UNIVERSIDAD D CÓRDOBA

Programa de Doctorado en Biomedicina

Título de la tesis:

Estudio longitudinal del estilo de vida y la actividad física de niños prepúberes en su desarrollo hacia la adolescencia, y su relación con la obesidad y otros factores de riesgo metabólico.

Longitudinal study of the lifestyle and physical activity of prepubertal children in their development towards adolescence, and their relationship with obesity and other metabolic risk factors

Director/ Directores:

Mercedes Gil Campos

Francisco Jesús Llorente Cantarero

Autor:

Francisco Javier Aguilar Gómez-Cárdenas

Fecha de depósito tesis en el Idep: 17 de Noviembre de 2021.

TITULO: *Estudio longitudinal del estilo de vida y la actividad física de niños prepúberes en su desarrollo hacia la adolescencia, y su relación con la obesidad y otros factores de riesgo metabólico*

AUTOR: *Francisco Javier Aguilar Gómez-Cárdenas*

Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

https://www.uco.es/ucopress/index.php/es/
ucopress@uco.es

TÍTULO DE LA TESIS:

ESTUDIO LONGITUDINAL DEL ESTILO DE VIDA Y LA ACTIVIDAD FÍSICA DE NIÑOS PREPÚBERES EN SU DESARROLLO HACIA LA ADOLESCENCIA, Y SU RELACIÓN CON LA OBESIDAD Y OTROS FACTORES DE RIESGO METABÓLICO

DOCTORANDO/A: Francisco Javier Aguilar Gómez-Cárdenas

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

El doctorando Francisco Javier Aguilar Gómez-Cárdenas ha cumplido en tiempo y de forma excelente todos los objetivos planteados. Estos han sido abordados progresivamente y actualmente se han publicado tres artículos para la tesis programada por compendio de artículos publicados en JCR con índice de impacto Q1-JCR.

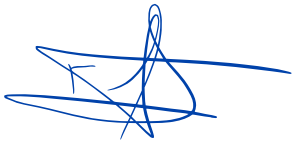
Además, ha participado en cursos de formación, ha publicado otro artículo de revisión en una revista internacional indexada, y ha participado en múltiples actividades complementarias tanto en investigación como en docencia. Destaca su participación en varios congresos nacionales con comunicaciones relativas a la temática de su programa de doctorado. Por tanto, el progreso ha sido excelente.

Se ha realizado un trabajo de tesis doctoral exhaustivo y de gran calidad. El doctorando ha aprendido metodología de la investigación, con aprendizaje de pruebas de campo y abordando distintas tecnologías aplicables a su ámbito profesional y de conocimiento.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 4 de NOVIEMBRE de 2021

Firma del/de los director/es



Fdo.: PROF. DRA. MERCEDES GIL CAMPOS

Fdo.: Prof. Dr. FRANCISCO JESÚS LLORENTE CANTARERO

ESTUDIO LONGITUDINAL DEL ESTILO DE VIDA Y LA ACTIVIDAD FÍSICA DE NIÑOS PREPÚBERES EN SU DESARROLLO HACIA LA ADOLESCENCIA, Y SU RELACIÓN CON LA OBESIDAD Y OTROS FACTORES DE RIESGO METABÓLICO

TESIS DOCTORAL

Francisco Javier Aguilar Gómez-Cárdenas

Departamento de Especialidades Médico-Quirúrgicas

Facultad de Medicina

Universidad de Córdoba

UNIVERSIDAD D CÓRDOBA

Departamento de Especialidades Médico-Quirúrgicas

ESTUDIO LONGITUDINAL DEL ESTILO DE VIDA Y LA ACTIVIDAD FÍSICA DE NIÑOS PREPÚBERES EN SU DESARROLLO HACIA LA ADOLESCENCIA, Y SU RELACIÓN CON LA OBESIDAD Y OTROS FACTORES DE RIESGO METABÓLICO

Presentada por:

Francisco Javier Aguilar Gómez-Cárdenas

Realizada bajo la dirección de los doctores:

Mercedes Gil Campos

Francisco Jesús Llorente Cantarero

UNIVERSIDAD D CÓRDOBA

“El estudioso es el que lleva a los demás a lo que él ha comprendido: la verdad”.

Santo Tomás de Aquino

A mis padres y hermanos.
A Gloria, el amor de mi vida.
Al resto de mi familia, en especial a mi abuelo Gaspar y Tía Carmen.
Gracias a todos por tanto cariño, paciencia, apoyo, inspiración, esfuerzo, enseñanzas y confianza en mí. Gracias de corazón.

Agradecimientos

A todos los niños y adolescentes que, junto con sus familias, han dedicado parte de su tiempo a participar en este estudio en favor del progreso científico.

A la Profesora Mercedes Gil Campos por todo su apoyo durante mi formación como pediatra y como investigador. Estoy muy agradecido por hacerme partícipe de los diferentes proyectos de investigación desde el primer minuto y por demostrar una inagotable confianza en mí.

Al Profesor Francisco Jesús Llorente Cantarero por su confianza y apoyo continuos durante todo este trabajo de investigación. Sus enseñanzas en el campo de la acelerometría y la actividad física han sido el pilar fundamental para llevar a cabo este trabajo.

Al Profesor Ángel Gil Hernández por revelarse para mi como un auténtico ejemplo a seguir en el ámbito de la investigación y el progreso científico. Numerosas han sido sus inestimables lecciones sobre estadística, estrés oxidativo o inflamación, entre muchas otras.

A todo el Grupo de Investigación del Estudio GENOBOX por incluirme en su proyecto de investigación como uno más, por colaborar en la elaboración de cada uno de los trabajos aquí presentados, por facilitarme el desarrollo de esta tesis doctoral y por celebrar conmigo todos los éxitos que hemos logrado hasta el momento.

ÍNDICE

ÍNDICE DE FIGURAS

ABREVIATURAS

AF: Actividad física

Cpm: Cuentas por minuto

EO: Estrés oxidativo

F2-IsoPs: F2-8-iso-prostaglandin F2α

FSH: Hormona folículo-estimulante

HA: Moderadamente activo

HDLc: Partículas de colesterol de alta densidad

HGF: Factor de crecimiento hepatocitario

IMC: Índice de masa corporal

IL-6: interleucina-6

IL-8: interleucina-8

LA: Poco activo

LDLc: Partículas de colesterol de baja densidad

LH: Hormona luteinizante

LPA: Actividad física ligera

MCP-1: Proteína quimioatrayente soluble tipo 1 de los macrófagos

MMP-9: Metalopeptidasa de la matriz tipo 9

MPA: Actividad física moderada

MPO: Mieloperoxidasa

MVPA: Actividad física moderada-vigorosa

NGF: Factor de crecimiento nervioso

NP: Normopeso

OB: Obeso

OMS: Organización Mundial de la Salud

sICAM: Molécula soluble de adhesión intercelular

SP: Sobrepeso

ST: Tiempo sedentario

sVCAM: Molécula soluble de adhesión celular vascular tipo 1

TAG: Triglicéridos

TNF-α: Factor de necrosis tumoral alfa

t-PAI: inhibidor tisular del activador de plasminógeno tipo 1

VLA: Muy poco activo

VHA: Muy activo

8-OHdG: 8-hidroxi-20-deoxiguanosina

RESUMEN

En la actualidad, la obesidad infantil continúa siendo uno de los grandes problemas de Salud Pública en nuestro país. Las principales estrategias para afrontarlo son el control dietético y la práctica de actividad física. En este sentido, los beneficios atribuidos a la actividad física quedan recogidos en las guías de actividad física y comportamiento sedentario de la Organización Mundial de la Salud (OMS) en las que se recomienda de forma específica la práctica de al menos 60 minutos al día de actividad física moderada-vigorosa junto con una limitación del tiempo sedentario. Sin embargo, las descripciones acerca de la práctica de actividad física de forma objetiva mediante acelerometría en población pediátrica española escasean.

En este sentido, este trabajo se propone objetivar en tiempo e intensidad cómo es la práctica de actividad física en una muestra de niños y adolescentes españoles y sus relaciones con diferentes factores de riesgo cardiovascular y de estrés oxidativo.

Este trabajo forma parte del proyecto GENOBOX, que es un estudio multicéntrico llevado a cabo en tres ciudades españolas (Córdoba, Santiago de Compostela y Zaragoza). De los 1444 niños y adolescentes participantes, se obtuvieron datos validos de acelerometría en 513 niños y adolescentes. En el primer estudio se describe de forma tanto transversal como longitudinal cómo es la práctica de actividad física de estos sujetos, y se interpretan las diferencias por sexo, estadio puberal e índice de masa corporal. En el segundo estudio, se analizan las relaciones entre la práctica de actividad física y diferentes parámetros que evalúan el estrés oxidativo. Por último, el tercer estudio, analiza las relaciones entre la práctica de actividad física y diferentes biomarcadores de riesgo cardiometabólico (adipocinas, citoquinas pro-inflamatorias y moléculas de daño endotelial).

Los principales resultados han sido:

- Durante la transición de la infancia (etapa prepuberal) a la adolescencia (etapa puberal) se produce un descenso generalizado de actividad física junto con aumento del tiempo sedentario.
- En descenso de actividad física durante este periodo es más acusado en varones que en mujeres, aunque los primeros se mantienen más activos que las mujeres en ambos periodos.
- Una mayor práctica de actividad física moderada y vigorosa junto con un menor tiempo sedentario se relacionan con una disminución de biomarcadores urinarios de estrés oxidativo y, por tanto, con un mejor perfil redox.

- Una mayor práctica de actividad física moderada-vigorosa junto con un menor IMC, se relacionan con una disminución de la concentración plasmática de biomarcadores de riesgo cardiometabólico.
- Los datos presentados en este trabajo apoyan las recomendaciones publicadas por la OMS e incluso sugieren el posible beneficio para salud de objetivos de actividad física y limitación del tiempo sedentario más estrictos. Por el contrario, la adecuación de nuestra muestra a las recomendaciones emitidas por la OMS es muy baja.

PRODUCCIÓN CIENTÍFICA

PRODUCCIÓN CIENTÍFICA

ARTÍCULOS

- Llorente-Cantarero FJ, **Aguilar-Gómez FJ**, Anguita-Ruiz A, et al. Changes in physical activity patterns from childhood to adolescence: Genobox longitudinal study. *Int J Environ Res Public Health*. 2020;17(19):1-13. doi:10.3390/ijerph17197227

- Llorente-Cantarero FJ, **Aguilar-Gómez FJ**, Leis R, et al. Relationship between physical activity, oxidative stress, and total plasma antioxidant capacity in spanish children from the genobox study. *Antioxidants*. 2021;10(2):1-14. doi:10.3390/antiox10020320

- Llorente-Cantarero FJ, **Aguilar-Gómez FJ**, Bueno-Lozano G, et al. Impact of physical activity intensity levels on the cardiometabolic risk status of children: the Genobox study. International Journal of Sport Nutrition and Exercise Metabolism. *Int J Sport Nutr Exerc Metab*. 2021.

- **Aguilar-Gómez FJ**, Bueno-Lozano G, Leis R, et al. Metabolic syndrome before puberty: Myth or reality? Curr Opin Endocr Metab Res. 2020;14(Table 1):97–103. Available from: https://doi.org/10.1016/j.coemr.2020.06.006

COMUNICACIONES

- Llorente-Cantarero FJ, **Aguilar-Gómez FJ**, Anguita-Ruiz A, et al. Changes in physical activity patterns from childhood to adolescence: genobox longitudinal study. XI Young Investigators Meeting. IMIBIC. Córdoba, 30 Octubre 2020.

- Llorente-Cantarero FJ, **Aguilar-Gómez FJ**, Leis R, et al. Relationship between physical activity, oxidative stress, and total plasma antioxidant capacity in spanish children from the genobox study. XII Young Investigators Meeting. IMIBIC. Córdoba, 29 Octubre 2021.

PREMIOS Y ESTANCIAS

- Mejor comunicación tipo póster: Llorente-Cantarero FJ, **Aguilar-Gómez FJ**, Anguita-Ruiz A, et al. Changes in physical activity patterns from childhood to adolescence: genobox longitudinal study. XI Young Investigators Meeting. IMIBIC. Córdoba, 30 Octubre 2020.

- Estancia: Hospital Universitario Niño Jesús de Madrid. Unidad de Endocrinología Pediátrica. Del 01/01/2021 al 25/04/2021.

INTRODUCCIÓN

I. INTRODUCCIÓN

La obesidad continúa siendo una de las enfermedades crónicas más prevalentes entre niños y adolescentes, considerándose como la gran pandemia del siglo XXI. La obesidad infantil, al igual que la desarrollada en la edad adulta, se ha relacionado con diversas comorbilidades entre las que destacan las alteraciones en el metabolismo lipídico, la pérdida de la homeostasis glucémica o el aumento de la presión arterial(1,2). La conjunción de dichas alteraciones con la obesidad (aunque en ocasiones sin ésta) en la edad adulta constituye lo que se conoce como *síndrome metabólico,* que se traduce de forma global en un aumento del riesgo de enfermedad cardiovascular. En este contexto, dado el aumento de la prevalencia de obesidad infantil durante los últimos años, desde comienzos de siglo se han propuesto diversas adaptaciones pediátricas del concepto *síndrome metabólico* con la intención de objetivar dicho riesgo en niños y adolescentes tanto a corto como a largo plazo(3). Por todo ello, durante los últimos años gran parte de la investigación en la población pediátrica se ha focalizado en la obesidad. En este sentido, los cambios en el estilo de vida, incluyendo la dieta y la actividad física (AF), siguen siendo los pilares fundamentales para la prevención y el tratamiento de la obesidad infantil(4,5). En este contexto, la práctica de AF se convierte así en una de las principales estrategias de Salud Pública para el abordaje de la obesidad en la edad pediátrica(6,7). Por ello, cobra especial interés detallar de forma precisa cómo es la práctica de AF en la infancia y la adolescencia en España, así como conocer la influencia que tiene dicha actividad sobre la obesidad y el sobrepeso, y sobre factores de riesgo cardiovascular clásicos (incluidos en las definiciones de síndrome metabólico) y no clásicos (adipoquinas, citoquinas inflamatorias, marcadores de daño vascular o marcadores de estrés oxidativo, entre otros). Todo este conocimiento permitiría elaborar programas específicos de AF a nivel estatal integrados dentro de las estrategias de Promoción de la Salud y Prevención en el Sistema Nacional de Salud.

Evaluación de la actividad física

La práctica de AF se ha medido de forma clásica mediante cuestionarios y/o diarios de AF. Los cuestionarios y/o diarios de AF presentan algunas ventajas entre las que destaca su bajo coste y, con ello, la posibilidad de reclutar un mayor número de sujetos, aumentando así el tamaño muestral. En cambio, son un método de evaluación de la AF subjetivo y, por tanto, no exento de múltiples sesgos. En cambio, desde finales de siglo XX disponemos de acelerómetros que son dispositivos capaces de medir de forma objetiva el movimiento, detectando la aceleración en uno, dos o tres ejes del espacio (uni-

o triaxial, respectivamente) y cuantificándolo en función del tiempo en cuentas por minuto (cpm)(8). El número de cpm detectado en un intervalo de tiempo determinado es procesado mediante programas informáticos específicos, permitiendo categorizar la AF realizada (por ejemplo, a lo largo de un día) en función de su intensidad en tiempo sedentario y actividades ligera, moderada o vigorosa (ST, LPA, MPA y VPA, respectivamente)(9).

Figura 1. Dispositivo Actigraph® GT3X+

La categorización objetiva de la AF medida mediante acelerometría en función de la intensidad (LPA, MPA o VPA) se ha convertido en el método estándar para cuantificar y expresar dicha actividad a nivel internacional. De hecho, las recomendaciones sobre la práctica de AF en niños y adolescentes (5-17 años) elaboradas por Organización Mundial de la Salud (OMS) incluyen en su última versión (y en la precedente) la recomendación de practicar al menos 60 minutos diarios de actividad física moderada-vigorosa (MVPA), así como la reducción del ST, para la consecución de diversos beneficios para salud(10). Entre estos beneficios se incluyen, la mejora de la aptitud física y cardiorrespiratoria, de la salud metabólica (presión arterial, perfil lipídico y glucémico, y resistencia a insulina), de la salud ósea, del perfil cognitivo (desempeño académico y funciones ejecutivas) y de la salud mental (reducción de los síntomas de depresión), junto con la reducción del tejido adiposo(10).

Partiendo de estas recomendaciones, se ha evaluado la adhesión de la población infantil a éstas. En este sentido, se han publicado diversos informes que evalúan el grado de adecuación de la población pediátrica española a la recomendaciones internacionales de práctica de AF (OMS). El más reciente, publicado en 2018 y basado en encuestas sobre AF, detalla que menos de la mitad de los niños/as y adolescentes (27-33%) cumplen las recomendaciones(11), ofreciendo datos similares a los de 2016 que, a diferencia del más reciente, incluía algunos estudios con AF recogida mediante acelerometría(12). Ambos

informes destacan diferencias por género con una mayor AF de los varones respecto a las mujeres.

Por otro lado, se ha descrito que el paso de la infancia a la adolescencia se acompaña de un descenso generalizado de la AF junto con un aumento del ST(13). Estos resultados provienen fundamentalmente de estudios de diseño transversal y/o basados en cuestionarios/diarios de AF, si bien disponemos de algunos de diseño longitudinal realizados en otros países, en concreto Estados Unidos de América o el Reino Unido, que parecen confirmar esta tendencia(13–16). No se conocen con exactitud las razones de estos cambios, aunque algunos autores han sugerido que la pubertad es, desde sus múltiples perspectivas, biológica (cambios en la composición corporal, aparición de caracteres sexuales secundarios, cambios hormonales), psicológica (percepción de sí mismos, autoestima, cambio de intereses) o social (responsabilidades, tiempo dedicado a trabajo o estudio), un periodo crítico para este descenso de AF(17).

La importancia de conocer de forma precisa cómo es y cómo cambia la práctica de AF en la infancia y en la adolescencia radica en la influencia que ésta tiene sobre la obesidad y otros factores de riesgo cardiometabólico. Entre las diferentes variables de riesgo cardiometabólico, la obesidad ha centrado la mayor parte de la atención durante los últimos años focalizando el interés en conocer si la intervención con la práctica de AF es útil en la prevención y/o el tratamiento de la obesidad(6,7). Por otro lado, la influencia de la AF sobre otros marcadores no tradicionales de riesgo metabólico como aquéllos relacionados con el estrés oxidativo o con el estado inflamatorio han recibido, sobre todo en la edad pediátrica, menor atención.

Actividad física y estrés oxidativo

El concepto de estrés oxidativo (EO) ha sido definido como "un desequilibro entre oxidantes y antioxidantes a favor de los oxidantes, llevando a una situación de interrupción de la señalización redox y de daño celular"(18). La obesidad se ha relacionado tanto en niños como en adultos con una alteración de la homeostasis redox, con aumento de los factores oxidantes y una disminución de los factores antioxidantes(19). Por otro lado, el ejercicio físico se ha relacionado con un aumento de los antioxidantes y un descenso de los oxidantes(20). Asimismo, el ejercicio físico parece mejorar el índice de masa corporal (IMC) en niños y adolescentes(6,7). Sin embargo, se desconoce si el efecto positivo del ejercicio físico sobre el equilibrio redox se produce debido a la mejora en el IMC. En este contexto, teniendo en cuenta que la OMS recomienda un tiempo e intensidad específicos de AF diaria (60 minutos al día de

MVPA), no hemos encontrado estudios que describan las relaciones entre la práctica de AF medida mediante acelerometría (tiempo e intensidad) con biomarcadores del equilibrio redox.

Para la evaluación de dicho estado redox, se procede mediante la medición de la diferentes moléculas, habitualmente en sangre y/u orina, que participan en los diversos sistemas de reducción-oxidación que se producen a nivel celular. En este sentido, el F2-8-iso-prostaglandin F2α (F2-IsoPs) y la 8-hidroxi-20-deoxiguanosina (8-OHdG), detectados en orina, son reconocidos marcadores de EO, habiéndose detectado aumentados en situaciones como obesidad, diabetes mellitus tipo 2 o enfermedades cardiovasculares(21,22). Por otro lado, el sistema de defensa antioxidante está compuesto por dos subsistemas: el enzimático y el no-enzimático. El sistema de defensa enzimático, está representado por enzimas como la peroxidasa de glutatión, la reductasa de glutatión, la s-transferasa de glutatión, la superóxido dismutasa o la catalasa. El sistema de defensa no-enzimático está compuesto por múltiples moléculas que pueden ser medidas en plasma como el ácido úrico, tocoferoles, ácido ascórbico, el glutatión o la ubiquinona. Este sistema de defensa no-enzimático puede ser medido de forma conjunta enfrentando al plasma a múltiples sustancias oxidantes obteniendo un parámetro llamado capacidad antioxidante no-enzimática, más conocido como capacidad antioxidante total (TAC)(23). Algunos autores inciden en la importancia de conocer que este parámetro no mide, como se ha explicado, de forma completa la capacidad antioxidante de un organismo. Sin embargo, se considera un parámetro de gran utilidad puesto que evalúa el sistema de defensa antioxidante de forma global, evitando la dificultad de interpretar de forma conjunta múltiples antioxidantes medidos de manera independiente(24).

Actividad física e Inflamación

La obesidad infantil se ha relacionado con el síndrome metabólico (dislipidemia, elevación de la presión arterial o alteración del metabolismo hidrocarbonado) y con alteraciones en la concentración plasmática de múltiples citoquinas relacionadas con la función del tejido adiposo (adipoquinas), la inflamación (citoquinas proinflamatorias) o con moléculas relacionadas con el daño vascular (marcadores de daño endotelial), que revelan un estado crónico de inflamación de bajo grado(25). En este contexto, mientras la práctica de AF parece mejorar las alteraciones incluidas dentro del concepto "síndrome metabólico", las relaciones entre la práctica de AF, especialmente aquella medida objetivamente mediante acelerometría, la composición corporal y los marcadores de dicho estado pro-inflamatorio crónico no han sido estudiadas en la población pediátrica.

El tejido adiposo constituye un órgano endocrino que produce múltiples adipocinas tales como leptina, adiponectina o resistina, las cuales participan en la homeostasis energética. Los niños con un IMC más alto o con masa grasa elevada presentar mayores niveles de leptina y resistina que aquellos con IMC normal. En cambio, la obesidad se relaciona con niveles bajos de adiponectina(26). En este contexto, las intervenciones con AF parecen mejorar el IMC y reducir las concentraciones plasmáticas de leptina(27,28). Sin embargo, estos estudios no proporcionan información específica acerca del tipo, intensidad o tiempo requerido para conseguir estos beneficios.

La concentración plasmática de diversas citoquinas pro-inflamatorias se ha asociado con obesidad en la edad pediátrica(29,30). Las citoquinas implicadas en las rutas de la inflamación como las interleucina 6 y 8 (IL-6 e IL-8) o el factor de necrosis tumoral alfa (TNF-α) se han encontrado elevadas en niños con IMC elevado(31). De la misma manera, algunos biomarcadores de disfunción endotelial como el inhibidor tisular del activador de plasminógeno (tPAI-1) o la sE-selectina parecen estar aumentados en niños con un IMC alto(31,32). Al igual que lo comentado anteriormente respecto a las adipocinas, no hay publicadas descripciones sobre la relación entre la concentración de estas citoquinas pro-inflamatorias y/o de daño endotelial, y la AF en términos de tiempo o intensidad en la población pediátrica.

HIPÓTESIS Y OBJETIVOS

II. HIPÓTESIS Y OBEJTIVOS

Desde que la OMS ha recomendado un objetivo específico en términos de tiempo e intensidad de práctica de MVPA y la reducción del ST, cobra especial interés conocer cómo es la práctica de AF en niños y adolescentes españoles, describir los cambios que se producen durante la transición de la infancia a la adolescencia y las relaciones entre dicha práctica y otras variables de riesgo cardiometabólico como el peso corporal, diversas variables de estrés oxidativo o ciertos marcadores inflamatorios y de riesgo cardiometabólico.

Al igual que las descripciones realizadas en otros países como el Reino Unido, nuestra hipótesis es que con la edad la práctica de AF disminuye junto con un aumento paralelo del tiempo sedentario. Desconocemos en qué grado, los niños y adolescentes cumplen la recomendación de los 60 minutos al día de MVPA, aunque es probable que sí existan diferencias por sexo.

En cuanto a las relaciones entre AF y variables de estrés oxidativo, es probable que una mayor actividad física se relacione con un mejor perfil redox, con aumento de la concentración plasmática de sustancias antioxidantes y reducción de sustancias oxidantes.

Por último, la práctica de AF, el peso corporal y la concentración de diversos biomarcadores cardiometabólicos son variables que están probablemente muy interrelacionadas. En este sentido, nuestra hipótesis es que una práctica regular de actividad física debería relacionarse con menores niveles plasmáticos de biomarcadores inflamatorios, incluso de manera independiente del IMC.

Con todo esto, los objetivos de nuestro estudio son:

- Describir cómo es la práctica de AF medida mediante acelerometría en los sujetos de nuestro estudio tanto en la infancia como en la adolescencia.

- Describir qué cambios se producen en dicha práctica en aquellos sujetos seguidos desde la infancia a la adolescencia.

- Describir las diferencias en la práctica de AF por sexo, índice de masa corporal y cambios en el índice masa corporal.

- Describir las relaciones entra la práctica de actividad física y el tiempo sedentario con biomarcadores de estrés oxidativo, junto con la influencia de la composición corporal.

- Describir las relaciones entre la práctica de actividad física y biomarcadores de riesgo cardiometabólico (adipocinas, marcadores de inflamación y moléculas de daño endotelial), junto con la influencia del peso corporal.

MATERIALES Y MÉTODOS

III. MATERIALES Y MÉTODOS

Población

Los participantes de este trabajo proceden del proyecto de investigación GENOBOX que es un estudio multicéntrico de diseño caso-control llevado a cabo en tres ciudades españolas (Córdoba, Santiago de Compostela y Zaragoza) entre los años 2012 y 2015. En este estudio se reclutaron un total de 1444 niños (738 mujeres y 706 varones) siguiendo los siguientes criterios generales de inclusión y exclusión. Los criterios generales de inclusión: raza caucásica y edad comprendida entre los 3 y los 17 años. Los criterios generales de exclusión: diagnóstico de cualquier enfermedad congénita, inflamatoria o crónica, incluyendo diabetes mellitus, discapacidad psicomotriz, utilización de medicación hormonal o cualquier otra medicación para el tratamiento de la tensión arterial o alteraciones del metabolismo lipídico o de la glucosa y haber participado en otro estudio de investigación durante los tres meses previos al reclutamiento. Los criterios generales de exclusión mencionados se mantuvieron invariados para los tres estudios mencionados. En cambio, los criterios de inclusión generales descritos se modificaron de acuerdo a los objetivos específicos de cada estudio.

Figura 2. Estudio multicéntrico GENOBOX

En el caso del primer estudio, se seleccionaron 213 niños (108 mujeres y 105 varones) con edades comprendidas entre los 5 y los 14 años, siguiendo los siguientes criterios de inclusión: caucásicos con datos de acelerometría válidos según el protocolo previamente establecido, y disponer de muestra de sangre y datos de exploración física para determinar estadio puberal (prepuberal o puberal) mediante analítica sanguínea (hormona folículo estimulante, hormona luteinizante, estradiol en mujeres o testosterona en hombres) y exploración física mediante el estadiaje de Tanner (diseño corte transversal). Estadio prepuberal fue definido mediante estadio de Tanner 1 y hormona folículo-estimulante (FSH) (<5.0 U/L), hormona luteinizante (LH) (<8 U/L), testosterona en niños (<0.5 ng/mL), y estradiol (<10 pg/mL) en niñas. Estadio puberal fue definido mediante estadio de Tanner ≥2, confirmado con medición de hormonas sexuales. De los 213 niños seguidos durante el periodo 2012 a 2015, un subgrupo de 75 niños proporcionó datos en ambos estadios, prepuberal y puberal (diseño longitudinal).

En el caso del segundo estudio, se seleccionaron 513 niños (268 mujeres y 245 varones) con edades comprendidas entre los 6 y los 14 años, siguiendo los siguientes criterios de inclusión: caucásicos con datos de acelerometría válidos según el protocolo previamente establecido, disponer de muestra de sangre y datos de exploración física para determinar estadio puberal (prepuberal o puberal), siguiendo para ello los mismos criterios previamente citados en el primer estudio, y medición en plasma de glucosa e insulina basales, partículas de colesterol de alta densidad (HDLc), partículas de colesterol de baja densidad (LDLc), triglicéridos (TAG), adiponectina, leptina, resistina, factor de crecimiento nervioso (NGF), factor de crecimiento hepatocitario (HGF), TNF-α, IL-6, IL-8, proteína soluble quimioatrayente (MCP-1), inhibidor de activador de plasminógeno tipo 1 tisular y activo (tPAI-1 and aPAI-1), sE-selectina, P-selectina, molécula soluble de adhesión celular vascular tipo 1 (sVCAM), molécula de adhesión intercelular tipo 1 (sICAM-1), metaloproteinasa de la matriz extracelular 9 (MMP-9) y mieloperoxidasa (MPO).

En el caso del tercer estudio se seleccionaron 216 niños (105 mujeres y 111 varones) con edades comprendidas entre los 6 y los 14 años, siguiendo los siguientes criterios de inclusión: caucásicos con datos de acelerometría válidos según el protocolo previamente establecido, medición de la composición corporal mediante análisis de bioimpedanciometría, disponer de muestra de sangre y datos de exploración física para determinar estadio puberal (prepuberal o puberal), siguiendo para ello los mismos criterios previamente citados en el primer estudio, y medición en plasma de la TAC y en

orina de 8-OHdG y F2-IsoPs. Adicionalmente, en un subgrupo de 74 niños (41 mujeres y 33 varones) se obtuvieron mediciones en plasma de carotenos, retinol y tocoferoles.

El protocolo del estudio fue aprobado por los respectivos comités de ética de los tres hospitales españoles participantes (Hospital Universitario Reina Sofía de Córdoba, Hospital Clínico Universitario de Santiago de Compostela y Hospital Universitario Lozano Blesa de Zaragoza; códigos: Córdoba 01/2017, Santiago de Compostela 2011/198, Zaragoza 12/2010) y se realizó siguiendo las guías de ética en investigación médica recogidas en la Declaración de Helsinki(33). Asimismo, tras explicar por completo los objetivos y detalles del estudio a los padres y a los participantes, se obtuvo el consentimiento informado de los padres de los participantes, así como la aceptación por parte de estos últimos para participar en el estudio.

Datos clínicos y antropométricos

En todos los sujetos participantes se realizó una completa anamnesis y exploración física, incluyendo la evaluación de la madurez sexual clasificando a los pacientes en función de los diferentes estadios de Tanner, confirmando el inicio o no de la pubertad mediante la medición sanguínea de gonadotropinas y hormonas sexuales (testosterona en varones o estradiol en mujeres). Estos datos fueron recogidos por un único examinador por cada centro. El peso corporal fue medido mediante una báscula digital, la talla mediante un tallímetro de precisión y la circunferencia abdominal fue medida en ayunas, en ortostatismo y al final de la espiración aplicando horizontalmente en torno al abdomen una cinta no elástica a la altura del punto medio entre el margen costal inferior y la cresta iliaca. Con estos datos, se calculó el IMC y se clasificó a los niños y adolescentes de nuestro estudio en normopeso (NP), sobrepeso (SP) u obesidad (OB) utilizando los puntos de corte específicos para edad y sexo definidos por la "International Obesity Task Force"(34). Asimismo, para estudiar los cambios producidos en los sujetos del primer estudio entre el estadio prepuberal y el estadio puberal, se crearon los siguientes grupos de cambio de IMC: NP-sin cambios, SP/OB-sin cambios, mejoría del IMC (aquellos que pasaron de OB a SP o NP, y de SP a NP) y empeoramiento del IMC (aquellos que pasaron de NP a SP y OB, y aquellos que pasaron de SP a OB). Para obtener los datos de composición corporal, se utilizó el análisis por bioimpedanciometría mediante un dispositivo específico (BC420SMA, Tanita®, Tokio, Japón). La presión arterial sistólica (PAS) y la diastólica (PAD) fue medida mediante un manómetro digital (Omrom, M6 AC) siguiendo las recomendaciones internacionales para la medición de la presión arterial(35).

Procesamiento de muestras sanguíneas

Las muestras sanguíneas fueron extraídas de la vena cubital entre las 08:00 y las 09:30h tras ayuno durante la noche. Los análisis rutinarios fueron analizados en los laboratorios generales de referencia de cada uno de los centros hospitalarios.

Parámetros generales

La determinación de glucosa (CV = 1.0%) fue realizada utilizando el método glucosa oxidasa de un analizador automático (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems, Mannheim, Alemania). La determinación de insulina (CV = 2.6%) se realizó mediante radioinmunoanálisis (RIA) usando un analizador automático de micropartículas (AxSYM; Abbott Laboratories, Abbott Park, IL, USA). La resistencia a insulina se calculó mediante el modelo homeostático de resistencia a insulina (HOMA-IR) utilizando la fórmula: HOMA-IR = glucemia basal (mmol) x insulinemia basal (mU/mL) /22.5 (36). Los TAG (CV = 1.5%), el colesterol total (CV = 0.9%), HDL-c (CV = 0.8%), y LDL-c (CV = 1.5%) fueron medidas utilizando un analizado automático (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems, Mannheim, Alemania). La FSH (CV = 3.6%), LH (CV = 3.1%), testosterona (CV= 2%), y estradiol (CV = 1.8%) fueron medidas mediante quimioluminiscencia usando un analizador automático (Architec I4000, Abbott Laboratories, Abbott Park, IL, USA).

Parámetros de estado redox

La determinación de la TAC se realizó mediante colorimetría utilizando un kit específico (Cat no. 709001, Cayman Chemical, Ann Arbor, MI, USA). La determinación urinaria de F2-IsoPs se realizó mediante la técnica ELISA utilizando un kit específico (EA85 Oxford Biomedical Research, Oxford, MI, USA) (CV): 14.13%). La determinación urinaria de 8-OHdG se realizó mediante la técnica ELISA utilizando un kit específico (KOG-200S/E JaICA, Fukuroi, Japan) (CV: 5.73%). Las concentraciones urinarias de estos biomarcadores fueron normalizadas y expresadas en función de las concentraciones urinarias de creatinina. La determinación de creatinina urinaria se realizó mediante colorimetría utilizando un kit específico (Ref. 1001115, Spinreact, Barcelona, Spain) (CV: 2.89%). Las mediciones de la concentración plasmática de retino, carotenos y tocoferoles se realizó mediante cromatografía líquida de alta presión acoplada a espectrometría de masas (UHPLC-MS).

Parámetros inflamatorios y de riesgo cardiovascular

Los siguientes biomarcadores seleccionados fueron analizados en plasma utilizando el kit específico de anticuerpos monoclonales humanos LINCOplex (Linco Research, St. Charles, MO) mediante el sistema Luminex 200 (Luminex Corporation, Austin, TX): adiponectina (CV = 7.9%), leptina (CV = 7.9%) (Cat. HADK2-61 K-B), resistina (CV = 6.0%), NGF (CV = 6%), HGF (CV = 7.7%), TNF-α (CV = 7.8%), IL-6 (CV = 7.8%), IL-8 (CV = 7.9%), MCP-1 (CV = 7.9%), inhibidor del activador de plasminógeno-1 (PAI-1) (CV = 6.6%) (Cat. HADK1-61 K-A), P-selectin (CV = 10.1%), sE-selectin (CV = 11.2%), sVCAM (CV = 11.1%), sICAM (CV = 7.9%), MMP-9 (CV = 6.8%), y MPO (CV = 12.3%) (Cat. HCVD1- 67 AK).

Acelerometría

Para el análisis cuantitativo de la AF se utilizaron acelerómetros ActiGraph GT3X+ (ActiGraph; Pensacola, FL, USA). Estos acelerómetros se colocaron sobre la cresta ilíaca derecha, quedando sujetos mediante un cinturón elástico en torno a la cintura. Dicho dispositivo debía ser llevado durante las 24 horas del día, exceptuando el momento de la ducha o el descanso nocturno (sólo si el dispositivo era molesto para dormir). La programación del dispositivo se realizó siguiendo las recomendaciones previamente publicadas, agrupando la información en periodos de 15 segundos(37). Los datos recogidos por el acelerómetro fueron procesados utilizando el programa Actilife v6.13.3 (ActiGraph; Pensacola, FL, USA). Durante dicho procesamiento, se aplicaron dos reglas en el procesamiento de la AF recogida tal y como se recomienda en la bibliografía(38): (a) todas las cuentas negativas se consideraron como datos perdidos, y (b) los periodos de 20 minutos o más de "cero cuentas por minuto" consecutivas se consideraron como datos perdidos. Posteriormente, aquellos sujetos que presentaban al menos 8 horas de monitorización al día, durante al menos 3 días, incluyendo al menos un día de fin de semana, se consideraron válidos para el análisis de la AF y el ST. El volumen final AF recogida por el dispositivo en cpm, se tradujo a los diferentes niveles de AF siguiendo la clasificación establecida por Evenson y su grupo: ST: ≤100 cpm, LPA: >100–<2296 cpm, MPA: >2296–<4012 cpm, y VPA: ≥4012 cpm(9).

Análisis estadístico

Todos los procedimientos estadísticos se llevaron a cabo mediante SPSS (IBM SPSS Statistics for MacOS, Version 25.0. Armonk, NY, USA).

Primer estudio: evaluación de la actividad física

La normalidad de todas las variables cuantitativas continuas fue analizada utilizando el test de Kolmogorov, y todas fueron apropiadamente normalizadas mediante transformación logarítmica, raíz cuadrada o el método de la transformada inversa. La heterocedasticidad entre grupos fue analizada mediante el test de Levene.

Las diferencias para diferentes variables entre los participantes en periodo prepuberal y puberal fueron evaluadas utilizando los test apropiados: Chi2 ó t-Student para datos emparejados.

En la parte del estudio de corte transversal, el ANOVA de dos vías y el test de Kruskal-Wallis se utilizaron para analizar las diferencias entre las mediciones obtenidas. Además, se aplicó el test de Dunn junto con los análisis *posth-hoc* ajustados por edad para

determinar qué grupos mostraban diferencias entre sí tras los resultados del ANOVA. Un valor $p< 0.05$ se consideró significativo.

En la parte del estudio de diseño longitudinal, se evaluaron las diferencias para cada nivel de AF entre los tiempo prepuberal y puberal para todos los sujetos y, de forma separada, por sexo, grupos de IMC y grupos de cambio de IMC, utilizando tests para datos emparejados t-Student, Wilcoxon y test de Dunn, ajustados por edad. Los valores absolutos de AF detectados en el tiempo prepuberal y puberal podrías ser diferentes, pudiendo infra- o sobreestimar la diferencias. Por ello se calcularon valores relativos de cada nivel de AF en función del tiempo total medido (por ejemplos, para LPA, el % de LPA = (minutos de LPA medidos/minutos totales de tiempo medido) x 100), siguiendo una metodología utilizada previamente para evitar este sesgo(14).

Por otro lado, se evaluaron las diferencias de IMC z-score y las variables de AF entre los tiempos prepuberal y puberal. Tras esto, dada la colinealidad encontrada entre las variables de AF, se realizaron test de regresión multivariable seleccionando los cambios en el IMC z-score como variable dependiente y los cambios en las diferentes intensidades de AF como variables independientes, así como la edad, el estadio puberal y el género, que también fueron incluidas en el modelo. Un valor $p<0.05$ se consideró como significativo.

Segundo estudio: actividad física y estrés oxidativo

La normalidad de todas las variables cuantitativas continuas fue analizada utilizando el test de Kolmogorov y/o de Shapiro-Wilk, y aquella que lo precisaban fueron apropiadamente normalizadas mediante su raíz cuadrada (TAC, 8-OHdG, F_2-IsoPs, MPA Y VPA) o mediante transformación logarítmica (ST). La heterocedasticidad entre grupos fue analizada mediante el test de Levene.

Las diferencias entre sujetos prepuberales y puberales fueron analizadas mediante t-Student para muestras independientes o U de Mann-Whitney. Para variables categóricas se utilizó la Chi2.

Para investigar las relaciones entre el IMC, la composición corporal, la resistencia de los tejidos periféricos a la insulina, los niveles de AF, las variables de EO y la TAC, se realizó un análisis de componentes principales (PCA). Tras la extracción del conjunto inicial de componentes no relacionados con el método del factor principal, posteriormente se utilizó la rotación ortogonal de componentes Varimax para facilitar la interpretación. Lo factores de carga más altos indicaban una relación más fuerte entre un factor y la variable

observada. Factores de carga menores 0.359 (factor crítico, $p<0.001$) indicaban correlaciones marginales.

Para estudiar los niveles globales de AF y sedentarismo, se diseñó una puntuación específica incluyendo ST, MPA y VPA que recibió el nombre de (PASS, *Physical Activity and Sedentarism Score*). Para ello, se calcularon los cuartiles de cada una de dichas variables, siendo los minutos de MPA y VPA mayores para el cuarto cuartil (Q4) que para el primer cuartil (Q1), y al revés en el caso del ST. Cada sujeto, obtuvo una puntuación entre 1 y 4 de acuerdo al cuartil para cada variable (por ejemplo, 1 por estar en el Q1 de MPA y 4 por estar en el Q4 de ST). Sumando la puntuación de las tres variables, cada sujeto obtuvo un PASS comprendido entre 3 (todas las variables en Q1) a 12 (todas las variables en Q4). Un mayor PASS indicaba un hábito más activo. Basándonos en esta puntuación, se crearon 4 grupos de PASS: muy poco activo (VLA: con una puntuación de 3), poco activo (LA: con una puntuación de 4 a 6), moderadamente activo (MA: con una puntuación de 7 a 9) y muy activo (HA: con una puntuación de 10 a 12). Para evaluar las diferencias entre los cuatro grupos para las diferentes variables del estudio se utilizó un MANOVA y un test de Box bajo el supuesto de matrices de covarianza iguales. Un valor de Pillai Trace menor de 0.05 fue considerado estadísticamente significativo para el MANOVA. Las diferencias entre los cuatro grupos basadas en el PASS fueron analizadas posteriormente utilizando ANCOVA univariante (UNIANCOVA), ajustado por edad y/o z-score de IMC. Las diferencias por pares se evaluaron mediante análisis *post-hoc* para determinar diferencias entre los grupos. Para que aquellas variables que no seguían la normalidad, las diferencias entre grupos se evaluaron mediante un test de Kruskal-Wallis. Se consideraron diferencias significativas cuando la $p<0.05$.

Tercer estudio: Actividad física y factores de riesgo cardiometabólico

El tamaño muestra para el estudio GENOBOX fue calculado teniendo en cuenta un error α de 0.05 y una potencia estadística del 80% (error $\beta = 0.20$), de acuerdo a la ecuación para el cálculo de tamaño muestral para el estudio de las diferencias de proporciones de una variable entre dos grupos independientes. El tamaño de muestra bajo estas condiciones alcanzó un total de 300 sujetos para asegurar el hallazgo de diferencias significativas para una diferencia mínima del 20% para cada variable entre los grupos de estudio. La normalidad de todas las variables cuantitativas continuas fue analizada utilizando el test de Kolmogorov y/o de Shapiro-Wilk, siendo las variables insulina, triacilglicéroles y los biomarcadores inflamatorios apropiadamente normalizados

mediante transformación logarítmica o raíz cuadrada. La heterocedasticidad entre grupos fue analizada mediante el test de Levene.

La muestra fue inicialmente dividida en cuartiles en función de la media de la variable MVPA: muy poco activo (VLA, desde 6.42 hasta 36.44 minutos/día), poco activo (LA, desde 36.52 hasta 49.45 minutos/día), moderadamente activo (MA, desde 49.80 hasta 63.65 minutos/día), y muy activo (HA, desde 63.73 hasta 129.04 minutos/día).

Para el estudio de las diferencias entre los grupos de MVPA para las diferentes variables incluidas en el estudio se utilizó el análisis de la varianza de una vía (ANOVA) y el test de Kruskal-Wallis. Asimismo, se utilizaron apropiadamente la t-Student o la U-Mann-Whitney, ajustados por z-score de IMC y edad, para el estudio de las diferencias por pares entre los grupos de MVPA. Asimismo, se utilizaron test apropiados para evaluar la tendencia y la relaciones lineales a lo largo de los cuartiles. La asociación entre MVPA y los biomarcadores inflamatorios se realizó mediante la correlación ρ de Spearman para las variables no paramétricas y la de Pearson para aquellas paramétricas. La regresión lineal múltiple se utilizó para evaluar las relaciones entre los cuartiles de MVPA y los biomarcadores inflamatorios.

RESULTADOS Y DISCUSIÓN

CHANGES IN PHYSICAL ACTIVITY PATTERNS FROM CHILDHOOD TO ADOLESCENCE: GENOBOX LONGITUDINAL STUDY

Francisco Jesús Llorente-Cantarero[1,2], Francisco Javier Aguilar-Gómez[3], Augusto Anguita-Ruiz[2,4,5], Azahara Iris Rupérez[2,6], Rocío Vázquez-Cobela[7], Katherine Flores-Rojas[3], Concepción M. Aguilera[2,4,5], Esther M. Gonzalez-Gil[2,4,6], Mercedes Gil-Campos[2,3], Gloria Bueno-Lozano[2,6,8] and Rosaura Leis[2].

Int. J. Environ. Res. Public Health 2020, 17, 7227

[1]Department of Specific Didactics, Faculty of Education, University of Córdoba, 14071 Córdoba, Spain.
[2]CIBEROBN, (Physiopathology of Obesity and Nutrition) Institute of Health Carlos III (ISCIII), 28029 Madrid, Spain.
[3]Metabolism and Investigation Unit, Reina Sofia University Hospital, Institute Maimónides of Biomedicine Investigation of Córdoba (IMIBIC), University of Córdoba, 14004 Córdoba, Spain.
[4]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Center of Biomedical Research, University of Granada, Armilla, 18016 Granada, Spain.
[5]Instituto de Investigación Biosanitaria IBS, 18012 Granada, Spain
[6]GENUD Research group, University of Zaragoza, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria (IIS), 50009 Aragón, Zaragoza, Spain
[7]Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Department of Pediatrics, University Clinical Hospital of Santiago, University of Santiago de Compostela, 15701 Santiago de Compostela, Spain.
[8]Pediatric Endocrinology Unit, Lozano Blesa University Clinical Hospital of Zaragoza, Faculty of Medicine, University of Zaragoza, 50009 Zaragoza, Spain.

International Journal of
Environmental Research and Public Health

MDPI

Article

Changes in Physical Activity Patterns from Childhood to Adolescence: Genobox Longitudinal Study

Francisco Jesús Llorente-Cantarero [1,2], Francisco Javier Aguilar-Gómez [3], Augusto Anguita-Ruiz [2,4,5], Azahara Iris Rupérez [2,6], Rocío Vázquez-Cobela [7], Katherine Flores-Rojas [3], Concepción M. Aguilera [2,4,5], Esther M. Gonzalez-Gil [2,4,6], Mercedes Gil-Campos [2,3,*], Gloria Bueno-Lozano [2,6,8] and Rosaura Leis [2]

1 Department of Specific Didactics, Faculty of Education, University of Córdoba, 14071 Córdoba, Spain; llorentefj@yahoo.es
2 CIBEROBN, (Physiopathology of Obesity and Nutrition) Institute of Health Carlos III (ISCIII), 28029 Madrid, Spain; augustoanguitaruiz@gmail.com (A.A.-R.); airuperez@unizar.es (A.I.R.); caguiler@ugr.es (C.M.A.); esthergg@unizar.es (E.M.G.-G.); gbuenoloz@yahoo.es (G.B.-L.); mariarosaura.leis@usc.es (R.L.)
3 Metabolism and Investigation Unit, Reina Sofia University Hospital, Institute Maimónides of Biomedicine Investigation of Córdoba (IMIBIC), University of Córdoba, 14004 Córdoba, Spain; fjaviagui1992@gmail.com (F.J.A.-G.); katherine1.flores@gmail.com (K.F.-R.)
4 Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Center of Biomedical Research, University of Granada, Armilla, 18016 Granada, Spain
5 Instituto de Investigación Biosanitaria IBS, 18012 Granada, Spain
6 GENUD Researchgroup, University of Zaragoza, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria (IIS), 50009 Aragón, Zaragoza, Spain
7 Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Department of Pediatrics, University Clinical Hospital of Santiago, University of Santiago de Compostela, 15701 Santiago de Compostela, Spain; cobela.rocio@gmail.com
8 Pediatric Endocrinology Unit, Lozano Blesa University Clinical Hospital of Zaragoza, Faculty of Medicine, University of Zaragoza, 50009 Zaragoza, Spain
* Correspondence: mercedes_gil_campos@yahoo.es

Received: 8 August 2020; Accepted: 29 September 2020; Published: 2 October 2020

Abstract: Longitudinal changes of physical activity (PA) from childhood into adolescence have not been accurately described yet for the Spanish population. The aim of this study is to evaluate the changes of PA, assessed by accelerometry and anthropometric measures in a cohort of 213 children from the prepubertal to pubertal period, focusing on those with valid data from both time points (n = 75). Sedentary time (ST) increased about 50%, while all PA intensities declined from the pre-pubertal to pubertal period. Light PA (LPA) was the major contributor, decreasing by about 30%. Boys were more active than girls in both periods, but they showed a higher decline in PA, especially moderate-to-vigorous PA (MVPA). The proportion who reached the recommendation of 60 min of MVPA decreased by 33.3% in boys and 4.6% in girls. Children with obesity or overweight had lower MVPA than those with normal-weight in the pre-pubertal period, but no differences were found in the pubertal period. This study shows a decrease of PA and an increase of sedentarism in the transition from childhood to adolescence, particularly in boys. Regardless of body weight, adolescents tend to be less active. Therefore, prevention programs should be implemented to achieve optimal PA and reduce sedentarism during infancy considering the differences found by sex.

Keywords: childhood; obesity; physical activity; pubertal status; sedentary time

1. Introduction

Physical activity (PA) improves cardiorespiratory fitness and strengthens the musculoskeletal system, contributing to maintain an adequate body composition and preventing childhood obesity [1,2].

In this context, the World Health Organization (WHO) and other entities [3–6] have recommended at least 60 min per day of moderate-to-vigorous physical activity (MVPA) for children and adolescents [7,8]. A recent systematic review also highlighted the potential benefits of total PA and light PA (LPA), especially in the improvement of cardio-metabolic biomarkers [9].

On the other hand, sedentarism has been proposed as an independent risk factor of unhealthy outcomes, such as overweight or obesity, especially in adults. However, evidence in youth is less conclusive to date [10,11]. Sedentary behavior is characterized by a very low energy expenditure (≤1.5 of metabolic equivalents of task (METS)) in a sitting, reclining, or lying posture [12]. However, there are no recommendations for sedentary time (ST) but a suggestion to limit screen time (a component of ST) to no more than 2 h per day. A recent review revealed that less than 50% of European children and adolescents meet the WHO recommendations regarding PA when measured subjectively [13], but even those who achieve the 60 min of MVPA may also spend a high proportion of their time being sedentary [14].

In addition to these findings, the practice of PA seems to decrease progressively during childhood and adolescence, coupled to the increase of ST [15]. Although the reduction of MVPA has always received attention, the latest research has focused also on the importance of LPA decline [9,15]. There are several factors associated with the PA decline: biological, psychosocial, and environmental. Specifically, the influence of gender, pubertal status, or body mass index (BMI) have not been accurately described yet. Moreover, most of these studies include self-reported PA and/or a cross-sectional design [16–19], being few those with longitudinal data.

During childhood and adolescence, boys seem to perform more PA than girls [13], although the rate of decline by gender varies between studies especially related with social factors and others. This reduction in adolescents seems to occur earlier in girls (9–12 years) and later in boys (13–16 years) [17,20], suggesting that it may be related with pubertal status more than with chronological age. It seems that young people become less physically active as they progress along the maturation process [16–19]; thus, puberty could be a critical lapse for PA [21]. The appearance of secondary sexual characteristics, the changes in body composition, hormonal imbalance, and self-perception are related to the practice of PA in boys and girls [22]. So, puberty timing (e.g., the age of menarche or peak of high velocity) related to age and gender may be relevant in explaining the decrease in the practice of exercise.

A recent review [23] revealed that the practice of MVPA is significantly lower in children and adolescents with obesity than in their normal-weight peers, although differences are relatively small and both groups are below the recommendations. Moreover, no differences have been found in ST between BMI groups, without any information regarding total PA or LPA. The relationships between changes in body composition, gender, or puberty and PA, remain under investigation. This is of special importance for addressing population-based interventions.

Based on these previous observations, it is important to describe changes of PA and ST according to the presence or absence of pubertal development, as well as to corroborate if the differences previously reported in other countries, such as United Kingdom [15], between genders and BMI groups remain similar for the Spanish population. The aim of the present study is to analyze the time spent on all intensities of PA, measured by accelerometry, and ST in a cohort of children followed from pre-pubertal to pubertal status, focusing also on gender differences and BMI changes.

2. Materials and Methods

2.1. The Cross-Sectional Study Design

The present study was carried out under the framework of the GENOBOX study [24,25]. GENOBOX is a cross-sectional case-control, multicentre study carried out in children from 2012–2015. After assessing

them in a first visit at the primary care centre, the children fulfilling the inclusion criteria and their parents were invited to the Endocrine Departments of the Reina Sofía University Hospital in Córdoba, University Clinical Hospital in Santiago de Compostela, and Lozano Blesa University Clinical Hospital in Zaragoza, obtaining a similar sample distribution among three regions.

Nine hundred and fifty-three prepubertal children were assessed based in the sample size estimation for the GENOBOX study [24,25]. Out of them, a subsample of 213 (27 from Córdoba, 104 from Santiago de Compostela, and 82 of Zaragoza) children (105 boys) was selected based on the following inclusion criteria for the present study: to have valid blood samples including sex hormones (follicle-stimulating hormone, luteinizing hormone, testosterone in boys, and estradiol in girls); being aged between 5–14 years and being in a pre-pubertal stage (Tanner I confirmed with sex hormones: follicle-stimulating hormone (<5.0 U/L), luteinizing hormone (<8 U/L), testosterone in boys (<0.5 ng/mL), and ostradiol (<10 pg/mL) in girls) at baseline, with an absence of endogenous obesity and metabolic diseases at recruitment, no use of medications for controlling blood pressure (BP), glucose, or lipid metabolism levels, and valid data for the present study variables; especially, with data from an accelerometer according to the protocol.

2.2. The Longitudinal Study Design

Two measurements were conducted on the selected children before and after the onset of puberty, being all of them part of the previously mentioned cross-sectional study population. All these children were first recruited as prepubertal children during the year period (2012–2015), baseline, and called again for follow-up medical consultation in 2018. All subjects with clinical signs of puberty at follow-up (at least Tanner II, confirmed with sex hormones), were included in the longitudinal study. Finally, 75 children presented valid data of PA, measured by accelerometers, at both prepubertal and pubertal stage. During the whole course of the study (2012–2018), children remained under regular medical monitoring by the same pediatricians.

Children and parents or custody holders were informed about the purpose and procedures of the study, giving the children their assent to participate. Signed written consents were obtained from the parents after the Ethics Committees of all participating institutions approved the study. We complied with the Declaration of Helsinki [26] and followed the recommendations of the Good Clinical Practice of the CEE (Central and Eastern Europe) (Document 111/3976/88 July 1990) and the legal, in-force Spanish regulation, which regulates Clinical Investigations in human beings (RD 223/04 on Clinical Assays).

2.3. Anthropometric and Clinical Measurements

Medical history and a physical examination including the evaluation of sexual maturity according to Tanner's five-stage scale [27] were assessed in both visits, at prepubertal and pubertal stages, and confirmed with sexual hormone measurements. Anthropometric measurements were taken by a single examiner within each hospital. Body weight was measured using a standard beam balance. Height was measured using a precision stadiometer. Waist circumference (WC) was measured in fasting state by applying an inelastic tape horizontally midway between the lowest rib margin and the iliac crest of the standing child at the end of a gentle expiration. BMI was calculated (kg/m^2), and overweight and obesity were defined using age and sex-specific BMI cut-off points of the International Obesity Task Force, equivalent to adult values of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity [28]. In this study, three BMI groups were created to test differences in PA between them in the two time points (baseline and follow-up): normal-weight (NW), overweight (OW), and with obesity (OB). For the analysis of the changes in PA between the two time points, BMI-change groups were created as follows: NW-no change group, OW/OB-no change group, improving-BMI group (for those who changed from OB to OW or NW, and from OW to NW), and worsening-BMI group (for those who changed from NW to OW or OB, and from OW to OB).

Systolic and diastolic blood pressure (BP) were measured three times by the same examiner using an electronic manometer (Omrom, M6 AC) and following international recommendations [29], and the mean of the three measurements was considered the current value.

2.4. Biochemical Analysis

Blood samples were drawn from the antecubital vein between 08:00 and 09:30 h after an overnight fast. Routine blood tests were analyzed at the general laboratory of each participating hospital. Glucose (CV = 1.0%) was analyzed using the glucose oxidase method in an automatic analyzer (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems, Mannheim, Germany), and plasma insulin was analyzed by radioimmunoassay (RIA) (CV = 2.6%) using an automatic microparticle analyzer (AxSYM; Abbott Laboratories, Abbott Park, IL, USA). Insulin resistance (IR) was calculated by the homeostatic model assessment of IR (HOMA-IR). Serum triacylglycerols (TAG) (CV = 1.5%), total cholesterol (CV = 0.9%), high density lipoprotein cholesterol (HDL-c) (CV = 0.8%), and low-density lipoprotein cholesterol (LDL-c) (CV = 1.5%) were measured using an automatic analyzer (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems, Mannheim, Germany). The sex hormones follicle-stimulant hormone (FSH) (CV = 3.6%); luteinizing hormone (LH) (CV = 3.1%), testosterone (CV = 2%), and estradiol (CV = 1.8%) were measured by chemiluminescence using an automatic analyzer (Architec I4000, Abbott Laboratories, Abbott Park, IL, USA).

2.5. Accelerometry

ActiGraph GT3X+ accelerometers (ActiGraph; Pensacola, FL, USA) were used to assess PA levels in this study. Accelerometers were placed over the right iliac crest and held in place using an adjustable elastic belt for 24 h a day and could be removed only to shower or for nocturnal rest (if the instrument caused discomfort in sleeping). It was programmed for 15 epochs (period of 15 s), as previously recommended [30].

Accelerometry data were processed using the Actilife v6.13.3 program. Two rules were used for excluding data: (a) all negative counts were replaced by a missing data code, and (b) periods of 20 min or more of consecutive zero counts were replaced by a missing data code prior to further analysis, as recommended by Treuth et al. [31]. The output generated by the ActiGraph GT3X+ included the total volume of PA and each PA intensity as defined by the cut-points of the following counts per minute (CPMs) based in Evenson et al. [32] classification: sedentary: ≤100 CPM, light (LPA): >100–<2296 CPM, moderate (MPA): >2296–<4012 CPM, and vigorous PA (VPA): ≥4012 CPM. A minimum of 8 h of monitoring per day for at least 3 days including at least 1 weekend day was considered acceptable for the evaluation of PA and sedentary time.

After meeting these conditions, differences in time measured between the two timepoints may have been different and over- or underestimated in absolute values, so relative values of each PA intensity were calculated as follows: % of LPA = (min of LPA measured/min of total time measured) × 100, as previously [33].

2.6. Statistical Analyses

All continuous variables were tested for normality using the Kolmogorov test, and all were transformed through natural log, or square root or rank-based inverse normal transformation. Heteroscedasticity between groups was explored with the Levene test. Differences in the characteristics of the participants for prepubertal and pubertal periods were tested using Chi-square or t-paired tests.

In the cross-sectional study, the two-way ANOVA and Kruskal–Wallis were employed to assess group differences in the measurements according to standard statistical assumptions. In addition, the Dunn tests were applied conveniently as post-hoc analyses adjusted by age to determine which experimental groups differed from each other for the ANOVA. A p-value ≤ 0.05 was considered significant.

In the longitudinal study, mean (SD) differences in the time of ST/PA (all intensities), between the two time points were assessed for all subjects and separately by gender, BMI groups, and BMI-change groups, using paired t-tests, paired Wilcoxon signed rank tests, and Dunn tests conveniently, adjusted by age. Absolute values would overestimate the differences between prepubertal and pubertal time; thus, relative values of each level of PA were also calculated (min of intensity level of PA with regard to the total measured time, expressed as percentage).

On the other hand, differences between prepubertal and pubertal stage (Δ) were calculated for BMI z-score and the PA variables. After that, given the co-linearity found between PA variables, several multivariable regression tests selecting changes in BMI-Z score as dependent variable, and changes in the different physical activity intensities as independent variables, as well as age, Tanner status, and gender were included in the model carried out; (Supplementary Table S1). A p-value ≤ 0.05 was considered as significant. All statistical procedures were conducted by using SPSS (IBM SPSS Statistics for MacOS, Version 25.0. Armonk, NY, USA).

3. Results

Measurements of PA with an Actigraph device were collected from 52.8% of children at baseline (n = 112), 67.6% of children at follow-up (n = 142), and 35.2% of children at both time points (prepubertal and pubertal stages) (n = 75) (Figure 1). Table 1 shows the characteristics of the population in the group with both measurements. The number of days with valid PA data recorded was lower in prepubertal time than in pubertal time, although both of them were above the recommendations. The proportion of girls was a little higher, and it remained around 50% for each BMI group (data not shown). At baseline, 69% were children with overweight and obesity. The mean of the BMIZ-score in prepubertal children showed no significant difference to that at pubertal stage. At the end of follow-up, about 3/4 of adolescents showed no BMI group changes and the others had an improvement to a normal weight, or a worsening to obesity.

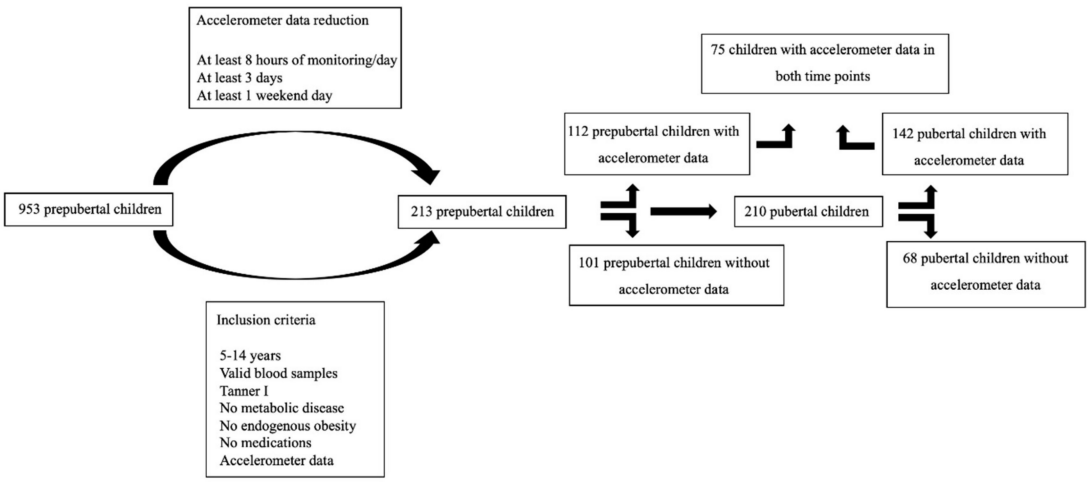


Figure 1. Design of the study.

Total min of PA detected by the accelerometer were significantly higher at the pubertal (891.1 ± 169.6 min) than at prepubertal stage (771.8 ± 79.4 min). In Table 2, PA is presented for both times as the mean of measured min/day and relative values of these measurements for both groups. Absolute and relative values of ST were significantly higher in pubertal time compared with basal time, with no gender differences. In the adolescents, average ST increased by 66.9 min. LPA, MPA, and MVPA were lower in pubertal measures than in prepubertal. At baseline, only MVPA shows statistical differences in absolute values (p = 0.03) and, MPA and MVPA for relative values (p = 0.027 and p = 0.025, respectively). At the pubertal stage, only VPA showed significant gender differences. The decline of MPA and MVPA

from prepubertal to pubertal period was higher in boys than girls. In contrast, VPA in boys was the only PA intensity which increased in absolute values between times (1.6 min/day).

Table 1. Characteristics of the longitudinal sample (n = 75) at prepubertal and pubertal periods and evolution in weight status.

	Prepubertal	Pubertal	p
Days of Physical activity	4.65 ± 0.70	6.52 ± 0.86	<000.1
Age (years)	8.46 ± 1.37	13.84 ± 1.88	<000.1
Females (%)	42 (56.0)		
BMI (Kg/m^2)	21.92 ± 4.86	25.80 ± 6.78	0.001
BMI Z-Score	1.79 ± 1.95	1.63 ± 1.89	0.610
Cole groups (%)			
Normal-weight	23 (30.6)	29 (38.6)	
Overweight	19 (25.3)	18 (24.0)	
With Obesity	33 (44.0)	28 (37.3)	
Changes in BMI			
No changes (%)	55 (73.3)		
Normal-weight	21		
Overweight	9		
Obesity	25		
Improvement (%)	15 (20)		
Obesity to overweight	7		
Overweight to normal-weight	7		
Obesity to normal-weight	1		
Worsening (%)	5 (6.7)		
Normal-weight to overweight	2		
Overweight to obesity	3		
Normal-weight to obesity	0		

Data are expressed as mean ± DS. Differences between groups are presented in *p* column.

As Table 3 shows, around 60% of boys accomplished the recommendation of 60 min/day of MVPA at prepubertal period, while only 28% of girls did. In pubertal period, the proportion of adolescents who met this recommendation decreased in both genders, however, the decline was greater for boys than girls (33.3% vs. 4.6%, respectively).

Table 4 shows PA data according to BMI groups. At baseline, there were no differences in ST between BMI groups. NW children showed higher MPA, VPA, and MVPA values than OW and OB children. In fact, only NW prepubertal children reached 60 min of MVPA. At the pubertal stage, there were no differences between BMI groups for ST or any PA intensity. As seen previously, ST increased about 16% in relative values for all BMI groups, with a decline in PA, especially in LPA. This reduction in LPA, MPA, VPA, and MPVA tended to be higher for NW children than OW and OB children, with a tendency of similarity between BMI groups in the pubertal period.

Table 5 shows the PA measurements from the longitudinal analysis according to BMI-change groups. Subjects who did not change their BMI increased their ST for up to 70% of the time measured (about 15% more in relative values), which means about 1 h more per day of ST in pubertal stage. Those who improved their BMI had the highest increase in ST (about 18.8% in relative values). In contrast, those whose BMI worsened showed the shortest increase in ST (about 6%).

Table 2. Differences in physical activity levels between prepubertal and pubertal periods measured in mean minutes and relative percentages in the longitudinal sample (n = 75).

	Mean Values (min ± SD)				Relative Values (% ± SD)			
	Prepubertal	**Pubertal**	**Δ (%)**	***p***	**Prepubertal**	**Pubertal**	**Δ (%)**	***p***
Sedentary								
All	443.3 ± 74.1	636.8 ± 164.2	+43.6	<0.001	56.0 ± 6.7	71.1 ± 8.2	+15.1	<0.001
Boys	445.2 ± 78.1	661.9 ± 146.4	+48.6	<0.001	56.1 ± 6.3	71.6 ± 7.0	+15.5	<0.001
Girls	441.8 ± 71.6	617.1 ± 176.1	+39.6	<0.001	55.9 ± 7.0	70.7 ± 9.1	+14.8	<0.001
Light PA								
All	286.8 ± 47.8	200.6 ± 50.1	−32.0	<0.001	36.7 ± 5.6	23.4 ± 6.9	−13.3	<0.001
Boys	281.5 ± 43.7	202.7 ± 60.1	−27.9	<0.001	35.9 ± 4.8	22.5 ± 5.6	−13.4	<0.001
Girls	291.0 ± 50.8	199.0 ± 51.5	−31.6	<0.001	37.3 ± 6.2	24.1 ± 7.8	−13.2	<0.001
Moderate PA								
All	40.9 ± 13.3	31.1 ± 12.3	−23.9	<0.001	5.2 ± 1.6	3.6 ± 1.5	−1.6	<0.001
Boys	43.7 ± 14.8	31.7 ± 13.3	−27.4	<0.001	5.7 ± 1.7	3.6 ± 1.5	−2.1	<0.001
Girls	38.6 ± 11.7	30.6 ± 11.6	−20.7	0.003	4.9 ± 1.3 $^{\delta}$	3.6 ± 1.5	−1.3 $^{\delta}$	<0.001
Vigorous PA								
All	15.3 ± 9.2	15.2 ± 11.0	−0.6	0.965	1.9 ± 1.1	1.7 ± 1.2	−0.2	0.255
Boys	17.4 ± 10.5	19.0 ± 10.2	+9.1	0.379	2.1 ± 1.2	2.1 ± 1.3	0	0.809
Girls	13.6 ± 7.8	12.2 ± 8.9 $^{\lambda}$	−10.2 $^{\kappa}$	0.296	1.7 ± 0.9	1.4 ± 1.0 $^{\delta}$	−0.3	0.139
MVPA								
All	56.1 ± 20.4	45.5 ± 18.0	−18.8	<0.001	7.1 ± 2.5	5.3 ± 2.3	−1.8	<0.001
Boys	61.4 ± 22.4	49.3 ± 18.9	−19.7	0.024	7.9 ± 2.8	5.6 ± 2.4	−2.3	0.001
Girls	52.1 ± 18.1 $^{\delta}$	42.6 ± 16.9	−18.2	0.008	6.6 ± 2.1 $^{\delta}$	5.1 ± 2.2	−1.5 $^{\delta}$	0.001

PA: physical activity; MVPA: moderate-to-vigorous PA; Data of PA for both periods and variation between them are presented for total sample with absolute (mean) and relative values. Mean values are expressed as mean of min/day of each level of PA ± SD. Relative values are expressed as ((mean of min/day of any level of PA measured/mean of total min/day of PA measured) × 100) ± SD. Differences between sex are indicated in girls' rows of "prepubertal", "pubertal", and "Δ" columns. Differences between periods for all, boys and/or girls are indicated in *p* column. Differences are expressed with: δ for $p < 0.05$; λ for $p < 0.01$; κ for $p < 0.001$.

Table 3. Distribution in percentage of children by gender in prepubertal and pubertal periods, related with moderate-to-vigorous physical activity in the longitudinal sample ($n = 75$).

	Boys (%)		Girls (%)	
MVPA (min)	**Prepubertal**	**Pubertal**	**Prepubertal**	**Pubertal**
<30	3 (9.0)	7 (21.2)	4 (9.5)	12 (28.5)
30–59.9	10 (30.3)	17 (51.5)	26 (61.9)	20 (47.6)
60–89.9	15 (45.4)	9 (27.2)	11 (26.1)	10 (23.8)
>90	5 (15.1)	0	1 (2.3)	0

Regarding PA, the biggest increase in ST was replaced by a decline in LPA (about 88% of ST, which means about 35–40 min less per day), being statistically significant for all of them, except the worsening BMI group. The improving and not-changing BMI groups decreased MPA and MVPA in absolute and relative values, but VPA did not show differences in any of them. The worsening group did not show any difference between prepubertal and pubertal time in any group of PA intensities.

Table 4. Comparison of physical activity levels and minutes of practice between prepubertal and pubertal periods according to BMI groups in the longitudinal sample (n = 75).

Total Sample (n = 75)	Mean Values				Relative Values			
	Prepubertal	Pubertal	Δ (%)	p	Prepubertal	Pubertal	Δ (%)	p
Sedentary								
NW	429.3 ± 63.1	641.3 ± 174.5 $^{\delta}$	+49.3	<0.001	55.5 ± 7.3	73.2 ± 8.1	+17.7	<0.001
OW	428.1 ± 68.1	695.5 ± 120.5 $^{\lambda}$	+62.4	<0.001	56.5 ± 8.1	72.9 ± 5.7	+16.4	<0.001
OB	444.9 ± 85.0	624.9 ± 152.6	+40.4 $^{\kappa}$	<0.001	55.9 ± 6.4	71.1 ± 8.0	+15.2	<0.001
Light PA								
NW	279.5 ± 46.6	186.2 ± 52.5 $^{\delta}$	−33.3 $^{\delta}$	<0.001	36.1 ± 5.3	21.7 ± 6.7	−14.4	<0.001
OW	279.1 ± 52.9	208.4 ± 58.2	−25.3	<0.001	36.8 ± 6.6	21.9 ± 5.1	−14.9	<0.001
OB	290.1 ± 54.7	204.1 ± 56.0 $^{\delta}$	−29.6	<0.001	37.1 ± 5.8	23.7 ± 6.4	−13.4	<0.001
Moderate PA								
NW	45.4 ± 13.6 $^{\kappa}$	27.6 ± 12 $^{\delta}$	−39.2 $^{\kappa}$	<0.001	5.8 ± 1.6 $_{\kappa}$	3.2 ± 1.5	−2.6	<0.001
OW	36.7 ± 15.4	31.7 ± 12.5	−13.6	0.004	4.8 ± 1.9	3.3 ± 1.2	−1.5	<0.001
OB	39.7 ± 12.4 $^{\lambda}$	30.5 ± 14.0	−23.1 $^{\delta}$	<0.001	5.1 ± 1.4 $_{\lambda}$	3.5 ± 1.5	−1.6	<0.001
Vigorous PA								
NW	18.8 ± 12.2 $^{\lambda}$	15.1 ± 10.2	−19.6 $^{\delta}$	0.009	2.4 ± 1.4 $_{\kappa}$	1.7 ± 1.2	−0.7	<0.001
OW	13.1 ± 9.7	16.6 ± 11.9	+26.7 $^{\delta}$	0.012	1.7 ± 1.1	1.7 ± 1.1	0	1.000
OB	14.2 ± 7.5 $^{\lambda}$	13.2 ± 10.8	−7.0	0.405	1.7 ± 0.9 $_{\kappa}$	1.5 ± 1.2	−0.2	0.143
MVPA								
NW	61.9 ± 21.2 $^{\lambda}$	42.0 ± 18.8	−32.1 $^{\delta}$	<0.001	8.2 ± 2.9 $_{\kappa}$	4.9 ± 2.4	−3.3	<0.001
OW	49.8 ± 23.6	47.9 ± 19.9	−3.8	0.482	6.5 ± 2.8	5.0 ± 1.9	−1.5	<0.001
OB	54.8 ± 17.6 $^{\delta}$	42.3 ± 19.7	−22.8	<0.001	6.9 ± 2.1 $_{\lambda}$	5.0 ± 2.4	−1.9	<0.001

PA: physical activity; MVPA: moderate-to-vigorous PA; NW: children normal-weight; OW: children with overweight; OB: children with obesity. Data of PA for each period and variation between them are presented with absolute (mean) and relative values. Mean values are expressed as mean of min/day of each level of PA ± SD. Relative values are expressed as ((mean of min/day of any level of PA measured/mean of total min/day of PA measured) × 100) ± SD. Differences between periods for NW, OW, and/or OB are indicated in Δ column. Differences between NW and OW are indicated in NW row. Differences between OW and OB are indicated in OW row. Differences between OB and NW are indicated in OB row. Differences are expressed with: **δ** for $p < 0.05$; **λ** for $p < 0.01$; **κ** for $p < 0.001$.

Table 5. Comparison of physical activity levels between prepubertal and pubertal periods according to BMI-change groups in the longitudinal sample (n = 75).

Total Sample (n = 75)	Mean Values				Relative Values			
	Prepubertal	Pubertal	Δ (%)	p	Prepubertal (%)	Pubertal (%)	Δ (%)	p
Normal-Weight No Changes (n = 21)								
Sedentary	423.0 ± 60.6	595.0 ± 183.1	+40.6	0.001	55.2 ± 6.3	69.9 ± 9.3	+14.7 $^{\beta}$	<0.001
Light	279.7 ± 42.0	196.2 ± 56.2	−29.8 $^{\beta}$	<0.001	3.6 ± 4.6	24.1 ± 7.7	−12.4	<0.001
Moderate	44.8 ± 10.5	30.5 ± 12.4	−31.9	0.001	5.8 ± 1.3	3.8 ± 1.7	−2.0 $^{\beta}$	0.001
Vigorous	17.9 ± 9.7	16.5 ± 10.0	−7.8	0.523	2.3 ± 1.2	2.0 ± 1.2	−0.2	0.384
MVPA	62.7 ± 19.2	47.0 ± 18.9	−25.0	0.007	8.2 ± 2.4	5.8 ± 2.6	−2.3	0.007
Overweight/With Obesity No changes (n = 34)								
Sedentary	445.8 ± 79.1	639.1 ± 162.1	+43.3	<0.001	55.4 ± 6.3	70.5 ± 7.4	+15.1 $^{\beta}$	<0.001
Light	300.7 ± 45.7	210.5 ± 51.8	−29.9 $^{\beta}$	<0.001	37.6 ± 6.2	24.0 ± 6.5	−13.5 $^{\beta}$	<0.001
Moderate	40.0 ± 12.4	32.2 ± 12.1	−19.5	0.016	5.0 ± 1.2	3.7 ± 1.3	−1.3	0.001
Vigorous	15.2 ± 9.6	14.9 ± 11.5	−1.9	0.885	1.8 ± 1.0	1.6 ± 1.1	−0.2	0.294
MVPA	56.3 ± 19.6	47.5 ± 18.8	−15.6	0.066	6.9 ± 2.2	5.3 ± 1.9	−1.6	0.005
Improving (n = 15)								
Sedentary	465.4 ± 70.4	728.6 ± 119.1	+56.5	<0.001	57.6 ± 5.4	76.5 ± 6.7	+18.8 $^{\beta}$	<0.001
Light	274.9 ± 44.2	178.1 ± 62.6	−35.2 $^{\beta}$	<0.001	35.8 ± 4.0	19.2 ± 6.2	−16.5 $^{\beta}$	<0.001
Moderate	37.5 ± 15.9	26.0 ± 10.0	−30.6	0.020	4.8 ± 2.1	2.6 ± 0.7	−2.1 $^{\beta}$	0.003
Vigorous	13.4 ± 7.8	13.9 ± 9.8	+3.7	0.813	1.6 ± 1.0	1.5 ± 1.1	−0.1	0.626
MVPA	50.6 ± 22.9	39.0 ± 15.9	−22.9	0.045	6.4 ± 3.0	4.1 ± 1.6	−2.2	0.010
Worsening (n = 5)								
Sedentary	444.6 ± 100.6	521.3 ± 85.3	+17.2	0.285	58.5 ± 12.8	64.4 ± 5.6	+5.8 $^{\delta,\lambda,\kappa}$	0.329
Light	263.1 ± 78.7	226.1 ± 20.7	−14.0 $^{\delta,\lambda,\kappa}$	0.354	34.5 ± 9.6	28.1 ± 2.8	−6.3 $^{\lambda,\kappa}$	0.252
Moderate	41.8 ± 20.8	43.1 ± 14.4	+3.1	0.834	5.4 ± 2.5	5.3 ± 1.7	−0.1 $^{\delta,\kappa}$	0.932
Vigorous	10.4 ± 7.2	16.0 ± 16.8	+53.8	0.306	1.3 ± 0.9	1.9 ± 2.0	+0.5	0.383
MVPA	42.3 ± 16.3	46.5 ± 13.0	+9.9	0.630	6.8 ± 3.2	7.3 ± 3.7	+0.5	0.595

MVPA: moderate-to-vigorous physical activity; Improving: Subjects with overweight or obesity in prepubertal time and changed to overweight or normal-weight, respectively, in pubertal time. Worsening: Subjects who were normal-weight or overweight in prepubertal time and changed to overweight or obesity, respectively, in pubertal time. Data of PA and variation between periods are presented for total sample with absolute (mean) and relative values. Mean values are expressed as mean of min/day of each level of PA ± SD. Relative values are expressed as ((mean of min/day of any level of PA measured/mean of total min/day of PA measured) × 100) ± SD. Differences between BMI-change groups ($p < 0.05$) according to "Δ" for each level of PA intensity are expressed in "Δ" column. Differences regarding "Normal-weight no changes" group are expressed with **δ**; differences regarding "OW/OB no changes group" are expressed with **λ**; differences regarding "Improving group" are expressed with **κ**; differences regarding "Worsening" group are expressed with **β**.

Finally, the results of the multivariable regression test are showed in the Table S1.

4. Discussion

Changes of PA and ST according to the presence or absence of pubertal development, as well as the differences between genders and BMI groups have been studied in this Spanish sample. The performance of PA in this Spanish cohort decreased from childhood to adolescence, being replaced by a rise in sedentarism. The time spent on all intensities of PA has been measured objectively by accelerometry, focused also on gender differences, and especially related with BMI changes.

This increase in ST which accounted for 72% of the total measured time in pubertal adolescents (Table 2), has been previously reported to be about 40 min per day than the baseline values, or reaching 90 min per day for British and North Americans [15,33–35]. Parallel to the increased ST, LPA was the main contributor in the reduction of PA, with a 14% decrease in relative values, while MPA, and especially VPA, remained stable (Table 2). Some researchers also found that the rise of ST matched the decrease in LPA in adolescence, while MVPA remained relatively constant during this stage [15,34–36]. Previous studies focused on MVPA as the most important contributor to the decrease of PA and its association with health benefits [1,20,37]. However, our data suggest that MVPA plays a small role in this reduction, at least in older children. It seems that VPA and MVPA levels were already low at the prepubertal time in our cohort, especially in girls, and only boys at baseline accomplished

the 60 min of MVPA recommended by the WHO (Table 2). The latest research proposes an earlier decline of MVPA during early childhood [38–40], which makes us think about a stepped decline of the different PA intensities. We hypothesize that the decline of PA is produced from early childhood to adolescence in a staggered manner, with a decrease of MPA and VPA from early to late childhood, and a decline of LPA from late childhood to adolescence. ST increases progressively along this process. Most longitudinal studies are performed in adolescents or in late childhood populations [15,33–35,41], while early childhood data comes from cross-sectional and non-objective PA measures. Thus, future research in this area, especially about MVPA decline, should focus on this population.

In the present study, gender differences in PA were found for MPA, VPA, and MVPA, both in pre-pubertal and pubertal stages, showing that boys are more active than girls regardless of pubertal status. However, PA in boys decreased more prominently than in girls as in line with previous reports [33,41]. In our cohort, the proportion of boys who accomplished the 60 min recommendation fell from 60% to one quarter after puberty. In contrast, the percentage of girls who reached the WHO recommendation was already low at prepubertal time, as previously reported [40], showing a lighter decrease with adolescence (Table 3).

On the other hand, min of MVPA were higher in boys than girls in both time points, but differences tended to decrease over time, especially due to the reduction of activity in boys, similar to the previously reported results [33]. The gap between boys and girls in MPA seems also to be higher in the prepubertal than pubertal period, but it is lower in the prepubertal than pubertal period for VPA. In summary, differences in MPA tend to be similar, while those for VPA tend to increase.

PA and ST measurements between BMI groups showed higher MPA, VPA, and MVPA values in prepubertal NW children than in OW and OB, while no differences were found for the different PA intensities between groups classified by BMI in pubertal stage. Noteworthy, NW children were the only group who reached the 60 min recommendation of MVPA (Table 4). A recent systematic review [23] revealed that MVPA was significantly lower in children and adolescents with obesity compared to controls, but differences were small and none of the participants accomplished the WHO recommendation of MVPA. All BMI groups increased their ST and decreased their PA intensities in absolute and relative values, but the decrease of NW subjects was higher than those for OW and OB, showing that even these groups perform similar min of PA of any intensity in pubertal time, and there was a tendency of similarity between BMI groups from childhood to adolescence (Table 4).This may be because in the adolescence, children with obesity are more aware of their excess of weight and some of them try to compensate by improving their habits. So, perhaps the smallest increase in sedentary lifestyle is compensated with a greater interest in exercise than in the rest of the normal weight population. Most subjects in this study (around 70%) did not change their baseline BMI status after reaching puberty, and nearly 20% of them improved it (Table 1). These results could be explained because overweight/obesity children were addressed to the pediatric endocrinologist at prepubertal time where the received general dietary recommendations for weight management. The proportion of subjects with overweight and obesity at baseline in the population study was higher than in the general population. This also may be linked with the recruitment, which took place on the pediatric endocrinologist. However, PA levels did not vary when we studied normal-weight subjects separately.

Some interesting results were found when the sample was divided regarding their BMI changes. Subjects who improved or did not change their BMI increased their ST in relative values, at the expense of the decline of PA, especially LPA (Table 5). The worsening BMI group showed the shortest increase in ST (Table 5), although this could be explained by the reduced sample size. Similarly, VPA did not show differences in any of the groups.

Minutes measured of PA for prepubertal children were fewer than for pubertal, so relative values were calculated. This difference is related with the minutes that the Actigraph device was worn and the amount of them interpreted as null. Children probably tolerated this worse, and this reduced the valid time measured. We found differences in the total min of measured PA in previous studies

without further explanation. Instead, Corder et al. [33,42] included several results as a percentage. Thus, differences in the proportion of PA levels relative to the time measured seem to be more informative than absolute mean differences.

The present study shows changes in objectively measured PA in prepubertal Spanish children who become adolescents. Most of the previous longitudinal data came from children or adolescents [15,33,34,41] and only a few studies have included children to follow until adolescence [40]. Subjects were classified regarding pubertal status instead of age, unlike previous literature [33,40]. This involved difficulties and losses in the follow-up, but allowed us to focus on the importance of the transition from childhood to adolescence in the decline of PA.

Previous studies used BMI as a static variable [33], so changes in PA behavior according to BMI and the bidirectional relationship between PA and weight status were difficult to interpret. This study brings a new approach with the inclusion of BMI as a dynamic variable, which allows to explore both changes in BMI and PA at the same time. However, a limitation to consider is that this subsample is from the GENOBOX study, so the proportion of subjects with overweight/obesity was higher than in the total population and trends of PA should be interpreted in that context. Moreover, in prepubertal time, the number of available devices for monitoring PA limited the sample size. As in pubertal time, some adolescents did not agree to wear the Actigraph device, and that restricted the sample size of the longitudinal group.

5. Conclusions

In conclusion, the results of this longitudinal study show a decrease of PA along with the increase of ST in the transition from childhood to adolescence, with differences by gender and BMI. Boys showed a higher decline in MVPA than girls, although remained more active. Regardless of body weight, teens tend to be less active. Therefore, it is necessary to implement measures at these stages to reduce sedentary lifestyle and at least maintain physical activity levels.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-4601/17/19/7227/s1, Table S1. Associations of ΔBMIz-score with with Δphysical activity in intensities, age, sex and pubertal stage during the follow up in Genobox study using multiple regression analysis.

Author Contributions: Conceptualization, M.G.-C., R.L., G.B.-L. and C.M.A.; methodology, M.G.-C., R.L., G.B.-L., C.M.A., and F.J.L.-C.; formal analysis, F.J.L.-C. and F.J.A.-G.; investigation, F.J.L.-C., F.J.A.-G., A.I.R., K.F.-R., E.M.G.-G. and R.V.-C.; resources, M.G.-C., R.L., G.B.-L. and C.M.A.; data curation, A.A.-R.; writing—original draft preparation, F.J.A.-G., F.J.L.-C., M.G.-C. and R.L.; writing—review and editing, all the group; visualization, F.J.A.-G., F.J.L.-C., M.G.-C. and R.L.; supervision, M.G.-C. and R.L.; project administration, M.G.-C., R.L., G.B.-L. and C.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I + D + I), Instituto de Salud Carlos III-Health Research Funding (FONDOS FEDER) (PI11/02042, PI11/02059, PI11/01425; PI16/00871, PI16/01301, PI16/01205) and RETIC (Redes temáticas de investigación cooperativa) (Red SAMID RD12/0026/0015). The authors also acknowledge Instituto de Salud Carlos III for personal funding of A.A.R: Contratos i-PFIS: doctorados IIS-empresa en ciencias y tecnologías de la salud de la convocatoria 2017 de la Acción Estratégica en Salud 2013–2016 (IFI17/00048). This paper will be included in F.J.A. doctorate, under the "Biomedicine Program" at the University of Córdoba, Spain.

Acknowledgments: The authors would like to thank the children and parents who participated in the study.

Conflicts of Interest: The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

References

1. Janssen, I.; LeBlanc, A.G. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. *Int. J. Behav. Nutr. Phys. Act.* **2010**, *7*. [CrossRef] [PubMed]
2. Psaltopoulou, T.; Tzanninis, S.; Ntanasis-Stathopoulos, I.; Panotopoulos, G.; Kostopoulou, M.; Tzanninis, I.G.; Tsagianni, A.; Sergentanis, T.N. *Prevention and Treatment of Childhood and Adolescent Obesity: A Systematic Review of Meta-Analyses*; Springer: Singapore, 2019; Volume 15, ISBN 0123456789.

3. Okely, T.D.; Salmon, J.; Vella, S.; Cliff, D.; Timperio, A.; Tremblay, M.; Trost, S.; Shilton, T.; Hinkley, T.; Ridgers, N.; et al. *A Systematic Review to Update the Australian Physical Activity Guidelines for Children and Young People*; Commonwealth of Australia: Canberra, Australia, 2012.
4. Tremblay, M.S.; Carson, V.; Chaput, J.-P.; Connor Gorber, S.; Dinh, T.; Duggan, M.; Faulkner, G.; Gray, C.E.; Gruber, R.; Janson, K.; et al. Canadian 24-Hour Movement Guidelines for Children and Youth: An Integration of Physical Activity, Sedentary Behaviour, and Sleep. *Appl. Physiol. Nutr. Metab.* **2016**, *41*, S311–S327. [CrossRef] [PubMed]
5. Weggemans, R.M.; Backx, F.J.G.; Borghouts, L.; Chinapaw, M.; Hopman, M.T.E.; Koster, A.; Kremers, S.; van Loon, L.J.C.; May, A.; Mosterd, A.; et al. The 2017 Dutch Physical Activity Guidelines. *Int. J. Behav. Nutr. Phys. Act.* **2018**, *15*, 1–12. [CrossRef] [PubMed]
6. Piercy, K.L.; Troiano, R.P.; Ballard, R.M.; Carlson, S.A.; Fulton, J.E.; Galuska, D.A.; George, S.M.; Olson, R.D. The physical activity guidelines for Americans. *JAMA J. Am. Med. Assoc.* **2018**, *320*, 2020–2028. [CrossRef]
7. World Health Organization. *Global Recommendations on Physical Activity for Health*; World Health Organization: Geneva, Switzerland, 2010.
8. McManus, A.M.; Mellecker, R.R. Physical activity and obese children. *J. Sport Health Sci.* **2012**, *1*, 141–148. [CrossRef]
9. Poitras, V.J.; Gray, C.E.; Borghese, M.M.; Carson, V.; Chaput, J.P.; Janssen, I.; Katzmarzyk, P.T.; Pate, R.R.; Connor Gorber, S.; Kho, M.E.; et al. Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Appl. Physiol. Nutr. Metab.* **2016**, *41*, S197–S239. [CrossRef]
10. Verloigne, M.; Loyen, A.; Van Hecke, L.; Lakerveld, J.; Hendriksen, I.; De Bourdheaudhuij, I.; Deforche, B.; Donnelly, A.; Ekelund, U.; Brug, J.; et al. Variation in population levels of sedentary time in European children and adolescents according to cross-European studies: A systematic literature review within DEDIPAC. *Int. J. Behav. Nutr. Phys. Act.* **2016**, *13*. [CrossRef]
11. Biddle, S.J.H.; García Bengoechea, E.; Wiesner, G. Sedentary behaviour and adiposity in youth: A systematic review of reviews and analysis of causality. *Int. J. Behav. Nutr. Phys. Act.* **2017**, *14*, 1–21. [CrossRef]
12. Tremblay, M.S.; Aubert, S.; Barnes, J.D.; Saunders, T.J.; Carson, V.; Latimer-Cheung, A.E.; Chastin, S.F.M.; Altenburg, T.M.; Chinapaw, M.J.M.; Aminian, S.; et al. Sedentary Behavior Research Network (SBRN)-Terminology Consensus Project process and outcome. *Int. J. Behav. Nutr. Phys. Act.* **2017**, *14*, 1–17. [CrossRef]
13. Van Hecke, L.; Loyen, A.; Verloigne, M.; van der Ploeg, H.P.; Lakerveld, J.; Brug, J.; De Bourdeaudhuij, I.; Ekelund, U.; Donnelly, A.; Hendriksen, I.; et al. Variation in population levels of physical activity in European children and adolescents according to cross-European studies: A systematic literature review within DEDIPAC. *Int. J. Behav. Nutr. Phys. Act.* **2016**, *13*, 1–22. [CrossRef]
14. Keane, E.; Li, X.; Harrington, J.M.; Fitzgerald, A.P.; Perry, I.J.; Kearney, P.M. Physical activity, sedentary behavior and the risk of overweight and obesity in school-aged children. *Pediatr. Exerc. Sci.* **2017**, *29*, 408–418. [CrossRef] [PubMed]
15. Mitchell, J.A.; Pate, R.R.; Dowda, M.; Mattocks, C.; Riddoch, C.; Ness, A.R.; Blair, S.N. A prospective study of sedentary behavior in a large cohort of youth. *Med. Sci. Sports Exerc.* **2012**, *44*, 1081–1087. [CrossRef] [PubMed]
16. Craggs, C.; Corder, K.; Van Sluijs, E.M.F.; Griffin, S.J. Determinants of change in physical activity in children and adolescents: A systematic review. *Am. J. Prev. Med.* **2011**, *40*, 645–658. [CrossRef] [PubMed]
17. Dumith, S.C.; Gigante, D.P.; Domingues, M.R.; Kohl, H.W. Physical activity change during adolescence: A systematic review and a pooled analysis. *Int. J. Epidemiol.* **2011**, *40*, 685–698. [CrossRef]
18. Gebremariam, M.K.; Bergh, I.H.; Andersen, L.F.; Ommundsen, Y.; Bjelland, M.; Lien, N. Stability and change in potential correlates of physical activity and association with pubertal status among Norwegian children in the transition between childhood and adolescence. *Int. J. Behav. Nutr. Phys. Act.* **2012**, *9*, 1–8. [CrossRef]
19. Lu, C.; Stolk, R.P.; Sauer, P.J.J.; Sijtsma, A.; Wiersma, R.; Huang, G.; Corpeleijn, E. Factors of physical activity among Chinese children and adolescents: A systematic review. *Int. J. Behav. Nutr. Phys. Act.* **2017**, *14*, 36. [CrossRef]
20. Nader, P.R.; Bradley, R.H.; Houts, R.M.; McRitchie, S.L.; O'Brien, M. Moderate-to-vigorous physical activity from ages 9 to 15 years. *JAMA J. Am. Med. Assoc.* **2008**, *300*, 295–305. [CrossRef]

21. Sherar, L.B.; Cumming, S.P.; Eisenmann, J.C.; Baxter-Jones, A.D.G.; Malina, R.M. Adolescent biological maturity and physical activity: Biology meets behavior. *Pediatr. Exerc. Sci.* **2010**, *22*, 332–349. [CrossRef]
22. Cumming, S.P.; Standage, M.; Gillison, F.; Malina, R.M. Sex Differences in Exercise Behavior During Adolescence: Is Biological Maturation a Confounding Factor? *J. Adolesc. Health* **2008**, *42*, 480–485. [CrossRef]
23. Elmesmari, R.; Martin, A.; Reilly, J.J.; Paton, J.Y. Comparison of accelerometer measured levels of physical activity and sedentary time between obese and non-obese children and adolescents: A systematic review. *BMC Pediatr.* **2018**, *18*. [CrossRef]
24. Anguita-Ruiz, A.; Mendez-Gutierrez, A.; Ruperez, A.I.; Leis, R.; Bueno, G.; Gil-Campos, M.; Tofe, I.; Gomez-Llorente, C.; Moreno, L.A.; Gil, Á. The protein S100A4 as a novel marker of insulin resistance in prepubertal and pubertal children with obesity. *Metabolism* **2020**, *105*. [CrossRef]
25. Leis, R.; Jurado-Castro, J.M.; Llorente-Cantarero, F.J.; Anguita-Ruiz, A.; Iris-Rupérez, A.; Bedoya-Carpente, J.J.; Vázquez-Cobela, R.; Aguilera, C.M.; Bueno, G.; Gil-Campos, M. Cluster Analysis of Physical Activity Patterns, and Relationship with Sedentary Behavior and Healthy Lifestyles in Prepubertal Children: Genobox Cohort. *Nutrients* **2020**, *12*, 1288. [CrossRef] [PubMed]
26. World Medical Association. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA J. Am. Med. Assoc.* **2013**, *310*, 2191–2194. [CrossRef] [PubMed]
27. Bornstein, M.H. Tanner Stages. In *The SAGE Encyclopedia of Lifespan Human Development*; SAGE Publications, Inc.: Thousand Oaks, CA, USA, 2018.
28. Cole, T.J.; Bellizzi, M.C.; Flegal, K.M.; Dietz, W.H. Establishing a standard definition for child overweight and obesity worldwide: International survey. *Br. Med. J.* **2000**, *320*, 1240–1243. [CrossRef] [PubMed]
29. McCrindle, B.W. Assessment and management of hypertension in children and adolescents. *Nat. Rev. Cardiol.* **2010**, *7*, 155–163. [CrossRef]
30. Migueles, J.H.; Cadenas-Sanchez, C.; Ekelund, U.; Delisle Nyström, C.; Mora-Gonzalez, J.; Löf, M.; Labayen, I.; Ruiz, J.R.; Ortega, F.B. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. *Sport. Med.* **2017**, *47*, 1821–1845. [CrossRef]
31. Treuth, M.S.; Schmitz, K.; Catellier, D.J.; McMurray, R.G.; Murray, D.M.; Almeida, M.J.; Going, S.; Norman, J.E.; Pate, R. Defining accelerometer thresholds for activity intensities in adolescent girls. *Med. Sci. Sports Exerc.* **2004**, *36*, 1259–1266. [CrossRef]
32. Evenson, K.R.; Catellier, D.J.; Gill, K.; Ondrak, K.S.; McMurray, R.G. Calibration of two objective measures of physical activity for children. *J. Sports Sci.* **2008**, *26*, 1557–1565. [CrossRef]
33. Corder, K.; Sharp, S.J.; Atkin, A.J.; Griffin, S.J.; Jones, A.P.; Ekelund, U.; van Sluijs, E.M.F. Change in objectively measured physical activity during the transition to adolescence. *Br. J. Sports Med.* **2015**, *49*, 730–736. [CrossRef]
34. Harding, S.K.; Page, A.S.; Falconer, C.; Cooper, A.R. Longitudinal changes in sedentary time and physical activity during adolescence. *Int. J. Behav. Nutr. Phys. Act.* **2015**, *12*, 44. [CrossRef]
35. Treuth, M.S.; Baggett, C.D.; Pratt, C.A.; Going, S.B.; Elder, J.P.; Charneco, E.Y.; Webber, L.S. A longitudinal study of sedentary behavior and overweight in adolescent girls. *Obesity* **2009**, *17*, 1003–1008. [CrossRef] [PubMed]
36. Kandola, A.; Lewis, G.; Osborn, D.P.J.; Stubbs, B.; Hayes, J.F. Depressive symptoms and objectively measured physical activity and sedentary behaviour throughout adolescence: A prospective cohort study. *Lancet Psychiatry* **2020**, *7*, 262–271. [CrossRef]
37. Ekelund, U.; Luan, J.; Sherar, L.B.; Esliger, D.W.; Griew, P.; Cooper, A. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. *JAMA J. Am. Med. Assoc.* **2012**, *307*, 704–712. [CrossRef] [PubMed]
38. Cooper, A.R.; Goodman, A.; Page, A.S.; Sherar, L.B.; Esliger, D.W.; van Sluijs, E.M.F.; Andersen, L.B.; Anderssen, S.; Cardon, G.; Davey, R.; et al. Objectively measured physical activity and sedentary time in youth: The International children's accelerometry database (ICAD). *Int. J. Behav. Nutr. Phys. Act.* **2015**, *12*, 1–10. [CrossRef]
39. Kwon, S.; Janz, K.F.; Letuchy, E.M.; Burns, T.L.; Levy, S.M. Developmental trajectories of physical activity, sports, and television viewing during childhood to young adulthood: Iowa bone development study. *JAMA Pediatr.* **2015**, *169*, 666–672. [CrossRef]

40. Farooq, M.A.; Parkinson, K.N.; Adamson, A.J.; Pearce, M.S.; Reilly, J.K.; Hughes, A.R.; Janssen, X.; Basterfield, L.; Reilly, J.J. Timing of the decline in physical activity in childhood and adolescence: Gateshead Millennium Cohort Study. *Br. J. Sports Med.* **2018**, *52*, 1002–1006. [CrossRef]
41. Collings, P.J.; Wijndaele, K.; Corder, K.; Westgate, K.; Ridgway, C.L.; Sharp, S.J.; Dunn, V.; Goodyer, I.; Ekelund, U.; Brage, S. Magnitude and determinants of change in objectively-measured physical activity, sedentary time and sleep duration from ages 15 to 17.5y in UK adolescents: The ROOTS study. *Int. J. Behav. Nutr. Phys. Act.* **2015**, *12*, 61. [CrossRef]
42. Corder, K.; Van Sluijs, E.M.F.; Ekelund, U.; Jones, A.P.; Griffin, S.J. Changes in children's physical activity over 12 months: Longitudinal results from the SPEEDY study. *Pediatrics* **2010**, *126*, e926–e935. [CrossRef]

RELATIONSHIP BETWEEN PHYSICAL ACTIVITY, OXIDATIVE STRESS AND TOTAL PLASMA ANTIOXIDANT CAPACITY IN SPANISH CHILDREN FROM THE GENOBOX

Francisco Jesús Llorente-Cantarero[1,2,†], Francisco Javier Aguilar-Gómez[3,†], Rosaura Leis[2,4], Gloria Bueno[2,5,6], Azahara I. Rupérez 6 , Augusto Anguita-Ruiz 2,7,8 , Rocío Vázquez-Cobela[2,4], María Dolores Mesa[7,8], Luis A. Moreno[2,6], Ángel Gil[2,7,8], Concepción María Aguilera[2,7,8,*] and Mercedes Gil-Campos[2,3]

[1]Department of Specific Didactics, Faculty of Education, Maim nides Institute of Biomedicine Research of Córdoba (IMIBIC), University of Córdoba, 14071 Córdoba, Spain; llorentefj@yahoo.es

[2]CIBEROBN, (Physiopathology of Obesity and Nutrition) Institute of Health Carlos III (ISCIII),28029 Madrid, Spain; mariarosaura.leis@usc.es (R.L.); gbuenoloz@yahoo.es (G.B.); augustoanguita@ugr.es (A.A.-R.); cobela.rocio@gmail.com (R.V.-C.); lmoreno@unizar.es (L.A.M.); agil@ugr.es (Á.G.); mercedes_gil_campos@yahoo.es (M.G.-C.)

[3]Metabolism and Investigation Unit, Reina Sofía University Hospital, Maim nides Institute of Biomedicine Research of Córdoba (IMIBIC), University of Córdoba, 14004 Córdoba, Spain; fjaviagui1992@gmail.com

[4]Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Pediatric Department, University of Santiago de Compostela, Clinic University Hospital of Santiago, Instituto de Investigación Sanitaria de Santiago (IDIS), 15706 Santiago de Compostela, Spain

[5]Pediatric Endocrinology Unit, Clinic University Hospital Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, 50009 Zaragoza, Spain

[6]GENUD Research Group, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria (IIS) Aragón, University of Zaragoza, 50009 Zaragoza, Spain; airuperez@unizar.es

[7]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Center of Biomedical Research, University of Granada, Armilla, 18016 Granada, Spain; mdmesa@ugr.es

[8]Instituto de Investigación Biosanitaria ibs Granada, 18014 Granada, Spain

† These authors contributed equally to this work.

antioxidants

MDPI

Article

Relationship between Physical Activity, Oxidative Stress, and Total Plasma Antioxidant Capacity in Spanish Children from the GENOBOX Study

Francisco Jesús Llorente-Cantarero [1,2,†], Francisco Javier Aguilar-Gómez [3,†], Rosaura Leis [2,4], Gloria Bueno [2,5,6], Azahara I. Rupérez [6], Augusto Anguita-Ruiz [2,7,8], Rocío Vázquez-Cobela [2,4], María Dolores Mesa [7,8], Luis A. Moreno [2,6], Ángel Gil [2,7,8], Concepción María Aguilera [2,7,8,*] and Mercedes Gil-Campos [2,3]

1 Department of Specific Didactics, Faculty of Education, Maimónides Institute of Biomedicine Research of Córdoba (IMIBIC), University of Córdoba, 14071 Córdoba, Spain; llorentefj@yahoo.es
2 CIBEROBN, (Physiopathology of Obesity and Nutrition) Institute of Health Carlos III (ISCIII), 28029 Madrid, Spain; mariarosaura.leis@usc.es (R.L.); gbuenoloz@yahoo.es (G.B.); augustoanguita@ugr.es (A.A.-R.); cobela.rocio@gmail.com (R.V.-C.); lmoreno@unizar.es (L.A.M.); agil@ugr.es (Á.G.); mercedes_gil_campos@yahoo.es (M.G.-C.)
3 Metabolism and Investigation Unit, Reina Sofía University Hospital, Maimónides Institute of Biomedicine Research of Córdoba (IMIBIC), University of Córdoba, 14004 Córdoba, Spain; fjaviagui1992@gmail.com
4 Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Pediatric Department, University of Santiago de Compostela, Clinic University Hospital of Santiago, Instituto de Investigación Sanitaria de Santiago (IDIS), 15706 Santiago de Compostela, Spain
5 Pediatric Endocrinology Unit, Clinic University Hospital Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, 50009 Zaragoza, Spain
6 GENUD Research Group, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria (IIS) Aragón, University of Zaragoza, 50009 Zaragoza, Spain; airuperez@unizar.es
7 Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Center of Biomedical Research, University of Granada, Armilla, 18016 Granada, Spain; mdmesa@ugr.es
8 Instituto de Investigación Biosanitaria ibs Granada, 18014 Granada, Spain
* Correspondence: caguiler@ugr.es; Tel.: +34-9-5824-1000 (ext. 20314)
† These authors contributed equally to this work.

Abstract: The World Health Organization has recommended performing at least 60 min a day of moderate-to-vigorous physical activity (MVPA) and reducing sedentarism in children and adolescents to offer significant health benefits and mitigate health risks. Physical fitness and sports practice seem to improve oxidative stress (OS) status during childhood. However, to our knowledge, there are no data regarding the influence of objectively-measured physical activity (PA) and sedentarism on OS status in children and adolescents. The present study aimed to evaluate the influence of moderate and vigorous PA and sedentarism on OS and plasma total antioxidant capacity (TAC) in a selected Spanish population of 216 children and adolescents from the GENOBOX study. PA (light, moderate, and vigorous) and sedentarism (i.e., sedentary time (ST)) were measured by accelerometry. A Physical Activity-Sedentarism Score (PASS) was developed integrating moderate and vigorous PA and ST levels. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) and isoprostane F2α (F_2-IsoPs), as markers of OS, were determined by ELISA; and TAC was estimated by colorimetry using an antioxidant kit. A higher PASS was associated with lower plasma TAC and urinary 8-OHdG and F_2-IsoPs, showing a better redox profile. Reduced OS markers (8-OHdG and F_2-IsoPs) in children with higher PASS may diminish the need of maintaining high concentrations of antioxidants in plasma during rest to achieve redox homeostasis.

Keywords: physical activity; accelerometry; oxidative stress; plasma total antioxidant capacity; 8-hydroxy-2′-deoxyguanosine; isoprostane F2α

Citation: Llorente-Cantarero, F.J.; Aguilar-Gómez, F.J.; Leis, R.; Bueno, G.; Rupérez, A.I.; Anguita-Ruiz, A.; Vázquez-Cobela, R.; Mesa, M.D.; Moreno, L.A.; Gil, Á.; et al. Relationship between Physical Activity, Oxidative Stress, and Total Plasma Antioxidant Capacity in Spanish Children from the GENOBOX Study. *Antioxidants* **2021**, *10*, 320. https://doi.org/10.3390/antiox10020320

Academic Editor: Barbara Tavazz

Received: 1 February 2021
Accepted: 18 February 2021
Published: 20 February 2021

1. Introduction

Physical activity improves cardiorespiratory fitness and strengthens the musculoskeletal system, helping to maintain proper body composition, both in children and adolescents [1,2]. Physical activity practice also seems to reduce lipid peroxidation and improve the antioxidant defense system, resulting in the maintenance of redox homeostasis [3].

The concept of "oxidative stress" has been defined as "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and molecular damage" [4]. Urinary isoprostanes and 8-hydroxy-2′-deoxyguanosine (8-OHdG), among other body-oxidized compounds, are well-recognized biomarkers of oxidative stress, which have been found to be increased in several situations, including obesity, type 2 diabetes, and cardiovascular diseases [5]. F2-8-iso-prostaglandin F2α, also known as isoprostane $F_2\alpha$ (F_2-IsoPs), is generated by free radical-induced peroxidation of arachidonic acid and is currently regarded as one of the most reliable biomarkers of in vivo oxidative stress [6]. In addition, 8-OHdG is considered to be a biomarker of generalized cellular oxidative stress [7]; it can be easily determined in urine with good reproducibility and recovery of untimed samples [8].

Two subsystems integrate the antioxidant defense system: the first is related to the activity of several enzymes such as glutathione peroxidases, glutathione reductase, glutathione S-transferase, superoxide dismutases, and catalase; the second is formed by non-enzymatic antioxidants such as uric acid, tocopherols, ascorbate, glutathione and ubiquinone [9]. The non-enzymatic component can be chemically measured in blood plasma by facing it to several oxidizing substances, obtaining the non-enzymatic antioxidant capacity (NEAC), total antioxidant status, or, henceforth, total antioxidant capacity (TAC). The use of indices of global redox status, such as plasma TAC, may be more appropriate than the comparison of single biomarkers to evaluate oxidative stress. In this context, TAC seems a useful parameter for assessing the global redox status in children and adolescents [10].

Regardless of dietary modifications, physical activity interventions seem to improve body mass index in children and adolescents [11,12]. Similarly, the practice of physical activity, both in adults and children, has been associated with an increase in antioxidants and a reduction of pro-oxidants [13]. Additionally, obesity has been related to an imbalanced redox status [14]. However, it is unclear whether the effect of exercise on redox status is mediated or not by changes in body weight status.

The effect of acute and chronic physical activity practice on oxidative stress responses in children and adolescents has been recently reviewed [15]. Acute exercise seems to induce a relevant, but transient, increase in markers of oxidative stress. In contrast, regular exercise appears to be associated with increased antioxidants and reduced systemic oxidative biomarkers, even independently of body weight status. However, the studies included in that review are heterogeneous in terms of the type of exercise, intensity, and time of physical activity practiced [15].

The World Health Organization (WHO) and other international institutions have recommended that children and adolescents should perform at least an average of 60 min per day of moderate to vigorous-intensity, mostly aerobic activities across the week, in order to achieve several positive outcomes regarding cardiovascular, metabolic, and musculoskeletal health [16]. However, the impact of physical activity, especially in terms of time and intensity, on redox status, has not been accurately described for children and adolescents. In fact, to our knowledge, evaluation of the redox status of children according to objectively-measured physical activity by accelerometry has not been investigated.

We hypothesized that physical activity practice, especially moderate-to-vigorous physical activity (MVPA), and sedentary time could be related to higher plasma levels of TAC in children and adolescents, showing a better global redox status. For this purpose, we aimed to compare plasma levels of TAC and biomarkers of oxidative stress (urinary F_2-IsoPs and 8-OHdG) according to a Physical Activity-Sedentarism Score (PASS), characterized by

moderate and vigorous physical activity and sedentary time measured by accelerometry, in a cross-sectional sample of Spanish children from the GENOBOX study.

2. Materials and Methods

2.1. Population

The present work was part of the GENOBOX study. GENOBOX is a case-control, multicenter study carried out in a total of 1444 children (706 males and 738 females), aged 3 to 17 years Spanish children during 2012–2015. Detailed inclusion and exclusion criteria as well as informed consent and approval by the local Ethics Committees of the three Spanish Hospitals (Hospital Universitario Reina Sofía, Córdoba; Hospital Clínico Universitario, Santiago de Compostela; and Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain; Code IDs: Córdoba 01/2017, Santiago 2011/198, Zaragoza 12/2010) where children were recruited have been reported elsewhere [14]. A subsample of 216 children (111 boys) was selected based on the following inclusion criteria for the present study: Caucasian children and adolescents aged 6 to 14 with body composition measured by bioelectrical impedance analysis, valid blood measurements including sex hormones (follicle-stimulating hormone, luteinizing hormone, testosterone in boys, and estradiol in girls), and accurate data from an accelerometry standardized protocol, as well as measured plasma levels of TAC and urinary F_2-IsoPs and 8-OHdG. Within the subsample of 216 subjects, a subgroup of 74 children (33 boys) also had measures of plasma carotenes, retinol, and tocopherols.

2.2. Clinical and Anthropometric Examination

Medical history and a physical exam including the evaluation of sexual maturity according to Tanner's five-stages were assessed and confirmed with sexual hormone measurements. Anthropometric measurements were taken by a single examiner, and details have been previously reported [17].

2.3. Blood Sampling

Blood samples were drawn from the antecubital vein between 08:00 and 09:30 h after an overnight fast. Routine blood tests (Glucose (CV = 3.0%), plasma insulin (CV = 2.6%), follicle-stimulant hormone (CV = 3.6%); luteinizing hormone (CV = 3.1%), testosterone (CV = 2%), and estradiol (CV = 1.8%) were measured as previously reported using automated analyzers [17] The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated based on the published equation: HOMA-IR = fasting glucose (mmol) × fasting insulin (mU/mL)/22.5 [18].

Plasma TAC was determined by colorimetry using a commercial antioxidant assay kit (Cat no. 709001, Cayman Chemical, Ann Arbor, MI, USA). Urinary F_2-IsoPs was determined by using a commercial competitive ELISA kit (EA85 Oxford Biomedical Research, Oxford, MI, USA) (CV): 14.13%). Urinary 8-OHdG was also determined by using a commercial competitive ELISA kit (KOG-200S/E JaICA, Fukuroi, Japan) (CV: 5.73%). The concentrations were normalized by urinary creatinine and expressed in ng/mg of creatinine. Creatinine concentration in urine samples was determined with a colorimetric kit (Ref. 1001115, Spinreact, Barcelona, Spain) (CV: 2.89%). Measurement of plasma concentrations of retinol, carotenes, and tocopherols were determined after extraction with 1-propanol by ultra-high-pressure liquid chromatography coupled to mass spectrometry UHPLC-MS as reported elsewhere [14].

2.4. Accelerometry

ActiGraph GT3X and GT3X+ accelerometers (ActiGraph; Pensacola, FL, USA) were used to assess physical activity levels in this study. Accelerometers were placed over the right iliac crest and held in place using an adjustable elastic belt for 24 h a day with a minimum of 8 h of monitoring per day for at least 3 days (at least one weekend day). It was programmed for 15 epochs (period of 15 s), as previously recommended [19]. Accelerometry data were processed using the Actilife v6.13.3 program (ActiGraph; Pensacola, FL, USA)

replacing, as a missing data code before further analysis, all negative counts and periods of 20 min or more of consecutive zero counts were replaced [20]. The output generated by the ActiGraph GT3X+ included the total volume of physical activity and each physical activity intensity as defined by the cut-points of counts per minute (CPMs) based in the classification by Evenson et al. [21].

2.5. Statistical Analysis

All continuous variables were tested for normality using the Shapiro–Wilk and Kolmogorov tests; the variables following a non-normal distribution were square-root (TAC, 8-OHdG, F_2-IsoPs, fat body mass (FM), moderate and vigorous physical activity) or logarithm transformed (sedentary time). The homogeneity of variances was estimated using Levene's test. Differences between pre-pubertal and pubertal children were analyzed by two-independent-sample t-tests or Mann–Whitney U tests. χ^2 tests were applied to categorical variables expressed in percentage.

Principal component analysis (PCA) was performed to investigate the relationships among body mass index, body composition, peripheral tissue insulin resistance—as a risk feature of metabolic syndrome—physical activity levels, and oxidative stress and TAC in the 210 children. Extraction of the initial set of uncorrelated components was accomplished with the principal factor method, and then Varimax orthogonal rotation of components was used to facilitate interpretation. High loading values indicate a stronger relationship between a factor and an observed variable. Factor loadings lower than 0.359 (critical factor, $p < 0.001$) revealed marginal correlations.

To estimate the overall physical activity and sedentarism levels, a composite activity score of sedentary time, moderate and vigorous intensities was calculated (i.e., PASS). For this purpose, quartiles of each variable were designed, being the minutes of moderate and vigorous physical activity higher for the fourth quartile compared to the first one, and the opposite for sedentary time. Each subject obtained 1 to 4 points according to the quartile of each variable (e.g., 1 point for being in Q1 for moderate physical activity or 4 points for being in Q4 for sedentary time). By summing the points of the three variables, the PASS was obtained for each subject. The PASS ranged from 3 (all variables in the first quartile) to 12 (all variables in the fourth quartile); a higher score indicated a higher active habit. Based on this score, four groups of PASS were established: very low active (VLA; a score of 3), low active (LA; score from 4 to 6), moderately active (MA; score from 7 to 9), and high active (HA; score from 10–12). MANOVA was used to test the difference between the four groups across several outcome variables/outcomes simultaneously and Box's test looks at the assumption of equal covariance matrices. Pillai's trace value lower than 0.05 was considered statistically significant for the MANOVA test. Differences between the four groups based on the PASS were later analyzed using univariant ANCOVA (UNIANCOVA), adjusting for age and/or body mass index (BMI) z-score; pairwise differences were assessed by post hoc analyses to determine differences between experimental groups. For those variables not following normality, a Kruskal–Wallis test was used to evaluate differences between groups. Values in the descriptive tables and results are expressed as means and standard deviations. Differences were considered significant when $p < 0.05$. All statistical procedures were conducted using SPSS (IBM SPSS Statistics, Version 25.0. Armonk, NY, USA).

3. Results

General demographic, anthropometric, physical activity, and peripheral insulin resistance variables in prepubertal and pubertal selected children within the GENOBOX study are shown in Table 1. Within the 216 subjects, 105 were at prepubertal and 111 at the pubertal stage. There were no differences between prepubertal and pubertal groups regarding BMI z-score and percentages of FM and fat free mass (FFM). No differences were also found for moderate physical activity, but it was closed to statistical significance. At the same time, HOMA-IR and sedentary time were significantly higher for those at pubertal status.

Table 1. General demographic, anthropometric, physical activity, and peripheral insulin resistance variables in prepubertal and pubertal Spanish children from the GENOBOX study.

Variables	All Participants (216)	Prepubertal (105)	Pubertal (111)	*p*-Value
Age (years)	10.8 ± 2.2	9.4 ± 1.7	12.1 ± 1.7	<0.001
Weight (kg)	50.3 ± 17.3	41.8 ± 12	58.2 ± 17.9	<0.001
Height (m)	1.47 ± 0.13	1.38 ± 0.10	1.55 ± 0.10	<0.001
BMI (kg/m^2)	22.9 ± 5.2	21.7 ± 4.5	23.9 ± 5.6	0.003
BMI z-score	1.15 ± 2.14	1.11 ± 2.38	1.15 ± 2.18	0.886
FM (kg)	15.2 ± 9.3	12.8 ± 7	17.3 ± 10.6	0.001
FM (%)	27.8 ± 9.9	27.9 ± 9.3	27.6 ± 10.4	0.836
FFM (kg)	35.1 ± 10.2	29.4 ± 6.4	40.4 ± 10.3	<0.001
FFM (%)	71.6 ± 11.1	71.5 ± 10.4	71.7 ± 11.8	0.887
Normal weight (%)	34.9	14.9	20.2	0.342 *
Overweight (%)	23	11.5	11.5	0.342 *
Obesity (%)	42.1	22.6	19.2	0.342 *
HOMA-IR	2.91 ± 1.81	2.32 ± 1.54	3.40 ± 1.83	<0.001
ST (min/d)	482 ± 97	467 ± 103	495 ± 89	0.018
PA Moderate (min/d)	38 ± 14	40 ± 13	36 ± 15	0.050
PA Vigorous (min/d)	15 ± 10	14 ± 8	16 ± 11	0.251
MVPA (min/d)	53 ± 21	54 ± 20	52 ± 23	0.494

BMI: body mass index; FM: fat mass; FFM: fat-free mass; HOMA-IR: homeostasis model assessment-insulin resistance; ST: sedentary time; PA: physical activity; MVPA: mean moderate-vigorous physical activity. Data are shown as mean ± SD. Student's *t*-test for parametric analysis and U de Mann–Whitney for non-parametric analysis was used to compare variables between prepubertal and pubertal stages. * represents *p* value for Chi-square test.

From the eight items included in the PCA (physical activity, body composition, insulin resistance, oxidative stress, and total plasma antioxidant capacity), three principal components were extracted (Table 2), which explained 64.4% of the total variance (30% of the variance was explained by the first factor, an additional 21% by the second factor, and another 13% by the third factor) (Table 3). The first principal component, termed "metabolic risk" showed a positive correlation between HOMA-IR, FM, and FFM, and humble correlations for sedentary time (negative) and MVPA (positive). The second component, termed "oxidative stress", included correlations among urinary F_2-IsoPs, 8-OHdG, and sedentary time. The third component named "physical activity" included a positive correlation between FFM and MVPA, and negative with sedentary time. TAC did not reach the critical value to be included in any of the components.

The differences between PASS levels for the variables included in the PCA are presented in Table 4 and Figures 1–3. Children with a higher PASS allocated in the high active group had a lower BMI z-score than those in the moderately active and low active groups.

Figure 1 depicts the differences between the PASS levels for the variables with a higher factor loading of the "metabolic risk" component (FM, FFM, and HOMA-IR). Children in the high active group showed the lowest levels of FM and HOMA-IR. No differences were found between groups for FFM.

The influence of PASS on redox status, assessed by plasma TAC, and urinary F_2-IsoPs and 8-OHdG, is represented in Figure 2. TAC's plasma level was lower for those children in the high active group compared to those in the moderately active and low active groups (UNIANCOVA $p = 0.035$, Figure 2a). Regarding the evaluation of the oxidative stress status, children in the high active group showed lower urine levels of 8-OHdG (Figure 2b) and F_2-IsoPs (Figure 2c) than the rest of the groups (UNIANCOVA $p = 0.005$ and $p = 0.036$, respectively).

Table 2. Principal component analysis for the GENOBOX study extracted from physical activity, body composition, peripheral tissue insulin resistance, oxidative stress, and total plasma antioxidant capacity variables.

Variables	Component matrix [a]		
	Factor *		
	Metabolic Risk	Oxidative Stress	Physical Activity
HOMA-IR	0.831		
FM (kg)	0.804		
FFM (kg)	0.798		0.388
TAC (mM)	0.356		
8-OHdG (ng/mL)		0.859	
F_2-IsoPs (ng/mL)		0.831	
MVPA (min/d)	−0.377		0.771
ST (min/d)	0.366	0.387	−0.422

HOMA-IR: homeostasis model assessment-insulin resistance; FM: fat mass; FFM: fat-free mass; TAC: plasma total antioxidant capacity; 8-OHdG: urinary 8-hydroxy-2′-deoxyguanosine; F_2-IsoPs: urinary F2α-isoprostanes; MVPA: mean moderate-vigorous physical activity; ST: sedentary time. [a] Extraction of the initial set of uncorrelated components was accomplished with the principal factor method, and then the Varimax orthogonal rotation of components was used to facilitate interpretation. The number of components retained was based on Scree plot analysis and eigenvalues greater than 1 (with the components accounting for more of the total variance than any single variable). * Factor loading is the product-moment correlation (a measure of linear association) between an observed variable and an underlying factor. A significant loading factor was defined as a value greater than 0.359 ($p < 0.01$).

Table 3. Eigen values and percentages of variance associated with each linear component (factor) before extraction, after extraction, and after rotation, in the principal component analysis for the GENOBOX study of children relating physical activity, body composition, and peripheral tissue insulin resistance to risk factors for oxidative stress and total plasma antioxidant capacity.

Component	Total Variance Explained								
	Initial Eigenvalues			Sums of Loads Squared from Extraction			Sums of Loads Squared of Rotation		
	Total	% of variance	% Accumulated	Total	% of Variance	% Accumulated	Total	% of Variance	% Accumulated
1	2.402	30.029	30.029	2.402	30.029	30.029	2.232	27.901	27.901
2	1.694	21.175	51.204	1.694	21.175	51.204	1.634	20.424	48.325
3	1.063	13.293	64.496	1.063	13.293	64.496	1.294	16.171	64.496

The first value in the row gives the proportion of variance (the degree of spread in the data set) explained by body composition and tissue peripheral resistance; the second value, the proportion explained by oxidative stress; and the third value, the proportion explained by the level of MVPA and sedentarism.

Table 4. Differences in body mass index (BMI) z-score, moderate-vigorous physical activity and sedentary time between groups of Physical Activity-Sedentarism Score (PASS) of the Spanish children from the GENOBOX study.

Variables	Physical Activity-Sedentarism Score Levels				*p*-Value	*p*-Value for Trend
	Very Low Active (12)	Low Active (65)	Moderate Active (82)	High Active (57)		
BMI z-score	$1.25 \pm 1.4^{a,b}$	$1.57 \pm 2.1^{a,b}$	$1.41 \pm 1.9^{a,b}$	$0.51 \pm 2.9^{a,c}$	0.046	0.029
PASS	3^{a}	5.27 ± 0.78^{b}	8 ± 0.84^{c}	10.88 ± 0.81^{d}	<0.001	<0.001
MVPA (min/d)	22 ± 5^{a}	34 ± 11^{b}	55 ± 12^{c}	76 ± 17^{d}	<0.001	<0.001
ST (min/d)	601 ± 70^{a}	517 ± 92^{b}	471 ± 95^{c}	397 ± 54^{d}	<0.001	<0.001

BMI: body mass index; PASS: Physical Activity-Sedentarism Score; PA: physical activity; MVPA: moderate-vigorous physical activity; ST: Sedentary time. Data are shown as mean ± SD. One-way ANOVA test was used to compare variables among the different PASS levels. No matching superscript letters (a, b, c, d) indicate significant differences ($p < 0.05$) by pairwise post hoc test adjusted for age and/or BMI z-score to determine which experimental groups differed from each other.

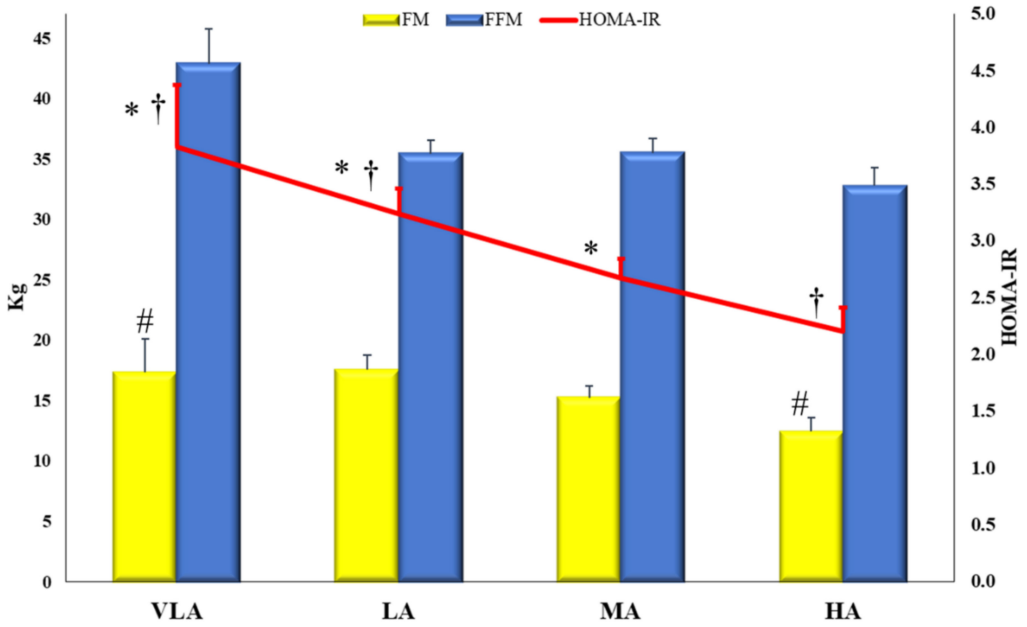


Figure 1. Fat mass, fat-free mass and HOMA-IR according to Physical Activity-Sedentarism Score (PASS) groups in Spanish children from the GENOBOX study. FM: fat mass; FFM: fat-free mass; HOMA-IR: homeostasis model assessment-insulin resistance; VLA: very low active; LA: low active; MA: moderately active; HA: high active. Differences between PASS levels were analyzed using one-way ANCOVA, adjusting for age and/or BMI z-score; pairwise differences were assessed by post hoc analyses to determine differences between experimental groups. For those variables not following normality, a Kruskal–Wallis test was used to evaluate differences between groups. FM: # Differences between the HA and VLA of (p = 0.003). HOMA-IR (UNIANCOVA, p=0.005): † Differences between HA vs. LA and VLA (p = 0.018 and p = 0.010, respectively); * Differences between A vs. LA and VLA (p = 0.014 and p = 0.010, respectively). p for trend for HOMA-IR (p = 0.001) and FM (p = 0.044).

Finally, the plasma concentration of some components of the non-enzymatic antioxidant defense system, retinol, beta-carotene, and tocopherols were measured in a subgroup of our study. Differences in the plasma levels of these biomarkers, according to the PASS groups, are presented in Figure 3. Children in the low active group showed the highest values of retinol (UNIANCOVA, p = 0.002) compared to those in other groups. Regarding tocopherols (UNIANCOVA, p = 0.022), children in the high active group showed lower levels compared with children with very low active and moderately active groups.

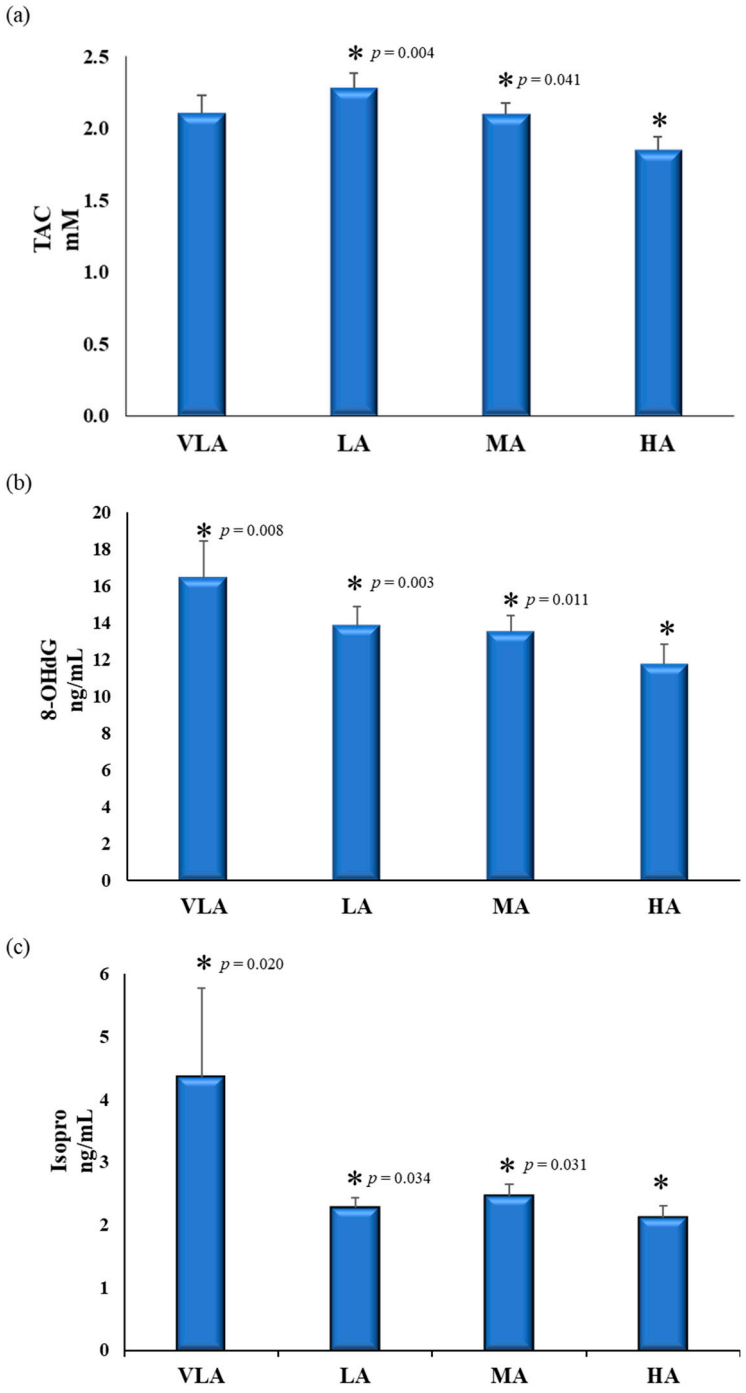


Figure 2. (**a**)Total plasma antioxidant capacity, (**b**) urinary 8-hydroxy-2′-deoxyguanosine and (**c**) $F_{2\alpha}$-Isoprostanes by Physical Activity-Sedentarism Score (PASS) groups in Spanish children from the GENOBOX study. TAC: total plasma antioxidant capacity; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; F_2-IsoPs: $F_{2\alpha}$-isoprostanes; PA: physical activity; VLA: very low active; LA: low active; MA: moderately active; HA: high active. Differences between PASS levels were analyzed using one-way ANCOVA, adjusting for age and/or BMI z-score; pairwise differences were assessed by post hoc analyses to determine differences between experimental groups. For those variables not following normality, a Kruskal–Wallis test was used to evaluate differences between groups. * Differences with HA score. *p* for trend for TAC ($p = 0.020$).

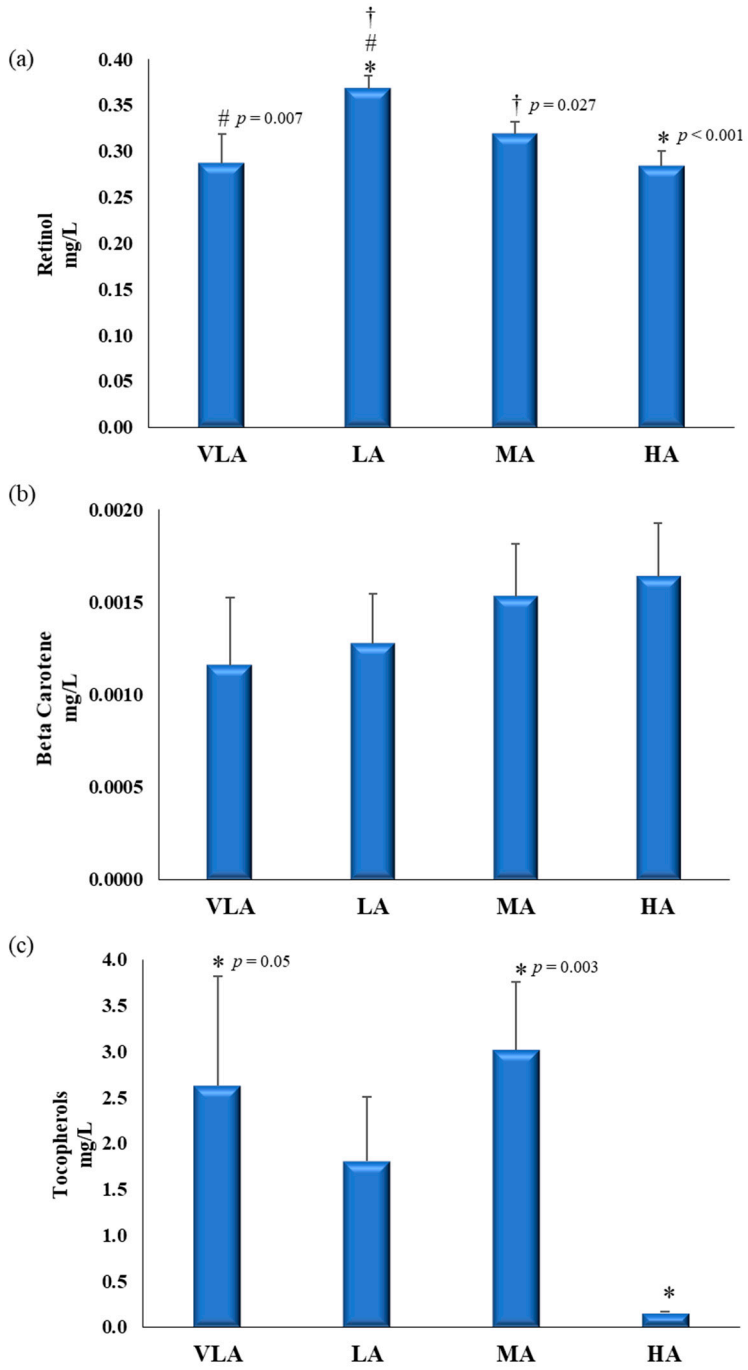


Figure 3. (**a**) Plasma retinol, (**b**) beta-carotene, and (**c**) tocopherols levels according to the Physical-Activity-Sedentarism Score (PASS) levels in Spanish children from the GENOBOX study. PA: physical activity; VLA: very low active; LA: low active; MA: moderately active; HA: high active. Differences between PASS levels were analyzed using one-way ANCOVA, adjusting for age and/or BMI z-score; pairwise differences were assessed by post hoc analyses to determine differences between experimental groups. For those variables not following normality, a Kruskal–Wallis test was used to evaluate differences between groups. * Differences with HA; # Differences with VLA; † Differences MA. *p* for trend for retinol ($p = 0.043$) and tocopherols ($p = 0.040$).

4. Discussion

The influence of objectively measured physical activity and sedentary time on redox status of children and adolescents has been scarcely described so far. In the present analysis, a high PASS, characterized by a high time spent on moderate and vigorous physical activity,

along with a low sedentary time, was associated with lower levels of plasma TAC, and urinary F_2-IsoPs and 8-OHdG and, therefore, to better redox status.

Differences in the redox profile (oxidants and antioxidants) of children and adolescents according to their physical fitness, sports practice, and physical activity collected by questionnaires have been previously reported [15,22,23]. However, since WHO has recommended to "practice at least an average of 60 min per day of moderate- to vigorous-intensity physical activity across the week" and to "limit the amount of sedentary time, particularly the amount of recreational screen time", we noticed that there are no data about the influence of physical activity, in terms of duration and intensity, and sedentarism on redox status [16]. In this context, our study showed that high active children, who performed 76 min of MVPA on average and spent significantly less time being sedentary, presented lower levels of plasma TAC, urinary F_2-IsoPs and 8-OHdG than moderately active children who performed a mean of MVPA of 55 min/day, close to the international recommendation. These results agree with the statements of the WHO about physical activity practice and sedentary time limitation in children and adolescents, but also suggest the possible benefit on redox status of increased MVPA practice.

In our study, contrary to what we expected, children with a higher PASS, especially high active children, showed the lowest plasma TAC concentrations. There are several possible factors that could explain this finding. On one hand, the effect of physical activity practice on redox status is different for acute or chronic exercise. Globally, acute exercise produces a transient increase in both pro-oxidant and antioxidant biomarkers, while a regular physical activity practice improves the antioxidant defense system and reduces the systemic levels of oxidative stress markers [15]. Focusing on TAC plasma levels, they seem to increase in response to acute exercise, while the effect of chronic exercise on these concentrations remains controversial [15,24,25]. Regarding the oxidative stress biomarkers measured in our study, urinary 8 F_2-IsoPs and 8-OHdG, the available evidence of the long-term effect of regular physical activity on these parameters is scarce [26,27]. Nasca et al. reported an increase in urinary F_2-IsoPs concentrations after a 5-week exercise program in scholars [26]. In contrast, no significant changes in urinary F_2-IsoPs concentrations were found after a 3-month exercise program in adolescent girls [27]. These results are controversial and none of these studies measured biomarkers of the antioxidant defense system to evaluate de redox status. However, in our study, we also found that those children with a high PASS and low TAC plasma concentration also showed the lowest urine concentrations of 8-OHdG and F_2-IsoPs. In this context, we hypothesize that concomitantly reduced oxidative stress markers in children with a high PASS may explain a diminished need of maintaining high concentrations of antioxidants in plasma during rest to achieve redox homeostasis. Regular practice of physical activity may reduce basal levels of oxidative stress biomarkers, improve the response of the antioxidant defense system to a stress situation, or even both. In this way, it would be interesting to evaluate the differences in the plasma levels of TAC in response to an acute planned exercise between groups with different PASS.

On the other hand, body composition (FM and FFM) is also related to differences in the redox status of children. Obesity and its associated comorbidities have been linked to imbalanced redox homeostasis both in adults and children [14,28]. In fact, plasma concentrations of TAC seem to be lower in children with obesity when compared to normal-weight peers [24,29,30]. However, Ruperez et al. [14] recently described different plasma TAC concentrations according to pubertal status. Thus, obese children at the prepubertal stage had lower TAC and pubertal children higher TAC than controls [14]. In this context, available evidence suggests that obesity may negatively affect the exercise-related antioxidant responses to acute exercise [15,24]. While the regular practice of physical activity seems to improve both, body composition [11,12] and oxidative stress status [13,15], as much in children as in adults. In order to shed light on the relationships between these variables, the PCA performed in the present study showed that body composition was associated with MVPA and sedentary time (first component: metabolic risk). In contrast,

oxidative stress biomarkers were associated with sedentary time (second component: oxidative stress) (Table 3). In addition, a higher score of PASS was associated with lower FM and HOMA-IR, but also with lower levels of TAC, urinary 8-OHdG and F_2-IsoPs, even after age- and BMI z-score-adjustments. These data suggest that regular practice of physical activity can influence oxidative stress improvement in both ways, indirectly (through changes in body composition) and directly.

Lastly, pubertal status has been related to differences in the redox response to exercise. Available evidence suggests that the transition from childhood to adolescence may promote a maturation of pro-oxidant and anti-oxidant mechanisms associated with the activation of somatotropic and gonadal axes [15]. Recently, Chaki et al. [31] compared the redox status, from baseline and after a high-intensity exercise, between sedentary pre- and post-pubertal boys. They found in both baseline and post-exercise that pro-oxidant and anti-oxidant biomarkers were higher for those with pubertal maturation. They suggested post-pubertal boys may face to oxidative stress more efficiently than their prepubertal peers [31]. Following the same trend, pubertal children of our sample exhibited higher levels of TAC than those who were prepubertal and the relationship between TAC and PASS levels remained negative for both groups but attenuated for pubertal children (data not shown).

Regarding sedentary time, there are no data about its effect on oxidative stress status in children and adolescents. Previous reports evaluating the influence of sedentarism are only focused in sports practitioners. In these studies, higher plasma TAC levels have been reported in professional sports practitioners (basketball players, judokas, kayakers, and canoeists) than in sedentary controls, both at rest and after exercise [32–34]. However, these professional sports practitioners also showed higher levels of OS biomarkers than sedentary controls, both at rest and after exercise.

TAC levels were lower in high active group compared with low active group. However, the concentrations of retinol, beta-carotene, and tocopherols, which contribute to determining TAC, had a different trend. The reason for these apparently contradictory results may be because plasma TAC is influenced not only by liposoluble antioxidants, namely α-tocopherol, β-carotene, which are mainly located in lipoproteins, and retinol, which is bounded to retinol-binding protein but for plasma proteins, notably albumin and other thiols rich proteins, urate and ascorbate. The contribution to TAC from urate has been reported to be (35–65%) and plasma proteins (10–50%), while ascorbate (0–24%) and tocopherol (5–10%) [35].

5. Strengths and Limitations

To our knowledge, this is the first study evaluating the influence of objectively measured physical activity and low active by accelerometry on oxidative stress status in children and adolescents. In addition, most previous studies analyzing redox status of children were focused on obesity and its comorbidities, while the role of physical activity in this relationship had not been adequately investigated. By last, the PASS, which integrates the main components of the WHO recommendations for physical activity and sedentary behavior, showed as a useful parameter to evaluate the health benefits of increased physical activity and reduced sedentary time.

In contrast, our study presents several limitations. First, it is not a longitudinal or intervention study, so we can only describe results and generate hypothesis of the relationship between physical activity and oxidative stress status. Second, among the multiple oxidative stress and antioxidant defense system biomarkers, the most appropriate parameters to evaluate redox status in children and adolescents have not been well established yet. The measurement of TAC is useful for evaluating the redox profile, but it is only a part of the antioxidant defense system. In the same way, F_2-IsoPs and 8-OHdG are two of the most common biomarkers of oxidative stress. In this context, we measured plasma TAC and urinary F_2-IsoPs and 8-OHdG in an acceptable sample of children and adolescents, while the sample size was substantially reduced when evaluating non-enzymatic biomarkers of

oxidative stress (retinol, beta-carotene, and tocopherols) due to the limited availability of blood samples. In this context, due to the variance of plasma TAC and oxidative stress biomarkers, it would be necessary a higher sample size to validate the results of the present work. Furthermore, some other factors not taken into account, such as nutrition or physical fitness, could also mediate the relationship between PA and oxidative stress status.

6. Conclusions

In conclusion, a high physical-activity-sedentarism score characterized by a high time devoted to moderate and vigorous physical activity, along with a low sedentary time, was associated with lower plasma TAC levels and reduced urinary F_2-IsoPs and 8-OHdG concentrations, contributing to a better redox profile. Future research should evaluate if these active children perform a better redox response to exercise than those who are less active.

Author Contributions: Conceptualization, M.G.-C., R.L., G.B., Á.G., L.A.M. and C.M.A.; methodology, M.G.-C., R.L., G.B., L.A.M., C.M.A., M.D.M. and F.J.L.-C.; formal analysis, F.J.L.-C., M.D.M. and F.J.A.-G.; investigation, F.J.L.-C., F.J.A.-G., A.I.R., and R.V.-C.; resources, M.G.-C., R.L., G.B. and C.M.A.; data curation, F.J.L.-C., F.J.A.-G. and Á.G.; writing—original draft preparation, F.J.A.-G., F.J.L.-C., Á.G., M.G.-C. and C.M.A.; writing—review and editing, all the group; visualization, F.J.A.-G., F.J.L.-C., Á.G., M.G.-C., M.D.M., A.A.-R. and C.M.A.; supervision, M.G.-C., Á.G. and C.M.A.; project administration, M.G.-C., R.L., G.B. and C.M.A. All authors have read and agreed to the published version of the manuscript. **Funding:** This research was funded by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I + D + I), Instituto de Salud Carlos III-Health Research Funding (FONDOS FEDER) (PI11/02042, PI11/02059, PI11/01425; PI16/00871, PI16/01301, PI16/01205) and RETIC (Redes temáticas de investigación cooperativa) (Red SAMID RD12/0026/0015). The authors also acknowledge Instituto de Salud Carlos III for personal funding of A.A.R: Contratos i-PFIS: doctorados IIS-empresa en ciencias y tecnologías de la salud de la convocatoria 2017 de la Acción Estratégica en Salud 2013–2016 (IFI17/00048). Á.G. was funded by the Research Plan of the Vice-Rectorate of Research and Transfer of the University of Granada, Spain This paper will be included in F.J.A. doctorate, under the "Biomedicine Program" at the University of Córdoba, Spain.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local Ethics Committee of each hospital (Code IDs: Córdoba 01/2017, Santiago 2011/198, Zaragoza 10/2010).

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

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Acknowledgments: The authors would like to thank the children and parents who participated in the study.

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

References

1. García-Hermoso, A.; Ezzatvar, Y.; Ramírez-Vélez, R.; Olloquequi, J.; Izquierdo, M. Is device-measured vigorous-intensity physical activity associated with health-related outcomes in children and adolescents? A systematic review and meta-analysis. *J. Sport Health Sci.* **2020**. [CrossRef]
2. Saunders, T.J.; Gray, C.E.; Poitras, V.J.; Chaput, J.P.; Janssen, I.; Katzmarzyk, P.T.; Olds, T.; Connor Gorber, S.; Kho, M.E.; Sampson, M.; et al. Combinations of physical activity, sedentary behaviour and sleep: Relationships with health indicators in school-aged children and youth. *Appl. Physiol. Nutr. Metab.* **2016**, *41*, S283–S293. [CrossRef]
3. Watson, T.A.; MacDonald-Wicks, L.K.; Garg, M.L. Oxidative stress and antioxidants in athletes undertaking regular exercise training. *Int. J. Sport Nutr. Exerc. Metab.* **2005**, *15*, 131–146. [CrossRef] [PubMed]
4. Sies, H. Oxidative stress: Concept and some practical aspects. *Antioxidants* **2020**, *9*, 852. [CrossRef]
5. Basu, S. F2-isoprostanes in human health and diseases: From molecular mechanisms to clinical implications. *Antioxid. Redox Signal.* **2008**, *10*, 1405–1434. [CrossRef]

6. Roberts, L.J.; Morrow, J.D. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. *Free Radic. Biol. Med.* **2000**, *28*, 505–513. [CrossRef]
7. Kasai, H.; Crain, P.F.; Kuchino, Y.; Nishimura, S.; Ootsuyama, A.; Tanooka, H. Formation of 8-hydroxyguanine moiety in cellular dna by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **1986**, *7*, 1849–1851. [CrossRef] [PubMed]
8. Saito, S.; Yamauchi, H.; Hasui, Y.; Kurashige, J.; Ochi, H.; Yoshida, K. Quantitative determination of urinary 8-hydroxydeoxyguanosine (8-OH-dg) by using ELISA—PubMed. *Res. Commun. Mol. Pathol. Pharmacol.* **2000**, *107*, 39–44. [PubMed]
9. Urso, M.L.; Clarkson, P.M. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* **2003**, *189*, 41–54. [CrossRef]
10. Morandi, A.; Corradi, M.; Piona, C.; Fornari, E.; Puleo, R.; Maffeis, C. Systemic anti-oxidant capacity is inversely correlated with systolic blood pressure and pulse pressure in children with obesity. *Nutr. Metab. Cardiovasc. Dis.* **2020**, *30*, 508–513. [CrossRef]
11. Mead, E.; Brown, T.; Rees, K.; Azevedo, L.B.; Whittaker, V.; Jones, D.; Olajide, J.; Mainardi, G.M.; Corpeleijn, E.; O'Malley, C.; et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese children from the age of 6 to 11 years. *Cochrane Database Syst. Rev.* **2017**, *6*, CD012651. [CrossRef]
12. Al-Khudairy, L.; Loveman, E.; Colquitt, J.L.; Mead, E.; Johnson, R.E.; Fraser, H.; Olajide, J.; Murphy, M.; Velho, R.M.; O'Malley, C.; et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese adolescents aged 12 to 17 years. *Cochrane Database Syst. Rev.* **2017**, *6*, CD012691. [CrossRef]
13. de Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. *Sport. Med.* **2017**, *47*, 277–293. [CrossRef]
14. Rupérez, A.I.; Mesa, M.D.; Anguita-Ruiz, A.; González-Gil, E.M.; Vázquez-Cobela, R.; Moreno, L.A.; Gil, Á.; Gil-Campos, M.; Leis, R.; Bueno, G.; et al. Antioxidants and oxidative stress in children: Influence of puberty and metabolically unhealthy status. *Antioxidants* **2020**, *9*, 618. [CrossRef]
15. Avloniti, A.; Chatzinikolaou, A.; Deli, C.K.; Vlachopoulos, D.; Marco, L.G.; Leontsini, D.; Draganidis, D.; Jamurtas, A.Z.; Mastorakos, G.; Fatouros, I.G. Exercise-induced oxidative stress responses in the pediatric population. *Antioxidants* **2017**, *6*, 6. [CrossRef]
16. WHO. *WHO Guidelines on Physical Activity and Sedentary Behaviour*; WHO: Geneva, Switzerland, 2020; ISBN 9789241550536.
17. Cole, T.J.; Bellizzi, M.C.; Flegal, K.M.; Dietz, W.H. Establishing a standard definition for child overweight and obesity worldwide: International survey. *Br. Med. J.* **2000**, *320*, 1240–1243. [CrossRef] [PubMed]
18. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412–419. [CrossRef] [PubMed]
19. Migueles, J.H.; Cadenas-Sanchez, C.; Ekelund, U.; Delisle Nyström, C.; Mora-Gonzalez, J.; Löf, M.; Labayen, I.; Ruiz, J.R.; Ortega, F.B. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. *Sport. Med.* **2017**, *47*, 1821–1845. [CrossRef] [PubMed]
20. Treuth, M.S.; Schmitz, K.; Catellier, D.J.; McMurray, R.G.; Murray, D.M.; Almeida, M.J.; Going, S.; Norman, J.E.; Pate, R. Defining accelerometer thresholds for activity intensities in adolescent girls. *Med. Sci. Sports Exerc.* **2004**, *36*, 1259–1266. [CrossRef] [PubMed]
21. Evenson, K.R.; Catellier, D.J.; Gill, K.; Ondrak, K.S.; McMurray, R.G. Calibration of two objective measures of physical activity for children. *J. Sports Sci.* **2008**, *26*, 1557–1565. [CrossRef]
22. Llorente-Cantarero, F.J.; Gil-Campos, M.; Benitez-Sillero, J.D.; Muñoz-Villanueva, M.C.; Túnez, I.; Pérez-Navero, J.L. Prepubertal children with suitable fitness and physical activity present reduced risk of oxidative stress. *Free Radic. Biol. Med.* **2012**, *53*, 415–420. [CrossRef]
23. Llorente-Cantarero, F.J.; Gil-Campos, M.; de Benitez-Sillero, J.D.; Muñoz-Villanueva, M.C.; Tasset, I.; Pérez-Navero, J.L. Profile of oxidant and antioxidant activity in prepubertal children related to age, gender, exercise, and fitness. *Appl. Physiol. Nutr. Metab.* **2013**, *38*, 421–426. [CrossRef]
24. Paltoglou, G.; Fatouros, I.G.; Valsamakis, G.; Schoina, M.; Avloniti, A.; Chatzinikolaou, A.; Kambas, A.; Draganidis, D.; Mantzou, A.; Papagianni, M.; et al. Antioxidation improves in puberty in normal weight and obese boys, in positive association with exercise-stimulated growth hormone secretion. *Pediatric Res.* **2015**, *78*, 158–164. [CrossRef] [PubMed]
25. Alghadir, A.H.; Gabr, S.A.; Iqbal, Z.A.; Al-Eisa, E. Association of physical activity, vitamin E levels, and total antioxidant capacity with academic performance and executive functions of adolescents. *BMC Pediatrics* **2019**, *19*, 1–8. [CrossRef] [PubMed]
26. Nasca, M.M.; Zhang, R.; Super, D.M.; Hazen, S.L.; Hall, H.R. Increased oxidative stress in healthy children following an exercise program: A pilot study. *J. Dev. Behav. Pediatrics* **2010**, *31*, 386–392. [CrossRef] [PubMed]
27. Youssef, H.; Groussard, C.; Lemoine-Morel, S.; Jacob, C.; Moussa, E.; Fazah, A.; Pineau, J.C.; Pincemail, J.; Cillard, J.; Delamarche, A. Aerobic training suppresses exercise-induced lipid peroxidation and inflammation in overweight/obese adolescent girls. *Pediatric Exerc. Sci.* **2015**, *27*, 67–76. [CrossRef]
28. Paltoglou, G.; Schoina, M.; Valsamakis, G.; Salakos, N.; Avloniti, A.; Chatzinikolaou, A.; Margeli, A.; Skevaki, C.; Papagianni, M.; Kanaka-Gantenbein, C.; et al. Interrelations among the adipocytokines leptin and adiponectin, oxidative stress and aseptic inflammation markers in pre- and early-pubertal normal-weight and obese boys. *Endocrine* **2017**, *55*, 925–933. [CrossRef]
29. Vehapoglu, A.; Turkmen, S.; Goknar, N.; Özer, Ö.F. Reduced antioxidant capacity and increased subclinical inflammation markers in prepubescent obese children and their relationship with nutritional markers and metabolic parameters. *Redox Rep.* **2016**, *21*, 271–280. [CrossRef]

30. Rowicka, G.; Dyląg, H.; Ambroszkiewicz, J.; Riahi, A.; Weker, H.; Chełchowska, M. Total Oxidant and Antioxidant Status in Prepubertal Children with Obesity. *Oxid. Med. Cell. Longev.* **2017**, *2017*. [CrossRef]
31. Chaki, B.; Pal, S.; Chattopadhyay, S.; Bandyopadhyay, A. High-intensity exercise-induced oxidative stress in sedentary pre-pubertal & post-pubertal boys: A comparative study. *Indian J. Med. Res.* **2019**, *150*, 167–174. [CrossRef]
32. Yilmaz, N.; Erel, Ö.; Hazer, M.; Bağci, C.; Namiduru, E.; Gül, E. Biochemical assessments of retinol, α-tocopherol, pyridoxal—5-phosphate oxidative stress index and total antioxidant status in adolescent professional basketball players and sedentary controls. *Int. J. Adolesc. Med. Health* **2007**, *19*, 177–186. [CrossRef]
33. Teixeira, V.; Valente, H.; Casal, S.; Pereira, L.; Marques, F.; Moreira, P. Antioxidant status, oxidative stress, and damage in elite kayakers after 1 year of training and competition in 2 seasons. *Appl. Physiol. Nutr. Metab.* **2009**, *34*, 716–724. [CrossRef] [PubMed]
34. Abed, K.; Rebai, H.; Bloomer, R.J.; Trabelsi, K.; Masmoudi, L.; Zbidi, A.; Sahnoun, Z.; Hakim, A.; Tabka, Z. Antioxidant status and oxidative stress at rest and in response to acute exercise in judokas and sedentary men. *J. Strength Cond. Res.* **2011**, *25*, 2400–2409. [CrossRef] [PubMed]
35. Wayner, D.D.; Burton, G.W.; Ingold, K.U.; Barclay, L.R.; Locke, S.J. The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochim. Biophys. Acta* **1987**, *924*, 408–419. [CrossRef]

IMPACT OF PHYSICAL ACTIVITY INTENSITY LEVELS ON THE CARDIOMETABOLIC RISK STATUS OF CHILDREN: THE GENOBOX STUDY

Francisco J. Llorente-Cantarero,[1,2] Francisco J. Aguilar-Gómez,[3] Gloria Bueno-Lozano,[2,4,5]Augusto Anguita-Ruiz,[2,6,7] Azahara I. Rupérez,[5] Rocío Vázquez-Cobela,[2,8] Katherine Flores-Rojas,[3]Concepción M. Aguilera,[2,6,7] Luis A. Moreno,[2,5] Ángel Gil,[2,6,7] Rosaura Leis,[2,8,9,10] and Mercedes Gil-Campos[2,3]

[1]Department of Specific Didactics, Faculty of Education, Maimónides Institute of Biomedicine Research of Córdoba (IMIBIC), University of Córdoba, Córdoba, Andalucía, Spain;
[2]CIBEROBN (Physiopathology of Obesity and Nutrition), Institute of Health Carlos III (ISCIII), Madrid, Spain;
[3]Metabolism and Investigation Unit, Reina Sofía University Hospital, Maimónides Institute of Biomedicine Research of Córdoba (IMIBIC), The University of Córdoba, Córdoba, Andalucía, Spain;
[4]Pediatric Endocrinology Unit, Clinic University Hospital Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Aragón, Spain;
[5]GENUD Research Group, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria de Aragón (IIS Aragón), University of Zaragoza, Zaragoza, Aragón, Spain;
[6]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix," Center of Biomedical Research, University of Granada, Armilla, Granada, Spain;
[7]Instituto de Investigación Biosanitaria ibs Granada, Granada, Spain;
[8]Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Pediatric Department, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain;
[9]Unit of Pediatric Gastroenterology, Hepatology and Nutrition, Pediatric Service, University Clinical Hospital of Santiago (CHUS), Santiago de Compostela, Galicia, Spain;
[10]Pediatric Nutrition Research Group, Institute of Sanitary Research of Santiago de Compostela (IDIS), University Clinical Hospital of Santiago (CHUS–USC), Santiago de Compostela, Galicia, Spain.

International Journal of Sport Nutrition and Exercise Metabolism, (Ahead of Print)
https://doi.org/10.1123/ijsnem.2021-0148

First Published Online: Dec. 1, 2021

Human Kinetics
ORIGINAL RESEARCH

Impact of Physical Activity Intensity Levels on the Cardiometabolic Risk Status of Children: The Genobox Study

Francisco J. Llorente-Cantarero,[1,2] Francisco J. Aguilar-Gómez,[3] Gloria Bueno-Lozano,[2,4,5] Augusto Anguita-Ruiz,[2,6,7] Azahara I. Rupérez,[5] Rocío Vázquez-Cobela,[2,8] Katherine Flores-Rojas,[3] Concepción M. Aguilera,[2,6,7] Luis A. Moreno,[2,5] Ángel Gil,[2,6,7] Rosaura Leis,[2,8,9,10] and Mercedes Gil-Campos[2,3]

[1]Department of Specific Didactics, Faculty of Education, Maimónides Institute of Biomedicine Research of Córdoba (IMIBIC), University of Córdoba, Córdoba, Andalucía, Spain; [2]Biomedical Research Networking Center for Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III (ISCIII), Madrid, Spain; [3]Metabolism and Investigation Unit, Reina Sofía University Hospital, Maimónides Institute of Biomedicine Research of Córdoba (IMIBIC), The University of Córdoba, Córdoba, Andalucía, Spain; [4]Pediatric Endocrinology Unit, Clinic University Hospital Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Aragon, Spain; [5]GENUD Research Group, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria de Aragón (IIS Aragón), University of Zaragoza, Zaragoza, Aragon, Spain; [6]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix," Center of Biomedical Research, University of Granada, Armilla, Granada, Spain; [7]Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [8]Unit of Investigation in Nutrition, Growth and Human Development of Galicia, Pediatric Department, University of Santiago de Compostela, Santiago de Compostela, Galicia, Spain; [9]Unit of Pediatric Gastroenterology, Hepatology and Nutrition, Pediatric Service, University Clinical Hospital of Santiago (CHUS), Santiago de Compostela, Galicia, Spain; [10]Pediatric Nutrition Research Group, Institute of Sanitary Research of Santiago de Compostela (IDIS), University Clinical Hospital of Santiago (CHUS–USC), Santiago de Compostela, Galicia, Spain

Childhood obesity has been related to metabolic syndrome and low-grade chronic inflammation. This study aimed to evaluate the impact of physical activity intensities and practice on inflammation, endothelial damage, and cardiometabolic risk factors in children. There were 513 participants, aged 6–14 years, recruited for the study. Physical activity was measured by accelerometry, and the children were classified into four groups according to quartiles of moderate to vigorous physical activity (MVPA) practice as very low active, low active, moderate active, and high active. Anthropometric measures, blood pressure, and plasma metabolic and proinflammatory parameters were analyzed. Very low active group presented a worse lipid profile and higher insulin, leptin, adiponectin, resistin, matrix metallopeptidase-9, and tissue plasminogen activator inhibitor-1, while lower levels of tumor necrosis factor-alpha, Type 1 macrophages, and interleukin 8 than high-active children. Regression analyses showed that a higher MVPA practice was associated with lower levels of triacylglycerols (β: –0.118; $p = .008$), resistin (β: –0.151; $p = .005$), tPAI (β: –0.105; $p = .046$), and P-selectin (β: –0.160; $p = .006$), independently of sex, age, and body mass index (BMI). In contrast, a higher BMI was associated with higher levels of insulin (β: 0.370; $p < .001$), Homeostasis Model Assessment (β: 0.352; $p < .001$), triacylglycerols (β: 0.209; $p < .001$), leptin (β: 0.654; $p < .001$), tumor necrosis factor-alpha (β: 0.182; $p < .001$), Type 1macrophages (β: 0.181; $p < .001$), and tissue plasminogen activator inhibitor (β: 0.240; $p < .001$), independently of sex, age, and MVPA. A better anthropometric, metabolic, and inflammatory profile was detected in the most active children; however, these differences were partly due to BMI. These results suggest that a higher MVPA practice and a lower BMI in children may lead to a better cardiometabolic status.

Keywords: obesity, moderate to vigorous physical activity, cardiovascular risk factors

Physical activity (PA) practice, especially moderate to vigorous PA (MVPA), in children and adolescents seems to improve physical fitness and cardiometabolic health, contributing to maintain an adequate body composition (Poitras et al., 2016). Indeed, the management of childhood obesity is based on lifestyle modifications, that is, dietary and PA recommendations (Mead et al., 2017). The World Health Organization (WHO) recommends a daily practice of 60 min of MVPA across the week to reduce adiposity and improve physical fitness and cardiometabolic health (World Health Organization, 2020).

Childhood obesity has been related to several disorders such as dyslipidemia, elevated blood pressure, and impaired glucose metabolism, included on the so-called "metabolic syndrome" (Ahrens et al., 2014; Guzzetti et al., 2019). Likewise, childhood obesity has also been associated with disturbances in the plasma concentration of several cytokines revealing a low-grade inflammatory status in these children (Singer & Lumeng, 2017). In this context, an increased MVPA practice in children has been associated with improvements in cardiometabolic risk factors within the metabolic syndrome framework (Ekelund et al., 2012; Júdice et al., 2020). However, the relationship between PA practice, particularly MVPA, and this low-grade inflammatory status is not well-known yet.

The adipose tissue constitutes an endocrine organ that produces several adipokines such as leptin, adiponectin, and resistin, which participate in energy homeostasis. Increased plasma concentrations of leptin and resistin in children with high body mass index (BMI) and high body fat mass have been reported compared

Leis (mariarosaura.leis@usc.es) is corresponding author, https://orcid.org/0000-0002-0540-4210.

with those with normal BMI; in contrast, obesity is related to low plasma levels of adiponectin (Martos-Moreno et al., 2014). In this context, PA interventions seem to improve BMI and reduce plasma concentrations of leptin in children (Sirico et al., 2018; Yu et al., 2017) and adults (Fedewa et al., 2018; Yu et al., 2017). However, these studies do not provide specific information about the type, intensities, or time of exercise required to achieve these benefits.

Plasma concentrations of some other inflammatory cytokines have also been associated with obesity in children (Olza et al., 2013) and adults (Schmidt et al., 2015). Cytokines implied on inflammation pathways such as interleukins (IL-6 and IL-8) or tumor necrosis factor-alpha (TNF-α) have also been found to be elevated in children with high BMI (Rupérez et al., 2018). Likewise, some biomarkers of endothelial dysfunction (plasminogen activator inhibitor 1 [PAI-1] and soluble endothelial leukocyte adhesion molecule-1 [sE-selectin]) seemed to be related to BMI but inconsistently (Rupérez et al., 2018; Utsal et al., 2012). Similar to adipocytokines, descriptions of the relationship between PA practice and the plasma concentrations of these parameters have been scarcely investigated.

Based on these previous data, it is unclear if PA, especially MVPA, is related to the cardiometabolic risk status of children with obesity by changes in selected hormones, inflammatory cytokines, and endothelial damage biomarkers. We hypothesize that higher practice of MVPA may be linked to a better cardiovascular risk status in children. To test this hypothesis, we evaluated differences in anthropometric, metabolic, and inflammatory variables according to the practice of MVPA, measured by accelerometry, in a selected sample of the GENOBOX study.

Methods

Study Design and Participants

The GENOBOX is a case-control, multicenter study carried out in 1,444 Spanish children (706 males and 738 females), aged 3–17 years, who were recruited at three Spanish Hospitals (Hospital Universitario Reina Sofía, Córdoba; Hospital Clínico Universitario, Santiago de Compostela, and Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain) during 2012–2015. The GENOBOX study protocol including detailed inclusion and exclusion criteria as well as informed consent and approval by the local ethics committees (Comité de Ética de la Investigación de Córdoba, de Santiago de Compostela y de Zaragoza) have been reported previously (Rupérez et al., 2020). The study was performed according to the ethical guidelines of the Edinburgh revision of the Declaration of Helsinki (2000). A subsample of 513 children (245 boys) was selected based on the following inclusion criteria for the present study: White children and adolescents aged 6–14 years with valid blood measurements including sex hormones (follicle-stimulating hormone, luteinizing hormone, testosterone in boys, and estradiol in girls), and accurate data from an accelerometry standardized protocol, as well as measured plasma levels of glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol, triacylglycerols (TAG), adiponectin, leptin, resistin, nerve growth factor, hepatocyte growth factor, TNF-α, IL-6 and IL-8, soluble chemoattractant protein of Type 1 macrophages (MCP-1), tissue PAI-1 (tPAI-1) and active PAI-1, sE-selectin, P-selectin, soluble vascular cell adhesion molecule-1, intercellular adhesion molecule-1, matrix metallopeptidase-9, and myeloperoxidase (MPO). Exclusion criteria were: Presence of diabetes mellitus, congenital, chronic, or inflammatory disease, psychomotor disability, use of hormonal medications, or other drugs that modify blood pressure, glucose, or lipid metabolism, having performed intense exercise in the 24 hr previous to the examination, or having participated in a research study in the last 3 months.

Anthropometric and Clinical Evaluation

Medical history and a physical exam, including the evaluation of sexual maturity according to Tanner's five stages, were assessed and confirmed with sexual hormone measurements. Anthropometric measurements were taken by a single examiner within each hospital. Bodyweight was measured using a standard beam balance. Height was measured using a precision stadiometer. Waist circumference (WC) was measured in the fasting state by applying an inelastic tape horizontally midway between the lowest rib margin and the iliac crest of the standing child at the end of a gentle expiration. BMI was calculated (in kilograms per meter square), and overweight and obesity were defined using age and sex-specific BMI cutoff points of the International Obesity Task Force, equivalent to adult values of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity (Cole et al., 2000). Systolic and diastolic blood pressure were measured three times by the same examiner using an electronic manometer (Omrom M6 AC; Omrom, Kyoto, Japan) and following international recommendations (McCrindle, 2010).

Accelerometry

ActiGraph GT3X and GT3X+ accelerometers (ActiGraph, Pensacola, FL) were used to assess PA levels in this study. Accelerometers were placed over the right iliac crest and held in place using an adjustable elastic belt for 24 hr a day and could be removed only to take a shower or for nocturnal rest (if the instrument caused discomfort in sleeping). It was programmed for 15 epochs (period of 15 s), as previously recommended (Migueles et al., 2017). Accelerometry data were processed using the ActiLife (version 6.13.3; Pensacola, FL) program. Two rules were used for excluding data: (a) A missing data code replaced all negative counts, and (b) periods of 20 min or more of consecutive zero counts were replaced by a missing data code before further analysis, as recommended by Treuth et al. (2004). The output generated by the ActiGraph GT3X+ included the total volume of PA and each PA intensity as defined by the cut points of the following counts per minute (CPMs) based in the classification by Evenson et al. (2008): sedentary: ≤100 CPM, light PA: >100 to <2,296 CPM, moderate PA: >2,296 to <4,012 CPM, and vigorous PA: <4,012 CPM. A minimum of 8 hr of monitoring per day for at least 3 days including at least 1 weekend day was considered acceptable for the evaluation of PA and sedentary time. The children were divided into four groups according to quartiles of MVPA as very low active (VLA, from 6.42 to 36.44 min), low active (from 36.52 to 49.45 min), moderate active (from 49.80 to 63.65 min), and high active (HA, from 63.73 to 129.04 min).

Sampling and Biochemical Measurements

Blood samples were drawn from the antecubital vein between 08:00 and 09:30 hr after an overnight fast. Routine blood tests were analyzed at the general laboratory of each participating hospital. Glucose (coefficient of variation [CV] = 1.0%) was analyzed using the glucose oxidase method in an automatic analyzer (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems, Mannheim, Germany), and plasma insulin was analyzed by radioimmunoassay (CV = 2.6%) using an automatic microparticle

analyzer (AxSYM; Abbott Laboratories, Abbott Park, IL). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated based on the published equation: HOMA-IR = fasting glucose (in millimoles) × fasting insulin (in milliunits per milliliter)/22.5 (Matthews et al., 1985). Total plasma cholesterol (CV = 0.9%), HDL-c (CV = 0.8%), LDL-c (CV = 1.5%), and TAG (CV = 1.5%) were measured using an automatic analyzer (Roche-Hitachi Modular P and D Autoanalyzer; Roche Laboratory Systems). The sex hormones follicle-stimulant hormone (CV =3.6%), luteinizing hormone (CV = 3.1%), testosterone (CV = 2%), and estradiol (CV = 1.8%) were measured by chemiluminescence using an automatic analyzer (Architec I4000; Abbott Laboratories, Abbott Park, IL).

Inflammation and Cardiovascular Risk Biomarkers

These selected biomarkers were analyzed in plasma with the LINCOplex kits of human monoclonal antibodies (Linco Research, St. Charles, MO) on a Luminex 200 System (Luminex Corporation, Austin, TX): adiponectin (CV = 7.9%), leptin (CV = 7.9%) (Cat. HADK2-61 K-B), resistin (CV = 6.0%), nerve growth factor (CV = 6%), hepatocyte growth factor (CV = 7.7%), TNF-α (CV =7.8%), IL-6 (CV = 7.8%), IL-8 (CV = 7.9%), MCP-1 (CV = 7.9%), PAI-1 (CV = 6.6%) (Cat. HADK1-61 K-A), P-selectin (CV =10.1%), sE-selectin (CV = 11.2%), soluble vascular cell adhesion molecule- 1 (CV = 11.1%), sICAM (CV = 7.9%), matrix metallopeptidase-9 (CV = 6.8%), and MPO (CV = 12.3%) (Cat. HCVD1-67 AK).

Statistical Analysis

The sample size estimation was calculated for the GENOBOX study based on the principal metabolic risk factors for cardiovascular disease associated with obesity. The calculation of the sample size was carried out for a 95% degree of confidence (Type I error α = .05) and a power of 80% (beta error = 0.20) according to the estimation equation of *n* by comparison of two proportions of one variable in two independent groups. The sample size under these conditions was raised to a total of 300 to be sure that significant differences can be found for a minimal difference, of 20% in each parameter, between children with obesity and normal-weight children. All continuous variables were tested for normality using the Shapiro–Wilk and Kolmogorov tests. Insulin, TAG, and inflammatory biomarkers were transformed through the natural log or square root to normalize these values. The sample was divided into four quartiles after assigning ranges based on the mean MVPA variable. The Levene test explored heteroscedasticity between experimental groups. According to standard statistical assumptions, one-way analysis of variance, and the Kruskal–Wallis test were employed to assess group differences for the considered variables. Pairwise *t* tests and pairwise Mann–Whitney *U* tests were applied conveniently as post hoc analyses, adjusted by BMI *z* score and age, to determine which experimental groups differed from each other. Polynomial test for trend was used to analyze the linear relationship within quartiles. Associations between biomarkers were studied with Spearman's ρ correlation coefficients for nonparametric variables and Pearson's for those parametric ones. Multivariable linear regression was used to evaluate the relationship between PA variables (MVPA by quartiles) and inflammatory biomarkers. Values in descriptive tables and results are expressed as means and standard deviations or mean relative differences (Δ). All statistical analyses were performed using the SPSS software (IBM SPSS, version 24; IBM Corp., Somers, NY).

Results

Demographic, anthropometric, blood pressure, and general metabolic parameters according to the level of MVPA practice are presented in Table 1.

The sample showed significant differences by sex. The majority of boys (63.3%) were moderate active or HA, while most girls (61.9%) were low active or VLA. Our sample was predominantly obese (47.2%) and overweight (23.8%), and increasing min of MVPA were associated with decreasing BMI *z* score in girls but not in boys (Table 1 and see Supplementary Table S1 [available online]).

Regarding metabolic parameters, glucose, insulin, HOMA-IR, and TAG levels tended to be higher in those groups with less min of MVPA. In contrast, HDLc levels were higher for the HA group than the rest of the less active groups. These differences remained statistically significant for glucose only in boys and for TAG in both sexes (see Supplementary Table S1 [available online]).

Table 2 shows plasma levels of cardiovascular risk biomarkers (adipokines, inflammation, and vascular damage biomarkers) according to MVPA quartiles. First, the plasma concentration of the adipokines leptin and resistin was higher for the VLA group than the HA group. Likewise, increasing leptin and resistin was significantly associated with decreasing min of MVPA. However, the concentration of adiponectin did not show differences between MVPA groups. Second, the levels of inflammatory biomarkers such as TNF-α, IL-8, and MCP-1 were higher for the HA group than the VLA group. Increased levels of those inflammatory biomarkers were significantly associated with decreasing minute of MVPA. Finally, plasma levels of vascular damage and cardiovascular risk biomarkers such tPAI-1 and matrix metallopeptidase-9 were higher for the VLA group compared with HA group, but only decreased levels of tPAI-1 were significantly associated to min of MVPA. Plasma levels of these cardiovascular risk biomarkers by sex are presented in Supplementary Table S2 (available online). Decreased levels of P-selectin and tPAI were significantly associated with higher min of MVPA in boys but not in girls.

Based on these results, correlations were explored between MVPA and the parameters studied, as shown in Table 3. Increasing min of MVPA were weakly but positively correlated to plasma levels of HDLc (r = .112), TNF-α (r = .118) and MCP-1 (r = .107), and negatively correlated to insulin (r = –.121), TAG (r = –.158), resistin (r = –.144), tPAI (r = –.128), and P-selectin (r = –.129). Regression analyses were performed on the parameters with previous significant correlations (Table 4). The plasma concentration of TAG (β: –0.118), resistin (β: –0.151), tPAI (β: –0.105), and P-selectin (β: –0.160) were negatively associated with the min of MVPA by quartiles, independently of age, sex, and BMI. Regression analyses also showed that BMI had stronger associations than MVPA with these parameters, and it was positively associated with the plasma concentration of insulin (β: 0.370), TAG (β: 0.209), leptin (β: 0.654), TNF-α (β: 0.182), MCP-1 (β: 0.181), and tPAI (β: 0.240).

Discussion

In the present study, a higher MVPA practice and a lower BMI are related to a better metabolic and inflammatory profile in children and adolescents. The MVPA variable was classified into quartiles (VLA, low active, moderate active, and HA) to explore whether any cutoff values of MVPA were associated with a healthier status,

Table 1 Characteristics of the Studied Children According to the Time Spent on MVPA

Variables	VLA *N*: 128	LA *N*: 128	MA *N*: 129	HA *N*:128	*p* (ANCOVA)	*p* (for trend)
MVPA (min/day)	27.1 ± 6.7	43.0 ± 3.7	56.9 ± 4.1	79.9 ± 14.8	<.001	<.001
Boys (*N*)[a]	40 (16.3%)	50 (20.4%)	70 (28.6%)	85 (34.7%)	<.001*	<.001
Girls (*N*)[a]	88 (32.8%)	78 (29.1%)	59 (22.0%)	43 (16%)	<.001*	<.001
Prepubertal (*N*)[a]	66 (22.9%)	73 (25.3%)	77 (26.7%)	72 (25%)	.321*	.838
Pubertal (*N*)[a]	62 (27.8%)	55 (24.7%)	52 (23.3%)	54 (24.2%)		
Prepubertal MVPA (min/day)	26.3 ± 7.5	43.1 ± 3.7	56.5 ± 4.1	78.9 ± 13.9	<.001	<.001
Pubertal MVPA (min/day)	27.9 ± 5.6	43.1 ± 3.7	57.5 ± 4	81.1 ± 16.3	<.001	<.001
Age (years)	11 ± 2[LA]	10 ± 2	11 ± 2	10 ± 2	.250	.364
Height (cm)	146.3 ± 13.	143.7 ± 14.	144.4 ± 14.	144.6 ± 15.4	.792	.432
Weight (kg)	52.6 ± 17.9	51.4 ± 20.3	51.4 ± 18.3	49.8 ± 19.2	.424	.266
BMI (kg/m^2)	24.1 ± 5.7	24.1 ± 6.1[HA]	23.9 ± 5.4	23 ± 5.4	.256	.150
BMI *z* score	1.6 ± 1.9	1.9 ± 2.3[HA]	1.7 ± 1.8	1.3 ± 2.4	.135	.146
WC (cm)	80.4 ± 15.3	80.6 ± 16.9	80.3 ± 15.5	78.1 ± 16.1	.309	.271
Normal weight[a]	37 (25.0%)	33 (22.3%)	33 (22.4%)	45 (30.4%)	.345*	.273
Overweight[a]	32 (26.2%)	30 (24.6%)	30 (24.6%)	30 (24.6%)	.345*	.273
Obesity[a]	59 (24.4%)	64 (26.4%)	66 (27.3%)	53 (21.9%)	.345*	.273
SBP (mmHg)	109.8 ± 13.1	109.4 ± 13.1	108.5 ± 12.6	109.1 ± 1	.894	.539
DBP (mmHg)	66.3 ± 8.9	65.6 ± 9.3	63.9 ± 10.2	65.1 ± 10.2	.287	.169
Glucose (mg/dl)	85.8 ± 8.1[MA]	84.1 ± 7.3	83.4 ± 7.2	83.9 ± 8.6	.101	.033
Insulin (mU/L)	12.7 ± 7.2[MA,HA]	12.3 ± 8.3[HA]	11.3 ± 10.8	10.1 ± 7.2	.007	<.001
HOMA-IR	2.7 ± 1.6[HA]	2.6 ± 1.8	2.3 ± 2.3	2.1 ± 1.6	.173	.008
CHOL (mg/dl)	168.9 ± 32.1[LA]	161.7 ± 31.7	162.5 ± 26.8	164.5 ± 2	.150	.288
TAG (mg/dl)	76.3 ± 34.3[MA,HA]	71.5 ± 33.3[HA]	63.3 ± 26.8	64.1 ± 37.3	.001	<.001
HDLc (mg/dl)	51.1 ± 13.1[HA]	50.8 ± 14.5[HA]	51.2 ± 13.1[HA]	55.4 ± 14.1	.092	.014
LDLc (mg/dl)	98.8 ± 27.9	94.2 ± 27.7	96.3 ± 25.5	93.6 ± 24.4	.329	.195

Note. All data are expressed as mean ± SD. VLA = very low active; LA = low active; MA= moderate active; HA = high active; MVPA = moderate to vigorous physicalactivity; BMI = body mass index; WC= waist circumference; SBP = systolic blood pressure; DBP = diastolic blood pressure; HOMA-IR = homeostatic model assessmentinsulinresistance; CHOL = cholesterol; TAG = triacylglycerols; HDLc = high-density lipoprotein cholesterol; LDLc = low-density lipoprotein cholesterol; ANCOVA = analysis of covariance.

[VLA, LA, MA, and HA]Significant differences ($p < .05$) by pairwise post hoc test adjusted for age or BMI *z* score to determine which experimental groups differed from each other.

[a]These variables are expressed as number of subjects within each group (proportion of subjects within each group expressed in percentage).

*p value for chi-square test.

following a similar approach we used previously (Alessa et al., 2017). Although WHO has recommended to "practice at least an average of 60 min/day of moderate- to vigorous-intensity physical activity across the week" as a general recommendation (World Health Organization, 2020), there is no consensus whether this threshold is the most appropriate to improve these metabolic or inflammatory biomarkers (Füssenich et al., 2016). In our sample, the widest differences in metabolic and inflammatory parameters occurred between the extreme quartiles of MVPA practice (VLA vs. HA), showing the HA group, with a mean of 80 min/day of MVPA, the best cytokine profile. These data suggest that the WHO general recommendations of 60 min/day of MVPA may be revised in the future to address a better metabolic and inflammatory status.

From the anthropometric variables, it is worth noting that the subjects in the present study were predominantly overweight and obese. Still, there were no differences in BMI, BMI *z* score and WC between groups of MVPA. However, there were notable sex differences, being boys more active than girls, especially conditioned by social factors and in agreement with similar previous reports (Corder et al., 2015).

Regarding metabolic status, VLA children (those who perform 27 min/day of MVPA on average) showed higher plasma levels of glucose, insulin, and HOMA-IR as well as a worse lipid profile than HA children. However, in regression analyses, changes in plasma concentrations of insulin and HOMA-IR were associated independently with age, sex, and BMI, but not with the level of PA. In contrast, plasma levels of TAG remained negatively associated with min of MVPA by quartiles, independently of sex, age, and BMI. Similarly, from cross-sectional data, Väistö et al. (2019) reported increased MVPA practice in children aged 6–8 years that was associated with lower insulin, HOMA-IR, TAG, low-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, and body fat percentage, adjusted for sex, age, and puberty. However, only insulin, HOMA-IR, and systolic blood pressure remained associated but attenuated with MVPA, after further adjustment for body fat percentage (Väistö et al., 2019). In turn, Cureau et al. (2017) found that a higher MVPA practice in adolescents is associated with a lower number of cardiometabolic risk factors (blood pressure, HOMA-IR, HDLc, and TAG), even after further adjustment for WC (Cureau et al., 2017). The

Table 2 Differences Between Adipokines and Inflammatory Cytokines With Respect to MVPA Levels Divided by Quartiles

Variables	VLA	LA	MA	HA	*p* (ANCOVA)	*p* (for trend)
Adiponectin (mg/L)	14.47 ± 7.63^{HA}	12.37 ± 6.67	14.25 ± 7.21^{HA}	12.34 ± 7.11	.020	.053
Leptin (μg/L)	15.74 ± 12.08^{HA}	17.50 ± 17.31	14.62 ± 10.70	13.82 ± 13.56	.123	.006
Resistin (μg/L)	26.44 ± 16.97^{HA}	23.71 ± 18.60	22.29 ± 14.79	20.13 ± 12.54	.045	.014
NGF (ng/L)	9.79 ± 4.96	16.93 ± 33.89	32.73 ± 63.35	15.85 ± 19.09	.291	.238
HGF (μg/L)	0.37 ± 0.25	0.51 ± 0.39	0.63 ± 0.56	0.52 ± 0.31	.299	.104
TNF-α (ng/L)	2.49 ± 1.28^{HA}	2.81 ± 1.45	3.02 ± 1.68	3.01 ± 1.88	.066	.029
IL6 (ng/L)	1.91 ± 1.81	1.88 ± 3.08	4.27 ± 10.17	2.76 ± 3.82	.488	.233
IL8 (ng/L)	1.70 ± 1.26^{HA,A}	2.06 ± 1.78	2.49 ± 2.97	2.19 ± 2.84	.029	.007
MCP-1 (ng/L)	81.69 ± 27.14^{HA,A}	93.87 ± 38.05	95.95 ± 33.91	93.34 ± 41.21	.075	.039
aPAI-1 (μg/L)	17.49 ± 16.03^{LA}	7.55 ± 3.46	13.06 ± 7.13	13.73 ± 9.04	.141	.878
tPAI-1 (μg/L)	26.39 ± 16.15^{HA}	23.43 ± 14.90	22.98 ± 14.03	21.01 ± 13.02	.045	.014
P-selectin (μg/L)	123.34 ± 151.39	80.08 ± 124.78	81.20 ± 123.11	72.79 ± 100.26	.246	.207
sE-selectin (μg/L)	29.30 ± 10.25	31.25 ± 21.63	35.54 ± 18.68	25.83 ± 12.24	.345	.421
sVCAM (mg/L)	1.06 ± 0.26	0.99 ± 0.25	1.01 ± 0.27	1.02 ± 0.21	.71	.665
sICAM1 (mg/L)	0.11 ± 0.05^{HA,LA}	0.11 ± 0.06^{A}	0.14 ± 0.16	0.12 ± 0.08	.021	.239
MMP-9 (μg/L)	121.38 ± 64.55^{HA}	95.55 ± 36.43	103.85 ± 45.47	90.32 ± 45.45	.203	.070
MPO (μg/L)	37.35 ± 44.81	39.40 ± 44.56	58.71 ± 92.92^{HA}	36.91 ± 40.79	.132	.856

Note. Data are expressed as mean ± *SD*. *p*: One-way ANCOVA. VLA = very low active; LA =low active; MA = moderate active; HA = high active; NGF=erve growth factor; HGF=hepatocyte growth factor; TNF- = tumor necrosis factor alpha; IL = interleukin; MCP-1 = chemoattractant marker of Type 1 macrophages; PAI-1 = plasminogen activator inhibitor-1; tPAI = tissue PAI; aPAI = active PAI; P-selectin = plasma-selectin; sVCAM = vascular endothelial cell adhesion molecule-1; sICAM-1 = soluble intercellular adhesion molecule-1; MVPA = moderate to vigorous physical activity; MMP-9 = matrix metalloproteinase-9; MPO = myeloperoxidase; ANCOVA = analysis of covariance.
VLA, LA, MA, and HASignificant differences ($p < .05$) by pairwise post hoc test adjusted for age or/and BMI *z* score to determine which experimental groups differed from each other.

relationship between MVPA and cardiometabolic biomarkers has been summarized in a previous systematic review, including a controlled trial, and longitudinal and cross-sectional studies. An overall beneficial effect of MVPA on cardiometabolic risk factors (adiposity, high blood pressure, insulin resistance, and dyslipidemia) was reported (Poitras et al., 2016).

Similar to cardiometabolic parameters, some disturbances in plasma concentrations of a number of adipokines, such as an increase in leptin and/or resistin, and a decrease in adiponectin, have been previously associated with a higher BMI. Physical exercise interventions, without dietary modifications, on adipocytokines in children with overweight and obesity, have been recently reported in three systematic reviews and meta-analyses of randomized controlled trials (García-Hermoso et al., 2017; Sirico et al., 2018; Yu et al., 2017). From the results published by García-Hermoso et al. (2017), there was no influence of PA on plasma concentrations of leptin or resistin, while those reported by Yu et al. (2017) and Sirico et al. (2018) showed a significant reduction in leptin levels after exercise interventions. All of them found that PA, especially aerobic exercise, induces an increase in adiponectin plasma concentrations (García-Hermoso et al., 2017; Sirico et al., 2018; Yu et al., 2017). Similar results have been reported for the adult population (Fedewa et al., 2018; Yu et al., 2017). However, the studies included in those meta-analyses had small sample sizes and high heterogeneity regarding the characteristics of PA interventions and methodological limitations.

The evidence about the effect of objectively measured PA by accelerometry on adipokines is scarce and contradictory. The present study results initially showed that HA children had lower levels of leptin, resistin, and adiponectin than VLA children. However, only resistin remained negatively associated with min of MVPA by quartiles after age, sex, and BMI adjustment. In contrast, BMI was positively associated with leptin and negatively with resistin and adiponectin. This suggests that BMI plays a more important role than MVPA on the adipokines profile. Regarding leptin concentrations, Martinez-Gomez et al. (2012) and Jiménez-Pavón et al. (2012) found that vigorous PA in adolescents, but not moderate PA or MVPA, was negatively associated with leptin levels, even after WC and total body fat adjustments, respectively (Jiménez-Pavón et al., 2012; Martinez-Gomez et al., 2012). Concerning adiponectin, while some studies have reported no association with MVPA (Martinez-Gomez et al., 2012), others have reported an inverse relationship in children (Metcalf et al., 2009; Nielsen et al., 2016). This paradoxical result has been attributed to an increased adiponectin secretion to maintain adequate insulin sensitivity in those children with insufficient MVPA practice (Metcalf et al., 2009; Nielsen et al., 2016). In this context, Alessa et al. (2017) reported that a decrease in serum leptin joined to an increase in serum adiponectin was associated with both the total min of PA and the min of MVPA (both by quartiles), even after BMI adjustment in adults (Alessa et al., 2017). Regarding resistin, this adipokine has been related negatively to PA and positively to BMI in adults (Marcelino-Rodríguez et al., 2017; Rava et al., 2020), while there is no information about resistin levels according to objectively measured PA.

Several inflammatory cytokines have been found to be elevated in children with obesity (Rupérez et al., 2018; Utsal et al., 2012). Beyond the effect of PA on BMI status, it has been hypothesized that benefits of PA may be at least partly due to an anti-inflammatory effect (Pedersen, 2017). Regarding inflammatory cytokines, we found that plasma concentrations of TNF-α,

IL-8, and MCP-1 were higher as the min of MVPA increased along with the groups. Similarly, these parameters were positively correlated to the min of MVPA by quartiles, except for IL-8. However, regression analyses showed to us that MVPA quartiles did not mediate differences in the plasma concentration of TNF-α and MCP-1, but BMI, sex, and age. Therefore, none of the inflammatory cytokines included in our study were independently associated with MVPA by quartiles, while TNF-α and MCP-1 were positively associated with BMI. Similar reports to ours found controversial results. On the one hand, Nielsen et al. (2016) found that higher min of MVPA were associated with lower plasma levels of IL-6 and C-reactive protein (only in 8–11 years old Danish girls and after adjustment by fat mass; Nielsen et al., 2016), while Strizich et al. (2018) did not find any association between minutes of MVPA and C-reactive protein, after BMI adjustment in Hispanic children and adolescents from 8 to 16 years (Strizich et al., 2018). Although BMI may only mediate the relationship between PA and inflammatory biomarkers, a recent systematic review and meta-analysis of randomized controlled trials showed that PA interventions on children and adolescents with overweight and obesity reduced C-reactive protein levels along with a decrease in BMI, weight, and percentage of body fat (Han et al., 2019). In fact, from longitudinal data in adults with overweight or obesity, the practice of MVPA reduced an "inflammatory score" composed by some of these parameters (IL-6, IL-8, and MCP-1), after adjusting by changes in BMI, in a prospective interventional study (Fuentes et al., 2020).

Finally, only tPAI-1 plasma concentrations were significantly lower among the vascular-damage biomarkers as the min of MVPA increased between quartiles (Table 2). In correlation analyses, tPAI-1 and P-selectin showed a negative correlation to MVPA (Table 3). In the same way, regression analyses showed that both parameters were negatively associated with min of MVPA by quartiles, independently of age, sex, and BMI (Table 4). On the other hand, regression analyses showed us again that BMI had stronger associations than MVPA with these parameters, and it was positively associated with the plasma concentration TNF-α,

Table 3 Correlations Between Biomarkers and MVPA in Studied Children

Variable	*r*	*p*
Insulin	−.121	.004
HOMA-IR	−.125	.003
TAG	−.158	<.001
HDLc	.112	.012
Resistin	−.144	.003
TNF-α	.118	.012
MCP-1	.107	.021
tPAI1	−.128	.007
P-selectin	−.129	.012

Note. MVPA = moderate to vigorous physical activity; TAG = triacylglycerols; HOMA-IR = homeostatic model assessment-insulin resistance; HDLc = high-density lipoprotein cholesterol; TNF-α = tumor necrosis factor alpha; MCP-1 = chemoattractant marker of Type 1 macrophages; tPAI-1 = tissue plasminogen activator inhibitor-1; P-selectin = plasma-selectin; *r* = Spearman's *r* correlation coefficient.

Table 4 Multiple Regression Analysis Between Studied Variables and Physical Activity

		Age		Sex		BMI		MVPA	
	R^2	β	*p*	β	*p*	β	*p*	β	*p*
Insulin	.236	0.299	<.001	0.134	.001	0.370	<.001	-0.054	.196
HOMA-IR	.233	0.304	<.001	0.109	.010	0.352	<.001	-0.068	.109
TAG	.086	0.129	.003	0.091	.041	0.209	<.001	-0.118	.008
Adiponectin	.033	-0.120	.025	-0.001	.982	-0.145	.007	-0.084	.119
Leptin	.430	0.270	<.001	0.155	<.001	0.654	<.001	0.007	.871
Resistin	.050	0.048	.362	-0.006	.908	-0.154	.004	-0.151	.005
NGF	.030	-0.005	.973	-0.051	.734	0.121	.408	0.083	.568
HGF	.109	0.103	.446	-0.197	.169	0.172	.215	0.099	.477
TNF-α	.103	-0.173	.001	-0.119	.023	0.182	<.001	0.086	.098
IL-6	.018	0.055	.457	0.005	.943	0.096	.195	0.102	.174
IL-8	.028	-0.125	.020	-0.071	.189	0.023	.662	0.058	.287
MCP-1	.094	-0.155	.003	-0.118	.025	0.181	<.001	0.078	.136
aPAI	.107	0.142	.296	0.302	.039	0.182	.191	0.024	.866
tPAI	.077	0.133	.011	0.027	.609	0.240	<.001	-0.105	.046
P-selectin	.069	0.038	.502	-0.091	.114	-0.202	<.001	-0.160	.006
sE-selectin	.045	-0.173	.220	-0.138	.354	-0.069	.630	-0.086	.555
sICAM	.011	-0.009	.864	-0.017	.757	0.083	.122	0.058	.285
sVCAM1	.251	-0.455	<.001	-0.245	.065	-0.257	.046	-0.127	.320
MMP9	.070	-0.084	.542	-0.165	.258	-0.016	.912	-0.253	.078
MPO	.011	-0.029	.593	-0.079	.147	-0.075	.165	-0.001	.984

Note. MVPA = moderate to vigorous physical activity; BMI = body mass index; TAG = triacylglycerols; HOMA-IR = homeostatic model assessment-insulin resistance; NGF = nerve growth factor; HGF = hepatocyte growth factor; TNF-α = tumor necrosis factor alpha; IL = interleukin; MCP-1 = chemoattractant marker of Type 1 macrophages; PAI-1 = plasminogen activator inhibitor-1; tPAI = tissue PAI; aPAI = active PAI; P-selectin = plasma-selectin; sVCAM = vascular endothelial cell adhesion molecule-1; sICAM-1 = soluble intercellular adhesion molecule-1; MMP-9 = matrix metalloproteinase-9; MPO = myeloperoxidase.

MCP-1, and tPAI, independently of sex, age, and MVPA. Like our approach, Strizich et al. (2018) found lower plasma concentrations of sE-selectin, but not tPAI-1, as the min of MVPA increased by tertiles, although these differences, between sE-selectin and MVPA, disappeared after adjustment for BMI and WC (Strizich et al., 2018). These results are in line with reported reductions in PAI-1 and sE-selectin after weight loss interventions, including PA practice in adolescents (Hasson et al., 2012; Huang et al., 2011).

As seen before, changes in metabolic parameters (glucose and lipid metabolism), adipokines, inflammatory cytokines, and biomarkers of endothelial function are intimately related to BMI, and at the end, with obesity. This suggests all these biomarkers, usually recognized as cardiovascular risk factors, probably link the relationship between obesity and cardiovascular disease.

The present work's main strength is the large homogeneous sample of children, considering BMI, age, and sex as possible confounding factors in the relationship between MVPA and metabolic and inflammatory biomarkers. In this way, statistical adjustments were carried out to avoid these influences. Moreover, there was a homogeneous distribution of children with obesity, overweight, and normal weight between MVPA groups. In all of them, accelerometry was used as an objective method to measure PA. Notwithstanding, our study presents several limitations. First, it is not a longitudinal or intervention study, so we can only describe associations and generate a hypothesis of the relationship between PA and cardiometabolic risk biomarkers but no causal relationships. Second, disturbances in some cardiometabolic risk biomarkers are usually related to a long-term clinical course of chronic low-grade inflammation in obesity; this may limit our chance to detect metabolic impairment early in children.

In summary, the present results support the relationship between moderate to high intensity of PA and a healthier metabolic and inflammatory status and highlight the importance of maintaining an adequate BMI for this purpose. So, these observations are consistent with the possibility that increasing MVPA in children beyond 60 min/day along with the maintenance of an adequate BMI may achieve health benefits. Further studies are needed to clarify the appropriate format and implementation of a healthcare system based on active behaviors, especially to avoid obesity with a low grade of inflammation and metabolic comorbidities.

Acknowledgments

F.J. Llorente-Cantarero and F.J. Aguilar-Gómez contributed equally to this work. The authors would like to thank the children and parents who participated in the study. Conceptualization was done by M. Gil-Campos, R. Leis, G. Bueno-Lozano, Á. Gil, L.A. Moreno, and C.M. Aguilera; methodology was done by M. Gil-Campos, R. Leis, G. Bueno-Lozano, L.A. Moreno, C.M. Aguilera, and F.J. Llorente-Cantarero; formal analysis was done by F.J. Llorente-Cantarero, M.D.M., and F.J. Aguilar-Gómez; investigation was done by F.J. Llorente-Cantarero, F.J. Aguilar-Gómez, A.I. Rupérez, and R. Vázquez-Cobela; resources were done by M. Gil-Campos, R. Leis, G. Bueno-Lozano, and C.M. Aguilera; data curation were done by F.J. Llorente-Cantarero, F.J. Aguilar-Gómez, A. Anguita-Ruiz, and Á. Gil; writing—original draft preparation was done by F.J. Llorente-Cantarero, F.J. Aguilar-Gómez, Á. Gil, M. Gil-Campos, and C.M. Aguilera; writing—review and editing, all the group; visualization was done by F.J. Llorente-Cantarero, F.J. Aguilar-Gómez, Á. Gil, M. Gil-Campos, M.D.M., A. Anguita-Ruiz and C.M. Aguilera; supervision was done by M. Gil-Campos, Á. Gil, and C.M. Aguilera; project administration was done by M. Gil-Campos, R. Leis, G. Bueno-Lozano, and C.M. Aguilera. All authors have read and agreed to the published version of the manuscript. This work was supported by the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I + D + I), Instituto de Salud Carlos III-Health Research Funding (FONDOS FEDER; PI051968, PI1102042, and PI1600871) and Redes temáticas de investigación cooperativa RETIC (Red SAMID RD12/0026/0015). The authors also acknowledge Instituto de Salud Carlos III for personal funding of A. Anguita-Ruiz: Contratos i-PFIS: doctorados IIS-empresa en ciencias y tecnologías de la salud de la convocatoria 2017 de la Acción Estratégica en Salud 2013–2016 (IFI17/00048). Á. Gil was funded by the Research Plan of the Vice-Rectorate of Research and Transfer of the University of Granada, Spain This paper will be included in Francisco Javier Aguilar-Gómez doctorate under the "Biomedicine Program" at the University of Córdoba, Spain.

References

Ahrens, W., Moreno, L., Mårild, S., Molnár, D., Siani, A., De Henauw, S., Böhmann, J., Günther, K., Hadjigeorgiou, C., Iacoviello, L., Lissner, L., Veidebaum, T., Pohlabeln, H., & Pigeot, I. (2014). Metabolic syndrome in young children: Definitions and results of the IDEFICS study. *International Journal of Obesity, 38,* S4–S14. https://doi.org/10.1038/ijo.2014.130

Alessa, H.B., Chomistek, A.K., Hankinson, S.E., Barnett, J.B., Rood, J., Matthews, C.E., Rimm, E.B., Willett, W.C., Hu, F.B., & Tobias, D.K. (2017). Objective measures of physical activity and cardiometabolic and endocrine biomarkers. *Medicine & Science in Sports & Exercise, 49*(9), 1817–1825. https://doi.org/10.1249/MSS.0000000000001287

Cole, T.J., Bellizzi, M.C., Flegal, K.M., & Dietz, W.H. (2000). Establishing a standard definition for child overweight and obesity worldwide: International survey. *British Medical Journal, 320*(7244), 1240–1243. https://doi.org/10.1136/bmj.320.7244.1240

Corder, K., Sharp, S.J., Atkin, A.J., Griffin, S.J., Jones, A.P., Ekelund, U., & van Sluijs, E.M.F. (2015). Change in objectively measured physical activity during the transition to adolescence. *British Journal of Sports Medicine, 49*(11), 730–736. https://doi.org/10.1136/bjsports-2013-093190

Cureau, F.V., Ekelund, U., Bloch, K.V., & Schaan, B.D. (2017). Does body mass index modify the association between physical activity and screen time with cardiometabolic risk factors in adolescents? Findings from a country-wide survey. *International Journal of Obesity, 41*(4), 551–559. https://doi.org/10.1038/ijo.2016.210

Ekelund, U., Luan, J., Sherar, L.B., Esliger, D.W., Griew, P., & Cooper, A. (2012). Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. *Journal of the American Medical Association, 307*(7), 704–712. https://doi.org/10.1001/jama.2012.156

Evenson, K.R., Catellier, D.J., Gill, K., Ondrak, K.S., & McMurray, R.G. (2008). Calibration of two objective measures of physical activity for children. *Journal of Sports Sciences, 26*(14), 1557–1565. https://doi.org/10.1080/02640410802334196

Fedewa, M.V., Hathaway, E.D., Ward-Ritacco, C.L., Williams, T.D., & Dobbs, W.C. (2018). The effect of chronic exercise training on leptin: A systematic review and meta-analysis of randomized controlled trials. *Sports Medicine, 48*(6), 1437–1450. https://doi.org/10.1007/s40279-018-0897-1

Fuentes, G.C., Castañer, O., Warnberg, J., Subirana, I., Buil-Cosiales, P., Salas-Salvadó, J., Corellacj, D., Serra-Majemck, L., Romagueracl, D., Estruchcm, R., Martínezcn, J.A., Pintóco, X., Vázquez, C., Vidalqr, J., Turcs, J.A., Arósct, F., Bullocu, M., Fitó, M., & Schröder, H. (2020). Prospective association of physical activity and inflammatory biomarkers in older adults from the PREDIMED-Plus study

with overweight or obesity and metabolic syndrome. *Clinical Nutrition, 39*(10), 3092–3098. https://doi.org/10.1016/j.clnu.2020.01.015

Füssenich, L.M., Boddy, L.M., Green, D.J., Graves, L.E.F., Foweather, L., Dagger, R.M., McWhannell, N., Henaghan, J., Ridgers, N.D., Stratton, G., & Hopkins, N.D. (2016). Physical activity guidelines and cardiovascular risk in children: A cross sectional analysis to determine whether 60 minutes is enough. *BMC Public Health, 16*(1), 1–7. https://doi.org/10.1186/s12889-016-2708-7

García-Hermoso, A., Ceballos-Ceballos, R.J.M., Poblete-Aro, C.E., Hackney, A.C., Mota, J., & Ramírez-Vélez, R. (2017). Exercise, adipokines and pediatric obesity: A meta-analysis of randomized controlled trials. *International Journal of Obesity, 41*(4), 475–482. https://doi.org/10.1038/ijo.2016.230

Guzzetti, C., Ibba, A., Casula, L., Pilia, S., Casano, S., & Loche, S. (2019). Cardiovascular risk factors in children and adolescents with obesity: Sex-related differences and effect of puberty. *Frontiers in Endocrinology, 10*(AUG), 591. https://doi.org/10.3389/fendo.2019.00591

Han, Y., Liu, Y., Zhao, Z., Zhen, S., Chen, J., Ding, N., Ma, Y., & Wen, D. (2019). Does physical activity-based intervention improve systemic proinflammatory cytokine levels in overweight or obese children and adolescents? Insights from a meta-analysis of randomized control trials. *Obesity Facts, 12*(6), 653–668. https://doi.org/10.1159/000501970

Hasson, R.E., Adam, T.C., Davis, J.N., Kelly, L.A., Ventura, E.E., Byrd-Williams, C.E., Toledo-Corral, C.M., Roberts, C.K., Lane, C., Azen, S.P., Chou, C.-P., Spruijt-Metz, D., Weigensberg, M.J., Berhane, K., & Goran, M.I. (2012). Randomized controlled trial to improve adiposity, inflammation, and insulin resistance in obese African-American and Latino youth. *Obesity, 20*(4), 811–818. https://doi.org/10.1038/oby.2010.343

Huang, F., Del-Río-Navarro, B.E., de Castro, G.T.M., Alcántara, S.T., Sienra Monge, J.J.L., Ontiveros, J.A.P., Olivos, E.N., Barron, M.F., Lopéz, A.R., Villafaña, S., & Hong, E. (2011). Weight loss induced by 6-month lifestyle intervention improves early endothelial activation and fibrinolysis in obese adolescents. *Child: Care, Health and Development, 37*(3), 377–384. https://doi.org/10.1111/j.1365-2214.2010.01173.x

Jiménez-Pavón, D., Ortega, F.B., Artero, E.G., Labayen, I., Vicente-Rodriguez, G., Huybrechts, I., Moreno, L.A., Manios, Y., Béghin, L., Polito, A., De Henauw, S., Sjöström, M., Castillo, M.J., González-Gross, M., & Ruiz, J.R. (2012). Physical activity, fitness, and serum leptin concentrations in adolescents. *Journal of Pediatrics, 160*(4), 598–603.e2. https://doi.org/10.1016/j.jpeds.2011.09.058

Júdice, P.B., Hetherington-Rauth, M., Northstone, K., Andersen, L.B., Wedderkopp, N., Ekelund, U., & Sardinha, L.B. (2020). Changes in physical activity and sedentary patterns on cardiometabolic outcomes in the transition to adolescence: International Children's Accelerometry Database 2.0. *Journal of Pediatrics, 225,* 166–173.e1. https://doi.org/10.1016/j.jpeds.2020.06.018

Marcelino-Rodríguez, I., Almeida Gonzalez, D., Alemán-Sánchez, J.J., Brito Díaz, B., Rodríguez Pérez, M.D.C., Gannar, F., Coello, S.D., Fernández, F.J.C., & Cabrera De León, A. (2017). Inverse association of resistin with physical activity in the general population. *PLoS One, 12*(8), 1–11. https://doi.org/10.1371/journal.pone.0182493

Martinez-Gomez, D., Eisenmann, J.C., Gomez-Martinez, S., Veses, A., Romeo, J., Veiga, O.L., Marcos, A., & AFINOS Study Group. (2012). Associations of physical activity and fitness with adipocytokines in adolescents: The AFINOS study. *Nutrition, Metabolism and Cardiovascular Diseases, 22*(3), 252–259. https://doi.org/10.1016/j.numecd.2010.07.010

Martos-Moreno, G.A., Gil-Campos, M., Bueno, G., Bahillo, P., Bernal, S., Feliu, A., Lechuga-Sancho, A.M., Palomo, E., Ruiz, R., & Vela, A. (2014). Las alteraciones metabólicas asociadas a la obesidad están ya presentes en los primeros años de vida: Estudio colaborativo español. *Nutricion Hospitalaria, 30*(4), 787–793. https://doi.org/10.3305/nh.2014.30.4.7661

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., & Turner, R.C. (1985). Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412–419. https://doi.org/10.1007/BF00280883

McCrindle, B.W. (2010). Assessment and management of hypertension in children and adolescents. *Nature Reviews Cardiology, 7,* 155–163. https://doi.org/10.1038/nrcardio.2009.231

Mead, E., Brown, T., Rees, K., Azevedo, L.B., Whittaker, V., Jones, D., Olajide, J., Mainardi, G.M., Corpeleijn, E., O'Malley, C., Beardsmore, E., Al-Khudairy, L., Baur, L., Metzendorf, M.-I., Demaio, A. & Ells, L.J. (2017). Diet, physical activity and behavioural interventions for the treatment of overweight or obese children from the age of 6 to 11 years. *Cochrane Database of Systematic Reviews, 6*. https://doi.org/10.1002/14651858.CD012651

Metcalf, B.S., Jeffery, A.N., Hosking, J., Voss, L.D., Sattar, N., & Wilkin, T.J. (2009). Objectively measured physical activity and its association with adiponectin and other novel metabolic markers. *Diabetes Care, 32*(3), 467–473. https://doi.org/10.2337/dc08-1329

Migueles, J.H., Cadenas-Sanchez, C., Ekelund, U., Delisle Nyström, C., Mora-Gonzalez, J., Löf, M., Labayen, I., Ruiz, R., & Ortega, F.B. (2017). Accelerometer data collection and processing criteria to assess physical activity and other outcomes: A systematic review and practical considerations. *Sports Medicine, 47*(9), 1821–1845. https://doi.org/10.1007/s40279-017-0716-0

Nielsen, M.S., Quist, J.S., Chaput, J.P., Dalskov, S.M., Damsgaard, C.T., Ritz, C., Astrup, A., Michaelsen, K.F., Sjödin, S., & Hjorth, M.F. (2016). Physical activity, sedentary time, and sleep and the association with inflammatory markers and adiponectin in 8- to 11-year-old Danish children. *Journal of Physical Activity and Health, 13*(7), 733–739. https://doi.org/10.1123/jpah.2015-0123

Olza, J., Aguilera, C.M., Gil-Campos, M., Leis, R., Bueno, G., Valle, M., Cañete, R., Tojo, R., Moreno, L.A., & Gil, A. (2013). Waist-to-height ratio, inflammation and CVD risk in obese children. *Public Health Nutrition, 17*(10), 2378–2385. https://doi.org/10.1017/S1368980013003285

Pedersen, B.K. (2017). Anti-inflammatory effects of exercise: Role in diabetes and cardiovascular disease. *European Journal of Clinical Investigation, 47*(8), 600–611. https://doi.org/10.1111/eci.12781

Poitras, V.J., Gray, C.E., Borghese, M.M., Carson, V., Chaput, J.P., Janssen, I., Katzmarzyk, P.T., Pate, R.R., Gorber, S.C., Kho, M.E., Sampson, M., & Tremblay, M. S. (2016). Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied Physiology, Nutrition and Metabolism, 41*(6), S197–S239. https://doi.org/10.1139/apnm-2015-0663

Rava, A., Pihlak, A., Kums, T., Purge, P., Pääsuke, M., & Jürimäe, J. (2020). Resistin concentration is inversely associated with objectively measured physical activity in healthy older women. *Aging Clinical and Experimental Research, 32*(3), 475–481. https://doi.org/10.1007/s40520-019-01222-6

Rupérez, A.I., Mesa, M.D., Anguita-Ruiz, A., González-Gil, E.M., Vázquez-Cobela, R., Moreno, L.A., Gil, A., Gil-Campos, M., Leis, R., Bueno, G., & Aguilera, C.M. (2020). Antioxidants and oxidative stress in children: Influence of puberty and metabolically unhealthy status. *Antioxidants, 9*(7), 1–19. https://doi.org/10.3390/antiox9070618

Rupérez, A.I., Olza, J., Gil-Campos, M., Leis, R., Bueno, G., Aguilera, C.M., Gil, A., & Moreno, L.A. (2018). Cardiovascular risk biomarkers

and metabolically unhealthy status in prepubertal children: Comparison of definitions. *Nutrition, Metabolism and Cardiovascular Diseases, 28*(5), 524–530. https://doi.org/10.1016/j.numecd.2018.02.006

Schmidt, F.M., Weschenfelder, J., Sander, C., Minkwitz, J., Thormann, J., Chittka, T., Mergl, R., Kirkby, K.C., Faßhauer, M., Stumvoll, M., Holdt, L.M., Teupser, D., Hegerl, U., & Himmerich, H. (2015). Inflammatory cytokines in general and central obesity and modulating effects of physical Activity. *PLoS One, 10*(3), 1–17. https://doi.org/10.1371/journal.pone.0121971

Singer, K., & Lumeng, C.N. (2017). The initiation of metabolic inflammation in childhood obesity. *Journal of Clinical Investigation, 127*(1), 65–73. https://doi.org/10.1172/JCI88882

Sirico, F., Bianco, A., D'Alicandro, G., Castaldo, C., Montagnani, S., Spera, R., Meglio, F.D., & Nurzynska, D. (2018). Effects of physical exercise on adiponectin, leptin, and inflammatory markers in childhood obesity: Systematic review and meta-analysis. *Childhood Obesity, 14*(4), 207–217. https://doi.org/10.1089/chi.2017.0269

Strizich, G., Kaplan, R.C., Sotres-Alvarez, D., Diaz, K.M., Daigre, A.L., Carnethon, M.R., Vidot, D.C., Delamater, A.M., Perez, L., Perreira, K., Isasi, C.R., & Qi, Q. (2018). Objectively measured sedentary behavior, physical activity, and cardiometabolic risk in Hispanic youth: Hispanic community health study/study of Latino youth. *Journal of Clinical Endocrinology and Metabolism, 103*(9), 3289–3298. https://doi.org/10.1210/jc.2018-00356

Treuth, M.S., Schmitz, K., Catellier, D.J., McMurray, R.G., Murray, D.M., Almeida, M.J., Going, S., Norman, J.E., & Pate, R. (2004). Defining accelerometer thresholds for activity intensities in adolescent girls. *Medicine & Science in Sports & Exercise, 36*(7), 1259–1266. https://doi.org/10.1249/01.MSS.0000074670.03001.98

Utsal, L., Tillmann, V., Zilmer, M., Mäestu, J., Purge, P., Jürimäe, J., Saar, M.A., Lätt, E.A., Maasalu, K., & Jürimäe, T. (2012). Elevated serum IL-6, IL-8, MCP-1, CRP, and IFN-γ levels in 10- to 11-year-old boys with increased BMI. *Hormone Research in Paediatrics, 78*(1), 31–39. https://doi.org/10.1159/000339831

Väistö, J., Haapala, E.A., Viitasalo, A., Schnurr, T.M., Kilpeläinen, T.O., Karjalainen, P., Westgate, K., Lakka, H.-M., Laaksonen, D.E., Ekelund, U., Brage, S., Lakka, T.A., & Lakka, T.A. (2019). Longitudinal associations of physical activity and sedentary time with cardiometabolic risk factors in children. *Scandinavian Journal of Medicine & Science in Sports, 29*(1), 113–123. https://doi.org/10.1111/sms.13315

World Health Organization. (2020). WHO Guidelines on physical activity and sedentary behaviour. *World Health Organization*. https://apps.who.int/iris/bitstream/handle/10665/325147/WHO-NMH-PND-2019.4-eng.pdf?sequence=1&isAllowed=y%0Ahttp://www.who.int/iris/handle/10665/311664%0Ahttps://apps.who.int/iris/handle/10665/325147

Yu, N., Ruan, Y., Gao, X., & Sun, J. (2017). Systematic review and meta-analysis of randomized, controlled trials on the effect of exercise on serum leptin and adiponectin in overweight and obese individuals. *Hormone and Metabolic Research, 49*(3), 164–173. https://doi.org/10.1055/s-0042-121605

CONCLUSIONES

V. CONCLUSIONES

Conclusión general

La práctica de actividad física de los niños prepúberes en su desarrollo hacia la adolescencia se relaciona con un mejor estado metabólico, mejor perfil de factores inflamatorios y de riesgo cardiovascular, y menor estrés oxidativo.

Conclusiones específicas:

I. Durante la transición de la infancia (fase prepuberal) a la adolescencia (fase puberal) se produce un descenso generalizado de la actividad física junto con un aumento del tiempo sedentario.

II. El descenso de actividad física durante este periodo se produce en ambos sexos, aunque es más acusado en varones, sobre todo en el caso de la actividad física moderada-vigorosa. Aún así, los varones se mantienen más activos que las mujeres en ambos periodos.

III. Durante el periodo prepuberal, los niños con normopeso realizan más actividad física moderada, vigorosa y moderada-vigorosa que los niños con sobrepeso y obesidad. En cambio, no se encontraron diferencias relevantes entre los grupos de índice de masa corporal durante el periodo puberal.

IV. Una mayor práctica de actividad física, en especial moderada y vigorosa, junto con un menor tiempo sedentario, representados por el PASS (*Physical Activity and Sedentarism Score*), se relacionan con una disminución de biomarcadores de estrés oxidativo y, por tanto, con un mejor estado redox.

V. Una mayor práctica de actividad física moderada-vigorosa junto con un menor índice de masa corporal se relacionan con un mejor perfil metabólico (metabolismo hidrocarbonado y lipídico) y una menor concentración de varias citoquinas pro-inflamatorias (adipocinas, citoquinas pro-inflamatorias y moléculas de daño endotelial).

VI. En su conjunto, estos datos apoyan la recomendación de la Organización Mundial de la Salud para niños y adolescentes de 5 a 17 años de practicar al menos 60 minutos de actividad física moderada-vigorosa al día y de limitar el tiempo sedentario. Asimismo, estos datos muestran el incumplimiento de gran parte de los sujetos de nuestra muestra de dicha recomendación, así como los posibles beneficios para salud, en términos de estrés oxidativo y perfil cardiometabólico, que observamos en aquellos niños que cumplen con creces dichas recomendaciones.

BIBLIOGRAFÍA

VI. BIBLIOGRAFÍA

1. Ahrens W, Moreno L, Mårild S, Molnár D, Siani A, De Henauw S, et al. Metabolic syndrome in young children: Definitions and results of the IDEFICS study. Int J Obes. 2014;38:S4–14.
2. Guzzetti C, Ibba A, Casula L, Pilia S, Casano S, Loche S. Cardiovascular Risk Factors in Children and Adolescents With Obesity: Sex-Related Differences and Effect of Puberty. Vol. 10, Frontiers in Endocrinology . 2019. p. 591. Available from: https://www.frontiersin.org/article/10.3389/fendo.2019.00591
3. Aguilar-Gomez FJ, Bueno-Lozano G, Leis R, Aguilera CM, Gil-Campos M. Metabolic syndrome before puberty: Myth or reality? Curr Opin Endocr Metab Res. 2020;14(Table 1):97–103. Available from: https://doi.org/10.1016/j.coemr.2020.06.006
4. Janssen I, LeBlanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int J Behav Nutr Phys Act. 2010;7.
5. Psaltopoulou T, Tzanninis S, Ntanasis-Stathopoulos I, Panotopoulos G, Kostopoulou M, Tzanninis IG, et al. Prevention and treatment of childhood and adolescent obesity: a systematic review of meta-analyses. Vol. 15, World Journal of Pediatrics. Springer Singapore; 2019. 350–381 p. Available from: https://doi.org/10.1007/s12519-019-00266-y
6. Mead E, Brown T, Rees K, Azevedo LB, Whittaker V, Jones D, et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese children from the age of 6 to 11 years. Vol. 2017, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2017. Available from: https://pubmed.ncbi.nlm.nih.gov/28639319/
7. Al-Khudairy L, Loveman E, Colquitt JL, Mead E, Johnson RE, Fraser H, et al. Diet, physical activity and behavioural interventions for the treatment of overweight or obese adolescents aged 12 to 17 years. Vol. 2017, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2017. Available from: https://pubmed.ncbi.nlm.nih.gov/28639320/
8. Aguilar Cordero MJ, Sánchez López AM, Guisado Barrilao R, Rodriguez Blanque R, Noack Segovia J, Pozo Cano MD. Descripción del acelerómetro como método para valorar la actividad física en los diferentes periodos de la vida: revisión

sistemática. Nutr Hosp. 2014;29(6):1250–61. Available from: https://scielo.isciii.es/scielo.php?script=sci_arttext&pid=S0212-16112014000600005&lng=es&nrm=iso&tlng=es

9. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. J Sports Sci. 2008;26(14):1557–65.
10. WHO. WHO Guidelines on physical activity and sedentary behaviour. World Health Organization. 2020. 4 p.
11. Roman Viñas B, Serra Majen L, Aznar Laín S, Zazo F MMJ. Informe 2018 Actividad Física en niños y adolescentes en España. Fund para la Investig Nutr. 2018;1–24.
12. Roman Viñas B, Serra Majem L, Leis R, González Gros M, Sánchez López M ALS. Informe 2016 Actividad Física en niños y adolescentes en España. 2016. :6–8.
13. Mitchell JA, Pate RR, Dowda M, Mattocks C, Riddoch C, Ness AR, et al. A prospective study of sedentary behavior in a large cohort of youth. Med Sci Sports Exerc. 2012 Jun;44(6):1081–7.
14. Corder K, Sharp SJ, Atkin AJ, Griffin SJ, Jones AP, Ekelund U, et al. Change in objectively measured physical activity during the transition to adolescence. Br J Sports Med. 2015 Jun;49(11):730–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24273308
15. Harding SK, Page AS, Falconer C, Cooper AR. Longitudinal changes in sedentary time and physical activity during adolescence. Int J Behav Nutr Phys Act. 2015 Apr 1;12(1):44.
16. Collings paul J, Wijndaele K, Corder K, Westgate K, Ridgway CL, Sharp SJ, et al. Magnitude and determinants of change in objectively-measured physical activity, sedentary time and sleep duration from ages 15 to 17.5y in UK adolescents: The ROOTS study. Int J Behav Nutr Phys Act. 2015 May 14;12(1):61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25971606
17. Sherar LB, Esliger DW, Baxter-Jones ADG, Tremblay MS. Age and gender differences in youth physical activity: Does physical maturity matter? Med Sci Sports Exerc. 2007;39(5):830–5.
18. Sies H. Oxidative stress: Concept and some practical aspects. Antioxidants. 2020;9(9):1–6.
19. Rupérez AI, Mesa MD, Anguita-Ruiz A, González-Gil EM, Vázquez-Cobela R,

Moreno LA, et al. Antioxidants and oxidative stress in children: Influence of puberty and metabolically unhealthy status. Antioxidants. 2020;9(7):1–19.

20. de Sousa CV, Sales MM, Rosa TS, Lewis JE, de Andrade RV, Simões HG. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. Sport Med. 2017;47(2):277–93.
21. Basu S. F2-isoprostanes in human health and diseases: From molecular mechanisms to clinical implications. Vol. 10, Antioxidants and Redox Signaling. Antioxid Redox Signal; 2008. p. 1405–34. Available from: https://pubmed.ncbi.nlm.nih.gov/18522490/
22. Roberts LJ, Morrow JD. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med. 2000;28(4):505–13.
23. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology. 2003;189(1–2):41–54.
24. Morandi A, Corradi M, Piona C, Fornari E, Puleo R, Maffeis C. Systemic anti-oxidant capacity is inversely correlated with systolic blood pressure and pulse pressure in children with obesity. Nutr Metab Cardiovasc Dis. 2020;30(3):508–13. Available from: https://doi.org/10.1016/j.numecd.2019.10.008
25. Singer K, Lumeng CN. The initiation of metabolic inflammation in childhood obesity. J Clin Invest. 2017;127(1):65–73.
26. Martos-Moreno GA, Kopchick JJ, Argente J. Adipoquinas en el niño sano y con obesidad. An Pediatr. 2013;78(3).
27. Yu N, Ruan Y, Gao X, Sun J. Systematic Review and Meta-Analysis of Randomized, Controlled Trials on the Effect of Exercise on Serum Leptin and Adiponectin in Overweight and Obese Individuals. Horm Metab Res. 2017;49(3):164–73.
28. Sirico F, Bianco A, D'Alicandro G, Castaldo C, Montagnani S, Spera R, et al. Effects of Physical Exercise on Adiponectin, Leptin, and Inflammatory Markers in Childhood Obesity: Systematic Review and Meta-Analysis. Child Obes. 2018;14(4):207–17.
29. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical Activity. PLoS One. 2015;10(3):1–17.
30. Olza J, Aguilera CM, Gil-Campos M, Leis R, Bueno G, Valle M, et al. Waist-to-height ratio, inflammation and CVD risk in obese children. Public Health Nutr. 2013;17(10):2378–85.

31. Rupérez AI, Olza J, Gil-Campos M, Leis R, Bueno G, Aguilera CM, et al. Cardiovascular risk biomarkers and metabolically unhealthy status in prepubertal children: Comparison of definitions. Nutr Metab Cardiovasc Dis. 2018;28(5):524–30. Available from: https://doi.org/10.1016/j.numecd.2018.02.006
32. Utsal L, Tillmann V, Zilmer M, Mäestu J, Purge P, Jürimäe J, et al. Elevated serum IL-6, IL-8, MCP-1, CRP, and IFN-γ levels in 10- to 11-year-old boys with increased BMI. Horm Res Paediatr. 2012;78(1):31–9.
33. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. Vol. 310, JAMA - Journal of the American Medical Association. American Medical Association; 2013. p. 2191–4.
34. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. Br Med J. 2000 May 6;320(7244):1240–3.
35. McCrindle BW. Assessment and management of hypertension in children and adolescents. Vol. 7, Nature Reviews Cardiology. 2010. p. 155–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20065950
36. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
37. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nyström C, Mora-Gonzalez J, Löf M, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. Sport Med. 2017;47(9):1821–45.
38. Treuth MS, Schmitz K, Catellier DJ, McMurray RG, Murray DM, Almeida MJ, et al. Defining accelerometer thresholds for activity intensities in adolescent girls. Med Sci Sports Exerc. 2004;36(7):1259–66.