio incluyen: 1) El diseño multicéntrico del estudio *HELENA* implicó la participación de adolescentes de 10 ciudades Europeas. Esto permitió a los investigadores utilizar una gran base de datos con información relevante y diversa de diferentes poblaciones de toda Europa. 2) Existen pocas publicaciones en la literatura que consideren los efectos de interacción genes x DM en términos de composición corporal en jóvenes Europeos; la mayoría de los estudios disponibles se centran exclusivamente en población adulta. 3) Una cantidad importante de los estudios previos se centraron en interacciones específicas con *SNPs* analizados de manera individual, mientras que la presente Tesis Doctoral ha incluido el desarrollo de *GRSs* específicos de sobrepeso y obesidad, con una mayor capacidad predictiva para evaluar el riesgo de obesidad y de SM en adolescentes Europeos. 4) Se incluyó el patrón de DM completo, desarrollando clústeres de diferentes grupos de alimentos típicamente Mediterráneos con un puntaje de adherencia, en lugar de considerar la ingesta de macronutrientes individualmente o grupos de alimentos específicos.

En conclusión, a pesar del creciente numero de estudios que evalúan la interacción genes x DM en población Europea adulta, el numero de estudios encontrados en población Europea joven fue escaso. Aun así, mediante el desarrollo de un índice para evaluar la predisposición genética a sobrepeso y obesidad, la presente Tesis Doctoral ha mostrado que, el genotipo relacionado con la obesidad podría modular la relación entre DM y adiposidad y SM en adolescentes Europeos. Además, considerando el mismo enfoque de análisis de interacción, valorando en este caso tiempo sedentario de pantalla x DM, se observó que, el tiempo sedentario de pantalla tiene un efecto modulador en la asociación entre DM y la adiposidad en adolescentes Europeas. Se necesita más investigación en esta línea, para entender mejor las diferencias interindividuales en la asociación entre DM y obesidad y SM,

así como los mecanismos del efecto protector de DM en la salud cardiovascular en general, a través de la integración de las ciencias ómicas y la estrategia de nutrición personalizada.

ABSTRACT

Obesity during childhood and adolescence is one of the most serious health problems worldwide, with physical and psychological negative implications, from early ages. Together with obesity, the prevalence of metabolic syndrome (MetS) has alarmingly increased among young people. Both obesity and MetS are complex and multifactorial diseases, influenced by the interaction of genetic and environmental factors. During the last two decades, new large scale studies (genome wide association studies (GWAS)) have revealed new variants of certain genes related to the risk of developing obesity in adult population. Studying the influence of single nucleotide polymorphisms (SNPs), either individually o combined (forming a genetic risk score (GRS)), has been considered a highly useful approach to predict the genetic predisposition to obesity or MetS, across different age groups. However, the majority of obesity related variants have been identified in adult population. Within the environmental factors, a low Mediterranean diet (MD) adherence has been associated to the risk of obesity and cardiometabolic diseases. The benefits associated to a high MD adherence are widely known in young and adult populations. However, the positive effects of the MD on health could be influenced by genetic variations. There are a number of studies assessing the influence of the MD on each individual´s genetic profile to modulate the risk of obesity in adult population. Based on a recent systematic search of the literature, the number of studies analyzing gene x MD interactions in European youth is scarce. On the other hand, the MD adherence has also been associated to lifestyle factors, such as screen time use, which plays a key role in the development of obesity among European adolescents. Considering the prevalence of chronic diseases from early age, together with the importance of promoting healthy

eating and lifestyle patterns, a series of objectives have been proposed in the present Doctoral Thesis:

1) To describe the potential gene x MD interaction effects which could modulate the risk of obesity and MetS development in European youth.

2) To construct a GRS to predict the genetic risk to develop overweight and obesity in European adolescents.

3) To assess whether interaction effects occur between the obesity-GRS and MD adherence on adiposity and MetS in European adolescents.

4) To assess whether screen-based sedentary time has a modulatory effect on the association between MD and adiposity markers.

In order to achieve these objectives, the results obtained in The Healthy Lifestyle in Europe by Nutrition in adolescence (HELENA) Study were considered. The HELENA study is a multi-centric cross-sectional study, that included ten European countries. The sample size of the HELENA study was 3.528 adolescents, of which approximately one third of the total, 1.172 adolescents, were randomly selected to draw a blood sample in order to obtain information on the serum parameters of interest. During the preparation of the original articles proposed within the HELENA study, different sample sizes were considered, depending on the availability of complete information on the variables of interest. For the development of Article II, a total of 1.069 adolescents with data on the genetic profile and adiposity were included; in Article III, a final number of 605 adolescents with dietary, genetic, adiposity and biochemical profile information including variables related to MS were considered; finally, Article IV included 2.047 adolescents with data on diet, sedentary screen time, and adiposity.

Within the limited scientific evidence found in the literature which considers the gene x MD interactions in European youth, one study showed that the MD beneficial effects, in relation with adiposity and MetS values, were only patent in individuals with specific genetic risk, obtaining in this way, a higher MD health-related benefits when the genetic risk was lower. Additionally, the effect of the genetic risk on the MD benefits was higher in female than in male adolescents.

Aiming to assess the genetic predisposition to overweight and obesity, a GRS formed by 21 SNPs associated to overweight and obesity was developed ($p<0.05$). In order to assess the predictive ability of the GRS, the area under the curve (AUC) was calculated. The weighted format (weighted genetic risk score (wGRS)) obtained a value of 0.734, while the unweighted (unweighted genetic risk score (uGRS)) AUC was 0.723; the GRS was internally validated. In addition, in the gene x MD interaction study, a higher MD adherence was associated to lower adiposity and MetS levels ($p<0.05$). The higher use of the MD benefits was observed when the uGRS value was lower (lower number of risk alleles), observing sex-related differences (stronger interaction effect in female than male adolescents) ($p<0.05$).

Moreover, the analysis including variables of body composition (BMI, WC and FMI) and MD, an inverse association was initially observed between MD and BMI (in both sex groups) and FMI (only in female adolescents) ($p<0.05$). In addition, in the sedentary screen time x MD interaction analysis, it was observed that, higher MD adherence was associated with lower levels of BMI, WC and FMI, when the screen sedentary time exposure was lower among female adolescents ($p<0.05$). This interaction effect was not observed in male adolescents.

The results shown in the original articles within the HELENA study framework, include a series of limitations and strengths inherent to the study design. The main limitations of the results shown in this Doctoral Thesis were: 1) Due to the cross-sectional nature of the HELENA study, no cause-effect relationships can be determined. 2) In the initial design of the HELENA study, only specific risk loci were selected, which, at that time, were considered as obesity risk factors. 3) Although the model to develop the GRS was internally validated, the results should be validated in larger children and adolescent study populations, using obesity incidence, to test the reliability of this obesity-specific GRS in other populations with similar ethnicity. 4) More common SNPs in non-European GRSs were found than in European GRSs. This finding could be due to the higher number of GRSs developed in other ethnicities in comparison to the number of GRS performed in European population. 5) There is no data available regarding the relatedness or the ethnic origins among the studied participants. 6) Due to the low MD adherence registered in general terms, and more particularly in the HELENA adolescents included in the analysis of the present Doctoral Thesis, the results should be carefully interpreted. On the other hand, the strengths of this study included: 1) The multicentric design of the HELENA study involved the participation of adolescents from 10 European cities. This allowed the researchers to use a large database with relevant and diverse information from different populations across Europe. 2) Scarce number of manuscripts were found in the literature considering gene x MD interaction effects in terms of body composition in European youth, where the majority of the studies were available exclusively on adult populations. 3) Most analyses from previous studies were focused on specific single SNP interactions, whereas the present Doctoral Thesis has included the development of an overweight and obesity-specific GRS, with higher predictive ability to evaluate the risk of obesity and MetS in European adolescents. 4) We included the whole MD pattern by

developing a cluster of different food groups typically consumed in the Mediterranean area through an adherence score, rather than considering single macronutrients or individual specific food groups´ intake.

To conclude, despite the growing number of studies evaluating gene x MD interaction in European adult populations, the number of studies found in European youth is scarce. Nevertheless, by developing a score to evaluate the genetic predisposition to overweight and obesity, the present Doctoral Thesis has shown that, the obesity related genotypes could modulate the relationship between MD and adiposity and MetS in European adolescents. Moreover, the same interaction analysis approach assessing, in this case, screen sedentary time x MD, has shown that screen sedentary time had a modulating effect on the association between MD and adiposity in female adolescents. Further research is needed to better understand the inter-individual differences in the association between MD and obesity and MetS, as well as the mechanisms behind the protective effects of MD in the overall cardiovascular health through the integration of omics and a personalized nutrition strategy.

1. INTRODUCCIÓN

1.1 Obesidad infantil y síndrome metabólico

La Organización Mundial de la Salud (OMS) considera la obesidad infanto-juvenil como uno de los problemas de salud pública más serios del siglo XXI (1). Durante los años 80 y 90 del siglo pasado, la prevalencia de obesidad infanto-juvenil experimentó un incremento rápido; esta tendencia parece consolidarse en niveles considerablemente altos en países de mayor desarrollo (2). La prevalencia de la obesidad infantil varía ampliamente entre los distintos países. Según los últimos datos presentados por la OMS a nivel mundial, la prevalencia de sobrepeso y obesidad en niños y adolescentes entre 5 y 19 años de edad ha aumentado desde un 4% en 1975 a un 18% en 2016, con proporciones similares en chicos (19%) y chicas (18%) (3). En Europa, de acuerdo con las estimaciones de la OMS, aproximadamente uno de cada tres preadolescentes (<11 años) tienen sobrepeso u obesidad (4). Existen diferencias entre las distintas regiones Europeas, observándose niveles más altos de obesidad en los países Mediterráneos que en los países del norte de Europa (5). En España, según el estudio ALADINO, en 2019, el porcentaje de sobrepeso registrado en menores de 10 años fue 23.3% y el de obesidad 17.3% (6). Recientemente, el impacto del confinamiento y las restricciones de movilidad causada por la pandemia por *coronavirus disease 2019 (COVID19)* se ha asociado a un cambio negativo en los estilos de vida en la población joven, con un incremento de la incidencia proyectada de obesidad infanto-juvenil (7). Los efectos sobre la salud en un ambiente obesogénico se ven exacerbados debido factores ambientales tales como la presencia a nivel mundial del virus *severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)* (8).

Las enfermedades cardiovasculares (ECV) son la principal causa de muerte a nivel mundial (9). Se estima que 7 de las 10 causas principales de muerte en 2019 fueron enfermedades no transmisibles, representando el 74% de las muertes a nivel global en 2019 (9). Esta tendencia empeora en países de ingresos bajos y medios, en los cuales, en los adolescentes con obesidad, el síndrome metabólico (SM) afecta entre el 24% y 56% de los jóvenes (10). En Europa, la prevalencia de SM oscila entre un 2% y 18% en jóvenes con normopeso, y un 21% y 35% en jóvenes con obesidad, dependiendo de la definición utilizada (11). En España, un estudio en niños y niñas con obesidad mostró una prevalencia total de SM del 19.6% (12). Finalmente, el efecto de la pandemia *COVID19* en relación al aumento de riesgo cardiometabólico ha sido estudiado en población europea adulta (13) y se encuentra actualmente en estudio en población joven.

1.1.1 Definiciones y valores de referencia

El sobrepeso y la obesidad se definen como la acumulación anormal o excesiva de grasa corporal que representa un riesgo para la salud, fruto de la interacción de distintos factores (14). Para valorar el sobrepeso y la obesidad en niños/as y adolescentes, los métodos más comúnmente utilizados son aquéllos basados en medidas antropométricas. Por ejemplo, medidas como el pliegue cutáneo tríceps o la circunferencia de cintura (CC) pueden predecir la cantidad total de grasa en niños/as y adolescentes (15). Sin embargo, el índice más ampliamente usado para determinar sobrepeso y obesidad es el índice de masa corporal (IMC) (16). Para calcular el IMC se divide el peso (en kg) por la altura (en metros) al cuadrado (kg/m^2). La valoración del IMC en niños/as y adolescentes es específico de la edad y el sexo (16) y se define a menudo cuando el IMC del niño/a se

encuentra entre los percentiles 85 y 95, mientras que la obesidad se define cuando el IMC está en el percentil 95 o más alto (17).

Entre los estándares internacionales de referencia del IMC más extensamente utilizados en adolescentes está el de la Federación Mundial de la Obesidad (*World Obesity Federation*), conocida anteriormente como *International Obesity Task Force (IOTF*) (16, 18). Definir puntos de corte a nivel internacional puede aportar numerosas ventajas y permite a los investigadores establecer comparaciones con otros estudios o poblaciones (18). Sin embargo, la falta de uniformidad en la metodología para establecer el criterio de desarrollo de valores de referencia de crecimiento, para su uso en el IMC, puede llevar a veces a disparidad en los resultados (19). Por ejemplo, los valores de referencia del IMC de la OMS pueden no ser adecuadas para todos los niños/as Europeos (19). Otros puntos de corte del IMC han sido propuestos por los Centros para el Control y la Prevención de Enfermedades *(U.S. Centers for Disease Control and Prevention*) *(CDC)* de EE. UU., donde las diferencias de la capacidad predictiva de la salud en la edad adulta, entre las referencias de los *CDC* y *World Obesity Federation* son mínimas (20). Un estudio en niños/as y adolescentes en edad escolar mostró una comparación de las estimaciones de obesidad basadas en diferentes valores de referencia (criterios de *CDC*, *IOTF* y OMS). Los resultados mostraron que la mayor subestimación de sobrepeso y obesidad se encontró en la referencia de la OMS (8.97% y 5.67% respectivamente) (21).

Sin embargo, el IMC no es reflejo directo de la cantidad de grasa en un individuo. No obstante, la investigación ha mostrado que el IMC se asocia con medidas más directas de la grasa corporal obtenidas mediante la medida de la hidrodensitometría, la densitometría de rayos X, la pletismografía por desplazamiento de aire, etc. tanto en población joven, como adulta (22, 23). Alternativamente, la obesidad también podría evaluarse considerando la circunferencia de cintura (CC) como una medida de estimación

de la grasa abdominal (24), ya que el IMC podría subestimar la prevalencia de obesidad central en población joven (25).

Por otro lado, la aparición conjunta en un mismo individuo de uno o varios factores de riesgo como obesidad (principalmente central), intolerancia a la glucosa, hipertensión y dislipemia aterogénica (niveles altos de triglicéridos (TG) y lipoproteínas de alta densidad *(high density lipoprotein (HDL))* se denominan conjuntamente síndrome metabólico (SM). Este término se utiliza de forma general para indicar una situación clínica en la que concurren trastornos metabólicos y cardiovasculares que son factores de riesgo para el desarrollo de enfermedades crónicas (26). Además, la presencia de interacciones entre estos componentes pueden variar según edad, sexo y etnia (26). La definición de SM integra diferentes aspectos a tener en cuenta; por ejemplo, los componentes del SM se expresan como variables continuas, con lo que se necesitan puntos de corte para definir el riesgo. Para aplicar estos umbrales, todavía no existe consenso para establecer el diagnostico de cada componente. Los componentes están relacionados entre sí, pero la fisiopatología de su relación todavía no se conoce con precisión. Finalmente, la inclusión de resistencia a la insulina o diabetes puede generar controversia, aunque fisiopatológicamente parece ser un factor central (27).

Existen numerosos criterios de clasificación de SM para niños/as y adolescentes. La mayoría de ellos, basados en clasificaciones de SM de adultos (28). Cabe destacar que estas definiciones en adultos no pueden ser directamente aplicadas en población infanto-juvenil, ya que, por ejemplo, la clasificación del SM en niños/as, requiere de percentiles específicos de edad y sexo, además de puntos de corte pediátricos (29). Una de las propuestas más destacadas para seleccionar estos puntos de corte procede del *National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII)* (30). Algunos

autores modificaron estos criterios de *NCEP* reemplazando los puntos de corte de población adulta por los correspondientes a población joven (Tabla 1).

Tabla 1. Definiciones del síndrome metabólico infanto-juvenil.

Definición	**Exceso de adiposidad**	**Presión arterial**	**Lípidos en sangre**	**Glucosa en ayunas**
IDF (31)	10-15 años: CC ≥ percentil 90 >15 años: CC ≥ 94 cm (♂) CC ≥ 80 cm (♀)	TAS ≥ 130mm Hg o TAD ≥ 85mm Hg	TG ≥ 150 mg dl^{-1} HDL < 40 mg/dl (♂); < 50 mg/dl (♀)	≥100 mg dl^{-1} o diagnóstico de DMT2
Cook et al. (32)	CC ≥ percentil 90	TAS o TAD ≥ percentil 90	TG ≥ 110 mg dl^{-1} o HDL ≤ 40 mg dl^{-1}	≥110 mg dl^{-1}
Ford et al. (33)	IMC ≥ percentil 90	TAS o TAD ≥ percentil 90	TG ≥ 110 mg dl^{-1} o HDL ≤ 40 mg dl^{-1}	≥110 mg dl^{-1} Análisis adicional ≥110 mg dl^{-1}
de Ferrati et al. (34)	CC ≥ percentil 75	TAS o TAD ≥ percentil 90	< 15 años: TG ≥100 mg/dl HDL ≤ 50 mg/dl > 15 años: TG ≥ 110 mg/dl HDL ≤ 45 mg/dl	≥110 mg/dl^{-1}

Abreviaciones: CC (Circunferencia de cintura); DMT2 (Diabetes Mellitus Tipo 2); HDL (colesterol de alta densidad); *IDF (International Diabetes Federation)*; IMC (Índice de Masa Corporal); TAD (Tensión Arterial Diastólica); TAS (Tensión Arterial Sistólica) y TG (Triglicéridos). Fuente: elaboración propia.

Debido a la falta de consenso mostrada en los criterios de clasificación del SM, otros estudios han desarrollado escalas de valoración cuantitativa de riesgo cardiovascular (35). Estas escalas suelen tomar valores de referencia por edad y sexo para distintos factores de riesgo cardiovascular, basados en estudios de grandes cohortes de poblaciones étnicamente diversas (36). La estimación de la prevalencia del SM puede variar según se consideren las distintas definiciones (31-34). Aunque los porcentajes de prevalencia son más bajos en población joven que en adultos, las tasas crecientes de obesidad infanto-

juvenil y los comportamientos sedentarios durante las últimas décadas podrían dar lugar a una mayor prevalencia de SM en niños/as y adolescentes a corto plazo (27).

1.1.2 Genética de la obesidad y el síndrome metabólico

La aparición de sobrepeso y obesidad es el resultado de la coexistencia de determinantes de salud complejos y sus interacciones a distintos niveles. Los factores genéticos que influencian el desarrollo de obesidad han sido ampliamente estudiados en las últimas décadas (37). El primer estudio de genoma completo (*genome wide association study* (*GWAS*)) relacionado con la obesidad se publicó en 2007, en el cual identificaron un grupo de variantes genéticas comunes en el gen *fat mass obesity-associated* (*FTO*) significativamente asociadas al IMC (38). El continuo progreso de los estudios genéticos ha extendido el número de variantes genéticas, polimorfismos de un solo nucleótido (*SNPs*), asociadas con la obesidad hasta más de 1.100 (39). El gen *FTO* (40) se ha asociado consistentemente con el riesgo de obesidad y alteraciones cardiometabólicas asociadas en población infantil (41) y adulta (42) Europea. Sin embargo, esos *SNPs* únicamente explican el 6% de la varianza en el IMC (43). Recientemente, el uso de un índice de riesgo poligénico (*polygenic risk score (PRS)*) que incorpora información genética de 2.1 millones de *SNPs*, incrementó la varianza explicada por el IMC hasta un 23% (44). Este *PRS* se desarrolló utilizando como variable el IMC de población adulta, aunque también muestra asociaciones fiables en población joven (44). Curiosamente, las variantes genéticas relacionadas con el IMC en adultos se asociaron más significativamente con el IMC de los niños/as durante su rebote adiposo (alrededor de los cinco años de edad), que en su punto máximo de adiposidad (menos de

9 meses de edad), donde las variantes genéticas tuvieron una mínima influencia sobre el IMC (45). Estos hallazgos fortalecen la idea del uso del *PRS* de adultos en niños/as y adolescentes. Sin embargo, el uso de un índice de riesgo genético (*genetic risk score (GRS)*) específico para población infantil podría incluso aumentar el porcentaje de varianza explicada y ayudar a comprender los mecanismos de la predisposición genética a la obesidad durante las primeras etapas de la vida (35).

Distintos autores han explorado la susceptibilidad genética al SM. Se estima que la heredabilidad del SM en población de ascendencia Europea varía entre el 10 y el 30% (36, 37). Además, se han observado numerosos *SNPs* asociados con componentes individuales del SM en estudios *GWAS* de diferentes grupos étnicos (38, 39). Sin embargo, el efecto de estos *SNPs* considerando todos los componentes del SM es muy poco conocido y la mayoría de estos estudios *GWAS* se han realizado en adultos (40, 41). Además, el estudio simultáneo de la genética de la obesidad y el SM en población infanto-juvenil podría llevar a observar un solapamiento parcial entre ambas, ya que, en ocasiones, la predisposición genética al SM podría estar dirigida por genes relacionados con obesidad (42).

Como se mencionó anteriormente, los *GRSs* pueden ser fundamentales para identificar a las personas con riesgo elevado de obesidad y/o desarrollo de SM (46). A la hora de combinar varios *SNPs* para formar un *GRS* se pueden seguir dos metodologías diferentes: sumando el número de alelos de riesgo y calculando un *GRS* no ponderado *(unweighted genetic risk score (uGRS)),* o multiplicando el número de alelos de riesgo por cada coeficiente de efecto estimado y calculando un *GRS* ponderado *(weighted genetic risk score (wGRS))* (47). Ambas metodologías han demostrado ser herramientas genéticas sólidas y útiles para predecir la susceptibilidad genética al desarrollo de obesidad y SM en población infantil y adolescente (33, 35, 42).

1.2 Dieta Mediterránea

Tanto un patrón dietético adecuado basado en alimentos y productos típicamente Mediterráneos como un estilo de vida físicamente activo se han asociado con una reducción significativa de la mortalidad asociada a una mayor adherencia a la dieta Mediterránea (DM) tradicional. *Trichopoulou, A. et al.* observaron asociaciones inversas entre una mayor adherencia a la DM y mortalidad por enfermedad coronaria y cáncer en población adulta en Grecia, en un período de seguimiento de 44 meses (48). El ensayo clínico multicéntrico español de 4.8 años de seguimiento PREDIMED (Prevención con Dieta Mediterránea) evaluó los efectos de la DM en términos de enfermedad cardiovascular en adultos, mediante diferentes intervenciones dietéticas incluyendo aceite de oliva virgen extra y/o frutos secos, y proporcionó pruebas sólidas de que la DM podría ser un modelo dietético óptimo para el manejo de la enfermedad cardiovascular (49).

Hoy en día, aunque con cierta variabilidad, el seguimiento del estilo de vida tradicional Mediterráneo todavía está presente entre las poblaciones más mayores (50). Sin embargo, los jóvenes se han alejando del patrón de alimentación Mediterráneo, hecho que repercute en su salud cardiovascular con aparición de enfermedades crónicas desde edades tempranas (51).

Para abordar y entender el patrón alimentario basado en productos típicamente Mediterráneos, es necesario contextualizar el origen de esta dieta, los alimentos característicos consumidos procedentes de la zona geográfica y los efectos beneficiosos sobre la salud asociados al consumo habitual de la DM en todas las etapas de la vida. Además, para valorar la adherencia a la DM, se procederá a describir los índices previamente propuestos por distintos autores, donde se cuantifica el grado de seguimiento del patrón Mediterráneo en función de los alimentos consumidos por cada individuo.

1.2.1 Contextualización, patrón alimentario y beneficios

El patrón de DM se caracteriza por la ingesta de aceite de oliva virgen, el consumo elevado de frutos secos y legumbres, cereales sin refinar, frutas y verduras, el consumo moderado de lácteos y pescado y el bajo consumo de carne roja y derivados (48). El concepto del patrón de alimentación siguiendo el estilo de vida Mediterráneo abarca una serie de elementos que van desde el estilo de producción, el consumo estacional y local de alimentos, la práctica de técnicas culinarias empleando como base el aceite de oliva, además de hierbas aromáticas y condimentos, comer socialmente, la práctica de actividad física diaria y el impacto que todos estos aspectos pueden tener en el estado general de salud (52).

La adherencia a la DM entre los más jóvenes varía dentro de los diferentes países Mediterráneos, mientras que hay escasa información sobre adherencia a la DM en países no Mediterráneos (53). Con la información disponible se observa, en términos generales, una baja adherencia a la DM entre la población joven, por lo que es probable que este hecho condicione algunos resultados, en los que se han observado asociaciones entre una alta adherencia a la DM y baja frecuencia de obesidad (51).

La influencia de la alimentación desde los primeros momentos de la vida sobre la salud podría ser especialmente relevante. La lactancia materna parece proteger contra la obesidad infantil (54), por lo que los hábitos dietéticos maternos durante la lactancia podrían influir sobre la composición de la leche, y por tanto, en la nutrición del lactante (55). Un estudio previo mostró que las concentraciones minerales de la leche materna podrían verse influenciadas positivamente por la adherencia materna a la DM (56).

En niños/as y adolescentes Europeos, se han identificado diversos trabajos que valoran la asociación entre la adherencia a la DM y la obesidad. Una mayor adherencia

al patrón de DM se asoció significativamente con un menor peso corporal (57), IMC (58, 59), CC (60-62) y porcentaje de grasa corporal (%GC) (63). Aunque menos estudiada, la relación entre la DM y el SM también se ha explorado en población joven. Así, una mayor adherencia a la DM dio como resultado una mejora en el perfil de lípidos y glucosa plasmática tras una intervención sobre el estilo de vida (64), además de una reducción global del riesgo de los componentes del SM (65). Por el contrario, una menor adherencia a la DM podría contribuir a una mayor obesidad central, hipertrigliceridemia y resistencia a la insulina (66). Sin embargo, no todos los estudios que consideraron la DM como un patrón dietético saludable obtuvieron resultados positivos en términos de obesidad y SM en jóvenes (53). Es por eso que, otras variables del estilo de vida Mediterráneo, como la actividad física (AF), pueden complementar las estrategias dietéticas implementadas, que parecen ser menos eficaces si se considera la intervención alimentaria de forma aislada (67). Existe amplia evidencia de que una insuficiente AF desempeña un papel clave en el desarrollo de obesidad (68) y del fenotipo cardiometabólico (69), más aún cuando la obesidad ya se encuentra presente en el individuo (70).

En adultos, los beneficios de una alta adherencia a la DM para prevenir eventos cardiovasculares son ampliamente conocidos (71). Entre otros, la DM mejora el perfil lipídico y los niveles de adiposidad (72), reduce el riesgo de sobrepeso y obesidad (73), SM (72), diabetes mellitus tipo 2 (DMT2) (74) e hipertensión (75).

1.2.2 Valoración de la adherencia a la dieta Mediterránea mediante índices

A lo largo de los años, se han construido diferentes índices de valoración del grado de adherencia a la DM que permiten evaluar cómo afecta la a la salud (76). Una de los primeros y más utilizados índices de valoración de adherencia a la DM fue el sugerido por

Trichopoulou, A. et al. (48, 77, 78). Los autores recogieron información dietética a través de un cuestionario semicuantitativo de 150 preguntas sobre la frecuencia de consumo de alimentos y bebidas comúnmente consumidos en Grecia. Seguidamente, establecieron un índice de 9 componentes, típicamente consumidos en ese área geográfica, para valorar el grado de adherencia a la DM. Se asignó un valor de 0 o 1 en función del punto de corte especifico de la mediana por sexo. A los componentes considerados beneficiosos (vegetales, legumbres, frutas y frutos secos, cereales y pescado) se les asignó el valor 1, si su consumo registrado estaba por encima de la mediana, mientras que a los productos considerados perjudiciales (carnes y derivados y productos lácteos) se les asignó el valor 0, si su consumo estaba por encima de la mediana. En el caso de consumo por debajo de la mediana, los valores fueron asignados inversamente en función del criterio establecido para cada componente como beneficioso o perjudicial para la salud (48).

Para evaluar la adherencia a la DM en población joven, han ido surgiendo progresivamente más versiones de este índice adaptado para niños/as (79) y adolescentes (80). Los diferentes índices de adherencia se han ido desarrollando en base a grupos de alimentos típicamente consumidos en la DM. Estos grupos de alimentos pueden variar ligeramente en función de las recomendaciones de cada país. La información recogida para construir los índices mencionados se ha obtenido principalmente usando cuestionarios de frecuencia de consumo de alimentos *(Food Frequency Questionnaire (FFQ))* (63) o recuerdos de 24 horas *(24h recall)* (81). Además, para calcular la ingesta dietética habitual de cada individuo, se pueden usar diferentes métodos de corrección para ajustar la variabilidad inter- e intra-individual (82, 83). Estos métodos permiten estimar mejor la adherencia del niño/a o adolescente a la DM a partir de la información proporcionada.

1.3 Tiempo sedentario de pantalla

Algunos comportamientos sedentarios como ver la televisión, jugar a los videojuegos o utilizar el teléfono móvil, entre otros, se presentan con elevada frecuencia durante la adolescencia (84). La AF y los comportamientos sedentarios son dos conceptos totalmente contrarios; sin embargo, el hecho de que habitualmente uno sea más sostenido en el tiempo que otro (ej. los comportamientos sedentarios son más fáciles de ser mantenidos que la AF), hace que a menudo sean analizados conjuntamente como comportamientos que coexisten, en lugar de como acciones independientes entre sí (85). La Academia Americana de Pediatría *(American Academy of Pediatrics (AAP))* aconseja a los niños/as y adolescentes no utilizar y/o estar expuesto a dispositivos de pantalla más de dos horas al día (86). Ver la televisión durante más de dos horas al día se asocia con concentraciones elevadas de colesterol, mientras que una o dos horas son suficientes para incrementar la tensión arterial sistólica (TAS) (87). De forma general, los datos procedentes de estudios transversales muestran que un tiempo de pantalla mayor a dos horas al día se asocia con mayores niveles de tensión arterial y con un riesgo incrementado de padecer SM (87). En adolescentes Europeos, la asociación entre el comportamiento sedentario ver la televisión y el riesgo cardiometabólico global estaba mediada por el grado de adiposidad (88). Además del tiempo de uso de televisión, existen otros dispositivos que progresivamente se han instaurado en la rutina diaria de los adolescentes, como la *Tablet*, el *Smartphone* y el uso de televisión para videojuegos, los cuales también están asociados a una elevada prevalencia de obesidad (89). En los últimos años, con la llegada de la pandemia provocada por el virus *SARS-CoV-2 (COVID 19)* y sus consecuentes restricciones de movilidad y aislamiento social, la práctica de actividades

sedentarias y tiempo de uso de pantalla ha aumentado de forma considerable entre los jóvenes (90), siendo éstos el grupo de edad más afectado (91).

1.3.1 Definición, valoración y recomendaciones internacionales

El tiempo de pantalla se define como el tiempo dedicado a comportamientos relacionados con el uso de pantallas. Dentro de estos comportamientos, se puede optar por adoptar una actitud sedentaria (ej. sentados, tumbados o con gasto energético mínimo) o físicamente activa (ej. en movimiento, con cierta actividad física o gasto energético). En este estudio, hacemos referencia al tiempo sedentario de pantalla, que es el tiempo invertido en usar un dispositivo de pantalla (*Smartphone, Tablet*, ordenador o televisión) mientras se es sedentario en cualquier contexto (ej. colegio, trabajo, lugar de ocio) (92).

Existen dos métodos para valorar el tiempo sedentario de pantalla: medidas subjetivas y objetivas. Las medidas subjetivas del tiempo sedentario de pantalla se obtienen mediante el uso de cuestionarios, siendo éste el método principal de valoración de tiempo sedentario de pantalla. Se pueden administrar por el investigador o pueden ser rellenados por los participantes sin presencia de evaluador. La información recogida permite distinguir entre varios tipos de comportamientos: ver la televisión, el uso de video consolas o el uso de Internet, entre otros. Sin embargo, las medidas subjetivas son propensas a sesgos dado que mayoritariamente dependen de la capacidad del individuo para recordar con precisión comportamientos pasados (93). No obstante, las mediciones subjetivas se utilizan ampliamente en estudios epidemiológicos, puesto que tienen un bajo coste y aportan información sobre comportamientos específicos (94).

Las mediciones objetivas proporcionan una evaluación detallada del tiempo de inactividad de los individuos, pero no distinguen entre los diferentes tipos de

comportamientos, entre ellos, el tiempo de pantalla. La inactividad física se mide a través de aparatos como el acelerómetro, monitores de postura o monitores de frecuencia cardiaca (95). El coste puede ser elevado dependiendo del número de sujetos a estudiar. Los acelerómetros se pueden utilizar para estimar el hábito total del comportamiento sedentario a través de la acumulación de niveles bajos de conteo de movimiento, utilizando puntos de corte específicos para la edad de la población estudiada (96). También se pueden usar para detectar breves interrupciones en el tiempo, definidas por períodos en los que los conteos de movimiento exceden el umbral especificado, que pueden no ser registradas mediante cuestionario (97).

Ambos métodos de medida de tiempo de pantalla, objetivo y subjetivo, están actualmente en proceso de desarrollo. La miniaturización de los dispositivos de seguimiento, la operatividad entre las tecnologías de uso en la medición y la comunicación y el enfoque analítico utilizado son alternativas potenciales para el desarrollo futuro en este campo (98).

La OMS recomienda que no exista tiempo sedentario de pantalla en niños menores a dos años, y propone limites inferiores a una hora al día (cuanto menos tiempo, mejor) para niños/as entre dos y cinco años (99). La *AAP* apuntó también restricciones similares de tiempo de pantalla en edad escolar (100). En cuanto a las recomendaciones de tiempo de pantalla para niños/as más mayores o adolescentes, la Sociedad Pediátrica Canadiense (*Canadian Peadiatric Society*) ofrece una guía de salud sobre el uso de pantalla basada en evidencia científica, y fija el uso máximo de pantalla para uso recreacional en jóvenes de 5 a 19 años de edad en dos horas (101). En la misma línea, estudios previos relazados en EE.UU sobre la valoración del tiempo de pantalla en adolescentes también fijan el límite de exceso de recomendaciones diarias en dos horas (102).

En la actualidad, existen estudios en los que se sugiere una nueva valoración en las recomendaciones de tiempo de pantalla debido al cambio de contexto derivado de la pandemia del *COVID19* y el reajuste constante que se lleva a cabo por parte de las instituciones de educación, con la implementación de tecnologías basadas en el uso de Internet, para acceder a una formación adecuada de los estudiantes trabajando remotamente (103).

1.4 Marco conceptual de la Tesis Doctoral

La presente Tesis Doctoral se ha realizado siguiendo el modelo conceptual (Figura 1), que presenta el contexto en el cual se valoran las principales asociaciones estudiadas dentro de esta compliación de artículos: primeramente, la relación de una serie de polimorfismos genéticos y su asociación con el IMC; además, su efecto modulador sobre la DM y su relación con parámetros de obesidad y SM en jóvenes Europeos. También, observar un posible efecto de interacción entre tiempo sedentario de pantalla x DM, y qué influencia tiene en términos de adiposidad en adolescentes Europeos.

Existen, no obstante, otros factores modificables que pueden interactuar con la DM que no han sido investigadas en el presente trabajo como son la práctica de AF, los hábitos alimentarios o el estatus socioeconómico, que de acuerdo con la literatura, parecen jugar un papel modulador en el desarrollo de enfermedades crónicas en población joven (104-106).

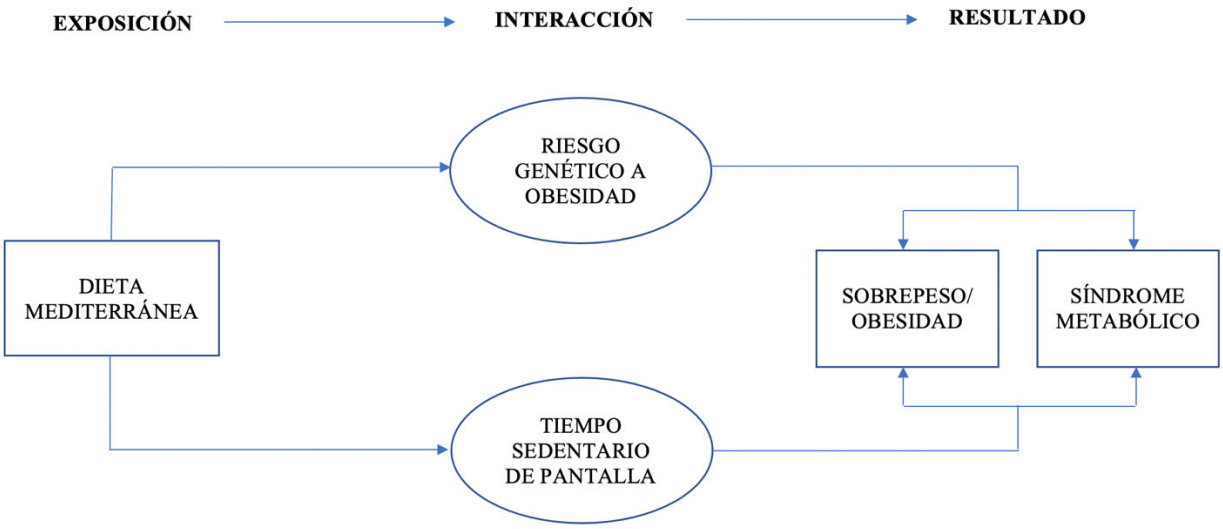

Figura 1. Marco conceptual (hipótesis). Fuente: elaboración propia.

1.5 Hipótesis de estudio

La hipótesis planteada en la presente Tesis Doctoral parte de la idea de que existen factores ambientales durante la adolescencia que parecen jugar un papel crítico en el desarrollo de enfermedades crónicas como obesidad y SM. Por ello, los beneficios de una alta adherencia a la DM pueden verse modulados por mecanismos de interacción que pueden modificar la respuesta a un patrón alimentario saludable y, por tanto, condicionar la aparición temprana de sobrepeso u obesidad y SM. Concretamente, la presente Tesis Doctoral evalúa las interacciones de la DM con factores, *a priori*, no modificables, como es el riesgo genético de obesidad, y con un factor ambiental modificable, el tiempo de pantalla, para valorar su influencia sobre el sobrepeso, la obesidad y el SM.

Dentro de la compilación de los estudios originales incluidos en la presente Tesis Doctoral, se plantean una serie de hipótesis, enmarcadas dentro de la cohorte de adolescentes participantes en el estudio *HELENA*: la elaboración de un índice de riesgo

genético, con polimorfismos previamente relacionados con la obesidad, podría servir para evaluar la predisposición genética a sobrepeso y obesidad (Artículo II). Además, la mayor predisposición al riesgo de obesidad podría atenuar el efecto protector de la adherencia a la DM sobre la adiposidad y el SM (Artículo III). Finalmente, se plantea que niveles altos de tiempo sedentario de pantalla podrían atenuar el efecto protector de la adherencia a la DM en indicadores de adiposidad (Artículo IV).

2. OBJETIVOS

Los objetivos generales de la presente Tesis Doctoral son explorar, describir y analizar los estudios previos de interacciones genes x DM, desarrollar un índice de riesgo genético (*GRS*) para valorar la predisposición genética a sobrepeso u obesidad, así como valorar el efecto de interacción entre genes x DM y tiempo sedentario de pantalla x DM en términos de SM y adiposidad en adolescentes Europeos.

Los objetivos específicos de los cuatro artículos que componen la Tesis Doctoral son los siguientes:

Artículo I. Proporcionar una visión general de los beneficios de la DM y su impacto en la composición corporal en niños/as y adolescentes Europeos y describir los posibles efectos de la interacción genes x DM que podría modular el riesgo de desarrollo de obesidad y SM en jóvenes Europeos.

Artículo II. Desarrollar un *GRS* para sobrepeso y obesidad en adolescentes Europeos del estudio *HELENA*.

Artículo III. Analizar la interacción entre la adherencia a la DM y el *GRS* de obesidad sobre la adiposidad y el SM en adolescentes Europeos del estudio *HELENA*.

Artículo IV. Analizar la asociación entre la adherencia a la DM y el tiempo sedentario en adolescentes Europeos del estudio *HELENA*. Además, se explorará si el tiempo sedentario de pantalla tiene un efecto modulador en la asociación entre la DM y los marcadores de adiposidad.

2. OBJECTIVES

The general objectives of this Doctoral Thesis are to explore, describe and analyze previous studies assessing gene x MD interaction effects on adiposity, to develop a genetic risk score (GRS) to assess the genetic predisposition to overweight or obesity, as well as assessing the gene x MD and screen sedentary time x MD interaction effects on obesity and MetS in European adolescents.

The specific objectives of the four articles included in the present Doctoral Thesis are the following:

Article I. To provide an overview of the MD benefits and its impact on body composition in European children and adolescents and to describe the potential gene x MD interaction effects that could modulate the risk of obesity and MetS development in European youth.

Article II. To develop a GRS for overweight and obesity in adolescents participating in the HELENA study.

Article III. To assess whether interaction effects occur between the MD adherence and obesity-GRS on adiposity and MetS in European adolescents from the HELENA study.

Article IV. To assess whether an association between MD and sedentary time exists in European adolescents from the HELENA study. Moreover, we intend to explore whether screen sedentary time has a modulatory effect on the association between MD and adiposity markers.

3. MATERIAL Y MÉTODOS

La presente Tesis Doctoral se ha elaborado teniendo en cuenta resultados del estudio *HELENA* (*The Healthy Lifestyle in Europe by Nutrition in adolescentes*).

3.1 Diseño y participantes del estudio HELENA

El estudio *HELENA* es un estudio transversal y multicéntrico que fue diseñado para obtener información fiable y comparable sobre los determinantes nutricionales y ambientales que influyen en la salud de adolescentes Europeos con el objeto de prevenir factores de riesgo de enfermedades crónicas del presente y futuro (107). El estudio incluyó 3.528 adolescentes (1.845 chicas) entre 12.5 y 17.5 años, en 10 ciudades de 9 países Europeos: Viena (Austria), Gante (Bélgica), Lille (Francia), Dortmund (Alemania), Atenas y Heraklion (Grecia), Pécs (Hungría), Roma (Italia), Zaragoza (España) y Estocolmo (Suecia), reclutados entre 2006 y 2007 a través de invitaciones a colegios seleccionados en cada ciudad participante, con el acuerdo del equipo directivo de cada centro (Figura 2) (108).

Los criterios de inclusión de los participantes en el estudio fueron: no estar participando simultáneamente en otro estudio; no haber padecido ninguna enfermedad o síntomas de la misma durante la semana anterior a las mediciones antropométricas, haber obtenido medidas de peso y altura y haber completado satisfactoriamente al menos el 75% del resto de pruebas designadas por el estudio. Esta información puede ser revisada dentro del capítulo 2 del *Manual de Operaciones del estudio HELENA* (109).

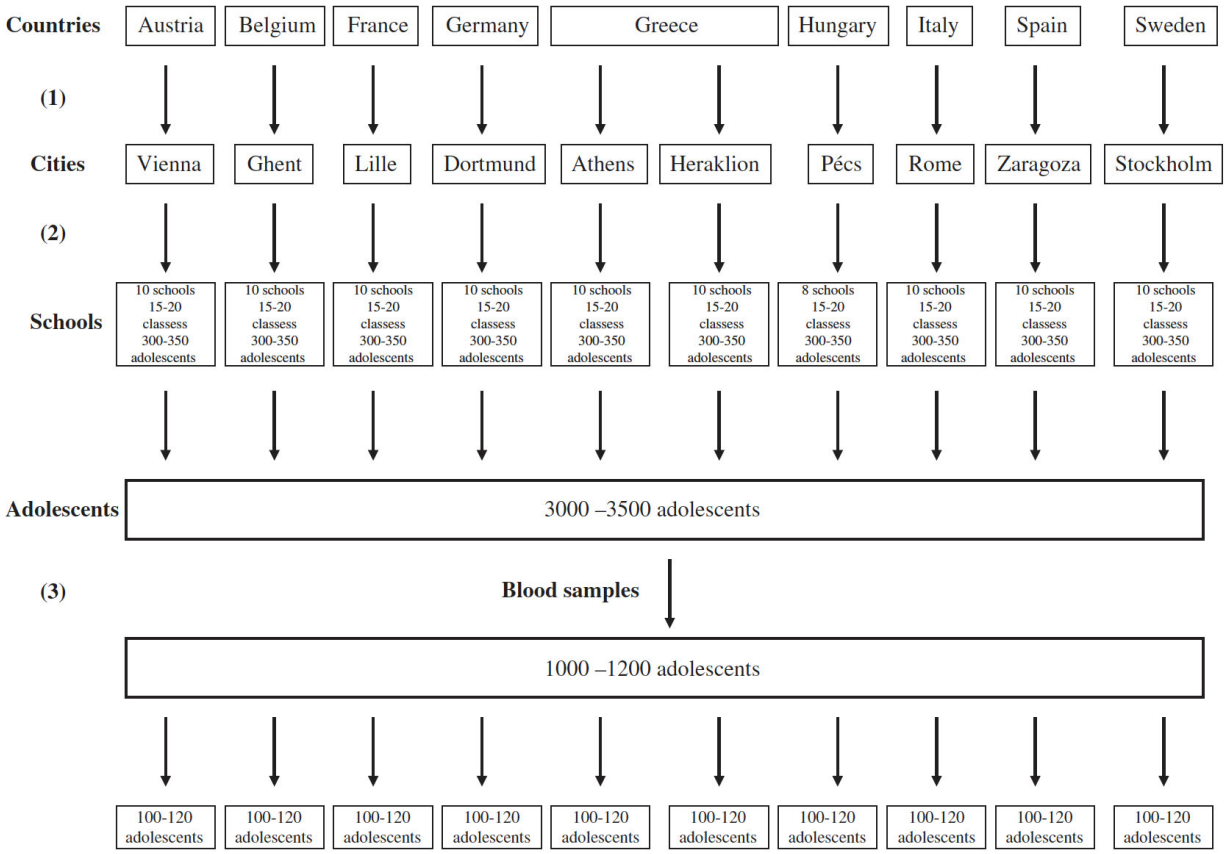

Figura 2: Esquema del procedimiento del reclutamiento aleatorio por conglomerados del estudio *HELENA*. (1): se considera el equilibrio geográfico y la existencia de un grupo de investigación experimentado en la zona. (2): para la selección de los participantes, se realiza estratificación por edad, sexo y centro educativo en todas las ciudades; (3): para la obtención de muestras biológicas, se realiza estratificación por edad, sexo y centro educativo en cada ciudad. Se decide obtener muestras de sangre solamente en un tercio de la muestra, lo cual es suficiente para describir las variables bioquímicas e inmunológicas que presentan una mayor variabilidad. Fuente: *Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study* (107).

Para la realización de la presente Tesis Doctoral se ha utilizado datos empíricos del estudio *HELENA* (excepto el Artículo I que describe los posibles efectos de interacción genes x DM sobre composición corporal en una revisión narrativa). Los Artículos II y III incluyen análisis con información genética, por lo que el tamaño de las muestras de adolescentes incluidos en los mismos se redujeron considerablemente. Para la elaboración del Articulo IV, que no precisa de información genética, el tamaño muestral fue mayor.

Los criterios de selección y el tamaño de la muestra final para cada uno de los artículos incluidos en la presente Tesis Doctoral se detallan a continuación (Figura 3):

El Artículo II contó con datos de 1.069 niños con información completa sobre la composición corporal relacionada con la obesidad y la genética.

El Artículo III, contó con un total de 605 adolescentes de los que se obtuvo información completa sobre consumo de alimentos, genética composición corporal relacionada con obesidad y SM.

El Artículo IV, incluyó datos de 2.047 adolescentes con información sobre consumo de alimentos, tiempo total de pantalla como medida de valoración del sedentarismo y composición corporal en términos de adiposidad.

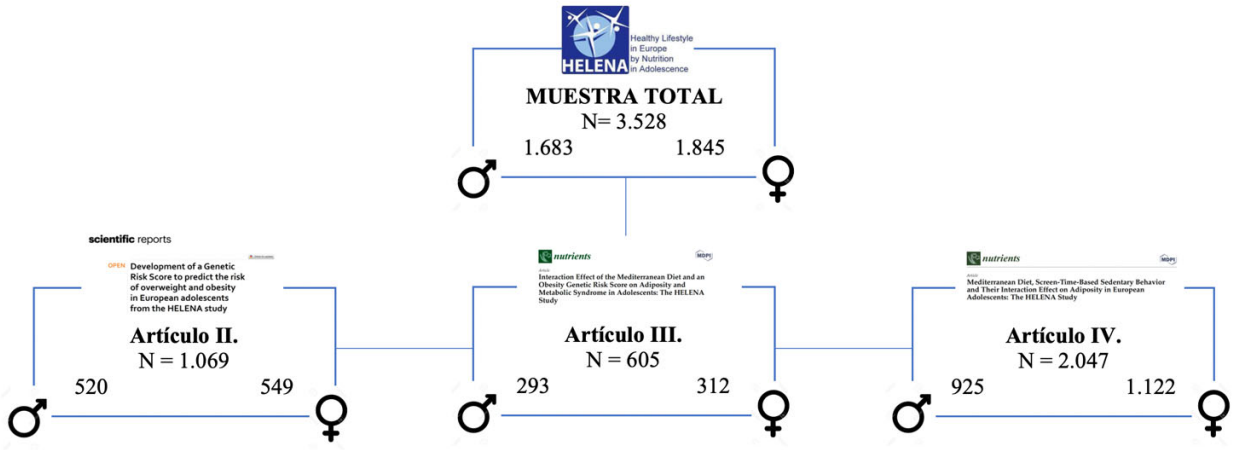

Figura 3: Tamaño muestral de los artículos incluidos en la presente Tesis Doctoral. Fuente: elaboración propia.

El estudio *HELENA* se realizó siguiendo las pautas éticas de la Declaración de Helsinki de 1961 (revisión de Edimburgo 2000), las normas de la Buena Práctica Clínica y la legislación sobre investigación clínica en humanos. El estudio fue aprobado por el Comité Ético de Investigación Clínica de Aragón (CEICA), en el caso de la Universidad

de Zaragoza (110). El protocolo del estudio en el resto de los países Europeos participantes en *HELENA* fue aprobado por los comités éticos locales correspondientes, para cada centro participante en el estudio. Los padres o tutores legales de los adolescentes participantes en el estudio firmaron un consentimiento informado, el cual fue leído y firmado, mostrando su aceptación para la participación en el mismo. Asimismo, los menores también dieron verbalmente su consentimiento antes de realizar las distintas pruebas de valoración.

3.2 Metodología de las valoraciones del estudio HELENA

3.2.1 Examen físico, medidas de adiposidad y de riesgo cardiometabólico

Las medidas antropométricas fueron realizadas por personal especializado siguiendo los protocolos estandarizados (111), descritos en la referencia establecida por la Sociedad Internacional para el avance de la Cineantropometría (*International Society for the Advancement of Kinanthropometry (ISAK*)) (112). La altura se midió con un instrumento telescópico de medición de altura *(Type SECA 225)* al 0.1 cm más cercano, rango 70 a 200 cm, con pies descalzos, con la cabeza alineada de manera que se mantiene horizontal el plano de *Frankfort*. El sujeto se mantuvo de pie, con los talones juntos, y los talones, glúteos y la parte superior de la espalda en contacto con la escala. La cabeza, cuando se posicionó manteniendo horizontal el plano de *Frankfort*, no necesitó estar tocando la escala. El plano de *Frankfort* se obtuvo cuando el punto *Orbitale*® (borde inferior de la cuenca del ojo) estaba en el mismo plano horizontal del punto del *Tragion*® (la muesca superior del trago de la oreja). El peso se midió con una báscula electrónica *(Type SECA 861)* al 0.1 kg más cercano, rango 0 a 150 kg estando los adolescentes en ropa interior y

sin calzado. Peso y altura fueron medidos en triplicado. El Índice de Masa Corporal (IMC) se calculó en función de la altura y del peso (kg/m^2) (16). La circunferencia de la cintura (CC) se midió por triplicado con una cinta de medición no elástica (*Type SECA 200*) al 0.1 cm más cercano, en el punto medio entre la última costilla y la cresta ilíaca. La media de las tres medidas fue la utilizada. Los pliegues cutáneos de bíceps, tríceps, subescapular, suprailíaco y muslo fueron medidos por triplicado con un LIPÓMETRO *Holtain* al 0.2mm más cercano. La media de las tres medidas fue la utilizada (111). Para valorar la contribución de la masa grasa relativa al tamaño corporal, el porcentaje de masa grasa se calculó mediante la *ecuación de Slaughter* (113), y posteriormente, se obtuvo el índice de masa grasa (IMG) mediante la ecuación masa grasa (kg)/altura (m^2) (114). La batería completa de medidas antropométricas realizadas puede consultarse en el capítulo 15 del *Manual de Operaciones de HELENA* (109)

La tensión arterial sistólica (TAS) y diastólica (TAD) se midieron en duplicado en posición sentada con un intervalo de 10 minutos, con el aparato oscilométrico de tensión arterial *OMRON HEALTHCARE® (M6- HEM7001; OMRON HEALTHCARE®, Kyoto,* Japón*).* La lectura más baja de la tensión arterial fue la medida utilizada. Seguidamente, la media de las tensiones arteriales (MTA) se obtuvo mediante la ecuación TAD+[(TAS-TAD)/3)]. Colesterol total (CT), colesterol unido a lipoproteinas de alta densidad (*High density lipoprotein* (*HDL*)), triglicéridos (TG) y glucosa fueron medidas utilizando métodos enzimáticos *(Dade Behring, Schwalbach,* Alemania*)*. Los niveles de insulina fueron obtenidos de suero congelado utilizando el analizador *Immulite 2000 analyzer (DPC Bierman GmbH, Bad Nauheim,* Alemania) (115). Como medida de la resistencia a la insulina, el índice de la valoración del modelo homeostático de resistencia a la insulina *(homeostatic model assessment insulin resistance (HOMA IR))* fue calculado a partir de

las medidas de glucosa e insulina, usando la fórmula propuesta por *Matthews, DR et al. HOMA IR* = Insulina ayuno (µUI/ml) x Glucosa ayuno (mmol/l) / 22.5 (115).

Además, se calculó un índice de riesgo cardiometabólico, a partir de la suma de valores *z-score* de CC, índice *HOMA,* CT/*HDL* y MTA. El *HDL* fue multiplicado por -1, caracterizado como bajo riesgo cardiometabólico con valores en incremento. Los índices cardiometabólicos estandarizados de las variables de interés fueron calculados en base a puntos de corte específicos de edad y sexo (36). Valores más bajos en el índice indican mejor perfil cardiometabólico. Como análisis de sensibilidad, un segundo índice de riesgo de SM fue calculado en el Artículo III, siguiendo las recomendaciones de la *International Diabetes Federation* (*IDF*) (31). Los resultados de ambos índices cardiometabólicos en adolescentes han sido incluidos en la presente Tesis Doctoral.

Finalmente, el desarrollo puberal fue evaluado durante el examen médico realizado por un pediatra, siguiendo la metodología descrita por *Tanner and Whitehouse* (116). El desarrollo puberal fue categorizado como estadíos de *Tanner* desde el estadío I (no maduración sexual) al estadío V (maduración sexual completa).

3.2.2 Ingesta de alimentos y patrón de dieta Mediterránea

Los hábitos dietéticos fueron determinados mediante un cuestionario de recuerdo de alimentos de 24 horas rellenado por los mismos adolescentes a través del software *HELENA dietary assessment tool* (*HELENA-DIAT*) (117). Esta herramienta fue previamente validada en adolescentes (118) y posteriormente adaptada para poder ser realizada en las 10 ciudades participantes (Anexo I). Los participantes completaron el recuerdo en dos días no consecutivos en un periodo de dos semanas (119). Para calcular la ingesta dietética habitual de cada individuo, se ha utilizado el método *multiple source*

(MSM) (82) que permite la corrección de la variabilidad de la información dietética inter- e intra- individuo.

Para el cálculo de la ingesta de alimentos típicamente Mediterráneos hemos usado el índice de DM *(Mediterranean Diet Score (MDS*)), basado en nueve componentes individuales, siguiendo la metodología propuesta por *Trichopoulou, A et al.:* (1) frutas y verduras, (2) frutos secos, (3) cereales y raíces, (4) legumbres, (5) pescado, (6) productos lácteos, (7) carne, (8) la relación de ácidos grasos mono insaturados y saturados y (9) la ingesta de alcohol (48). El presente *MDS* fue validado y adaptado para los adolescentes de *HELENA*, el cual mostró las asociaciones más fuertes con los indicadores dietéticos y nutricionales y el estilo de vida Mediterráneo (80). El consumo de verduras, frutas y frutos secos, cereales, legumbres, pescado, productos lácteos, y el ratio de ácidos grasos mono insaturados y saturados contribuyen positivamente a la adherencia a la DM, mientras que carne (incluyendo carne procesada) y alcohol se consideraron inversamente (70). Los productos lácteos se consideran positivamente, ya que se recomiendan durante los períodos de crecimiento y desarrollo, como la adolescencia (120). La evaluación de la ingesta de cada uno de los subgrupos del *MDS* por sexo se calcula mediante la mediana de la ingesta en gramos al día. En caso de que la ingesta de alimentos en gramos al día en cada uno de los subgrupos considerados beneficiosos estuvo por encima de la mediana, se asignó el valor 1, mientras que si la ingesta se encontraba por debajo de la mediana, se asignó valor 0. Este criterio se recalculó en sentido opuesto en el caso de los alimentos considerados como negativos para la salud (valor 0 por encima de la mediana, valor 1 por debajo de la mediana). La ingesta de alcohol se consideró como hábito poco saludable entre los adolescentes. Por lo tanto, en una situación donde no hubo consumo alcohólico, se asignó el valor 1, mientras que cualquier ingesta de alcohol se calculó como valor 0.

La valoración de la adherencia a la DM se consideró mediante una escala de 0 a 9 puntos, donde las puntuaciones más altas indican una mayor adherencia (121).

3.2.3 Información genética y desarrollo del índice de riesgo genético

Aproximadamente un tercio de los adolescentes reclutados de las 10 ciudades participantes fueron seleccionados aleatoriamente para la extracción de sangre y así obtener información sobre los parámetros séricos de interés (*N*=1.172). El tamaño de los subgrupos (alrededor de 100 adolescentes por ciudad) fue elegido teniendo en cuenta las medias de los parámetros inmunológicos, ya que fueron las medidas sanguíneas que representaban una mayor variabilidad entre todos los parámetros incluidos en el estudio (Tabla 2). La extracción de las muestras se realizó en ayunas desde la noche anterior. Un laboratorio certificado, bajo metodología estandarizada, se encargó de la recolección, el transporte y procesado de muestras (122). Para la extracción de ADN de las muestras, se utilizaron tubos *EDTA K3* y se almacenó en el *Instituto de Ciencias de la Alimentación y la Nutrición (IEL)* de la *Universidad de Bonn* (Alemania), y se envió al *Laboratoire d'Analyse Genomique Centre de Ressources Biologiques (LAG-CRB) e BB- 0033-00071 Institut Pasteur de Lill*e, (Francia). El ADN se extrajo de los glóbulos blancos con el kit *Puregene (QIAGEN, Courtaboeuf*, Francia) y se almacenó a -20 ºC. El genotipado se realizó mediante un sistema *Illumina (Illumina, Inc, San Diego, California,* EE.UU*)* utilizando la tecnología *Golden-Gate* (esquema de procedimiento de muestreo, *GoldenGate; Software, Inc, San Francisco, California,* EE.UU*).*

Tabla 2: Parámetros sanguíneos incluidos en el estudio *HELENA*.

IEL/UPM		*INRAN*	*CSIC*	*Institute Pasteur Lille*
Insulin	Albumin	Ferritin	Adhesion molecules	Genetic
Vitamins A and E	Creatinine	AGP	s-VCAM, S-ICAM, E-selectin, L-selectin	Phenotypes
Vitamin C	Glucose	C-reactive	Cytokines	*In situ*
Vitamin D	Uric acid	protein	IL-2,4,6,10, IFN-γ,	Hemogram
		Soluble	TNF-α, TGF-β1	
		transferring	Inflammatory proteins	
		receptor (sTFR)	Ceruloplasmin, C3,C4	
TEAC	Lipoprotein(a)		Immunoglobulins	
Vitamin B12	Apo A, Apo B		IgA, M, G	
Total homocysteine	Cholesterol, HDL,LDL,			
Plasma and RBC folate	triacylglycerols			
Holo-transcobalamin	GGT,GOT,GPT			
	Fatty acids (e.g. oleic acid, α-linoleic acid, linoleic acid, "Mead" acid, arachidonic acid, docosahexaenoic acid)			
Vitamin B6			Lymphocyte subpopulations	
Adiponectin, leptin			CD3,CD4,CD8,CD16/56,CD19	
Cortisol				

Fuente: *Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study* (107).

El análisis de las variantes genéticas del estudio *HELENA* se realizó mediante la selección genes candidatos. En primer lugar, se identificaron ciertas conductas y vías metabólicas relacionadas con la salud en la adolescencia. Así se incluyeron la ingesta de alimentos, el comportamiento alimentario, las elecciones y preferencias alimentarias, el metabolismo energético y del tejido adiposo, el metabolismo de la glucosa, la insulina, los lípidos y las lipoproteínas, entre otros. Finalmente, se seleccionaron los *SNPs* que desempeñan un papel clave en los genes que codifican las vías mencionadas. Se utilizó la base de datos *HapMap* para seleccionar *SNPs* marcadores e independientes. Los *SNPs* se seleccionaron con una frecuencia alélica menor *(minor allele frequency (MAF))* por encima de 0.1 y *SNPs* marcadores con r^2 por encima de 0.8. Si los *SNPs* marcadores descritos para un solo gen excedían en número (más de ~ 20), sólo se seleccionaron los *SNPs* significativamente asociados con fenotipos de estudios anteriores, si estaban disponibles. Por último, los *SNPs* de la base de datos *National Center for Biotechnology*

Information (NCBI) se utilizaron cuando un número limitado de *SNPs* estaba disponible en la base de datos *HapMap.*

La base de datos de *HELENA* cuenta con un total de 611 *SNPs* relacionados con la obesidad y fenotipos asociados a la obesidad. Estos *SNPs* fueron considerados en la elaboración del Articulo II para construir un *GRS*. Se tuvo en cuenta el IMC como la variable referencia en términos de adiposidad, con el fin de predecir la predisposición al sobrepeso u obesidad en adolescentes Europeos (123). Cada *SNP* se recodificó como 0, 1 o 2 según el número de alelos de riesgo definidos previamente en la literatura. Seguidamente, se realizó una selección adicional de *SNPs* utilizando un modelo lineal generalizado *(generalized linear model (GLM))* para establecer un punto de corte inicial (p<0.20) para refinar el número de *SNPs* incluidos hasta un total de 104. Posteriormente, se aplicó un algoritmo paso a paso hacia atrás para seleccionar los *SNPs* significativos por debajo del umbral p<0.05 en un modelo multivariante, y obtener finalmente 21 *SNPs* asociados significativamente con el IMC.

La correspondencia entre las probabilidades reales y previstas de este modelo se analizó mediante una curva de calibración. El *GRS* no ponderado (*uGRS*) se calculó sumando el número de alelos de riesgo de los 21 *SNPs*, considerando también aquéllos que aparecen como factores protectores mediante el reajuste de sus valores. El *GRS* ponderado (*wGRS*) fue el resultado de multiplicar el número de alelos de riesgo en cada locus (0, 1, 2) por cada coeficiente estimado del modelo multivariante. Los participantes que no contaban con información genética fueron excluidos del análisis del *GRS*. La curva de características operativas del receptor *(receiver operating characteristic (ROC))* (124) se utilizó para comprobar la precisión diagnóstica del *GRS* para clasificar a los posibles participantes por alteraciones relacionadas con la obesidad (125). El área bajo la curva *(area under curve (AUC))* se calculó tanto para el *uGRS* como para el *wGRS* considerando

las categorías de peso corporal, no sobrepeso *vs*. sobrepeso/obesidad, como variable binaria. La decisión de considerar el *uGRS* o *wGRS* para proceder con el diseño del modelo final se tomó mediante la comparación del valor más alto del *AUC* utilizando la *prueba de Delong*. El modelo fue validado internamente realizando un análisis de validación cruzada *10-fold*. Para este análisis, todo el conjunto de datos se dividió en 10 grupos, usando 9 de ellos para construir el modelo predictivo y el restante, para validar el modelo. Este procedimiento se repitió teniendo en cuenta todas las formas posibles de seleccionar los 9 subgrupos, asegurando que las diferentes formas para validar el *GRS* han sido con datos no utilizados en proceso de construcción del modelo. Además, se evaluó la distribución de los valores de *uGRS* y *wGRS* para los grupos no sobrepeso y sobrepeso/obesidad para interpretar el funcionamiento del *GRS*. Con el fin de proporcionar el mejor punto de corte para el uso del *GRS* como variable dicotómica se utilizó la maximización del *índice de Youden* (126).

Por último, para probar la fiabilidad de *GRS* con otras variable de adiposidad distinta del IMC, se realizaron modelos de regresión lineal simple *(linear regression model (LRM))* para evaluar la asociación de *wGRS* y *uGRS* con el IMG.

3.2.4. Comportamientos sedentarios basados en tiempo de uso de pantalla

Para recoger información sobre el tiempo habitual dedicado a una pantalla entre los adolescentes, se utilizó un cuestionario de comportamientos sedentarios basado en el tiempo sedentario de pantalla, rellenado por los adolescentes y previamente validado (Anexo II) (94). Se valoró el tiempo dedicado a ver televisión, juegos de ordenador, videojuegos y uso de Internet no relacionado con el estudio durante días entre semana y los fines de semana en categorías, en una escala categórica que va de 0 a 240 minutos al

día. Se obtuvo el tiempo medio diario para cada categoría y el tiempo total se calculó sumando días laborables y días de fin de semana, obteniendo tiempo total de pantalla en minutos por día. Por último, el tiempo total sedentario se obtuvo sumando el tiempo anotado en cada categoría.

Los *coeficientes K ponderados de Cohen* se utilizaron para evaluar la fiabilidad *test-retest* del cuestionario del tiempo de pantalla utilizado en el estudio *HELENA.* Los valores más comunes observados fueron moderados, sustanciales o acuerdo casi perfecto (>0.7). Excepcionalmente, el uso de Internet por motivos de estudio mostró valores de 0.46 entre semana y 0.33 los fines de semana, respectivamente (127).

Además, en el Artículo IV, se realizó un análisis de sensibilidad, con el fin de descartar posibles diferencias potencialmente significativas dentro de los modelos de interacción planteados, considerando por un lado, los valores atípicos encontrados en la variable tiempo de pantalla, y por otro, eliminando los valores atípicos dentro de la misma variable.

3.2.5. Estatus socioeconómico

La valoración del estatus socioeconómico se realizó mediante cuestionario y fue rellenado por los padres/tutores de los participantes del estudio (Anexo III). Se consideró la escala de afluencia familiar *(family affluence scale (FAS)),* que es un indicador de los recursos materiales de la familia. La evaluación del estatus socioeconómico se realizó a través de una escala numérica, que varía desde 0 (valor más bajo) a 8 (valor más alto). Además, la escala se categoriza en distintos niveles: 0 a 2 (bajo), 3 a 5 (medio) y 6 a 8 (alto) (128). Se consideran parámetros como la propiedad de un automóvil, tener un dormitorio propio, la disponibilidad de Internet y disponer de ordenador en casa. Esta

información se evaluó mediante un cuestionario, que se utilizó como predictor de salud en los adolescentes (129).

3.3. Análisis estadístico

Los estadísticos descriptivos permitieron analizar las características generales de los participantes. Las características descriptivas fueron mostradas en forma de mediana y rango intercuartílico, para el caso de las variables continuas, y como frecuencias absolutas y relativas en el caso de las variables categóricas (Artículos II, III y IV).

El *test no paramétrico Saphiro-Wilk* se utilizó para comprobar la normalidad de las variables. Los test estadísticos utilizados para comparar diferencias entre sexos fueron el *Pearson´s chi cuadrado* para variables categóricas y el *Mann–Whitney* para variables continuas.

El *test estadístico Pearson´s chi cuadrado* fue utilizado para analizar el *equilibrio de Hardy-Weinberg*. Además, la parte estadística correspondiente al desarrollo del *GRS* para elucidar la predisposición a la obesidad es explicada en el apartado *3.2.3 Información genética y desarrollo del índice de riesgo genético* (Artículo II).

Modelos de regresión linear múltiple, específicos de cada sexo, fueron llevados a cabo para valorar la asociación entre adiposidad y parámetros cardiometabólicos, además del efecto de interacción entre el *GRS* de obesidad y la DM, añadiendo el efecto de la DM individualmente dentro del mismo modelo (Artículo III).

Para observar la asociación entre DM y tiempo sedentario de pantalla, se utilizaron modelos de regresión lineal múltiple por sexo. Primero, un modelo crudo de regresión

linear simple fue construido para observar las asociaciones entre DM y tiempo sedentario de pantalla. Después, un modelo de regresión lineal múltiple inicial fue ejecutado, considerando ingesta energética, estatus socioeconómico y los estadios de *Tanner*, como variables de ajuste. Se aplicó un algoritmo paso a paso hacia atrás para seleccionar las variables significativas en el modelo multivariante para preseleccionar aquéllas que están significativamente asociadas de forma independiente con parámetros de adiposidad en el modelo final. Además, un nuevo análisis de regresión lineal múltiple fue creado para valorar la asociación entre adiposidad y DM, añadiendo el efecto de interacción del tiempo sedentario de pantalla, el efecto de la DM de forma individual y el de las variables de ajuste mencionadas anteriormente. Finalmente, se observaron valores extremos en la distribución de la variable tiempo sedentario de pantalla, así que se llevó a cabo un análisis de sensibilidad excluyendo estos valores atípicos del extremo superior de la distribución (Artículo IV).

Todos los análisis estadísticos llevados a cabo en la presente Tesis Doctoral fueron realizados con *RStudio Version 1.2.5001 (RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/)* y el nivel de significación se estableció en $p<0.05$.

4. RESULTADOS

Los resultados y discusión específica de la presente Tesis Doctoral quedan reflejados en los siguientes artículos científicos.

4. RESULTS

The results and specific discussion of this Doctoral Thesis are shown in the following research manuscripts.

Artículo I [Article I]: Mediterranean Diet and Genetic Determinants of obesity and Metabolic Syndrome in European children and Adolescents.

Seral-Cortes M, Larruy-García A, De Miguel-Etayo P, Labayen I, Moreno L.

Genes. 2022;13(3)

genes

MDPI

Review

Mediterranean Diet and Genetic Determinants of Obesity and Metabolic Syndrome in European Children and Adolescents

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Abstract: Childhood obesity and metabolic syndrome (MetS) are multifactorial diseases influenced by genetic and environmental factors. The Mediterranean Diet (MD) seems to modulate the genetic predisposition to obesity or MetS in European adults. The FTO gene has also been shown to have an impact on the MD benefits to avoid obesity or MetS. Since these interaction effects have been scarcely analyzed in European youth, the aim was to describe the gene–MD interplay, analyzing the impact of the genetic factors to reduce the obesity and MetS risk through MD adherence, and the MD impact in the obesity and MetS genetic profile. From the limited evidence on gene–MD interaction studies in European youth, a study showed that the influence of high MD adherence on adiposity and MetS was only observed with a limited number of risk alleles; the gene–MD interplay showed sex-specific differences, being higher in females. Most results analyzed in European adults elucidate that, the relationship between MD adherence and both obesity and MetS risk, could be modulated by obesity genetic variants and vice versa. Further research is needed, to better understand the inter-individual differences in the association between MD and body composition, and the integration of omics and personalized nutrition considering MD.

Keywords: genetic risk score; single nucleotide polymorphism; interaction effect; Mediterranean Diet; obesity; metabolic syndrome; children and adolescents

Citation: Seral-Cortes, M.; Larruy-García, A.; De Miguel-Etayo, P.; Labayen, I.; Moreno, L.A. Mediterranean Diet and Genetic Determinants of Obesity and Metabolic Syndrome in European Children and Adolescents. *Genes* **2022**, *13*, 420. https://doi.org/10.3390/genes13030420

Academic Editor: Hirokazu Takahashi

Received: 28 January 2022
Accepted: 23 February 2022
Published: 25 February 2022

1. Introduction

Obesity is defined as excess of body fat, and its prevalence has alarmingly increased over the last decades, with negative implications for the population's health status [1]. In 2016, more than 6% of children and adolescents had obesity worldwide, with similar numbers in males and females [2]. Nowadays, the expected incidence of childhood obesity could be negatively escalating due to the COVID-19 pandemic [3,4]. Childhood overweight increases the risk of persistent obesity and related cardiometabolic events in adulthood [5]. Together with obesity, metabolic syndrome (MetS) is known to be a major health challenge in youth with increasing prevalence and a high risk of developing cardiovascular diseases in adulthood [6,7]. Both diseases could coexist within a given individual, acting in negative synergy to be fully established in a permanent status later in life [8]. The definition of MetS for children and adolescents comprises a number of cardiometabolic risk factors, such as total and/or central adiposity, dyslipidemia (high triglycerides and low high density lipoprotein (HDL)-cholesterol concentrations), hypertension, and insulin resistance [9]. Since obesity and MetS are complex and multifactorial diseases, they are

influenced by genetic and environmental factors [10,11]. It seems that these factors do not act independently [12]. Instead, they either interact [13,14] or mediate [15,16] with each other to influence overweight and obesity risk.

In adults, the benefits of a high adherence to the Mediterranean Diet (MD) to prevent cardiovascular events are widely known [17]. It improves the lipid profile and adiposity levels [18], reduces the risk of overweight and obesity [19], MetS [18], type 2 diabetes mellitus (T2DM) [20] and hypertension [21], among others. Although less studied, the MD adherence is also relevant from the early stages in life. Breastfeeding could protect against childhood obesity [22], so maternal dietary habits could have an impact in the milk composition, thus, a balanced nutrition for the infant [23]. In fact, a study showed that breast milk mineral concentrations could be positively influenced by maternal MD adherence [24]. Moreover, in young age populations, MD has been observed to have inverse associations with obesity and MetS indicators such as high body mass index (BMI) [25], increased waist circumference (WC) [26], insulin resistance and high lipid profile [27]. As it is rather challenging to measure a whole diet [28], several diet scores assessing the degree of adherence to a certain dietary pattern have been developed to estimate their impact in a given population [29]. In this sense, the MD adherence has been extensively studied [30] through item-scales in different population groups [31,32].

Interestingly, the MD has previously shown to attenuate the genetic predisposition to obesity or MetS in European adults [33,34]. Individual single nucleotide polymorphisms (SNPs) or a combination of a number of SNPs have been considered as useful genetic tools to predict the predisposition to obesity and MetS in different age groups [35,36]. It is highly relevant studying gene-MD interaction effects from early stages in life, knowing that the development and implementation of chronic diseases could happen from preschool age due to low adherence to MD [37]. In European youth, the individual's genetic profile has been previously observed to modulate the effect of MD in terms of obesity and MetS [38]. Although different approaches have previously considered MD as the dietary factor associated with reduction of obesity or MetS in children and adolescents [39,40], gene-MD interaction effects have scarcely been examined in youth. Therefore, the aims of the present study are: (i) to provide an overview of the MD benefits and its impact on body composition in European children and adolescents; (ii) to describe the potential gene-MD interaction effects that could modulate the risk of obesity and MetS development in European youth. Regarding the gene–MD interplay, we intend to analyze both interaction pathways, the impact of the genetic factors in the ability of MD to reduce the obesity and MetS risk, and to observe the MD impact on the genetic predisposition to obesity and MetS in European youth (Figure 1).

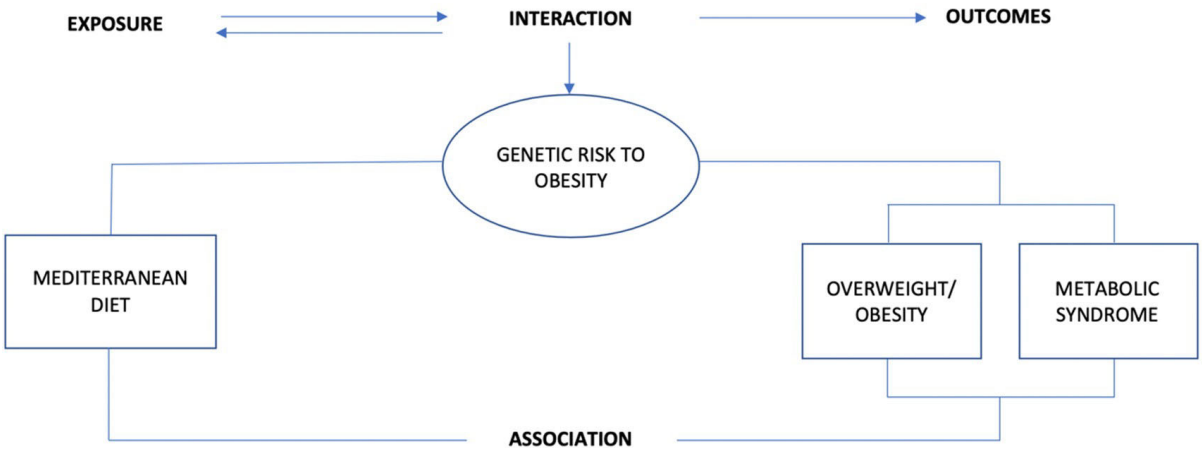

Figure 1. Conceptual framework of the present narrative review.

2. Obesity and Metabolic Syndrome: Definitions and Reference Values for Risk Estimation

To identify obesity in children and adolescents, the most commonly used methods are those based on anthropometric measurements. On one hand, single measurements such as triceps skinfold and waist circumference (WC) can predict total fat content in youth [41]. However, BMI is the most widely used anthropometric index. The World Obesity Federation (former International Obesity Task Force (IOTF)) BMI cut-off points are widely used to assess the prevalence of overweight and obesity in children [42]. Defining international cut-offs provides numerous advantages and it allows researchers to make comparisons with other BMI references [43]. However, the lack of uniformity in the methodological criteria to develop growth references for childhood BMI could sometimes lead to disparity in the results [44]. For instance, the World Health Organization's (WHO) BMI references values might not me suitable for all European children [44]. Other BMI thresholds have been proposed by the U.S. Centers for Disease Control and Prevention (CDC), although the differences in the predictive capacity between the CDC reference and the World Obesity Federation's reference are minimal [45]. A study of school aged children and adolescents showed a comparison of obesity estimations based on different reference values (CDC, IOTF and WHO criteria). The results showed the highest overweight and obesity underestimation in the WHO reference (8.97% and 5.67% respectively) [46]. More so, in a large cohort of Mediterranean children and adolescents, different BMI classification systems were assessed. The World Obesity Federation's threshold showed higher specificity in assessing overweight and obesity whereas the WHO values had the highest sensitivity among all considered references [47]. As mentioned, obesity could also be assessed considering WC as a measurement of abdominal fatness estimation [48], since BMI could underestimate central obesity prevalence in young populations [49].

MetS comprises a cluster of anthropometric and biological markers which could include triglycerides, HDL cholesterol, systolic and diastolic blood pressure, waist circumference and homeostatic model assessment (HOMA) levels or fasting glucose, including obesity as a precondition of MetS development in children and adolescents [50]. The selection of the corresponding components depends on the elaboration of each MetS definition. A number of MetS definitions have been proposed throughout the years. The most widely used are the ones suggested by the WHO, National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) and International Diabetes Federation (IDF), all to be thereafter adjusted versions to be applied in children and adolescents [9,51–53]. Different reference values have been displayed to develop MetS risk scores in children and adolescents [54,55]. This unified strategy increases the possibility to estimate and compare the prevalence and trends of cardiometabolic risk in youth when using continuous cardiometabolic risk scores.

3. Mediterranean Diet Assessment

Previous studies have shown a significant reduction of mortality associated with a greater adherence to the traditional MD, which considers both an adequate dietary pattern based on Mediterranean foods/products and a physically active lifestyle [56]. Trichopoulou, A. et al. observed inverse associations with greater MD adherence for death due to coronary heart disease and cancer in the adult population of Greece during a median of 44 month follow up [56]. The PREDIMED study in Spain, a multicenter randomized controlled trial of 4.8 years follow up, assessed the long term effects of the MD in terms of cardiovascular disease in adults, through different diet interventions supplemented with extra virgin olive oil or nuts. The study provided strong evidence that the MD could be an optimal dietary model for the management of cardiovascular events [57]. Nowadays, the traditional Mediterranean lifestyle is still fairly followed in the elderly, with some variability in this age group [58]. However, younger populations are moving away from the MD pattern/adherence [59]. Following a MD pattern involves the intake of extra virgin olive oil, high consumption of nuts and legumes, unrefined cereals, fruits and vegetables, moderate

consumption of dairy products, fish and low consumption of meat and meat products [56]. The current understanding of MD is not only explained by the foods constituting the dietary pattern, but also on the whole process encompassing food production to food consumption (harvesting process), food consumption characteristics (seasonally and locally consumed), cooking techniques (extended use of olive oil with different condiments), eating behaviors (eating socially), daily physical activity and the impact that all these aspects might have on the overall health status [30].

In order to measure the degree of the adherence to the MD, different MD scores have been constructed to assess how food affects health [60]. One of the first and most extendedly used MD scores of all time was suggested by Trichopoulou, A. et al. [61]. Further adapted versions for children [62] and adolescents [63] were progressively emerging, resulting in useful tools to evaluate MD adherence in younger population. The scores were formed based on a number of typically consumed MD food groups, which varied from one another/from their recommendations. The information collected to build the mentioned indexes was performed through food frequency questionnaires (FFQ) [64] or 24 h recalls [65]. In order to calculate individual usual dietary intake, different correction methods could be used to adjust for between and within individuals' variability [66,67]. These methods allow us to better estimate the individual's adherence to MD based on the information provided.

4. Genomics of Obesity and Metabolic Syndrome

The development of overweight or obesity is the result of coexisting complex health determinants and their interactions at different levels. The genetic factors influencing the development of obesity have been consistently studied for the last decades [68]. The constant progress in genetic studies have extended the number of genetic variants, single nucleotide polymorphisms (SNPs), associated with BMI to 751 [69]. The *FTO* gene, known to be the first genome-wide association study (GWAS)-identified obesity gene [70], has previously been shown to have a relationship with obesity and metabolic pathways in European children [71] and adults [72]. Nevertheless, those SNPs only explain 6% of BMI variance. Recently, the use of a polygenic risk score (PRS) incorporating all information from 2.1 million SNPs regardless of their genome-wide significance, increased the variance explained by BMI to 23% [73]. Thus, the emphasis is now to understand the underlying mechanisms by which obesity related SNPs could influence body composition parameters. The mentioned PRS proposed by Khera et al. was based on adult BMI, but it also shows reliable associations in children [73]. Generally, the majority of GWAS loci for obesity related outcomes were identified in adult population. However, most of these mentioned loci are also associated with obesity in children and adolescents, suggesting that the genetic profile for obesity remains constant during the life course [74]. Interestingly, a study showed that adults BMI-related genetic variants were more significantly associated with child BMI during their adiposity rebound (around 5 years old), than in their adiposity peak (below 9 months old), with genetic variants slightly influencing BMI in the latter [75]. These findings strength the idea of the adult-based PRS use in children and adolescents. However, the use of a specific PRS based on children's BMI could even increase the percentage of variance explained. Hence, the development of PRSs and genetic risk scores (GRS) (wide-genome significant) in early age populations would help to understand the expression mechanisms of obesity predisposition during childhood [76].

In the same way, MetS genetic susceptibility pathways have been evaluated. It was estimated that MetS heritability from European ancestry ranges between 10% and 30% [77,78]. In addition, numerous SNPs associated with individual MetS components have been reported in GWAS studies in different ethnic populations [79,80]. However, the effect of these SNPs on MetS with all included components remains understudied. Moreover, the majority of GWAS studies have been conducted in adult populations [81,82]. Nevertheless, understanding childhood obesity and MetS genetic pathways simultaneously could lead

to observing partial overlapping, as sometimes genetic predisposition to MetS could be driven by obesity genes [83].

As mentioned before, GRSs might be fundamental for identifying individuals to be at high risk of obesity and/or MetS development [84]. In order to combine a number of SNPs to form a GRS, different methodologies can be followed: by summing the number of risk alleles we obtain an unweighted GRS (uGRS); by multiplying the number of risk alleles to each estimated coefficient, a weighted GRS (wGRS) is derived [36]. Although PRSs could contribute in a greater way to explain genetic variance to be at risk of a certain disease, a combination of genome-wide associated SNPs in a GRS form could contribute, a priori, to adding as much precision in the predictive ability to assess the risk of obesity and MetS as non-genome wide significant approaches (PRS), for clinical utility [85]. In any case, both methodologies have been shown to be strong and useful genetic tools to predict obesity and MetS genetic susceptibility in children and adolescents [73,76,83].

5. Association between Mediterranean Diet and Body Composition and Metabolic Syndrome in Youth

When evaluating MD dietary pattern/adherence in terms of obesity and MetS, it has been observed that MD adherence varies between Mediterranean countries within young population while little evidence is available in non-Mediterranean countries [32]. Thus, some non-interventional MD-obesity associations are likely to be conditioned by a generally poor MD adherence among youth [59]. Focusing our scope on European children and adolescents, diverse associations between MD and obesity related outcomes have been found in the literature. Higher adherence to the MD pattern was significantly associated with lower weight status [86], BMI [87], WC [88] and body fat percentage (%BF) [64].

Although less studied, the relationship between MD and MetS was also explored in youth. A high MD adherence resulted in glucose and lipid profile improvement after a lifestyle intervention [27] as well as a lower risk of overall MetS components [39]. In contrast, low MD adherence could contribute to higher central obesity, hypertriglyceridemia and insulin resistance [89]. However, not all studies considering MD as a healthy dietary pattern reported positive outcomes in terms of obesity and MetS in youth [32]. The inclusion of other Mediterranean lifestyle variables, such as physical activity, could complement the ineffective strategies following a dietary intervention alone, as it has been shown to play a key role on shaping the obesity [90] and cardiometabolic phenotype [91], more so when obesity is already established [92].

6. The Gene-Mediterranean Diet Interaction Effect in Obesity and Metabolic Syndrome

As it was the main focus of this narrative review, the present chapter is based on a systematic literature search considering gene–MD interaction effects and its relationship with body weight and composition in European children and adolescents. The focus was on assessing all studies considering the gene–MD interaction effects and its relationship with changes in body composition and biological parameters: BMI, WC or any of the MetS components (HOMA or glucose levels, HDL/Total cholesterol, systolic or diastolic BP) in children and adolescents. PUBMED was the electronic database searched. Mesh® terms were used during the search strategy, based on medical subject headings and text words of peer papers identified. The search terms and words used were combined as follows: ("Obesity"[MeSH Terms] OR "Pediatric obesity"[MeSH Terms] OR "Metabolic syndrome"[MeSH Terms]) AND (diet, Mediterranean[MeSH Terms] OR "Diet score"[All Fields] OR "Diet indices"[All Fields]) AND ("Environmental exposure"[MeSH Terms] OR "Genetic predisposition to disease"[MeSH Terms]) AND ("child*"[Title/Abstract] OR "child"[MeSH Terms] OR "Preschool*"[Title/Abstract] OR "Child, Preschool*"[MeSH Terms] OR "adolescen*"[Title/Abstract] OR "adolescent"[MeSH Terms] OR "Youth"[Title/Abstract] OR "Teen*"[Title/Abstract] OR "Young people"[Title/Abstract]) AND (humans[Filter]). The following terms were also considered to screen genomic related studies: ("Genetic susceptibility"[MeSH Terms]) OR "Single nucleotide polymorphism"[MeSH Terms]) OR "genetic

screening"[MeSH Terms]) OR "gene"[MeSH Terms]) OR "nutrigenomics"[MeSH Terms]) OR "nutrigenetics"[MeSH Terms]) OR "genetic interaction[MeSH Terms]", although no results were found adding these terms to the intended search criteria. Additional filters were applied: written in the English language, population based-studies from Europe or individuals of European ancestry and age range from birth to 18 years old within targeted individuals. A total of three articles were obtained with the mentioned inclusion criteria, although after proofreading, only one article met the inclusion criteria proposed later in this this review [38]. The reference lists of all included manuscripts were double checked in order to identify potential missing articles that could have been ignored through the initial search.

This systematic search could set a milestone for a future in depth systematic review with the same established criteria when studies considering genomics and its impact on the benefits of MD among European youth will be more commonly found in the literature. The main findings of the identified study showed that the influence of high MD adherence on adiposity and MetS was only observed if a limited number of risk alleles were present. In addition, the gene–MD interaction effect showed sex-specific differences, being higher in females than in males (Figure 2). The analysis was carried out under the HELENA study, a cross-sectional multicentric study in European adolescents [93]. A cohort of 605 individuals aged 12.5–17.5 years old was used. To assess the MD adherence, a nine single component MD score was used [56,92], collected from 24 h recall questionnaires. Obesity-specific GRSs were developed in order to measure the genetic predisposition to adiposity and MetS [76]. The interaction effect resulted in being significantly associated to the main outcomes in both sex groups ($p < 0.05$).

No further studies assessing gene–MD interaction effects in terms of body composition or MetS in European children and adolescents were found. However, similar approaches of gene–MD interplay were observed in adult populations of European origin in relation to obesity and MetS. The studies shown in Tables 1 and 2 were obtained as a result of removing the age filter and opening the search to all age categories. After selection of candidate articles, a total of 16 studies in European adult populations were considered; they were published from 2009 to 2021. Table 1 provides an overview of the impact of the genetic factors to influence the association between MD and obesity and MetS risk while Table 2 shows some examples observing the MD impact on the genetic predisposition to obesity and MetS. The assessed studies in European adults have considered different genetic approaches in order to evaluate the predisposition to obesity and MetS. Individual SNPs have predominantly been used as interacting genetic factor, to assess potential differences between individuals carrying different risk alleles. The *FTO*rs9939609 was the most common SNP considered in the obesity and MetS development [33,94–97]. Other previously obesity related SNPs were also included in other studies, such as *TCF7L2*rs7903146 [34,94] and *MC4R*rs17782313 [33,97]. In addition, the combined effect of a number of SNPs comprising a GRS was also considered in the gene–MD interaction analyses. The GRSs observed in the studies analyzed were formed from 2 [11] to 77 obesity related SNPs [98]. The mentioned GRS formed by 77 SNPs, was built from loci associated to BMI in adults of European descent. A weighted method was applied to calculate the GRS on the basis of the selected SNP's relative effect size. The GRS ranged from 0–154, with each unit corresponding to one risk allele and higher GRS indicating higher predisposition to obesity [98]. This GRS was applied in a large combined prospective cohort (N = 14,046) of individuals of European ancestry during a 20 year follow up, where it was observed that the benefits of an improved diet quality were more pronounced in those individuals at higher genetic risk of obesity [98]. As this manuscript considered both genes and MD as modulating factors, it was suitable to be included in duplicate, so further description of this study's characteristics can be found in Tables 1 and 2. In this sense, the description of the main outcomes and the statistical models analyzing the gene–diet interplay were interpreted, in order to better understand the strategy used to assess the interaction effect in each studied article.

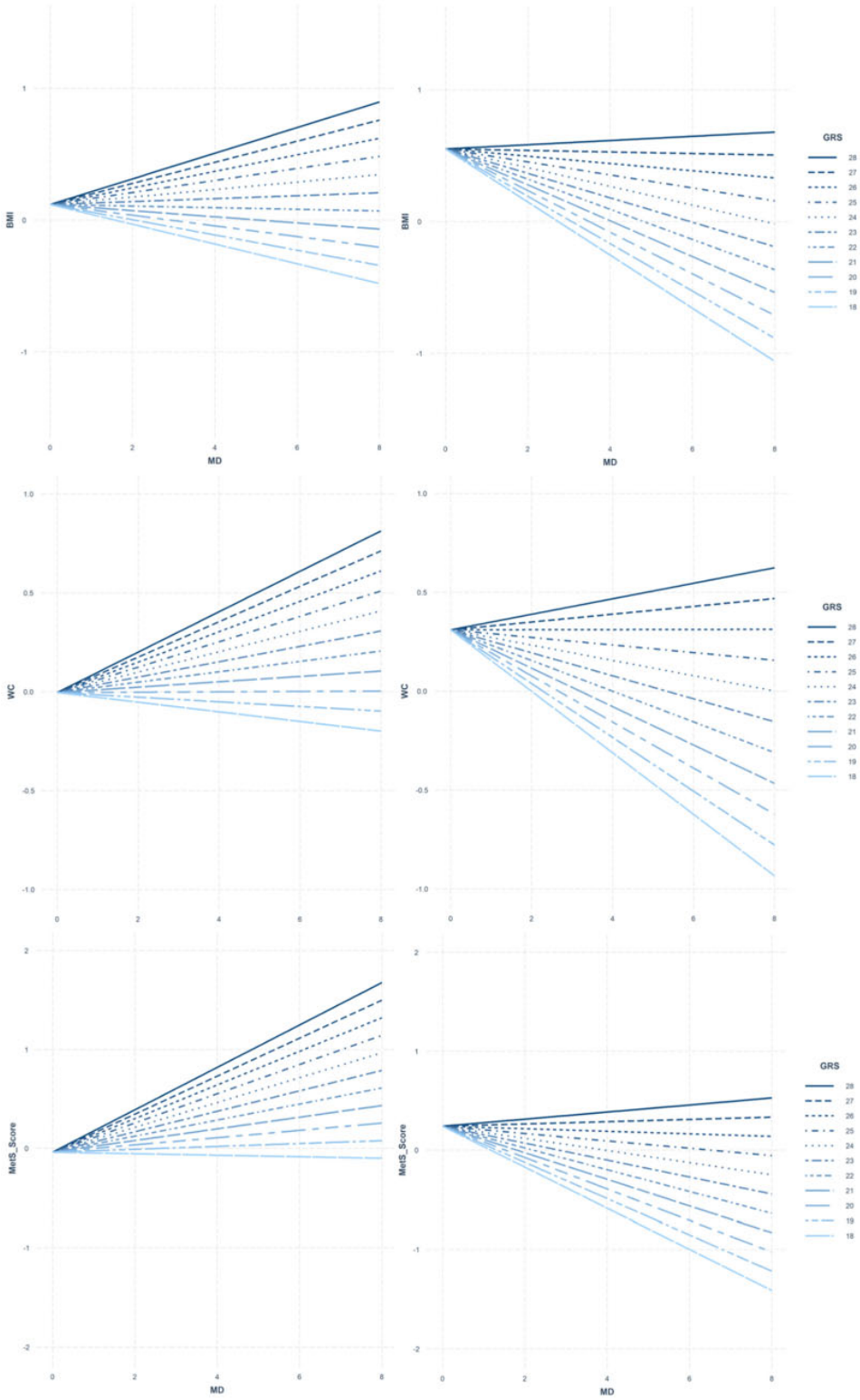

Figure 2. Interaction models between body mass index (BMI), waist circumference (WC), and metabolic syndrome score (MetS Score) and the Mediterranean diet (MD) according to the obesity genetic risk score (Obesity-GRS) modulation compared by sex (males left panel, females right panel). The Obesity-GRS values (18–28) are displayed according to our population distribution. Legend: The population distribution design follows different line patterns with reference points to observe the trend of the adolescent cohort according to the genetic risk for obesity. To analyze these results represented in the figure, a positive gradient shows the MD acting as arisk factor, while a negative gradient shows the protective role of the MD.

Table 1. Studies considering the individuals genetic profile to modulate the benefits of MD in relation to obesity and MetS in European adults. Articles ordered by outcome (starting with obesity, then MetS) and publication year (starting by most recent).

Author	Outcome	Year	Country	Age Group	Study Design	Sample Size	Diet Assessment †	Genetic Input	Results
Wang, T. et al. [98]	Obesity	2018	US (European ancestry)	Adults. 30–55 yo	Prospective cohort study. 20 year follow-up	14,046 (62.8% females)	FFQ. Traditional MDS (9-point score)	77 obesity related SNP GRS	The beneficial effect of improved diet quality on weight management was particularly pronounced in people at high genetic risk for obesity.
Roswall, N. et al. [94]	Obesity	2014	Multicentric: 5 European countries.	Adults. 47.6 ± 7.5 yo	Longitudinal. Median follow-up 6.8 years	11,048 (55.1% females)	FFQ. Traditional MDS (18-point score)	2 SNPs: *FTO*rs9939609 and *TCF7L2*rs7903146	High MDS was associated with lower changes in WC and BMI, regardless of *FTO* and *TCF7L2* risk alleles. In weight, the effect may depend on the T*CF7L2*rs7903146 variant (beneficial effect only in subjects with 1 or 2 risk alleles).
Lowry, E. et al. [11]	MetS	2018	Canada (European ancestry)	Adults 60.7 ± 0.73 yo	Longitudinal. 1 year intervention	159 (51.6% females)	24 h recall. Mediterranean based Canadian Healthy eating Index. (Range 0–100) [99]	2 SNPs: *APOA5*rs662799 and *ADIPOQ*rs1501299 GRS	Participants carrying none of the risk alleles in the 2 SNPs (GRS = 0) showed the greatest reduction in MetS score during the intervention.

Legend. List of abbreviations: Apolipoprotein A5 (APOA5); Adiponectin (ADIPOQ); Body Mass Index (BMI); Food Frequency Questionnaire (FFQ); Fat Mass and Obesity-Associated (FTO); Genetic Risk Score (GRS); Mediterranean Diet Score (MDS); Metabolic Syndrome (MetS); Randomized clinical trial (RCT); Single Nucleotide Polymorphism (SNP); Transcription Factor 7 Like 2 (TCF7L2); United States (US); Waist Circumference (WC) and years old (yo). †: Type of dietary questionnaire and type of MD score used were displayed. FFQ questionnaires were used in Wang, T. et al. and Roswall, N. et al. to measure the adherence to typically consumed items in the MD based on the traditional MDS recommendations [56]. Lowry, E. et al. used a 24 h recall to then consider the Canadian eating index, adapted to the Mediterranean lifestyle [100]. The higher the score obtained from the information provided in the questionnaire, the greater the adherence to the Mediterranean Diet. Applicable to all MD scores evaluated in the present table.

Table 2. Studies considering the MD as interaction factor to modulate the association between the genetic risk and obesity or MetS related outcomes in European adults. Articles ordered by outcome (starting by obesity, then MetS) and publication year (starting by the most recent).

Author	Outcome	Year	Country	Age Group	Study Design	Sample Size	Diet Assessment †	Genetic Input	Results
Baratali, L. et al. [101]	Obesity	2021	Switzerland	Adults. CS: 58.4 ± 10.6 yo PS: 58.0 ± 10.4 yo	Cross-sectional and prospective. 5.3 year follow-up	CS: 3033 PS: 2542 (CS: 53.2% females; PS: 54.7% females)	FFQ. 2 MDS: Traditional MDS I (8-point score) Swiss MDS II (9-point score)	2 obesity GRSs based on 31 and 68 SNPs.	No gene-diet interaction was found for changes in obesity markers, suggesting that diet exerts the same effect irrespective of the genetic background of the participants.
Wang, T. et al. [98]	Obesity	2018	US (European ancestry)	Adults. 30–55 yo	Prospective cohort study. 20 year follow-up	14,046 (62.8% females)	FFQ. Traditional MDS (9-point score)	77 obesity related SNP GRS	MD could not significantly attenuate the genetic association with increases in BMI and body weight.

Table 2. *Cont.*

Author	Outcome	Year	Country	Age Group	Study Design	Sample Size	Diet Assessment †	Genetic Input	Results
Livingstone, K.M. et al. [95]	Obesity	2016	Multicentric. 7 European countries	Adults. 40.4 ± 13.0 yo	RCT. 6 month follow-up	1607 (58.0% females)	FFQ. PREDIMED MDS (14-point score)	1 SNP: *FTO*rs9939609	No evidence of interactions between *FTO* genotype and dietary intakes on BMI and WC were found.
Corella, D. et al. [102]	Obesity	2014	Spain	Adults. 67.0 ± 6.2 yo	RCT. Median follow up 4.8 years	7161 (57.4% females)	FFQ. PREDIMED MDS (14-point score)	1 SNP: *FAIM2*rs7138803	No statistically significant gene-diet interactions between MD and *FAIM2*rs7138803 were found in determining BMI.
Corella, D. et al. [33]	Obesity	2012	Spain	Adults. 67.0 ± 6.2 yo	RCT. Median follow up 4.8 years	7052 (57.3% females)	FFQ. PREDIMED MDS (14-point score)	2 SNPs: *MC4R*rs17782313 and *FTO*rs9939609	Statistical and biological interactions with MD modulate the effects of *FTO* and *MC4R* polymorphisms on obesity.
Razquin, C. et al. [96]	Obesity	2010	Spain	Adults. 55–80 yo	RCT. 3 year follow-up	776 (54.9% females)	FFQ. Not specified	1 SNP: *FTO*rs9939609	After a nutritional intervention with MD, A-allele carriers had lower body weight gain than wild type subjects.
Razquin, C. et al. [103]	Obesity	2010	Spain	Adults. 55–80 yo	RCT 3 year follow-up. MD + virgin olive oil	737 (55% females)	FFQ. Not specified	1 SNP: −174G/C on the *IL*6 gene	After a nutritional intervention with MD + olive oil, CC subjects for the −174G/C had the greatest reduction in body weight.
Razquin, C. et al. [104]	Obesity	2009	Spain	Adults. 55–80 yo	RCT 3 year follow-up MD + virgin olive oil	774 (59.3% females)	FFQ. Not specified	1 SNP: Pro12Ala of the *PPARγ*gene	After a nutritional intervention with MD, reduced WC was observed among the population studied, reversing the negative effect of the 12Ala allele carriers
Coltell, O. et al. [105]	MetS	2021	Spain	Adults 67.0 ± 0.2 yo	Cross-sectional	954 (63.5% females)	FFQ. Spanish short screener (14-point score)	GWAS identified *OPCML*rs2917570	If MD is low, the minor allele of the rs2917570 is associated with higher adiponectin concentration. However, when adherence to MD is high, the minor allele is associated with lower adiponectin concentration. *
Coltell, O. et al. [106]	MetS	2020	Spain	Adults 65.1 ± 0.2 yo	Cross-sectional	426 (56.5% females)	FFQ. PREDIMED PLUS MDS (17-point score)	13 SNPs from GWAS in the *ME*1 gene in relation to serum omega- 3 PUFA	When MD adherence to is low, the minor allele is associated with an increase in serum omega-3 PUFA concentrations. If MD adherence is high, the minor allele is associated with a decrease in serum omega-3 PUFA concentrations. **

Table 2. *Cont.*

Author	Outcome	Year	Country	Age Group	Study Design	Sample Size	Diet Assessment †	Genetic Input	Results
San Cristobal, R. et al. [107]	MetS	2017	Multicentric. 7 European countries	Adults. 40.8 ± 13.0 yo	RCT. 6 month follow-up. On-line	1263 (57.1% females)	FFQ. PREDIMED MDS (14-point score)	14 SNPs GRS of MetS related traits	Higher GRS may reduce MD adherence benefits on total cholesterol concentration.
Corella, D. et al. [108]	MetS	2016	Spain	Adults. 66.9 ± 6.2 yo	RCT. Median follow up 4.8 years	7098 (58.2% females)	FFQ. PREDIMED MDS (14-point score)	1 SNP: *CLOCK*rs4580704	The interaction between the SNP and MD did not reach the statistical significance and the heterogeneity by MD is not confirmed.
Ortega-Azorín, C.S. et al. [109]	MetS	2014	Spain	Adults. 66.9 ± 6.2 yo	RCT. Median follow up 4.8 years	7166 (58.1% females)	FFQ. PREDIMED MDS (14-point score)	1 SNP: *MLXIPL*rs3812316	MD enhances the triglyceride lowering effect of the *MLXIPL*rs3812316 variant.
Corella, D. et al. [34]	MetS	2013	Spain	Adults. 67.0 ± 6.2 yo	RCT. Median follow up 4.8 years	7018 (57.4% females)	FFQ. PREDIMED MDS (14-point score)	1 SNP: TCF7L2rs7903146	MD may reduce increased fasting glucose and lipids in TT individuals.
Ortega-Azorín, C.S. et al. [97]	MetS	2012	Spain	Adults 66.9 ± 6.2 yo	RCT. Median follow up 4.8 years	7052 (57.3% females)	FFQ. PREDIMED MDS (14-point score)	2 SNPs: *FTO*rs9939609 and *MC4R*rs17782313	The *FTO*rs9939609 and the *MC4*R-rs17782313 association with T2DM depends on diet. High MD adherence counteracts the genetic predisposition.

Legend. List of abbreviations. Body Mass Index (BMI); Clock circadian regulator (CLOCK); Cross-sectional (CS); Fas Apoptotic Inhibitory Molecule 2 (FAIM2); Fat Mass and Obesity-Associated (FTO); Food Frequency Questionnaire (FFQ); Genetic Risk Score (GRS); Genome Wide Association Study (GWAS); Interleukin 6 (IL6); Malic Enzyme 1 (ME1); Mediterranean Diet (MD); Mediterranean Diet Score (MDS); Max-like protein X interacting protein-like (MLXIPL); Melanocortin-4 Receptor (MC4R); Opioid Binding Protein/Cell Adhesion Molecule Like (OPCML); Polyunsaturated fatty acid (PUFA); Prospective (PS); Randomized clinical trial (RCT); United States (US); Transcription factor 7-like 2 (TCF7L2); Type 2 Diabetes Mellitus (T2DM); Waist circumference (WC) and years old (yo). †: Type of dietary questionnaire and type of MD score used were displayed. FFQ questionnaires were used in all cases to measure the adherence to typically consumed items in the MD. In terms of MDS, Baratalli et al. considered 2 MDS: one following the traditional recommendations (MDS I) [56] and another one considering dairy products as beneficial items (MDS II) [110]. Wang, T. et al. used traditional MDS recommendations [56]. Livingstone, K.M. et al., Corella, D. et al. (2012, 2013, 2014 and 2016), San Cristobal, R. et al. and Ortega-Azorín, C.S. et al. (2012 and 2014) manuscripts used the MDS estimation based on the adapted version of the PREDIMED study [17,57]. Dietary assessment in Razquin, C. et al. (2010) considered PREDIMED recommendations, although no measuring MDS was reported. Coltell, O. et al. (2020) considered the MDS developed in the PREDIMED-PLUS trial [111]. Coltell, O. et al. (2021) considered a validated short screener in Spanish population [112]. The higher the score obtained from the information provided in the questionnaire, the greater the adherence to the Mediterranean Diet. Applicable to all MD scores evaluated in the present table. * Adiponectin: possible protective role against insulin resistance and arteriosclerosis. ** Omega-3 PUFA: potential beneficial effects in cardiovascular and metabolic risk factors.

7. Evaluation of the Genomic Role in the Mediterranean Diet Impact: Children, Understudied Population

Although some authors have shown the ability of the MD and the genetic profile to interact, modulating the risk associated with obesity and MetS parameters in adults of European origin, almost no studies assessing gene–diet interaction effects have been identified in European youth. Therefore, the present review has shown that gene–diet interaction effects in early life remain deeply understudied in young individuals of European origin.

From the only study extracted from the targeted inclusion criteria of this review's search, promising results were shown in terms of the influence of the individual's genetic risk to obesity, acting as a modulating factor in the benefits that a high MD adherence exerts on European adolescents of the HELENA study. The obesity-GRS developed to predict the risk of adiposity and MetS in this cohort of European adolescents showed that when adherence of MD was high, certain individuals, based on their genotype, had lower BMI, WC or MetS score. On the other hand, a small fraction of the selected sample was not genetically predisposed to have better body composition or metabolic profile despite a high MD adherence, or they could even see their health status worsen at that point [38]. Another important finding in the mentioned study was the sex-specific differences observed when the genotype was influencing the relation to MD and adiposity and MetS, as the interaction effect was higher in females than in males [38]. Similarly, a study performed in European adults explored gene–MD and gene–sex interactions in terms of polyunsaturated fatty acids (PUFAs) levels, showing that sex could be another relevant factor explaining differences in the effect of genetics on PUFAs [106].

The importance of assessing gene–diet interactions in adults relies on the fact that the associations of childhood BMI with adult diseases are explained by shared genetic factors [113], meaning that a partial genetic overlap exists in the biological processes under- lying children's BMI with those underlying adults' BMI and cardiometabolic traits [113]. However, it is also possible that this transition might be explained through phenotypic continuity of BMI from childhood into adulthood [113]. Since there is evidence of ge- netic correlations between child and adult BMI, some authors carried out a pleiotropy test (shared genetics) and functional enrichment of SNPs associated with childhood BMI and 15 adult cardiometabolic traits [114]. This study showed that pleiotropic genetic effects and enrichment of functional annotations in genetic variants were significantly associated with both childhood obesity and cardiometabolic diseases in adulthood.

In light of the above, a number of studies have been identified, observing how the genes and MD interplay act to modify body composition in relation to obesity and MetS in European adults. For instance, the previously mentioned PREDIMED study has contributed to disseminate a series of gene–MD interaction results in European adults to prevent cardiovascular events through an MD-based intervention [57]. The consumption of MD products was assessed through a 14-point scale extracted from FFQs when the greater the score, the greater the MD adherence estimation [112]. Additionally, different individual SNPs have been explored to assess whether the MD could modulate the genetic predisposition to certain components related to obesity or MetS. In this sense, not all the gene-MD interactions studied resulted in a significant effect where the primary outcome was improved by MD adherence. Instead, although some studies have shown positive interaction effects in terms of obesity [33] and MetS [34,98,110], no statistical significance was reached in other studies. Therefore, the MD adherence could not always be confirmed to be beneficial for obesity [102] and MetS [108] respectively. More so, GWAS studies in European adults have established novel associations between SNPs and metabolic variables where MD was acting as an interaction factor [106] whereas other known obesity related SNPs, such as *FTO*rs9939609, have also proved to reduce the weight gain of certain allele carriers of different cohorts after nutritional intervention [33,96]. However, no consistency was found in other multicentric studies in this regard [94,95].

A combination of SNPs in a GRS format was also considered in other analysis to predict the genetic risk of obesity and MetS in European adults. The studies evaluated

showed diversity in their results. On one hand, significant interactions, suggesting that a certain genetic profile might predict an adverse response to what one would normally expect from a healthy eating pattern. On the other hand, non-significant ones [11,107] suggest otherwise [101]. One of the studies analyzed in the present review (Wang, T. et al.), used an obesity-GRS to detect individuals with a genetic predisposition to obesity in two prospective cohorts [98]. MD could not significantly attenuate the association between genetic susceptibility and increases in BMI and body weight, although improving adherence to the Alternate Healthy Eating Index 2010 (AHEI-2010) [115] or Dietary Approach to Stop Hypertension (DASH) [116] patterns could attenuate the genetic association with weight gain. At the same time, they assessed whether the beneficial effect of improved diet quality on weight management was particularly pronounced in individuals at high genetic risk of obesity. In this case, the beneficial effect of improved diet quality on weight management was particularly pronounced in people at high genetic risk for obesity. The role of the interaction factor was doubly considered, depending on whether the main goal was to observe the influence of MD on the genetic predisposition to obesity, or whether the individuals' genotype could influence the use of health benefits from the MD to reduce the risk of obesity [117]. The role of MD attenuating the genetic susceptibility to obesity and MetS of certain individuals is the commonly found approach in gene–diet interaction studies in the literature [57]. However, the benefits of the adherence to a healthy diet, such as the MD, are not universally influenced in all possible genotypic profiles. Therefore, not all possible allele combinations would benefit from the same pattern. The mentioned study strengthens the idea of evaluating all possible interaction pathways at the same time, so the role of both, MD and genetic risk to a certain disease, acting as modulative factors, is fully understood.

Further studies considering the MD and genetic susceptibility as attenuating factor were also explored in studies which main outcome was partially related to our exposure. For instance, women carrying the T allele of the *TCF7L2*rs7903146 who also had high MD adherence early in pregnancy, had lower risk of developing gestational diabetes mellitus than CC carriers [118]. The same SNP-diet interaction was analyzed in terms of weight status [94] and fasting glucose and lipids [34] in European adults, where the effect of MD could depend on the *TCF7L2*rs7903146. Selected SNPs from GWAS were observed to interact with MD in relation to serum bilirubin concentrations (cardiovascular risk biomarker) [119]. At candidate gene level, *PPARγ2*rs1801282-MD interactions were observed to favor telomeres length (obesity and cardiovascular disease are linked to shortened telomeres) [120] and no MD effect was observed in terms of inflammation markers regardless of the inflammation related *COX-2* -765G>C and *IL-6* -174G>C polymorphisms [121].

In order to find relevant gene–diet interaction analysis in relation to obesity and MetS in European children and adolescents, we need to consider different dietary patterns or specific nutrient intakes other than MD. Such is the case of the α-linolenic acid dietary intake, which interacted with FADS1 genetic variability to affect serum non-HDL-cholesterol concentrations in European adolescents [122], or another study where the genetic liability to obesity, assessed by a obesity polygenic risk score (PRS), was attenuated by a higher fiber intake [123].

The present review showed some limitations. First, the targeted topic of the present review represented a small fraction in the literature, as the gene-MD interaction effect analysis in terms of body composition in European children and adolescents are currently under development. Second, due to the narrow scope of the systematic search, little evidence was found with the intended search criteria in young population. Therefore, the majority of the studies evaluated in the present review were conducted in European adults. Lastly, due to the consistently low adherence of the MD among different age populations, the results showed in the mentioned studies of this review should be interpreted carefully.

The optimal use of the benefits of MD for a more effective prevention and treatment programs of obesity and MetS in youth is through a personalized diet. In this sense, some intervention studies considering a personalized approach with MD have been put into

practice in recent years. For example, the MED4youth study, an ongoing multicenter RCT aimed to reduce obesity levels through MD vs. a traditional low-fat diet in European adolescents [124]. One of the main goals of the study is to apply an omics approach to observe whether the proposed interventions could modulate gut microbiota and derived metabolites in order to investigate their mechanisms and maximize the beneficial effect of a high MD adherence. In addition, the Food4me study recruited European adults to perform an internet-based nutritional intervention to evaluate the effect of personalized interventions on dietary changes associated with MD [125]. When participants were randomly assigned to receive different kinds of intervention advice, the personalized nutrition advice at diet, phenotype and genotype level showed the largest differences in terms of MD score [125]. These types of tailored interventions highlight the relevance of understanding the molecular basis of the MD effect. The integration of post-GWAS, cross-disciplinary collaborations combining omics technologies and computational techniques enable us to incorporate a significant number of biological markers using genomics, epigenomics, metagenomics, metabolomics and so forth, which better reflect the interaction processes taking place at molecular and cellular levels [126–129]. These contributions so far have helped researchers to understand the MD effects on the intermediate or final phenotypes of cardiovascular health [130]. However, dealing not only with a single MD component, but with a combination of foods, nutrients and phytochemicals, can provide separate or combined effects at different levels. This rather complex and multidimensional task becomes the next challenge for the forthcoming years of research [131].

8. Conclusions

The present review confirms that the numerous approaches carried out considering MD as healthy dietary pattern had, in general, an association with low obesity and MetS frequencies in children and adolescents. The number of gene–MD interaction analyses in relation to the risk of obesity or MetS performed in young populations are considerably limited. However, the few results analyzed in European youth suggest the possibility that obesity related genotypes modulate the relationship between MD adherence and obesity and MetS risk. Further research is needed to better understand the inter-individual differences in the association between MD and obesity and MetS, as well as the mechanisms behind the protective effects of MD in the overall cardiovascular health through the integration of omics and a personalized nutrition approach considering MD.

Author Contributions: L.A.M. is M.S.-C.'s supervisor. P.D.M.-E. and I.L. are M.S.-C.'s co-supervisors. M.S.-C., A.L.-G., L.A.M. and P.D.M.-E. contributed to the conceptualization, narrative design and writing original draft preparation. M.S.-C., P.D.M.-E. and A.L.-G. did editing of the first draft. L.A.M. and I.L. provided critical comments on the paper and draft. All authors have read and agreed to the published version of the manuscript.

Funding: Miguel Seral-Cortes (M.S.C.), the corresponding author, has received funding from the Iberus Talent Pre-doctoral fellowships 2018, under the European Union's H2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 801586.

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. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The content of this article reflects the authors' views alone, and the European Community is not liable for any use that may be made of the information contained herein.

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Artículo II [Article II]: Development of a Genetic Risk Score to predict the risk of overweight and obesity in European adolescents from the HELENA study.

Seral-Cortes M, Sabroso-Lasa S, De Miguel-Etayo P, Gonzalez-Gross M, Gesteiro E, Molina-Hidalgo C, et al.

Scientific reports. 2021;11(1).

scientific reports

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Development of a Genetic Risk Score to predict the risk of overweight and obesity in European adolescents from the HELENA study

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Obesity is the result of interactions between genes and environmental factors. Since monogenic etiology is only known in some obesity-related genes, a genetic risk score (GRS) could be useful to determine the genetic predisposition to obesity. Therefore, the aim of our study was to build a GRS able to predict genetic predisposition to overweight and obesity in European adolescents. A total of 1069 adolescents (51.3% female), aged 11–19 years participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study were genotyped. The sample was divided in non-overweight (non-OW) and overweight/obesity (OW/OB). From 611 single nucleotide polymorphisms (SNP) available, a first screening of 104 SNPs univariately associated with obesity ($p < 0.20$) was established selecting 21 significant SNPs ($p < 0.05$) in the multivariate model. Unweighted GRS (uGRS) was calculated by summing the number of risk alleles and weighted GRS (wGRS) by multiplying the risk alleles to each estimated coefficient. The area under curve (AUC) was calculated in uGRS (0.723) and wGRS (0.734) using tenfold internal cross-validation. Both uGRS and wGRS were significantly associated with body mass index (BMI) ($p < .001$). Both GRSs could potentially be considered as useful genetic tools to evaluate individual's predisposition to overweight/obesity in European adolescents.

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Childhood obesity is a major public health problem[1]. Pediatric obesity increases the risk of physical and psychological health problems already in childhood, and later in adulthood[2]. More so, adiposity related disorders predominantly diagnosed in adults such as type 2 diabetes mellitus (T2DM) and cardiovascular diseases might originate in early life, and potentially reduce life expectancy. Over the last two decades, large-scale studies have been unveiling new common variants in locus of certain genes related with childhood and adult obesity[3–5]. At least 97 loci have been associated with obesity[6]. Currently, the *FTO* gene still remains the locus explaining the largest association with obesity in adults, children and adolescents[7,8]. In this regard, previous studies have shown that each copy of the *FTO* rs9939609 polymorphism A allele is associated with 2.8% higher body fat in European adolescents[9,10]. Some studies have found some associations between single nucleotide polymorphisms (SNPs) with obesity risk factors, being potentially useful as early life risk indicators in children and adults[11]. However, individual SNPs can explain little of disease variance[12]. Several studies have demonstrated the potential value of other genetic approaches that combine a number of SNPs to develop a genetic risk score (GRS) by summing the number of risk alleles: unweighted GRS (uGRS) or by multiplying the number of risk alleles to each estimated coefficient: weighted GRS (wGRS)[13–15]. The creation and validation of obesity-specific GRS sets a landmark in personalised genetic risk prediction for obesity and obesity-related diseases[16]. Different obesity-related GRS have been constructed in adults[15–18] and children[19,20] with significant obesity-gene associations, being implemented on a variety of ethnic population backgrounds. Within European populations, Seyednasrollah et al.[21] computed two weighted GRS (wGRS) of 97 and 19 SNPs previously related to the risk of obesity in two cohorts including 2262 Finnish children and adolescents (3–18 years). Further, Viljakainen et al.[22] developed a wGRS to predict the risk of overweight and obesity in a cohort of 1142 Finnish preadolescents (11.3 ± 0.2 years) considering body mass index (BMI) and 30 BMI-related SNPs from previous genome-wide association studies (GWAS). As only few studies testing obesity risk in European adolescents with GRSs have been conducted, the aim of the present study was to develop a GRS for overweight and obesity in adolescents participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study.

Methods

Study design and population. The data were extracted from the HELENA multicentric and cross-sectional study containing a total sample of 4356 adolescents (51.6% females), aged 11–19 years old, from 10 European cities located in separated geographical points in Europe in 2006–2007. Their size of the cities was large enough to ensure participants diversity[23]. The main objective of the HELENA study was to obtain comparable data of a large sample of European adolescents on nutrition and health-related parameters by a standardised procedure[24]. More so, the study was performed following the ethical guidelines of the Declaration of Helsinki 1964 (revision of 2013), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries and was approved by the Ethics Committee of each city participating in the study[25]. The protocol was approved by the Ethical Committee (Comité de Ética de la Investigación de la Comunidad Autónoma de Aragón: CEICA). Written informed consent and assent to participate in the study were obtained from adolescents and their parents before being enrolled. One third of the subjects (N = 1172) from the total sample were randomly selected for blood sampling[24]. After including specific inclusion criteria from genomic parameters (SNPs) and anthropometry (BMI), a total of 1069 adolescents (51.3% females) were finally considered for the analysis in the present study. The flow chart of the selected sample is displayed in Supplementary Fig. 1.

Physical examination. All measurements were performed by trained researchers following standard protocols. Weight and height were measured following standard procedures[26]. BMI was calculated from height and weight (kg/m^2)[27] and categorised into non-overweight (non-OW) and overweight, including obesity (OW/OB), according to the age- and sex-specific BMI international cut-offs proposed by the World Obesity Federation[28]. FMI was calculated was calculated dividing fat mass (FM) by height squared (in meters).

Blood collection and genotyping. The blood samples were collected in overnight fasting state. A standardised methodology for blood collection, transport and analysis was performed by a certified laboratory[29]. Blood for DNA extraction was collected in EDTA K3 tubes and stored at the Institute of Nutritional and Food Sciences (IEL) of the University of Bonn, and sent to the Laboratoire d'Analyse Genomique Centre de Ressources Biologiques (LAG-CRB) e BB- 0033-00071 Institut Pasteur de Lille, F-59000 Lille, France. DNA was extracted from white blood cells with the Puregene kit (QIAGEN, Courtaboeuf, France) and stored at − 20 °C. The genotyping was done by an Illumina system (Illumina, Inc, San Diego, California) using the Golden- Gate technology (Sampling procedure scheme, GoldenGate; Software, Inc, San Francisco, California). In terms of gene selection within the HELENA study, a candidate gene approach was used. First, a certain number of behaviour and metabolic pathways related to the adolescence´s health were identified. These included included food intake, eating behaviour, food choices and preferences, energy and adipose tissue metabolism, glucose, insulin, lipid and lipoprotein metabolism among others. Finally, SNPs playing a key role in genes coding for the abovementioned pathways were selected. The HapMap database was used to select tag and independent SNPs. SNPs were selected with a minor allele frequency (MAF) above 0.1 and tag SNPs with r^2 above 0.8. If tag SNPs described for a single gene exceeded in number (more than ~ 20), only SNPs significantly associated with appropriate phenotypes in previous studies were selected, if available. Lastly, SNPs from the NCBI database were used when a limited number of SNPs were available in the HapMap database.

Statistical analysis. Descriptive characteristics by sex are shown as median and interquartile range (IQR) for continuous variables and as absolute and relative frequency for categorical ones. The statistical tests used to

compare differences by sex were Pearson´s chi-square for categorical variables and Mann–Whitney-Wilcoxon test for continuous variables. Pearson's chi-square statistic test was used to analyse the Hardy–Weinberg equilibrium. Shapiro–Wilk non-parametric test to check normality of variables was performed.

All statistical analyses were performed using RStudio Version 1.2.5001 (*RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL* http://www.rstudio.com/) and the significance level was set at $p < 0.05$.

Development of the genetic risk score. Candidate gene approach was the procedure based to select the genes in the HELENA study. First, relevant adolescent´s behaviours and metabolic pathways related to health were identified. Second, key proteins that, according to the literature, play a role in these pathways were also identified. Third, a selection of SNPs coding for these proteins was performed. Fourth, in order to select and tag SNPs independently, the HapMap database (2007 release) was used. SNPs with a minor allele frequency (MAF) above 0.1 and tag SNPs with r^2 above 0.8 were selected. If too many tag SNPs described for a single gene (more than ~ 20) were identified, only SNPs significantly associated with appropriate phenotypes in previous publications were selected, if available. Finally, SNPs from the NCBI database were included when a too limited number of SNPs were available in the HapMap database. Based on the above, a total of 611 SNPs related to obesity and obesity-related phenotypes available in the HELENA dataset[30] were used to build a GRS considering BMI as obesity-related variable in order to predict a major predisposition of overweight/obesity in European adolescents[24]. Each SNP was recoded as 0, 1, or 2 depending on the number of risk alleles defined in previous literature, respectively. A further selection of SNPs was performed using generalised linear model (GLM) to establish an initial cut off point ($p < 0.20$) to refine the search to 104 SNPs. Then, a step by step algorithm was applied to select the significant SNPs under the $p < 0.05$ threshold in a multivariate model to shortlist a final number of 21 SNPs significantly associated with BMI. The correspondence between actual and predicted probabilities of this model was analysed by a calibration curve. The unweighted GRS (uGRS) was calculated by summing the number of risk alleles from the 21 SNP variants with a rescaling, considering the SNPs that appear as protector factors. The wGRS was the result of multiplying the number of risk alleles at each locus (0, 1, 2) for each estimated coefficient of the multivariate model. Participants with missing data were dismissed in the GRS analysis (N = 3287). Receiver operating characteristics (ROC) curve analysis[31] was applied to test the diagnostic accuracy of the GRS to classify potential participants for obesity associated disturbances[32]. The area under curve (AUC) was calculated in uGRS and wGRS considering weight status as binary variable (i.e., non-OW vs. OW/OB). Selection of uGRS over wGRS to proceed with the design of the final model was performed by the higher value of the AUC compared using the Delong test. The model was internally validated performing tenfold cross validation analysis. For this analysis, the whole dataset was divided in 10 groups, using 9 of them to build the predictive model and the one to remains to validate this model. This procedure was repeated taking into account all possible ways to select the 9 subgroups, ensuring different forms to validate GRS with data not used in the building model process. Moreover, we evaluated the distribution of uGRS and wGRS values for NON-OW and OW/OB in a boxplot to graphically analyse the performance of the GRS. In order to provide the best cut-off for the use of the GRS as a dichotomic variable, the maximisation of the Youden index[33] was explored (see Table 3). Lastly, to test the GRS reliability with a general adiposity estimate other than BMI, simple linear regression models (LRM) were performed to evaluate the association between fat mass index (FMI) and both wGRS and uGRS.

Informed consent. Informed consent was signed by parents of all participants. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Results

Description of the study sample: demographics. The sample was composed of 520 boys and 549 girls. Main characteristics of study participants are shown in Supplementary Table 1. The median age of participants barely differed between males (14.6 years, IQR: 13.6—15. 7) and females (14.6 years, IQR: 13.5–15.7) ($p = 0.722$). The prevalence of OW/OB was higher in males (22.2%), than in females (18.8%) ($p = 0.009$).

Associations between SNPs and overweight/obesity: Building and validation of GRS. Initially, 104 SNPs potentially associated with BMI were selected (Supplementary Table 2 and were entered in the multivariate model to build the GRS. From them, we found a final number of 21 SNPs significantly associated with OW/OB in the HELENA study Table 1. Table 2 shows the univariate and multivariate model´s odds ratio (OR) of each of the selected SNPs for the GRS build up. A forest plot is displayed in Fig. 1 to present the OR´s direction (protective/risk) of each SNP. Within our GRS, *AMPD1* rs2010899, *PPARG* rs4135275, *NR3C1* rs4912905, *LPA*rs 9,355,296, *IL-6* rs1524107, *CNTFR* rs2183013, *IGF1* rs1019731, *THRA* rs1568400 and *FASN* rs4246444 had a protective role in the prediction of OW/OB whereas *NR3C1* rs7701443, *NR3C1* rs13182800, *CD36* rs3211867, *CNTF* rs2515362, *DRD2* rs1800497, *FTO* rs9939609, *CETP* rs4783961, *NOS2A* rs8068149, *THRA* rs7502966, *ANGPTL4* rs1044250, *LXRβ* rs17373080 and *PTPN1* rs2143511 increased the risk of OW/OB. Supplementary Fig. 2 shows the calibration curves analysing the correspondence between probabilities of overweight and the real outcome. It can be observed that there is a good agreement between predicted and actual probabilities,thus, the panel of SNPs shows a good adjustment in order to predict OW/OB.

Using the predictABEL R package[34], from the multivariate logistic regression model built to predict OW/OB, an uGRS and a wGRS were derived. The predictive ability of the GRS by means of the ROC curve, AUCs and Youden index of uGRS and wGRS models are displayed in Fig. 2. The results of the GRSs´ AUC (uGRS: 0.7198,wGRS: 0.7336) showed a moderate ability to discriminate OW/OB status. AUC´s comparisons displayed statistically significant differences between uGRS and wGRS to predict OW/OB ($p = 0.043$). The discrimination

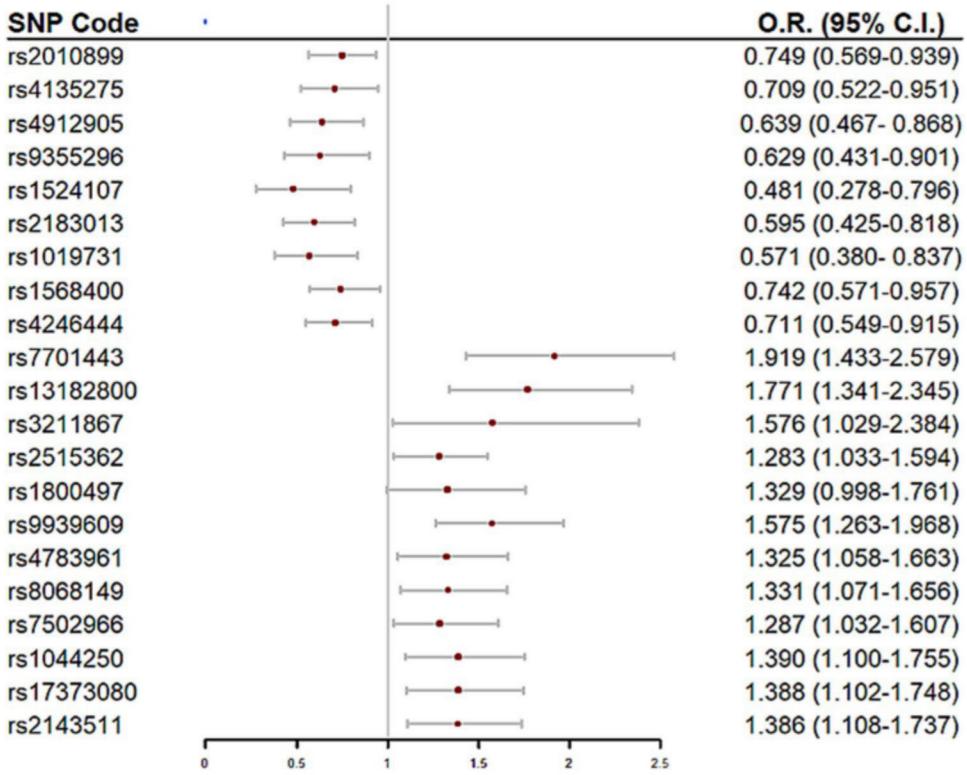

Figure 1. Forest plot of single nucleotide polymorphisms (SNPs) negatively (OR < 1) and positively (OR > 1) associated with risk of OW/OB. Legend: SNPs ordered by chromosome number. Protective SNPs against risk of overweight/obesity are shown in the upper part of the forest plot; SNPs with predisposition risk to overweight/obesity are shown in the bottom part. Multivariate model Odds Ratio (O.R.) and 95% confidence intervals (C.I.) displayed.

ability of wGRS and uGRS was internally validated by cross-validation using 10 folds. Both GRS provide robust predictions as showed by the AUC results (0.723 and 0.734 for uGRS and wGRS respectively). The distribution of uGRS and wGRS values for the groups of NON-OW and OW/OB by boxplots is displayed in Fig. 3. Both GRS discriminate between groups, but there is no a cut-off point that can clearly separate NON-OW and OW/OB groups. The Youden index was 23.5 for uGRS (specificity 69.4%, sensitivity 63.6%), and − 0.126 for wGRS (specificity 61.1%, sensitivity 74.6%). A more general analysis of sensitivity, specificity, positive and negative predictive value, and accuracy is shown in Table 3.

Associations between GRS and adiposity. LRM showed a significant association between GRS and FMI ($p \leq 2e{-}16$) in both weighted ($\beta = 0.877$) and unweighted ($\beta = 0.312$) variants for the total sample.

Discussion

In the present study, two GRSs, uGRS and wGRS, including 21 SNPs associated with BMI, were successfully developed to assess the risk of overweight and obesity in European adolescents. Hence, to the best of our knowledge, this are the first GRSs with these characteristics in a diversely distributed sample of European adolescents.

There are few previous studies focusing on BMI-specific GRSs with overweight and obesity in European pediatric populations and none exclusively in European adolescents, which reinforces the potential of our GRS analysis. In a cohort of 1142 Finnish preadolescents, Viljakainen et al.[22] constructed a wGRS to predict the risk of overweight (1.39-fold increased odds) and obesity (1.41-fold increased odds) using 30 BMI-related SNPs, stating that their GRS was poor in predicting short-term longitudinal changes in BMI. In two Finnish children and adolescent cohorts, Seyednasrollah et al.[21] developed two wGRS of 97 and 19 SNPs previously related to the risk of obesity, and obtained a lightly better prediction accuracy with the 19-SNP GRS than in our study (AUC = 0.769 *vs.* 0.734). However, none of the SNPs used in the two GRSs in European adolescents above mentioned concur with the SNPs utilised to develop our GRS.

The majority of the GRSs developed in pediatric and adolescent populations have been performed in non-European subjects, based on SNPs associated with obesity risk from previous GWAS. Comparatively to the European GRSs, the non-European studies provided a fewer number of selected SNPs related to obesity risk to develop their GRS. In a cross-sectional study of Brazilian children and adolescents (mean age 11.9 ± 2.8 years)[19], the BMI-specific wGRS composed of 3 SNPs was associated with a 2.65-fold increased risk of overweight and obesity. In a Chinese cohort of children aged 7.3 to 11.1 years, Fang J et al.[35] developed an uGRS of 11 BMI-related SNPs which explained 0.11 kg/m^2 BMI increase in those children carrying BMI susceptibility alleles. Lv D et al.[36]

rs code	Nearest gene	Alleles (major/minor)	MAF	*p*	Genotyping success rate	HWE
rs2010899	*AMPD1*	A/C	0.44	0.012	99.8	0.349
rs4135275	*PPARG*	A/G	0.19	0.024	99.9	0.655
rs4912905	*NR3C1*	C/G	0.23	0.004	100.0	0.665
rs7701443	*NR3C1*	A/G	0.40	1.35×10^{-4}	100.0	0.127
rs13182800	*NR3C1*	C/A	0.24	5.96×10^{-4}	99.9	0.183
rs9355296	*LPA*	G/A	0.13	0.013	100.0	0.216
rs1524107	*IL-6*	G/A	0.07	0.006	100.0	0.879
rs3211867	*CD36*	C/A	0.07	0.033	100.0	0.728
rs2183013	*CNTFR*	C/G	0.17	0.001	100.0	0.934
rs2515362	*CNTF*	A/G	0.44	0.024	99.9	0.184
rs1800497	*DRD2*	G/A	0.18	0.049	99.8	0.763
rs1019731	*IGF1*	C/A	0.11	0.005	100.0	0.744
rs9939609	*FTO*	T/A	0.40	5.81×10^{-4}	100.0	0.322
rs4783961	*CETP*	A/G	0.50	0.014	100.0	0.558
rs8068149	*NOS2A*	G/A	0.46	0.010	100.0	0.136
rs7502966	*THRA*	A/G	0.44	0.025	99.7	0.264
rs1568400	*THRA*	A/G	0.26	0.023	100.0	0.349
rs4246444	*FASN*	C/A	0.27	0.008	94.7	0.461
rs1044250	*ANGPTL4*	G/A	0.29	0.005	99.6	0.051
rs17373080	*LXRβ*	G/C	0.32	0.005	99.7	0.523
rs2143511	*PTPN1*	A/G	0.43	0.004	99.9	0.337

Table 1. Main characteristics of the 21 single nucleotide polymorphisms (SNPs) included in the genetic risk score. Association of SNPs in relation to body mass index (BMI) displayed in p values (*p*). *AMPD1* (Adenosine Monophosphate Deaminase 1); *ANGPTL4* (Angiopoietin like protein 4); BMI (Boy Mass Index); *CD36* (cluster of differentiation 36); *CETP* (Cholesteryl ester transfer protein); *CNTF* (Ciliary Neurotrophic Factor); *CNTFR* (Ciliary Neurotrophic Factor Receptor); *DRD2* (Dopamine Receptor D2); *FASN* (Fatty acid synthase); *FTO* (Fat mass and obesity-associated gene); HWE (Hardy–Weinberg Equilibrium); *IGF1* (Insulin Like Growth Factor 1); *IL-6* (Interleukin-6); *LPA* (Lipoprotein A); *LXRB* (Liver X receptor beta); MAF (Minor allele frequency); *NOS2A* (Nitric oxide synthase 2); *NR3C1* (Nuclear receptor subfamily 3, group C, member 1); *PPARG* (Peroxisome Proliferator Activated Receptor Gamma); *PTPN1* (Protein Tyrosine Phosphatase Non-Receptor Type 1); *THRA* (Thyroid Hormone Receptor Alpha).

positively associated the cumulative effect of 5 BMI-related SNPs GRS to obesity risk by morethan sevenfold increased odds in individuals carrying 5–7 risk alleles (age 11.6±2.5, N=2977). Finally, SNPs previously related to other cardiometabolic risk factors (hypertension) in a Chinese adolescent population (aged 12.2±3.0 years) were used to develop a 3 SNP GRS positively associated to obesity risk[37]. The increased risk of obesity associated to the mentioned GRSs in non-European subjects seem to have a consistency to some SNPs showed in the present study, despite acknowledging that the subjects origin does not allow to make comparisons in this regard.

The elaboration of a BMI-GRS comprised protector and risk in the same model. Some of those SNPs have been significantly associated with the risk of obesity in previous studies. The A allele of *FTO* rs9939609 polymorphism has been consistently associated with higher BMI and waist circumference in several studies in adults[11], adolescents[9] and children[38]. In cohorts of children and adults with European ancestry, Frayling et al.[11] and Willer et al.[39] found the strongest associations of the A risk allele of *FTO* rs9939609 with BMI. These findings confirm the role of *FTO* rs9939609 in our GRS as risk factor to OW/OB. In a study by Bokor et al.[40], *CD36* rs3211867 increased the risk of obesity by almost two folds in a cohort of Hungarian obese (N = 307) and normal weight (N=339) adolescents. Although the study had two independent samples with limited sample size, the findings are consistent with the results of our study. Moreover, in a pooled-study by Solaas et al.[41], authors observed significant association between *LXRβ* rs17373080 and the risk of T2DM and OW/OB by 1.59-fold increased odds. Equally, in our study, *LXRβ* rs17373080 SNP was associated with a 1.38 fold higher risk of OW/OB. Of note, the last two studies included participants from the HELENA cohort. On the other hand, the present study showed that *THRA rs*1568400 SNP was negatively associated with the risk of OW/OB whereas the same SNP was associated with higher BMI in a cohort of Spanish adults[42].

Other SNPs included in the GRS developed in the present study have also been associated with obesity-related cardiometabolic risk factors in ethnically diverse adults, but not with overweight or obesity risk. Thus, in a European population, *DRD2 rs*1800497[43] modified the relationship between birth weight and adulthood educational attainment in Finnish subjects and *FASN rs*4246444[44] attenuated the effect on low density lipoproteins (LDL) peak particle diameter when consuming a high amount of fat in a Canadian cohort. Within non-European background, in Chinese population, *NR3C1* rs7701443[45] was significantly associated with a higher risk of metabolic syndrome and CC alleles of *IL-6* rs1524107[46] had a higher risk of developing nephropathy in T2DM subjects. In addition, *PPARG* rs4135275[47] was positively associated with glycated hemoglobin and fasting plasma glucose in Taiwanese mental health patients. In pregnant Turkish women, *LPA* rs9355296[48] was positively related to vascular

		Univariate*		Multivariate*	
rs code	Chromosome	O.R. (95% C.I.)	*p*	O.R. (95% C.I.)	*p*
rs2010899	1	0.822 (0.665–1.014)	0.068	0.749 (0.596–0.939)	0.012
rs4135275	3	0.738 (0.556–0.967)	0.031	0.709 (0.522–0.951)	0.012
rs4912905	5	0.810 (0.630–1.034)	0.096	0.639 (0.467–0.867)	0.024
rs9355296	6	0.636 (0.448–0.886)	0.009	0.629 (0.430–0.901)	< 0.001
rs1524107	7	0.602 (0.363–0.950)	0.038	0.481 (0.278–0.795)	0.014
rs2183013	9	0.674 (0.495–0.903)	0.010	0.595 (0.425–0.818)	0.033
rs1019731	12	0.681 (0.468–0.967)	0.038	0.571 (0.379–0.837)	0.049
rs1568400	17	0.794 (0.623–1.006)	0.059	0.742 (0.571–0.957)	0.023
rs4246444	17	0.756 (0.592–0.957)	0.022	0.711 (0.548–0.914)	0.009
rs7701443	5	1.170 (0.946–1.447)	0.146	1.919 (1.432–2.579)	0.005
rs13182800	5	1.374 (1.094–1.721)	0.006	1.771 (1.340–2.344)	< 0.001
rs3211867	7	1.487 (1.009–2.159)	0.040	1.576 (1.029–2.384)	0.006
rs2515362	11	1.189 (0.973–1.452)	0.090	1.283 (1.032–1.594)	0.002
rs1800497	11	1.204 (0.924–1.559)	0.162	1.329 (0.998–1.761)	0.024
rs9939609	16	1.496 (1.219–1.838)	< 0.001	1.575 (1.263–1.968)	0.005
rs4783961	16	1.272 (1.034–1.568)	0.023	1.325 (1.058–1.663)	0.014
rs8068149	17	1.190 (0.974–1.456)	0.088	1.331 (1.071–1.656)	0.010
rs7502966	17	1.165 (0.952–1.427)	0.138	1.287 (1.031–1.607)	0.025
rs1044250	19	1.211 (0.976–1.500)	0.079	1.390 (1.100–1.754)	0.006
rs17373080	19	1.313 (1.061–1.624)	0.012	1.388 (1.102–1.748)	0.005
rs2143511	20	1.294 (1.056–1.588)	0.013	1.386 (1.108–1.736)	0.004

Table 2. Association of the 21 single nucleotide polymorphisms (SNPs) of the genetic risk score with OW/OB. SNPs ordered by chromosome number and protector/risk nature of the SNP. *Univariate (individual model of SNP-Overweight/Obesity association) and multivariate model (multiple model SNP-Overweight/Obesity association) displayed with Odds Ratio (O.R.) and 95% confidence interval (C.I.).

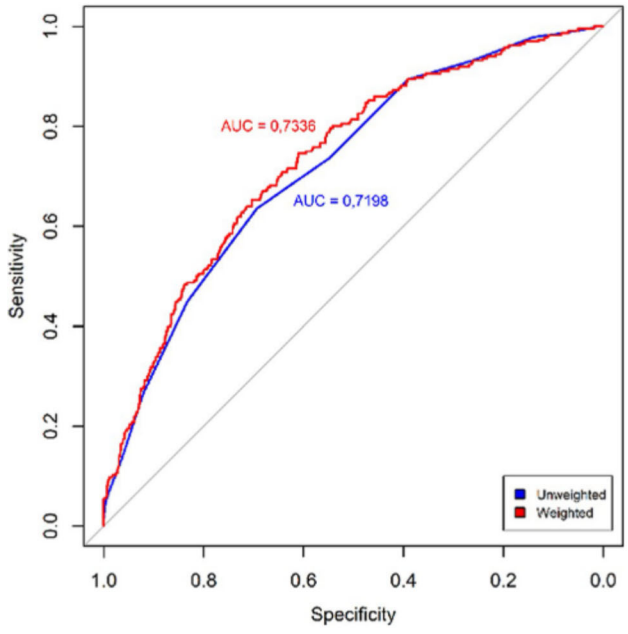

Figure 2. Receiver operating characteristics (ROC) curves of the two genetic risk scores, unweighted (uGRS) and weighted (wGRS). Areas under curves (AUC) are indicated. The straight line represents the ROC expected by chance only.

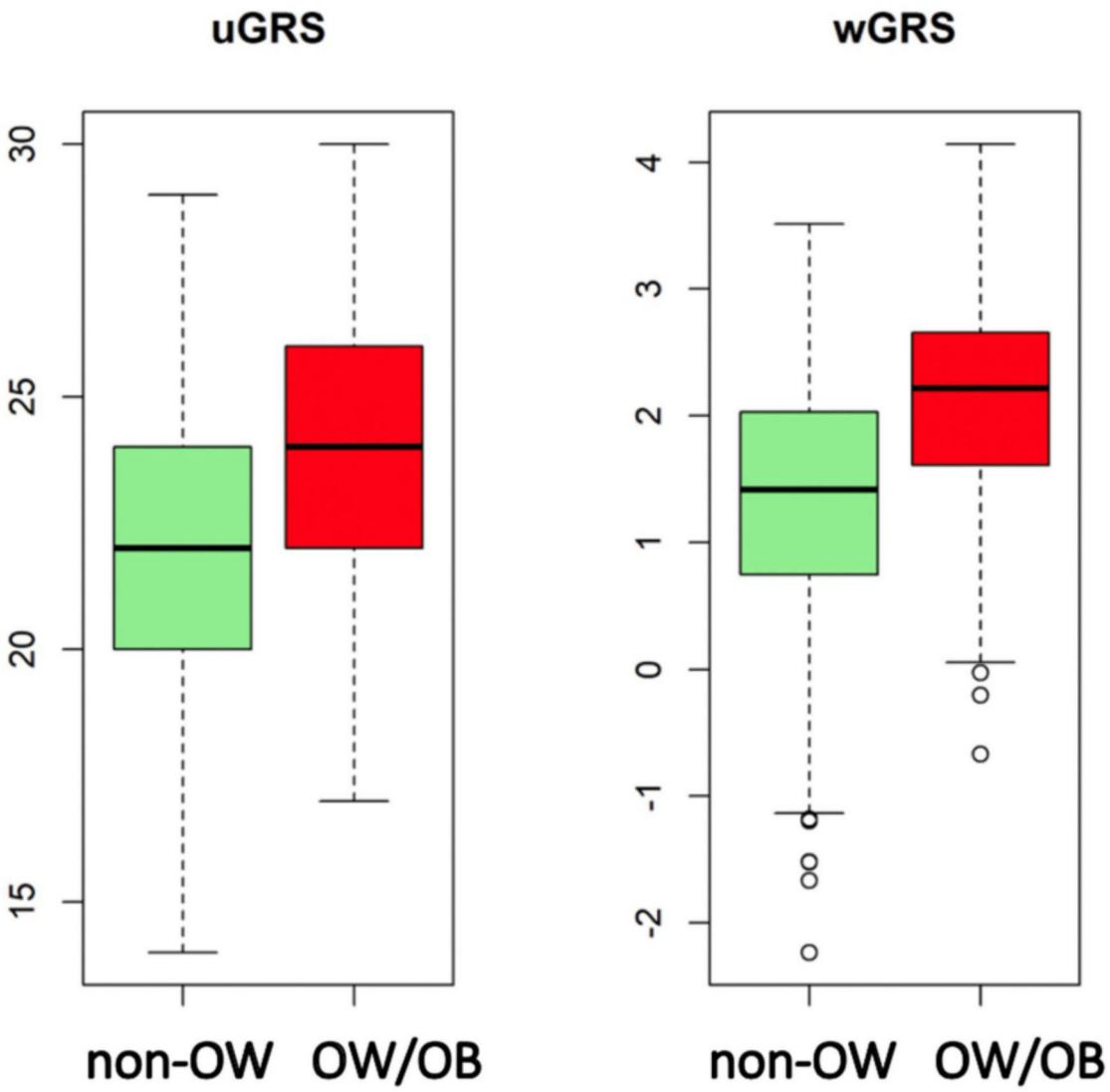

Figure 3. Boxplot of the distribution of unweighted genetic risk score (uGRS) and weighted genetic risk score (wGRS). Legend: Values for the groups of Non-overweight (NON-OW) and Overweight/Obesity (OW/OB).

inflammation as future cardiovascular event indicator. Finally, in a large adult cohort of 5 different ancestry groups, *CETP* rs4783961[49] was involved in sleep-associated adverse high density lipoproteins (HDL) profiles.

In contrast, as far as we know, several SNPs included our GRSs (i.e., *AMPD1* rs2010899, *NR3C1* rs4912905, *CNTFR* rs2183013, *IGF1* rs1019731 as protector factors and *NR3C1* rs13182800, *CNTF* rs2515362, *NOS2A* rs8068149, *THRA* rs7502966, *ANGPTL4* rs1044250 and *PTPN1* rs2143511 as risk factors) are new predictive factors, as they had not previously been associated with obesity or obesity related diseases nor had been significantly relevant in previous studies.

Additionally, the present GRS was positively tested to evaluate its ability to predict the risk of overweight and obesity in other adiposity estimates (FMI). Previous studies have also identified potential interactions between an obesity-GRS and diet on FMI in English children (9yrs)[50]. Monnereau et al.[51] constructed 15 SNPs-wGRS related to child BMI in children (6yrs) from Netherlands significantly associated to total fat mass. More so, another study[52] showed a BMI-based GRS significantly associated to higher body fat mass in Finnish children and adolescents.

Although using the external weight from meta-analyses is the gold standard to build a GRS, when the external weights are not available, the uGRS is commonly used[35,53]. In the present approach, internal weights from the genetic effects of the same study were used. The wGRS outperformed the uGRS in terms of statistical power (0.734 *vs*. 0.723). Conventionally, it is accepted that the AUC in a ROC analysis should be >0.8 to be of clinical value for screening[54]. When constructing the GRS models, AUC fell short of this threshold combining genetic factors alone. As SNPs themselves have little predictor capacity, we should consider the results obtained to construct the uGRS and wGRS as statistically acceptable, so our wGRS could be replicated in other cohorts with similar characteristics. Thus, our findings add a significant contribution to obesity-specific GRS that may improve the predictive values of obesity biomarkers in adolescents.

Cut-off	Spec	Sens	NPV	PPV	Acc	Cut-off	Spec	Sens	NPV	PPV	Acc
uGRS						wGRS					
30	100	0.42	77.99	100	78.01	4.14	100	0.42	77.99	100	78.01
29	99.88	3.81	78.56	90.00	78.67	3.51	99.88	5.50	78.86	92.85	79.04
28	99.15	6.35	78.89	68.18	78.67	3.30	99.15	8.89	79.34	75.00	79.23
27	96.39	13.55	79.74	51.61	78.11	2.98	96.63	15.25	80.09	56.25	78.67
26	92.07	26.69	81.59	48.83	77.64	2.61	92.07	27.54	81.76	49.61	77.82
25	83.31	44.91	84.22	43.26	74.83	2.26	83.31	48.72	85.15	45.27	75.67
24	69.38	63.55	87.04	37.03	68.10	1.88	69.38	65.25	87.57	37.65	68.47
23	54.74	73.72	88.03	31.57	58.93	1.50	54.74	79.23	90.29	33.15	60.14
22	39.13	89.40	92.87	29.38	50.23	1.10	39.13	89.40	92.87	29.38	50.23
21	26.17	93.22	93.16	26.34	40.97	0.77	26.17	93.22	93.16	26.34	40.97
20	14.04	97.88	95.90	24.39	32.55	0.35	14.04	97.03	94.35	24.23	32.36
19	7.92	98.72	95.65	23.30	27.97	0.03	7.92	98.72	95.65	23.30	27.97
18	2.88	99.57	96.00	22.50	24.22	-0.54	2.88	99.57	96.00	22.50	24.22
17	1.32	100	100	22.30	23.10	-0.82	1.32	100	100	22.30	23.10
16	0.48	100	100	22.15	22.45	-1.20	0.48	100	100	22.15	22.45
15	0.00	100	100	22.09	22.17	-1.67	0.12	100	100	22.09	22.17

Table 3. Specificity, sensitivity, negative and positive predictive value and accuracy analysis presented in percentages (%). Abbreviations: uGRS (Unweighted Genetic Risk Score); wGRS (Weighted Genetic Risk Score); Acc (accuracy); NPV (negative predictive value); PPV (positive predictive value); Sens (sensitivity); Spec (specificity).

Other authors[55] suggest that traditional predictors, such as family history and childhood obesity have stronger predictive power than models based on the established genetic variants. Nonetheless, the limited predictive ability of genetic variants does not undervalue the role of gene discovery for obesity as, based on the literature, genetic analyses have already provided with promising insights involving BMI regulation[6]. As such, the present GRSs, or future GRS comprising additional SNPs from other genes which do not have (a priori) previous reported associations with obesity, could yield promising results to minimise the risk of cardiovascular events related to obesity.

However, the present study has some limitations. The results should be validated in larger pediatric study populations, also using obesity incidence, in order to test the reliability of this obesity-specific GRS in other populations with similar ethnicity. Despite that some studies on children reporting the prediction of adulthood obesity efficiently 20 to 30 years later[21], different genetic factors might affect the short-term changes in BMI, especially during periods of rapid growth[56]. Due to the cross-sectional nature of the study, no cause-effect relationship can be determined. Additionally, although the model to develop the GRSs was internally validated performing tenfold cross validation analysis, we understand that the optimal situation would have been an external validation in an independent cohort. Moreover, only selected risk loci are available in the HELENA study. Since the established common variants from GWAS explain a small proportion of the BMI variation[6], it is likely that other loci from rarer variants, still to be discovered, will emerge when larger sample sizes are included in GWAS. Furthermore, there is no data available regarding the relatedness or the ethnic origins among the studied participants; the allele frequencies and their effect size might be different from non-European populations and the outcome should not be reproduced to other ethnicities. Since the HELENA study selected genes based on candidate genes instead of GWAS, the overlapping effect observed between SNPs of European and non-European adolescents could be possible. More common SNPs in non-European GRSs were found than in European GRSs. This finding could be due to the higher number of GRSs developed in other ethnicities in comparison to the number of GRS performed in European population. Also, we used the same data in the SNPs selection process and in the building model, thus little bias can be produced. Therefore, the results showed in the present study should be considered carefully. Further studies with larger sample size could provide key information of this potential genetic predisposition to obesity. On the other hand, the present study has also some strengths. The multicentric design of HELENA study involved the participation of adolescents from 10 European cities. This allowed the researchers to use a large database with relevant and diverse information from different populations across Europe. Additionally, only few GRSs to predict the overweight and obesity risk have been developed particularly in European adolescents, an understudied population from the early treatment and prevention perspective[21,22]. Similarly to Viljakainen et al.[22], the proposed genetic score of predisposition to obesity defined in this study might efficiently contribute to discern population at risk for overweight and obesity and not just obesity alone.

In conclusion, our findings suggest that the GRSs developed in the present study (uGRS and wGRS) could be considered as a useful genetic tool to evaluate individual's predisposition to OW/OB, allowing to advance in the prevention and management of the disease from early stages in life. Future GRS development with larger samples will be able to detect new variants previously not related to obesity that could influence the genetic risk of obesity, other than the common ones.

Received: 21 July 2020; Accepted: 22 January 2021
Published online: 04 February 2021

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Acknowledgements

This work was part of the HELENA Study (http://www.helenastudy.com/). We gratefully acknowledge financial support of the European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034).The data for this study were gathered under the auspices of the HELENA project, and further analysis was additionally supported by the Spanish Ministry of Economy and Competitiveness (Grants RYC-2010-05957 and RYC-2011-09011), the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn). Miguel Seral-Cortes, the corresponding author, has received funding from the Iberus Talent Pre-doctoral fellowships 2018, under the European Union's H2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 801586. Pilar De Miguel-Etayo was supported by ISCIII – CB15/00043 (Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición, CIBERObn). The study was performed following the ethical guidelines of the Declaration of Helsinki 1964 (revision of 2013), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries[25]. We are grateful for the support provided by school boards, headmasters, teachers, school staff and communities, and for the effort of all study nurses and our data managers.

Author contributions

L.M. was coordinator of the HELENA project, and together with F.G., M.G.G. and S.H. formed the Core Management Group of the project. M.G.G. was responsible for the blood sampling and analysis procedure. P.D.M.-E. and I.L. are co-supervisor of MS. M.S., I.L., L.M.E. and S.S. contributed to the GRS analysis and development. I.L. supervised the genomic data. L.M., I.L., P.D.M.-E., L.M.E. and S.S. did editing of the first draft. E.G., A.M., M.G.G., C.M.H., S.H., F.G., C.M., Y.M., M.P., K.W., A.K., E.E., D.S.T. and J.R. provided critical comments on the paper, draft, and analysis. All authors read the draft and agreed on the final version.

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/s41598-021-82712-4.

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Artículo III [Article III]: Interaction Effect of the Mediterranean Diet and an Obesity Genetic Risk Score on Adiposity and Metabolic Syndrome in Adolescents: The HELENA Study.

Seral-Cortes M, Sabroso-Lasa S, De Miguel-Etayo P, Gonzalez-Gross M, Gesteiro E, Molina-Hidalgo C, et al.

Nutrients. 2020;12(12).

nutrients

MDPI

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Interaction Effect of the Mediterranean Diet and an Obesity Genetic Risk Score on Adiposity and Metabolic Syndrome in Adolescents: The HELENA Study

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Received: 5 November 2020; Accepted: 14 December 2020; Published: 16 December 2020

Abstract: Obesity and metabolic syndrome (MetS) are worldwide major health challenges. The Mediterranean diet (MD) is associated with a better cardiometabolic profile, but these beneficial

effects may be influenced by genetic variations, modulating the predisposition to obesity or MetS. The aim was to assess whether interaction effects occur between an obesity genetic risk score (obesity-GRS) and the MD on adiposity and MetS in European adolescents. Multiple linear regression models were used to assess the interaction effects of an obesity-GRS and the MD on adiposity and MetS and its components. Interaction effects between the MD on adiposity and MetS were observed in both sex groups ($p < 0.05$). However, those interaction effects were only expressed in a certain number of adolescents, when a limited number of risk alleles were present. Regarding adiposity, a total of 46.8% males and 98.1% females had lower body mass index (BMI) as a result of higher MD adherence. Concerning MetS, only 4.8% of males with higher MD adherence had lower MetS scores. However, the same effect was observed in 95.2% of females. In conclusion, obesity- related genotypes could modulate the relationship between MD adherence and adiposity and MetS in European adolescents; the interaction effect was higher in females than in males.

Keywords: metabolic syndrome; Mediterranean diet; genetic risk score; HELENA; adolescents; sex

1. Introduction

Metabolic syndrome (MetS) is known to be a major world health challenge, with increasing prevalence together with obesity and cardiovascular diseases [1]. The prevalence of overweight and obesity worldwide has drastically increased among youth in recent years, with similar numbers in males and females [2]. The prevalence of obesity and metabolic syndrome in European children and adolescents continue in the same increasing line despite the efforts of prevention programs in recent years [3,4]. The definition of MetS features a number of cardiometabolic risk factors, including total and/or central adiposity, dyslipidemia, hypertension, and insulin resistance [5]. Clustering of cardiometabolic risk factors is increasingly considered in children's and adolescents' health rather than single risk factors [6,7]. In European children, an inverse association between the Mediterranean diet (MD) and childhood obesity has been observed [8] and showed that high MD adherence at early age is associated with a lower risk of developing overweight and obesity during childhood [9]. Moreover, in children and adolescents, MD was associated with lower body mass index (BMI) and improved glucose and lipid profiles [10]. The beneficial effects associated with a high MD adherence may be influenced by the interaction with other factors, such as genetic variations, which could modulate the predisposition/risk to obesity and MetS [11]. In adults, a systematic review [12] showed that the interaction between the melanocortin 4 receptor (*MC4R)* gene (a protein-coding gene previously associated to BMI [13]) and MD modulates the development of obesity and type 2 diabetes mellitus (T2DM) phenotypes. In Chinese children and adolescents, interactions between genetic variants and dietary behaviors in relation to obesity have been observed [14].

Previous studies have shown the potential of genetic approaches that identify individuals at high risk of developing a disease. That is the case of genetic risk scores (GRS), that combine a number of single nucleotide polymorphisms (SNPs) by summing the number of risk alleles [15]. In order to try to prevent the development of obesity and MetS in European adolescents it is crucial to improve our understanding of the predisposing genetic factors [16,17].

To our knowledge, no studies have examined the interaction effect between MD adherence and an obesity-related GRS on adiposity and MetS in European adolescents. Therefore, the aim of our study is to assess whether interaction effects occur between the MD adherence and obesity-GRS on adiposity and MetS in European adolescents. We hypothesize that higher predisposition to obesity risk may attenuate the protective effect of MD adherence on adiposity and MetS in European adolescents.

2. Materials and Methods

2.1. Study Design and Population

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) multicentric and cross- sectional study included a total sample of 4356 adolescents (51.6% females), aged 11–19 years [18]. Data were obtained from 10 European cities located in different geographical points within Europe, during 2006–2007. The HELENA study was designed to obtain reliable and comparable data on nutrition and health-related parameters, applying standardized procedures [19]. The HELENA study was approved by the Research Ethics Committees of each study site and followed the ethical guidelines of the Declaration of Helsinki 1964 (revision of 2000), good clinical practice, and the legislation about clinical research in humans in each one of the countries involved in the study [20]. Written informed consent was obtained by the parents/legal guardians of all participants. Blood sampling was performed in one third of the individuals randomly selected from the total sample (N = 1172) [19]. Inclusion of specific parameters to develop the present study (genomic, adiposity, cardiometabolic risk factors, and dietary data) provided a final number of 605 adolescents (51.6% female) meeting the selection criteria. Information of selection procedure is displayed in a flow chart (Figure 1).

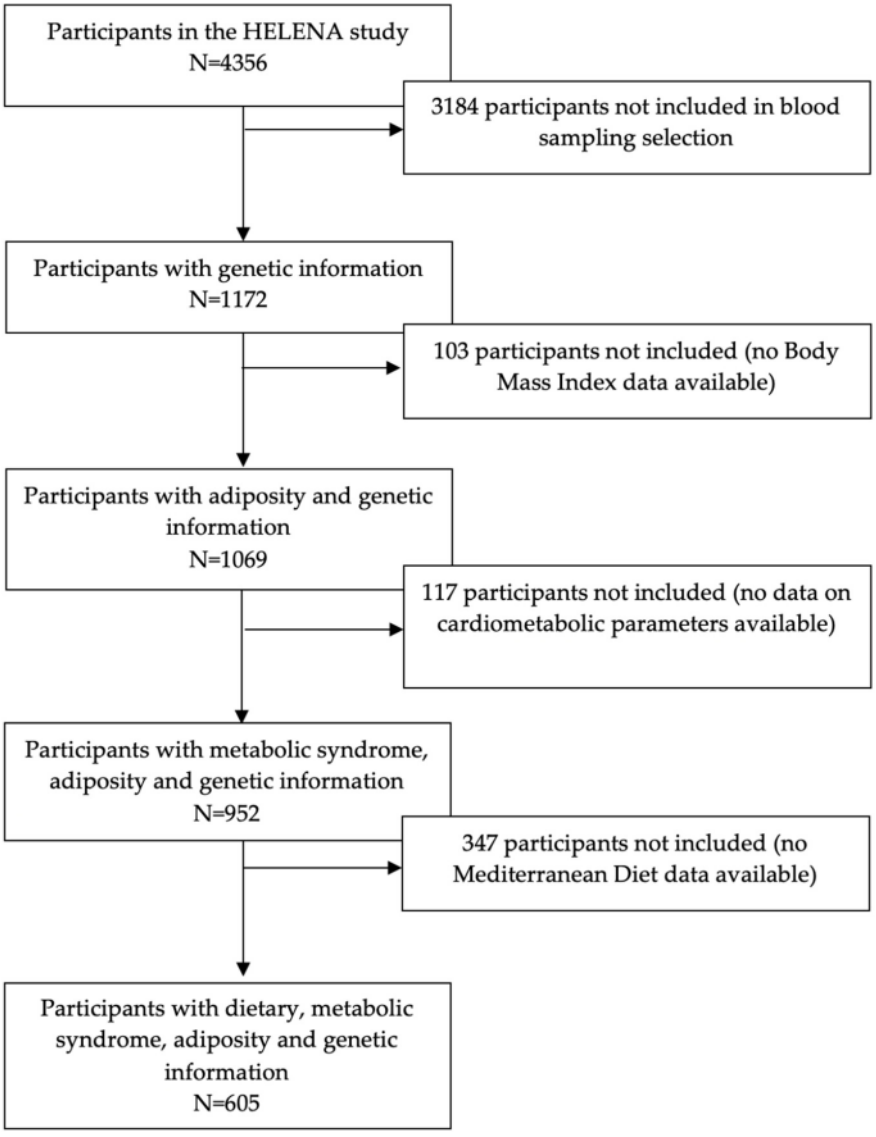

Figure 1. Flow chart of the sample selection process.

2.2. Physical Examination, Adiposity Measurements and Cardiometabolic Risk Score

Anthropometric measurements were performed by trained researchers following standard protocols [21]. Height was measured barefoot in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm, and weight was measured in underwear and without shoes with an electronic scale (Type SECA 861) to the nearest 0.1 kg. BMI was calculated from weight and height (kg/m^2) [22]. Waist circumference (WC) was measured in triplicate with a nonelastic tape (SECA 200) to the nearest 0.1 cm as the mid-point between the lowest rib and the iliac crest [21], and the average of the three measures was used. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in sitting position separated in a 10- minute interval with a blood pressure oscillometric monitor device OMRON HEALTHCARE® (M6- HEM7001 ; OMRON HEALTHCARE®, Kyoto, Japan) The lowest blood pressure (BP) reading was used. Thereafter, the mean of arterial pressures (MAP) of all participants was obtained from the DBP+[(SBP-DBP)/3)] formula. Total cholesterol (TC), HDL-cholesterol (HDL-c), triglycerides (TG), and glucose were measured using enzymatic methods (Dade Behring, Schwalbach, Germany). Insulin levels were obtained from frozen serum using an Immulite 2000 analyzer (DPC Bierman GmbH, Bad Nauheim, Germany). As measurement of insulin resistance, the homeostatic model assessment (HOMA) index was calculated from glucose and insulin measurements [23]. Moreover, a clustered cardiometabolic risk score was computed from the sum of the standardized z-scores of TC/HDL ratio, WC, HOMA index, and MAP [7]. The standardized z-scores of intended variables were calculated from the age and sex specific cut-off points [24]. Lower values in the score indicate better cardiometabolic risk profile. As sensitivity analyses, a second MetS risk score, comprising HDL-c, WC, Glucose, SBP and TG, was obtained from the International Diabetes Federation (IDF) guidelines [5]. HDL-c was multiplied by -1 as characterized by lower metabolic risk with increasing values. Results obtained with both cardiometabolic risk scores in adolescents have been included in the present analyses.

2.3. Dietary Intake Assessment and Mediterranean Diet Score

Dietary habits were determined from a self-administered, computerized, validated 24-h dietary recall called the HELENA Dietary Assessment Tool (HELENA-DIAT) [25,26], a tool validated first in Flemish adolescents [26] and then adapted to be used in the 10 cities [27]. Participants completed the HELENA-DIAT on two non-consecutive days within a time span of two weeks. This method has been used and recommended to assess dietary intake in European children and adolescents [28]. In order to calculate individual usual dietary intake, the multiple source method (MSM) was used [28]. This method allows correction of dietary data for between and within individuals' variability.

We used a Mediterranean diet score (MDS) based on nine single components: vegetables, fruits and nuts, cereals and roots, pulses, fish, monounsaturated/saturated fatty acids ratio, dairy products, meat, and alcohol. A scale indicating the degree of adhesion/adherence to the traditional MD was developed [29]. The description of MD food subgroup components is described elsewhere [30]. Vegetables, fruits and nuts, cereals, legumes, fish, dairy products, and unsaturated to saturated fat ratio positively contributed to the MD adherence, whereas meat (including processed meat) and alcohol consumption were inversely considered. Of note is that dairy products are positively considered as they are recommended during growth and development periods, such as adolescence [31]. Alcohol intake was regarded as an unhealthy habit among adolescents. Therefore, in a no- alcohol consumption situation, the value 1 was assigned, while any alcohol intake was computed as value 0. The MD adherence was constructed by a 0 to 9 points scale, with higher scores indicating greater adherence [32]. The sex-specific median intake (g/day) of all subgroups forming the MDS is shown in Supplementary Table S1.

2.4. Genomic Information and Genetic Risk Score

Standard methods for blood collection, transport, and analysis was performed by a certified laboratory [33]. Blood sampling (EDTA K3 tubes) for DNA extraction, collection, and storage was

performed at the Institute of Nutritional and Food Sciences (IEL) of the University of Bonn, and sent to the Laboratoire d'Analyse Genomique Centre de Ressources Biologiques (LAG-CRB) BB- 0033- 00071 Institut Pasteur de Lille, F-59000 Lille, France. DNA was obtained from white blood cells with the Puregene kit (QIAGEN, Courtaboeuf, France) and stored at −20 °C. The genotyping was done by an Illumina system (Illumina, Inc., San Diego, CA, USA) using the Golden Gate technology (sampling procedure scheme, GoldenGate; Software, Inc, San Francisco, CA, USA).

To analyze the influence of genetic information on the association between MD and adiposity and cardiometabolic biomarkers, we used the obesity-GRS developed from HELENA adolescents (submitted but not yet accepted) [34] using 21 SNPs significantly associated with overweight/obesity, defined as the equivalent to BMI >25kg/m². The main characteristics of SNPs forming the obesity- GRS are displayed in Supplementary Table S2. Also, a comparative analysis by sex is shown in Supplementary Table S3.

2.5. Statistical Analysis

To test the variables´ normality, the Shapiro–Wilk test was performed. As not all variables follow a normal distribution, the descriptive sex-specific characteristics are displayed as median and interquartile range (IQR) for continuous variables; absolute and relative frequencies are shown for categorical variables. In order to compare differences by sex, the statistical Pearson´s chi-square test was used for categorical variables and the Mann–Whitney–Wilcoxon test for continuous variables.

Sex-specific multiple linear regression models were used to assess the association between adiposity and cardiometabolic parameters and the interaction effect between the obesity-GRS and MD, adding to the effect of MD alone in the same model.

RStudio Version 1.2.5001 (RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/) was used to perform all statistical analyses and $p<0.05$ was the significance level set in the present analysis.

3. Results

3.1. Descriptive Characteristics of the Study Sample

The main characteristics of participants are shown in Table 1. In summary, there were significant differences between males and females for weight and height ($p \leq 0.001$) although no significant differences were found for BMI. Regarding cardiometabolic risk factors, girls had higher WC ($p \leq 0.001$) and HOMA ($p = 0.044$), whereas boys showed higher SBP ($p \leq 0.001$), MAP ($p \leq 0.001$), and MetS ($p \leq 0.001$) than girls. There were no significant differences for the remaining cardiometabolic parameters. The distribution of obesity-GRS among participants is displayed by sex in Figure 2. Focusing in the SNPs included in the obesity-GRS, no statistically significant differences between sex were found in Supplementary Table S3.

Table 1. Demographics and cardiometabolic characteristics of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) participants displayed by sex.

	Total	**Male**	**Female**	***p*-Value**
	***n* = 605**	***n* =293**	***n* =312**	
Age (years)	14.7 (13.8–15.6)	14.8 (13.8–15.6)	14.8 (13.7–15.7)	0.948
Height (cm)	166.0 (159.5–172.2)	170.0 (163.9–177.0)	162.2 (157.0-167.2)	**<0.001**
Weight (kg)	58.4 (49.9–64.5)	61.1 (51.1–68.8)	55.8 (49.2–61.6)	**<0.001**
BMI (kg/m^2)	21.1 (18.6–22.9)	21.0 (18.5–22.7)	21.2 (18.7–23.0)	0.194
WC (cm)	72.1 (66.7–75.8)	65.75 (46.0–79.0)	71.5 48.7–83)	**<0.001**
HOMA index	2.2 (1.3–2.6)	2.2 (1.3–2.5)	2.3 (1.4–2.7)	**0.044**
SBP (mmHg)	116 (108–124)	120 (112–129)	112 (105–120)	**<0.001**
DBP (mmHg)	64.4 (59.0–70.0)	64.0 (59.0-69.0)	64.8 (60.0–70.0)	0.331
MAP	0.6 (−0.01–1.1)	1.1 (0.5–1.6)	0.2 (−0.4–0.7)	**<0.001**
HDL-c (mmol/L)	55.7 (49–63)	53.3 (47-59)	57.9 (50–65)	**<0.001**
TG (mmol/L)	68.7 (47.0–80.0)	65.7 (46.0–79.0)	71.5 (48.7–83.0)	0.056
TC:HDL ratio	2.3 (2.5–1.1)	2.3 (2.5–3.2)	2.9 (2.5–3.3)	0.839
PA (mins/day)	54.8 (40.7–71.5)	65.4 (51.8–82.4)	47.3 (35.2–59.8)	**<0.001**
MetS Score *	0.02 (-1.2–1.0)	0.3 (−0.7–1.3)	−0.3 (−1.5–0.8)	**<0.001**
MDS **	4 (0–8)	4 (0–8)	4 (0–8)	0.495
Obesity-GRS ***	23 (21–24)	23 (21–25)	22 (21–24)	0.087

Abbreviations: BMI (body mass index); WC (waist circumference); HOMA index (homeostatic model assessment index); SPB (systolic blood pressure); DBP (diastolic blood pressure); MAP (mean arterial pressure); HDL-c (high density lipoprotein cholesterol); TG (triglycerides); TC:HDL ratio (total cholesterol/HDL cholesterol ratio); PA (physical activity); (MetS Score (Metabolic Syndrome score); MDS (Mediterranean diet score); and obesity-GRS (obesity-related genetic risk score). Median values (p. 25–p. 75) expressed. * Metabolic Syndrome score resulting from the mean of WC, HOMA, MAP, and TC-HDL variables combined. ** Mediterranean diet score resulting from the sum of nine food subgroups compliance. *** Genetic risk score resulting from the sum of risk alleles of HELENA participants. Boldface values indicate sig *p*-value Sig *p*-value < 0.05.

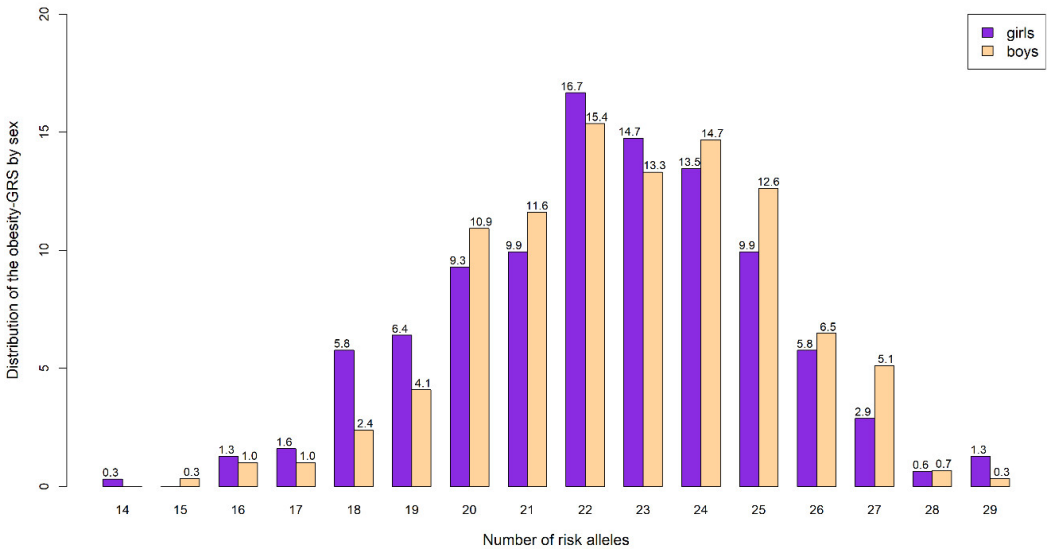

Figure 2. Distribution of the obesity-GRS (obesity-related genetic risk score) by sex (% displayed) in the HELENA cohort according to the number of risk alleles.

3.2. Interaction between MD and Obesity-GRS on Adiposity/Cardiometabolic Variables

Table 2 shows the association between the cardiometabolic parameters in relation to MD and the interaction between MD and the obesity-GRS in the additive model, by sex groups.

Considered within the additive model (Table 2), MD adherence had a protective role over BMI (male $p < 0.01$ vs. female $p \leq 0.001$), WC (male $p < 0.05$ vs. female $p \leq 0.001$), and MetS (male $p < 0.05$ vs. female $p \leq 0.01$) in both sex groups. However, the association of the MD with HOMA ($p < 0.05$) and SBP ($p < 0.05$) was only observed in females, and the association of the MD with MAP ($p \leq 0.05$) and DBP ($p < 0.05$) only in males.

Furthermore, both the MD and the interaction between MD and obesity-GRS were significant in the case of BMI for both sex groups; the inverse association between the MD and BMI was higher in females than in males (females $p \leq 0.001$ vs. males $p < 0.01$). When studying the cardiometabolic variables, we observed significant interactions between obesity-GRS and MD in both sex groups for WC ($p < 0.05$) and MetS ($p < 0.05$). For both adiposity and cardiometabolic variables, females showed stronger interactions than males. Moreover, the obesity-GRS and MD showed a significant interaction on MAP ($p < 0.05$) and DBP ($p < 0.01$) for males only, whereas the interaction on HOMA was only significant for females ($p < 0.05$).

Table 2. Multiple linear regression models of obesity-related genetic risk score (obesity-GRS) and Mediterranean diet (MD) interaction, and the MD effect alone on adiposity and cardiometabolic parameters by sex.

p-Values	Male		Female	
	Obesity-GRSxMD	**MD**	**Obesity-GRSxMD**	**MD**
BMI (kg/m^2)	**0.003**	**0.008**	**<0.001**	**<0.001**
WC (cm)	**0.009**	**0.030**	**<0.001**	**<0.001**
HOMA	0.495	0.836	**0.027**	**0.013**
SBP (mmHg)	0.994	0.739	0.310	**0.047**
DBP (mmHg)	**0.005**	**0.014**	0.795	0.626
MAP	**0.031**	**0.045**	0.872	0.325
TG (mmol/L)	0.421	0.413	0.587	0.689
TC:HDL	0.465	0.530	0.184	0.118
MetS Score	**0.014**	**0.047**	**0.006**	**0.002**

Figure 3 shows the interaction effects of the obesity-GRS and the MD on BMI, WC, and MetS for male and female participants. The relations between the MD and BMI, WC, and MetS were modulated by the obesity-GRS values. In order to interpret the abovementioned variables, different sub-figures have been displayed in a matrix panel according to each sex group for BMI, WC, and MetS. Different lines were drawn to relate MD and adiposity and cardiovascular biomarkers modulated by the distribution of the genetic predisposition to obesity in our population. It must be remarked that the majority of the adolescents were concentrated in the central parts of the graph, within 20-26 risk alleles (82.3% of the total population); therefore, extreme values should be interpreted cautiously. Thus, for those participants represented with a negative slope, a higher MD adherence could act as a protective factor in relation to cardiometabolic factors despite their genetic predisposition to obesity (high or low).

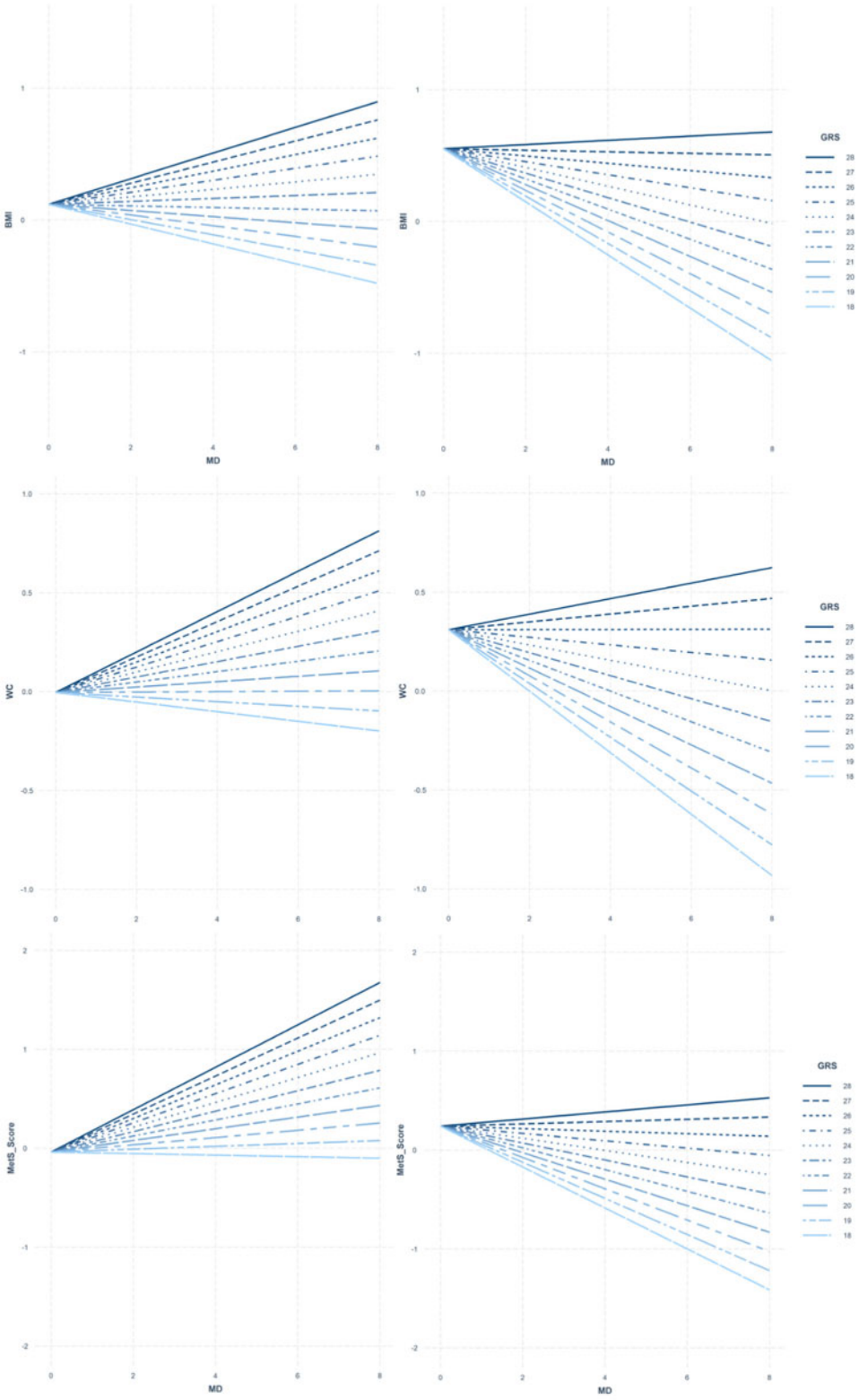

Figure 3. Matrix panel of interaction models between body mass index (BMI), waist circumference (WC), and metabolic syndrome score (MetS Score); and the Mediterranean diet (MD) according to the obesity genetic risk score (Obesity-GRS) modulation compared by sex (males left panel, females right panel). Obesity-GRS values (18–28) displayed according to our population distribution. Legend: when designing the population distribution representation, different lines were drawn as reference points to observe the trend of the studied population according to the genetic predisposition to obesity. When analyzing the results represented in these figures, a positive gradient shows the MD acting as risk factor, whereas a negative gradient indicates the protective role of the MD.

Regarding the adiposity parameters, a total of 46.8% of males (those with 22 risk alleles or below) with higher MD adherence had lower BMI; in 98.1% of females (in those individuals scoring 27 risk alleles or below), a higher MD adherence was associated with lower BMI, attenuating the genetic risk to obesity. For WC, we also observed that 19.8% males (≤20 risk alleles) with higher MD adherence, had lower WC; however, 95.2% of females (≤26 risk alleles), had also lower WC levels.

Concerning MetS, 4.8% of males with higher MD adherence had lower cardiometabolic risk score if the risk score was ≤18. More so, 95.2% of females having higher MD adherence showed higher MetS scores if the risk score was ≤26. In terms of sensitivity analyses, the MetS analyses were repeated using the MetS score following the IDF recommendations. In males, the obesity-GRS and MD showed a significant interaction on MetS ($p = 0.001$) but not on the IDF MetS score ($p = 0.092$). However, the interaction was significant for both MetS scores in females ($p = 0.005$ vs. $p = 0.003$).

There were sex-related differences in the interaction between genes and diet on other cardiometabolic parameters, such as HOMA, DBP, and MAP. Specifically, in females with higher MD adherence, those having ≤25 risk alleles had lower HOMA levels (89.4% of the adolescents). Male participants with higher MD adherence with an obesity-GRS ≤21 (31.4% of the adolescents) had lower DBP. Likewise, males with higher MD adherence and an obesity-GRS ≤22 (46.8% of the adolescents) had a lower MAP. The interaction effects of the obesity-GRS and MD on HOMA, DBP, and MAP for male and female participants are displayed in Supplementary Figure S1.

4. Discussion

The main findings of the present study indicate that the influence of high MD adherence on adiposity and MetS was only expressed when a limited number of risk alleles were present. As a result, the gene–diet interaction effect was higher in females than in males.

The MD has previously been shown to provide numerous health benefits [35], such as the reduction of risk factors for non-communicable diseases [36,37]. However, little is known about how the genetic variations among individuals determine the response to MD adherence [38]. To our knowledge, no previous studies have assessed their interaction effects using an obesity-related GRS. Instead, isolated SNPs from candidate genes previously associated in the literature with adiposity or MetS were examined. More so, no gene–diet interaction studies on adiposity and cardiometabolic parameters considering the MD were found in adolescents, as the majority of studies have been conducted in adult populations.

Concerning adiposity, similar findings were observed in a study where the *FTO*rs9939609 and *MC4R*rs17782313 polymorphisms showed an interaction with the MD adherence, which reduced the risk of obesity and T2DM [39]. In line with our findings, one study also showed low adiposity levels in relation with the interaction effect with different allele combinations, and considering other dietary approaches different to the MD: low polyunsaturated fatty acids (PUFA) intake showed an inverse association with obesity risk (BMI ≥30 kg/m^2) when the *ADAM*17i33708A polymorphism was present [40]. Another study, considering high saturated fat intake, showed a significantly higher BMI in the GG carriers of the *THRA*rs1568400 than in the A carriers [41], suggesting counter-productive effects in comparison to our study. In the current study, not only BMI, but also WC, a surrogate marker of abdominal adiposity, showed lower levels in both male and female adolescents, as a result of the obesity-GRS–MD interaction; to our knowledge, no similar findings have been reported in other studies with similar characteristics.

Regarding MetS, we found no studies assessing a gene–diet interaction effect on a MetS risk score. We have also assessed cardiometabolic parameters individually. Our results showed low HOMA levels in the majority of females modulated by the obesity-GRS when adhering to the MD. One study assessing the effect of increasing the ratio of saturated fat to carbohydrate intake, showed higher HOMA levels in carriers of the minor allele (*PLIN*11482G>A)[42].

Concerning DBP, the male adolescents with higher MD adherence had lower levels of DBP when having 21 or fewer risk alleles. Our findings are coincident to one intervention study promoting the Dietary Approaches to Stop Hypertension (DASH) diet, where AA carriers of the Angiotensinogen

genotype (G-6A ANG polymorphism) showed the greatest reduction in DBP [43]. No interaction studies considering the MAP levels were reported in the literature.

Despite the fact that we did not observe an effect on HDL-c, we included the TC:HDL ratio variable in our MetS score, showing a significant association with the gene–diet interaction. In this sense, Ordovás et al. observed that women carriers of the A allele of the *APOA1* gene (G-A polymorphism) responded with higher HDL-c concentrations to a high PUFA intake, whereas the opposite effect was observed in the G carriers [44]. Other authors have also pointed novel genes– nutrients interactions with high-carbohydrate diets in GG carriers of *KCTD10*i5642G>C and *MMAB*3U3527G>C and C allele carriers of *KCTD10*V206VT>C, contributing to lower HDL-c concentrations [45].

When adhering to the MD, no associations were found in terms of TG levels. However, another study showed that, after 12 months of a MD-based intervention, higher levels of HDL-c and TG were seen in those individuals carrying the T allele of the *CETP*rs3764261 than in those with the GG genotype [46]. Another study showed that the *TNF-alpha*rs1800629 GG subjects had higher levels of TG than A carriers in MetS patients after another MD intervention [47]. Finally, other authors assessed the interaction between *PPARA*L162V and PUFA intake, noticing lower TG levels with higher intake in the V carriers [48].

In order to compare the present results to other cardiometabolic definitions, the development of our MetS score was compared to another MetS score, following the IDF recommendations. The IDF score showed no association with the GRS–MD interaction in males. This fact could be due to the different age and sex specific criteria selected to define the cut-off points between authors. Nevertheless, positive associations of the GRS–MD interaction were seen in the female group for both MetS scores. The consensus to use unified criteria to identify adolescents at risk of MetS remains under development.

The present study has some limitations. As the HELENA project is a cross-sectional study, cause–effect relationships cannot be established. Moreover, only selected risk loci are available in the HELENA study. When constructing the obesity-GRS, it has been calculated that BMI changes can be explained by a small proportion of genetic variants discovered so far [49], so potential rarer variants yet to be found might emerge when Genome-Wide Association Studies (GWAS) are carried out.

On the other hand, the study presents several strengths. Most analyses from previous studies were focused on specific single SNPs interactions, whereas the present study used an obesity-GRS, considered as a useful genetic tool [50], to predict adiposity and MetS in European adolescents. More so, the development of the cardiometabolic risk score was considered appropriate for the present study as it provides higher sensitivity and low susceptibility to errors compared to other approaches [51]. Furthermore, we included the whole MD pattern developing a cluster of different food groups in an adherence scale rather than considering single macronutrients or individual specific food groups´ intake. Additionally, different effects were identified in males and females. There is different behavior dependent on sex, not attributable to genetic predisposition, maybe associated with physical activity. Due to the multicentric design of the HELENA study, congregating participants from 10 European cities, the researchers have been provided with large datasets from diversely distributed populations across Europe. Finally, the study is focused on adolescents, a population age that is understudied and where early detection plays a key role in the development of obesity and metabolic syndrome. Little has been found in the literature using a similar approach, where most similar studies are available on adult populations.

As MetS and excess of adiposity may occur at any stage from childhood to adulthood, early detection and diagnosis is fundamental to elaborate on health prevention programs among youth to effectively reduce the risk of cardiovascular diseases and T2DM [52,53]. At the same time, it has been previously shown that greater adherence to MD was associated with a significant improvement in overall health status among youth, suggesting the implementation of a MD dietary pattern for primary prevention of major chronic diseases [54]. The effect of the interventions would be heterogeneous depending on the genetic background of the individuals and should be considered in the efficacy analyses.

5. Conclusions

Obesity-related genotypes had a modulation effect in the relationship between MD adherence and obesity and MetS risk in European adolescents. The genes–diet interaction effect on MetS was stronger in females than in males. These observations strengthen the idea of applying genomic information to promote targeted dietary advice.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/12/12/3841/s1, Table S1: Mediterranean diet score items specified in g/day displayed in sex-specific median intake in HELENA participants with dietary information available, Table S2: Main characteristics of the 21 single nucleotide polymorphisms (SNPs) included in the obesity genetic risk score (obesity-GRS), Table S3: Comparative analysis of the 21 single nucleotide polymorphisms (SNPs) included in the obesity genetic risk score (obesity-GRS) by sex, Figure S1: Interaction models between HOMA, diastolic blood pressure (DBP) and mean arterial pressure (MAP) and Mediterranean Diet (MD) according to the obesity genetic risk score (obesity-GRS) modulation in female. Obesity-GRS values (18–28) are displayed according to our population distribution.

Author Contributions: L.A.M. is M.S.-C.'s supervisor. P.D.M.-E. and I.L. are M.S.-C.'s co-supervisors. M.S.-C., S.S.-L., P.D.M.-E., L.M.E., L.A.M., and I.L. contributed to the conceptualization, methodology, formal analysis and writing—original draft preparation. I.L. supervised the genomic data. M.G.-G. was responsible for the blood sampling and analysis procedure. M.G.-G., E.G., C.M.-H., S.D.H., É.E., L.C., Y.M., E.K., K.W., A.K., L.B., A.M., D.S.-T., and J.R.R. participated in the writing—review and editing. L.A.M. was coordinator of the HELENA project, and together with M.G.-G. and S.D.H. formed the Core Management Group of the project. All authors have read and agreed to the published version of the manuscript.

Funding: Miguel Seral-Cortes, the corresponding author, has received funding from the Iberus Talent Pre-doctoral fellowships 2018, under the European Union's H2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 801586. Pilar De Miguel-Etayo was supported by ISCIII – CB15/00043 (Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición, CIBERObn). This work was part of the HELENA Study (http://www.helenastudy.com/). We gratefully acknowledge financial support of the European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034).The data for this study were gathered under the auspices of the HELENA project, and further analysis was additionally supported by the Spanish Ministry of Economy and Competitiveness (Grants RYC-2010-05957 and RYC-2011- 09011), the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn).

Acknowledgments: The autors acknowledge the Laboratoire d'Analyse Génomique Centre de Ressources Biologiques (LAG-CRB) BB-0033-00071 Institut Pasteur de Lille, F-59000 Lille, France for managing human samples. We are grateful for the support provided by school boards, headmasters, teachers, school staff and communities, and for the effort of all study nurses and our data managers.

Conflicts of Interest: The authors declare no conflict of interest. The funding sources had no role in the design, conduct, and analysis of the study or in the decision to submit the manuscript for publication.

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Artículo IV [Article IV]: Mediterranean Diet, Screen-Time-Based Sedentary Behavior and Their Interaction Effect on Adiposity in European Adolescents: The HELENA Study.

Seral-Cortes M, Sabroso-Lasa S, Bailo-Aysa A, Gonzalez-Gross M, Molnár D, Censi L, et al.

The HELENA Study.

Nutrients. 2021;13(2)

nutrients

MDPI

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Mediterranean Diet, Screen-Time-Based Sedentary Behavior and Their Interaction Effect on Adiposity in European Adolescents: The HELENA Study

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Citation: Seral-Cortes, M.; Sabroso-Lasa, S.; Bailo-Aysa, A.; Gonzalez-Gross, M.; Molnár, D.; Censi, L.; Molina-Hidalgo, C.; Gottrand, F.; Henauw, S.D.; Manios, Y.; et al. Mediterranean Diet, Screen-Time-Based Sedentary Behavior and Their Interaction Effect on Adiposity in European Adolescents: The HELENA Study. *Nutrients* **2021**, *13*, 474. https://doi.org/10.3390/nu13020474

Academic Editor: Maria Annunziata Carluccio
Received: 31 December 2020
Accepted: 27 January 2021
Published: 30 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Childhood obesity is a worldwide epidemic. Mediterranean diet (MD) is inversely associated with childhood obesity, but the interaction with other environmental factors, such screen time, might influence the health benefits of a high MD adherence in adolescents. The aim of the present study was to assess whether an association between MD and screen time exists in European adolescents. Moreover, we also explored whether sedentary time has a modulatory effect on the association between MD and adiposity. Adherence to the MD (24 h recalls), screen time (questionnaire), pubertal development, body mass index (BMI), fat mass index (FMI) and waist circumference (WC) were evaluated in 2053 adolescents (54.7% females), aged 12.5–17.5 years. In females, MD adherence was associated with lower BMI and FMI only when they were exposed to less than 338 min/day of screen time (81.8% of females); MD adherence was also associated with lower WC only when females were exposed to less

than 143 min/day of screen time (31.5% of females). No significant MD-screen time interaction was observed in males. In conclusion, screen-time-based sedentary behaviours had a modulatory effect in the association between MD adherence and adiposity in European female adolescents.

Keywords: Mediterranean diet; sedentary time; adiposity; adolescents; sex and HELENA

1. Introduction

Childhood overweight and obesity's prevalence has been rising worldwide in recent years [1]. Recent studies showed that overall, European children continue to struggle with high prevalence of obesity despite the effort in terms of prevention programs in previous years [2]. Metabolic syndrome and type 2 Diabetes are more likely to occur in adulthood in those children and adolescents where obesity is gradually establishing [3]. The Mediterranean dietary pattern is inversely associated with childhood obesity [4]. In fact, a high Mediterranean diet (MD) adherence from an early age is related to a lower risk of overweight and obesity development in childhood [5]. However, the interaction with other environmental factors, such as screen time, might influence the health benefits of a high MD adherence in adolescents. Sedentary behaviours, such screen time and physical inactivity were found to increase the risk of overweight and obesity in European adolescents [6,7]. Current recommendations suggest limiting recreational screen time to less than 2 h per day [8]. However, previous studies have shown that more than half of all children exceed screen time recommendations [9–11]. Additionally, increasing sedentary time is associated with unhealthy dietary patterns in European adolescents [12]. Furthermore, there is a growing evidence of transition from the traditional MD pattern into consumption of energy- dense foods, such as in the Western diets, especially in Mediterranean countries [13–16].

Previous data showed an inverse association between MD adherence and sedentary time, including screen time [17,18]. However, to our knowledge, a potential interaction effect between MD and screen time on adiposity remains unknown. Therefore, the aim of the present study is to assess whether an association between MD and sedentary time exists in European adolescents in the Healthy Lifestyle in Europe by Nutrition in Adoles- cence (HELENA) study. Moreover, we intend to explore whether sedentary time has a modulatory effect on the association between MD and adiposity markers. We hypothesize that high levels of screen time may attenuate the protective effect of MD adherence on adiposity parameters.

2. Materials and Methods

2.1. Study Design and Population

HELENA is a multicentre and cross-sectional study. Description of the study sampling and recruitment, standardization and harmonization methodology, data collection, analysis strategies and quality control procedures was published elsewhere [19,20]. The HELENA study was designed to obtain reliable and comparable information on adolescents' nu- tritional, environmental and health-related influences to prevent risk factors for present and future nutrition-related chronic diseases [21]. Each one of the participating countries involved in the HELENA study approved the protocol by the local Research Ethics Com- mittees and followed the ethical guidelines of the Declaration of Helsinki 1964 (revision of 2000), the Good Clinical Practice and the legislation about clinical research in humans [22]. A written consent was provided to the parents or guardians of all individuals participating in the study, which was read and signed. The present study comprises 2047 adolescents (54.7% females), aged 12.5–17.5 years, with valid and specific data on adherence to MD, screen time and adiposity. Description of the selection process is shown in a flow chart (Figure 1).

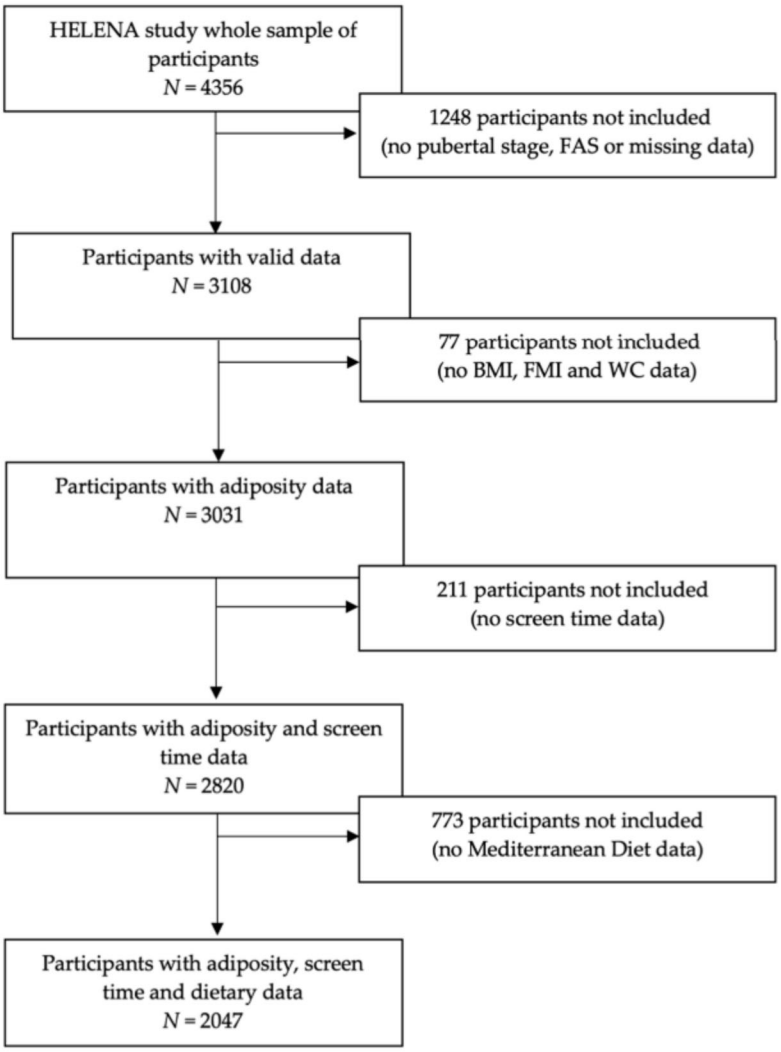

Figure 1. Flow chart of the sample selection process. Abbreviations: FAS, family affluence scale; BMI, body mass index; FMI, fat mass index and WC, waist circumference.

2.2. Physical Examination and Adiposity Measurements

Anthropometric measurements were strictly controlled and performed following stan- dard protocols [23]. Body height was measured barefoot with a telescopic stadiometer (SECA 225) to the nearest 0.1 cm. Body weight was measured in underwear and with no footwear to the nearest 0.1 kg with an electronic scale (SECA 861, Hamburg, Germany). Height and weight were measured in triplicate. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m) [24]. Waist circumference (WC) measurements were performed with a non-elastic tape (SECA 200) to the nearest 0.1 cm at the mid-point be- tween the lowest rib and the iliac crest. Subscapular and tricipital skinfold thicknesses were measured in triplicate. In order to assess the contribution of fat mass relative to body size, the body fat percentage was calculated using the Slaughter's equation [25], and then, FMI was calculated as body fat in relation to height squared [FM (kg)/height (m^2)] [26]. Pubertal status was evaluated during a medical examination by a physician/paediatrician following the methodology described by Tanner and Whitehouse [27]. Pubertal status was categorized as Tanner stages from no sexual maturation (stage I) to complete sexual maturation (stage V).

2.3. Dietary Intake and Mediterranean Diet Score (MDS) Assessment

The HELENA Dietary Assessment Tool (HELENA-DIAT) is a self-administered com- puterized 24 h dietary recall used to collect all the adolescents' dietary intake [28,29]. This tool was first validated in Flemish adolescents [29] and then adapted to be implemented in

the participating centres of each country [30]. Participants provided twice dietary information through the HELENA-DIAT on 2 non-consecutive days within a space of 2 weeks. Previous authors considered this method as an useful procedure to evaluate the dietary intake in European children and adolescents [31]. The multiple source method (MSM) allowed us to calculate usual dietary intake of each individual, which enables the possibility to correct the dietary information for between and within individuals' variability [32].

A Mediterranean diet score (MDS) was computed from the sum of 9 single subcomponents that were described elsewhere [33]. In short, vegetables, fruits and nuts, cereals, legumes, fish, dairy products (recommended during growth and development periods [34]) and unsaturated to saturated fat ratio were considered healthy food subgroups of MD, whereas meat products (including processed meat) and alcohol consumption were classified as unhealthy factors. Therefore, a participant consuming a healthy MD-associated food group was designated with 1 point, whereas the unhealthy food subgroups contributed with 0 points. A Mediterranean diet score (MDS), showing the degree of adhesion to the MD for each individual, was developed using a 0–9 point scale, with low values (0–4) indicating poor adherence and high values (5–9) greater adherence, respectively [35,36]. Supplementary Table S1 shows the median intake in g/day by sex of each subgroup from the MDS and the adherence levels to the MD.

2.4. Screen-Time-Based Sedentary Behavior Assessment

In order to report the habitual time devoted to screen time among adolescents, a vali- dated self-reported screen-time-based sedentary behaviours questionnaire was used [37]. The time spent in TV viewing, computer games, video games and internet for non-study reasons during both week and weekend days was collected in categories in a scale ranging from 0–240 min per day. The daily mean time for each category was obtained and the final time was calculated summing weekdays and weekend days, obtaining the total screen time in minutes per day (min/day). Lastly, a total sedentary time value was obtained by summing up the time reported in each category. The weighted Cohen's κ-coefficients were used to assess the test–retest reliability of the screen-time-based questionnaire used in the HELENA study. The most common values observed were moderate, substantial or almost perfect agreement (>0.7). Exceptionally, internet for study reasons showed 0.46 in weekdays and 0.33 in weekends, respectively [38]. Furthermore, a sensitivity analysis was carried out in order to discard potential disparities in the interaction models due to those outlier individuals considered (or not) in the screen time variable.

2.5. Socioeconomic Status

The family affluence scale (FAS) is an indicator of material affluence, which ranges from 0 (lowest) to 8 (highest) and further recategorized in low (0–2), medium (3–5) and high (6–8) levels [39]. The scale considers parameters such as car ownership, having an own bedroom, internet availability and computer ownership. This information was assessed through a questionnaire, and it was used as a predictor of the adolescents' health outcomes [40].

2.6. Statistical Analysis

The normality of the variables was assessed with the Shapiro–Wilk non-parametric test. Not all variables followed a normal distribution, so the descriptive sex-specific charac- teristics are shown as median and interquartile range (IQR) for continuous variables, while categorical variables are shown as absolute and relative frequencies. Moreover, Pearson's chi-square statistical test was used to obtain comparative sex-related differences for categor- ical variables; the Mann–Whitney–Wilcoxon test was performed for continuous variables. In order to observe the association between MD and screen time, sex-specific multiple linear regression models were performed. First, a raw simple linear regression model was constructed to observe associations between MD and screen time. Then, an initial multiple linear regression model was performed considering energy intake, socioeconomic status and Tanner stage as confounders. A step-by-step algorithm was applied to select the signif-

icant variables in a multivariate model to shortlist the independent variables significantly associated with the adiposity parameters in the final model. Furthermore, a new multiple regression analysis was created to assess the association between adiposity parameters and MD, adding the screen time interaction effect, the MD effect alone and the abovementioned confounders. Finally, as we observed extreme values for screen time in the highest end of the distribution, we performed a sensitivity analysis, excluding the outliers in the top end of the distribution. Level of significance was set at $p < 0.05$. RStudio Version 1.2.5001 (RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA, URL http://www.rstudio.com/) was used to perform all statistical analyses.

3. Results

3.1. Descriptive Characteristics of the Study Sample

Table 1 shows the main characteristics of the HELENA participants included in the present study. Summarizing, males had higher weight, height and WC ($p \leq 0.001$), and lower FMI than females (all $p < 0.001$). Moreover, males were more exposed to screen time ($p \leq 0.001$) and had higher energy intake ($p \leq 0.001$) than females, although females were in more advanced pubertal stages than males ($p \leq 0.001$). Finally, there were no significant differences regarding FAS and MDS.

Table 1. Demographics and behavioural characteristics of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) participants displayed by sex.

	Total	**Male**	**Female**	***p***
	***n* = 2047**	***n* = 925**	***n* = 1122**	
Age (years)	14.7 (13.7–15.7)	14.8 (13.7–15.7)	14.7 (13.7–15.7)	0.590
Height (cm)	165.9 (159.3–172.0)	170.1 (163.9–177.1)	162.4 (157.7–167.0)	**<0.001**
Weight (kg)	58.4 (50.3–64.3)	61.5 (52.3–68.9)	55.8 (49.0–61.2)	**<0.001**
BMI (kg/m^2)	21.1 (18.7–22.8)	21.1 (18.6–22.8)	21.1 (18.8–22.8)	0.282
WC (cm)	71.8 (66.2–75.8)	73.8 (67.8–78.3)	70.2 (65.0–74.4)	**<0.001**
FMI (kg/m^2)	5.15 (3.1–6.3)	4.5 (2.4–5.3)	5.7 (4.0–6.8)	**<0.001**
Pubertal stage [*n* (%)]				**<0.001**
I	7 (0.3%)	7 (0.8%)	0 (0%)	
II	134 (6.5%)	84 (9.1%)	50 (4.5%)	
III	502 (24.5%)	226 (24.4%)	276 (24.6%)	
IV	881 (43.0%)	381 (41.2%)	500 (44.6%)	
V	523 (25.5%)	227 (24.5%)	296 (26.4%)	
FAS [*n* (%)]				0.112
Low	199 (9.7%)	76 (8.2%)	123 (11.0%)	
Medium	1133 (55.3%)	519 (56.1%)	614 (54.7%)	
High	715 (35.0%)	330 (35.7%)	385 (34.3%)	
MDS * (points)	4 (3–5)	4 (3–5)	4 (3–5)	0.071
Energy intake (kcal/day)	2180.1 (1634.9–2569.7)	2517.9 (1921.0–2984.3)	1901.6 (1492.4–2244.9)	**<0.001**
Screen time (min/day)	256.2 (139.3–330.0)	288.1 (171.4–367.4)	229.9 (126.4–300.0)	**<0.001**

Median values (*p*25–*p*75) expressed. Abbreviations: BMI, body mass index; WC, waist circumference; FMI, fat mass index; FAS, family affluence scale; MDS, Mediterranean diet score. * Mediterranean diet score resulting from the sum of 9 food subgroups compliance. Score ranging from 0–9 points. Significant values ($p < 0.05$) expressed in bold font.

The screen time distribution (min/day) between HELENA participants is shown in Figure 2.

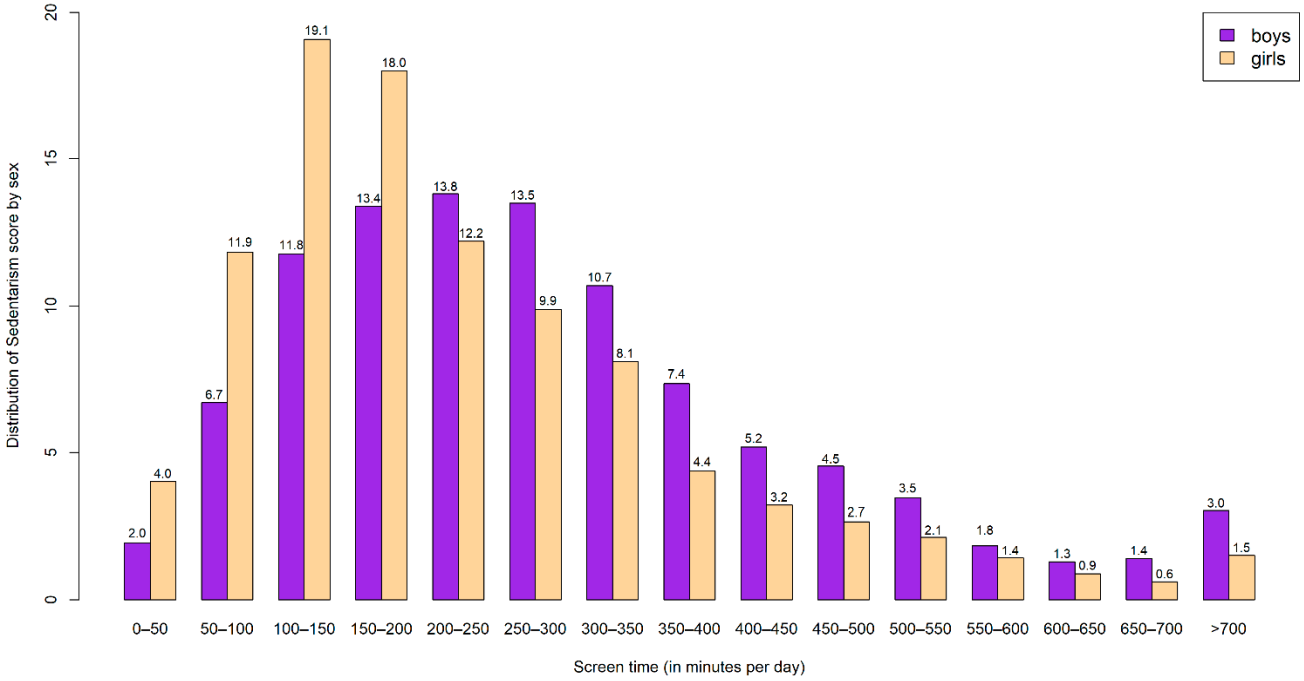

Figure 2. Distribution of screen time (% displayed) among HELENA participants by sex.

3.2. Association between MD Adherence and Screen-Time-Based Sedentary Behaviors

The associations between MD and screen time are shown in Table 2. The univariate model (Model I) showed that MD and screen time were inversely associated in both males and females ($p < 0.001$). These relationships were maintained in the initial multivariate model ($p < 0.001$) and after adjusting by confounders (Model II; $p < 0.001$).

Table 2. Multiple linear regression models showing the associations of the Mediterranean diet score (MDS) with screen time.

	Screen Time (min/day)				
	Model I [a]		**Model II** [b]		
	β	***p***	**β**	***p***	**R^2**
Male					0.029
MDS (point)	−12.535	**<0.001**	−12.402	**<0.001**	
Energy Intake (kcal/day)	-	-	0.026	**<0.001**	
Female					0.016
MDS (point)	−12.402	**<0.001**	−12.402	**<0.001**	
Energy Intake (kcal/day)	-	-	-	-	

[a] Model I, unadjusted model, studies the association between screen time and MDS. [b] Model II presents the variables statistically significant in relation to sedentary time as follows: an initial model was constructed between screen time and MD considering Tanner stage, FAS categories and energy intake as covariates. Furthermore, a step-by-step algorithm was applied to discard non-significant associations. Only statistically significant variables are shown in the present table. Significant values ($p < 0.05$) expressed in bold font.

3.3. Interaction between MD Adherence and Screen-Time-Based Sedentary Behaviors on Adiposity

The interaction effects between MD and screen time on adiposity parameters by sex group are displayed in Table 3. In males, the screen-time–MD interaction was not significantly associated to any adiposity index. However, in females, there were significant interaction effects between the screen time and MD on BMI ($p < 0.05$), WC ($p < 0.01$) and FMI ($p < 0.05$) (Table3).

Table 3. Multiple linear regression models of screen time and Mediterranean diet score (MDS) interaction and covariates to predict body mass index, waist circumference and fat mass index displayed by sex.

	Males	***(p-***		**Females**	***(p-***	
	BMI (kg/m^2)	**WC (cm)**	**FMI (kg/m^2)**	**BMI (kg/m^2)**	**WC (cm)**	**FMI (kg/m^2)**
Covariates						
Pubertal Stage						
II *	0.502	0.139	**0.048**	-	-	-
III	0.547	0.209	**0.010**	**0.026**	**0.012**	**0.126**
IV	0.534	0.936	**0.047**	**<0.001**	**<0.001**	**<0.001**
V	0.638	0.866	**0.008**	**<0.001**	**<0.001**	**<0.001**
FAS						
Medium	**0.048**	0.604	**0.045**	**<0.001**	0.138	**<0.001**
High	**0.024**	0.695	**0.019**	**<0.001**	0.058	**<0.001**
Energy Intake (kcal/day)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Studied Variables						
MDS (point)	**0.049**	0.260	0.198	**0.043**	0.160	**0.042**
Screen time: MDS	0.062	0.617	0.122	**0.025**	**0.002**	**0.022**

Abbreviations: BMI, body mass index; WC, waist circumference; FMI, fat mass index; FAS, family affluence scale; MDS Mediterranean diet score. * Tanner II *p*-values were not considered to be estimated in the female group, as a statistically small number of females were present in this stage for the current analysis. Significant values ($p < 0.05$) expressed in bold font.

In order to interpret the modulation effect of screen time on the relationship between MD and adiposity indices in females, a set of figures is displayed in a matrix panel related to each adiposity index (Figure 3). A number of lines were drawn to represent the MD and adiposity variables modulated by the distribution of the screen time. Most participants were located in lower and central parts of the distribution, corresponding to 50–350 min/day (74.5% of the total population); considering this distribution, the impact of outliers was carefully considered. Despite the observed screen time habits, those individuals represented by a negative slope could benefit from the protective role of MD in relation to adiposity indices when the MD adherence is high. Therefore, a high MD adherence was associated with lower BMI only in those females being exposed to screen time less than 338 min/day (81.8% of the total females). Moreover, high MD adherence was associated with lower WC only in those females being exposed to screen time less than 143 min/day (31.5% of total females). Finally, a high MD adherence was associated with lower FMI only in those females being exposed to screen time less than 338 min/day (81.8% of total females).

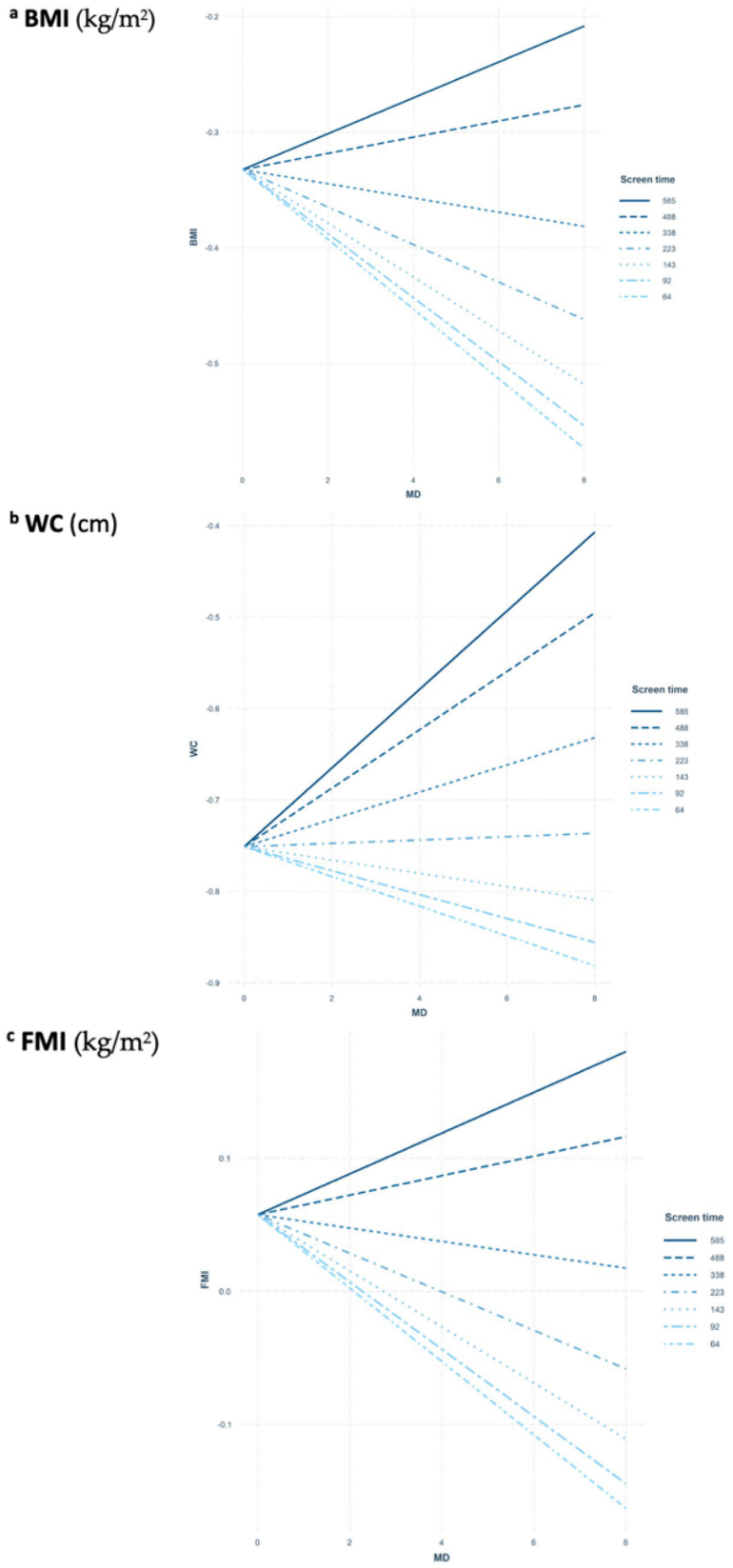

Figure 3. Matrix panel of interaction models on (**a**) BMI (body mass index), (**b**) WC (waist circumference), (**c**) FMI (fat mass index); and MD (Mediterranean diet) according to screen time modulation in females. In order to design the representation of the distribution in HELENA adolescents, different lines were traced as reference points to observe the slope of the studied population according to the sedentary time. A positive gradient represents the MD acting as risk factor, while a negative gradient shows the MD acting as protective factor.

3.4. Sensitivity Analysis for Screen-Time-Based Sedentary Behaviors Plausible Data

For the significant interaction models (females), sensitivity analysis was performed considering screen time outliers (highest value: 856 min/day) vs. interaction models not considering outliers (highest value: 632 min/day) on adiposity indices. Minimal differences were observed in the two interaction models of each adiposity parameter ($\beta < 0.001$ vs. $\beta < 0.001$ in BMI, WC and FMI).

4. Discussion

The main findings of the present study are the observed inverse association between MD adherence and screen time, and the joint interaction effect between both factors on adiposity in European female adolescents. Thus, the benefits associated with a high MD adherence were only observed in those females with lower screen time. On the other hand, no MD–screen-time interaction effect was observed in males.

In line with our findings, previous studies reported an inverse association between MD adherence and sedentary time among youth. In a population of Mediterranean European adolescents, self-reported inactivity [41] was inversely related to MD adherence in both sex groups [42]. In non-Mediterranean European adolescents, a low MD adherence was associated with higher sedentary time, although their sedentary time assessment only considered sitting time during weekdays [43].

To our knowledge, no other studies have examined screen time as modulatory factor in the MD effect on adiposity indices. However, a combined effect of different lifestyle patterns in cluster studies assessing their relationship to adiposity has been reported in similar age populations [44–47]. Regarding the combined effect of unhealthy habits, in European children, a cluster including high sedentary activities (including screen time), low PA, sweet beverages and low fruits and vegetables intake was associated with high BMI and WC [44]. Similar results were found in non-European children, where an unhealthy cluster of TV viewing with energy dense foods was associated to high BMI [45]. Although we are considering cross-sectional studies, similar associations were also obtained in longitudinal studies in European [46] and non-European children [45]. In the same way, associations between clusters of healthy lifestyle patterns and low levels on adiposity indices were observed in Spanish youth [47].

Cluster analysis could be useful to evaluate the combined effect of environmental and behavioural factors on overweight and obesity among children and adolescents [47,48]. However, we found no studies that have considered the interaction effect between the factors included. Yet, a recent study conducted in a large sample of adolescents showed that screen time was associated with increased risk of overweight/obesity, regardless of being combined with other healthy or unhealthy behaviours [49]. This finding suggests that high levels of screen time might mask the beneficial effects of a healthy diet, such as the MD, on decreasing the obesity risk. Previous studies in children have reported similar co-existing effects among behaviours [50,51], which supports the findings of our study; low levels of PA might also counteract the beneficial effect of a healthy dietary pattern [52]. It remains challenging to find the potential mechanisms responsible for sex dispar- ities in the obtained results. As in other studies in European adolescents [53], we found that the time spent in screen time activities was higher in males than in females. However, conflicting data were found in this regard [43]. Screen time could be an indicator of sex variability, but further investigation is required to obtain comparable data to explain gen- der differences in the present analysis. Another hypothesis that might explain the sex differences could be the poorer sleep duration and thus increased adiposity in females than males, previously observed in other similar age cohorts [54]. Short sleep duration was already observed to be associated with increased adiposity biomarkers in HELENA ado- lescents, particularly in females [55], and with lower dietary quality [56]. Further sex differences were also observed in a cluster study comprising PA, sedentary behaviours and sleep duration according to endocrine, metabolic and immunological biomarkers, which suggests new pathways to focus our future research [57].

In the current study, a sensitivity analysis was carried out in order to detect potential differences in the results obtained considering vs. not considering outliers in the number of hours of screen time. There were no significant differences between the two proposed models assuming vs. not assuming those extreme values; therefore, the final results were not affected by those specific differences in screen time.

In addition, a different effect was observed in the studied adiposity indices depending on the amount of screen time, requiring less screen time in those females to obtain lower WC levels than BMI and FMI, while higher levels were observed in those females with higher screen time. Similar findings were observed in terms of BMI and WC differences in previous studies in adolescents [6].

The current study presents some limitations. Due to the cross-sectional nature of the HELENA study, cause–effect relationships cannot be concluded. We assume the challenging task of collecting dietary data through the self-administered computerized 24 h dietary recall in adolescents; therefore, reliability of answer depends completely on the responders' interpretation. Although the two non-consecutive days assessment of dietary habits might not consider certain periods of time such as weekends or holidays, this method has previously shown good validity and accuracy in similar populations [58]. An identical situation occurs for the self-reported questionnaire to obtain measures of screen-time-based sedentary behaviours, where we also acknowledge that screen time might vary between weekdays and weekend days; yet, this method was adapted and validated to be used in adolescents [37]. Additionally, other screen time devices such as tablets, phones and consoles are becoming more dominant over the traditional TV viewing routines; so, future studies should consider them in their screen-time-based sedentary behaviours assessment. However, some strengths should also be acknowledged. Firstly, it is important to highlight the high standard of implementation in terms of methodology and design of the HELENA study [19,21], as well as the large number of sedentary behaviours considered to estimate screen time in the present cohort of participants. Furthermore, datasets with a large number of adolescents diversely recruited across Europe have been provided as a result of the multicentric nature of the HELENA study. This fact allows researchers to focus their analysis on an age range which is rarely studied, the adolescence period, where early detection of adverse sedentary and dietary habits might influence health status in adulthood. Finally, little has been found in the literature considering the same interaction approach, where screen time plays an important role in the association of MD and adiposity among adolescents.

As we have observed, the negative association between screen time and MD adherence on adiposity indices, joint public health implementation programs oriented to both, avoid- ing excessive screen time and promoting the MD dietary pattern for primary prevention of major chronic diseases, should be considered in the near future. In previous childhood obesity prevention programs, the greatest changes were observed in sedentary time [59]. At the same time, previous literature has shown that the most effective interventions to prevent childhood obesity are the ones combining diet and PA/sedentarism [60]. The evidence of co-existing effects might help to understand how screen time and dietary behaviours should be approached simultaneously in prevention programs.

5. Conclusions

Screen time and MD adherence are inversely associated in European adolescents. Moreover, in females, screen time had a modulatory effect in the association between MD adherence and adiposity in European adolescents. These findings support the idea of applying more personalized public health recommendations in reducing screen time to benefit from the MD, in order to decrease the adiposity levels among youth.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/2/474/s1, Table S1: Mediterranean diet score (MDS) subgroups displayed in g/day and sex-specific median intake in those HELENA adolescents with dietary data.

Author Contributions: L.A.M. is M.S.-C.'s supervisor. P.D.M.-E. and I.L. are M.S.-C.'s co-supervisors. M.S.-C., S.S.-L., A.B.-A., L.A.M., L.M.E., I.L. and P.D.M.-E. contributed to the conceptualization, methodology, formal analysis and writing—original draft preparation. D.M., L.C., C.M.-H., F.G., S.D.H., Y.M., C.M., K.W., A.K. and J.D. participated in the writing—review and editing. L.A.M. was coordinator of the HELENA project, and together with M.G.-G., S.D.H. and J.D. formed the core management group of the project. All authors have read and agreed to the published version of the manuscript.

Funding: Miguel Seral-Cortes (M.S.-C.), the corresponding author, has received funding from the Iberus Talent Pre-doctoral fellowships 2018, under the European Union's H2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 801586. Pilar De Miguel-Etayo (P.D.M.-E.) was supported by ISCIII-CB15/00043 (Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición, CIBERObn). This work was part of the HELENA Study (http://www.helenastudy.com/). We gratefully acknowledge financial support of the European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034). The data for this study were gathered under the auspices of the HELENA project, and further analysis was additionally supported by the Spanish Ministry of Economy and Competitiveness (Grants RYC-2010-05957 and RYC-2011-09011), the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn).

Institutional Review Board Statement: The HELENA study was approved by the Research Ethics Committees of each study site and followed the ethical guidelines of the Declaration of Helsinki 1964 (revision of 2000), good clinical practice, and the legislation about clinical research in humans in each one of the countries involved in the study in February 2006.

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 for further scientific analysis on request from the coordinator of the HELENA study to the following e-mail: lmoreno@unizar.es.

Acknowledgments: We are grateful for the support provided by school boards, headmasters, teachers, school staff and communities, and for the effort of all study nurses and our data managers.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The content of this article reflects the authors' views alone, and the European Community is not liable for any use that may be made of the information contained herein.

Appendix A

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5. DISCUSIÓN

En la presente Tesis Doctoral, se han descrito los beneficios de la DM sobre la obesidad y el SM, y se pone en valor la importancia de desarrollar estudios centrados en la interacción genes x dieta en niños/as y adolescentes Europeos. A pesar de que en población adulta Europea se han desarrollado trabajos en esta línea desde hace más de dos décadas, su desarrollo en población Europea joven ha sido muy escasa hasta el presente. La presente Tesis Doctoral muestra dos *GRS*s, ponderado y no ponderado, asociados significativamente con el IMC para predecir el riesgo genético de sobrepeso y obesidad en adolescentes Europeos, así como un análisis de interacción genes x DM sobre la obesidad y el SM. Los resultados muestran que los efectos beneficiosos derivados de una alta adherencia a la DM sobre la adiposidad y el SM sólo se producían cuando un número limitado de alelos de riesgo estaban presentes. Es decir, los beneficios de una mayor adherencia a la DM se observaron únicamente en aquellos adolescentes con menor puntuación en el *GRS* no ponderado. Además, dada la importancia de los determinantes ambientales que pueden modificar los efectos de la DM sobre la composición corporal, realizamos otro análisis de interacción considerando el tiempo sedentario de pantalla. Los resultados muestran que los beneficios asociados a una alta adherencia a la DM se observan únicamente en chicas adolescentes con menor tiempo de uso de pantallas.

5.1. Evaluación de la genética en la influencia de la dieta Mediterránea en términos de salud

El efecto de la interacción genes x dieta sobre la obesidad en edades tempranas de la vida ha sido escasamente estudiado en población Europea, lo que dificulta establecer

comparaciones entre nuestros resultados y trabajos previos. A través de una búsqueda sistematizada, se exploraron una serie de estudios que valoraban la influencia de los factores genéticos en la asociación que existe entre la DM y obesidad o SM en jóvenes Europeos. Tras obtener escasos resultados en el grupo de edad de 0 a 18 años (130), se procedió a ampliar la búsqueda a todos los rangos de edad. De este modo, se seleccionaron diferentes estudios de interacción genes x MD en población Europea adulta. A la hora de evaluar el efecto de la interacción en cada artículo, se encontraron, por un lado, estudios que valoraban el efecto modulador del perfil genético sobre los beneficios de la DM en relación con obesidad y SM (131-133) y, por otro lado, estudios que consideran la DM como factor de interacción para modular la asociación entre el riesgo genético y la obesidad, el SM o los componentes individuales del SM (131, 134-147). En ambos casos, se obtuvieron resultados dispares en relación con el impacto del efecto modulador. Por ejemplo, *Wang, T et al.* analizaron los dos posibles enfoques de interacción: primero, se consideró la utilización de un *GRS* formado por 77 *SNPs* relacionados con obesidad, el cual mostró que, el efecto beneficioso de la DM en términos de peso corporal fue más pronunciado en aquellos individuos con alto riesgo genético de obesidad (131); paralelamente, se mostró que la DM no pudo atenuar la predisposición genética a la obesidad, resultando en un incremento significativo de IMC y peso corporal (131). De esta forma, el impacto del *GRS* y la DM se estudió utilizando dos posibles enfoques metodológicos en función de la variable seleccionada como factor de interacción, obteniendo, en este caso, resultados dispares.

Para evaluar la susceptibilidad genética a obesidad o SM de los individuos estudiados en la revisión narrativa (Artículo I), se utilizaron diferentes estrategias. Por un lado, la capacidad predictiva de los *SNPs* estudiados de manera individual, donde se valoran las posibles diferencias entre sujetos que portan diferentes alelos de riesgo. El

FTOrs9939609 fue el *SNP* más utilizado para estudiar el riesgo de desarrollo de obesidad y SM (132, 135, 137, 138, 145). Otros *SNPs* previamente relacionados con la obesidad, como el *TCF7L2rs7903146* (132, 146) y el *MC4Rrs17782313* (137, 145), también fueron incluidos en los estudios que han considerado la interacción genes x DM en población Europea adulta. Por otro lado, el efecto combinado de una serie de *SNPs* formando un *GRS,* fue otra estrategia considerada para valorar los estudios que integraron en sus análisis la interacción genes x DM. Se han encontrado en la literatura diversos *GRSs*, que van desde los 2 hasta los 77 *SNPs*, construidos a partir de variantes asociadas con IMC en adultos de ascendencia Europea. Los mencionados *Wang, T et al.* aplicaron un método ponderado para la construcción de un *GRS,* en un rango de puntuación que oscilaba entre 0 a 154, con cada unidad correspondiendo a un alelo de riesgo. Una puntuación de *GRS* más alta indicaba una mayor predisposición a la obesidad. Este *GRS* se utilizó en una cohorte prospectiva combinada (*N*=14.046) de individuos con descendencia Europea durante un periodo de seguimiento de 20 años (131).

El estudio de la influencia genética en el desarrollo de enfermedades crónicas en la edad adulta se fundamenta en la existencia de una serie de factores genéticos compartidos a lo largo del curso de la vida (148), lo que significa que existe una superposición genética parcial en los procesos biológicos que subyacen al IMC de población joven, con los que subyacen al IMC de población adulta. Alternativamente, es posible que esta transición se explique a través de la continuidad fenotípica del IMC desde la infancia hasta la edad adulta (148).

5.2. Índice genético predictivo del riesgo de obesidad

La presente Tesis Doctoral incluye el desarrollo de dos índices de riesgo genético de obesidad en adolescentes Europeos: un *GRS* no ponderado *(uGRS)* y un *GRS* ponderado *(wGRS),* los cuales incluyen 21 *SNPs* significativamente asociados con el IMC. Hasta la fecha, son los primeros *GRSs* con estas características en una muestra geográficamente distribuida de adolescentes Europeos. Existen pocos estudios previos centrados en *GRSs* basados específicamente en el IMC para valorar sobrepeso y obesidad en población adolescente, y ninguno que sea exclusivo para adolescentes Europeos, lo que refuerza el interés de nuestro análisis.

En la literatura científica se pueden encontrar algunos estudios similares en población infantil. En una cohorte de 1.142 preadolescentes de Finlandia, *Viljakainen et al.* (149) construyeron un *wGRS* para predecir el riesgo de sobrepeso (probabilidades aumentadas 1.39 veces) y obesidad (probabilidades aumentadas 1.41 veces), utilizando 30 *SNPs* relacionados con el IMC. Los autores indicaron, sin embargo, que su *GRS* tenía una baja capacidad predictiva sobre los cambios longitudinales del IMC a corto plazo. De manera similar, *Seyednasrollah et al*. (150) desarrollaron dos *wGRS* de 97 y 19 *SNPs* relacionados con el riesgo de obesidad en dos cohortes de niños/as y adolescentes de Finlandia. Comparativamente, el *GRS* formado por 19 *SNPs* mostró una capacidad predictiva ligeramente más alta que el *wGRS* de nuestro estudio (*AUC*=0.769 *vs*. 0.734).

La mayoría de los *GRSs* desarrollados en población infantil y adolescente se han hecho en sujetos de procedencia no Europea, fundamentados en *SNPs* asociados con riesgo de obesidad procedentes de *GWAS* anteriores. Comparativamente con los *GRSs* Europeos, los estudios no Europeos se desarrollaron con menor número de *SNPs* que pueden predecir el riesgo de obesidad. En un estudio transversal en niños/as y

adolescentes de Brasil (edad media=11.9 ± 2.8 años) (151) un *wGRS* de tres *SNPs*, basado en el IMC, se asoció con un incremento de la probabilidad del riesgo de sobrepeso y obesidad de 2.65. En una cohorte de jóvenes en edad escolar de China (7.3 a 11.1 años), *Fang J et al.* (152) desarrollaron un *uGRS* con 11 *SNPs* relacionados con obesidad que explicó un incremento de 0.11 kg/m^2 en el IMC. Por otra parte, *Lv, D et al.* (153) propusieron un *GRS* con 5 *SNPs* relacionados con obesidad que, en aquellos individuos con cinco o más alelos de riesgo, aumentaba el riesgo de obesidad en más de siete veces (edad 11.6 ± 2.5, *N*=2.977). En relación con los *GRSs* mencionados, cabe destacar que algunos de los *SNPs* utilizados en los *GRSs* de sujetos no Europeos coinciden con los mostrados en nuestro *GRS*, a pesar de asumir que el origen de los sujetos no permite hacer consideraciones de este tipo.

La construcción de nuestro *GRS* basado en el IMC comprende la inclusión de *SNPs* protectores y de riesgo en el mismo modelo. Algunos de esos *SNPs* han sido asociados significativamente con el riesgo de obesidad en estudios previos. El alelo A del *FTO*rs9939609 ha sido consistentemente relacionado con valores más altos de IMC y CC en varios estudios en adultos (38), adolescentes (154) y niños/as (155). En cohortes de niños/as y adultos con ascendencia Europea, *Frayling et al.* (38) y *Willer et al.* (156) encontraron las asociaciones más estrechas del alelo A del *FTO*rs9939609 con el IMC. Estos hallazgos confirman el papel del *FTO*rs9939609 como factor de riesgo de desarrollo de sobrepeso y obesidad. Además del FTOrs9939609, en un estudio de *Bokor et al.* (157), el *CD36*rs3211867 incrementó el riesgo de obesidad en casi dos veces en una cohorte de adolescentes con obesidad (*N*=307) y con normopeso (*N*=339) de Hungría. Aunque el estudio tenía dos muestras independientes de tamaño limitado, los hallazgos eran consistentes con los resultados de nuestro estudio. En otro estudio combinado de *Solaas et al.* (158), se observaron asociaciones significativas entre *LXRβ*rs17373080 y el riesgo de desarrollar sobrepeso u

obesidad, siendo 1.59 veces más probable. Cabe destacar que los dos últimos estudios incluyeron participantes de la cohorte del estudio *HELENA*. Por otro lado, el presente estudio muestra que el *THRA*rs1568400 se asocia negativamente con el riesgo de sobrepeso u obesidad, mientras el mismo *SNP* se relacionó con un IMC más elevado en un cohorte de adultos en España (159).

Otros *SNPs* incluidos en el *GRS* desarrollado en este estudio también han sido asociados con factores de riesgo cardiometabólico relacionados con la obesidad en adultos de diversas etnias, aunque en estos casos no se considera el riesgo de sobrepeso u obesidad. Por ejemplo, en población Europea, el *DRD2*rs180049743 modificó la relación entre el peso al nacer y el rendimiento académico en sujetos de Finlandia (160), mientras que el *FASN*rs424644444 atenuó el efecto sobre el diámetro máximo de las partículas de lipoproteínas de baja densidad *(low density lipoprotein (LDL))*, al consumir altas cantidades de grasa en una cohorte de Canadá (161). En población de China, el *NR3C1*rs770144345 mostró asociaciones significativas con un mayor riesgo de SM (162), mientras que los alelos CC del *IL-6*rs152410746 se relacionaron con mayor riesgo de desarrollar nefropatía en sujetos con DMT2 diagnosticada (163). Adicionalmente, el *PPARG*rs413527547 se asoció positivamente con las concentraciones de hemoglobina glicosilada y glucosa en plasma en pacientes con problemas de salud mental de Taiwán (164). En mujeres embarazas de Turquía, el *LPA*rs935529648 se asoció con la inflamación vascular como futuro indicador de accidentes cardiovasculares (165). Finalmente, el *CETP*rs478396149 mostró un efecto de interacción con el tiempo total de sueño, modulando los niveles de colesterol *HDL* en una gran cohorte de adultos de cinco etnias diferentes (166).

Por otro lado, varios *SNPs* incluidos en nuestro *GRS* (ej., *AMPD1*rs2010899, *NR3C1*rs4912905, *CNTFR*rs2183013, *IGF1*rs1019731 como factores protectores y

*NR3C1*rs13182800, *CNTF*rs2515362, *NOS2A*rs8068149, *THRA*rs7502966, *ANGPTL4*rs1044250 y *PTPN1*rs2143511 como factores de riesgo) son nuevos factores predictores, ya que no se habían asociado previamente con obesidad, con enfermedades relacionadas con la obesidad, ni habían sido significativamente relevantes en otros estudios previos. Conviene destacar que nuestro *GRS* mostró una buena capacidad predictiva de riesgo de sobrepeso y obesidad cuando se utilizaron otros parámetros de adiposidad como el IMG además del IMC, lo que refuerza nuestros hallazgos.

El *gold standard* para la construcción de un *GRS* ponderado es el que hace uso de los coeficientes provenientes de un meta-análisis. Sin embargo, cuando estos no están disponibles, el *uGRS* es la herramienta genética más comúnmente usada (167). En nuestro trabajo, los coeficientes considerados proceden del efecto genético interno de este mismo estudio. El *wGRS* superó ligeramente al *uGRS* en términos de potencia estadística (0.734 *vs*. 0.723). Convencionalmente, se acepta que el *AUC* en un análisis *ROC* debe ser de >0.8 para tener valor clínico en cuanto a la detección de una enfermedad (168). Cuando los modelos del *GRS* fueron construidos, el *AUC* no alcanzó el umbral propuesto combinando únicamente factores genéticos. Ya que los *SNPs* por sí mismos tienen una capacidad predictiva limitada, debemos considerar que los resultados obtenidos para construir el *wGRS* y el *uGRS* son estadísticamente aceptables y que contribuyen significativamente a mejorar la capacidad predictiva de los biomarcadores de obesidad en adolescentes. Sin embargo, nuestros *GRSs* deberían ser replicados en otras cohortes de características similares. Otros autores (169) sugieren que los predictores tradicionales, como la historia familiar y la obesidad infantil tienen un mayor poder predictivo que modelos basados en variantes genéticas establecidas. A pesar de la limitada capacidad predictiva de las variantes genéticas, el descubrimiento de nuevos polimorfismos relacionados con

obesidad han proporcionado información relevante sobre las distintas variantes que intervienen en la regulación del IMC (37). Dado que estas variantes comunes establecidas a través de *GWAS* explican una pequeña proporción de la variación del IMC, es probable que surjan otros lugares de riesgo cromosómicos de variantes más raras, aún por descubrir, cuando se incluyan muestras de mayor tamaño en estudios *GWAS*. Como tal, los presentes *GRSs*, o *GRSs* futuros incorporando *SNPs* de otros genes que no tienen, *a priori*, asociaciones previas con obesidad, podrían proporcionar resultados prometedores para minimizar el riesgo de accidentes cardiovasculares relacionados con obesidad.

5.3. Interacción genes x dieta y su efecto en composición corporal

Tras la construcción y validación de *GRSs* específicos de obesidad, hemos puesto en práctica esta herramienta genética para valorar su efecto modulador sobre la asociación entre DM y adiposidad y SM en los adolecentes del estudio *HELENA*. Los principales hallazgos de este estudio muestran que, la influencia de una alta adherencia a la DM sobre la adiposidad y el SM solo se hacía patente cuando un número limitado de alelos de riesgo estaban presentes. Además, el efecto de la interacción genes x DM fue más alto en chicas que en chicos adolescentes.

La DM se asocia con numerosos beneficios para la salud (52), como la reducción de factores de riesgo de enfermedades no transmisibles (77, 170). Sin embargo, se sabe poco sobre cómo la genética individual determina la influencia de la adherencia a la DM sobre la salud (171). Hasta donde sabemos, ningún estudio previo ha valorado el efecto de la interacción genes x DM utilizando un *GRS* específico de sobrepeso y obesidad en adolescentes Europeos. Los estudios previos disponibles se han centrado en el análisis de

la interacción entre la DM y *SNPs* aislados extraídos de genes candidatos que previamente se habían asociado con la obesidad o el SM (135, 146). Además, no se han encontrado estudios de interacción genes x DM sobre adiposidad y SM en adolescentes, ya que la inmensa mayoría de los estudios se han realizado en población adulta.

En línea con nuestros hallazgos, los *FTO*rs9939609 y *MC4R*rs17782313 mostraron una interacción significativa con la adherencia a la DM, lo que redujo el riesgo de obesidad y DMT2 en adultos Europeos (145). Asimismo, los niveles de obesidad se vieron atenuados en otros estudios que evaluaron la interacción entre el riesgo genético a padecer obesidad y la adherencia a patrones alimentarios diferentes a la DM . La ingesta baja de ácidos grasos poliinsaturados (*polyunsaturated fatty acid (PUFA)*) mostró una asociación inversa con el riesgo de obesidad (IMC $\geq$ 30 kg/m^2) cuando el polimorfismo *ADAM17i*33708A estaba presente (172). Otro estudio que estudió la interacción de dietas con alto contenido de grasas saturadas con el *THRA*rs1568400, mostró un IMC más alto en individuos portadores del alelo de riesgo (159), aunque su efecto de interacción era de sentido inverso en contraste con nuestros hallazgos. Además, nuestro estudio consideró la CC como marcador de la adiposidad abdominal, obteniendo niveles más bajos en chicos y chicas adolescentes, debido al efecto del *GRS* en los beneficios de la adherencia a la DM; hasta donde sabemos, no se han reportado hallazgos similares en otros estudios con características similares.

Con respecto a los parámetros que forman el SM, la presente Tesis Doctoral muestra niveles bajos de *HOMA* en la mayoría de las chicas adolescentes, que son modulados por el *GRS* de obesidad en el caso de adherirse a la DM. En línea con nuestros hallazgos, un estudio que evaluó los niveles de *HOMA* en portadores del *PLIN*11482, vio como la ingesta de grasas saturadas e hidratos de carbono resultaba en niveles más altos de *HOMA* en portadores del alelo menor (G>A) (173). En cuanto a la TAD, nuestros

adolescentes chicos con mayor adherencia a la DM presentaron niveles más bajos de TAD cuando tenían 21 o menos alelos de riesgo. Nuestros hallazgos muestran resultados similares con un estudio de intervención de 8 semanas de duración que promovía el patrón dietético *DASH* (*Dietary Approaches to Stop Hypertension*), donde los portadores del genotipo AA del gen del angiotensinógeno (polimorfismo *G-6A ANG*) mostraron la mayor reducción en la TAD (174). En términos de MTA, no se encontraron estudios de interacción en la literatura.

En cuanto al perfil lipídico, nuestro estudio incluye concentraciones séricas de colesterol *HDL* y TG en un clúster para valorar el riesgo cardiometabólico, que muestra una puntuación más baja de riesgo al SM cuando la puntuación del *GRS* es más baja, modulando los beneficios de una alta adherencia a la DM. Diversos estudios de la literatura aplican un enfoque similar al nuestro. *Ordovás et al.* observaron que las mujeres portadoras del alelo A del gen *APOA1* (polimorfismo G-A) respondían con mayores concentraciones de *HDL* a una ingesta elevada de *PUFA*, mientras que en las portadoras del alelo G se observaba el efecto contrario (175). Otro estudio mostró que, tras 12 meses de intervención basada en DM, se observaron concentraciones más altas de *HDL* y TG en aquellos individuos que portaban el alelo T del *CETP*rs3764261 que en aquellos con el genotipo GG [46]. En un estudio en pacientes con SM, se mostró que los sujetos portadores GG del *TNFα*rs1800629 tenían concentraciones más altas de TG que los portadores de A después de intervención con DM [47].

Finalmente, no ha sido posible contrastar nuestros resultados de interacción de genes x SM de forma global, como índice continuo en población Europea, por no haber encontrado trabajos similares en la literatura científica.

Como análisis de sensibilidad, y con el objeto de comparar nuestros resultados con otros trabajos previos, se calculó un segundo índice de SM de acuerdo con la definición

de la *IDF*. El índice de SM basado en los criterios de la *IDF* mostró que sus valores eran menores como resultado de la interacción del perfil genético sobre el aprovechamiento de la DM en chicas, pero no en chicos adolescentes. Este hecho podría deberse a los diferentes criterios específicos de edad y sexo seleccionados para definir los puntos de corte entre autores. Sin embargo, se observaron asociaciones positivas de la interacción *GRS* x DM en chicas adolescentes para ambos índices de SM. A día de hoy, se sigue apelando a la importancia del establecimiento de unos valores universales de valoración del riesgo a padecer SM en adolescentes. A pesar de ello, la unificación de criterios todavía permanece en desarrollo.

Dado que el SM y el exceso de adiposidad pueden ocurrir en cualquier etapa de la vida desde la niñez hasta la edad adulta, la detección y el diagnóstico tempranos son fundamentales para elaborar programas de prevención de la salud entre los jóvenes para reducir de manera efectiva el riesgo de enfermedades cardiovasculares y DMT2 (176, 177). Al mismo tiempo, se ha mostrado que una mayor adherencia a la DM se asoció con una mejora significativa en el estado de salud general entre los jóvenes, lo que sugiere que la DM es un patrón dietético apto para su implementación en la prevención de enfermedades crónicas desde edades tempranas (178).

5.4. Interacción sedentarismo x dieta y su efecto en composición corporal

Finalmente, en el análisis de interacción entre la adherencia a la DM y el tiempo sedentario de pantalla, la presente Tesis Doctoral muestra, inicialmente, una asociación inversa entre la DM y el tiempo sedentario de pantalla; además, hemos observado un efecto de modulación de la exposición al tiempo sedentario de pantalla sobre el aprovechamiento de la DM en los niveles de adiposidad de adolescentes Europeos. Así,

los beneficios asociados con una alta adherencia a la DM sobre la adiposidad solo se observaron en aquellas chicas adolescentes con menor tiempo sedentario de pantalla. Por otro lado, no se observó ningún efecto de interacción tiempo sedentario de pantalla x DM sobre la adiposidad en chicos adolescentes.

En línea con nuestros hallazgos, estudios previos reportaron una asociación inversa entre adherencia a la DM y tiempo sedentario entre los jóvenes. En una población de adolescentes Europeos del Mediterráneo, la inactividad (autorreportada) (179) estaba inversamente relacionada con la adherencia a la DM en ambos sexos (180). En adolescentes Europeos no Mediterráneos, se observó que una baja adherencia a la DM estaba asociado con mayor tiempo sedentario, aunque la evaluación de tiempo sedentario solo consideró tiempo sentado durante los días entre semana (181).

Hasta donde llega nuestro conocimiento, ningún otro estudio ha examinado el tiempo de pantalla como factor modulador en el efecto de la DM sobre los índices de adiposidad. Sin embargo, el efecto combinado de diferentes patrones de estilos de vida que evalúan su relación con la adiposidad se han reportado en estudios de clúster con poblaciones de edades similares (182-185). En cuanto al efecto combinado de hábitos no saludables, en niños/as Europeos, un clúster que incluyó un conjunto de actividades sedentarias (incluido el tiempo de pantalla), baja actividad física, bebidas dulces y poca ingesta de frutas y verduras se asoció con un IMC y una CC elevados (182). Resultados similares se encontraron en jóvenes no Europeos, donde un clúster no saludable formado por ver televisión y consumo de alimentos de alta densidad energética se asoció con un IMC alto (183). También se obtuvieron asociaciones similares en estudios longitudinales en niños/as Europeos (184) y no Europeos (183). Del mismo modo, se observaron asociaciones entre clústeres de patrones de estilo de vida saludable y niveles bajos de índices de adiposidad en niños/as Españoles (185).

Los análisis de clúster podrían ser útiles para evaluar el efecto combinado de factores ambientales y factores de comportamiento relacionado con el sobrepeso y la obesidad en niños/as y adolescentes (186). Sin embargo, no hemos encontrado estudios que hayan considerado el efecto de la interacción entre los factores incluidos. Aun así, un estudio reciente, realizado en una gran muestra de preadolescentes de 19 países (N=63.215), mostró que el tiempo sedentario de pantalla se asociaba con un mayor riesgo de sobrepeso u obesidad, independientemente de la combinación con otros comportamientos saludables no saludables (187). Este hallazgo sugiere que niveles altos de tiempo sedentario de pantalla podrían enmascarar los efectos beneficiosos de una dieta saludable, como la DM, en la disminución del riesgo de obesidad. En este sentido, se ha observado en estudios previos desarrollados en adolescentes, que comportamientos perjudiciales para la salud, como el consumo de dietas de alto contenido energético, pueden aparecer enmascarados debido a la coexistencia de estos factores con otros favorecedores de la promoción de salud, como la AF (188, 189); estos hallazgos respaldan los observados en nuestro estudio. Unos niveles bajos de AF también podrían contrarrestar el efecto beneficioso de un patrón dietético saludable (190).

Por otro lado, resulta complejo elucidar los posibles mecanismos responsables de la disparidad en cuanto al sexo de los resultados obtenidos. Al igual que en otros estudios en adolescentes Europeos (191), se observó que el tiempo dedicado a las actividades de pantalla era mayor en los chicos que en chicas adolescentes. Sin embargo, hay datos contradictorios al respecto en la literatura (181). El tiempo sedentario de pantalla podría ser un indicador de variabilidad en cuanto a sexo, pero necesita más investigación para obtener datos comparables que expliquen estas diferencias en el presente análisis. Otra alternativa que podría explicar las diferencias en cuanto al sexo podría ser la menor duración del sueño y, por tanto, una mayor adiposidad en chicas que en chicos. Esta

hipótesis fue observada previamente en otras cohortes de edades similares (192). La duración corta del sueño se observó que estaba asociada con un aumento de los biomarcadores de adiposidad en los adolescentes *HELENA*, particularmente en chicas adolescentes (106); también se observaron asociaciones entre la duración corta del sueño y una menor calidad de la dieta (193). Adicionalmente, también se observaron diferencias de sexo en un estudio de clústeres formado por AF, comportamientos sedentarios y duración del sueño según biomarcadores endocrinos, metabólicos e inmunológicos, lo que sugiere nuevas vías para enfocar nuestra investigación en el futuro (194).

Entre los adolescentes incluidos para desarrollar el análisis de interacción tiempo sedentario de pantalla x DM, se identificaron una serie de valores extremos en el número de horas del tiempo sedentario de pantalla. Para descartar posibles diferencias en los resultados obtenidos, se llevó a cabo un análisis de sensibilidad considerando *vs.* no considerando esos valores atípicos extremos. No hubo diferencias significativas entre los dos modelos propuestos (asumiendo *vs.* no asumiendo esos valores extremos); por lo tanto, los resultados finales no se vieron afectados por esas diferencias específicas en el tiempo sedentario de pantalla.

Además, dentro de los índices de adiposidad que se valoraron en nuestro estudio, se observó cierta variabilidad entre ellos en función de la exposición al tiempo de pantalla. Es decir, con una mayor adherencia a la DM, se observaron niveles más bajos de CC que IMC e IMG, en aquellas chicas adolescentes con menor tiempo sedentario de pantalla, mientras que se observó mayor CC en aquellas chicas adolescentes con mayor tiempo sedentario de pantalla, en comparación con el IMC y el IMG. Hallazgos similares fueron encontrados en cuanto a diferencias de IMC y CC en estudios previos con adolescentes (195).

Como consideraciones generales, hay que tener en cuenta que otros dispositivos de tiempo de pantalla, como *Tablet*, *Smartphone* y consolas, se están volviendo más dominantes que otros dispositivos más tradicionales como es la televisión; por lo tanto, estudios futuros deberían incluir estos dispositivos en su evaluación de comportamientos sedentarios basados en el tiempo de pantalla. Como hemos observado, existe una asociación negativa entre el tiempo sedentario de pantalla y la adherencia a la DM sobre los índices de adiposidad entre los jóvenes. De este modo, se deberían considerar en programas conjuntos de implementación de salud pública orientados tanto a evitar el exceso de tiempo de pantalla, como a promover el patrón dietético de DM para la prevención primaria de las principales enfermedades crónicas. En programas anteriores de prevención de obesidad infantil, los cambios más pronunciados se observaron en el tiempo sedentario (196); de ahí la importancia de incluir este tipo de variables en futuros estudios que valoren estilos de vida.

5.5. Nutrición personalizada y perspectivas de futuro

El uso óptimo de los beneficios de la DM en los programas de prevención y tratamiento del sobrepeso, la obesidad y el SM en población joven, se realizan a través del enfoque personalizado de la dieta y los hábitos de vida de cada individuo. En este sentido, en los últimos años se han puesto en marcha algunos estudios de intervención que contemplan un abordaje personalizado con la DM. Por ejemplo, el estudio *MED4youth*, un estudio controlado aleatorizado (ECA) multicéntrico que actualmente está curso, cuyo objetivo es reducir los niveles de obesidad mediante la promoción de la DM frente a la dieta tradicional baja en grasas en adolescentes Europeos (197). Uno de los objetivos principales del estudio es aplicar un enfoque denominado ómico para observar si las intervenciones propuestas

podrían modular la microbiota intestinal y los metabolitos derivados para investigar sus mecanismos y maximizar el efecto beneficioso de una alta adherencia a la DM (197). Además, otro estudio, el *Food4me*, reclutó a adultos Europeos para realizar una intervención nutricional online para evaluar el efecto de las intervenciones personalizadas en los cambios dietéticos asociados con la DM (198). Los participantes fueron asignados aleatoriamente para recibir diferentes tipos de asesoramiento durante la intervención. Uno de los hallazgos más relevantes del estudio fue que el asesoramiento nutricional personalizado que consideraba la dieta, el fenotipo y el genotipo fue el que obtuvo mejores resultados en la puntuación del índice de DM (198). Estos tipos de intervenciones personalizadas resaltan la relevancia de comprender las bases moleculares que participan en los efectos de la DM sobre la salud.

La integración de colaboraciones interdisciplinares post *GWAS* que combinan tecnologías ómicas y técnicas computacionales, nos permite incorporar un número importante de marcadores utilizando genómica, epigenómica, metagenómica, metabolómica, entre otras, que reflejan mejor los procesos de interacción que tienen lugar a nivel molecular y celular (171, 199-202). Estas contribuciones han ayudado a comprender los efectos de la DM en los fenotipos intermedios o finales de la salud cardiovascular (203). Sin embargo, abordar nuevos análisis con una combinación de alimentos, nutrientes y fitoquímicos en lugar de componentes individuales de la DM, puede proporcionar diferentes efectos, ya sean separados o combinados, en diferentes niveles. Esta línea de investigación compleja y multidimensional se ha convertido en el siguiente desafío para los próximos años (204).

6. APORTACIONES PRINCIPALES DE LA TESIS

Existen multitud de estudios que han mostrado una fuerte asociación entre la adherencia a la DM y los parámetros de composición corporal en población joven. No obstante, el papel que juegan otros factores, como el perfil genético del individuo y el tiempo sedentario de pantalla, interactuando con los efectos beneficiosos de la DM en términos de composición corporal, permanece poco estudiado. La presente Tesis Doctoral ha investigado el posible efecto de estos factores de interacción sobre la salud, y así contribuir a evitar el establecimiento de enfermedades crónicas desde edades tempranas.

A continuación, se explican las principales contribuciones de cada uno de los artículos incluidos en la presente Tesis Doctoral:

Artículo I. Se describe y se pone en valor la importancia de los estudios de interacción genes x MD y su influencia sobre la obesidad y el SM. A destacar el escaso número de estudios genes x DM en adolescentes en comparación con realizados en población adulta. Se destaca la importancia de la investigación en nutrición personalizada desde edades tempranas.

Artículo II. Se desarrollan con éxito dos *GRSs* (*uGRS y wGRS*) en adolescentes Europeos, que incluyen *SNPs* protectores y de riesgo como predictores del riesgo de sobrepeso y obesidad, para poder ser utilizados como herramienta genética en otras cohortes de edad y etnia similares.

Artículo III. Se observa que en el estudio de interacción genes x MD, el perfil genético tiene una influencia directa modulando el aprovechamiento de los efectos de la DM sobre la adiposidad y SM en adolescentes Europeos.

Artículo IV. Se comprueba que otros factores ambientales modificables, como el tiempo sedentario de pantalla, pueden modular el aprovechamiento de la DM en términos de adiposidad en adolescentes Europeos.

Es importante considerar que los efectos de las intervenciones basadas en DM sobre la obesidad y el SM pueden variar dependiendo del perfil genético de cada individuo, hecho que debería ser considerado en la valoración de la eficacia de las intervenciones. Además, la evidencia de que existen efectos que coocurren como la DM y tiempo sedentario de pantalla, pueden ayudar a entender cómo los comportamientos sedentarios y dietéticos deben ser abordados simultáneamente en programas de prevención de obesidad y SM.

6. MAIN THESIS CONTRIBUTIONS

Several studies have shown a strong association between MD adherence and body composition parameters in young population. However, the role of other factors, such as the genetic profile and screen sedentary time, which directly interact with the benefits that MD exerts in terms of body composition, remains scarcely studied. The present Doctoral Thesis has assessed the potential effects of these interaction factors and their impact in the preservation of health status against the establishment of chronic diseases at young age.

In summary, the main contributions of each of the articles included in the present Doctoral Thesis are:

Article I. The importance of gene x diet interaction studies and how they behave in relation with obesity and MetS is described and put into value. It is relevant to mention the scarce contribution of gene x MD studies in adolescents compared to the ones found in adult population, and the importance of applying personalized nutrition and omics sciences from early age.

Article II. Both GRSs (uGRS and wGRS) are successfully developed with protector and risk SNPs in European adolescents, which predict the risk to overweight and obesity, so it can be used as genetic tool to assess the genetic predisposition to overweight and obesity in other cohorts of similar age and ethnicity.

Article III. It is observed that, in gene x diet interaction studies, the genetic profile has a direct influence modulating the MD benefits in terms of adiposity and MetS in European adolescents.

Article IV. There are other modifiable environmental factors, such as screen sedentary time, which, in addition to genes, can modulate the MD benefits in terms of adiposity in European adolescents.

It is important to consider that the effect of future interventions could vary depending on the genetic profile of each individual and should be considered in assessing the efficacy of future analyses. In addition, the evidence that there are co-occurring effects such as MD and screen sedentary time, can help to understand how sedentary and dietary behaviors should be addressed simultaneously in obesity and MetS prevention programs.

7. CONSIDERACIONES METODOLÓGICAS

El estudio *HELENA* incluyó una amplia muestra de adolescentes Europeos, que es representativa de las ciudades seleccionadas (que no de los países de procedencia), donde los adolescentes se estratificaron por edad y sexo. Las selección de las ciudades no fue al azar, sino que se siguieron una serie de criterios, siendo el más importante, la existencia de un grupo de investigación con capacidad para realizar este estudio. Las ciudades debían tener una población de más de 100.000 habitantes y ser equivalentes y comparables en relación con cada uno de los países y con una muestra suficientemente grande como para asegurar la diversidad de la población (107). Además, las áreas rurales no se vieron representadas. Las escuelas fueron seleccionadas al azar tras estratificación por distrito escolar. La captación de adolescentes a través de las escuelas se realizó mediante el contacto con padres y/o tutores y los propios adolescentes interesados en el estudio, lo que puede suponer un sesgo al incluir a aquellos individuos que tienen una preocupación real por la salud de sus hijos adolescentes (205).

Además, en todos los artículos incluidos en la presente Tesis Doctoral se ha trabajado con la muestra de inicial de 3.528 adolescentes, menos en el desarrollo del Artículo II, que se incluyeron 4.356 adolescentes, ya que se consideró que para los análisis genéticos era más importante aumentar el tamaño de la muestra que la influencia que podía tener la edad o el desarrollo puberal.

La compilación de artículos originales enmarcados dentro del estudio *HELENA*, recoge una serie limitaciones y fortalezas relacionadas con el diseño del propio estudio. Las principales limitaciones son:

1) En el estudio *HELENA,* solo están disponibles lugares específicos del cromosoma que son seleccionados como asociados a un mayor riesgo. Dado que las

variantes comunes establecidas de *GWAS* sólo explican una pequeña proporción de la variación del IMC (37), es probable que surjan otros lugares de riesgo cromosómicos de variantes más raras, aún por descubrir, cuando se incluyan muestras de mayor tamaño en estudios *GWAS*.

2) No hay datos disponibles sobre el parentesco o los orígenes étnicos entre los participantes estudiados; las frecuencias alélicas y el tamaño de su efecto pueden ser diferentes de las poblaciones no Europeas. Por tanto, los resultados obtenidos no deben extrapolarse a otras etnias.

3) Para probar la validez de este *GRS* específico de sobrepeso y obesidad, los resultados obtenidos deben confirmarse en poblaciones de estudio infanto-juveniles más grandes, de etnia similar, valorando también la incidencia de obesidad. Aunque el modelo para desarrollar los *GRS* se validó internamente, realizando un análisis de validación cruzada de 10 veces, entendemos que la situación óptima hubiera sido una validación externa en una cohorte independiente.

4) En el desarrollo del *GRS* de sobrepeso y obesidad, encontraron *SNPs* más comunes en *GRSs* no Europeos que en *GRSs* Europeos. Este hallazgo podría deberse a la mayor cantidad de *GRSs* desarrollados en otras etnias en comparación con la cantidad de *GRSs* realizados en la población Europea.

5) Al utilizar medidas informadas por los padres, existe riesgo de que se reporten datos sesgados, por ejemplo en los informes sobre las dietas de los adolescentes, los patrones de actividad, el tiempo sedentario de pantalla y otros aspectos del balance energético, que no se pudieron medir objetivamente.

6) La valoración de los hábitos alimentarios reportados en dos días no consecutivos puede no tener en cuenta determinados periodos de tiempo, como fines de semana o vacaciones o la variación estacional del consumo de alimentos.

Las principales fortalezas de este estudio incluyen:

1) El diseño multicéntrico del estudio *HELENA* implicó la participación de adolescentes de 10 ciudades Europeas. Esto permitió a los investigadores utilizar una gran base de datos con información relevante y diversa de diferentes poblaciones de todo el continente Europeo.

2) Se han desarrollado pocos índices para predecir el riesgo de sobrepeso y obesidad, particularmente en adolescentes Europeos, una población poco estudiada desde la perspectiva de la prevención y el tratamiento temprano.

3) El índice genético propuesto para predecir el riesgo de sobrepeso y obesidad podría contribuir de manera eficiente a discernir la población con riesgo de sobrepeso y obesidad, y no la obesidad únicamente.

4) Existen pocos artículos en la literatura que consideren los efectos de interacción genes x DM en términos de composición corporal en jóvenes Europeos. La mayoría de los estudios similares están disponibles exclusivamente en población adulta.

5) El desarrollo de un índice de riesgo cardiometabólico se consideró adecuado para la valoración del riesgo a padecer SM, ya que proporciona mayor sensibilidad y baja susceptibilidad a errores en comparación con el estudio de parámetros cardiovasculares a nivel individual (206).

6) Hemos incluido el patrón de DM completo, desarrollando un índice derivado del consumo de diferentes grupos de alimentos en una escala de adherencia en lugar de considerar la ingesta de macronutrientes individualmente o grupos de alimentos específicos (118).

7) El alto estándar de implementación en términos de metodología y diseño del estudio *HELENA*, así como la gran cantidad de comportamientos sedentarios considerados para estimar el tiempo de pantalla en la presente cohorte de participantes.

El diseño transversal del estudio *HELENA* ha permitido establecer asociaciones entre variables de interés en cada análisis o hipótesis planteado, pero no así establecer relaciones de causalidad. Para dar un paso más allá en las relaciones descritas hasta el momento, sería conveniente replicar estos resultados en muestras Europeos de igual o mayor tamaño, en adolescentes Europeos, y de un modo prospectivo longitudinal.

En 2011, la *Communication Star* de la Unión Europea premió al estudio *HELENA* dentro de la categoría de *proyectos pequeños* entre 25 candidatos, por ser el proyecto enmarcado dentro del sexto Programa Marco que más decisivamente había contribuido dentro del sector de la nutrición, además de la difusión de sus resultados de una manera efectiva dentro de la comunidad científica (207).

7. METHODOLOGICAL CONSIDERATIONS

The HELENA study included an important sample of European adolescents, which are representative of the selected cities (not to the countries of origin), where the adolescents were stratified by age and sex. The selection of cities was not random. A series of criteria were followed, being the existence of a research group in the area with capacity to carry out the project, one the most important considerations. The selected city required to have more than 100.000 habitants, and to be equivalent and comparable in relation to the ones in each of the other countries, as well as having a sufficiently large sample size to ensure the diversity of the studied population (107). Furthermore, rural areas were not represented. Schools were randomly selected after stratification by school district. Recruitment of adolescents through school was carried out contacting parents and/or guardians and those adolescents interested in the study, which could lead to risk of bias by only including those individuals who have a real concern of their health status.

The cross-sectional design of the HELENA study has made it possible to establish associations between variables of interest in each analysis or hypothesis proposed, not to establish causal relationships. Therefore, to go beyond the relationships described so far, it would be convenient to replicate these results in European samples of equal or larger sample size, and following a longitudinal prospective approach (205).

In addition, an initial sample of 3.528 adolescents has been considered in all articles included in the present Doctoral Thesis, with the exception of Article II, which included a total of 4.356 adolescents, since it was considered being more important to increase the sample size with genetic information available than the potential influence that age or pubertal development could have in the analysis.

The compilation of original articles within the HELENA study framework has a series of limitations and strengths related to the study design.

The main limitations are:

1) Only selected risk loci are available in the HELENA study. Since the established common variants from GWAS explain a small proportion of the BMI variation (37), it is likely that other loci from rarer variants, still to be discovered, will emerge when larger sample sizes are included in GWAS.

2) There is no data available regarding the relatedness or the ethnic origins among the studied participants; the allele frequencies and their effect size might be different from non-European populations and the outcome should not be extrapolated to other ethnicities.

3) In order to test the reliability of this obesity-specific GRS, the results should be validated in larger children and adolescents study populations with similar ethnicity, also considering obesity incidence. Additionally, although the model to develop the GRSs was internally validated performing 10-fold cross validation analysis, we understand that the optimal situation would have been an external validation in an independent cohort.

4) During the development of the overweight and obesity specific GRS, more common SNPs in non-European GRSs were found than in European GRSs. This finding could be due to the higher number of GRSs developed in other ethnicities in comparison to the number of GRS performed in European population.

5) By relying on measurement reports filled by parents, there is a risk of bias in the data reported, for example in reports on adolescent´s food consumption, activity patterns, screen time and other aspects of energy balance, which could not be objectively measured.

6) Although the two non-consecutive days assessment of dietary habits might not consider certain periods of time such as weekends or holidays, this method has previously shown good validity and accuracy in similar populations (118).

The main strengths in the study include:

1) The multicentric design of the HELENA study considered the participation of adolescents from 10 European cities. This allowed the researchers to use a large database with relevant and diverse information from different populations across the European contient.

2) Only few GRSs to predict the overweight and obesity risk have been developed particularly in European adolescents, an understudied population for the prevention and the early treatment perspectives.

3) The proposed genetic score of predisposition to obesity defined in this study might efficiently contribute to discern population at risk for overweight and obesity, not just obesity alone.

4) There are a few manuscripts in the literature considering gene x MD interaction effects in terms of body composition in European youth; however, the majority of studies with similar approach are exclusively available in adult populations.

5) The development of the cardiometabolic risk score was considered appropriate for the present study as it provides higher sensitivity and low susceptibility to errors compared to other approaches (206).

6) We included the whole MD pattern developing a cluster of different food groups in an adherence scale rather than considering single macronutrients or individual specific food groups´ intake.

7) It is important to highlight the high standard implementation in terms of methodology and design of the HELENA study, as well as the large number of sedentary behaviors considered to estimate screen time in the present cohort of participants (108).

The cross-sectional design of the HELENA study has made it possible to establish associations between variables of interest in each analysis or hypothesis proposed, but not to establish causal relationships. To take a step further in the relationships described so far, it would be convenient to replicate these results in European samples of equal or larger sample size, in European adolescents, and in a longitudinal prospective approach.

In 2011, the Communication Star awarded the HELENA study, in the small project´s category, with other 25 participating studies, for being the project within the Sixth Framework Program that had contributed most decisively in the nutrition sector, as well as putting into value the dissemination of its results in an effective way within the scientific community (207).

8. CONCLUSIONES

Artículo I.

Los estudios analizados en jóvenes Europeos que consideran la interacción genes x DM, sugieren la posibilidad de que el genotipo relacionado con obesidad podría modular la relación que existe entre la adherencia a la DM, la obesidad y el SM.

Artículo II.

Los *GRSs* desarrollados (*wGRS* y *uGRS*) pueden ser considerados como herramientas genéticas útiles para evaluar la predisposición al sobrepeso u obesidad en un individuo, permitiendo avanzar en la prevención y el manejo de esta enfermedad desde etapas tempranas de la vida.

Artículo III.

Los genotipos relacionados con la obesidad tuvieron un efecto modulador en la relación entre la adherencia a la DM y el riesgo de obesidad y SM en adolescentes Europeos. El efecto de la interacción genes x dieta sobre el SM fue más fuerte en chicas que en chicos adolescentes.

Artículo IV.

En adolescentes Europeos, el tiempo sedentario de pantalla y la adherencia a la DM se encuentran asociados inversamente. Además, en chicas adolescentes Europeas, el tiempo sedentario de pantalla tuvo un efecto modulador en la asociación entre adherencia a la DM y adiposidad.

En conclusión, el número de trabajos que estudian la interacción genes x DM en relación con el riesgo de obesidad o SM desarrollados en población joven es

considerablemente limitado. El desarrollo de futuros *GRSs* específicos en muestras con mayor número de participantes jóvenes de origen Europeo facilitaría la detección de variantes genéticas nuevas, no relacionadas previamente con la obesidad, que puedan influir en el riesgo genético de desarrollo de la enfermedad, además de las variantes previamente conocidas. De esta forma, se podrá facilitar la observación de un efecto modulador del genotipo sobre la asociación entre patrones dietéticos y parámetros de composición corporal, como es el caso de la DM y los indicadores de obesidad y SM. Además, el tiempo sedentario de pantalla también puede actuar como factor modulador de la asociación entre DM y adiposidad. Estos hallazgos apoyan la idea de aplicar más recomendaciones personalizadas en programas de salud pública para introducir la DM como patrón de dieta saludable, así como controlar la exposición a dispositivos de pantalla entre los jóvenes, con el fin de disminuir los niveles de adiposidad y el riesgo de padecer SM en edades tempranas. Se necesita más investigación en esta línea, para entender mejor las diferencias inter-individuales en la asociación entre DM y obesidad y SM, así como los mecanismos que subyacen a los efectos protectores de la DM sobre la salud cardiovascular en general, mediante la integración de las ciencias ómicas y la nutrición personalizada.

8. CONCLUSIONS

Article I.

The studies considering gene x MD interactions, which were analyzed in European youth, suggest the possibility that obesity related genotypes could modulate the relationship between MD adherence and obesity and MetS risk.

Article II.

The GRSs developed (uGRS and wGRS) could be considered as a useful genetic tool to evaluate individual's predisposition to overweight and obesity, allowing to advance in the prevention and management of the disease from early stages in life.

Article III.

Obesity-related genotypes had a modulation effect in the relationship between MD adherence and obesity and MetS risk in European adolescents. The gene x diet interaction effect on MetS was stronger in female than in male adolescents.

Article IV.

Screen sedentary time and MD adherence are inversely associated in European adolescents. Moreover, in female European adolescents, screen sedentary time had a modulatory effect in the association between MD adherence and terms of adiposity.

In conclusion, the number of gene x MD interaction analyses in relation to the risk of obesity or MetS performed in young populations are considerably limited. Future GRS development with larger samples involving subjects of European origin will be able to detect new variants previously not related to a disease that could influence the genetic risk of that

disease, other than the common ones. In this way, it will be possible to facilitate the observation of a modulating effect of the genotype on the association between dietary patterns and body composition parameters, such as MD and obesity and MetS variables. Furthermore, screen sedentary time can also act as a modulating effect in the association between MD and adiposity. These findings support the idea of applying more personalized recommendations in public health programs to introduce MD as a healthy dietary pattern, as well as controlling exposure to screen devices among youth, in order to reduce the levels of adiposity and MetS risk from early ages. Further research is needed, to better understand the inter-individual differences in the association between MD and obesity and MetS, as well as the mechanisms behind the protective effects of MD in the overall cardiovascular health through the integration of omics and a personalized nutrition.

9. REFERENCIAS

9. REFERENCES

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APÉNDICE

APPENDIX

Factor de impacto de las revistas en las cuales se han publicado los artículos incluidos y ranking en *"ISI Web o Knowledge – Journal Citation Reports (JCR)"* dentro de sus áreas temáticas correspondientes.

[Impact factor of the Journals in which the included articles have been published and their ranking in *"ISI Web o Knowledge – Journal Citation Reports (JCR)"* considered by subject categories].

Artículos publicados [Published manuscripts]:

Revista [Journal]	**Factor de impacto [Impact Factor]**	**Cuartil [Quartile]**
Artículo I [Article I] Genes	4.096	Q2
Ranking in 2020 ISI JCR: 66/176 (Genetics & Heredity)		
Artículo II [Article II] Scientific Reports	4.380	Q1
Ranking in 2020 ISI JCR: 17/72 (Multidisciplinary Sciences)		
Artículo III [Article III] Nutrients	5.719	Q1
Ranking in 2020 ISI JCR: 17/88 (Nutrition & Dietetics)		
Artículo IV [Article IV] Nutrients	5.719	Q1
Ranking in 2020 ISI JCR: 17/88 (Nutrition & Dietetics)		

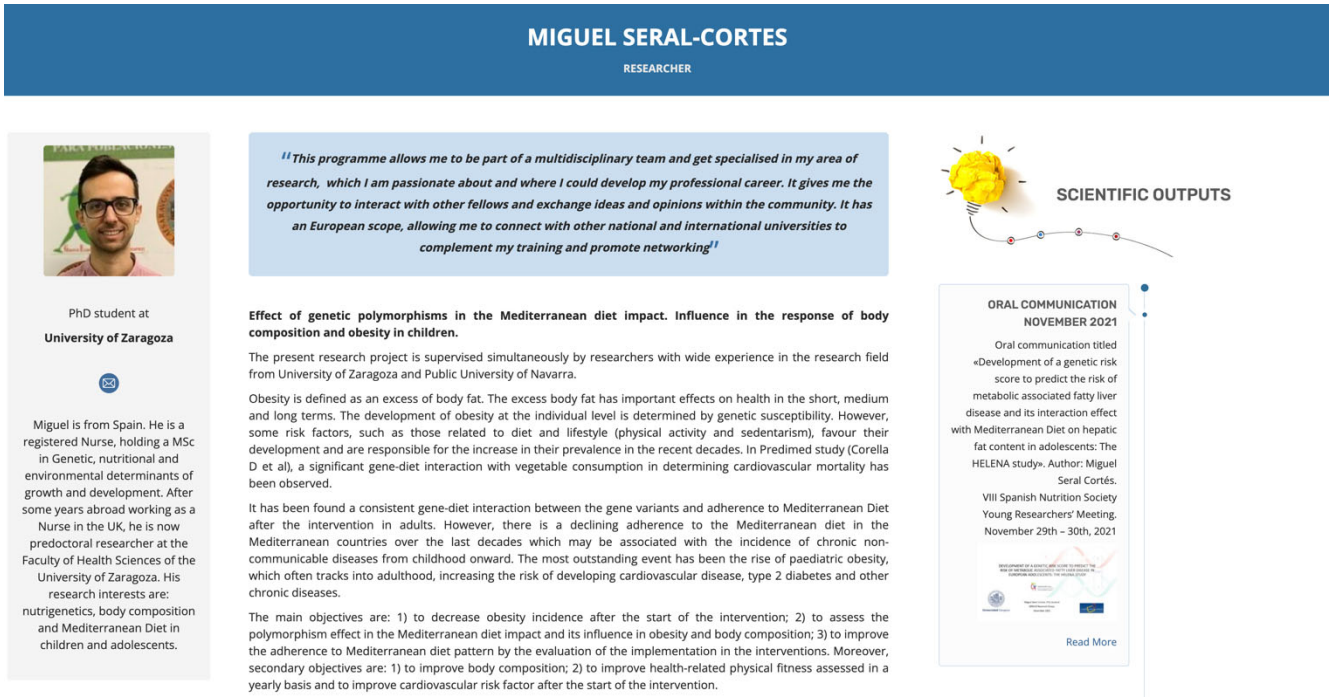
SOBRE EL AUTOR

ABOUT THE AUTHOR

A través del enlace mostrado a continuación, se puede obtener información sobre el perfil investigador del Doctorando Miguel Seral Cortés:

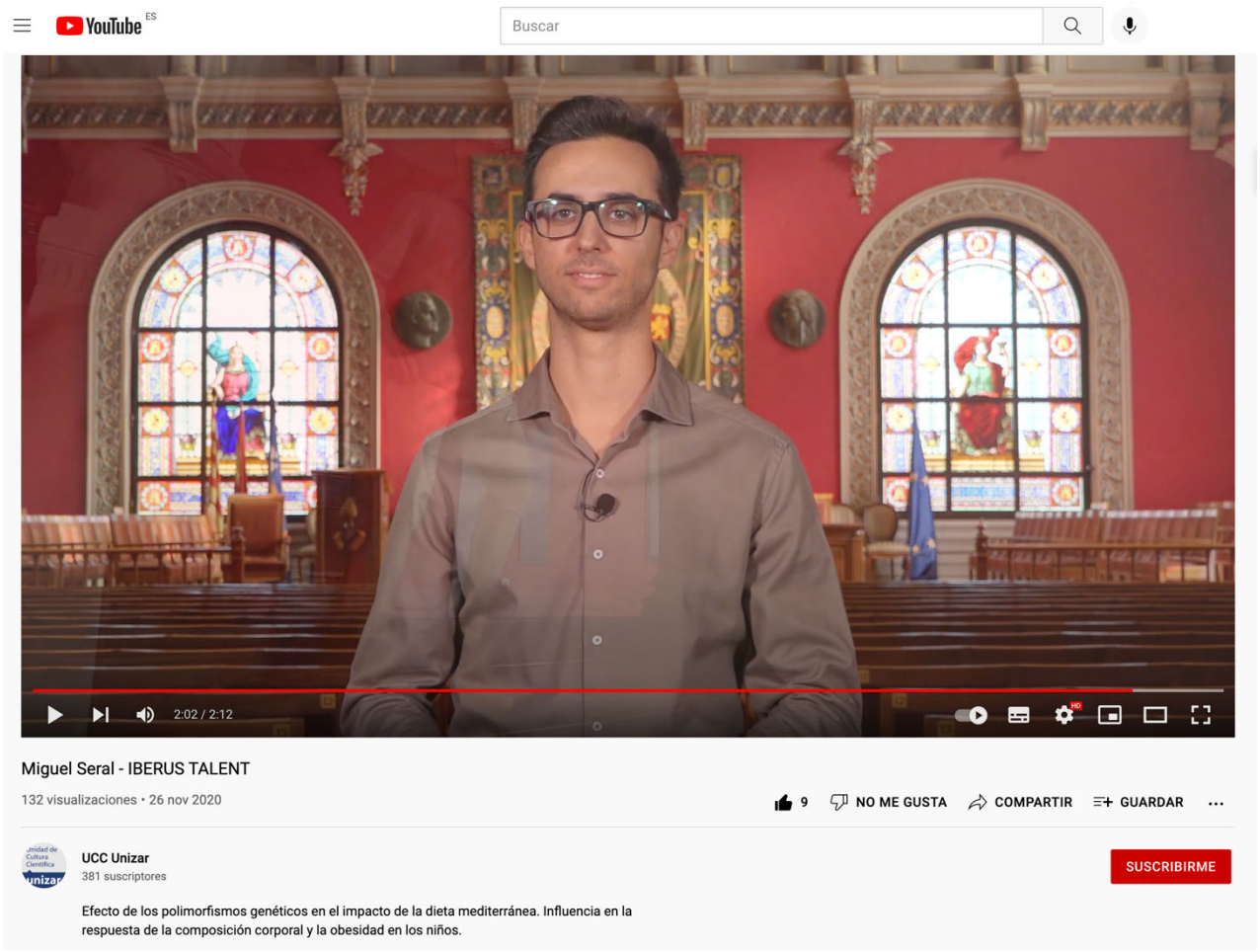
- Descripción personal.
- Línea de investigación: supervisión, estado del arte, objetivos principales.
- Publicaciones.
- Asistencia y participación en congresos.
- Divulgación científica en medios de comunicación.

https://www.iberustalent.com/researcher/miguel-seral-cortes/



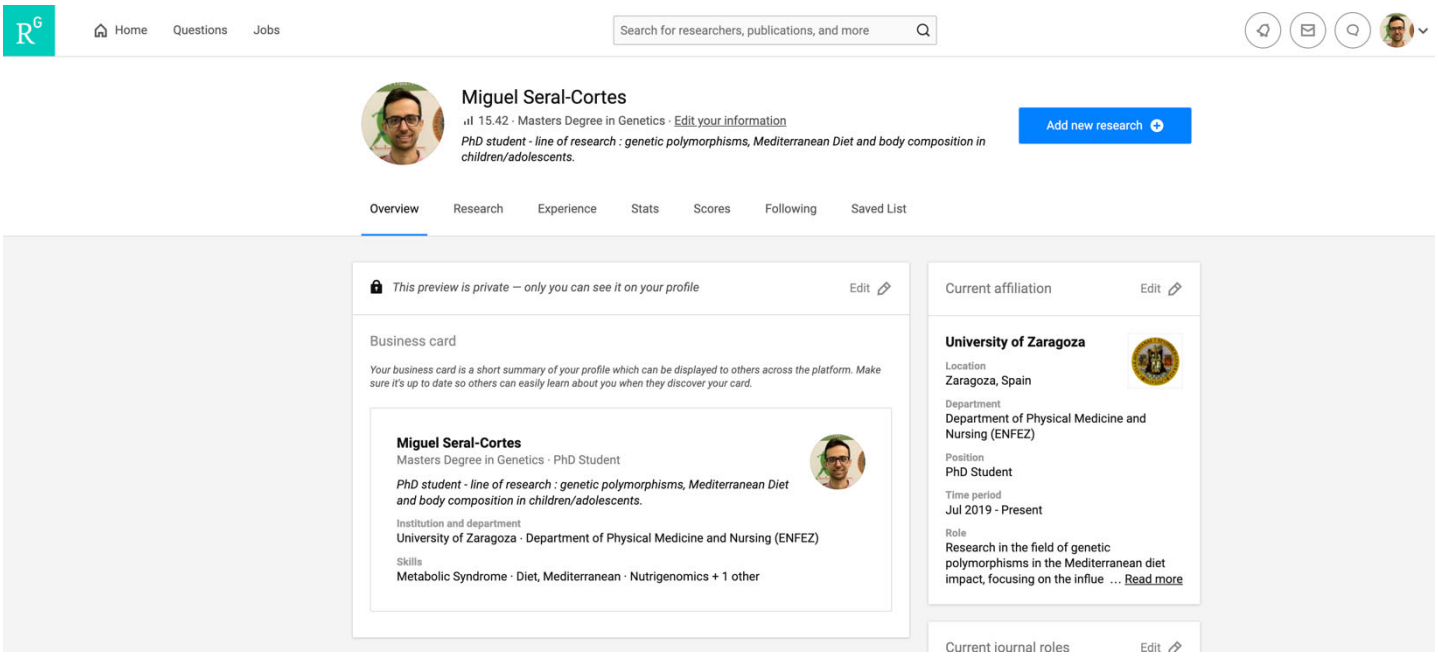
Además, se muestra a continuación la participación del Doctorando en la Noche Europea de los Investigadores e Investigadoras en Noviembre de 2020, mostrando un breve resumen de la línea de investigación seguida en el desarrollo de la presente Tesis Doctoral:

https://www.youtube.com/watch?v=PCxLH5C_QzU



Finalmente, el enlace para obtener información sobre el perfil investigador y estadísticas sobre el impacto de las publicaciones dentro de la comunidad científica:

https://www.researchgate.net/profile/Miguel-Seral-Cortes



AGRADECIMIENTOS

ACKNOWLEDGEMENTS

Quisiera no dejarme a nadie para agradecer la aportación de tantas personas que han hecho posible el desarrollo y finalización de esta Tesis Doctoral, durante los últimos 5 años.

Para empezar, agradecer a Luis la oportunidad que me brindó de poder formarme como investigador bajo su amparo y supervisión. Sin saber con certeza qué línea de investigación seguir o si podríamos conseguir algún tipo de financiación, él con la bondad que le caracteriza, accedió a comprometerse conmigo en este viaje, para mi tan desconocido por aquel entonces. Con el tiempo, me di cuenta de que había acertado de lleno en mi decisión. Tener como jefe a un investigador de referencia a nivel internacional en el ámbito de la nutrición es un privilegio a los que pocas personas pueden acceder. Muchas gracias Luis por tu tiempo y dedicación, por tu profesionalidad y por ser fuente de inspiración, ahora soy mejor profesional y persona.

Tan importante como la dirección de Tesis de Luis, han sido las figuras de mis codirectoras, Idoia y Pilar. Ambas han compartido todos los conocimientos y experiencia que poseen para progresar de manera efectiva en cada uno de los obstáculos que se presentaban a lo largo de esta etapa. Idoia, muchas gracias por tus consejos, tu cercanía y tu sinceridad, sin duda esta Tesis no hubiera posible sin tu ayuda. Gracias Pilar, por acogerme desde el primer día y enseñarme el funcionamiento de GENUD, y por tu supervisión en todas las tareas que he realizado estos últimos años.

Además, gracias a todas las personas que han compartido este progreso en GENUD conmigo, tanto la rama de nutrición como INEF. Mención especial a las fantásticas, Mary,

Paloma y Natalia, con las que he pasado buenas y malos momentos, y siempre han estado ahí para brindarme su apoyo.

Para terminar con el capítulo académico, agradecer al Campus Iberus, en particular al personal en cargo de Iberus Talent, por darme la oportunidad de formarme con un contrato Europeo Marie Curie, y poner todo de su parte para asegurarse de que tanto los compañeros como yo estemos formados y atendidos por profesionales de alto nivel; a la Universidad de Helsinki, en especial a Jaakko Kaprio, por darme la oportunidad de vivir la experiencia de participar en un grupo de investigación internacional durante más de tres meses, a pesar de atravesar las peores fases de la pandemia. Por último, gracias a Luis Mariano y Sergio, unas bestias de la estadística y la programación, imposible terminar esto sin vuestro apoyo.

En lo personal, indispensable el apoyo incondicional de mi familia, mis padres, Máximo e Inmaculada; mi hermano, Alejandro y mi pareja, María. No sólo durante el periodo como estudiante de Doctorado, sino desde que tengo uso de razón. Sin ellos no podría ser nada de lo que soy ahora. Esta vez, os doy las gracias por escucharme y apoyarme, a pesar de soy consciente que es difícil de imaginar muchas veces lo que hago cuando trabajo. Precisamente, es en estos momentos cuando me doy cuenta que, independientemente de mis éxitos o fracasos, siempre vais a estar ahí.

Y por último, gracias a todas aquellas personas, que en mayor o menor medida, han contribuido a que por fin, tras mucho esfuerzo y sacrificio, pueda escribir estas líneas como colofón a otra etapa de mi vida que voy a recordar con mucho cariño y emoción.

Miguel Seral-Cortes, has received funding from the Iberus Talent Pre-doctoral fellowships 2018, under the European Union's H2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 801586.

ANEXOS

ANNEXES

Para obtener una información más precisa de la metodología llevada a cabo en el estudio *HELENA* se adjunta el siguiente artículo:

Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study

LA Moreno, S De Henauw, M González-Gross, M Kersting, D Molnár, F Gottrand, L Barrios, M Sjostrom, Y Manios, CC Gilbert, C Leclercq, K Widhalm, A Kafatos and A Marcos, on behalf of the HELENA Study Group. Int J Obes (2008) 32, S4–S11.

Objective: To provide an overview of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) design, with particular attention to its quality control procedures. Other important methodological aspects are described in detail throughout this supplement.

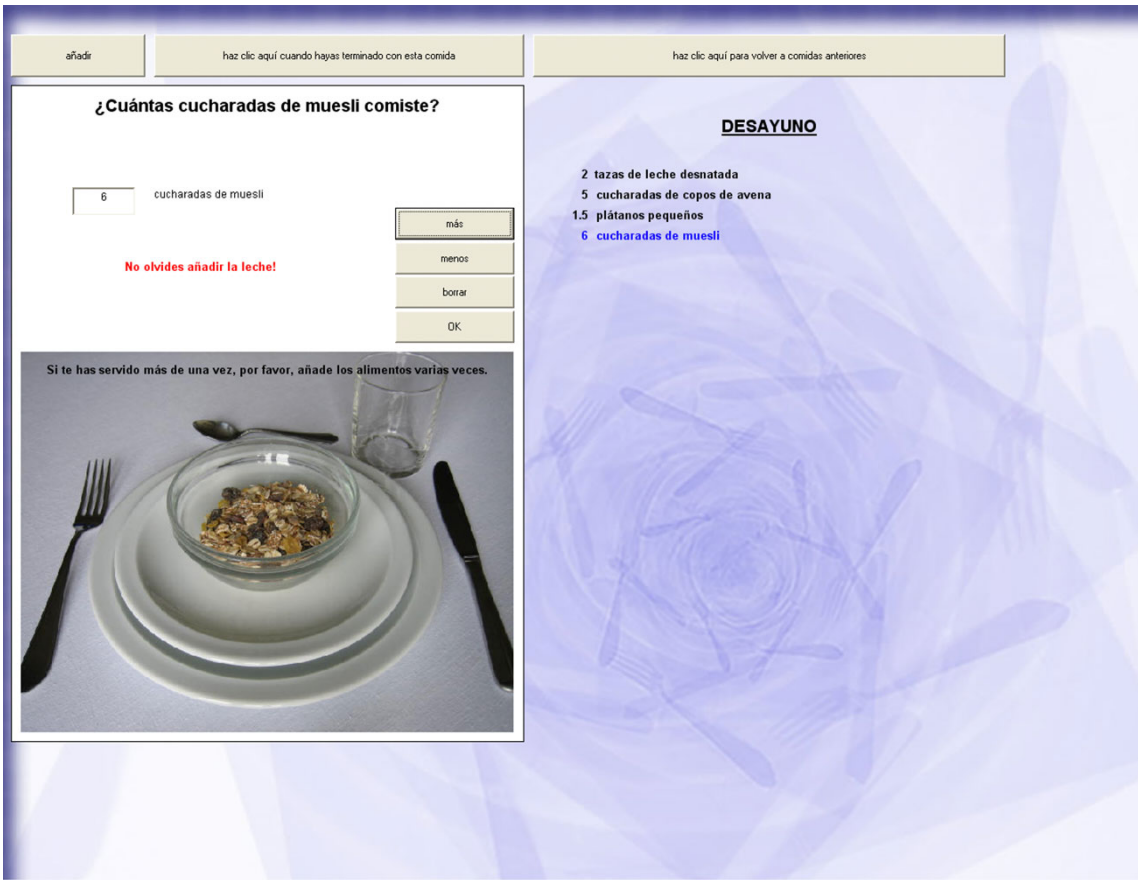
Design: Description of the HELENA-CSS sampling and recruitment approaches, standardization and harmonization processes, data collection and analysis strategies and quality control activities.

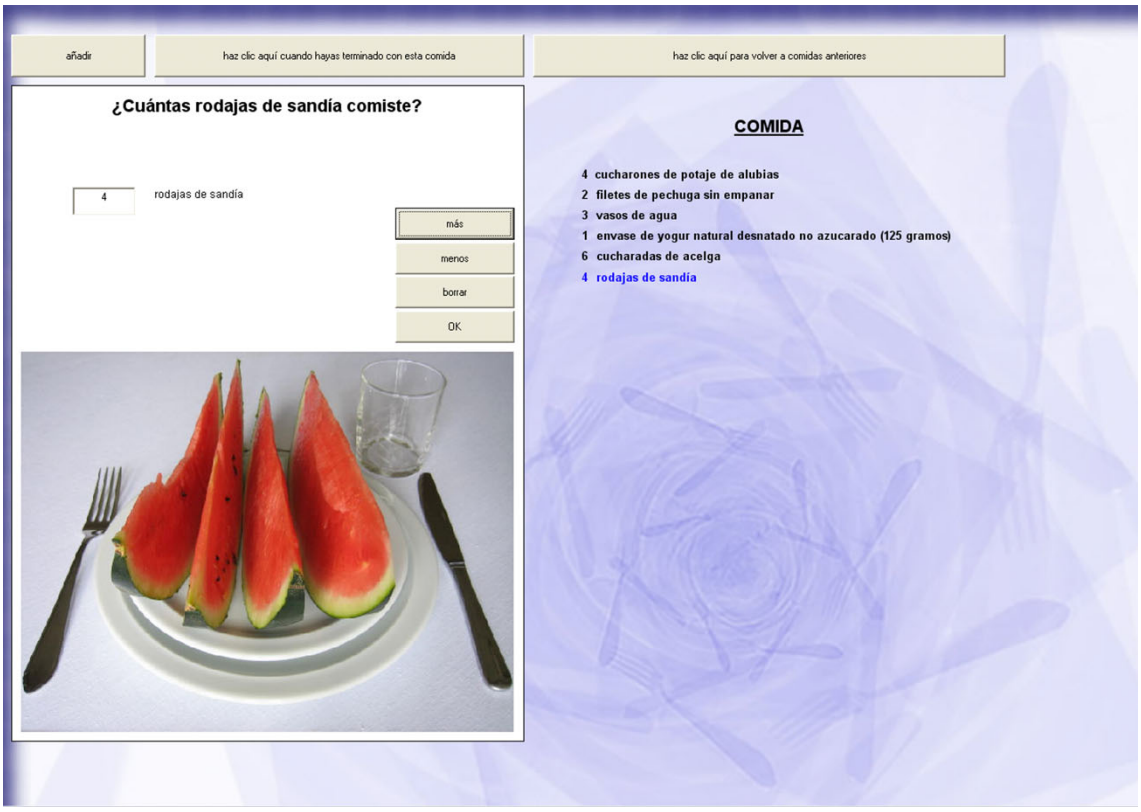
Results: The HELENA-CSS is a multi-center collaborative study conducted in European adolescents located in urban settings. The data management systems, quality assurance monitoring activities, standardized manuals of operating procedures and training and study management are addressed in this paper. Various quality controls to ensure collection of valid and reliable data will be discussed in this supplement, as well as quantitative estimates of measurement error.

Conclusion: The great advantage of the HELENA-CSS is the strict standardization of the fieldwork and the blood analyses, which precludes to a great extent the kind of immeasurable confounding bias that often interferes when comparing results from isolated studies.

1. Cuestionario sobre ingesta dietética HELENA-DIAT

Dietary intake questionnaire HELENA-DIAT


añadir
haz clic aquí cuando hayas terminado con esta comida
haz clic aquí para volver a comidas anteriores
¿Cuántas cucharadas de muesli comiste?
6
cucharadas de muesli
más
menos
borrar
OK
No olvides añadir la leche!
Si te has servido más de una vez, por favor, añade los alimentos varias veces.
DESAYUNO
2 tazas de leche desnatada
5 cucharadas de copos de avena
1.5 plátanos pequeños
6 cucharadas de muesli


añadir
haz clic aquí cuando hayas terminado con esta comida
haz clic aquí para volver a comidas anteriores
¿Cuántas rodajas de sandía comiste?
4
rodajas de sandía
más
menos
borrar
OK
COMIDA
4 cucharones de potaje de alubias
2 filetes de pechuga sin empanar
3 vasos de agua
1 envase de yogur natural desnatado no azucarado (125 gramos)
6 cucharadas de acelga
4 rodajas de sandía

2. Cuestionario sobre sedentarismo y tiempo de pantalla

Sedentary and screen time questionnaire

7788399663

Healthy Lifestyle in Europe by Nutrition in Adolescence — HELENA — Sixth Framework Programme 2002 - 2006

1. Cuántas horas al día pasas ...

	ninguna	menos de media hora	de media a una hora	de una a dos horas	de dos a tres horas	de tres a cuatro horas	cuatro o más horas
viendo la televisión							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
jugando con juegos en el ordenador							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
jugando con la videoconsola							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
navegando en internet por razones que no están relacionadas con el estudio							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
navegando en internet por motivos de estudio							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○
estudiando sin utilizar internet							
un día de colegio:	○	○	○	○	○	○	○
un día de fin de semana:	○	○	○	○	○	○	○

2. cuántas horas duermes normalmente por la noche ...

durante los días de semana: ☐☐,☐ horas por noche

durante el fin de semana: ☐☐,☐ horas por noche

3. si realizas alguna actividad académica o de ocio complementaria al colegio (idiomas, ajedrez clases de repaso, clases the música) aparte del tiempo de estudio personal ¿cuántas horas supone a la semana?

☐☐,☐ horas por semana

4. Cuando estás comiendo con tu familia ¿coméis delante de la televisión?

○ todos los días en cada comida
○ todos los días en 1 0 2 comidas
○ no todos los dias pero mas de 2 comidas a la semana
○ no más de 1 0 2 comidas a la semana
○ escasas veces
○ nunca

0340399660

5. tienes en casa ...

televisión:	○ no	○ si, 1	○ si, 2	○ si, 3 o más
ordenador:	○ no	○ si, 1	○ si, 2	○ si, 3 o más
videoconsola:	○ no	○ si, 1	○ si, 2	○ si, 3 o más

tienes en tu habitación ...

televisión:	○ no	○ si
ordenador:	○ no	○ si
videoconsola:	○ no	○ si

tiene tu hermano / hermana en su habitación ...

televisión:	○ no	○ si	○ no tengo hermanos viviendo en casa
ordenador:	○ no	○ si	○ no tengo hermanos viviendo en casa
videoconsola:	○ no	○ si	○ no tengo hermanos viviendo en casa

6. sin contar las comidas principales, cuántas veces ...	**nunca**	**menos de una vez a la semana**	**1-2 días por semana**	**3-4 días por semana**	**(casi) todos los días**	**varias veces al día**
bebes algo mientras ves la televisión	○	○	○	○	○	○
comes algo mientras ves la televisión	○	○	○	○	○	○

¿qué comes y bebes mientras ves la televisión? (marca todas las respuestas oportunas de esta lista)

○ normalmente no bebo nada	☐ refrescos light	☐ infusiones
☐ agua	☐ refrescos azucarados	☐ café
☐ leche o productos derivados	☐ cerveza	☐ otras bebidas: .....
☐ zumo de frutas		
○ normalmente no como nada	☐ snack salado (patatas)	☐ bocadillo
☐ fruta	☐ bollería	☐ productos lácteos
☐ frutos secos	☐ caramelos, chocolates y chocolatinas	☐ otros: .....

7. sin contar las comidas principales, cuántas veces ...	**nunca**	**menos de una vez a la semana**	**1-2 días por semana**	**3-4 días por semana**	**(casi) todos los días**	**varias veces al día**
bebes algo mientras juegas con videojuegos:	○	○	○	○	○	○
comes algo mientras juegas con videojuegos:	○	○	○	○	○	○

¿qué comes y bebes mientras juegas con videojuegos? (marca todas las respuestas oportunas de esta lista)

○ normalmente no bebo nada	☐ refrescos light	☐ infusiones
☐ agua	☐ refrescos azucarados	☐ café
☐ leche o productos derivados	☐ cerveza	☐ otras bebidas: .....
☐ zumo de frutas		
○ normalmente no como nada	☐ snack salado (patatas)	☐ bocadillo
☐ fruta	☐ bollería	☐ productos lácteos
☐ frutos secos	☐ caramelos, chocolates y chocolatinas	☐ otros: .....

8. sin contar las comidas principales, cuántas veces ...	**nunca**	**menos de una vez a la semana**	**1-2 días por semana**	**3-4 días por semana**	**(casi) todos los días**	**varias veces al día**
bebes algo mientras navegas en internet:	○	○	○	○	○	○
comes algo mientras navegas en internet:	○	○	○	○	○	○

¿qué comes y bebes mientras navegas en internet? (marca todas las respuestas oportunas de esta lista)

○ normalmente no bebo nada	☐ refrescos light	☐ infusiones
☐ agua	☐ refrescos azucarados	☐ café
☐ leche o productos derivados	☐ cerveza	☐ otras bebidas: .....
☐ zumo de frutas		
○ normalmente no como nada	☐ snack salado (patatas)	☐ bocadillo
☐ fruta	☐ bollería	☐ productos lácteos
☐ frutos secos	☐ caramelos, chocolates y chocolatinas	☐ otros: .....

3. Cuestionario sobre el nivel socioeconómico

Socioeconomic status questionnaire

6750042059

¿Has fumado tabaco alguna vez?

- O Sí
- O No

¿Con qué frecuencia fumas tabaco actualmente?

- O Cada día
- O Al menos una vez a la semana, pero no cada día
- O Menos de una vez a la semana
- O No fumo

¿Cuántos cigarros fumas por semana?

- O Ninguno
- O Menos de 5
- O Entre 5 y 10
- O Entre 11 y 20
- O Más de 20

¿Cuánto mides descalzo? 1 m ☐☐ cm

¿Cuánto pesas sin ropa? ☐☐☐,☐

Por favor, indícanos la afirmación más apropiada para tu madre:

- O tiene sobrepeso/obesidad
- O tiene peso normal
- O esta delgada/muy delgada
- O no lo sé

Por favor, indicanos la afirmación más apropiada para tu padre:

- O tiene sobrepeso/obesidad
- O tiene peso normal
- O esta delgado/muy delgado
- O no lo sé

El término familia se refiere a miembros viviendo juntos en la misma casa: padre, madre, hermanos
Para aquellos que vivan en dos familias, contesta acerca de la familia con la que vivas la mayor parte del tiempo.

¿Con quien vives la mayoría del tiempo?

- O con tus dos padres
- O con tu madre sóla
- O con tu madre y su nuevo compañero
- O con tu padre sólo
- O con tu padre y su nueva compañera
- O con tu madre la mitad del tiempo y tu padre la otra mitad
- O con tus abuelos
- O con padres adoptivos
- O en un centro de acogida
- O algún otro lugar

¿Cuantos de tus hermanas y/o hermanos incluyendo hermanastras y/o hermanastros viven en casa (excluyéndote a ti)?

O 0 O 1 O 2 O 3 O 4 O más de 4

¿Dispones de tu propia habitación para ti sólo?

O no O si

¿Cuantos coches posee tu familia? (por "familia" queremos decir miembros que viven juntos: padre, madre y hermanos)

O 0 O 1 O 2 O más de 2

¿Tienes conexión a internet en casa?

O no O si

¿Cómo le va a tu familia económicamente?

- O Fantásticamente bien
- O Muy bien
- O Normal
- O No muy bien
- O Mal

8965042059

Las siguientes preguntas son respecto a tu madre y a tu padre.
Si tienes madre y madrastra o padre y padrastro, contesta en relación a la persona más importante en tu educación.

Por favor indicar el nivel educativo más alto de tu madre y tu padre :

	PADRE	MADRE
educación elemental	O	O
terminó la ESO (BUP)	O	O
terminó Bachiller (COU)	O	O
termino educación superior (universitaria)	O	O

¿Cuál es la ocupación de tu padre ?

- O trabaja jornada completa
- O trabaja media jornada
- O amo de casa
- O retirado o enfermo
- O aprendiz/estudiante
- O en paro
- O temporalmente sin trabajar (ej: baja paternal)
- O nunca le veo
- O falleció
- O no lo sé

¿Cuál es la ocupación de tu madre ?

- O trabaja jornada completa
- O trabaja media jornada
- O ama de casa
- O retirada o enferma
- O aprendiz/estudiante
- O en paro
- O temporalmente sin trabajar (ej: baja maternal)
- O nunca la veo
- O falleció
- O no lo sé

Por favor, anota el tipo de trabajo que tienen tus padres. Si tienen más de un trabajo, indícalos

	PADRE	MADRE
1. Personal administrativo Presidente, Director de administración pública, Consejo de Administración (Jefe de Departamento o equivalente)	O	O
2. profesiones intelectuales y científicas Personal cualificado (matemático o especialista en ciencias y salud, especialistas técnicos :arquitectos, ingenieros, informáticos, biólogos, famrmacéuticos, médicos, abogados, profesor universitario, psicólogo, sociólogo, etc.)	O	O
3 Profesiones intermedias: Técnicos o peritos y otros trabajos intermedios (electricista, mecánico, enfermero/a, dietista, empleado de oficina, maestro, representante comercial y profesionales asociados)	O	O
4. Administración/Oficinas Banca, contabilidad, seguros, bibliotecarios, etc.	O	O
5. Empresas de negocios Ventas, marketing, publicidad, comunicaciones, etc.	O	O
6. Trabajadores cualificados de agricultura y pesca Granjeros, pescadores, guardabosques, etc.	O	O
7. Artesanos, manufactura y oficios relacionados Peluquero/a, mecánico, operario, artesano, mecánico, operario en industria textil, calzado, etc.	O	O
8. Operarios de maquinaria y montadores Trabajadores industriales y operarios de máquinas, conductores de grúas, etc.	O	O
9. Trabajos y ocupaciones elementales vendedores, empleados del hogar, albañiles, vigilantes de seguridad, limpiadores, etc.	O	O
10. fuerzas armadas	O	O
11. otro nombre (describelo detalladamente)	O	O
12. no trabaja	O	O

4. Lista de investigadores estudio HELENA

4. List of researchers HELENA study

HELENA Study Group

Co-ordinator: Luis A. Moreno.

Core Group members: Luis A. Moreno, Fréderic Gottrand, Stefaan De Henauw, Marcela González-Gross, Chantal Gilbert.

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