VALORACIÓN DE MARCADORES PLASMÁTICOS METABÓLICOS, INFLAMATORIOS Y DE ESTRÉS ÓXIDATIVO EN NIÑOS SANOS SEGÚN LA CONDICIÓN FÍSICA

Francisco Jesús Llorente Cantarero

Departamento de Especialidades Médico Quirúrgicas
Facultad de Medicina
Universidad de Córdoba

TITULO: *VALORACIÓN DE MARCADORES PLASMÁTICOS METABÓLICOS, INFLAMATORIOS Y DE ESTRÉS OXIDATIVO EN NIÑOS SANOS SEGÚN LA CONDICIÓN FÍSICA*

AUTOR: *FRANCISCO JESÚS LLORENTE CANTARERO*

© Edita: Servicio de Publicaciones de la Universidad de Córdoba.
Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

www.uco.es/publicaciones
publicaciones@uco.es

Departamento de Especialidades Médico Quirúrgicas

VALORACIÓN DE MARCADORES PLASMÁTICOS METABÓLICOS, INFLAMATORIOS Y DE ESTRÉS ÓXIDATIVO EN NIÑOS SANOS SEGÚN LA CONDICIÓN FÍSICA

"Assessment of Metabolic, Inflammation and Oxidative Stress biomarkers in prepubertal healthy children in relation to Cardiorespiratory Fitness"

Memoria para optar al título de doctor con mención internacional por la Universidad de Córdoba

INTERNATIONAL PhD THESIS

Presentada por:
Francisco Jesús Llorente Cantarero

Realizada bajo la dirección de los doctores:
María Mercedes Gil Campos
Juan de Dios Benítez Sillero

Córdoba, 2013

TÍTULO DE LA TESIS: VALORACIÓN DE MARCADORES PLASMÁTICOS METABÓLICOS, INFLAMATORIOS Y DE ESTRÉS ÓXIDATIVO EN NIÑOS SANOS SEGÚN LA CONDICIÓN FÍSICA.

DOCTORANDO/A: Francisco Jesús Llorente Cantarero

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).

Dña. MARIA MERCEDES GIL CAMPOS, con DNI 44286271R, como directora y D. JUAN DE DIOS BENÍTEZ SILLERO como director de esta tesis doctoral, con DNI 30827602N informamos que se ha realizado un trabajo de tesis doctoral exhaustivo y de gran calidad. El doctorando ha aprendido metodología de la investigación, y ha trabajado profundamente tanto en el trabajo de selección, estudio físico, clínico y muestreo, como en el aprendizaje de pruebas de campo y técnico en diferentes laboratorios abordando distintas tecnologías aplicables a su ámbito profesional y de conocimiento, y en la preparación y redacción de las publicaciones que se exponen en esta tesis doctoral.

Los artículos publicados a partir de esta Tesis, así como comunicaciones en congresos nacionales e internacionales, varios premios recibidos, y las dos estancias en centros externos, uno de ellos en Dinamarca, indican el excelente trabajo de investigación realizado. Por ello, solicito que el doctorando Francisco Jesús Llorente Cantarero pueda optar al grado de Doctor por la Universidad de Córdoba y autorizo la presentación de la tesis doctoral.

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

Córdoba, 5 de noviembre de 2012

Firma del/de los director/es

Fdo.:_____ Fdo.: _____

"La adquisición de cualquier conocimiento es siempre útil al intelecto, que sabrá descartar lo malo y conservar lo bueno".

Leonardo Da Vinci

A mis padres, a quienes debo lo que soy y sin cuyo apoyo este trabajo no hubiera sido posible.

A mi familia, con especial recuerdo a mis abuelos que supieron inculcar en mi el tesón y la tenacidad necesaria para afrontar la vida.

A Ana, mi gran amor, mi mayor apoyo cuando he estado lejos y la luz en los momentos más duros.

A Todos los participantes y sus familias que han colaborado en este estudio.

Al Prof. Dr. Juan Luís Pérez Navero por confiar y creer que la unión entre un pediatra y un deportista podría dar grandes frutos. Por su tesón y entrega sin importar las horas que hubiera que dedicar a la culminación de algún artículo.

A la Dra. Mercedes Gil Campos por despertar en mí la pasión por la investigación y mostrarme cual es el camino correcto hacia la excelencia. Por esa crítica acertada que todo trabajo de investigación necesita. Y por su ánimo y apoyo cuando todo parecía complicarse.

Al Dr. Juan de Dios Benítez Sillero por entender que las líneas de investigación que se inician han de tener una continuidad, dando como resultado un mayor conocimiento y el consecuente beneficio para la sociedad.

To Prof. Dr. Jørn Wulff Helge to allow me development part of my carrier with him and show me as is understood research in Europe. To make my stay very comfortable and mainly to become a reference for me both as a research as a person.

A la Prof. Dra. Clara Prats por descubrirme los entresijos de la microscopía y el funcionamiento más desconocido de la mitocondria. Por hacer que mi estancia en Copenhague fuera en muchos momentos como estar en casa.

Al Prof. Dr. Isaac Túnez por abrirme las puertas de su laboratorio e instruirme en un campo, hasta la fecha, poco conocido como es el Estrés Oxidativo.

A la Dra. Mari Carmen Muñoz Villanueva por su profesionalidad en el análisis estadístico, siendo un aspecto clave para la aceptación de los artículos.

Al Grupo de Bioquímica y Biología Molecular de Granada por su profesionalidad y ayuda inestimable en el análisis de parámetros inflamatorios.

Al Prof. Dr. Manuel Guillén del Castillo por sus enseñanzas y apoyo en el inicio de esta andadura.

Al Colegio Maristas Córdoba por aceptar participar en este proyecto, creer siempre en su culminación y facilitar todos los medios que estaban a su alcance.

Al Prof. Paco Pepe por su ayuda inestimable, haciendo que una tarea tan compleja como la organización y logística, pareciera algo sencillo y cotidiano, convirtiéndose en un pilar fundamental durante la recogida de muestras.

A Lourdes por su profesionalidad, por estar dispuesta a ayudar siempre que hiciera falta y por convertir algo tan complejo para mí como la extracción sanguínea en cotidiano.

A Sebastián del Rey y Carlos García por su ayuda durante los test físicos.

A Ana Giraldo por su ayuda en el análisis de muestras.

A Luisma por entender mejor que nadie las complicaciones que vienen ligadas al desarrollo de una tesis. Por encontrar un hueco para ayudarme o apoyarme cuando tenía algún problema a pesar de los que él se estaba encontrado en el camino de la vida y que requerían su presencia.

A Mi Primo Francisco por ser como un hermano para mí y hacer en muchas ocasiones más liviana la carga del camino a recorrer.

A Mi Tío Juan por su apoyo y convencimiento de que este día llegaría.

A Mis hermanos de Granada Zacarias, Manolo y Fede por el cariño y el apoyo que me han procesado desde la distancia o cuando he estado bajo su techo.

A Sergio, Elena, Laura (Makina), Pepa…….., no menos importantes que los que preceden. Por hacerme sentir, en estos pocos años, como uno mas del grupo, por preocuparos de que todo fuera bien y por estar ahí siempre que lo he necesitado.

A María Ortiz, Juan José y Juan Ramón por su apoyo en los momentos en que los ánimos no estaban muy arriba.

A Todas aquellas personas que no he nombrado aquí y que en mayor o menor medida han sido participes en la culminación de esta tesis.

I. INTRODUCCIÓN 29

Condición Física 32

Actividad Física 32

Síndrome Metabólico 33

Relación entre el síndrome metabólico y la condición física 34

Relación entre el síndrome metabólico y la actividad física 35

Estrés Oxidativo 36

Relación entre el estrés oxidativo y la condición física 37

Relación entre el estrés oxidativo y la actividad física 37

Inflamación 38

Relación entre la inflamación y la condición física 39

Relación entre la inflamación y la actividad física 39

II. HIPÓTESIS Y OBJETIVOS 41

Hipótesis 43

Objetivos 45

Aims 47

III. MATERIAL Y MÉTODOS 49

Participantes 51

Criterios de inclusión y de exclusión 51

Grupos de estudio 52

Reconocimiento físico 53

Historia clínica y exploración física 53

Determinaciones somatométricas 53

Determinaciones hemodinámicas 53

Valoración del nivel de condición física 54

Test de Course Navette 54

Abdominales en 30 segundos 55

Salto en largo sin impulso o salto horizontal 56

Dinamometría manual 57

Valoración del nivel de actividad física 58
Parámetros generales y metabólicos 58
Toma de muestras 58
Hematimetría y análisis de parámetros bioquímicos generales y metabólicos 58
Parámetros de inflamación 60
Parámetros de estrés oxidativo 61
Determinación de marcadores de estrés oxidativo 61
Determinación de biomarcadores antioxidantes 62
Actividad de las enzimas antioxidantes 62
Análisis estadístico 63

IV. RESULTADOS Y DISCUSIÓN 65

Non-traditional markers of metabolic risk in prepubertal children with different levels of cardiorespiratory fitness 67
Evaluation of metabolic risk in girls *vs* boys in relation with fitness and physical activity 77
Prepubertal children with suitable fitness and physical activity present reduced risk of oxidative stress 89
Profile of oxidant and antioxidant activity in prepubertal children related to age, gender, exercise and fitness 97
Plasma adipokines in prepubertal children with different levels of cardiorespiratory fitness and physical activity 125
Evaluation of solar exposure, intake and physical activity in relation with vitamin D serum status in Spanish prepubertal girls 155

V. CONCLUSIONES 163
VI. BIBLIOGRAFÍA 169

Figura 1. Esquema de la relación de la condición física y la actividad física con alteraciones bioquímicas en la infancia. 31

Figura 2. Alteraciones vasculares producidas en el síndrome metabólico y la diabetes tipo 2 34

Figura 3. Esquema del mecanismo de acción de moléculas con acción oxidante y antioxidante..... 37

Figura 4. Actuación de las adipoquinas sobre diferentes órganos. 38

Figura 5. Representación gráfica de la prueba de Course Navette. 55

Figura 6. Representación gráfica del test de abdominales 56

Figura 7. Representación del salto horizontal. 57

Figura 8. Representación de la técnica de dinamometría manual. 57

Figura 9. Representación gráfica de los autoanalizadores utilizados 59

Figura 10. Esquema de la tecnología Luminex® X MAP™ 60

AF	Actividad física
ALT	Alanina aminotransferasa
Apo-A1	Apolipoproteinas A1
Apo-B	Apolipoproteinas B
AST	Aspartato aminotransferasa
CA	Capacidad aeróbica
CF	Condición física
CV	Cardiovascular
CCV	Capacidad CV
ECV	Enfermedad cardiovascular
EO	Estrés oxidativo
FC	Frecuencia cardiaca
GPx	Glutatión peroxidasa
GSH	Glutatión reducido
GSSG	Glutatión oxidado
GT	Glutatión total
HDL-c	Colesterol de alta intensidad
HF	Alta condición física
HGF	Factor de crecimiento hepatocitario
HOMA-IR	Índice de resistencia a la insulina
IFG	Índice de fuerza general
IL-1alpha	Interleuquina-1alpha
IL-1RA	Interleuquina-1RA
IL-4	Interleuquina-4
IL-6	Interleuquina-6
IL-8	Interleuquina-8
IL-17	Interleuquina-17
IL-12p70	Interleuquina-12p70
IMC	Índice de masa corporal
LDL-c	Colesterol de baja densidad

LF	Baja condición física
LPL	Lipoproteina lipasa
MCP-1	Factor quimioatractivo de macrófagos
MIP-1alpha	Proteína inflamatoria de macrófagos 1alpha
MIP-1beta	Proteína inflamatoria de macrófagos 1beta
NGF	Factor de crecimiento neuronal
NO	Óxido nítrico
NOx	Nitritos totales
NPAF	Grupo sedentario
PAF	Grupo de práctica de actividad física
PAI 1	Inhibidor del activador del plasminógeno-1
PC	Perímetro de cintura
PCR	Proteína C-reactiva
ROS	Especies reactivas de oxígeno
SM	Síndrome metabólico
SOD	Superóxido dismutasa
TAD	Tensión arterial diastólica
TAG	Trigliceridos
TAS	Tensión arterial sistólica
TNF-α	Factor de necrosis tumoral alfa
VO_2 máx.	**C**onsumo máximo de oxigeno
4-HAD	4-hydroxyalkenals
20-MSRT	20m shuttle run test "*Course Navette"*

Actualmente, aún no hay suficiente información sobre el efecto de la condición física y el ejercicio en la infancia, y su impacto en la edad adulta. Existen datos contradictorios en la literatura acerca de la influencia del género, la condición cardiorrespiratoria y la actividad física sobre los cambios metabólicos, de estrés oxidativo e inflamatorios. De hecho, aún no se han identificado biomarcadores específicos en relación con una mejor o peor condición física, que condicionen un papel en el desarrollo o la prevención de las enfermedades pediátricas.

El objetivo general de esta tesis ha sido evaluar el impacto de la condición física y la actividad física sobre determinados biomarcadores tradicionales y no tradicionales de riesgo metabólico, de estrés oxidativo e inflamatorios, en niños prepúberes sanos.

El estudio se llevó a cabo en centros de educación primaria de Córdoba, España. Se seleccionaron 141 niños sanos (88 niños y 53 niñas) de 7-12 años, en estadio puberal de Tanner I. Se clasificaron en dos grupos en función de su nivel de condición física tras realizar el test de 20m "shuttle run test": un grupo de baja condición física (LF) y otro grupo con alta condición física (HF). Por otro lado, se dividieron en dos grupos en relación al ejercicio que realizaban en un programa de actividades extraescolares, o si eran sedentarios, tras realizar un cuestionario adaptado para obtener información sobre la práctica de actividad física. Paralelamente se midieron parámetros antropométricos, de presión arterial, y se analizaron biomarcadores en sangre de riesgo metabólico clásicos y no tradicionales (incluidas adipoquinas) y marcadores de estrés oxidativo.

Los principales resultados fueron:

- El grupo con baja condición física, con respecto al HF, presentó: a) mayores niveles de triglicéridos, glucosa, insulina e índice de resistencia a la insulina HOMA; b) menor producción de HDL-colesterol y apolipoproteinas A; c) similar producción de oxidantes y menor producción de antioxidantes; d) menor producción de leptina y mayor de interleuquina 6. No hubo diferencia entre grupos en el resto de biomarcadores medidos.

- El grupo que no practicaba actividad física con respecto al que si lo hacía presentó: e) similar producción de oxidantes y menor de antioxidantes; f) mayores niveles de factor quimioatractivo de macrófagos (MCP1) y valores similares en el resto de biomarcadores de inflamación.

- Al comparar por género, el grupo de niñas con baja condición y práctica física presentó: g) mayor nivel de glucosa, colesterol total y de LDL colesterol que los niños.

- El grupo de niñas presentó mayor estrés oxidativo que los niños.

Los resultados mostraron que una adecuada condición física apoyada por una práctica regular de actividad física realizada durante la infancia podría ayudar a evitar el desarrollo de patologías en la adolescencia y/o en la edad adulta como el síndrome metabólico y mayor estrés oxidativo, favoreciendo también un equilibrio más saludable en la producción de adipocitoquinas.

Currently, there is not yet enough information about the effect of fitness and exercise in childhood, and its impact in adulthood. There are conflicting data in the literature regarding the influence of gender, cardiorespiratory fitness and physical activity on the metabolic changes, oxidative stress and inflammation. In fact, specific biomarkers in relation with a good or a poor physical condition have not been identified yet. The identification of some biomarkers could condition the development or prevention of pediatric diseases.

The aim of this Thesis was to assess the impact of fitness status and physical activity on classical and non-classical plasma metabolic risk biomarkers, oxidative stress and inflammatory parameters in healthy prepubertal children.

The study was conducted in primary schools in Cordoba, Spain. We selected 141 healthy children (88 boys and 53 girls) aged 7-12 years, at Tanner I pubertal stage. They were classified into two groups according to their level of fitness after doing the 20m shuttle run test: low fitness (LF) and high fitness (HF) groups. In addition, these children were divided into two groups after an evaluation about physical practice or sedentary activities. For this evaluation, and a questionnaire was adapted for information on physical activity or sedentary situation and the exercise practice in a school program. Anthropometric parameters and blood pressure were measured. Blood classical and nontraditional metabolic risk biomarkers, adipocitokines and oxidative stress markers were analyzed.

The main results were:

- The group with low fitness, with respect to the HF group, presented: a) higher levels of triglycerides, glucose, insulin and insulin resistance index HOMA, b) lower production of HDL-cholesterol and apolipoproteins A, c) similar oxidant production and lower production of antioxidants; d) lower production of leptin and higher of interleukin-6. There were no differences in the other biomarkers measured, between thes groups.
- The group that did not practice physical activity with respect to practice it showed: e) similar production of oxidants and lower of antioxidants, f) higher levels of macrophage chemoattractant factor (MCP1) and similar values in the rest of biomarkers.
- Comparing by gender, the group of girls with low fitness and no practice of physical activity presented: g) higher levels of glucose, total cholesterol and LDL-cholesterol than boys.
- The group of girls showed higher oxidative stress than boys.

Results showed that an adequate physical condition supported by regular practice of physical activity during childhood could help to prevent the development of diseases in adolescence and/or in adulthood as metabolic syndrome and higher oxidative stress, promoting a healthy balance in the production of adipocytokines.

PRODUCCION CIENTIFICA

ARTICULOS

- **Llorente-Cantarero FJ**, Pérez-Navero JL, de Dios Benítez-Sillero J, Muñoz-Villanueva MC, Guillén-Del Castillo M, Gil-Campos M. Non-traditional markers of metabolic risk in prepubertal children with different levels of cardiorespiratory fitness. Public Health Nutr. 2012; 16:1-8. Factor de Impacto 2011: 2.169.

- **Llorente-Cantarero FJ**, Pérez-Navero JL, de Dios Benítez-Sillero J, Muñoz-Villanueva MC, Gil-Campos M. Evaluation of Metabolic Risk in Prepubertal Girls Versus Boys in Relation to Fitness and Physical Activity. Gender Medicine. 2012; VOL. 9, NO. 6. Factor de Impacto 2011: 2.101.

- **Llorente-Cantarero FJ**, Gil-Campos M, de Dios Benítez-Sillero J, Muñoz-Villanueva MC, Túnez I, Pérez-Navero JL. Prepubertal children with suitable fitness and physical activity present reduced risk of oxidative stress. Free Radic Biol Med. 2012; 53:415-20. Factor de Impacto 2011: 5.423.

- **Llorente-Cantarero FJ**, Gil-Campos M, de Dios Benítez-Sillero J, Muñoz-Villanueva MC, Tasset I, Pérez-Navero JL. Profile of oxidant and antioxidant activity in prepubertal children related to age, gender, exercise and fitness. Applied Physiology, Nutrition, and Metabolism. 2012; VOL. XX, NO. X. Factor de Impacto 2011: 2.131.

- **Llorente-Cantarero FJ**, Gil-Campos M, de Dios Benítez-Sillero J, Olza J, Muñoz-Villanueva MC, Aguilera CM, Pérez-Navero JL. Plasma adipokines in prepubertal children with different levels of cardiorespiratory fitness and physical activity.

- Ramirez-Prada D, de la Torre Aguilar MJ, **Llorente-Cantarero FJ**, Pérez-Navero JL, Gil-Campos M. Evaluation of solar exposure, intake and physical activity in relation with vitamin D serum status in spanish prepubertal girls. Nutr Hosp. 2012; vol 27, no. 6. Factor de Impacto 2011: 1.120.

COMUNICACIONES PRESENTADAS A CONGRESOS

Internacional

- **Llorente-Cantarero FJ**, Gil-Campos M, Muñoz-Villanueva MC, Pérez-Navero JL. Prepubertal children with suitable fitness and physical activity present reduced risk of oxidative stress. Sport Science in the heart of Europe. 17th annual Congress of the ECSS, 4-7 July 2012; Bruges–Belgium.

Nacional

1. Pérez-Navero JL, **Llorente-Cantarero FJ**, Benítez Sillero JD, Mata Rodríguez C, Gil Campos M. Biomarcadores de resistencia a la insulina en niños prepuberales sanos en relación con su condición física. XVIII Congreso de las Sociedades de Pediatría de Andalucía Oriental, Occidental y Extremadura. Granada. 2010.

2. Pérez-Navero JL, **Llorente-Cantarero FJ**, Benítez Sillero JD, Mata Rodríguez C, Gil-Campos M. Riesgo metabólico en preadolescentes sanos en relación con su condición física. XVIII Congreso de las Sociedades de Pediatría de Andalucía Oriental, Occidental y Extremadura. Granada. 2010.

3. **Llorente-Cantarero FJ**, Pérez-Navero JL, Benítez-Sillero JD, Muñoz-Villanueva MC, Gil-Campos M. Efectos del nivel de condición física y de la práctica de actividad física sobre el riesgo de dislipidemia en niños prepuberales. 60 Congreso de la Asociación Española de Pediatría. Valladolid. 2011.

4. **Llorente-Cantarero FJ**, Gil-Campos M, Benítez-Sillero JD, Muñoz-Villanueva MC, Pérez-Navero JL. Relación entre la condición física y biomarcadores no-tradicionales de riesgo metabólico en niños. 60 Congreso de la Asociación Española de Pediatría. Valladolid. 2011.

5. **Llorente-Cantarero FJ**, Pérez-Navero JL, Benítez-Sillero JD; Muñoz-Villanueva MC, Gil-Campos M. Importancia de la práctica de actividad en las niñas para prevenir el riesgo metabólico. Vox Pediátrica. 2011; XVIII: 85- 86.

6. **Llorente-Cantarero FJ**, Pérez-Navero JL, Benítez-Sillero JD, Muñoz-Villanueva MC, Gil-Campos M. Riesgo metabólico en relación con diferente sexo en niños prepúberes dependiente de la condición física. Vox Pediátrica. 2011; XVIII: 86.

7. **Llorente-Cantarero FJ**, Muñoz-Villanueva MC, Gil-Campos M, Pérez-Navero JL. Suitable fitness and physical activity in prepubertal children reduced risk of oxidative stress. III Jornadas de Jóvenes Investigadores del IMIBIC. Córdoba, 16 de abril 2012.

8. **Llorente-Cantarero FJ**, Gil-Campos M, Muñoz-Villanueva MC, Pérez-Navero JL. Una adecuada condición física o mayor práctica de actividad física condicionan un menor estrés oxidativo en el niño prepúber. 61º Congreso Nacional Asociación Española de Pediatría, Granada, mayo 2012.

9. **Llorente-Cantarero FJ**, Pérez-Navero JL, Muñoz-Villanueva MC, Olza Meneses J, M. Aguilera C, Gil-Campos M. Relación entre condición física y actividad física con niveles plasmáticos de adipocitoquinas en niños prepúberes sanos. 61º Congreso Nacional Asociación Española de Pediatría, Granada, mayo 2012.

BECAS, AYUDAS, ESTANCIAS Y PREMIOS RECIBIDOS

- I Convocatoria "Estancias Cortas Investigadores Emergentes". Concedida por el Instituto Maimónides de Investigación Biomédica (IMIBIC), diciembre 2011.

- Ayuda para la realización de estancias para la obtención de la Mención Internacional en el título de Doctor. Concedida el 19/5/2011 por la UCO.

- Estancia en: Center for Healthy Aging. Department of Biomedical Sciences, University of Copenhagen (Denmark). Del 1/08/2011 al 1/11/2011.

- Estancia en: Grupo GENUD (Growth, exercise, nutrition and development), Universidad de Zaragoza, España. Del 6/02/2012 al 12/02/2012.

- Premio a la mejor Comunicación Libre.60 Congreso de la Asociación Española de Pediatría. Valladolid. 18 de Junio de 2011.

INTRODUCCION

I

La sociedad actual, condicionada en gran medida por el estrés, está sufriendo una transformación metabólica tras el abandono de dietas saludables, como la dieta mediterránea, así como tras la reducción de otros hábitos como la actividad física (AF) (*Pate et al., 2009*), y aumento del sedentarismo. Desde edades tempranas, el sedentarismo parece estar relacionado con el desarrollo de determinados factores de riesgo de enfermedad cardiovascular (ECV) (*Martínez-Gómez et al., 2011*). El nivel actual de práctica de AF es cada vez menor, siendo especialmente relevante en la población escolar. La presencia de estos factores y hábitos de riesgo ya durante la niñez y la adolescencia incrementa de forma notable la probabilidad de desarrollar determinadas patologías en la vida adulta. Estas patologías incluyen: obesidad, diabetes tipo 2, dislipidemia, aterosclerosis, trastornos del comportamiento alimentario, osteoporosis, y ciertos tipos de cáncer (*González-Gross et al., 2003*). De hecho, aunque las manifestaciones clínicas indicativas de estas enfermedades suelen aparecer en la edad adulta, se sabe que en gran parte de ellas, su patogenia se establece en la infancia o en la etapa de la adolescencia (*Warnberg et al., 2004*).

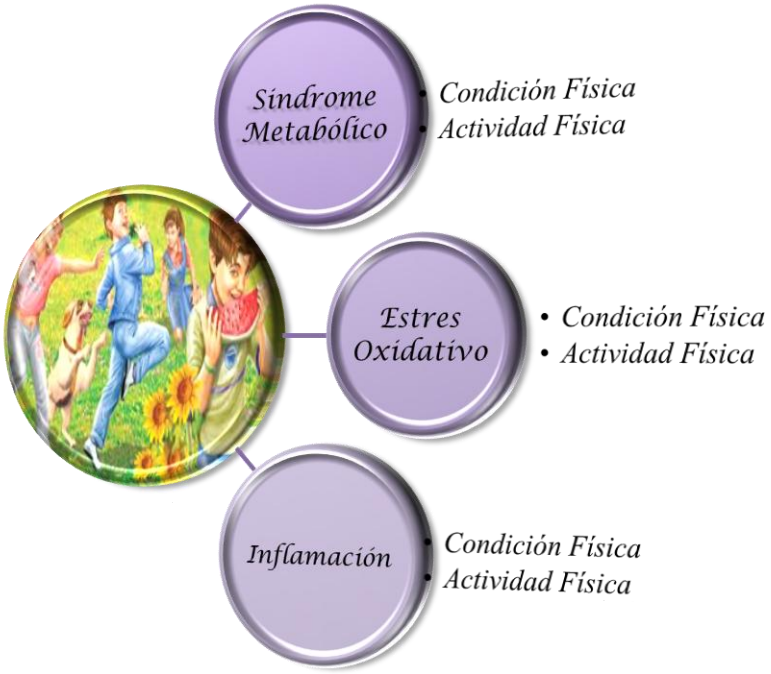


Figura 1. Esquema de la interacción entre la condición física y actividad física y el desarrollo de Síndrome Metabólico, Estrés Oxidativo e Inflamación.

CONDICION FISICA

La condición física (CF) se define como la capacidad para hacer ejercicio, entendida como una medida integrada de las funciones y estructuras que intervienen en la realización de ésta: muscular esquelética, cardiorrespiratoria, hemato-circulatoria, psiconeurológica y endocrino-metabólica (*Moliner-Urdiales et al., 2010*). La capacidad cardiovascular (CCV) es un marcador directo del estado fisiológico y refleja la capacidad general de los aparatos cardiovascular (CV) y respiratorio. En la actualidad, un índice bajo de CF se considera un fuerte predictor de riesgo de ECV no sólo en sujetos con sobrepeso u obesidad, sino también en sujetos con normopeso (*Naylor et al., 2009; Ortega et al., 2011*).

En importantes estudios longitudinales se ha constatado que el nivel de CF que se posee en la vida adulta, así como la presencia de otros factores de riesgo CV ya conocidos (hipercolesterolemia, hipertensión arterial y otros), están condicionados por el nivel de forma física que se tiene en la infancia o la adolescencia, especialmente por la capacidad aeróbica (CA) y la fuerza muscular (*Twisk et al., 2002*). Además, la realización de forma habitual de ejercicio prolongado está inversamente relacionada con la aparición de factores de riesgo CV (*Ortega et al., 2007*). En consecuencia, para valorar el riesgo de enfermedad futura derivada del sedentarismo de la forma más precoz posible, debe comenzarse necesariamente estudiando a la población infantil y adolescente (*Ortega et al., 2005*).

ACTIVIDAD FISICA

La AF regular es considerada una de las estrategias más eficaces para prevenir las principales causas de morbimortalidad en los países occidentales. Así, en EEUU se ha considerado el aumentar la práctica de la AF como el primero de sus objetivos en el año 2010. Ello está motivado por los importantes riesgos que conlleva la falta de AF para la salud individual y social (*Naylor et al., 2009*). En adultos, el mayor grado de AF se ha relacionado de forma positiva con la salud CV, obteniéndose algunos resultados similares en niños y adolescentes (*Kelishadi et al., 2008; Ruiz et al., 2006*). La relación aparentemente obvia entre la CCV y la AF requiere aún más estudios, principalmente

debido a la complejidad implícita en la valoración de la AF. No obstante, cuando se utilizan mediciones objetivas de ésta, se observa que existe una relación directa entre la AF y la CCV (*Ruiz et al., 2006*). La práctica de ejercicio físico es uno de los requisitos fundamentales para lograr un perfil lipídico-metabólico saludable, suponiendo que se consigue una mejora de la CF, y por tanto, de la salud (*García- Artero et al., 2007*).

Hay que considerar que en algunos estudios en adolescentes, la CF se asocia en mayor medida a factores de riesgo CV que el grado de AF (*García- Artero et al., 2007*) aunque no existe unanimidad respecto a qué variables son más influyentes. Es interesante constatar que no se han realizado estudios en los que la variable AF sea evaluada de forma independiente, ya que los trabajos realizados hasta el momento han considerado sólo diferentes niveles de CF. Se ha indicado que los niños que practican AF tienen niveles superiores de CF que los que no practican (*Ruiz et al., 2006*). Sin embargo, se pueden encontrar niños con un alto nivel de CF y que no realicen AF habitualmente, o por el contrario, niños que practiquen AF pero que tengan una baja CF. Por todo ello, continúa sin conocerse con certeza si en los adolescentes, y sobre todo en la infancia, se cumplen las recomendaciones actuales de AF en frecuencia e intensidad, para lograr una CCV saludable.

SINDROME METABOLICO

El síndrome metabólico (SM) se podría definir como la existencia de un grupo de factores de riesgo CV impulsado por la resistencia a la insulina periférica. Los datos de estudios recientes realizados sobre una población de niños y adolescentes indican que la prevalencia de SM varía entre 3% y 12% (*Tailor et al., 2010*).

Las sugerencias que se están estableciendo para futuras investigaciones, son determinar qué componentes individuales que condicionan la aparición del SM confieren el mayor riesgo en una futura morbilidad en los niños. No hay una definición universalmente aceptada de SM en la infancia (*Weiss et al., 2010*). Sin embargo, un consenso de la Federación Internacional de Diabetes define el SM en niños y adolescentes como la presencia de obesidad abdominal más dos de los parámetros clásicos siguientes: (I) aumento de los niveles de triglicéridos (TAG), (II) reducción en

los niveles de colesterol de alta densidad (HDL-c), (III) hipertensión arterial o (IV) niveles elevados de glucosa en ayunas (*Zimmet et al., 2007*).

Por otra parte, para algunos autores la alteración de ciertos factores no clásicos, tales como la elevación en plasma del ácido úrico, o la disminución de la adiponectina (*Gil-Campos et al., 2011*), se han asociado con características de resistencia a la insulina, de hecho, se ha sugerido que el ácido úrico puede ser un vínculo clave para el diagnóstico del SM en niños obesos prepúberes (*Gil-Campos et al., 2009*).

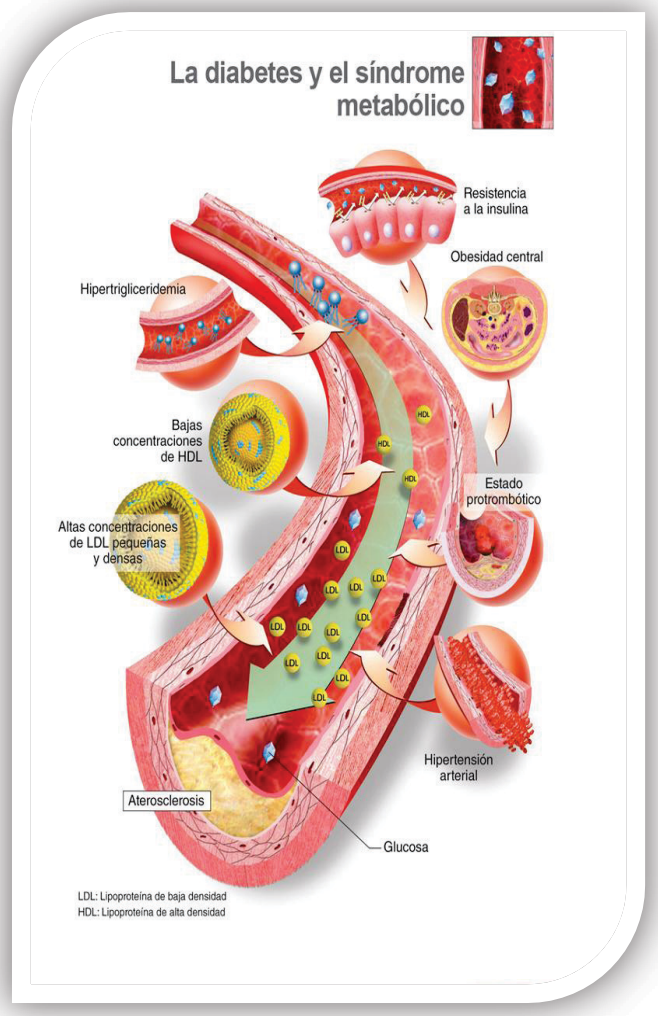


Figura 2. Alteraciones vasculares producidas en el síndrome metabólico y la diabetes tipo 2.

Además, también se han visto alterados en los niños con SM, incluso prepúberes, el índice de resistencia a la insulina (HOMA-IR), los niveles de colesterol total, colesterol de baja densidad (LDL-c), apolipoproteinas A1 (apo-A1) y apolipoproteinas B (apo-B) (*Gil-Campos et al., 2009; Bueno et al., 2006; Sellers et al., 2009*), transaminasas (*Bouglé et al., 2010*) y la proteína C-reactiva (PCR) (*Mauras et al., 2010*). Todos estos factores de estudio se han incluido dentro de los denominados "no tradicionales" de SM (*Gil-Campos et al., 2011*).

Relación entre el Síndrome Metabólico y la Condición Física

Respecto a los beneficios asociados a la CF, se ha demostrado una relación entre la capacidad aeróbica (CA) y determinados factores de riesgo CV en adolescentes (*Ruiz et al., 2006; Mesa et al., 2006*). Una alta CA (VO_2 máx. > 51,6 ml/kg/min) se asocia con

un menor índice lipídico-metabólico de riesgo CV, independientemente del grado de AF y fuerza muscular. Además, aquéllos sujetos que poseen un alto grado de fuerza muscular (tercil 3) presentan un perfil lipídico-metabólico más saludable que los que tienen un bajo nivel (tercil 1), independientemente de su CA (*García- Artero et al., 2007*). El análisis conjunto de CA y fuerza muscular muestra que una alta CA se corresponde con un riesgo lipídico-metabólico bajo, sea cual sea el grado de fuerza. De forma similar, un grado de fuerza muscular alto se corresponde con un riesgo lipídico-metabólico bajo a cualquier grado de CA. Sin embargo, una baja CA se corresponde con un alto índice lipídico-metabólico, excepto cuando el grado de fuerza es alto, y a su vez, un bajo grado de fuerza muscular se corresponde con un alto índice lipídico-metabólico, excepto cuando la CA es alta (*García-Artero et al., 2007*).

Relación entre el Síndrome Metabólico y la Actividad Física

Entre los beneficios para la salud que se derivan de la práctica regular de AF destacan la mejora del perfil lipídico con aumento de las concentraciones séricas de colesterol HDL-c y la reducción de los TAG. Uno de los mecanismos para la reducción de los TAG es el aumento de la actividad de la lipoproteína lipasa (LPL), inducida por la AF. En adultos sanos sometidos a ejercicio físico se ha observado una reducción de la carga aterogénica reduciendo los niveles de las apo B o del índice apoB/ApoA-I, aunque no se reduzcan los niveles de LDL-c. Diferentes estudios muestran también que el ejercicio físico promueve la mejora del metabolismo de la glucosa mediado por insulina. Después de un ejercicio crónico o agudo, la absorción de glucosa en el músculo es mayor, ya que los receptores son más sensibles a la acción de la insulina, lo que facilita la resíntesis de glucógeno. En niños sanos, el nivel de AF se ha relacionado inversamente con el índice HOMA-IR (*Platat et al., 2006*). Sin embargo, aún es desconocido el papel de la AF sobre otros biomarcadores metabólicos no evaluados tradicionalmente.

Por otra parte, todavía no está claro hasta qué punto las relaciones entre la AF, la CF y los factores de riesgo metabólico están influenciados por el género. Varios estudios han sugerido que el impacto de una elevada masa grasa, una baja CF y una baja AF sobre cambios en los niveles de insulina es mayor en los niños; mientras que en las niñas, los cambios en los niveles de insulina se asocian sólo con cambios en la masa

grasa (*Imperatore et al., 2002; Gutin et al., 2005*). Otros autores, sin embargo, han informado de que la resistencia a la insulina varía con la CF en ambos sexos (*Steele et al., 2008*).

ESTRES OXIDATIVO

La sobreproducción de radicales libres de oxígeno pueden dañar moléculas esenciales tales como ácidos nucleicos, proteínas, lípidos e hidratos de carbono, en un proceso llamado estrés oxidativo (EO) (*Isik et al., 2007; Mamiya et al., 2008; Antoncic-Svetina et al., 2010*). Para evitar la interacción de especies reactivas de oxígeno (ROS) con macromoléculas, las células han desarrollado diversos mecanismos de desintoxicación, así como un sistema de defensa antioxidante (*Urso et al., 2003*). El sistema de defensa antioxidante se puede subdividir en antioxidantes enzimáticos, tales como: la glutatión peroxidasa (GPx), glutatión reductasa y glutatión S-transferasa, y cascadas enzimáticas incluyendo la superóxido dismutasa (SOD) y la catalasa; y por otro lado, antioxidantes no enzimáticos tales como ácido úrico o glutatión (*Rietjens et al., 2007; Ji et al., 2008*).

Los efectos de la oxidación se pueden predecir a través de marcadores biológicos de EO, que puede servir de base para el diseño de intervenciones apropiadas para prevenir o aliviar el daño oxidativo (*Fisher-Wellman y Bloomer, 2009*). La detección de más de un biomarcador de EO es crucial, ya que la de uno solamente, puede inducir a resultados engañosos (*Tsukahara 2007*).

Los niveles de ciertos marcadores de EO, pueden ser predictores de ECV, y aunque éstas se presenten en la edad adulta, parece que dichos factores de riesgo pueden aparecer durante la infancia (*Roberts et al., 2007*). Tsukahara (2007) indica que en condiciones fisiológicas normales, las personas más jóvenes (especialmente niños) tienen más probabilidades de estar expuestos a mayores concentraciones de ROS y nitritos totales (NOx), como marcador del óxido nítrico (NO), que las personas mayores. Por otra parte, los autores han asociado la patogenia y evolución de numerosas enfermedades a esta edad con ROS inducidas por el daño oxidativo (*Casado et al., 2007*). Además, el uso de antioxidantes ha presentado nuevas perspectivas terapéuticas para las enfermedades que están relacionadas con mejorar el EO (*Tsukahara, 2007*). No obstante, los niveles de EO en una población sana con diferente CF y en edades diferentes no ha sido suficientemente investigado.

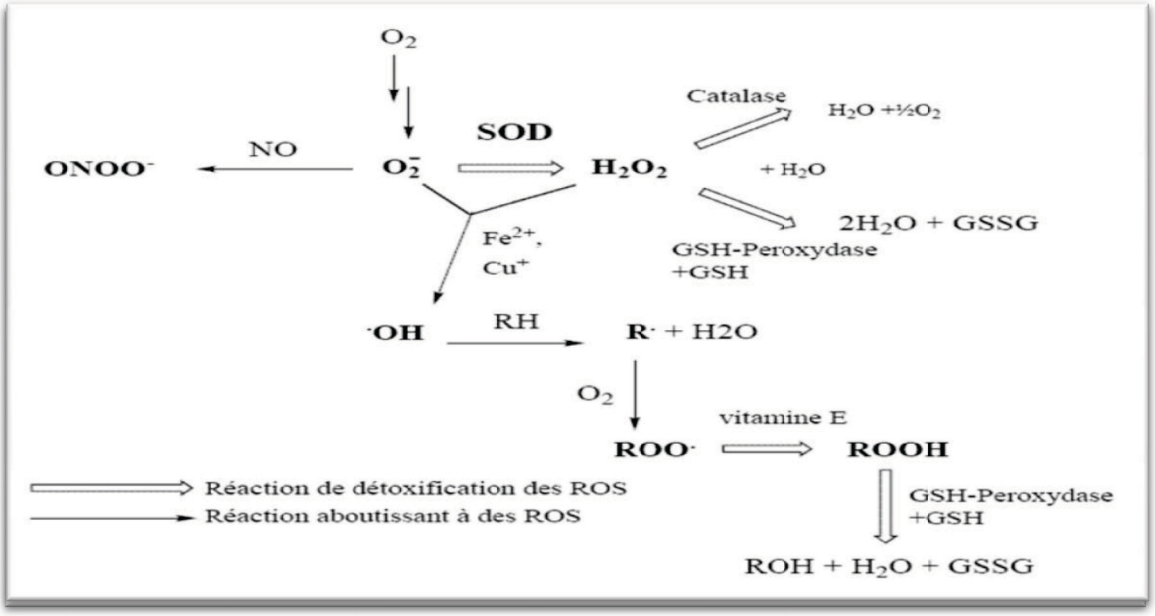


Figura 3. Esquema del mecanismo de acción de moléculas con acción oxidante y antioxidante.

Relación entre el Estrés Oxidativo y la Condición Física

Parece que los niños y adolescentes que practican AF fuera del horario escolar, o los que se someten a un periodo de entrenamiento sin tener en cuenta su CF, presentan concentraciones menores de antioxidantes o elevaciones de ROS en reposo respecto a los que no practican (*Gougoura et al., 2007*). No obstante, existen estudios que presentan resultados contradictorios dependiendo de la variable estudiada y del tipo de AF practicada, en función del nivel de entrenamiento y de la intensidad (*Gonenc et al., 2000; Cavas et al., 2004*). Se han observado descensos en los niveles de determinados biomarcadores de EO tras periodos de entrenamiento (*Gonenc et al., 2000*). Sin embargo, en otros trabajos al estudiar éstos en relación a una AF aguda o de alta intensidad, se ha observado un aumento de EO.

Relación entre el Estrés Oxidativo y la Actividad Física

Aunque la AF puede tener efectos beneficiosos sobre la salud, algunos estudios han divulgado que el ejercicio físico induce tensión oxidativa, aumentando la generación de ROS (*Bloomer et al., 2009*). El ejercicio aerobio causa un aumento del consumo de oxígeno y puede dar lugar a niveles elevados de radicales libres. Se ha demostrado que durante el ejercicio aerobio, el consumo de oxígeno de todo el organismo aumenta de 10 a 20 veces más en relación al estado de reposo (*Konig et al., 2001*). Esta situación lleva a un mayor flujo de electrones a través de la cadena de

transporte de electrones mitocondrial durante la actividad muscular y por tanto, a una mayor producción de ROS. Ello puede inducir a que durante el ejercicio extenuante aeróbico se observen alteraciones en los mecanismos de defensa antioxidante (*Chevion et al., 2003*).

INFLAMACIÓN

Actualmente, es conocida la función endocrino-metabólica del tejido adiposo. Entre otras hormonas, los adipocitos secretan un grupo diverso de proteínas que se denominan adipoquinas, que participan en diferentes funciones biológicas (*Koerner et al. 2005*).

Las citoquinas proinflamatorias tales como la interleuquina-6 (IL-6), factor de necrosis tumoral alfa (TNF-α), y otras como la PCR, elevadas en plasma, juegan un papel en el desarrollo y la aparición de complicaciones de la ECV, la diabetes tipo 2 o la obesidad (*Kiecolt-Glaser et al., 2002; Raison et al., 2006; Dandona et al., 2004*). Además, varias adipoquinas, como la leptina y adiponectina, intervienen en la regulación del metabolismo hidrocarbonado, lipídico y la homeostasis energética (*Körner et al. 2007*). Concentraciones elevadas de leptina y bajas de adiponectina parecen correlacionarse con otros componentes del SM (*Valle et al., 2005; Gil-Campos et al., 2011*), aunque todavía no es bien conocido el papel de las adipoquinas en la obesidad y el desarrollo del SM en los niños. Sin embargo, los factores subyacentes a estas enfermedades podrían originarse ya durante etapas precoces de la vida.

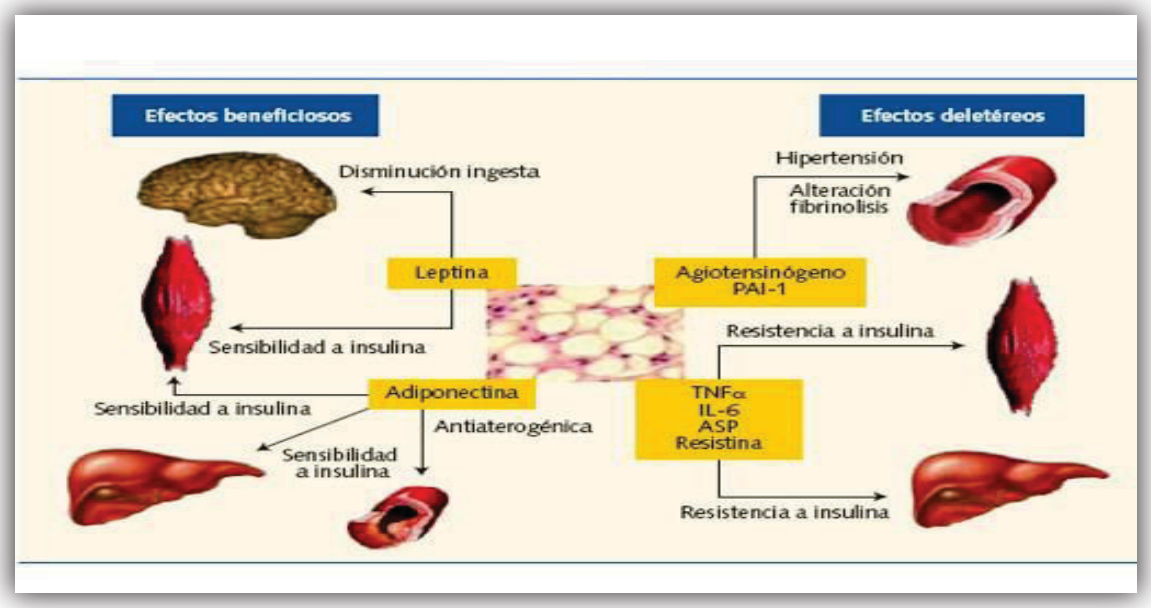


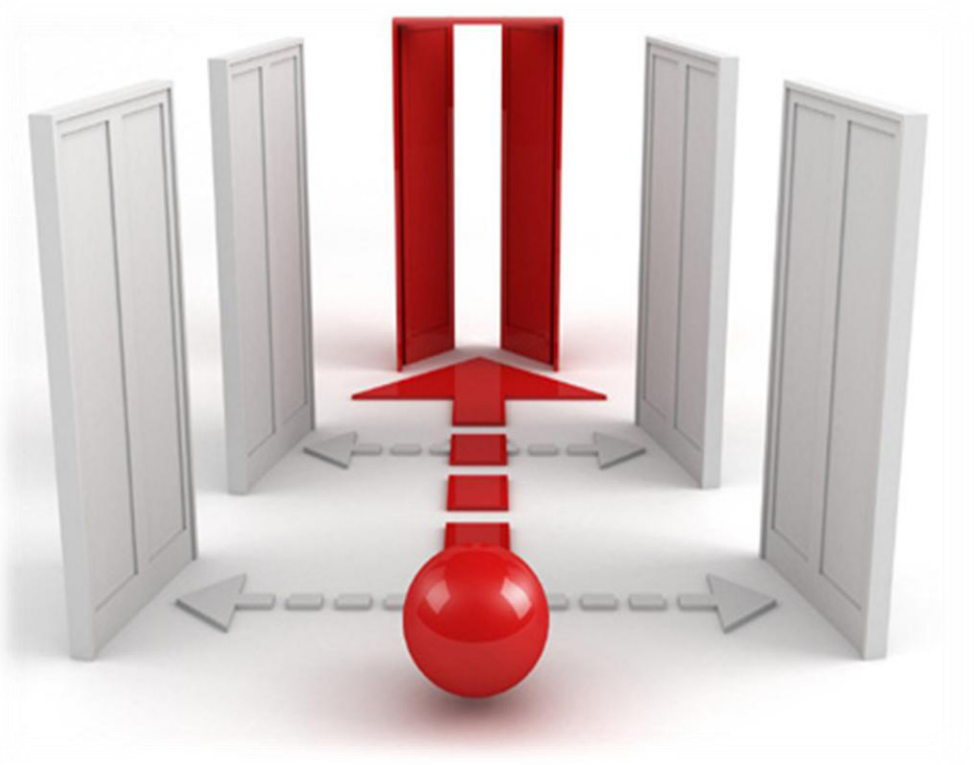
Figura 4. Actuación de las adipoquinas sobre diferentes órganos.

Relación entre Inflamación y la Condición Física

En la infancia, existen escasos estudios que relacionan el nivel de CF con marcadores inflamatorios, destacando algunos realizados en niños con patologías (*Tirakitsoontorn et al., 2001*) u obesos sometidos a programas de entrenamiento (*Barbeau et al., 2003*). En niños y adolescentes sanos, parece que niveles altos de AF y de CF se relacionan con mayores niveles plasmáticos de adiponectina, y menores valores de resistina y de TNF-α, sin evidenciarse asociación con un aumento en otras citoquinas o con resistencia a la insulina (*Rubin et al., 2008*). Sacheck (2008) afirma que algunos de los marcadores inflamatorios actuales pueden no ser sensibles para determinar el riesgo inflamatorio en niños y adolescentes, siendo necesarios más estudios de los parámetros conocidos así como estudiar nuevos biomarcadores para que nos permitan aumentar el conocimiento en la relación entre los procesos inflamatorios, la AF y la CF en la infancia.

Relación entre el Inflamación y la Actividad Física

La escasa práctica de AF, asociada a otros factores como el cambio en los hábitos de alimentación, ha condicionado el aumento en la prevalencia de enfermedades inflamatorias como la obesidad en la infancia. En recientes estudios se indica que la AF está inversamente asociada con el grado de inflamación (*Platat et al., 2006*), y por tanto, puede ser una medida efectiva para prevenir la aparición de complicaciones como el SM y la ECV asociada (*Devaraj et al., 2004*). Recientemente, se ha descrito que mayores niveles de AF y de CF podrían modificar diversos parámetros de inflamación, como la PCR, independientemente de los niveles de adiposidad (*Sacheck, 2008*). La mayor parte de los estudios que analizan el efecto de la AF sobre mediadores inflamatorios en plasma como las interleuquinas IL-1α, IL-6, IL-17, IL-8, MIP-1α, el MIP-1β, IL-12p70, IL-1RA, IL-4, TNF-α, eotaxinas, y el MCP-1 lo hacen sobre el ejercicio agudo (*McMurray et al., 2007*). En sujetos obesos jóvenes se ha estudiado el efecto del ejercicio físico utilizando programas de entrenamiento, y analizandola IL-6 (*Barbeau et al., 2002*). Sin embargo, son escasos los estudios que analizan el estado inflamatorio y sus mecanismos en función de la práctica de AF en niños y adolescentes (*Platat et al., 2006*). De hecho, en niños sanos sólo se ha descrito una relación inversa entre la práctica regular de AF y los niveles de PCR (*Cook et al., 2000*) o de IL-6 (*Platat et al., 2006*).



HIPOTESIS y OBJETIVOS

II

Hipótesis

Los hábitos de vida actuales y el sedentarismo incrementan la probabilidad de desarrollar determinadas patologías en la vida adulta. Estas patologías incluyen: obesidad, diabetes tipo 2, dislipidemia, aterosclerosis o trastornos del comportamiento alimentario (*González-Gross et al., 2003*). De hecho, aunque las manifestaciones clínicas suelen aparecer en la edad adulta, en muchas ocasiones, su inicio patogénico e incluso la propia enfermedad se establece en la infancia o la adolescencia (*Warnberg et al., 2004*). El nivel actual de práctica de AF es cada vez menor, siendo especialmente relevante en la población escolar aunque aún no se han establecido claramente qué niños pueden ser de riesgo. La AF es uno de los requisitos fundamentales para lograr un perfil lipídico-metabólico saludable, suponiendo una mejora de la CF (*García-Artero et al., 2007*). No obstante, aún existen datos escasos sobre si los niños, en especial los prepúberes, cumplen las recomendaciones actuales de práctica de AF, en frecuencia e intensidad, para lograr un buen estado de salud. La realización de este trabajo de tesis doctoral puede permitir aclarar si existen determinados niveles de CF que puedan favorecer la aparición de factores de riesgo o enfermedades, o que protejan de éstos. Para ello, se evaluará la AF habitual en niños prepúberes y se objetivará la CF. Además se realizarán análisis en muestras de sangre de determinados parámetros de riesgo CV, inflamación y EO que aportarán una novedosa e importante información sobre el estado metabólico de niños aparentemente sanos en edad prepuberal. Además, estos nuevos datos podrían utilizarse para la programación de estrategias futuras en el control y la prevención de la obesidad infantil y EVC.

II

Objetivo General:

Evaluar el impacto de la condición física y la actividad física sobre los biomarcadores tradicionales y no tradicionales de riesgo metabólico, estrés oxidativo y procesos inflamatorios en niños prepúberes sanos.

Objetivos Específicos:

I. Evaluar biomarcadores tradicionales y no-tradicionales de riesgo metabólico en niños sanos prepúberes con diferentes niveles de condición física y práctica de actividad física.

II. Estudiar la influencia del género en los cambios metabólicos en relación con la condición física y la actividad física.

III. Determinar el impacto de la condición física y la actividad física sobre el estrés oxidativo en niños prepúberes sanos, medido a través de los marcadores biológicos seleccionados en sangre.

IV. Analizar marcadores oxidativos en sangre en prepúberes de ambos sexos, y analizar si estos biomarcadores están influenciados por la edad, el índice de masa corporal, la capacidad cardiorrespiratoria y la actividad física, y el sexo.

V. Analizar adipoquinas y biomarcadores inflamatorios plasmáticos en niños prepúberes sanos con diferentes niveles de condición física y actividad física.

II

General Aim:

To assess the impact of fitness status and physical activity on classical and non-classical metabolic risk biomarkers, oxidative stress and inflammation process in healthy prepubertal children.

Specific Aims:

I. To assess classical and non-classical metabolic risk biomarkers in prepubertal healthy children with different levels of cardiorespiratory fitness and physical activity.

II. To study the influence of gender in relation to cardiorespiratory fitness and physical activity on metabolic changes.

III. To determine the impact of fitness status and physical activity on oxidative stress in healthy prepubertal children, measured by selected biomarkers in blood.

IV. To analize oxidative markers in prepubertal of both genders, and to evaluate whether these biomarkers are influenced by age, body mass index, cardiorespiratory fitness and physical activity, and sex.

V. To assess some plasma adipokines and inflammatory biomarkers in prepubertal healthy children with different levels of cardiorespiratory fitness and physical activity.

MATERIAL y METODOS

III

PARTICIPANTES

Se seleccionaron inicialmente 450 niños españoles de dos escuelas primarias locales de Córdoba con opción a participar en el estudio. Del total de alumnos/as, finalmente accedieron a participar 156 niños/as. Algunos de ellos fueron excluidos en última instancia, ya fuera porque no cumplían los criterios de inclusión, decidieran no seguir en el estudio, o porque la extracción de muestras sanguíneas no pudo realizarse o fue insuficiente. Finalmente, el estudio se llevo a cabo con 141 niños (88niños, 53niñas).

Se obtuvo consentimiento informado, por escrito, de un padre/madre o tutor legal. Los procedimientos del estudio fueron explicados verbalmente a todos los niños, y expresaron su consentimiento para participar en éste. Se obtuvo aprobación del comité local de investigación y ética para la realización del estudio y se realizó de acuerdo con la Declaración de Helsinki.

Criterios de inclusión

Se incluyeron niños/as sanos de entre 7 y 12 años con la certeza de estar en etapa prepuberal (estadío puberal de Tanner I), y confirmado tras obtener niveles plasmáticos de hormonas sexuales.

Criterios de exclusión

Se descartaron los niños menores a 7 años o mayores a 12, con percentiles de medidas antropométricas en rango inferior o superior a 1DS, así como aquéllos que presentaran algún signo de desarrollo puberal clínico o analítico. También se excluyeron aquellos niños con patología conocida o detectada durante el estudio, o con largos periodos de reposo previos por inmovilización, así como aquéllos que tomaran fármacos que pudieran interferir en las medidas de los biomarcadores.

Grupos de estudio

Tras la selección de los sujetos se realizaron dos divisiones atendiendo a la CF o la práctica de AF. Así, se obtuvieron dos subgrupos en cada categoría:

- *Grupos según su Condición Física*

  Los niños/as fueron divididos en dos grupos en relación a la medida obtenida tras la realización del test de *Course Navette* (20-MSRT). Fueron divididos en dos grupos de acuerdo a la metodología utilizada en estudios anteriores (*García-Artero et al., 2007; McGavock et al., 2009; Benitez-Sillero et al., 2011.*):

  - Los niños/as que registraron una puntuación igual o superior al valor medio de referencia establecido por *Olds et al. (2006)* se asignaron al grupo designado como de "igual o superior condición física" (high fitness): **HF**.

  - Aquéllos que obtuvieron una puntuación menor a la media establecida por *Olds et al. (2006)* se asignaron al grupo denominado como de "baja condición física" (low fitness): **LF**.

- *Grupos según la Actividad Física*

  Tras la evaluación de los hábitos de ejercicio mediante un cuestionario validado, los niños/as fueron clasificados en dos grupos:

  - Un grupo de práctica de actividad física (physical activity): **PAF**.

  - Un grupo con escasa práctica de actividad física o sedentario (non-physical activity): **NPAF**.

RECONOCIMIENTO FISICO

Historia clínica y exploración física

La anamnesis y la evaluación del estado de salud de los niños fueron realizadas por pediatras del Servicio de Pediatría del Hospital Universitario Reina Sofía de Córdoba. Se preguntó por los antecedentes personales y familiares, así como la existencia de patología previa, largos periodos en reposo o tratamientos previos. También se evaluó el nivel socio-económico. Se realizó una exploración física exhaustiva por sistemas y aparatos evaluando también la etapa de desarrollo puberal según Tanner (1962), y escogiendo a aquellos sujetos que se encontraban con desarrollo genital y de vello pubiano en el estadio I. Además, se determinaron parámetros antropométricos y medidas de frecuencia cardiaca (FC) y tensión arterial.

Determinaciones somatométricas

En todos los sujetos se midieron, mediante técnicas estandarizadas, el peso, la talla y el perímetro de la cintura. Para ello se utilizó una balanza y un estadiómetro de precisión (SC700; Seca, Hamburgo, Alemania), en la que los participantes estaban vestidos con ropa ligera y descalzos. Estas medidas fueron tomadas el mismo día del reconocimiento médico.

El índice de masa corporal (IMC) fue calculado como el peso (kg) / [talla (m)]^2. Se realizaron dos medidas del perímetro de cintura (PC) con una cinta elástica siguiendo los métodos estandarizados. Estas mediciones antropométricas se compararon con los estándares de referencia españoles para IMC y PC respectivamente (*Sobradillo et al., 2004; Moreno et al., 2002*) y con los puntos de corte específicos por edad y sexo propuestos por Cole et al. (2000) para definir la obesidad, y asegurarnos de estar incluyendo a niños sin obesidad, mayoritariamente.

Determinaciones hemodinámicas

Se realizó la medición de la FC en latidos/minuto y de la tensión arterial sistólica y diastólica (TAS y TAD) en mmHg, con un monitor de signos vitales 8100T Critikon-Dinamap Vital (Dinamap V-100, GE Healthcare, España), según método estandarizado.

VALORACION DEL NIVEL DE CONDICION FISICA

En una primera sesión se valoró la CF mediante varias pruebas validadas: el test de 20-MSRT para evaluar la resistencia, y otros 3 tests para medir la fuerza muscular: a) test de salto en longitud sin impulso y con pies juntos para evaluar la fuerza explosiva del tren inferior; b) dinamometría manual, y c) test de abdominales, mediante la realización del máximo número de abdominales completas durante un periodo de 30" (*Guillén del Castillo et al., 2007*).

Todos estos tests están incluidos en la batería EUROFIT validada y estandarizada por el Consejo de Europa (1993). Cada una de estas variables será transformada dividiendo cada uno de los valores observados por el valor máximo de dicha variable. El promedio de las 3 variables transformadas se utiliza para establecer una única variable denominada índice de fuerza general (IFG), con valores comprendidos entre 0 y 1 (*García-Artero et al., 2007*).

A partir de los resultados obtenidos en las diferentes pruebas se obtuvo un nivel de CF de cada niño.

Test de Course Navette (20-MSRT)

- *Objetivo*

  Medir la capacidad aeróbica máxima de los niños mediante un test de campo indirecto e incremental descrito por *Leger et al. (1988)*.

- *Material*

  Pista polideportiva plana, con dos líneas paralelas separadas entre sí 20 metros, señaladas con una cinta adhesiva y medida con una cinta métrica. El ritmo es marcado por los zumbidos emitidos desde una pista de audio con el protocolo de la prueba grabada en un CD-Rom el cual es reproducido por un radio-CD.

- *Desarrollo*

  El desarrollo de la prueba comienza cuando los sujetos se colocan detrás de la línea, a un metro de distancia unos de otros. Al oír la señal de partida comienzan a desplazarse hasta la línea opuesta y deben sobrepasarla. Allí esperan a oír la señal siguiente para volver a la línea inicial. Deben intentar seguir el ritmo de las señales acústicas. Al finalizar cada estadío de un minuto (palier), aumenta el

ritmo de desplazamiento (velocidad) desde 8,5 km/h en el primer palier a razón de 0,5 km/h progresivamente en cada palier sucesivo.

- Valoración de la prueba

  Cada sujeto repite estos desplazamientos constantemente hasta que no pueda llegar a sobrepasar la línea en el momento en que suene la señal. Entonces se retira de la prueba y se registra el último palier que haya completado en su totalidad. Cada palier representa un minuto. Para estratificar los niveles de CF en este test, se utiliza la escala desarrollada y validada por *Olds et al. (2006)* para niños según edad y sexo de todos los

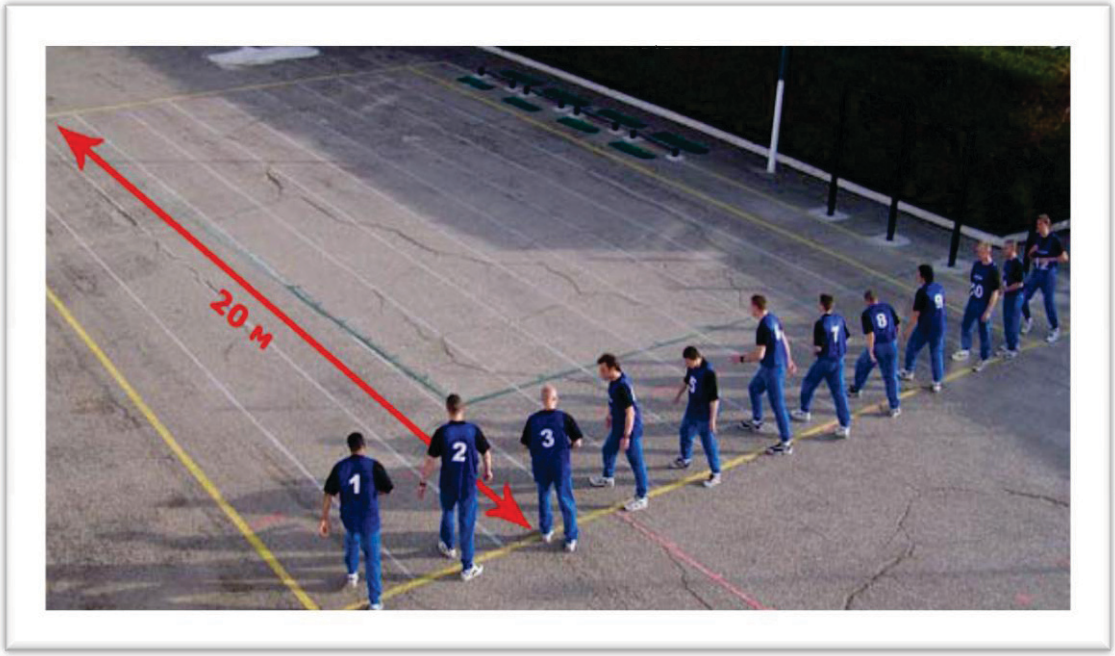


Figura 5. Representación gráfica de la prueba de Course Navette.

Abdominales en 30 segundos

- *Objetivo*

  Medir la fuerza resistencia de los músculos abdominales.

- *Material*

  Cronómetro digital con décimas de segundo.

- *Descripción*

El ejecutante, se coloca decúbito dorsal con las piernas flexionadas a 90°, los pies ligeramente separados, ubicando las manos entrelazadas detrás de la nuca. El ayudante le sostiene los pies y cuenta las repeticiones.

- *Desarrollo*

  A la señal debe intentar realizar el mayor número de ciclos de flexión y extensión de la cadera, tocando con los codos las rodillas en la flexión y el suelo con la espalda en la extensión.

- *Valoración de la prueba*

  Se registrará el número de repeticiones bien ejecutadas.

Figura 6. Representación gráfica del test de Abdominales.

Salto en largo sin impulso o salto horizontal

- *Objetivo*

  Determinar la potencia de las piernas.

- *Material*

  Cinta métrica con precisión en centímetros – preferiblemente utilizar una colchoneta fina.

- *Descripción*

  El ejecutante se coloca derecho con los pies separados detrás de la línea.

- *Desarrollo*

  Toma impulso con flexión de piernas y balanceo de brazos, saltando hacia delante manteniendo los pies firmes en el lugar que cayó. Se realizan 2 ó 3 intentos.

- *Valoración de la prueba*

  Se registra en centímetros el mejor intento.

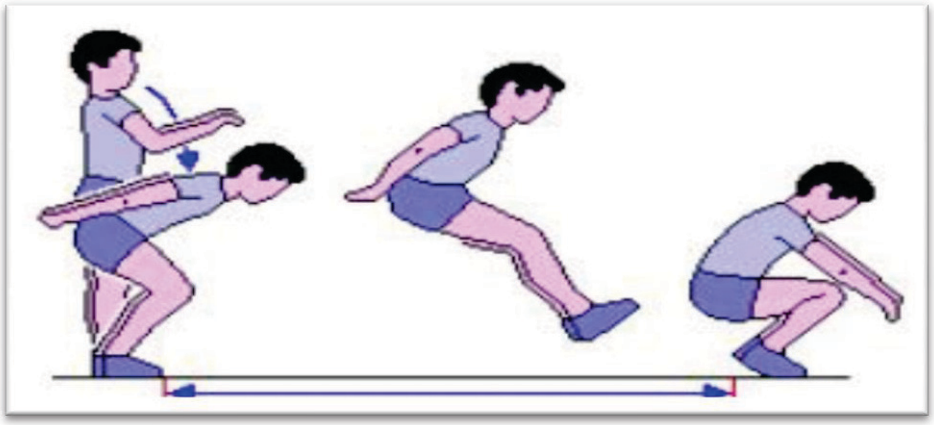


Figura 7. Representación del salto horizontal.

Dinamometría manual

- Objetivo

  El objetivo de la prueba es medir la fuerza estática de las extremidades superiores por medio de un dinamómetro de precisión.

- Material

  Dinamómetro digital de precisión modelo Takei digital TKK 5110, con precisión 0,1 kg y rango de (5 a 100 kg).

Figura 8. Representación de la técnica de dinamometría manual.

VALORACION DEL NIVEL DE ACTIVIDAD FISICA

Para estimar el nivel de práctica de AF que presentaban los sujetos se realizaron dos evaluaciones. Por una parte, se cuantificaron cuántos niños participaban en un programa de actividades extraescolares, y si la práctica que realizaban era al menos de tres días por semana, con una duración mínima de una hora y treinta minutos, durante al menos 1 año, como estaba previamente establecido en la programación de los colegios. Además, los niños rellenaron un test modificado basado en el cuestionario validado por el Instituto Nacional de Salud Infantil y Desarrollo Humano (*Pianta, 2007*), a través del cual se obtuvo información adicional sobre la práctica de AF y hábitos sedentarios.

PARAMETROS GENERALES Y METABOLICOS

Toma de muestras

Las extracciones de sangre se realizaron en situación de reposo y se llevaron a cabo por un facultativo especializado. Las muestras sanguíneas para las determinaciones bioquímicas se obtuvieron entre las 9 y las 10 de las mañana, tras 12 horas de ayuno, utilizando una vía venosa del brazo.

Todas las muestras se procesaron en las 2h siguientes a la extracción. Dicho proceso consistió en una centrifugación a 3500g durante 10 min. Una vez centrifugadas, el plasma y la capa leucocitaria se retiraron en diferentes tubos Eppendorf. A continuación, los eritrocitos se lavaron tres veces con suero fisiológico, tras los cuales fueron divididos en alícuotas. Todas las muestras fueron almacenadas a -80 °C hasta su análisis posterior.

Hematimetría y análisis de parámetros bioquímicos generales y metabólicos

Las muestras para obtener la hematimetría se colocaron en un autoanalizador que realiza el contaje por tamaño y colorimetría en el Servicio de Análisis Clínicos del hospital. Los datos obtenidos para cada muestra fueron: hematíes (103/µl), hemoglobina (g/dl), hematocrito (%), plaquetas (103/µl), leucocitos (103/µl) y fórmula leucocitaria [neutrófilos, linfocitos, monocitos, eosinófilos y basófilos (%)].

Los parámetros bioquímicos generales determinados fueron: glucosa (mg/dl), urea (mg/dl), creatinina (mg/dl), urato (mg/dl), calcio (mg/dl) y calcio corregido con proteínas (mg/dl), proteínas (g/dl), sodio (mEq/l), potasio (mEq/l), cloruro (mEq/l), hierro (µg/dl), ferritina (ng/ml), aspartato aminotransferasa (AST) (U/l) y alanina aminotransferasa (ALT) (U/l), TAG (mg/dl), colesterol total (mg/dl), HDL-c (mg/dl), LDL-c (mg/dl), apo A-I (mg/dl) y apo B (mg/dl) e insulina (mU/L), así como PCR ultrasensible. Todos estos parámetros se determinaron mediante métodos colorimétricos, enzimáticos, cinéticos, por potenciometría indirecta o inmunoturbidimetría previamente normalizados, utilizando un autoanalizador automático (Autoanalizadores Architect c16000 (A) e i2000SR (B) de Abbott Diagnostics®, y equipo Advia 120 Hematology System (C) de Bayer®). A partir de los datos obtenidos, se calcularon las siguientes relaciones: HDL-c/ LDL-c, HDL-c/ colesterol total, y apo A-I/ apo-B. La resistencia a la insulina se calculó según la ecuación HOMA-IR= fasting glucose (G0) (mM) x fasting insulin (I0) (µU/mL)/ 22.5 (*Matthews et al., 1985*).

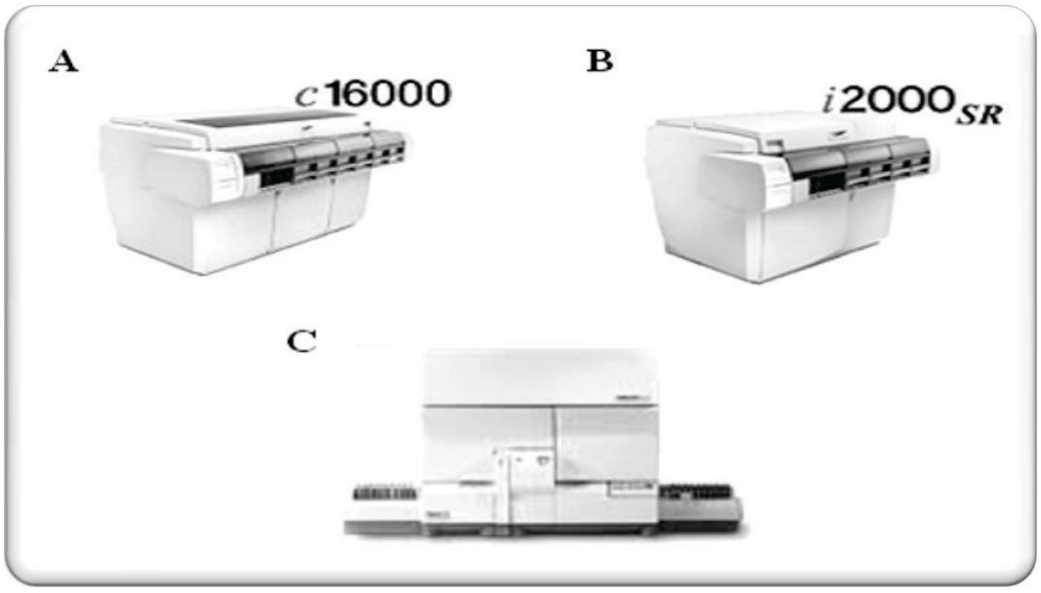


Figura 9. Representación gráfica de los autoanalizadores utilizados.

PARAMETROS DE INFLAMACION

El análisis de determinados biomarcadores de riesgo CV e inflamación se llevó a cabo en el Departamento de Bioquímica de la Facultad de Farmacia de la Universidad de Granada mediante detección simultánea de multi-analitos utilizando Kits de ensayo LINCO plex y la Tecnología de detección Luminex xMAP. Esta metodología permite el análisis cuantitativo mediante ELISA de varios biomarcadores utilizando una cantidad de muestra muy reducida (25-50 microlitros de plasma o suero).

Los componentes de cada kit incluyen los estándares, los controles de calidad, los anticuerpos conjugados de captura a microesferas, los anticuerpos de detección, el sistema indicador estreptavidina-ficoeritrina, el tampón de ensayo y el tampón de lavado. Cada kit utilizado está validado para la sensibilidad, recuperación, linealidad, precisión y especificidad. Asimismo, todos los analitos han sido testados individualmente y en combinación para asegurar la compatibilidad del sistema multiplex.

La tecnología Luminex xMAP se realiza a través de un proceso que colorea microesferas de polietileno de 5.2 nm con dos fluorocromos. La utilización de diferentes proporciones de estos fluorocromos permite colorear de manera diferente hasta 100 tipos diferentes de microesferas a las cuales se une un anticuerpo de captura específico para un biomarcador y que pueden ser identificadas con un láser (*Kellar and Douglass, 2003*).

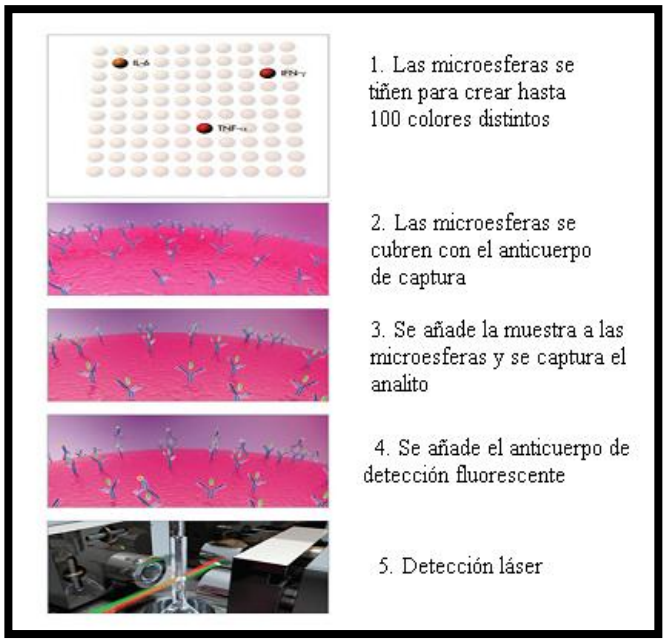


Figura 10. Esquema de la tecnología Luminex® X MAP™.

Se utilizó el kit Human adipokine Panel A, que permite la determinación simultánea de adiponectina, resistina y inhibidor del activador del plasminógeno-1 (PAI-1) activo y total; y el kit Human adipokine Panel B, que permite la determinación simultánea de leptina, IL-1 beta, IL-6, IL8, factor de crecimiento hepatocitario (HGF), factor de crecimiento neuronal (NGF), MCP-1 y TNF-alfa.

PARAMETROS DE ESTRES OXIDATIVO

Determinación de marcadores de estrés oxidativo

- *Productos de peroxidación de lípidos*

  Los lipoperóxidos [malonildialhehído (MDA) y 4-hidroxialquenales (4-HDA)] se midieron en plasma usando el método descrito por *Erdelmeier et al. (1998)*. Este método utiliza un reactivo que reacciona con chromatogenic MDA+4-HDA a 45ºC, dando un cromóforo estable con máxima absorbancia a 586 nm.

- *Proteínas Carboniladas*

  La concentración plasmática de proteínas carboniladas se midió usando el método de *Levine et al. (1990)*. Las muestras se incubaron con 2,4-dinitrofenilhidrazina en ácido clorhídrico durante 60 min. Entonces, las proteínas se precipitaron a partir de las soluciones utilizando 500 ml de ácido tricloroacético (20%). Después, las proteínas se lavaron con una solución de etanol y acetato de etilo (1/1 v / v) y se disolvió en 1 ml de guanidina clorhidrato de (6 M) a 37 1C. Los carbonilos se evaluaron en un espectrofotómetro (UV-1630; Shimadzu) a una longitud de onda de 360 nm (*Luo et al. 2009*).

- *Nitritos totales (nitritos y nitratos)*

  NOx se utilizó como un marcador de los niveles NO y ensayó siguiendo el método de Griess (*Ricart-Jané et al., 2002*) en plasma. Este ensayo utiliza la determinación del nitrito como un indicador de la producción de NO en muestras biológicas. El NO se transforma en nitrato y nitrito. Esta es una práctica común para utilizar, ya sea la reducción enzimática o química, para convertir todos los nitratos localizados en una muestra en nitritos y medir los nitritos totales como un indicador de la producción de NO. Cuando la reducción de nitrato se completó, el nitrito total se determinó espectrofotométricamente usando la reacción de Griess. La reacción se controló a 540 nm. La absorbencia se evaluó en un espectrofotómetro (UV-1603; Shimadzu) y expresada en gramos por mililitro.

Determinación de biomarcadores antioxidantes

- *Glutatión total (GT), glutatión oxidado (GSSG) y glutatión reducido (GSH)*

Estos biomarcadores antioxidantes fueron obtenidos de las células rojas de la sangre y medidos utilizando Bioxytech aop-490 TM (Oxis International, Portland, OR, EE.UU.). Se basa en la reducción de Cu^{2+} a Cu^{+} por la acción combinada de los antioxidantes de la muestra. Por lo tanto, los reactivos cromogénicos resultan en un complejo con Cu^{+}, que tiene una absorbencia a 490 nm (*Price et al., 2006*). Los niveles de GT y GSH se evaluaron utilizando el Bioxytech GSH y GSH-420-400 kits, respectivamente. La determinación de los niveles del GT se basa en la formación de un cromóforotiona, que tiene la absorbencia a 420 nm. La concentración de GSH se basa en una reacción que conduce a la formación de un cromóforo con absorbencia a 400 nm (*Rahman et al., 2006*). Niveles de GSSG fueron calculados restando GSH a GT.

Actividad de las enzimas antioxidantes

- Superóxido dismutasa (SOD)

Actividad SOD se determinó en eritrocitos utilizando un kit colorimétrico de ensayo adquirido de BioVision de Productos de Investigación (Mountain View, CA, EE.UU.). SOD cataliza la dismutación del anión superóxido en peróxido de hidrógeno y oxígeno molecular. La velocidad de la reducción con un anión superóxido esta linealmente relacionado con la actividad de la xantina oxidasa y se inhibe por la SOD. Por lo tanto, la inhibición de la actividad SOD se determina por un método colorimétrico.

- *Glutatión peroxidasa (GPx)*

La actividad de la GPx se evaluó en las células rojas de la sangre por el método de Flohé y Günzler (*Flohé et al., 1984*) utilizando un kit de ensayo de glutatión peroxidasa (Cayman Chemical). Este ensayo se basa en la oxidación de NADPH a $NADP^{+}$, que es catalizada por una concentración limitante de la glutatión reductasa, con absorbencia máxima a 340 nm. La actividad fue medida basada sobre la formación de GSSG a partir de la oxidación catalizada por GPx de GSH por H_2O_2, junto con el consumo de NADPH, en presencia de glutatión reductasa exógenamente añadido, con absorbencia máxima a 340 nm.

ANÁLISIS ESTADÍSTICO

Los análisis estadísticos se realizarán con el Paquete Estadístico para las Ciencias Sociales (SPSS) 18.0 para Windows. Los datos se expresaron como medias ± desviaciones estándar. Los valores con una $P \leq 0.05$ se consideraron estadísticamente significativos. En el apartado de metodología de los artículos se describe los métodos estadísticos empleados en cada uno de ellos. De forma general:

- La distribución normal de los datos se evaluó mediante el test de Shapiro-Wilk, y la homogeneidad de varianzas se estimaron utilizando el test de Levene.

- La comparación de medias entre los grupos, para variables continuas con distribución normal, se realizó mediante la prueba de la t de Student para muestras no apareadas. Para aquellos con una distribución asimétrica se utilizó el test de la U de Mann-Whitney.

- La prueba de χ^2 se aplicó para la comparación de proporciones.

- Las comparaciones entre los dos grupos de niños/as tras las pruebas se realizaron mediante análisis de la covarianza tras ajustar por la edad, el sexo y el IMC.

- Para estudiar las asociaciones entre biomarcadores de estrés oxidativo se realizó un análisis no paramétrico de correlación a través del cálculo de coeficientes de correlación con el test de Spearman´s ρ.

- Las correlaciones entre variables fueron evaluadas mediante la prueba de Pearson.

RESULTADOS y DISCUSION

NON-TRADITIONAL MARKERS OF METABOLIC RISK IN PREPUBERTAL CHILDREN WITH DIFFERENT LEVELS OF CARDIORESPIRATORY FITNESS

Francisco Jesús Llorente-Cantarero[1], Juan Luis Pérez-Navero[2], Juan de Dios Benitez-Sillero[1], Maria Carmen Muñoz-Villanueva[3], Manuel Guillén-del Castillo[1] and Mercedes Gil-Campos[2]

Public Health Nutr. 2012; 15:1827-34

[1]Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain.
[2]Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Córdoba, Spain.
[3]Unit of Methodology in Investigation, IMIBIC, Córdoba, Spain

 doi:10.1017/S1368980011003533

Non-traditional markers of metabolic risk in prepubertal children with different levels of cardiorespiratory fitness

Francisco Jesús Llorente-Cantarero[1], Juan Luis Pérez-Navero[2], Juan de Dios Benitez-Sillero[1], Maria Carmen Muñoz-Villanueva[3], Manuel Guillén-del Castillo[1] and Mercedes Gil-Campos[2,*]

[1]Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain: [2]Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Av de Menéndez Pidal, s/n 14004, Córdoba, Spain: [3]Unit of Methodology in Investigation, IMIBIC, Córdoba, Spain

Submitted 23 May 2011: Accepted 8 December 2011

Abstract

Objective: To assess classical and non-classical metabolic risk biomarkers in prepubertal children with different levels of cardiorespiratory fitness (CRF).

Design: CRF was assessed by the 20 m shuttle run test. To estimate physical activity, participants were observed while engaged in an after-school programme. Additionally, a short test based on a validated questionnaire was used to obtain information about physical activity practice and sedentary habits. Anthropometric parameters, blood pressure, and classical and non-traditional metabolic risk biomarkers – plasma lipid profile, glucose and insulin, homeostasis model assessment–insulin resistance index (HOMA-IR), plasma uric acid, transaminases and C-reactive protein (CRP) – were measured.

Setting: The study was conducted in local elementary schools in Córdoba, Spain.

Subjects: One hundred and forty-one healthy children (eighty-eight boys, fifty-three girls) aged 7–12 years, in Tanner stage I, were recruited. They were divided into two groups after they performed the 20 m shuttle run test: equal or higher cardiovascular fitness (EHCF) group and low cardiovascular fitness (LCF) group.

Results: The LCF group displayed significantly higher TAG ($P = 0{\cdot}004$) and lower HDL cholesterol levels ($P = 0{\cdot}001$), as well as significantly lower values for the non-traditional lipid marker apo-A1 ($P = 0{\cdot}001$) compared with the EHCF group. The LCF children displayed higher plasma glucose ($P = 0{\cdot}003$) and insulin levels, higher HOMA-IR scores ($P < 0{\cdot}001$) and higher plasma uric acid and CRP levels ($P < 0{\cdot}05$). After adjustment for BMI, age and sex, no statistically significant differences were found between groups for the biomarkers analysed.

Conclusions: The study provides new information to understand the role not only of weight status but also of the level of CRF on the metabolic health profile of prepubertal children.

Keywords
Physical fitness
Physical activity
Metabolic biomarkers
Childhood

The metabolic syndrome (MetS) refers to the clustering of cardiovascular risk factors driven by peripheral insulin resistance. Data from recent studies involving a population of children and adolescents indicate that the prevalence of MetS varies between 3 % and 12 %[1]. Suggestions for future research include establishing which individual components of the MetS cluster confer the greatest risk on future morbidity in children. There is no universally accepted definition of MetS in childhood[2] but for investigating associations between cardiorespiratory fitness (CRF) and metabolic risk, a total risk score has been calculated in various studies as the sum of *Z*-scores for the single risk factors defining MetS[3].

However, the International Diabetes Federation consensus defines MetS in children and adolescents as central obesity plus any two of the following classical parameters: (i) raised TAG levels, (ii) reduced HDL cholesterol (HDL-C) levels, (iii) hypertension and (iv) elevated fasting plasma glucose[4]. Moreover, some non-classical factors such as plasma uric acid elevation have been associated with features of insulin resistance; indeed, it has been suggested that uric acid could be a key link for the diagnosis of MetS in obese prepubertal children[5]. The homeostasis model assessment–insulin resistance index (HOMA-IR) and levels of total cholesterol, LDL cholesterol (LDL-C), apo-A1 and apo-B[5–7], transaminases[8] and C-reactive protein (CRP)[9] are also altered in children with MetS, but they are considered non-traditional features of MetS.

Going from moderate to high levels of CRF has consistently been associated with a lower risk of developing

*Corresponding author: Email mercedes_gil_campos@yahoo.es

NS Public Health Nutrition

MetS, CVD or type 2 diabetes[10]. CRF involves a set of health or skill-related attributes with a strong genetic component that remains relatively static, needing some time to change, and it is assessed using a battery of field tests. Nevertheless, variability in CRF is known to influence metabolic status[3]. Physical activity (PA) is often used interchangeably with energy expenditure and physical fitness and has been defined as any bodily movement produced by skeletal muscles which results in energy expenditure. Some authors suggest that CRF and PA may affect metabolic risk but the relationship is weak and they may act through different pathways[11,12].

In children, some studies have shown that CRF is independently associated with clustered metabolic risk factors[11,13]. Non-traditional factors have been also researched in children in relation to CRF[8,14]. Nevertheless, further research is needed to ascertain whether changes in fitness in the general population of children predict changes in cardiovascular risk profile and whether fitness and fatness independently influence metabolic risk[8,15]. Thus the aim of the present study was to assess classical and non-classical metabolic risk biomarkers, including insulin resistance, plasma lipid profile, uric acid, liver transaminases and CRP, in prepubertal healthy children with different levels of CRF.

Experimental methods

Participants and design

A group of 141 healthy children, eighty-eight boys (≤10 years, *n* 52; >10 years, *n* 36) and fifty-three girls (all ≤10 years), at prepubertal stage was selected from local elementary schools in Córdoba, Spain. Children were asked to perform a 20 m shuttle run test (20-mSRT) in order to evaluate their CRF.

Inclusion criteria were age 7–12 years and prepubertal stage (Tanner stage I), as validated by appropriate plasma sex hormone levels. Exclusion criteria were as follows: presence of pubertal development, disease, long periods of rest after illness, use of any medication that alters blood pressure or glucose or lipid metabolism, consumption of any special diet and failure to get the same record reached in the first attempt in the 20-mSRT[16]. Written informed consent was obtained from parents or legal guardians and the study was approved by an institutional ethics committee at the University Reina Sofia Hospital.

The validated scale developed by Olds *et al.*[17] was used to measure CRF after the 20-mSRT. The test performances are expressed as mean and standard deviation relative to all children of similar age and sex from all countries. In the present study, children were split into two groups according to the methodology used in previous studies[18,19]: (i) the children recording a score equal to or greater than the average reference value (seventy-five participants) were assigned to the group designated 'equal or higher cardiovascular fitness' (EHCF); and (ii) those with less-than-average scores (sixty-six participants) were assigned to the group 'low cardiovascular fitness' (LCF).

Furthermore, children were divided into quintiles after estimating their V_{O2max} obtained by cross-referencing the final level and shuttle number completed, using the formula developed by Leger *et al.*[20] (*Y*, ml/kg body weight per min), from the speed (*X*, km/h) corresponding to that stage (speed = 8+0·5 stage no.) and age (*A*, years): $Y = 31{\cdot}025 + 3{\cdot}238X - 3{\cdot}248A + 0{\cdot}1536AX$. V_{O2max} correlates oxygen consumption with exercise duration, and estimated relative V_{O2max} and extrapolated absolute V_{O2max} are used to determine fitness. Thus top and bottom groups were compared.

Physical examination and measurements

Anamnesis and a physical examination were assessed by paediatricians despite illness in all of the children. Sexual maturity was observed by physical examination according to the Tanner five-stage scale[21].

Weight and height were measured by standard techniques, using a beam balance and a precision stadiometer (SC700; Seca, Hamburg, Germany), with participants lightly dressed and barefooted. BMI was calculated as weight (kg)/[height (m)]^2. Waist circumference was measured in duplicate with an inelastic tape according to standardized methods. These anthropometric measurements were compared with Spanish reference standards[22,23] and with the age- and sex-specific cut-off points proposed by Cole *et al.*[24] to define obesity. Thus we ensured that most of the children of the study were healthy.

Systolic and diastolic blood pressures (BP) were measured in the right arm in a sitting position, using a random-zero sphygmomanometer (Dinamap V-100; GE Healthcare, Spain) after the participants had rested without changing position for at least 5 min.

Children were observed while engaged in an after-school programme at least three times weekly for at least 1 year to estimate PA. Additionally, a short test based on the validated questionnaire of the National Institute of Child Health and Human Development[25] was used for both groups to obtain information about PA practice and sedentary habits.

Evaluation of cardiorespiratory fitness

Standardized Eurofit battery tests[20,26] were performed to evaluate fitness; the 20-mSRT was used to assess CRF, and upper and lower body strength were also evaluated.

The 20-mSRT required participants to run back and forth between two lines set 20 m apart. Running speed started at 8·5 km/h and increased by 0·5 km/h each minute, reaching 18·0 km/h at minute 20. Running speed cues were indicated by signals emitted by a commercially available CD-ROM. Participants were allowed to voluntarily withdraw from the test after being verbally encouraged to maximally perform during each assessment. The test finished when the participant failed to reach the finishing lines concurrent with the audio signals on two consecutive occasions[27].

PHN Public Health Nutrition

Upper-body muscular strength was assessed by means of the handgrip strength and the bent arm hang tests with a digital hand dynamometer (Takei TKK Q4-5110, precision 0·1 kg and range 5 to 100 kg; Takei Scientific Instruments Co., Ltd, Niigata, Japan) and another test evaluating the number of abdominals performed within 30 s. Lower-body muscular strength was assessed by using the standing broad jump test.

Sampling

Baseline fasting blood samples were obtained from all children using an indwelling venous line to draw a 3 ml sample for the measurement of plasma glucose and insulin levels, lipid profiles and uric acid levels.

Biochemical analysis

Fasting gonadotrophins and sex hormones – follicle stimulating hormone (CV = 3·6 %), luteinizing hormone (CV = 3·1 %), testosterone (CV = 2 %) and oestradiol (CV = 1·8 %) – were measured by chemiluminescence using an automatic analyser (Architect I4000; Abbott Laboratories, Abbott Park, IL, USA) to validate that the children selected by clinical signs and Tanner stage were truly prepubertal.

Glucose was analysed using the glucose oxidase method in an automatic analyser (CV = 1 %). Plasma TAG (CV = 1·5 %), total cholesterol (CV = 0·9 %), HDL-C (CV = 0·8 %), LDL-C (CV = 1·5 %), apo-A1 (CV = 1·7 %), apo-B (CV = 2·6 %), uric acid (CV = 1·9 %), aspartate aminotransferase (CV = 1·7 %), alanine aminotransferase (CV = 3 %) and CRP were also measured using an automatic analyser (Accelerator APS system, Architect-c16000; Abbott Laboratories). CRP (CV = 4 %) was measured by particle-enhanced turbidimetric immunoassay (Dade Behring Inc., Deerfield, IL, USA). Plasma insulin was analysed by RIA with an automatic analyser for microparticles (Axsym; Abbott Laboratories). Insulin resistance was calculated by means of HOMA-IR, defined by the equation: HOMA-IR = [fasting glucose (mmol/l) × fasting insulin (μU/ml)]/22·5.

The criteria using classical features agreed by the International Diabetes Federation in 2007[4] were used in the present study to estimate the presence of MetS in the sample of prepubertal children.

Statistical analysis

Data were expressed as means and standard deviations. Normal distribution of data was assessed by the Shapiro–Wilk test. Homogeneity of variances was estimated using the Levene test. Comparison of means between groups for continuous variables with normal distribution was done using Student's *t* test for unpaired samples and for those with an asymmetric distribution by the Mann–Whitney *U* test. The χ^2 test was applied for comparison of proportions. Comparisons between the two groups of children after tests were performed using analysis of covariance after adjusting for age, sex and BMI. Correlations between variables were assessed using Pearson's test.

Cohen typified differences (*d*) in the studied parameters between children in the top and bottom quintiles of fitness and effect size correlation were calculated in order to quantify the magnitude of the difference (effect size).

The statistical software package PASW® Statistics 18 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Anthropometric and BP measurements and PA performance between groups of children with different levels of CRF are shown in Table 1. BMI and waist circumference values were significantly higher in the LCF group than in the EHCF group but none of these children were obese. There were no differences in BP levels between both groups.

For the Eurofit physical test battery, the EHCF group displayed significantly higher results in the abdominals test and the horizontal jump test; no between-group differences were observed in the dynamometry test. Most children (72 %) in the EHCF group practised PA regularly in an after-school programme (Table 1). A positive significant correlation was observed between CRF and PA ($r = 0{\cdot}346$; $P < 0{\cdot}001$).

Results for classical MetS biochemical parameters were as follows: the LCF group displayed significantly higher TAG and lower HDL-C levels, as well as significantly lower values for the non-traditional lipid marker apo-A1 (Fig. 1). The LCF group also displayed significantly higher plasma glucose (Fig. 2(a)) and insulin (Fig. 2(b)) and HOMA-IR scores (Fig. 2(c)).

Table 1 Anthropometric parameters, blood pressure and physical activity as a function of cardiorespiratory fitness level in healthy prepubertal children (*n* 141) aged 7–12 years, Córdoba, Spain

	EHCF group (*n* 75)		LCF group (*n* 66)		
Variable	Mean	SD	Mean	SD	*P**
Age (years)	9·57	1·20	9·68	1·09	0·5708
Weight (kg)	38·35	9·58	45·89	12·54	<0·0001
Height (cm)	142·33	9·51	144·74	8·85	0·1229
BMI (kg/m^2)	18·69	2·89	21·55	3·99	<0·0001
WC (cm)	63·20	7·64	71·21	11·56	<0·0001
SBP (mmHg)	120·00	10·36	123·25	16·09	0·1527
DBP (mmHg)	66·94	9·14	67·32	10·53	0·8213
20-mSRT (periods)	4·91	1·79	2·12	0·87	<0·0001
Abdominals (no.)	14·55	5·52	11·94	5·22	0·0047
HJ (m)	1·35	0·19	1·16	0·17	<0·0001
RHD (kg)	16·00	4·47	16·90	5·12	0·2734
LHD (kg)	15·16	4·23	16·34	6·22	0·1869
PPA (%)	72		42		0·0007†

EHCF, equal or higher cardiovascular fitness; LCF, low cardiovascular fitness; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; 20-mSRT, 20 m shuttle run test; abdominals, abdominal exercises; HJ, horizontal jump; RHD, right hand dynamometry; LHD, left hand dynamometry; PPA, performed physical activity.

*Statistical significance after application of a Student *t* test to data expressed as mean and SD.

†Statistical significance after application of a χ^2 test to data expressed as a percentage.

NS Public Health Nutrition

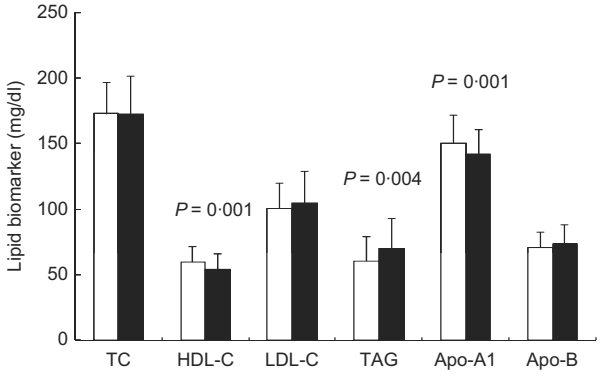


Fig. 1 Lipid biomarkers (TC, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol) in healthy prepubertal children (*n* 141) aged 7–12 years, Córdoba, Spain, according to cardiorespiratory fitness (□, equal or higher cardiovascular fitness (EHCF) group; ■, low cardiovascular fitness (LCF) group). Values are means and standard deviations represented by vertical bars; *P* values indicate statistical significance after application of a Student *t* test to the data

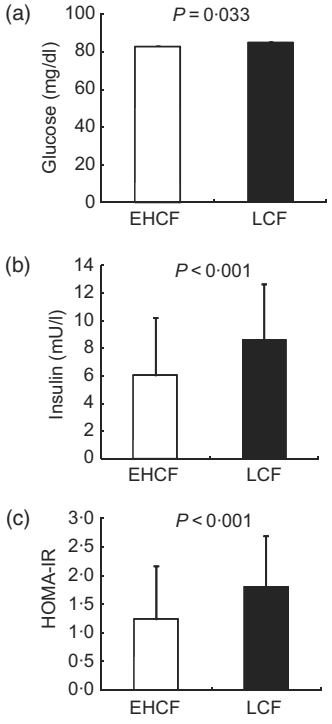


Fig. 2 Insulin resistance biomarkers in healthy prepubertal children (*n* 141) aged 7–12 years, Córdoba, Spain, according to cardiorespiratory fitness (□, equal or higher cardiovascular fitness (EHCF) group; ■, low cardiovascular fitness (LCF) group): (a) plasma glucose; (b) plasma insulin; (c) homeostasis model assessment–insulin resistance index (HOMA-IR). Values are means and standard deviations represented by vertical bars; *P* values indicate statistical significance after application of a Student *t* test to the data

With regard to non-classical MetS biomarkers, the EHCF group had significantly higher aspartate aminotransferase levels (Fig. 3(a)), and CRF was associated with this transaminase ($r = 0{\cdot}266$; $P = 0{\cdot}002$). Plasma uric acid (Fig. 3(b)) and CRP levels (Fig. 3(c)) were significantly higher in the LCF group than in the EHCF group.

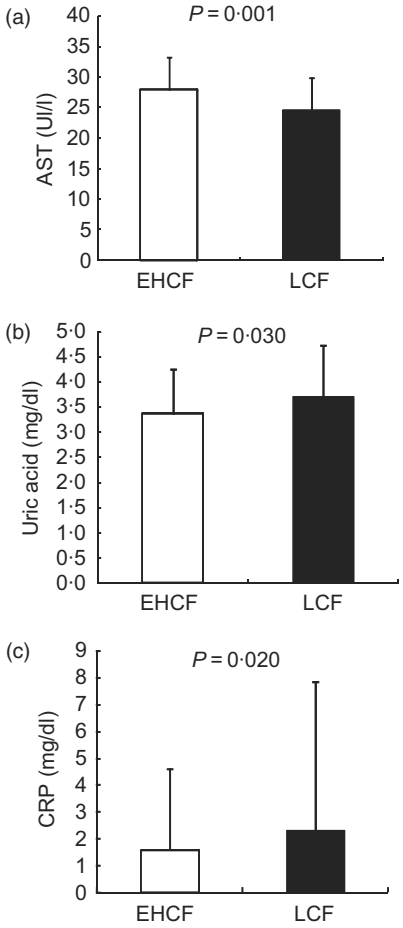


Fig. 3 Non-traditional biomarkers in healthy prepubertal children (*n* 141) aged 7–12 years, Córdoba, Spain, according to cardiorespiratory fitness (□, equal or higher cardiovascular fitness (EHCF) group; ■, low cardiovascular fitness (LCF) group): (a) aspartate aminotransferase (AST); (b) uric acid; (c) C-reactive protein (CRP). Values are means and standard deviations represented by vertical bars; *P* values indicate statistical significance after application of a Student *t* test to the data

After adjustment for BMI, age and sex, no significant differences were found between groups for the biomarkers analysed. However, using the criteria suggested by Zimmet *et al.*[4], 4·25 % of the children were diagnosed as having MetS and all of them belonged to the LCF group. In the correlation analysis, CRF was inversely associated with HOMA-IR ($r = -0{\cdot}240$; $P = 0{\cdot}05$), TAG ($r = -0{\cdot}196$; $P = 0{\cdot}023$), LDL-C ($r = -0{\cdot}227$; $P = 0{\cdot}008$) and uric acid ($r = -0{\cdot}178$; $P = 0{\cdot}042$).

Moreover, when we compared the top and the bottom quintiles after estimating V_{O2max} data, we found differences between these groups regarding age, height and systolic and diastolic BP. Children included in the bottom quintile were older and taller and showed higher BP levels than the children in the top quintile, who presented better CRF levels. Apart from the results obtained in

PHN Public Health Nutrition

Table 2 Comparison between the top and bottom quintiles of the studied parameters after estimating V_{O2max}: healthy prepubertal children (*n* 141) aged 7–12 years, Córdoba, Spain

	Quintile 1 (*n* 30)		Quintile 5 (*n* 29)				
Variable	Mean	SD	Mean	SD	*P**	Typified difference (Cohen *d*)	Effect size
Age (years)	10·33	0·96	9·37	1·30	0·002	0·84	0·39
Weight (kg)	51·72	13·73	35·03	6·29	<0·001	1·56	0·62
Height (cm)	148·72	9·88	140·40	6·83	<0·001	0·98	0·44
WC (cm)	76·33	11·17	60·73	5·45	<0·001	1·78	0·66
SBP (mmHg)	129·60	15·30	118·03	11·45	0·002	0·86	0·39
DBP (mmHg)	71·27	9·23	65·14	9·61	0·015	0·76	0·35
BMI (kg/m^2)	23·04	4·27	17·69	2·38	<0·001	1·55	0·61
TC (mg/dl)	176·34	35·62	169·10	23·38	0·364	0·24	0·12
HDL-C (mg/dl)	52·96	10·54	61·48	10·18	0·003	−0·82	−0·38
LDL-C (mg/dl)	111·54	25·92	95·62	18·56	0·010	0·71	0·33
Apo-A1 (mg/dl)	139·10	18·84	152·83	15·88	0·001*	−0·79	−0·37
Apo-B (mg/dl)	77·55	15·05	67·00	11·20	0·004	0·79	0·37
TAG (mg/dl)	72·43	20·53	57·96	17·38	0·003*	0·76	0·35
Glucose (mg/dl)	82·44	7·38	82·26	6·84	0·884	0·03	0·01
HOMA-IR	2·07	1·01	1·05	0·58	<0·001	1·24	0·53
CRP (mg/l)	1·61	1·33	1·29	1·64	0·115*	0·21	0·11
AST (UI/l)	22·48	5·70	27·50	4·63	<0·001*	−0·97	−0·44
ALT (UI/l)	22·31	28·05	17·38	6·85	0·779*	0·24	0·12
Uric acid (mg/dl)	4·02	1·13	3·15	0·75	0·001	0·91	0·41

Quintile 5, top quintile with the best fitness; quintile 1, bottom quintile with the worst fitness; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; HOMA-IR, homeostasis model assessment–insulin resistance index; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*Statistical significance after application of the Student *t* test or the Mann–Whitney *U* test to data expressed as mean and SD; Cohen *d* and effect size analysis has been done for these groups.

relation to EHCF and LCF groups, we also found higher LDL-C and apo-B levels in the lower quintile (Table 2).

As far as the top and bottom quintiles are concerned, we did not find relevant differences between groups due to the effect size when Cohen's *d* and effect size correlations were applied. Most of these values were between 0·2 and 0·8 (Table 2), which indicates a moderate effect size.

Discussion

In the present study, the major finding was that prepubertal children in the LCF group (with low CRF) displayed higher levels for some MetS risk factors such as lipid profile and insulin resistance and for certain non-traditional markers such as uric acid and CRP than did children in the EHCF group. After adjustment for BMI, age and sex, there were no significant between-group differences, suggesting a probable influence of body mass on fitness condition. Moreover, the correlations between traditional and non-traditional risk factors of MetS suggest that individual components of MetS might be influenced by CRF.

Paediatric data are inconsistent regarding whether fitness or fatness is most relevant to health outcomes in children. Only recently, a small number of prospective studies systematically examined the independent and joint associations of CRF and body mass with health outcomes[28]. Some research in children suggests that CRF has a protective influence on metabolic health[11,29] whereas other studies report that the effects of fitness may be indirect, mediated through its relationship to fatness[29,30]; fatness appears to be more predictive of cardiovascular risk than CRF[12,13].

In the present study, CRF was assessed using the 20-mSRT[27]; since this is a weight-bearing activity, the test might conceivably prompt overestimation of the relationship between CRF and both fatness and MetS risk factors. In other studies, however, measures were not modified by weight status[10] and others support the validity of using clinical measurements of physical fitness to predict insulin resistance. Low CRF is strongly associated with high BMI and recent investigations suggest that further research is required to explore more fully this relationship[15,27,31]. The results obtained in the current study provide additional information about non-traditional metabolic factors in prepubertal healthy children in relation to different levels of CRF and the influence of fatness.

In the present work, children in the EHCF group had lower BMI and a better metabolic profile (lower levels of TAG and higher levels of HDL-C and apo-A1, and lower levels of insulin and HOMA-IR) than did children from the LCF group, suggesting that children with good CRF seem to have better weight status and enjoy better metabolic health. Moreover, this group had lower uric acid and CRP levels. Similarly to our study, higher cardiovascular fitness has been associated with a favourable metabolic profile[12].

The inverse association seems to be consistent with some findings in adolescent studies[32–34] although others do not find these associations with insulin resistance markers[35]. The clinical value of fasting glucose as a risk biomarker has been questioned, although in our study there were significant differences between CRF groups.

PHN Public Health Nutrition

In the LCF group, glucose, insulin and HOMA-IR were higher than in the EHCF group, although neither group reached pathological levels (Fig. 2(c)). After adjustment for BMI, however, there were no significant between-group differences, suggesting an influence of body mass on glucose metabolism. In a sample of 1140 children, Ruiz *et al.*[13] found a positive association between these parameters and body fat after adjusting for CRF, and a negative association between both HOMA-IR and fasting insulin and CRF. This inverse association appears to be consistent with findings in adolescent research[32–34] although these associations with insulin resistance markers are not reported in other studies[36]. Indeed, HOMA-IR has been independently associated with lower CRF in children[15,37,38] and an inverse correlation between HOMA-IR and PA has been also reported[32].

High CRF is associated with low levels of TAG, suggesting that CRF more than PA influences the lipid profile[8,15]. Another remarkable result in the present study was that 72 % of the children who performed PA were in the EHCF group. PA has been inversely associated with metabolic risk factors[39] independently of CRF and adiposity[1,4,11]. However, in other studies, its relationship was attenuated[40] or unchanged[41] even after further adjustment according to adiposity[13] and CRF. Research suggests that exercise training improves glucose metabolism[42], inflammation[43] and both lipid and lipoprotein profiles, thus highlighting the impact of PA on cardiovascular risk[44]. In our results, the positive significant correlation between PA and CRF may indicate the influence of the different pathways involved in either PA or CRF. PA may thus exert an independent beneficial effect on health regardless of fatness[11].

To study these relationships, several studies have used mixed populations of children and adolescents[45,46], thus ignoring to evaluate the pubertal state. Puberty is associated with a decrease in insulin sensitivity[47]; due to this, working only with prepubertal children can reduce this effect. Nevertheless, little research has been done with homogeneous sub-populations[10,11,37]. In fact, the low significance in the results of the present study may partly be due to the early age of our groups.

With regard to non-traditional markers, a decline in plasma apo-A1 levels has been associated with low levels of HDL-C, and thus with increased cardiovascular risk[7]. In the present study, the LCF group displayed lower levels for these lipid parameters than the EHCF group (Fig. 1). Moreover, although uric acid has been put forward as a new biomarker for metabolic risk in childhood obesity[5,48], its relationship to CRF or PA has not been analysed. Only a study in patients with type 2 diabetes mellitus showed that uric acid was negatively associated with CRF[49]. Children from the LCF group of the present study had higher uric acid levels than EHCF children (Fig. 3(b)), suggesting that CRF may improve this metabolic alteration; a significant negative correlation was also noted between uric acid and CRF. Nevertheless, aspartate aminotransferase was higher in the EHCF group (Fig. 3(a)). The increase in liver transaminases has usually been associated with other components of MetS, particularly insulin resistance; some authors have found a relationship between alanine aminotransferase and CRF or PA in youth[8,34] despite no changes being reported by others[50].

An increase in pro-inflammatory cytokines and acute-phase reactants has been associated with cardiovascular risk factors. CRP is an indicator of a range of inflammatory processes of both vascular and non-vascular origin; elevated CRP may reflect a pro-atherogenic metabolic state that predisposes to atherothrombotic events[51]. Recent studies in children have shown strong evidence for the association between CRP increase and various markers for MetS[11,52], diabetes mellitus and CVD[35], and it has been reported that other potential factors and processes such as reduced CRF could contribute to low-grade inflammation in apparently healthy individuals. Here, the EHCF group had lower CRP levels than the LCF group (Fig. 3(c)). In prepubescent children, CRF has been inversely associated with CRP[43].

Concerning effect size results, there was little or moderate effect size in the present study. Moreover, none of the *P* values for the parameters compared between top and bottom quintiles groups were near significance. So, perhaps effect size did not have had an important role.

Finally, the International Diabetes Federation consensus report was used to evaluate associations of CRF with MetS, leaving aside the summarized metabolic risk score due to some limitations[4]. In a longitudinal study by McMurray *et al.*[53], children with MetS (4·6 %) had lower PA levels and CRF outcomes than the non-MetS group. Some 4·25 % of prepubertal children in the current study presented MetS; these children belonged to the LCF group and did not perform PA. A similar percentage has been reported in another study of children[1]. The classical features for MetS appear to be present even in our general prepubertal paediatric sample and to be related to low CRF. It is therefore important to clarify the impact of CRF and PA on non-traditional risk factors in children.

In relation to overall public health, new research is needed to evaluate metabolic risk as well as the factors related to it in children with low fitness measured by V_{O2max}, in different stages and ages. This would contribute to establish the groups with health risk in childhood that could benefit from scheduled PA programmes.

Conclusions

The metabolic health profile of prepubertal children displaying high levels of CRF is characterized by low TAG, HOMA-IR, uric acid and CRP levels, and higher levels of HDL-C and apo-A1, compared with children with low CRF. The present study provides new information to

PHN Public Health Nutrition

understand the role not only of weight status but also of the level of CRF on the metabolic health profile in prepubertal children in relation to traditional and non-traditional metabolic risk factors.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The authors have no conflict of interest. The contribution of each author to the manuscript was as follows: F.J.L.-C., J.d.D.B.-S. and M.G.-d.C. coordinated the fitness and physical activity tests; J.L.P.-N. and M.G.-C. performed the paediatric evaluations; F.J.L.-C. and M.G.-C. processed the biochemical samples; M.C.M.-V. carried out the statistical analyses. All authors contributed to the evaluation of results and writing of the manuscript. Each author has seen and approved the contents of the submitted manuscript.

References

1. Tailor AM, Peeters PH, Norat T *et al.* (2010) An update on the prevalence of the metabolic syndrome in children and adolescents. *Int J Pediatr Obes* **5**, 202–213.
2. Weiss R (2010) Metabolic syndrome in childhood – causes and effects. *Endocr Dev* **19**, 62–72.
3. Steele RM, Brage S, Corder K *et al.* (2008) Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. *J Appl Physiol* **105**, 342–351.
4. Zimmet P, Alberti KG, Kaufman F *et al.*; IDF Consensus Group (2007) The metabolic syndrome in children and adolescents – an IDF consensus report. *Pediatr Diabetes* **8**, 299–306.
5. Gil-Campos M, Aguilera CM, Cañete R *et al.* (2009) Uric acid is associated with features of insulin resistance syndrome in obese children at prepubertal stage. *Nutr Hosp* **4**, 607–613.
6. Bueno G, Bueno O, Moreno LA *et al.* (2006) Diversity of metabolic syndrome risk factors in obese children and adolescents. *Physiol Biochem* **62**, 125–133.
7. Sellers EA, Singh GR & Sayers SM (2009) Apo-B/A1 ratio identifies cardiovascular risk in childhood: the Australian Aboriginal Birth Cohort study. *Diab Vasc Dis Res* **6**, 94–99.
8. Bouglé D, Zunquin G, Sesbouë B *et al.* (2010) Relationships of cardiorespiratory fitness with metabolic risk factors, inflammation, and liver transaminases in overweight youths. *Int J Pediatr* 580897.
9. Mauras N, Delgiorno C, Kollman C *et al.* (2010) Obesity without established comorbidities of the metabolic syndrome is associated with a proinflammatory and prothrombotic state, even before the onset of puberty in children. *J Clin Endocrinol Metab* **95**, 1060–1068.
10. Kriemler S, Manser-Wenger S, Zahner L *et al.* (2008) Reduced cardiorespiratory fitness, low physical activity and an urban environment are independently associated with increased cardiovascular risk in children. *Diabetologia* **51**, 1408–1415.
11. Ekelund U, Anderssen SA, Froberg K *et al.* (2007) Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European Youth Heart Study. *Diabetologia* **50**, 1832–1840.
12. Froberg K & Andersen LB (2005) Mini review: physical activity and fitness and its relations to cardiovascular disease risk factors in children. *Int J Obes (Lond)* **29**, Suppl. 2, S34–S39.
13. Ruiz JR, Ortega FB, Rizzo NS *et al.* (2007) High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. *Pediatr Res* **61**, 350–355.
14. Invitti C, Maffeis C, Gilardini L *et al.* (2006) Metabolic syndrome in obese Caucasian children: prevalence using WHO-derived criteria and association with nontraditional cardiovascular risk factors. *Int J Obes (Lond)* **30**, 627–633.
15. Suriano K, Curran J, Byrne SM *et al.* (2010) Fatness, fitness, and increased cardiovascular risk in young children. *J Pediatr* **157**, 552–558.
16. Hasselstrøm H, Hansen SE, Froberg K *et al.* (2002) Physical fitness and physical activity during adolescence as predictors of cardiovascular disease risk in young adulthood. Danish youth and sports study. An eight-year follow-up study. *Int J Sports Med* **23**, 27–31.
17. Olds T, Tomkinson G, Léger L *et al.* (2006) Worldwide variation in the performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle run test in 37 countries. *Sports Sci* **24**, 1025–1038.
18. García-Artero E, Ortega FB, Ruiz JR *et al.* (2007) Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study). *Rev Esp Cardiol* **60**, 581–588.
19. McGavock JM, Torrance BD, McGuire KA *et al.* (2009) Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. *Obesity (Silver Spring)* **17**, 1802–1807.
20. Léger LA, Mercier D, Gadoury C *et al.* (1988) The multistage 20 meter shuttle run test for aerobic fitness. *J Sports Sci* **6**, 93–101.
21. Tanner JM (1962) *Growth at Adolescence*. Oxford: Blackwell.
22. Sobradillo B, Aguirre A, Aresti U *et al.* (2004) *Curvas y tablas de crecimiento (estudios longitudinal y transversal)*. Madrid: Fundación Faustino Orbegozo Eizaguirre.
23. Moreno LA, Pineda I, Rodríguez G *et al.* (2002) Waist circumference for the screening of the metabolic syndrome in children. *Acta Paediatr* **91**, 1307–1312.
24. Cole TJ, Bellizzi MC, Flegal KM *et al.* (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* **320**, 1240–1243.
25. Pianta RC; National Institute of Child Health and Human Development (2007) Developmental science and education: the NICHD study of early child care and youth development findings from elementary school. *Adv Child Dev Behav* **35**, 253–296.
26. Committee of Experts on Sports Research EUROFIT (1993) *Handbook for the EUROFIT Tests of Physical Fitness*. Strasburg: Council of Europe.
27. Vale S, Santos R, Soares-Miranda L *et al.* (2010) The relationship of cardiorespiratory fitness, birth weight and parental BMI on adolescents' obesity status. *Eur J Clin Nutr* **64**, 622–627.
28. LaMonte MJ & Blair SN (2006) Physical activity, cardiorespiratory fitness, and adiposity: contributions to disease risk. *Curr Opin Clin Nutr Metab Care* **9**, 540–546.
29. Allen DB, Nemeth BA, Clark RR *et al.* (2007) Fitness is a stronger predictor of fasting insulin levels than fatness in overweight male middle-school children. *J Pediatr* **150**, 383–387.
30. Thomas NE, Cooper SM, Williams SP *et al.* (2007) Relationship of fitness, fatness, and coronary-heart-disease risk factors in 12- to 13-year-olds. *Pediatr Exerc Sc* **19**, 93–101.
31. Aires L, Silva P, Silva G *et al.* (2010) Intensity of physical activity, cardiorespiratory fitness, and body mass index in youth. *J Phys Act Health* **7**, 54–59.

NS Public Health Nutrition

32. Twisk JW, Kemper HC & van Mechelen W (2002) The relationship between physical fitness and physical activity during adolescence and cardiovascular disease risk factors at adult age. The Amsterdam Growth and Health Longitudinal Study. *Int J Sports Med* **23**, Suppl. 1, S8–S14.
33. Andersen LB, Harro M, Sardinha LB *et al.* (2006) Physical activity and clustered cardiovascular risk in children: a cross sectional study (The European Youth Heart Study). *Lancet* **368**, 299–304.
34. Kelishadi R, Cook SR, Amra B *et al.* (2009) Factors associated with insulin resistance and non-alcoholic fatty liver disease among youths. *Atherosclerosis* **204**, 538–543.
35. Shaibi GQ, Ball GD, Cruz ML *et al.* (2006) Cardiovascular fitness and physical activity in children with and without impaired glucose tolerance. *Int J Obes (Lond)* **30**, 45–49.
36. Katzmarzyk PT, Church TS & Blair SN (2004) Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all cause and cardiovascular disease mortality in men. *Arch Intern Med* **164**, 1092–1097.
37. Telford RD, Cunningham RB, Shaw JE *et al.* (2009) Contrasting longitudinal and cross-sectional relationships between insulin resistance and percentage of body fat, fitness, and physical activity in children – the LOOK study. *Pediatr Diabetes* **10**, 500–507.
38. Puder JJ, Schindler C, Zahner L *et al.* (2011) Adiposity, fitness and metabolic risk in children: a cross-sectional and longitudinal study. *Int J Pediatr Obes* **6**, e297–e306.
39. Ostojic SM & Stojanovic MD (2010) High aerobic fitness is associated with lower total and regional adiposity in 12-year-old overweight boys. *J Sports Med Phys Fitness* **50**, 443–449.
40. Rizzo NS, Ruiz JR, Hurtig-Wennlöf A *et al.* (2007) Relationship of physical activity, fitness, and fatness with clustered metabolic risk in children and adolescents: the European Youth Heart Study. *J Pediatr* **150**, 388–394.
41. Brage S, Wedderkopp N, Ekelund U *et al.* (2004) Features of the metabolic syndrome are associated with objectively measured physical activity and fitness in Danish children: the European Youth Heart Study (EYHS). *Diabetes Care* **27**, 2141–2148.
42. Pedersen BK & Saltin B (2006) Evidence for prescribing exercise as therapy in chronic disease. *Scand J Med Sci Sports* **16**, 3–63.
43. Parrett AL, Valentine RJ, Arngrímsson SA *et al.* (2010) Adiposity, activity, fitness, and C-reactive protein in children. *Med Sci Sports Exerc* **42**, 1981–1986.
44. Ring-Dimitriou S, von Duvillard SP, Paulweber B *et al.* (2007) Nine months aerobic fitness induced changes on blood lipids and lipoproteins in untrained subjects versus controls. *Eur J Appl Physiol* **99**, 291–299.
45. Anderssen SA, Cooper AR, Riddoch C *et al.* (2007) Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. *Eur J Cardiovasc Prev Rehabil* **14**, 526–531.
46. Kwon S, Burns TL & Janz K (2010) Associations of cardiorespiratory fitness and fatness with cardiovascular risk factors among adolescents: the NHANES 1999–2002. *J Phys Act Health* **7**, 746–753.
47. Hannon TS, Janosky J & Arslanian SA (2006) Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. *Pediatr Res* **60**, 759–763.
48. Choi HK & Ford ES (2007) Prevalence of the metabolic syndrome in individuals with hyperuricemia. *Am J Med* **120**, 442–447.
49. Kadoglou NP, Iliadis F, Angelopoulou N *et al.* (2009) Cardiorespiratory capacity is associated with favourable cardiovascular risk profile in patients with Type 2 diabetes. *J Diabetes Complications* **23**, 160–166.
50. Lee YH, Song YW, Kim HS *et al.* (2010) The effects of an exercise program on anthropometric, metabolic, and cardiovascular parameters in obese children. *Korean Circ J* **40**, 179–184.
51. Wijnstok NJ, Twisk JW, Young IS *et al.* (2010) Inflammation markers are associated with cardiovascular diseases risk in adolescents: the Young Hearts project 2000. *J Adolesc Health* **47**, 346–351.
52. Martos R, Valle M, Morales RM *et al.* (2009) Changes in body mass index are associated with changes in inflammatory and endothelial dysfunction biomarkers in obese prepubertal children after 9 months of body mass index SD score loss. *Metabolism* **58**, 1153–1160.
53. McMurray RG, Bangdiwala SI, Harrell JS *et al.* (2008) Adolescents with metabolic syndrome have a history of low aerobic fitness and physical activity levels. *Dyn Med* **7**, 5.

NS Public Health Nutrition

II

EVALUATION OF METABOLIC RISK IN GIRLS VS BOYS IN RELATION WITH FITNESS AND PHYSICAL ACTIVITY

Llorente-Cantarero FJ[a,b], Pérez-Navero JL[b], Benitez-Sillero JD[a], Muñoz-Villanueva MC[c], Gil-Campos M[b]

GENDER MEDICINE. 2012; VOL. 9, NO. 6

[a]Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain.
[b]Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Córdoba, Spain.
[c]Unit of Methodology in Investigation, IMIBIC, Córdoba, Spain

Evaluation of Metabolic Risk in Prepubertal Girls Versus Boys in Relation to Fitness and Physical Activity

Francisco Jesus Llorente-Cantarero, BS[1,2]; Juan Luis Pérez-Navero, MD, PhD[2]; Juan de Dios Benitez-Sillero, PhD[1]; Maria Carmen Muñoz-Villanueva, MD, PhD[3]; and Mercedes Gil-Campos, MD, PhD[2]

[1]Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain; [2]Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Cordoba Spain; and [3]Unit of Methodology in Investigation, Maimonides Institute for Biomedical Research (IMIBIC), Córdoba, Spain

ABSTRACT

Background: Low levels of cardiorespiratory fitness (CRF) and physical activity (PA) are associated with a risk of the development of metabolic syndrome. Contradictory findings are reported in the literature regarding the influence of sex and CRF and PA on metabolic changes.

Objective: The aim of this study was to analyze the effects of CRF and PA on lipid and carbohydrate metabolism biomarkers in boys and girls.

Methods: A total of 82 prepubertal boys and 55 girls (7–12 years of age) were classified according to sex, low or high CRF, and performance or nonperformance of PA. Anthropometric and blood pressure (BP) measurements, plasma lipid profile values, glucose and insulin levels, and homeostasis model assessment for insulin resistance were analyzed.

Results: The percentage of boys with high CRF and performance of PA was higher than that of girls ($P < 0.05$). When children of the same sex were compared, higher values for body mass index and waist circumference z-scores were found for boys with low CRF compared with boys with high CRF ($P < 0.001$) without differences between girls, and in all groups classified by PA. Systolic and diastolic BPs were higher in boys than in girls, in both CRF and PA groups ($P < 0.05$). In the low CRF and no PA groups, girls had higher plasma glucose, total cholesterol, and low-density lipoprotein cholesterol levels than boys, with higher high-density lipoprotein cholesterol and apolipoprotein A levels ($P < 0.05$).

Conclusions: Sex in relation to CRF and PA could affect the plasma lipid profile. These changes in girls are associated with low CRF and low levels of PA. Considering these results, we suggest the need to improve CRF and promote PA, especially in girls, to reduce metabolic risk. (*Gend Med*. 2012;9:436–444)

Key words: childhood, fitness, metabolic biomarkers, physical activity.

INTRODUCTION

Low levels of cardiorespiratory fitness (CRF) and physical activity (PA) have been associated with a greater risk of the development of metabolic syndrome (MS),[1] cardiovascular disease (CVD), and type 2 diabetes.[2] However, the literature is contradictory regarding whether CRF and BP are related or act via different mechanisms.[3,4] Although the association between CRF and metabolic risk factors appears to be mediated by adiposity,[5] an independent inverse relationship has been reported between PA and cardiovascular risk.[3] In children, it has been reported that PA and CRF are separately and independently associated with individual and clustered metabolic risk factors.[4] It would be important to note which of these 2 variables is more associated with metabolic parameters to propose strategies to prevent or treat diseases. The approach is to know how we should act, whether to achieve good physical condition or greater PA.

http://dx.doi.org/10.1016/j.genm.2012.08.006

1550-8579/$ - see front matter

Regular PA in young people may help to reduce overweight/obesity and insulin resistance (IR),[6] leading to an improvement in CRF.[5] In adolescents, IR appears to be inversely related to regular PA, independently of adiposity and fat localization.[7] Moreover, a correlation has been reported between CRF and changes in insulin levels in children.[2,5]

It is not yet clear to what extent the relationships among PA, CRF, and metabolic risk factors are influenced by sex. Some studies have suggested that the impact of high fat mass, low CRF, and low PA on increased insulin levels is greater in boys, whereas in girls, changes in insulin levels are associated only with changes in fat mass.[2,6] On the other hand, in another study, increased PA and CRF helped to control IR in boys.[8] An earlier study of children 5 to 8 years of age highlighted a correlation between PA and homeostasis model assessment for insulin resistance (HOMA-IR) in girls but not in boys.[9] Other authors, however, have reported that IR varies with CRF in both sexes.[3] Age and pubertal status can initiate transient metabolic and hormonal changes as well as changes in fat mass, which may be responsible, at least in part, for the differences observed in other studies.[4,5,7] With respect to this, it has been suggested that estrogen may protect against the damaging effects of low CRF on IR in young women.[10]

Given the contradictory findings reported in the literature regarding the influence of sex and CRF and PA on metabolic changes, this study sought to analyze the effects of CRF and PA on lipid and carbohydrate metabolism biomarkers in prepubertal boys and girls.

PATIENSTS AND METHODS

Subjects and Design

We encouraged 450 children from 2 local elementary schools to participate in the study. All the children attending school were of middle socioeconomic status. Initially, a total of 156 prepubertal children were to take part in the study; however, some of them were excluded because they did not meet the inclusion criteria, decided not to participate, or did not complete the study, or vein puncture blood sampling was not possible. Finally, a group of 137 Spanish children (82 boys, 55 girls) were selected. Children were asked to perform a 20-m shuttle run test (SRT) to evaluate their CRF.[11] Inclusion criteria were age 7 to 12 years and definite prepubertal stage Tanner I[12] validated by appropriate plasma sex hormone levels. Exclusion criteria were as follows: the presence of pubertal development, disease, long recuperation period after illness, use of any drug that could alter blood pressure (BP) or glucose or lipid metabolism, or intake of a particular type of diet. Written informed consent was obtained from a parent or guardian, and the study procedures were verbally explained to all the children. Approval of the study was obtained from the local research ethics committee in accordance with the Declaration of Helsinki.

Children were classified into 2 groups according to the methodology used in other studies.[13,14] Those children with a score equal to or greater than the average reference value for the 20-m SRT were assigned to the high CRF (HCRF) group, using a validated scale developed by Olds et al,[11] defined for each age and sex. Those with lower than average scores were assigned to the low CRF (LCRF) group. Regarding PA, the same sample was divided into 2 groups without randomization: one that regularly participated in an after-school program of PA (PPA) and another that did not perform PA and had sedentary habits (no PA).

Anthropometric and BP Measurements

Medical history was obtained, a physical examination was performed by pediatricians, and sexual maturity was established by physical examination according to the Tanner 5-stage scale.[12]

For anthropometric measurements, children were barefoot and in their underwear. Body weight (in kilograms) and height (in meters) were measured using a standard beam balance (Seca, Hamburg, Germany). Body mass index (BMI) was calculated as weight (kg)/height (m^2). Waist circumference (WC) (in centimeters) was precisely measured with a tape measure with the subject standing. Moreover, the z-scores for BMI and WC were calculated. Anthropometric measurements were compared with the Spanish reference stan-

dards[15,16] for age and with sex-specific cutoff points to estimate obesity.[17]

BP was measured with a random-zero sphygmomanometer (Dinamap V-100; GE Healthcare, Milwaukee, Wisconsin) after resting, with the subject remaining immobile for at least 5 minutes in a sitting position, and using the right arm, unless there was a deformity.

CRF and PA

Fitness

To evaluate CRF, the 20-m SRT and other procedures included in the standardized Eurofit battery test[18] were used. This test requires participants to run back and forth between 2 lines set 20 m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at 20 minutes. Running speed cues were indicated by signals emitted by a commercially available CD-ROM (Multistage fitness test [Bleep Test], CD version 2009; Sport Coach, Armley, Leeds, United Kingdom). Subjects were allowed to voluntarily withdraw from the test after being verbally encouraged to perform maximally during each assessment. The test ended when the subject failed to reach the finish lines concurrent with the audio signals on 2 consecutive occasions.[19]

Physical Activity

Children in the PPA group were observed while engaged in an after-school program to evaluate PA. Previous information from the staff was elicited to select the children for this group. These after-school programs are designed to encourage them to take up some PA due to the increase in sedentary habits in children. With the objective of preventing childhood obesity, the government has established some PA programs in which children can choose the activities they like. These were performed 2 or 3 days per week. Thus, all the children in this study who performed PA had similar activities conducted by the professional staff at the 2 schools. However, children in the present study included in the no PA group did not voluntarily participate in these programs. For the PPA group to participate in the study, the children had to attend the program at least 3 times per week for at least 1 year. Additionally, a short test based on the National Institute of Child Health and Human Development–validated questionnaire[20] and an interview were used for both groups to obtain information about PA performance or sedentary habits. Diversity in activities/programs attended was sought. All the interviews were conducted during the school hours, taking approximately 30 minutes. Sample questions in the individual interview included the following. "Do you participate in the after-school program?" "How many times per week do you spend in the after-school program?" "Describe a typical day in your after-school program or in your after-school time." "Why do (don't) you come to the after-school program?"

Sampling and Biochemical Analysis

Baseline blood samples were obtained from all children after a 12-hour overnight fast, using an indwelling venous line to draw a 3-mL sample to measure plasma glucose and insulin levels and obtain a lipid profile. All samples were processed within 2 hours of sampling and divided into aliquots for immediate analysis.

Glucose was analyzed using the glucose oxidase method in an automatic analyzer (% CV: 1%). Plasma triacylglycerols (TGs) (% CV: 1.5%), total cholesterol (Chol) (% CV: 0.9%), high-density lipoprotein cholesterol (HDL-c) (% CV: 0.8%), low-density lipoprotein cholesterol (LDL-c) (% CV: 1.5%), apolipoprotein (apo) AI (% CV: 1.7%), and apo B (% CV: 2.6%) were measured using an automatic analyzer (Accelerator APS system, Architect-c16000, Abbott Laboratories, Abbott Park, Illinois). Plasma insulin was analyzed by radioimmunoassay with an automatic analyzer for microparticles (Axsym, Abbott Laboratories). IR was assessed using the equation HOMA = fasting glucose (mM) × fasting insulin (μU/mL)/22.5.[21]

Statistical Analysis

Data were expressed as mean (SD). Normality of data distribution was assessed by the Shapiro-Wilk test. Homogeneity of variance was estimated using the Levene test. A Student *t* test for unpaired samples was used for mean comparisons between groups for continuous variables with normal dis-

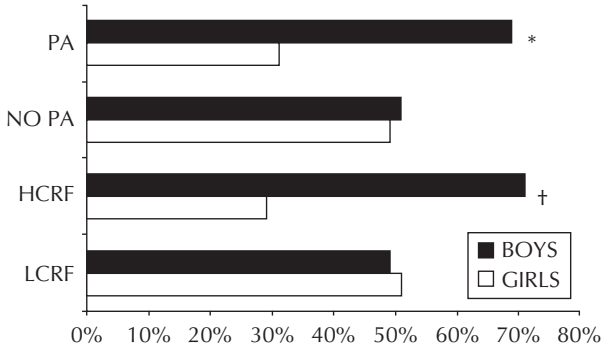


Figure 1. Percentages of prepubertal boys and girls according to different levels of cardiorespiratory fitness (CRF) and physical activity (PA). HCRF = high cardiorespiratory fitness group; LCRF = low cardiorespiratory fitness group; PPA = group that performed PA; NO PA = group that did not perform PA. Statistical significance after application of χ^2 test. Data are expressed as percentages. Significant differences between boys and girls in the HCRF group and PA group: $^*P \leq 0.01$; $^\dagger P \leq 0.05$.

tribution and the Mann-Whitney *U* test for those with an asymmetric distribution. Comparisons between the groups of children after the SRT were performed after adjusting for age and BMI. SPSS software version 18 (SPSS, Inc, Chicago, Illinois) was used for all statistical analyses.

RESULTS

Subject percentages by sex for CRF and PA levels in boys compared with girls are shown in **Figure 1**. There was a similar percentage of girls and boys in the LCRF and no PA groups. The percentage of boys with high CRF and who exercised regularly was strikingly higher than that of girls.

Cardiovascular Fitness

Results for anthropometric measurements, BP, carbohydrate metabolism, and lipid profile parameters according to the CRF levels are shown in **Table I**. Higher z-scores for BMI and WC were recorded for more prepubertal boys than girls in LCRF groups. After comparing the sex cutoff points of Cole et al,[17] only 14 girls were overweight and 7 were obese, and 37 boys were overweight and 12 were obese.

Systolic and diastolic BPs were higher in boys than in girls in the CRF groups. In the HCRF group, girls had higher blood glucose levels than boys, whereas in the LCRF group, girls had higher glucose, insulin, and HOMA-IR values.

After analyzing the lipid profile, in the LCRF group, girls had higher plasma Chol and LDL-c levels than boys. However, HDL-c levels were also higher in girls than boys in both CRF groups.

Direct comparisons between girls (and between boys) with LCRF and HCRF were also done. Higher BMI and WC z-scores, systolic BP, insulin, and HOMA-IR, cholesterol, and LDL-c and lower HDL-c and apo A levels were found for boys with LCRF than boys with HCRF. Girls with HCRF compared with girls with LCRF had higher HDL-c and apo A levels and lower apo B levels.

Physical Activity

The results of anthropometric measurements, BP, carbohydrate metabolism, and lipid profile according to the levels of PA are shown in **Table II**. Systolic and diastolic BPs were higher in boys than in girls in these PA groups. In children in the no PA group, girls had higher glucose, HOMA-IR, Chol, LDL-c, and TG levels than boys. Moreover, HDL-c levels were also higher in girls than in boys of these groups. Nonetheless, in the PPA group, blood glucose values were higher in boys (**Table II**).

Comparing children of the same sex classified by PA (girls vs girls and boys vs boys), there were higher Chol, TG, LDL-c, HDL-c, apo A, and apo B levels in girls in the no PA group compared with girls in the PPA group. In boys, there were no significant differences comparing the no PA and PA groups. After adjusting data for BMI and age, the analysis showed that BP values were influenced by CRF, PA, and sex. All lipid parameters were also affected by these 3 factors, with the exception of Chol ($P \leq 0.05$) and TGs ($P \leq 0.05$), which were not affected by sex. Glucose and insulin levels as well as the HOMA-IR also varied because of these factors but independently of sex ($P < 0.001$).

DISCUSSION

In this study, we provide new information regarding the influence of sex on changes in metabolic parameters, which, in turn, is also related to the CRF and PA in each sex. Although we cannot es-

Table I. Demographic data, anthropometric measurements, blood pressure, carbohydrate metabolism, and plasma lipid profile biomarkers in prepubertal girls and boys according to different levels of cardiorespiratory fitness (CRF).

	Low CRF (N = 63)			High CRF (N = 74)		
Variables	Girls (n = 32)	Boys (n = 31)	*P**	Girls (n = 23)	Boys (n = 51)	*P**
Age, y	9.00 (0.67)	10.35 (1.02)	<0.001	8.48 (1.08)	10.04 (0.96)	<0.001
BMI z-score, kg/m^2	0.72 (1.18)	1.67 (1.05)†	0.001	0.11 (0.81)	0.41 (0.93)	0.821
WC z-score, cm	0.59 (1.47)	1.39 (1.26)†	0.048	0.01 (0.98)	−0.14 (0.97)	0.998
SBP, mm Hg	114.53 (15.57)	131.74 (11.74)†	<0.001	116.19 (9.45)	121.68 (10.57)	<0.001
DBP, mm Hg	63.53 (11.57)	70.87 (7.9)	0.002	64.14 (8.03)	68.20 (9.55)	0.001
Glucose, mmol/L	4.89 (0.38)	4.51 (0.32)	<0.001	4.75 (0.33)	4.51 (0.42)	0.008
Insulin, mU/L	8.54 (4.18)	8.82 (4.05)‡	0.834	6.40 (2.08)	5.87 (4.82)	0.054
HOMA-IR, μU/mL	1.86 (0.93)	1.79 (0.87)‡	0.773	1.35 (0.48)	1.19 (1.07)	0.025
Chol, mg/dL	182.72 (30.74)	159.43 (23.61)‡	0.001	177.71 (31.47)	170.92 (20.65)	0.371
TGs, mg/dL	71.62 (20.93)	68.76 (26.09)	0.418	63.71 (17.17)	59.14 (19.99)	0.236
LDL-c, mg/dL	110.59 (29.68)	97.1 (15.48)	0.029	101.57 (20.52)	100.57 (18.81)	0.843
HDL-c, mg/dL	57.41 (11.59)‡	50.55 (10.63)†	0.026	63.00 (15.53)	58.14 (9.42)	0.007
Apo A, mg/dL	145.94 (16.98)‡	137.47 (19.34)§	0.155	150.43 (32.72)	150.1 (15.71)	0.270
Apo B, mg/dL	75.16 (18.08)‡	71.43 (9.98)	0.494	66.81 (11.29)	72.65 (12.24)	0.127

Apo AI = apolipoprotein AI; Apo B = apolipoprotein B; BMI = body mass index; Chol = total cholesterol; CRF = cardiorespiratory fitness; DBP = diastolic blood pressure; HDL-c = high-density lipoprotein; HOMA-IR = homeostasis model assessment for insulin resistance; LDL-c = low-density lipoprotein; SBP = systolic blood pressure; TGs = triacylglycerols; WC = waist circumference.
Data are expressed as mean (SD).
*Statistical significance between groups of girls and boys after application of Student's *t* test for unpaired samples and the Mann-Whitney *U* test for those with an asymmetric distribution.
†$P \leq 0.001$.
‡$P \leq 0.05$.
§$P \leq 0.01$.
Significant differences between groups of the same sex (boys compared with boys and girls compared with girls) with different CRF.

tablish exactly what determines that girls have metabolic differences in these variables compared with boys, this study shows that girls have lower CRF and perform less PA. The girls in the LCRF and no PA groups had higher plasma glucose, Chol, LDL-c, HDL-c, and apo A levels compared with boys, despite being younger than boys and having lower BMI and BP.

CRF reflects the overall capacity of the cardiovascular and respiratory systems and the ability to perform exercise for prolonged periods. It expresses a set of attributes associated with either health or skill, and it has a strong genetic component that remains relatively static, needing some time to change.[3,22] However, PA is often used interchangeably with energy expenditure and physical fitness, but its definition implicates any body movement produced by skeletal muscles that results in energy expenditure.[4,23] Therefore, PA may exert an independent beneficial effect on health regardless of fitness.

Exercise training has been inversely associated with metabolic risk factors[24] and with an improvement in glucose metabolism, inflammation, and lipid profile; this highlights the impact of PA on cardiovascular risk.[25] Independent associations of PA with CRF and fatness have been reported, suggesting the involvement of different pathways.[3,4] Other studies, however, report a weaker correlation, or no correlation at all, even after further adjustment for fatness and CRF.[5] On the other hand, a relationship between high CRF and low TG levels has been also noted, suggesting that CRF, more than PA, could influence the lipid profile.[26,27]

With regard to sex, its influence on the CRF and PA in relation to metabolism remains somewhat unclear; in fact, few studies have been published

Table II. Demographic data, anthropometric measurements, blood pressure, carbohydrate metabolism, and plasma lipid profile biomarkers in prepubertal girls and boys according to different levels of physical activity (PA).

	No PA (N = 59)			PPA (N = 78)		
Variables	Girls (n = 30)	Boys (n = 29)	*P**	Girls (n = 25)	Boys (n = 53)	*P**
Age, y	8.61 (0.74)	9.93 (1.01)	**<0.001**	9.00 (1.00)	10.29 (0.95)	**<0.001**
BMI z-score, kg/m^2	0.46 (1.19)	0.76 (1.09)	0.907	0.49 (0.97)	1.04 (1.24)	0.265
WC z-score, cm	0.18 (1.40)	0.31 (1.21)	**1.000**	0.56 (1.21)	0.58 (1.41)	1.000
SBP, mm Hg	11.86 (15.74)	122.03 (11.06)	**0.017**	117.80 (9.84)	128.26 (13.69)	**0.001**
DBP, mm Hg	61.82 (10.68)	67.10 (9.59)	**0.024**	65.96 (9.44)	70.41 (8.81)	**0.05**
Glucose, mmol/L	4.90 (0.36)	4.47 (0.45)	**<**0.001	4.77 (0.37)	4.53 (0.37)	**0.006**
Insulin, mU/L	7.24 (2.95)	5.92 (3.25)	**0.066**	8.15 (4.24)	7.66 (5.44)	0.312
HOMA-IR, μU/mL	1.58 (0.68)	1.17 (0.65)	**0.017**	1.75 (0.95)	1.56 (1.19)	0.207
Chol, mg/dL	191.71 (25.27)†	167.27 (21.56)	<0.001	168.44 (32.29)	166.36 (22.93)	0.775
TGs, mg/dL	71.89 (22.96)	61.34 (19.87)	0.098	64.68 (14.94)	66.53 (30.24)	0.407
LDL-c, mg/dL	113.82 (26.93)‡	99.79 (17.03)	**0.024**	99.4 (24.52)	98.82 (18.32)	0.909
HDL-c, mg/dL	63.07 (10.43)‡	54.79 (10.78)	**0.006**	55.76 (15.47)	55.35 (10.46)	0.550
Apo A, mg/dL	154.57 (14.99)‡	144.10 (18.90)	**0.035**	140.04 (30.11)	145.71 (17.76)	0.490
Apo B, mg/dL	76.21 (17.02)	72.10 (11.68)	0.363	66.96 (13.87)	72.61 (11.34)	0.071
	154.57 (14.99)‡					

Apo AI= apolipoprotein A1; Apo B = apolipoprotein B; BMI = body mass index; Chol = total cholesterol; DBP = diastolic blood pressure; HDL-c = high-density lipoprotein; HOMA-IR = homeostasis model assessment for insulin resistance; LDL-c = low-density lipoprotein; PA = physical activity; PPA = performance of physical activity; SBP = systolic blood pressure; TGs = triacylglycerols; WC = waist circumference.

Data are expressed as mean (SD).

*Statistical significance between groups of girls and boys after application of Student's *t* test for unpaired samples and the Mann-Whitney *U* test for those with an asymmetric distribution.

$^{\dagger}P \leq 0.05$.

$^{\ddagger}P \leq 0.01$.

Bold values represent significant differences between groups of the same sex (boys compared with boys and girls compared with girls) with different PA.

on this topic in children. It has been reported that sex may be a significant predictor of CRF, although other studies report no association. In children at various stages of puberty (Tanner stages I–III),[4,5] and in healthy adolescents (Tanner stage IV),[5,22–28] it has been reported that CRF is higher in boys than in girls. In the present study, similar results were found: 70% of boys had better CRF compared with girls (**Figure 1**).

Although some authors attribute this disparity to differences in sexual maturity,[29] with girls generally maturing earlier than boys, this explanation does not hold here because only prepubertal children were involved. It is clear that age is an important predictor because it contributes to the improvement in running economy that occurs during growth and development. When running time is considered in 20mSRT, the values increase with stage of maturity. In this work, the effect of puberty on metabolic processes was excluded by avoiding a mixed sample of children and adolescents, which might have distorted the results. The age difference between the groups of boys and girls was ~1 year, and all participants were at Tanner sexual development stage I to avoid the effect of increased body fat associated with sexual maturity,[29] usually attributed to the decrease in CRF reported in girls during adolescence.

Different authors use BMI and WC when referring adiposity. In the present study, we did not measure fat mass directly, but adiposity was evaluated using BMI and WC measurements.[30] We also used the widely accepted classification of childhood obesity proposed by Cole et al[17] to estimate whether our sample was healthy or overweight or obese. In the present study, however, BMI and WC

z-scores and BP values were higher in boys than in girls in the LCRF groups (**Table I**). Moreover, these variables were also higher in boys with LCRF than in other boys with better CRF without changes due to performing PA. Similar findings were reported in other studies involving adolescents and prepubertal children,[4,19] and a positive correlation was reported between age and CRF in boys but not girls.[4] Similar results were obtained in this study.

With respect to metabolic markers, boys with HCRF had significantly lower glucose, insulin, and HOMA-IR values than girls in the HCRF group, suggesting a lower risk of IR in this group of boys (**Table I**).

Similarly, most of the boys in the study performed PA (**Figure 1**), and the number of girls who chose to participate in the after-school program was lower than boys. In other studies of children of different ages, lower fitness and lower exercise levels in girls have been attributed to genetic and sociocultural factors.[4] In the present work, the selection criteria were similar for boys and girls because all of them were invited to participate in this program at the same time and with the same physical conditions. The socioeconomic level of the boys and girls was similar. However, it has been reported that girls are generally devalued in society and are more vulnerable to physical and emotional abuse and assault, which undermines the perception of personal agency.[31]

In the no PA group, girls exhibited higher glucose levels and higher HOMA-IR values than boys (**Table II**). However, after adjusting for sex, the results were independent of this factor. Telford et al[8] reported that the HOMA-IR increased between the ages of 8 and 10 years in both boys and girls, the latter displaying not only greater absolute values of HOMA-IR in both age groups but also greater changes over a 2-year period. These data complement previous cross-sectional findings indicating higher HOMA-IR values in girls than in boys, even in 5 year olds not selected because of obesity or illness.[32] These authors found significant longitudinal relationships between the HOMA-IR and both CRF and PA in boys, fewer of whom showed signs of advanced physical development compared with girls, and no variation was found at Tanner stage I.[8] However, physical maturation is known to be an influential factor in the widening sex gap in the HOMA-IR, and the accelerating growth in this parameter could be greater in girls because of advanced maturation compared with boys.[8]

With regard to the lipid profile, Chol and LDL-c plasma levels were higher in girls with LCRF (**Table I**) and in girls the no PA group (**Table II**) compared with their male counterparts. Nonetheless, these sex-related differences were noticeably absent in the HCRF groups (**Table I**) and in the PPA groups (**Table II**). Several studies with adolescents found lower CRF levels and higher lipid marker levels in girls, even when the prevalence of overweight is lower than in boys.[28] Overall, HDL-c was higher in girls, probably due to other factors such as sex, age, and eating habits. HDL-c has not been associated with PA,[4] but in the present study, after adjusting for CRF, PA, and sex, neither Chol nor TG was sex dependent, whereas other lipid parameters were dependent on CRF and PA. Moreover, no PA girls had higher levels of most of the lipid parameters with respect to girls who perform PA, suggesting that sedentary habits could influence this profile in girls (**Table II**).

The limitations of this study, despite the rigorous inclusion criteria applied, are mainly due to the refusal of some children to take part in the study as well as the influence of some socioeconomic and demographic factors, which may influence the results of PA, CRF, or BMI evaluation. In fact, the prevalence of overweight and obesity in Spain has been increasing, higher than in other European countries. Following World Health Organization standards, the prevalence of overweight in Spain found in a recent multicenter study with 7500 children 6 to 9.9 years of age from 2010 to 2011 (ALADDIN) was 26.3%, whereas the prevalence in girls was 25.9%.[33] The prevalence of obesity was 22% in boys and 16.2% in girls. Therefore, the registered data are similar to the sample in the present study.

In summary, these results suggest that low CRF and no PA prompt changes in the lipid profile and carbohydrate metabolism biomarkers. The atten-

dant metabolic risk seems to be greater in girls, particularly in those not performing PA. However, improvement of these conditions could eliminate the risk of metabolic disease.

CONCLUSIONS

Sex, in relation to CRF and PA, seems to condition the metabolic status increasing the risk of alterations in the lipid profile in girls versus boys. These changes are associated with low CRF and the lack of PA. Taking these results into account, we suggest the need to promote PA, especially in girls, to improve their fitness and consequently reduce the risk of metabolic disease.

ACKNOWLEDGMENTS

We thank the nursery group and physical trainers who provided help during the research. We also acknowledge the language review by some English-speaking members of our clinical group. Mr. Llorente-Cantarero, Dr. Pérez-Navero, and Dr. Gil-Campos contributed substantially to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and final approval of the version to be published. Dr. Benitez-Sillero contributed to the conception and design and revised the manuscript. Dr. Muñoz-Villanueva contributed to the statistical analysis and interpreted the results.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

REFERENCES

1. LaMonte MJ, Blair SN. Physical activity, cardiorespiratory fitness, and adiposity: contributions to disease risk. *Curr Opin Clin Nutr Metab Care*. 2006; 540–546.
2. Imperatore G, Cheng YJ, Williams DE, et al. Physical activity, cardiovascular fitness, and insulin sensitivity among U.S. adolescents: the National Health and Nutrition Examination Survey, 1999–2002. *Diabetes Care*. 2006;29:1567–1572.
3. Steele RM, Brage S, Corder K, et al. Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. *J Appl Physiol*. 2008;105: 342–351.
4. Ekelund U, Anderssen SA, Froberg K, et al. European Youth Heart Study Group. Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. *Diabetologia*. 2007;50: 1832–1840.
5. Ruiz JR, Ortega FB, Rizzo NS, et al. High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. *Pediatr Res*. 2007;61:350–355.
6. Gutin B, Yin Z, Humphries MC, et al. Relations of moderate and vigorous physical activity to fitness and fatness in adolescents. *Am J Clin Nutr*. 2005;81: 746–750.
7. Platat C, Wagner A, Klumpp T, et al. Relationships of physical activity with metabolic syndrome features and low-grade inflammation in adolescents. *Diabetologia*. 2006;49:2078–2085.
8. Telford RD, Cunningham RB, Shaw JE, et al. Contrasting longitudinal and cross-sectional relationships between insulin resistance and percentage of body fat, fitness, and physical activity in children-the LOOK study. *Pediatr Diabetes*. 2009;10: 500–507.
9. Metcalf BS, Voss LD, Hosking J, et al. Physical activity at the government-recommended level and obesity-related health outcomes: a longitudinal study (Early Bird 37). *Arch Dis Child*. 2008;93: 772–777.
10. Munoz J, Derstine A, Gower BA. Fat distribution and insulin sensitivity in postmenopausal women: influence of hormone replacement. *Obes Res*. 2002;10:424–431.
11. Olds T, Tomkinson G, Léger L, et al. Worldwide variation in the performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle run test in 37 countries. *Sports Sci*. 2006; 24:1025–1038.
12. Tanner JM. Growth at adolescence. Oxford: Blackwell; 1962.
13. García-Artero E, Ortega FB, Ruiz JR, et al. Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study). *Rev Esp Cardiol*. 2007;60:581–588.

14. McGavock JM, Torrance BD, McGuire KA, et al. Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. *Obesity*. 2009; 17:1802–1807.
15. Sobradillo B, Aguirre A, Aresti U, et al. *Curvas y Tablas de Crecimiento (Estudios Longitudinal y Transversal*. Madrid, Spain: Fundación Faustino Orbegozo Eizaguirre, 2004.
16. Moreno LA, Pineda I, Rodríguez G, et al. Waist circumference for the screening of the metabolic syndrome in children. *Acta Paediatr*. 2002;91, 1307–1312.
17. Cole TJ, Bellizzi MC, Flegal KM, et al. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;320:1240–1243.
18. Committee of Experts on Sports Research EUROFIT. *Handbook for the EUROFIT Tests of Physical Fitness*. Strasburg, France: Council of Europe, 1993.
19. Vale S, Santos R, Soares-Miranda L, et al. The relationship of cardiorespiratory fitness, birth weight and parental BMI on adolescents' obesity status. *Eur J Clin Nutr*. 2010;64:622–627.
20. Pianta RC; NICHD. Developmental science and education: the NICHD study of early child care and youth development findings from elementary school. *Adv Child Dev Behav*. 2007;35:253–296.
21. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
22. Moliner-Urdiales D, Ruiz JR, Ortega FB, et al. AVENA and HELENA Study Groups. Secular trends in health-related physical fitness in Spanish adolescents: the AVENA and HELENA studies. *J Sci Med Sport*. 2010;13:584–588.
23. Froberg K, Andersen LB. Mini review: physical activity and fitness and its relations to cardiovascular disease risk factors in children. *Int J Obes*. 2005;29:34–39.
24. Ostojic SM, Stojanovic MD. High aerobic fitness is associated with lower total and regional adiposity in 12-year-old overweight boys. *J Sports Med Phys Fitness*. 2010;50:443–449.
25. Parrett AL, Valentine RJ, Arngrímsson SA, et al. Adiposity, activity, fitness, and C-reactive protein in children. *Med Sci Sports Exerc*. 2010;42:1981–1896.
26. Suriano K, Curran J, Byrne SM, et al. Fatness, fitness, and increased cardiovascular risk in young children. *J Pediatr*. 2010;157:552–558.
27. Bouglé D, Zunquin G, Sesbouë B, et al. Relationships of cardiorespiratory fitness with metabolic risk factors, inflammation, and liver transaminases in overweight youths. *Int J Pediatr*. 2010;580897.
28. Moreira C, Santos R, Ruiz JR, et al. Comparison of different VO(2max) equations in the ability to discriminate the metabolic risk in Portuguese adolescents. *J Sci Med Sport*. 2011;14:79–84.
29. Mota J, Guerra S, Leandro C, et al. Association of maturation, sex, and body fat in cardiorespiratory fitness. *Am J Hum Biol*. 2002;14:707–712.
30. Olza J, Gil-Campos M, Leis R, et al.; Presence of the Metabolic Syndrome in Obese Children at Prepubertal Age. *Ann Nutr Metab*. 2011;58:343–350.
31. Carruthers C. Processes and outcomes of an afterschool program for adolescent girls. *JPRA*. 2006;24: 127–152.
32. Murphy MJ, Metcalf BS, Voss LD, et al.; EarlyBird Study (EarlyBird 6). Girls at five are intrinsically more insulin resistant than boys: the Programming Hypotheses Revisited The EarlyBird Study (EarlyBird 6). *Pediatrics*. 2004;113:82–86.
33. NAOS strategy: Spanish strategy for nutrition, physical activity and the prevention of obesity. Ministry of Health, Social Services and Equality of Spain. http://www.naos.aesan.mspsi.gob.es/naos/investigacion/aladino. Accessed October 10, 2012.

Address correspondence to: Mercedes Gil-Campos, PhD, Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), C.P. 14004, Córdoba, Spain. E-mail: mercedes_gil_campos@yahoo.es

III

PREPUBERTAL CHILDREN WITH SUITABLE FITNESS AND PHYSICAL ACTIVITY PRESENT REDUCED RISK OF OXIDATIVE STRESS

F.J. Llorente-Cantarero[a,b], M. Gil-Campos[b], J.D. Benitez-Sillero[a], M.C. Muñoz-Villanueva[c], I. Túnez[d], J.L. Pérez-Navero[b]

Free Radic Biol Med. 2012; 53:415-20

[a]*Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain.*
[b]*Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Córdoba, Spain.*
[c]*Unit of Methodology in Investigation, IMIBIC, Córdoba, Spain*

Free Radical Biology and Medicine 53 (2012) 415–420

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

Prepubertal children with suitable fitness and physical activity present reduced risk of oxidative stress

F.J. Llorente-Cantarero [a,b], M. Gil-Campos [b,*], J.D. Benitez-Sillero [a], M.C. Muñoz-Villanueva [c], I. Túnez [d], J.L. Pérez-Navero [b]

[a] Department of Corporal Expression, Faculty of Education, Instituto Maimónides de Investigación Biomédica de Córdoba, University of Córdoba, 14071 Córdoba, Spain
[b] Department of Pediatrics, University Reina Sofia Hospital, Instituto Maimónides de Investigación Biomédica de Córdoba, University of Córdoba, 14071 Córdoba, Spain
[c] Unit of Methodology in Investigation, Instituto Maimónides de Investigación Biomédica de Córdoba, University of Córdoba, 14071 Córdoba, Spain
[d] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Instituto Maimónides de Investigación Biomédica de Córdoba, University of Córdoba, 14071 Córdoba, Spain

ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form
23 March 2012
Accepted 14 May 2012
Available online 24 May 2012

Keywords:
Oxidative stress
Physical activity
Physical fitness
Preadolescents
Health status measurement
Free radicals

ABSTRACT

To assess the impact of fitness status and physical activity on oxidative stress in prepubertal children, we measured selected biomarkers such as protein carbonyls (PC), lipid peroxidation products, and total nitrites, as well as the antioxidant system: total glutathione (TG), oxidized glutathione (GSSG), reduced glutathione (GSH), superoxide dismutase activity, and glutathione peroxidase. A total of 132 healthy children ages 7–12, at prepubertal stage, were classified into two groups according to their fitness level: low fitness (LF) and high fitness (HF). They were observed while engaged in an after-school exercise program, and a questionnaire was created to obtain information on their physical activity or sedentary habits. Plasma and red blood cells were obtained to analyze biomarkers. Regarding oxidative stress markers, the LF group and the sedentary group showed higher levels of TG and GSSG and a lower GSH/GSSG ratio than the HF group and the children engaged in physical activity. A negative association was found between PC and GSSG and TG and between TG and the GSH/GSSG ratio. Moreover, a negative correlation was found between GSSG and fitness, with a positive correlation with the GSH/GSSG ratio. TG, GSSG, and the GSH/GSSG ratio seem to be reliable markers of oxidative stress in healthy prepubertal children with low fitness or sedentary habits. This research contributes to the recognition that an adequate level of fitness and recreational physical activity in childhood leads to better health and oxidative status.

For many years, cardiorespiratory fitness (CRF) has been thought to be an important marker of physiological status, as it reflects the overall capacity of the cardiovascular and respiratory systems and the ability to perform exercise for prolonged periods [1]. The variability between subjects influences the metabolic outcomes [2] and presence of oxidative stress [3].

Overproduction of oxygen free radicals can damage essential molecules such as nucleic acids, proteins, lipids, and carbohydrates, in a process called oxidative stress (OS) [4–6]. To avoid the interaction of reactive oxygen species (ROS) with macromolecules, cells have developed various detoxification mechanisms and an antioxidant defense system (ADS) [7]. The antioxidant defense system can be subdivided into enzymatic antioxidants, such as glutathione peroxidase (GPx), glutathione reductase, and glutathione *S*-transferase, and enzymatic cascades including superoxide dismutase (SOD), catalase, and nonenzymatic antioxidants—such as uric acid or glutathione [8,9]. However, the behavior of OS in a healthy population with various fitness levels and at different ages has not been sufficiently investigated. In adults, changes in the ADS have been assessed in trained and untrained subjects, suggesting lower OS in trained subjects [7,10]. Similarly, it has been observed that training status in adolescents can influence not only their antioxidant capacity but also the oxidative molecular damage degree [11].

Physical inactivity has been associated with physiological dysfunctions and reduced overall body resistance to OS [12,13]. Generally, at rest, the ADS is effective at controlling circulating free radicals, limiting cellular damage [14]. However, it has been reported that acute aerobic exercise induces OS, whereas regular aerobic exercise decreases oxidant markers and increases antioxidant enzyme activities [15]. Nonetheless, a substantial debate still exists on this matter, mainly because of controversial findings; in fact, most studies undertaken analyzed the OS profile in

Abbreviations: ADS, antioxidant defense system; BMI, body mass index; CRF, cardiorespiratory fitness; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; 4-HDA, 4-hydroxyalkenals; HF, high fitness; LF, low fitness; LPO, lipid peroxidation products; MDA, malondialdehyde; NO_x, total nitrites; OS, oxidative stress; PA, physical activity; PC, protein carbonyls; ROS, reactive oxygen species; SOD, superoxide dismutase; 20-mSRT, 20-m shuttle run test; TG, total glutathione

* Corresponding author. Fax: +34957010017.
E-mail address: mercedes_gil_campos@yahoo.es (M. Gil-Campos).

0891-5849/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2012.05.017

professional athletes or sports players habituated to high-intensity agonistic competitions [16–19], but not in regular practice of exercise or in the general population.

Although metabolic and physiologic differences exist between children and adults [3,20], there are few studies investigating OS induced by fitness and exercise in children. Moreover, it has been shown that children rely less on anaerobic metabolism than adults. So, oxygen flow to working muscles may be greater in children and, consequently, the exercise-induced OS response in children may be higher than that of adults [21]. On the other hand, the effects of low fitness and physical inactivity on OS remain uninvestigated, especially in childhood. The aim of this study was to assess the impact of fitness status and physical activity (PA) on OS in healthy prepubertal children, measured by selected biomarkers such as protein carbonyls (PC), lipid peroxidation products (LPO), and total nitrites (NO_x), as well as on the antioxidant system: total glutathione (TG), oxidized glutathione (GSSG) and reduced glutathione (GSH), SOD activity, and GPx.

Participants and methods

Subjects and design

A total of 132 Spanish children (81 boys, 51 girls) were selected from local elementary schools in Córdoba, Spain, to perform a 20-m shuttle run test (20-mSRT) [22] to evaluate their CRF. Additionally, they were asked about their physical activity and sedentary habits. Afterward, they were observed while engaged in an after-school exercise program to obtain information on their PA.

The inclusion criteria of this study were healthy subjects ages 7–12, at prepubertal stage (Tanner I) [23], confirmed by appropriate plasma sex hormone levels. Exclusion criteria were as follows: presence of pubertal development, disease, long periods of rest after illness, use of any medication that alters blood pressure or metabolism, and failure to repeat the same time reached in the first attempt in the 20-mSRT.

The validated scale developed by Olds et al. [24] was used to measure CRF after the 20-mSRT. The 20-m test performances are indicated as *Z* scores relative to all children of the same age and sex from all countries. In this study, the children were classified into two groups according to the methodology used in previous studies [25,26]: the children recording a score equal to or greater than the average reference value were assigned to one group (70 subjects), designated as "equal or higher fitness" (HF), and those with less-than-average scores (62 subjects) were assigned to the "low fitness" group (LF) (Table 1).

After assessment of exercise habits, the children were classified into two groups: the physical activity practice group (in an after-school program), PAG (76 subjects), and the sedentary group, SG (56 subjects).

Table 1
Demographic, anthropometric, and blood pressure measurements in prepubertal children with different levels of fitness.

Variable	LF ($N=62$)	HF ($N=70$)	*P*
Age (years)	9.62 ± 1.06	9.60 ± 1.23	0.846
Sex (% boys)	48%	71%	0.012
BMI (kg/m^2)	21.48 ± 3.99	18.7 ± 2.82	< 0.001
SBP (mm Hg)	122.3 ± 15.85	120.3 ± 10.54	0.409
DBP (mm Hg)	66.83 ± 10.59	67.23 ± 9.27	0.820

LF, low fitness group; HF, equal or higher fitness group; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. Statistical significance after application of Student's *t* test (or *U* of Mann–Whitney) to data is expressed as the mean $\pm$ SD and χ^2 was used for comparison of proportions.

Written informed consent was obtained from parents or legal guardians, and the study procedures were verbally explained to all children. Ethical approval of the study was given from the local human research and ethics committees.

Physical examination and anthropometric and blood pressure measurements

Anamnesis and physical examination including the evaluation of sexual maturity according to Tanner's 5-stage scale [23] were assessed. Anthropometric measurements (weight, height) were measured using standard techniques. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. Systolic and diastolic blood pressures were measured at rest, three times, on the right arm and while subjects were sitting, using a random-zero sphygmomanometer (Dinamap V-100).

Evaluation of cardiorespiratory fitness and physical activity

To evaluate CRF, the 20-mSRT [22] and other procedures included in the standardized Eurofit battery test [27] were measured [28]. This test requires subjects to run back and forth between two lines set 20 m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute reaching 18.0 km/h at minute 20. Running speed cues were indicated by signals emitted by a commercially available CD–ROM. Subjects were allowed to voluntarily withdraw from the test after being verbally encouraged to maximally perform during each assessment. The test came to its end when the subject failed to reach the finish lines concurrent with the audio signals on two consecutive occasions [29].

To estimate physical activity, it was evaluated whether children engaged in an after-school exercise program at least three times per week for at least 1 year or they were sedentary, and a short test based on the NICHD validated questionnaire [30] was used for both groups to obtain information about PA practice and sedentary habits.

Sampling and biochemical analysis

For all children, only one analytical evaluation was done at a time. Blood samples were collected after a 12-h fasting period and at rest, using an indwelling venous line to draw a 3-ml sample into a tube containing EDTA. After centrifugation at 3500*g* for 10 min, plasma and the buffy coat were removed into different Eppendorf tubes. The erythrocytes were washed three times. All samples—divided into aliquots—were frozen at -80 °C until they were analyzed.

Determination of oxidative stress biomarkers

Lipid peroxidation products

Plasma MDA and 4-hydroxyalkenals (4-HDA) were estimated using the method described by Erdelmeier et al. [31]. This method uses a chromatogenic reagent that reacts with MDA+4-HDA at 45 °C, yielding a stable chromophore with maximum absorbance at 586 nm.

Protein carbonyls

Plasma concentration of PC was measured using the method of Levine et al. [32]. Samples were incubated with 2,4-dinitrophenylhydrazine in HCl for 60 min. Then, proteins were precipitated from the solutions using 500 µl of trichloroacetic acid (20%). Afterward, the proteins were washed with a solution of ethanol and ethyl acetate (1/1 v/v) and dissolved in 1 ml of guanidine

hydrochloride (6 M) at 37 °C. The carbonyls were evaluated in a spectrophotometer (UV-1630; Shimadzu) at a wavelength of 360 nm [33].

Total nitrites (nitrites and nitrates)

NO_x was used as a marker of nitric oxide (NO) levels and assayed following the Griess method [34] in plasma. This assay uses the determination of nitrite as an indicator of NO production in biological samples. NO is transformed into nitrate and nitrite. It is common practice to use either enzymatic or chemical reduction to convert all nitrates into nitrite in a sample and measure total nitrite as an indicator of NO production. When nitrate reduction was complete, total nitrite was spectrophotometrically determined using the Griess reaction. The reaction was monitored at 540 nm. The absorbance was evaluated in a spectrophotometer (UV-1603; Shimadzu) and expressed as grams per milliliter.

Determination of antioxidant biomarkers

Total glutathione, oxidized glutathione, and reduced glutathione

These antioxidant biomarkers in red blood cells were measured using Bioxytech aop-490 TM (Oxis International, Portland, OR, USA). It is based on the reduction of Cu^{2+} to Cu^{+} by the combined action of the antioxidants of the sample. Thus, the chromogenic reagent results in a complex with Cu^{+}, which has an absorbance at 490 nm [35]. TG and GSH levels were evaluated using the Bioxytech GSH-420 and GSH-400 kits, respectively. The determination of TG levels was based on the formation of a chromophoric thione, which has absorbance at 420 nm. The GSH concentration is based on a reaction that leads to the formation of a chromophore with absorbance at 400 nm [36]. GSSG levels were calculated by subtracting GSH from TG.

Activity of antioxidant enzymes: superoxide dismutase and glutathione peroxidase

SOD activity was determined in erythrocytes using a colorimetric assay kit purchased from BioVision Research Products (Mountain View, CA, USA). SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase activity and is inhibited by SOD. Therefore, the inhibition activity of SOD is determined by a colorimetric method.

The activity of GPx was evaluated in red blood cells by the Flohé and Günzler method [37] using a glutathione peroxidase assay kit (Cayman Chemical). This assay is based on the oxidation of NADPH to $NADP^{+}$, which is catalyzed by a limiting concentration of glutathione reductase, with maximum absorbance at 340 nm. Activity was measured based on the formation of GSSG from the GPx-catalyzed oxidation of GSH by H_2O_2, coupled with NADPH consumption, in the presence of exogenously added glutathione reductase, with maximum absorbance at 340 nm.

Statistical analysis

Data are expressed as means ± SD. Normal data distribution was assessed by the Shapiro–Wilk test. Homogeneity of variances was estimated using the Levene test. Mean comparisons between groups of continuous variables with normal distribution were compared by Student's *t* test for unpaired samples, whereas those with an asymmetric distribution were compared using the Mann–Whitney *U* test. Mean ± SD and median ± interquartile range were calculated for each parameter. The χ^2 test was applied for proportion comparisons.

To study associations between oxidative stress biomarkers, a nonparametric correlation analysis was performed. Spearman's ρ correlation coefficients were calculated. The Statistical Package for Social Science software (PASW Statistic 18; SPSS, Chicago, IL, USA) was used for all statistical analyses.

Results

Demographic, anthropometric, and blood pressure measurements in this group of prepubertal children classified according to CRF are described in Table 1. There were no differences in age or blood pressure levels between groups. BMI was higher in the LF group and there were more boys than girls in the HF group (Table 1).

Regarding markers of OS, the LF group showed higher levels of TG and GSSG and lower GSH/GSSG ratio compared with the HF group (Table 2). Nevertheless, there were no significant differences between groups in some oxidant parameters such as LPO, PC, and NO_x and no differences were found in antioxidant biomarkers (GSH, SOD, and GPx).

When the children were studied basing on their PA, no significant changes were observed between the PAG and the SG in oxidant and antioxidant biomarkers, except for TG and GSSG, which were higher in the SG, with a lower GSH/GSSG ratio, compared with PAG children (Table 3).

Table 2
Biomarkers of oxidative stress in prepubertal children with different levels of fitness.

Biomarker of OS	LF ($N=62$)	HF ($N=70$)	*P*
PC (nM) (plasma)	3.71 ± 2.68	3.98 ± 2.9	0.710
LPO (μM) (plasma)	0.21 ± 0.1	0.23 ± 0.12	0.288
NO_x (μM) (plasma)	14.6 ± 3.66	14.63 ± 5.42	0.729
GSH (μM/g hemoglobin) (RBC)	22.7 ± 3.69	22.42 ± 3.81	0.621
GSSG (μM/g hemoglobin) (RBC)	4.67 ± 2.87	3.66 ± 2.54	0.051
TG (μM/g hemoglobin) (RBC)	27.37 ± 4.55	26.08 ± 5.25	0.023
GSH/GSSG (μM/g hemoglobin) (RBC)	9.54 ± 10.66	10.91 ± 10.79	0.048
SOD (U/ml/g hemoglobin) (RBC)	3.44 ± 2.62	3.62 ± 3.94	0.460
GPx (U/ml/g hemoglobin) (RBC)	0.21 ± 0.44	0.21 ± 0.33	0.340

LF, low fitness group; HF, equal or higher fitness group; PC, protein carbonyls; LPO, lipid peroxidation products; NO_x, total nitrites (nitrites and nitrates); GSH, reduced glutathione; GSSG, oxidized glutathione; TG, total glutathione; GSH/GSSG, reduced glutathione/oxidized glutathione ratio; SOD, activity of superoxide dismutase; GPx, glutathione peroxidase; RBC, red blood cells. Statistical significance after application of Student's *t* test (or *U* of Mann–Whitney) to data is expressed as the mean ± SD.

Table 3
Biomarkers of oxidative stress in two groups of prepubertal children: a physical activity practice group in a school program and a sedentary group.

Biomarker of OS	SG ($N=56$)	PAG ($N=76$)	*P*
PC (nM) (plasma)	3.59 ± 2.62	3.98 ± 2.92	0.504
LPO (μM) (plasma)	0.22 ± 0.1	0.21 ± 0.12	0.416
NO_x (μM) (plasma)	14.74 ± 3.89	14.47 ± 5.17	0.547
GSH (μM/g hemoglobin) (RBC)	22.3 ± 3.24	22.75 ± 4.05	0.974
GSSG (μM/g hemoglobin) (RBC)	4.81 ± 2.81	3.59 ± 2.55	0.013
TG (μM/g hemoglobin) (RBC)	27.11 ± 4.04	26.34 ± 5.51	0.050
GSH/GSSG (μM/g hemoglobin) (RBC)	8 ± 7.51	12.2 ± 13.01	0.008
SOD (U/ml/g hemoglobin) (RBC)	3.74 ± 3.18	3.35 ± 3.51	0.359
GPx (U/ml/g hemoglobin) (RBC)	0.15 ± 0.17	0.25 ± 0.47	0.182

PAG, physical activity practice group in school program; SG, sedentary group; PC, protein carbonyls; LPO, lipid peroxidation products; NO_x, total nitrites (nitrites and nitrates); GSH, reduced glutathione; GSSG, oxidized glutathione; TG, total glutathione; GSH/GSSG, reduced glutathione/oxidized glutathione ratio; SOD, activity of superoxide dismutase; GPx, glutathione peroxidase; RBC, red blood cells. Statistical significance after application of Student's *t* test (or *U* of Mann–Whitney) to data is expressed as the mean ± SD.

Table 4
Correlations between various biomarkers of oxidative stress and with fitness test measurements in prepubertal healthy children.

Variable	*r*	*P*
GSH//PC	−0.198	0.024
GSH//LPO	−0.168	0.055
TG//PC	−0.345	<0.001
TG//LPO	−0.227	0.009
TG//GSH	0.738	<0.001
GSSG//PC	−0.321	<0.001
GSSG//LPO	−0.170	0.052
GSSG//TG	0.737	<0.001
GSH/GSSG//PC	0.294	0.001
GSH/GSSG//TG	−0.611	<0.001
GPx//LPO	−0.290	0.001
SOD//GSSG	0.265	0.002
SOD//GSH/GSSG	−0.293	0.001
GSSG//20-mSRT	−0.440	<0.001
GSH/GSSG//20-mSRT	0.333	<0.001

PC, protein carbonyls; LPO, lipid peroxidation products; GSH, reduced glutathione; GSSG, oxidized glutathione; TG, total glutathione; GSH/GSSG, reduced glutathione/oxidized glutathione ratio; SOD, activity of superoxide dismutase; GPx, glutathione peroxidase; 20-mSRT, 20-m shuttle run test. *r*, Spearman's ρ correlation coefficient; *P*, probability.

There were negative and significant associations between PC and LPO with TG, PC with GSSG, and TG with the GSH/GSSG ratio. Positive correlations were found between TG and GSH and between TG and GSSG. Moreover, a negative correlation was found between GSSG and CRF, with a positive correlation with the GSH/GSSG ratio (Table 4).

Discussion

In this study, the biomarkers TG, GSSG, and GSH/GSSG ratio seem to be precocious and sensitive parameters for measuring changes in OS in prepubertal children, in relation to their CRF and PA. Children with low CRF or sedentary habits showed higher levels of TG and GSSG and a lower ratio of GSH/GSSG (Tables 2 and 3).

To evaluate OS and CRF after training, free radical and other OS biomarker levels were found to be lower than before training, except in agonistic training, which exhibits a chronic oxidative insult [38]. Moreover, most of the studies comparing trained and untrained adults indicate that trained adults have higher blood antioxidant enzyme levels both at rest and immediately after exercise [39–41]. Similarly, it has been reported that untrained individuals show a greater rise in OS biomarkers after exercise than trained subjects [42,43]. These observations suggest that appropriate fitness levels might positively reduce OS and partially attenuate its aging effects and that training results in an adaptation to exercise-induced OS [39]. In this work, GSSG and the CRF score measured by 20-mSRT were negatively correlated, and a positive correlation was found between this test and the GSH/GSSG ratio (Table 4). These results agree with other associations described for CRF and OS [3,41].

Specifically, in a few studies in young people and children [38,44], athletes exhibit increased OS and less antioxidant capacity compared with untrained children, and the authors suggest that, at these ages, subjects may be more susceptible to OS induced by chronic exercise. In a recent study, after 6 weeks of resistance exercise training in young males, GSH levels increased when measured at rest, independent of the training intensity [15]. In light of the results obtained, we may suggest that after-school exercise programs have lower intensity and are healthier, as they limit the OS production.

Researchers have regularly studied GSH status as a marker of OS in biological systems, as this seems to be one of the most relevant indices of exercise-induced oxidant production [45]. It is probable that repeated exercise can reduce GSH in the long term and increase GSSG in the blood of child athletes [44]. In contrast, in a study of young people with low training frequency, a well-balanced profile was observed at rest, but they were more susceptible to exercise-induced variations in GSSG/GSH with respect to others with a higher level of training. In fact, and agreeing with our results (Tables 2 and 3), the present study indicates that the GSH/GSSG ratio remains the most sensitive and reliable marker of OS, according to the data reported in scientific literature [38].

LPO have been established as a major pathogenetic mechanism of cellular injury in humans [46]. In these prepubertal children, we found no differences in LPO, PC, and NO_x levels between the groups, probably indicating that OS is minimum and that CRF and PA produce no changes. However, studies in trained adults [10,47,48] have shown lower levels of circulating lipid peroxidation markers than in untrained.

In addition, it has been reported that plasma concentrations of NO_x increased significantly with exercise in elderly women [49]. However, in a study of postmenopausal women, NO_x was not significantly different in the fit group compared with the sedentary group, most likely because of the relatively small sample size and wide interindividual variability [41]. In the present study, the absence of differences in these oxidant parameters may be due to the fact that the children of the sample were healthy and the PAG children were engaged in a non-competitive and less intensive exercise program than other cohorts [10,44]. On the other hand, the prepubertal stage and the young age of the studied subjects could be relevant factors that could explain the absence of OS. Aging [41] and puberty [50] are factors known to influence OS status. In fact, we selected prepubertal children to avoid this factor, including girls, although they were in the LF group in higher proportion. It has been reported that girls are usually more sedentary because of psychological factors. In other studies, genetic and sociocultural factors seem to be involved in this situation [51]. In the present work, the selection criteria were similar for both boys and girls because all of them were encouraged to participate in the same program at the same time. The socioeconomic levels of the boys and girls were similar; all of them were in the same school and lived near. Girls are generally devalued in society and are more vulnerable to physical and emotional abuse, which undermine their perception [52]. Maybe this is the reason the number of girls who chose to participate in the after-school program was lower than that of the boys.

Also, obesity is associated with increased inflammation and OS. Moreover, obese children have distinct patterns of dysregulation in baseline and adaptive oxidative responses to acute exercise that can also increase inflammation and OS [53]. In this work, children with LF had higher BMI than the HF group although they were not obese and, although this situation could influence the results, maybe it was not a main factor.

In this context, the high levels of the antioxidant enzyme GPx and catalase found in fit populations and the positive relationship between GPx and fitness variables support the paradigm that regular exercise upregulates the antioxidant defense, in response to the acute increase in ROS generation during a single bout of exercise [54]. When studying GPx and SOD levels, we found no differences between the low- and the high-CRF group or between the PAG and the SG. These results may indicate that, in prepubertal children, there is a balance between oxidation and reduction mechanisms, regardless of the subject's CRF and PA; this conclusion is also supported in this study by the results obtained for LPO, PC, and NO_x, in that no changes were found

between the groups. This could indicate that, in children at these ages, those with good CRF and practicing regular exercise do not show any OS, whereas sedentary children and those with low CRF show slight OS levels.

On the other hand, negative and significant correlations between PC and LPO with TG, PC with GSSG, and TG with GSH/GSSG ratio show a physiologic answer. In sedentary children and those in poor physical condition, OS is expressed by higher levels of TG and GSSG and a lower GSH/GSSG ratio. These changes could be modulated by a higher activity of the glutathione synthesis enzyme, which results in no significant differences in plasma levels of GSH. However, these inverse relationships could indicate a compensation of the enzymatic system. If oxidation were higher, greater levels of TG, glutathione reductase enzymatic activity, and GSH would be expected to act as an antioxidant to compensate for this situation. Finally, in a situation of OS, the enzymatic system could induce TG consumption in an environment of high levels of other oxidant markers as PC or LPO.

Conclusions

GT, GSSG, and GSH/GSSG ratio seem to be reliable markers of oxidative stress in healthy prepubertal children with low CRF or sedentary habits. The correlations found could indicate that the enzymatic system would act to compensate for OS to avoid an increase in oxidants, although further studies in children are necessary. This research contributes to the recognition that an adequate level of fitness and recreational physical activity in childhood leads to better health and oxidative status.

References

[1] Moliner-Urdiales, D.; Ruiz, J. R.; Ortega, F. B.; Jiménez-Pavón, D.; Vicente-Rodriguez, G.; Rey-López, J. P.; Martínez-Gómez, D.; Casajús, J. A.; Mesana, M. I.; Marcos, A.; Noriega-Borge, M. J.; Sjöström, M.; Castillo, M. J.; Moreno, L. A. AVENA and HELENA Study Groups. Secular trends in health-related physical fitness in Spanish adolescents: the AVENA and HELENA studies. *J. Sci. Med. Sport.* **13:**584–588; 2010.

[2] Steele, R. M.; Brage, S.; Corder, K.; Wareham, N. J.; Ekelund, U. Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. *J. Appl. Physiol.* **105:**342–351; 2008.

[3] Benitez-Sillero, J. D.; Perez-Navero, J. L.; Tasset, I.; Guillen-Del Castillo, M.; Gil-Campos, M.; Tunez, I. Cardiorespiratory fitness and oxidative stress: effect of acute maximal aerobic exercise in children and adolescents. *J. Sports Med. Phys. Fitness* **51:**204–210; 2011.

[4] Isik, A.; Koca, S. S.; Ustundag, B.; Selek, S. Decreased total antioxidant response and increased oxidative stress in Behcet's disease. *Tohoku J. Exp. Med.* **212:**133–141; 2007.

[5] Mamiya, T.; Katsuoka, F.; Hirayama, A.; Nakajima, O.; Kobayashi, A.; Maher, J. M.; Matsui, H.; Hyodo, I.; Yamamoto, M.; Hosoya, T. Hepatocyte-specific deletion of heme oxygenase-1 disrupts redox homeostasis in basal and oxidative environments. *Tohoku J. Exp. Med.* **216:**331–339; 2008.

[6] Antoncic-Svetina, M.; Sentija, D.; Cipak, A.; Milicic, D.; Meinitzer, A.; Tatzber, F.; Andrisic, L.; Zelzer, S.; Zarkovic, N. Ergometry induces systemic oxidative stress in healthy human subjects. *Tohoku J. Exp. Med.* **221:**43–48; 2010.

[7] Urso, M. L.; Clarkson, P. M. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* **189:**41–54; 2003.

[8] Rietjens, S. J.; Beelen, M.; Koopman, R.; Van Loon, L. J.; Bast, A.; Haenen, G. R. A single session of resistance exercise induces oxidative damage in untrained men. *Med. Sci. Sports Exerc.* **39:**2145–2151; 2007.

[9] Ji, L. L.; Radak, Z.; Goto, S. Hormesis and exercise: how the cell copes with oxidative stress. Am. J. *Pharmacol. Toxicol.* **3:**44–58; 2008.

[10] Falone, S.; Mirabilio, A.; Pennelli, A.; Cacchio, M.; Di Baldassarre, A.; Gallina, S.; Passerini, A.; Amicarelli, F. Differential impact of acute bout of exercise on redox- and oxidative damage-related profiles between untrained subjects and amateur runners. *Physiol. Res.* **59:**953–961; 2010.

[11] Santos-Silva, A.; Rebelo, M. I.; Castro, E. M.; Belo, L.; Guerra, A.; Rego, C.; Quintanilha, A. Leukocyte activation, erythrocyte damage, lipid profile and oxidative stress imposed by high competition physical exercise in adolescents. *Clin. Chim. Acta* **306:**119–126; 2001.

[12] Manna, I.; Jana, K.; Samanta, P. K. Intensive swimming exercise-induced oxidative stress and reproductive dysfunction in male Wistar rats: protective role of alpha-tocopherol succinate. *Can. J. Appl. Physiol.* **29:**172–185; 2004.

[13] Radak, Z.; Chung, H. Y.; Goto, S. Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic. Biol. Med.* **44:**153–159; 2008.

[14] Dixon, C. B.; Robertson, R. J.; Goss, F. L.; Timmer, J. M.; Nagle, E. F.; Evans, R. W. The effect of acute resistance exercise on serum malondialdehyde in resistance-trained and untrained collegiate men. *J. Strength Cond. Res.* **20:**693–698; 2006.

[15] Cakir-Atabek, H.; Demir, S.; PinarbaŞili, R. D.; Gündüz, N. Effects of different resistance training intensity on indices of oxidative stress. *J. Strength Cond. Res.* **24:**2491–2497; 2010.

[16] Gomez-Cabrera, M. C.; Domenech, E.; Viña, J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic. Biol. Med.* **44:**126–131; 2008.

[17] Knez, W. L.; Coombes, J. S.; Jenkins, D. G. Ultra-endurance exercise and oxidative damage: implications for cardiovascular health. *Sports Med* **36:**429–441; 2006.

[18] Kostaropoulos, I. A.; Nikolaidis, M. G.; Jamurtas, A. Z.; Ikonomou, G. V.; Makrygiannis, V.; Papadopoulos, G.; Kouretas, D. Comparison of the blood redox status between long-distance and short-distance runners. *Physiol. Res.* **55:**611–616; 2006.

[19] Skenderi, K. P.; Tsironi, M.; Lazaropoulou, C.; Anastasiou, C. A.; Matalas, A. L.; Kanavaki, I.; Thalmann, M.; Goussetis, E.; Papassotiriou, I.; Chrousos, G. P. Changes in free radical generation and antioxidant capacity during ultra-marathon foot race. *Eur. J. Clin. Invest.* **38:**159–165; 2008.

[20] Benitez-Sillero, J. D.; Perez-Navero, J. L.; Tasset, I.; Guillen-Del Castillo, M.; Gil-Campos, M.; Tunez, I. Influence of intense exercise on saliva glutathione in prepubescent and pubescent boys. *Eur. J. Appl. Physiol.* **106:** 181–186; 2009.

[21] Cooper, D. M.; Nemet, D.; Galassetti, P. Exercise, stress, and inflammation in the growing child: from the bench to the playground. *Curr. Opin. Pediatr.* **16:**286–292; 2004.

[22] Léger, L. A.; Mercier, D.; Gadoury, C.; Lambert, J. The multistage 20 m shuttle run test for aerobic fitness. *J. Sports Sci.* **6:**93–101; 1988.

[23] Tanner, J. M. *Growth at Adolescence*. Oxford: Blackwell; 1962.

[24] Olds, T.; Tomkinson, G.; Léger, L.; Cazorla, G. Worldwide variation in the performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle run test in 37 countries. *Sports Sci* **24:**1025–1038; 2006.

[25] García-Artero, E.; Ortega, F. B.; Ruiz, J. R.; Mesa, J. L.; Delgado, M.; González-Gross, M.; García-Fuentes, M.; Vicente-Rodríguez, G. M.; Gutiérrez, A.; Castillo, M. J. Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study). *Rev. Esp. Cardiol.* **60:**581–588; 2007.

[26] McGavock, J. M.; Torrance, B. D.; McGuire, K. A.; Wozny, P. D.; Lewanczuk, R. Z. Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. *Obesity* **17:**1802–1807; 2009.

[27] Committee of Experts on Sports Research EUROFIT *Handbook for the EUROFIT Tests of Physical Fitness*. Strasburg: Council of Europe; 1993.

[28] Keane, A.; Scott, M. A.; Dugdill, L.; Reilly, T. Fitness test profiles as determined by the Eurofit Test Battery in elite female Gaelic football players. *J. Strength Cond. Res.* **24:**1502–1506; 2010.

[29] Vale, S.; Santos, R.; Soares-Miranda, L.; Mota, J. The relationship of cardiorespiratory fitness, birth weight and parental BMI on adolescents' obesity status. *Eur. J. Clin. Nutr.* **64:**622–627; 2010.

[30] Pianta, R. C. NICHD. Developmental science and education: the NICHD study of early child care and youth development findings from elementary school. *Adv. Child Dev. Behav* **35:**253–296; 2007.

[31] Erdelmeier, I.; Gérard-Monnier, D.; Yadan, J. C.; Chaudière, J. Reactions of N-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals: mechanistic aspects of the colorimetric assay of lipid peroxidation. *Chem. Res. Toxicol.* **11:**1184–1194; 1998.

[32] Levine, R. L.; Garland, D.; Oliver, C. N.; Amici, A.; Climent, I.; Lenz, A. G.; Ahn, B. W.; Shaltiel, S.; Stadtman, E. R. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* **186:**464–478; 1990.

[33] Luo, S.; Levine, R. L. Methionine in proteins defends against oxidative stress. *FASEB J.* **23:**464–472; 2009.

[34] Ricart-Jané, D.; Llobera, M.; López-Tejero, M. D. Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess method. *Nitric Oxide* **6:**178–185; 2002.

[35] Price, J. A.; Sanny, C. G.; Shevlin, D. Application of manual assessment of oxygen radical absorbent capacity (ORAC) for use in high throughput assay of total antioxidant activity of drugs and natural products. *J. Pharmacol. Toxicol. Methods* **54:**56–61; 2006.

[36] Rahman, I.; Kode, A.; Biswas, S. K. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat. Protoc.* **1:**3159–3165; 2006.

[37] Flohé, L.; Günzler, W. A. Assays of glutathione peroxidase. *Methods Enzymol.* **105:**114–121; 1984.

[38] Pittaluga, M.; Parisi, P.; Sabatini, S.; Ceci, R.; Caporossi, D.; Valeria Catani, M.; Savini, I.; Avigliano, L. Cellular and biochemical parameters of exercise-induced oxidative stress: relationship with training levels. *Free Radic. Res.* **40:**607–614; 2006.

[39] Covas, M. I.; Elosua, R.; Fitó, M.; Alcántara, M.; Coca, L.; Marrugat, J. Relationship between physical activity and oxidative stress biomarkers in women. *Med. Sci. Sports Exerc.* **34:**814–819; 2002.

[40] Elosua, R.; Molina, L.; Fito, M.; Arquer, A.; Sanchez-Quesada, J. L.; Covas, M. I.; Ordoñez-Llanos, J.; Marrugat, J. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis* **167:**327–334; 2003.

[41] Pialoux, V.; Brown, A. D.; Leigh, R.; Friedenreich, C. M.; Poulin, M. J. Effect of cardiorespiratory fitness on vascular regulation and oxidative stress in postmenopausal women. *Hypertension* **54:**1014–1020; 2009.
[42] Balog, T.; Sobocanec, S.; Sverko, V.; Krolo, I.; Rocić, B.; Marotti, M.; Marotti, T. The influence of season on oxidant–antioxidant status in trained and sedentary subjects. *Life Sci.* **78:**1441–1447; 2006.
[43] Andrews, A. M.; Kantor, M. A. Oxidative stress increases in overweight individuals following an exercise test. *Mil. Med.* **175:**1014–1019; 2010.
[44] Gougoura, S.; Nikolaidis, M. G.; Kostaropoulos, I. A.; Jamurtas, A. Z.; Koukoulis, G.; Kouretas, D. Increased oxidative stress indices in the blood of child swimmers. *Eur. J. Appl. Physiol.* **100:**235–239; 2007.
[45] Sen, C. K. Update on thiol status and supplements in physical exercise. *Can. J. Appl. Physiol.* **26:**S4–12; 2001.
[46] Dmitriev, L. F.; Titov, V. N. Lipid peroxidation in relation to ageing and the role of endogenous aldehydes in diabetes and other age-related diseases. *Ageing Res. Rev* **9:**200–210; 2010.
[47] Shin, Y. A.; Lee, J. H.; Song, W.; Jun, T. W. Exercise training improves the antioxidant enzyme activity with no changes of telomere length. *Mech. Ageing Dev.* **129:**254–260; 2008.
[48] Mergener, M.; Martins, M. R.; Antunes, M. V.; da Silva, C. C.; Lazzaretti, C.; Fontanive, T. O.; Suyenaga, E. S.; Ardenghi, P. G.; Maluf, S. W.; Gamaro, G. D. Oxidative stress and DNA damage in older adults that do exercises regularly. *Clin. Biochem.* **42:**1648–1653; 2009.
[49] Maeda, S.; Tanabe, T.; Otsuki, T.; Sugawara, J.; Iemitsu, M.; Miyauchi, T.; Kuno, S.; Ajisaka, R.; Matsuda, M. Moderate regular exercise increases basal production of nitric oxide in elderly women. *Hypertens. Res.* **27:**947–953; 2004.
[50] Pérez-Navero, J. L.; Benítez-Sillero, J. D.; Gil-Campos, M.; Guillén-del Castillo, M.; Tasset, I.; Túnez, I. Changes in oxidative stress biomarkers induced by puberty. *An. Pediatr.* **70:**424–428; 2009.
[51] Ekelund, U.; Anderssen, S. A.; Froberg, K.; Sardinha, L. B.; Andersen, L. B.; Brage, S.; European Youth Heart Study Group Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European Youth Heart Study. *Diabetologia* **50:**1832–1840; 2007.
[52] Carruthers, C. Processes and outcomes of an after-school program for adolescent girls. *JPRA* **24:**127–152; 2006.
[53] Rosa, J. S.; Oliver, S. R.; Flores, R. L.; Ngo, J.; Milne, G. L.; Zaldivar, F. P.; Galassetti, P. R. Altered inflammatory, oxidative, and metabolic responses to exercise in pediatric obesity and type 1 diabetes. *Pediatr. Diabetes* **12:**464–472; 2011.
[54] Clarkson, P. M.; Thompson, H. S. Antioxidants: what role do they play in physical activity and health? *Am. J. Clin. Nutr.* **72:**637S–6346S; 2000.

PROFILE OF OXIDANT AND ANTIOXIDANT ACTIVITY IN PREPUBERTAL CHILDREN RELATED TO AGE, GENDER, EXERCISE AND FITNESS

IV

Llorente-Cantarero Francisco Jesus[1,2], Gil-Campos Mercedes[2], Benitez-Sillero Juan de Dios[1], Muñoz-Villanueva Mari Carmen[3], Tasset Inmaculada[4], Pérez-Navero Juan Luis[2]

Applied Physiology, Nutrition, and Metabolism 2012; VOL. XX, NO. X.

[1]Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain.
[2]Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Córdoba, Spain.
[3]Unit of Methodology in Investigation, IMIBIC, Córdoba, Spain

Applied Physiology, Nutrition, and Metabolism
Physiologie appliquée, nutrition et métabolisme

PROFILE OF OXIDANT AND ANTIOXIDANT ACTIVITY IN PREPUBERTAL CHILDREN RELATED TO AGE, GENDER, EXERCISE AND FITNESS

Journal:	*Applied Physiology, Nutrition, and Metabolism*
Manuscript ID:	apnm-2012-0219.R3
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	LLORENTE-CANTARERO, FRANCISCO JESUS GIL-CAMPOS, MERCEDES BENITEZ-SILLERO, JUAN DE DIOS MUÑOZ-VILLANUEVA, MARIA CARMEN TASSET, INMACULADA Perez-Navero, Juan Luis
Keyword:	pediatrics < medicine, stress, body mass index < body composition, physical activity < exercise, exercise endocrinology < exercise

PROFILE OF OXIDANT AND ANTIOXIDANT ACTIVITY IN PREPUBERTAL CHILDREN RELATED TO AGE, GENDER, EXERCISE AND FITNESS

Llorente-Cantarero Francisco Jesus[1,2], Gil-Campos Mercedes[2]*, MD, PhD, Benitez-Sillero Juan de Dios[1], PhD, Muñoz-Villanueva Maria Carmen[3], MD, PhD, Tasset Inmaculada[4], PhD, Pérez-Navero Juan Luis[2] MD, PhD.

1: Department of Corporal Expression. Faculty of Education. University of Cordoba, Avda San Alberto Magno s/n. 14004 Córdoba, Spain. 2: Department of Pediatrics. University Hospital Reina Sofia, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Avda Menéndez Pidal s/n. 14004, Córdoba, Spain. 3: Unit of Research Methodology, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Avda Menéndez Pidal s/n. 14007, Córdoba, Spain. 4: Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Córdoba; Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)/Universidad de Córdoba, Córdoba, Spain.

Short title: Oxidative stress in children related to age

*Corresponding author and reprint requests:
Gil-Campos M, PhD.
Servicio de Pediatría. Hospital Universitario Reina Sofia.
Avda. Menéndez Pidal s/n. C.P. 14004,
Córdoba, Spain
E-mail: mercedes_gil_campos@yahoo.es
Tel: +34957010497 Fax: +34957010017

ABSTRACT

Tissue damage resulting from oxidative stress induced by a pathological condition might have more serious consequences in children than in adults. Researchers still have not identified particular markers -alone or in combination with others- of oxidative stress, and their role in pediatric diseases.

Aim: To identify gender-based biomarkers for measuring oxidative stress status.

Methods: Oxidative biomarkers were studied in 138 healthy Spanish children (85 boys, 53 girls) aged between 7-12 years at prepubertal stage (Tanner I) independently of body mass index (BMI), age, fitness (measured by 20-mSRT) and physical activity (PA) (measured by enrolment in an after-school program of exercise).

Oxidative biomarkers measured: lipid peroxidation products (LPO), total nitrites (NOx), protein carbonyls (PC) and oxidized glutathione (GSSG). Antioxidant biomarkers: total glutathione (TG), reduced glutathione (GSH), superoxide dismutase activity (SOD) and glutathione peroxidase (GPx) activity.

Results: Girls presented lower height, weight and waist circumference values, and a lower BMI than boys. In relation to oxidative biomarkers, boys presented higher levels of PC as compared to girls ($P<0.001$). In spite of this, the group of girls presented higher levels of GSSG ($P<0.001$) and TG ($P=0.001$), and a lower GSH/GSSG ratio ($P<0.001$) as compared to the group of boys. As for the antioxidant response, girls showed higher levels of SOD ($P=0.002$) than boys. All analyses were adjusted for BMI, age, fitness and physical activity.

Conclusion: Prepubertal girls presented higher oxidative stress than boys, a situation that was parallel joint with elevated levels of SOD, independently of age, BMI, fitness and physical activity.

Key words: paediatric, healthy, oxidative stress, gender, fitness, physical activity

INTRODUCTION

An over-production of free radicals may alter the endogenous antioxidant defense system, which has been associated with an increase in oxidative stress (OS). OS biomarkers determine the extent of oxidative injury (Jakus and Rietbrock 2004; Noiri and Tsukahara 2005); this occurs in many pathological processes and contributes to disease mechanisms significantly (Heitzer et al. 2001). The effects of oxidation can be predicted through OS biomarkers, which can provide the basis for designing appropriate interventions to prevent or alleviate oxidative damage (Fisher-Wellman and Bloomer 2009). The detection of more than one OS biomarker is crucial, as a single biomarker might yield misleading results (Tsukahara 2007).

Tsukahara (2007) states that under normal physiological conditions, younger people (especially children) are more likely to be exposed to higher concentrations of reactive oxygen species (ROS) and total nitrites (NOx, as marker of nitric oxide, NO, formation) than older people. Moreover, authors have associated the pathogenesis and evolution of numerous diseases at this age with ROS-induced oxidative damage (Casado et al. 2007). The reason is infants' need for subsequent tissue growth to match somatic growth, and survival rates in children are normally higher. Furthermore, the use of antioxidants has presented new therapeutic perspectives for diseases that are related to enhance OS (Tsukahara 2007).

Women appear to have greater resistance to inflammatory and oxidative processes than men (Kerksick et al. 2008), which might influence the prevalence and severity of certain diseases –especially cardiovascular diseases (Miller et al. 2007). In fact, the gender longevity gap is associated with lower OS (Ali et al. 2006, Pepe et al. 2009).

In animal models, females have been shown to display higher concentrations of antioxidants and greater resistance to oxidative damage (Bureau et al. 2003). Similarly, in humans, higher glutathione peroxidase (GPx) (Rush and Sandiford 2003) and lower lipid and DNA oxidation (Pansarasa et al. 2000) have been reported in young women as compared to

men (Proteggente et al. 2002), while higher glutathione has been reported in newborn baby girls as compared to boys (Lavoie and Chessex 1997), thus suggesting that females might have some type of protection against oxidant insults at birth. Moreover, there is no convincing evidence on an existing correlation between OS and some diseases (Pavlova et al. 2005), and few evaluation studies are available on the oxidative status of healthy children (Tsukahara 2007).

On the other hand, obesity-related alterations in OS markers have been reported to be largely gender-independent in adolescents (Oliver et al. 2010). Physical fitness is known to exert independent positive effects on oxidative homeostasis regardless of the adiposity status (Kasapis and Thompson 2005). However, the oxygen flow into working muscles may be greater in children and, consequently, exercise-induced OS response may be higher in children than in adults (Cooper et al. 2004). Acute aerobic exercise has been reported to induce OS, while regular aerobic exercise has been associated with a decrease in oxidants and an increase in antioxidants (Cakir-Atabek et al. 2010).

Therefore the aim of this study is to evaluate the status of a series of oxidative markers: lipid peroxidation products (LPO), NOx and proteins carbonyl (PC) and oxidized glutathione (GSSG), and antioxidant biomarkers such as: total glutathione (TG), reduced glutathione (GSH), superoxide dismutase activity (SOD), and GPx, in prepubertal of both genders, and to analyze whether these biomarkers are influenced by age, body mass index (BMI), cardiorespiratory fitness (CRF) and physical activity (PA).

MATERIALS AND METHODS

Subjectsand Design

We encouraged 450 children from two local elementary schools to participate in the study. At the beginning, we numbered a total of 156 prepubertal children for the study though some of

them were lastly excluded as these did not meet the inclusion criteria, or decided not to participate or not complete the study, or vein puncture blood sampling was not possible. Finally, a total of 138 healthy Spanish children (85 boys, 53 girls) aged between 7-12 years at prepubertal stage (Tanner I) were included. Exclusion criteria were: presence of pubertal development, disease, long periods of rest after illness or use of any medication altering blood pressure or metabolism.

The study was conducted at the Department of Paediatrics. Written informed consent was obtained from parents or legal guardians, and the study procedures were verbally explained to all children. Ethical approval of the study was given from the local human research and Hospital ethical committees. The study methodologies conformed to the standards set by the Declaration of Helsinki.

Physical Examination, Anthropometric and Blood Pressure Measurements

Anamnesis and physical examination including the evaluation of sexual maturity according to Tanner's five-stage scale (Tanner 1962) were assessed. Prepubertal stage was established as Tanner stage I and confirmed by identification of appropriate plasma sex hormone levels. Anthropometric measurements (weight, height) were taken using standard techniques. BMI was expressed as weight (kg)/ height (m^2). Systolic and diastolic blood pressure (BP) were measured at rest by the same person to the right arm and while subjects were sitting, using a random-zero sphygmomanometer (Dinamap V-100). An average of three consecutive measurements was taken for analysis.

Evaluation of Fitness and Physical Activity

A validated scale developed by Olds et al. (2006) was used to measure fitness after performing a 20-meter shuttle run test (20-mSRT) described in detail below (Léger et al. 1988). This test is one of the most commonly used field tests to assess fitness in children and adolescents. At first, there was a learning development to do the test correctly. Afterwards,

participants ran as long as possible back and forth across a 20-m space at a specified audio signal protocol that increased by 0.5 km/h each minute, one time, at a running speed of 8.0 km/h. The 20-MST is a maximal running test starting Subjects were allowed to voluntarily withdraw from the test after being verbally encouraged to perform maximally during each assessment. The test is completed when the participant fails to reach the end lines concurrent with the audio signals on two consecutive occasions. The last lap completed was considered the individual fitness level for being the raw variable obtained. The 20-m test performances are indicated as z-scores relative to all children of the same age and sex from all countries.

To estimate PA, we investigated whether children were engaged in an after-school exercise program at least three times per week for at least 1 year, or on the contrary they were sedentary; a short test based on the NICHD validated questionnaire (Pianta 2007) was used in both groups to obtain information about PA practice and sedentary habits. Prior information from the staff was also elicited to select the children for each group. These after-school programs are designed to encourage them to take up some physical activity due to the increase in sedentary habits in children. Thus all the children in this study who practiced PA had similar activities driven by the professional staff of the two schools.

Sampling and Biochemical Analysis

Blood samples were collected between 9:00-9:30h am after a 12h-fasting period and at rest, using an indwelling venous line to draw a 3-ml sample in tubes containing 1 mg/ml EDTA-K3 as anticoagulant (for plasma and erythrocytes) . Samples were placed in chilled tubes with and were stored in containers with ice and kept in dark. Particular care was taken to avoid exposure to air light and ambient temperature. Plasma was separated from erythrocytes by centrifugation at 3500g for 10min. within 1 h of extraction. Aliquots of supernatant (1 ml)

were immediately frozen to -82ºc until analysis, one month later. ***Determination of Oxidative Stress and Antioxidant Biomarkers***

Lipid Peroxidation Products (LPO): Plasma malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA) were estimated using the method described by Eldermeier et al. (1998). This method uses a chromatogenic reagent that reacts with MDA+4-HDA at 45ºC, yielding a stable chromophore with maximum absorbance at 586 nm.

Protein Carbonyls (PC): plasma PC concentrations were measured using the method described by Levine et al. (1990). Samples were incubated with 2.4-dinitrophenylhydrazine in HCl for 60 min. Then, proteins were precipitated from the solutions using 500 µl of trichloroacetic acid (20%). Subsequently, proteins were washed with a solution of ethanol and ethylacetate (1:1 v/v), and dissolved in 1 ml of guanidine hydrochloride (6M) at 37ºC. Carbonyls were evaluated in a spectrophotometer (UV-1630; Shimadzu) at a wavelength of 360 nm (Luo and Levine 2009.

Total Nitrites (nitrites and nitrates; NOx): NOx was used as markers of NO levels, and were assayed following the Griess method (Ricart-Jané et al. 2002) in plasma. This assay uses the determination of nitrite as an indicator of NO production in biological samples. NO is transformed into nitrate and nitrite. It is common practice to use either enzymatic or chemical reduction to convert all nitrates into nitrite in a sample and measure total nitrite as an indicator of NO production. When nitrate reduction was completed, total nitrite was spectrophotometrically determined by using the Griess reaction. Reaction was monitored at 540nm. Absorbance was evaluated in a spectrophotometer (UV-1603; Shimadzu) g/ml.

Total Glutathione (TG,) Oxidized Glutathione (GSSG) and Reduced Glutathione (GSH): TG and GSH levels were evaluated in red blood cells using the Bioxytech GSH-420 and GSH-400 kits, respectively from, BIOXYTECH® aop-490 TM (Oxis International, Portland, OR, USA). It is based on the reduction of Cu^{2+} to Cu^{+} by the combined action of the

antioxidants of the sample. Thus, the chromogenic reagent results in a Cu^{+} complex with absorbance at 490nm (Price et al. 2006). The determination of TG levels was based on the formation of a chromophoric thione with absorbance at 420 nm. The GSH concentration is based on a reaction which leads to the formation of a chromophore with absorbance at 400 nm (Rahman et al. 2006). GSSG levels were calculated by subtracting GSH from TG.

Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx): SOD activity in erythrocytes was determined using a colorimetric assay kit from BioVision Research Products (Mountain View, CA, USA). SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. The reduction rate by a superoxide anion has a linear relationship with xanthine oxidase activity and is inhibited by SOD. Therefore, the inhibitory activity of SOD is determined by a colorimetric method.

GPx activity in red blood cells was evaluated by the Flohé and Gunzler method (1984) using the Glutathion Peroxidase assay kit (Cayman Chemical). This assay is based on the oxidation of NADPH to $NADP^{+}$, which is catalyzed by a limited concentration of glutathione reductase, with maximum absorbance at 340 nm. Activity was measured basing on the formation of GSSG from the GPx-catalysed oxidation of GSH by H_2O_2, coupled with NADPH consumption, in the presence of exogenously added glutathione reductase, with maximum absorbance at 340 nm.

Statistical Analysis

Data were expressed as mean ± SD. Normal data distribution was assessed by the Shapiro-Wilk test. Homogeneity of variances was estimated using Levene's test. The group means for continuous variables with normal distribution were compared by Student's t-test in unpaired samples, while variables with asymmetric distribution were compared using the Mann-Whitney U test.

Finally, differences between boys and girls were determined by analysis of covariance (ANCOVA) after adjustment for age, BMI, fitness and PA. All statistical analyses were performed using the Statistical Package for Social Science software (PASW Statistic 18. Inc. Chicago, IL, USA).

RESULTS

Anthropometric differences were found between girls and boys. Prepubertal girls were younger than prepubertal boys, and presented lower height, weight and waist circumference, and a lower body mass index, as compared to boys. Similarly, girls showed lower levels of BP (Table 1).

As concerns oxidative and antioxidant biomarkers, differences were observed between sexes before adjustments by age, BMI, fitness and physical activity (Table 2). After adjustments, as far as oxidant biomarkers are concerned, boys presented higher levels of PC than girls (girls: 1.74 *vs* boys: 5.27 nM; P: <0.001), while no differences were found in LPO (girls: 0.21 μM *vs* boys: 0.22 μM; P: 0.632) and NOx (girls: 14.67 μM *vs* boys: 14.63 μM; P: 0.975) markers between groups.

Relating to the antioxidant response, boys showed lower levels of TG (P=0.001) (Figure 1) and SOD as compared to the group of girls (Figure 2).

On the contrary, girls presented higher GSSG levels and a lower GSH/GSSG ratio than boys (Figure 1). No differences were found in GPx (Figure 2).

DISCUSSION

The main results of the present study suggest that there is an independent effect of gender on OS, even at prepubertal age. Girls showed higher levels of TG, GSSG and SOD and a lower GSH/GSSG ratio, as compared to the group of boys.

Concerning the related literature reviewed, scarce research has been conducted on prepubertal subjects or newborns (Casado et al. 2007, Lavoie and Chessex 1997). Most studies are focused on the adult population and are not gender-based (Picot et al. 1992). Nevertheless, the present study comprises a larger sample of children than previous studies.

Albeit the results obtained in anthropometric differences between sexes, we think these differences probably do not have an influence on OS. In fact, the analysis was adjusted for age and BMI to avoid such effects. In addition, to avoid the effect of puberty on OS (Pérez-Navero et al. 2009), our study is focused on the prepubertal stage (ages between 7-12-year-old) rather than on age-matched groups, as compared to previous studies that were conducted regardless of the physical development stage of infants (Erden-Inal et al. 2002).

By the other hand, good fitness (Santos-Silva et al. 2001) and moderate exercise have been proposed to have an antioxidant effect (Gomez-Cabrera et al. 2008; Llorente et al, 2012). Some authors have found that boys and girls did not differ in the redox response to training (Cavas and Tarhan 2004, Kabasakalis et al. 2009) or acute exercise (Nikolaidis et al. 2007). Similarly, we found no differences in OS biomarkers between boys and girls in relation to CRF and PA levels.

TG is a low-molecular-mass, thiol-containing tripeptide, glutamic acid-cysteine-glycine. It plays a major role in the detoxification of a wide range of chemicals. It acts as a cofactor for the enzyme peroxidase, thus serving as an indirect antioxidant donating electrons. It also exhibits a non-selenium-dependent GPx activity against organic hydroperoxides (Guemouri et al. 1991). A high TG activity has been observed during the first year of life, later it decreases and remains constant in childhood, adulthood and old age (Picot et al. 1992). The results of the present study in childhood are in agreement with those obtained by other authors as Habif et al. (2001) who found that females showed higher levels of TG than males.

GSH is converted to oxidized glutathione (GSSG) by seleno-dependent GPx. GSSG is subsequently reduced back to GSH by glutathione reductase (GSH-Rd). These two glutathione-dependent coupled enzymes (GPx and GSH-Rd) maintain the GSH/GSSG ratio within the cell, and an imbalance in this ratio generates OS (Al-Turk 1987). Erden-Inal et al. (2002) found no differences between females and males in GSH and GSSG biomarkers at the ages studied. However, they found differences in the GSH/GSSG ratio in a small group (28 subjects aged between 2-11 years old) where females presented lower GSH/GSSG ratios than males. The results obtained by Erden-Inal et al. (2002), match those obtained in this study – where a larger sample was used– where girls showed lower GSH/GSSG ratios and higher GSSG values than boys.

Our results might suggest that healthy girls may be more prone to OS than boys; although boys showed higher PC levels. However, there are numerous different types of protein oxidative modification, and there is no single universal marker for protein oxidation (Dalle-Donne et al. 2003). By the other hand, the ratio of reduced GSH to oxidized GSH (GSSG) is an indicator of cellular health, with reduced GSH constituting up to 98% of cellular GSH under normal conditions. So, the GSH/GSSG ratio is reduced in some diseases and it is an excellent biomarker to measure the cellular redox potential (Owen and Butterfield, 2010). So, the significant increase in the GSH/GSSG ratio resulting from lower GSSG levels in boys suggests that boys may have a lower oxidant status.

The body has developed a complex defense strategy to minimize the damaging effects of oxidants. Central to this defense are antioxidant enzymes, which include SOD and GPx (Franco et al. 2007). A study conducted on a Turkish population established a relationship between age, gender and physical exercise, and SOD and GPx. The Turkish study found no gender-based differences in any antioxidant enzyme. However, the results showed higher levels of SOD and GPx in children-adolescents and adults than in elderly people, and lower

levels of SOD and GPx in adults after acute exercise (Ozbay et al. 2002). In the present study, girls showed higher levels of SOD as compared with boys, though no differences were found in GPx levels. These results were not dependent on age, exercise or CRF. Other studies in children have observed an elevated erythrocyte SOD activity (Aydin et al. 2001). This result has been explained to be a compensatory mechanism against superoxide radical overproduction. This protective mechanism can be also observed in some disorders characterized by the presence of OS, as obesity (Erdeve et al. 2004) or atherosclerosis (Sierakowska-Fijałek et al. 2008) in children.

In this study, as other authors have referred (Habif et al. 2001), it is still unknown the role of gender in relation to physiological changes in the oxidation system. In neonates it has been suggested that gender-related differences could exist in the maturation of the enzymatic systems in different tissues (Lavoie and Chessex, 1997). Different studies similar to ours, also describe the possible relationship of different factors such as age, environment or lifestyle and its influence on the results in both sex (Nikolaidis et al, 2007). For this reason, interference variables introduced as BMI, CRF or PA, being the gender the only factor that has been shown to have an independent relation with the production of oxidative stress. Therefore, further studies are needed in subgroups of age and sex to assess the possible influence and try to know the physiological mechanism that induces this situation.

CONCLUSION

Prepubertal girls presented higher oxidative stress than boys, a situation that was parallel joint with elevated levels of SOD, independently of age, BMI, fitness and physical activity. Future paediatric research could give more information about the influence of gender in oxidative stress mechanisms.

REFERENCES

Ali, S.S., Xiong, C., Lucero, J., Behrens, M.M., Dugan, L.L., Quick, K.L. 2006. Gender differences in free radical homeostasis during aging: shorter-lived female C57BL6 mice have increased oxidative stress. Aging. Cell. **5**: 565–74. doi:10.1111/j.1474-9726.2006.00252.x.

Al-Turk, W.A., Stohs, S.J., el-Rashidy, F.H., Othman, S. 1987. Changes in glutathione and its metabolizing enzymes in human erythrocytes and lymphocytes with age. J. Pharm. Pharmacol. **39**: 13–6. doi:10.1111/j.2042-7158.1987.tb07154.x.

Aydin, A., Orhan, H., Sayal, A., Ozata, M., Sahin, G., Işimer, A. 2001. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. Clin. Biochem. **34**: 65–70. http://dx.doi.org/10.1016/S0009-9120(00)00199-5.

Bureau, I., Gueux, E., Mazur, A., Rock, E., Roussel, A.M., Rayssiguier, Y. 2003. Female rats are protected against oxidative stress during copper deficiency. J. Am. Coll. Nutr. **22**: 239–46.

Cakir-Atabek, H., Demir, S., PinarbaŞili, R.D., Gündüz, N. 2010. Effects of different resistance training intensity on indices of oxidative stress. J. Strength Cond. Res. **24**: 2491–7. doi:10.1519/JSC.0b013e3181ddb111.

Casado, A., de la Torre, R., López-Fernández, M.E. 2007. Copper/zinc superoxide dismutase activity in newborns & young people in Spain. Indian J. Med. Res. **125:** 655–60.

Cavas, L. and Tarhan, L. 2004. Effects of vitamin–mineral supplementation on cardiac marker and radical scavenging enzymes, and MDA levels in young swimmers. Int. J. Sport. Nutr. Exerc. Metab. **14**: 133–46.

Cheeseman, K.H. 1993. Lipid peroxidation in biological systems. In: DNA and Free Radicals, edited by B. Halliwell, O.I. Auroma, Ellis Harward, London.

319 Cooper, D.M., Nemet, D., Galassetti, P. 2004. Exercise, stress, and inflammation in the
320 growing child: from the bench to the playground. Curr. Opin. Pediatr. **16**: 286–92.
321 Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., Colombo, R. Protein carbonyl groups
322 as biomarkers of oxidative stress. 2003. Clin. Chim. Acta. **329**: 23–28.
323 Erdelmeier, I., Gérard-Monnier, D., Yadan, J.C., Chaudière, J. 1998. Reactions of N-methyl-
324 2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the
325 colorimetric assay of lipid peroxidation. Chem. Res. Toxicol. **11**: 1184–94.
326 doi:10.1021/tx970180z.
327 Erden-Inal, M., Sunal, E., Kanbak, G. 2002. Age-related changes in the glutathione redox
328 system. Cell Biochem. Funct. **20**: 61–6. doi:10.1002/cbf.937.
329 Erdeve, O., Siklar, Z., Kocaturk, P.A., Dallar, Y., Kavas, G.O. 2004. Antioxidant superoxide
330 dismutase activity in obese children. Biol. Trace. Elem. Res. **98**: 219–28.
331 doi:10.1385/BTER:98:3:219.
332 Esterbauer, H. and Cheeseman, K.H. 1987. Lipid peroxidation: Pathological implications.
333 Chem. Phys. Lipids. **45**: 103–107.
334 Fisher-Wellman, K. and Bloomer, R.J. 2009. Acute exercise and oxidative stress: a 30 year
335 history. Dyn. Med.**13**: 8:1. doi:10.1186/1476-5918-8-1.
336 Flohé, L., Günzler, W.A. 1984. Assays of glutathione peroxidase. Methods Enzymol. **105**:
337 114–21.
338 Franco, M.C., Kawamoto, E.M., Gorjão, R., Rastelli, V.M., Curi, R., Scavone, C. et al. 2007.
339 Biomarkers of OS and antioxidant status in children born small for gestational age:
340 evidence of lipid peroxidation. Pediatr. Res. **62**: 204–8.
341 doi:10.1203/PDR.0b013e3180986d04.

342 Gomez-Cabrera, M.C., Domenech, E., Viña, J. 2008. Moderate exercise is an antioxidant:
343 upregulation of antioxidant genes by training. Free. Radic. Biol. Med. **44**: 126–31.
344 http://dx.doi.org/10.1016/j.freeradbiomed.2007.02.001.
345 Guemouri, L., Artur, Y., Herbeth, B., Jeandel, C., Cuny, G., Siest, G. 1991. Biological
346 variability of superoxide dismutase, glutathione peroxidase and catalase in blood. Clin.
347 Chem. **37**: 1932.
348 Habif, S., Mutaf, I., Turgan, N., Onur, E., Duman, C., Ozmen, D. et al. 2001. Age and gender
349 dependent alterations in the activities of glutathione related enzymes in healthy subjects.
350 Clin. Biochem. **34**: 667–71. http://dx.doi.org/10.1016/S0009-9120(01)00279-X.
351 Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., Münzel, T. 2001. Endothelial dysfunction,
352 oxidative stress, and risk of cardiovascular events in patients with coronary artery
353 disease. Circulation. **104**: 2673–8. doi:10.1161/hc4601.099485.
354 Jakus, V. and Rietbrock, N. 2004. Advanced glycation end-products and the progress of
355 diabetic vascular complications. Physiol. Res. **53**: 13–42.
356 Kabasakalis, A., Kalitsis, K., Nikolaidis, M.G., Tsalis, G., Kouretas, D., Loupos, D. et al.
357 2009. Redox, iron, and nutritional status of children during swimming training. J. Sci.
358 Med. Sport. **12**: 691–6. doi:10.1016/j.jsams.2008.05.005.
359 Kasapis, C. and Thompson, P.D. The effects of physical activity on serum C-reactive protein
360 and inflammatory markers: a systematic review. J. Am. Coll. Cardiol. **45**: 1563–9.
361 http://dx.doi.org/10.1016/j.jacc.2004.12.077.
362 Kerksick, C., Taylor, L., Harvey, A., Willoughby, D. 2008. Gender-related differences in
363 muscle injury, oxidative stress, and apoptosis. Med. Sci. Sports. Exerc. **40**: 1772–80.
364 doi:10.1249/MSS.0b013e31817d1cce.

365 Lavoie, J.C. and Chessex, P. 1997. Gender and maturation affect glutathione status in human
366 neonatal tissues. Free. Radic. Biol. Med. **23**: 648–57. http://dx.doi.org/10.1016/S0891-
367 5849(97)00011-7.
368 Léger, L.A., Mercier, D., Gadoury, C., Lambert, J. 1988. The multistage 20 meter shuttle run
369 test for aerobic fitness. J. Sports Sci. **6**: 93–101. doi:10.1080/02640418808729800.
370 Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G. et al. 1990.
371 Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol.
372 **186**: 464–78. http://dx.doi.org/10.1016/0076-6879(90)86141-H.
373 Llorente-Cantarero, F.J., Gil-Campos, M., Benitez-Sillero, J.D., Muñoz-Villanueva, M.C.,
374 Túnez ,I., Pérez-Navero, J.L. 2012. Prepubertal children with suitable fitness and physical
375 activity present reduced risk of oxidative stress. Free Radical Biology and Medicine. **53**:
376 415–420.
377 Luo, S. and Levine RL. 2009. Methionine in proteins defends against oxidative stress. FASEB
378 J. **23**: 464–72. doi:10.1096/fj.08-118414.
379 Miller, A.A., De Silva, T.M., Jackman, K.A., Sobey, C.G. 2007. Effect of gender and sex
380 hormones on vascular oxidative stress. Clin. Exp. Pharmacol. Physiol. **34**: 1037–43.
381 doi:10.1111/j.1440-1681.2007.04732.x.
382 Nikolaidis, M.G., Kyparos, A., Hadziioannou, M., Panou, N., Samaras, L., Jamurtas, A.Z. et
383 al. 2007. Acute exercise markedly increases blood oxidative stress in boys and girls.
384 Appl. Physiol. Nutr. Metab. **32**: 197–205. doi:10.1139/h06-097.
385 Noiri, E. and Tsukahara, H. 2005. Parameters for measurement of oxidative stress in diabetes
386 mellitus: applicability of enzyme-linked immunosorbent assay for clinical evaluation. J.
387 Investig. Med. **53**: 167–75. doi:10.2310/6650.2005.00403.

388 Olds, T., Tomkinson, G., Léger, L., Cazorla, G. 2006. Worldwide variation in the
389 performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle
390 run test in 37 countries. Sports Sci. **24**: 1025–38. doi:10.1080/02640410500432193.
391 Oliver, S.R., Rosa, J.S., Milne, G.L., Pontello, A.M., Borntrager, H.L., Heydari, S. et al. 2010.
392 Increased oxidative stress and altered substrate metabolism in obese children. Int. J.
393 Pediatr. Obes. **5**: 436–44. doi:10.3109/17477160903545163.
394 Owen, J.B., Butterfield, D.A. 2010. Measurement of oxidized/reduced glutathione ratio.
395 Methos. Mol. Biol. **648**: 269–77.
396 Ozbay, B. and Dülger, H. 2002. Lipid peroxidation and antioxidant enzymes in Turkish
397 population: relation to age, gender, exercise, and smoking. Tohoku J. Exp. Med. **197**:
398 119–24. http://dx.doi.org/10.1620/tjem.197.119.
399 Pansarasa, O., Castagna, L., Colombi, B., Vecchiet, J., Felzani, G., Marzatico, F. 2000. Age
400 and sex differences in human skeletal muscle: role of reactive oxygen species. Free.
401 Radic. Res. **33**: 287–93.
402 Pavlova, E.L., Lilova, M.I., Savov, V.M. 2005. Oxidative stress in children with kidney
403 disease. Pediatr. Nephrol. **20**: 1599–604. doi:10.1007/s00467-005-1990-x.
404 Pepe, H., Balci, S.S., Revan, S., Akalin, P.P., Kurtoglu, F. 2009. Comparison of oxidative
405 stress and antioxidant capacity before and after running exercises in both sexes. Gend.
406 Med. **6**: 587–95. http://dx.doi.org/10.1016/j.genm.2009.10.001.
407 Pérez-Navero, J.L., Benítez-Sillero, J.D., Gil-Campos, M., Guillén-del Castillo, M., Tasset, I.,
408 Túnez, I. 2009. Changes in oxidative stress biomarkers induced by puberty. An. Pediatr.
409 **70**: 424–8. doi:10.1016/j.anpedi.2009.01.019.
410 Pianta, RC. 2007. NICHD. Developmental science and education: the NICHD study of early
411 child care and youth development findings from elementary school. Adv. Child Dev.
412 Behav. **35**: 253–96.

413 Picot, I.C., Trivier, J.M., Nicole, A., Sinet, P.M., Thevenib, M. 1992. Age correlated
414 modifications of coppper-zinc superoxide dismutade and glutathione related enzyme
415 activities in human erythrocytes. Clin. Chem. **38**: 66.
416 Price, J.A., Sanny, C.G., Shevlin, D. 2006. Application of manual assessment of oxygen
417 radical absorbent capacity (ORAC) for use in high throughput assay of "total" antioxidant
418 activity of drugs and natural products. J. Pharmacol Toxicol Methods. **54**: 56–61.
419 http://dx.doi.org/10.1016/j.vascn.2005.11.002.
420 Proteggente, A.R., England, T.G., Rehman, A., Rice-Evans, C.A., Halliwell, B. 2002. Gender
421 differences in steady-state levels of oxidative damage to DNA in healthy individuals.
422 Free Radic. Res. **36**: 157–62. doi:10.1080/10715760290006475.
423 Rahman, I., Kode, A., Biswas, S.K. 2006. Assay for quantitative determination of glutathione
424 and glutathione disulfide levels using enzymatic recycling method. Nat. Protoc. **1**: 3159–
425 65. doi:10.1038/nprot.2006.378.
426 Ricart-Jané, D., Llobera, M., López-Tejero, M.D. 2002. Anticoagulants and other
427 preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess
428 method. Nitric Oxide. **6**: 178–85. http://dx.doi.org/10.1006/niox.2001.0392.
429 Rush, J.W. and Sandiford, S.D. 2003. Plasma glutathione peroxidase in healthy young adults:
430 influence of gender and physical activity. Clin. Biochem. **36**: 345–51.
431 http://dx.doi.org/10.1016/S0009-9120(03)00039-0.
432 Santos-Silva, A., Rebelo, M.I., Castro, E.M., Belo, L., Guerra, A., Rego, C. et al. 2001.
433 Leukocyte activation, erythrocyte damage, lipid profile and oxidative stress imposed by
434 high competition physical exercise in adolescents. Clin. Chim. Acta. **306**: 119–126.
435 http://dx.doi.org/10.1016/S0009-8981(01)00406-5.

436 Sierakowska-Fijałek, A., Fijałkowski, P., Błaszczyk, J., Baj, Z., Stepień, M., Wosik-Erenbek,
437 M. et al. 2008. Estimation of selected parameters antioxidant barrier and lipid parameters
438 in children with atherosclerosis risk factors. Pol. Merkur Lekarski. **24**: 14–7.
439 Tanner, J.M. 1962. Growth at adolescente. Oxford, Blackwell.
440 Toyokuni, S. 1999. Reactive oxygen species-induced molecular damage and its application in
441 pathology. Pathol. Int. **49**: 91–102. doi:10.1046/j.1440-1827.1999.00829.x.
442 Tsukahara, H. 2007. Biomarkers for oxidative stress: clinical application in pediatric
443 medicine. Curr. Med. Chem. **14**: 339–51.
444 Working group on High Blood Pressure in Children and Adolescents. 2004. National High
445 Blood Pressure Education Program. Pediatrics. **114** (2): 555–76.
446

447 **Table 1.** Demographic, anthropometric and blood pressure measurements in prepubertal boys
448 and girls.
449

	Girls	Boys	P
	53	85	
Age (years)	8.78±0.90	10.16±0.97	<0.001
SBP (mmHg)	115.20±13.64	125.89±12.65	<0.001
DBP (mmHg)	63.92±10.36	69.14±9.15	0.003
Weight (kg)	36.94±9.14	45.89± 12.84	<0.001
Height (cm)	138.41±6.31	147.19±9.97	<0.001
BMI (kg/m^2)	19.08±3.68	20.83±3.74	0.010
WC (cm)	63.62±9.11	69.56±11.09	0.002
CRF (low) (%)	60.4	37.8	0.170
Non PA practice (%)	52.8	34.5	0.520

450
451 BMI: Body Mass Index; CRF: cardiorespiratory fitness; DBP: Diastolic Blood Pressure, PA:
452 physical activity; SBP: Systolic Blood Pressure; WC: waist circumference.
453 Data in percentiles for BP: (SBP: P90-95 in girls vs >P95 in boys; DBP: P75 in girls vs P90 in
454 boys). The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile
455 is 2.326 SD over the mean, in boys and girls (Task Force, 2004).
456 Statistical significance after application of Student's test (or U of Mann-Whitney) to data
457 expressed as mean ±SD.
458 For CRF and PA, statistical significance after application of Chi square test. Data expressed
459 as percentages.
460

461 Table 2. Plasma and erythrocyte levels of oxidative stress biomarkers in prepubertal boys and
462 girls before adjustments by age, BMI, fitness and physical activity.

	Girls	Boys	P
PC	2.00±1.05	4.94±2.93	<0.001
LPO	0.21±0.11	0.22±0.11	0.554
GSH	22.63±3.52	22.52±3.83	0.572
TG	29.01±3.88	25.17±4.95	<0.001
GSSG	6.39±2.33	2.65±1.83	<0.001
GSH/GSSG	4.99±5.36	14.1±12.65	<0.001
SOD	5.43±4.21	2.28±1.85	<0.001
Nox	15.08±3.01	14.27±5.43	0.082
GPx	0.14±0.14	0.25±0.47	0.316

463

464 Reduced glutathione (GSH); glutathione peroxidase (GPx); lipid peroxidation products
465 (LPO); oxidized glutathione (GSSG); reduced glutathione/ oxidized glutathione ratio
466 (GSH/GSSG); proteins carbonyl (PC); superoxide dismutase (SOD), total glutathione (TG),
467 total nitrites (NOx).
468 Statistical significance after application of Student's test (or U of Mann-Whitney) to data
469 expressed as mean ±SD.

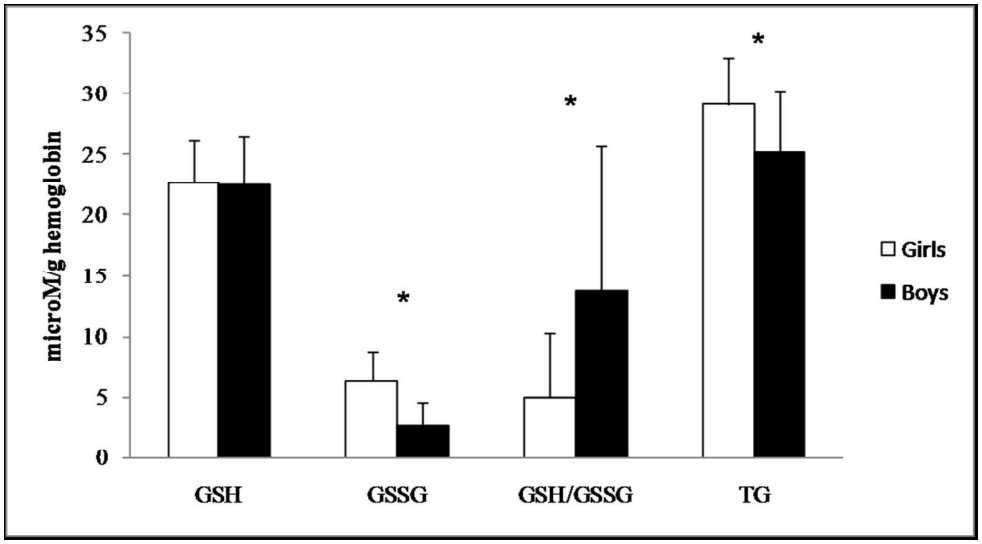
FIGURE LEGENDS

Figure 1. Levels of oxidized glutathione (GSSG), reduced glutathione (GSH), reduced glutathione/ oxidized glutathione ratio (GSH/GSSG) and total glutathione (TG) in erythrocytes, in prepubertal boys and girls.

Data are expressed as mean ±SD. *P<0.001: Statistical significance after application of ANCOVA (analysis of covariance) after adjusting for age, BMI, fitness and physical activity.

Figure 2. Levels of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activity in erythrocytes in prepubertal boys and girls.

Data are expressed as mean ±SD. *P<0.001: Statistical significance after application of ANCOVA (analysis of covariance) after adjusting for age, BMI, fitness and physical activity.



185x102mm (150 x 150 DPI)

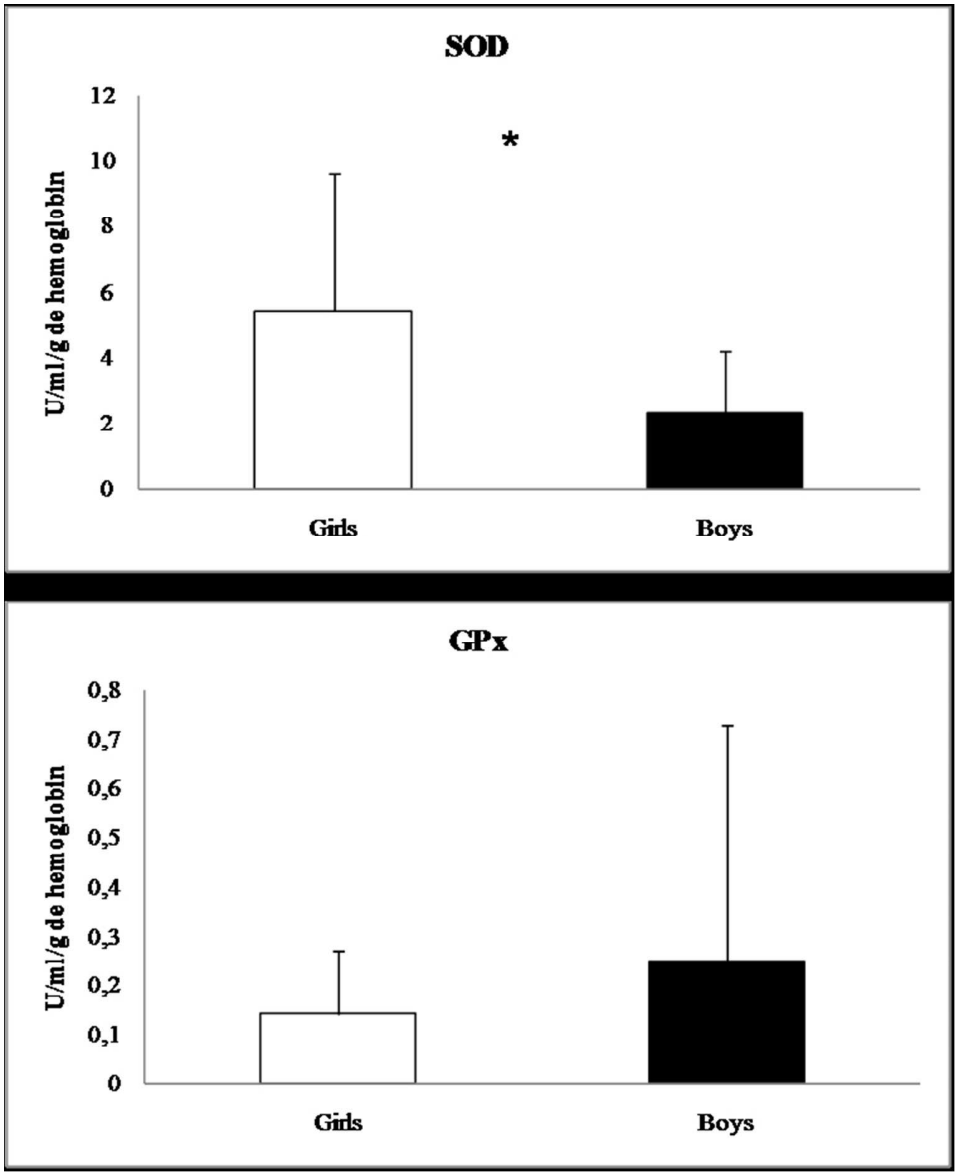


Figure 2
127x157mm (150 x 150 DPI)

PLASMA ADIPOKINES IN PREPUBERTAL CHILDREN WITH DIFFERENT LEVELS OF CARDIORESPIRATORY FITNESS AND PHYSICAL ACTIVITY

Llorente-Cantarero FJ[ab], Gil-Campos M[b], Benitez-Sillero JD[a], Olza J[d], Muñoz-Villanueva MC[c], Aguilera CM[d], Pérez-Navero JL[b]

V

[a]Department of Corporal Expression, Faculty of Education, University of Cordoba, Córdoba, Spain.
[b]Department of Pediatrics, University Reina Sofia Hospital, Maimonides Institute for Biomedical Research (IMIBIC), Córdoba, Spain.
[c]Unit of Methodology in Investigation, IMIBIC, Córdoba, Spain.
[d]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology, Centre of Biomedical Research, University of Granada, Granada, Spain.

PLASMA ADIPOKINE CONCENTRATIONS IN PREPUBERTAL CHILDREN WITH DIFFERENT LEVELS OF CARDIORESPIRATORY FITNESS AND PHYSICAL ACTIVITY

Llorente-Cantarero FJ[ab], Gil-Campos M[b*], MD, PhD, Benitez-Sillero JD[a], PhD, Olza J[d], Muñoz-Villanueva MC[c] MD, PhD, Aguilera CM[d], PhD, Pérez-Navero JL[b] MD, PhD.

[a]: Department of Corporal Expression. Faculty of Education. University of Cordoba, Avda San Alberto Magno s/n. 14004 Córdoba, Spain. llorentefj@yahoo.es; juande_dios@hotmail.com

[b]: Department of Pediatrics. University Reina Sofia Hospital, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Avda Menéndez Pidal s/n. 14004, Cordoba (Spain). mercedes_gil_campos@yahoo.es; ucip.hrs.sspa@juntadeandalucia.es,

[c]: Unit of Methodology in Investigation, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Avda Menéndez Pidal s/n. 14007, Córdoba, Spain. mc.munoz.exts@juntadeandalucia.es

[d]: Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology, Centre of Biomedical Research, University of Granada, Avda del Conocimiento, 18071 Granada, Spain. jolza@ugr.es

Short title: Adipokines in children according to fitness

Key words: Inflammation, adipocytokines, physical activity, fitness, childhood

***Corresponding author and reprint requests:**

Gil-Campos Mercedes, PhD.

Servicio de Pediatría. Hospital Universitario Reina Sofia.

Avda. Menéndez Pidal s/n. C.P. 14004,

26 Córdoba, Spain

27 E-mail: mercedes_gil_campos@yahoo.es

28 Tel:+3495701049; Fax:+34957010017

Abstract

Objective: To assess some adipokines and inflammatory biomarkers in prepubertal healthy children with different levels of cardiorespiratory fitness (CRF) and physical activity (PA).

Subjects: A total of 132 healthy children (78 boys/54 girls) aged between 7 and 12 years, in Tanner I sexual maturity stage (prepubertal) were recruited from local schools.

Design: Children were divided into two groups (equal or high fitness (HF) group and low fitness group (LF)) according to their performance in a 20-meter shuttle run test. To estimate the children's fitness level and exercise habits, participants were observed while engaged in an after-school program and were asked to answer a questionnaire on exercise and sedentary habits. After assessment, children were assigned either to the regular physical activity group (PAG) or to the sedentary group (SG). Anthropometric parameters, blood pressure, and the following plasma adipokines were measured: leptin, resistin, adiponectin, tumor necrosis factor alpha, hepatic growth factor, interleukin 6 (IL-6), IL 8, macrophage chemoattractant type 1 (MCP1), nerve growth factor, and plasminogen activator inhibitor 1.

Results: After adjustment for BMI, age and sex, the LF group showed higher leptin levels and lower IL-6 levels as compared to the HF group. In relation to PA, adiponectin and MCP1 levels were higher in the SG as compared to the PAG. However, after adjustment for BMI, age and sex, only MCP1 remained significant. When boys and girls were compared, no differences were found after adjustment for BMI, age, CRF and PA.

Conclusion: This study provides new information about the association between plasma levels of some adipokines and the level of fitness and physical activity in healthy prepubertal children after adjustment for age, sex or BMI.

Introduction

Currently, it is known that adipocytes secrete a diverse group of proteins called adipokines, which are involved in different biological functions. When proinflammatory cytokines –such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and other proteins as C-reactive protein (CRP) – are elevated in plasma, they may induce cardiovascular diseases (CVD), type II diabetes or obesity. Moreover, several adipokines –such as leptin and adiponectin– appear to play a role in glucose and lipid metabolism and energy homeostasis[1]. Although elevated leptin and low adiponectin concentrations seem to be correlated with a range of metabolic syndrome (MS) components, the role of adipokines in the development of metabolic changes in children is not yet fully understood. So, the underlying factors of obesity and MS could appear in these stages of life and even in healthy prepubertal children[2].

Physical activity (PA), cardiorespiratory fitness (CRF) and muscular fitness (MF) are key constructs in exercise science and have been shown to be negatively associated with death from many causes, including cardiovascular mortality[3]. CRF involves a set of health- or skill-related attributes with a strong genetic component that remains relatively static, needing some time to change, and which is assessed using a battery of field tests[4]. The term *physical activity* is often used interchangeably with *energy expenditure* and *physical fitness*. PA has been defined as any bodily movement produced by skeletal muscles which results in energy expenditure. CRF and PA may affect metabolic risk but the relationship is weak and they may act through different pathways[3,5].

In childhood, PA and CRF have also been negatively associated with traditional CVD risk factors[4,6] and low-grade inflammation[7]. One of the mechanisms through which physical fitness might promote cardiovascular health is by supporting anti-

inflammatory processes[8]. Although screening for inflammatory biomarkers in children is important, screening for another risk factor –low CRF– is much more compelling[9]. PA is also an important behavioural co-factor; in fact, people describing themselves as active have lower levels of inflammatory biomarkers than their sedentary counterparts[7]. Indeed, when physical or CRF is thoroughly and objectively assessed by maximal exercise testing, fitness is found to be inversely associated with inflammation, even after adjustment for confounds including age, smoking habits, medication, and visceral fat[8,10]. However, the relationship between PA and inflammation biomarkers in young people has been scarcely studied.

With respect to adipokines, an inverse relationship has been observed between regular PA and leptin levels, independently of adiposity[11]. Changes in leptin levels have also been found to be associated with changes in fitness induced by a training programme in obese adolescents[12]. Similarly, IL-6 also seems to be negatively associated with PA. Regular exercise seems to have the potential to decrease systemic proinflammatory cytokines as IL-6 serum levels[13], or during exercise, to decrease TNF-α or macrophage chemoattractant protein-1 (MCP1)[14]. On the other hand, low plasma adiponectin levels have been related to decreased insulin sensitivity and adipocyte dysfunction but the effects of PA on adiponectin levels in children are still unclear[15-16].

PA and physical fitness could be protective against low-grade inflammation, but further research is needed on children to clarify the associations between PA, CRF, adiposity and inflammation and other CVD risk factors[7,14]. Therefore, the aim of this study is to assess the plasma concentrations of a set of adipokines and inflammatory biomarkers in healthy prepubertal children with different CRF and PA levels.

Material / subjects and Methods

Subjects and Design

104 A total of 156 healthy children at prepubertal age were selected from local
105 elementary schools in Spain. Inclusion criteria were: subjects aged 7-12 years, at
106 prepubertal stage (Tanner I)[18] validated by appropriate plasma sex hormone level
107 assessment. Exclusion criteria were : presence of pubertal development, disease, long
108 periods of rest after illness, use of any medication altering blood pressure or glucose or
109 lipid metabolism, consumption of any diet, and failure to get the same record reached in
110 the first attempt in the 20-mSRT[19]. Finally, 132 children participated in the study: 78
111 boys and 54 girls. Written informed consent was obtained from parents or legal
112 guardians and the study was approved by the Institutional Ethics Committee at the
113 University Reina Sofia Hospital.

114 Children were asked to perform a 20-meter shuttle run test (20-mSRT) in order
115 to evaluate their CRF. The validated scale developed by Olds et al. (2006)[20] was used to
116 measure CRF after the 20-mSRT. Test performances are expressed as mean ± SD for all
117 children of similar age and sex from all countries. In the present study, children were
118 assigned to two groups according to the methodology used in previous studies[21-22]. The
119 children recording a score equal or greater than the average reference value were
120 assigned to the group designated “equal or higher cardiovascular fitness group” (HF)
121 (70 subjects); and those with less-than-average scores (62 subjects) were assigned to the
122 “low cardiovascular fitness” group (LF).

123 After assessment of exercise habits, children were classified into two groups: the
124 regular physical activity group (in an after-school program) (PAG) (76 subjects), and
125 the sedentary group (SG) (56 subjects).

126 **Clinical and Physical Activity Evaluation**

127 Anamnesis and physical examination were assessed by paediatricians to discard any
128 illness. Sexual maturity was assessed by physical examination according to Tanner five-

129 stage scale[18]. Weight and height were measured using standard techniques, beam
130 balance and a precision stadiometer (Seca) with participants lightly dressed and
131 barefooted. Body mass index (BMI) was calculated as weight (kg)/ height (m^2). Waist
132 circumference (WC) was measured in duplicate with an inelastic tape according to
133 standardized methods. These anthropometric measurements were compared with
134 Spanish reference standards[23], and with the age- and sex-specific cutoff points proposed
135 by Cole et al. (2000)[24] to define obesity.

136 Systolic and diastolic blood pressures (BPs) were measured in the right arm in a
137 sitting position using a random-zero sphygmomanometer (Dinamap V-100) after the
138 subjects had rested without changing position for at least five minutes.

139 To estimate PA, children were observed while engaged in an after-school
140 program. This program involved physical exercise at least three times per week for at
141 least one year. Additionally, a short test based on the NICHD validated questionnaire[25]
142 was used for both groups to obtain information about PA practice and sedentary habits.

143 **Evaluation of Cardiorespiratory Fitness**

144 Standardized Eurofit battery tests[26] were performed to evaluate fitness. A 20mSRT was
145 used to assess CRF, and upper and lower body strength was assessed by hand
146 dynamometry and standing broad jump (SBJ) tests respectively. The 20mSRT test
147 requires subjects to run back and forth between two lines set 20m apart. Running speed
148 started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at
149 minute 20. Running speed cues were indicated by signals emitted by a commercially-
150 available CD–ROM. Subjects were allowed to voluntarily withdraw from the test after
151 being verbally encouraged to maximally perform during each test. The test finished
152 when the subject failed to reach the finishing lines concurrent with the audio signals on
153 two consecutive times.

In addition, upper body muscular strength was assessed by means of the hand-grip strength and bent-arm hang tests (Takei TKK-5110, 0.1 kg precision and 5 to 100 kg average) and by doing crunches for 30 seconds. Lower body muscular strength was assessed by the standing broad-jump test.

Analytical -Procedures

Sampling. Children were assessed at the hospital between 0800h and 0930h after a 12-h overnight fasting. Blood samples were obtained from all children using an indwelling venous line to draw a 3-ml sample. After centrifugation, aliquots of plasma were frozen immediately and stored at -80º until analyzed.

Hematimetry, plasma hormones and metabolic biomarkers. Hematimetry and general biochemical parameters were also measured to evaluate participants' health state using an automatic analyzer (Accelerator APS system. Architect-c16000. Abbott Laboratories, S.A., Illinois, U.S.A.). Fasting gonadotropins and sex hormones: follicle stimulating hormone (FSH: CV: 3.6%); lutein hormone (LH: CV: 3.1%); testosterone (CV: 2%) and estradiol (CV: 1.8%) were measured by chemiluminescence using an automatic analyzer (Architect I4000, Abbott Laboratories) to validate that the children selected by clinical signs and Tanner stage were truly prepubertal. A radioimmunoassay with an automatic analyzer for microparticles (Axsym, Abbott Laboratories, Chicago, IL, USA) was used to measure insulin.

Plasma inflammatory biomarkers and adipokines. CRP was measured in a particle-enhanced turbidimetric immunoassay (PETIA) (Dade Behring Inc., IL) to prevent potential inflammatory complications (i.e. infections) and establish the relationship with between CRP and adipokine levels.

LINCOplex™ kits of human monoclonal antibodies (Linco Research, MO, USA) were analyzed on a Luminex ® 200™ System (Luminex Corporation, Austin, TX,

179 USA) to determine: adiponectin (CV: 9.2%) (Cat.#HCVD1-67AK), resistin (CV: 6.0%)
180 (Cat. HADK1-61K-A), leptin (CV: 7.9%) (Cat.#HADK2-61K-B), plasma hepatic
181 growth factor (HGF) (CV: 7.7%), IL-6 (CV: 7.8%), interleukin 8 (IL-8) (CV: 7.9%),
182 MCP1 (CV: 7.9%), nerve growth factor (NGF) (CV: 6%), plasminogen activator
183 inhibitor-1 (PAI1) (CV: 11.8%), and TNF-α (CV: 7.8%) levels, according to
184 manufacturer's instructions[27].
185
186 **Statistical Analysis**
187 Data were expressed as mean ± SD. Normal data distribution was assessed by the
188 Shapiro-Wilk test. Homogeneity of variances was estimated using the Levene test. The
189 mean values for continuous variables with normal distribution were compared using
190 Student's *t*-test for unpaired samples and Mann-Whitney U test for variables with
191 asymmetric distribution. Comparative analysis of test results for the two groups was
192 performed using ANCOVA methods (analysis of covariance) after adjustment for age,
193 sex, BMI, CRF and PA. Correlations between variables were assessed using a non-
194 parametric correlation analysis. Rho Spearman's correlation coefficients were
195 calculated. A simple logistic regression analysis was realized to evaluate the
196 associations between CRF and PA with inflammation biomarkers. Statistical
197 significance was considered when $P < 0.05$. All statistical analyses were performed
198 using Statistical Package for Social Science software (PASW Statistic 18. Inc. Chicago,
199 IL, USA).
200
201 **Results**
202 Demographic, anthropometric and blood pressure levels in the prepubertal children of
203 this study –classified by CRF– are displayed in Table 1. We found no differences in age

and blood pressure between these groups. BMI was higher in the LF group, which components did not have obesity.

Gender-based differences were found as regards age and BMI, which were greater in boys: age: 10.16±0.97 and BMI: 20.83±3.74 *vs* girls: age: 8.78±0.90 and BMI: 19.08±3.68; P<0.01.

Regarding inflammation biomarkers and adipokines in plasma, the LF group showed greater levels of PAI1 (18.97±10.64 *vs* 13.98±9.63) and leptin (21691.29±17675.84 *vs* 9012.28±8194.91) as compared to the HF group, with no differences in the remaining parameters. After adjustment for BMI, age and sex, leptin levels remained higher in the LF as compared to the HF group, and IL-6 levels were lower in the LF group (Table 2). Differences in IL-8 levels were near significance as they were higher in the LF and SG groups, as compared to the HF and PPA groups, respectively.

When children were studied according to their PA, it was observed that plasma adiponectin (21.01±10.4 *vs* 17.93±8.62) and MCP1 (183.23±100.72 *vs* 144.82±74.58) levels were higher in the SG as compared to the PAG. However, after adjustment for BMI, age and sex only the difference in MCP1 levels remained significant (Table 2).

When boys and girls were compared, we found greater levels of adiponectin (21.71±10.97 *vs* 17.66±8.13) and lower levels of TNFα (6.29±1.84 vs 7.62±3.17) in girls. However, such differences between girls and boys disappeared after adjustment for BMI, age, CF and PA (Table 3).

Table 4 shows relevant correlations between inflammation biomarkers and other metabolic parameters. There were strong positive correlations between leptin levels and TA, insulin, CRP, IL-6 and PAI1 levels. IL-6 was also correlated with CRP and weakly

correlated with TNF-α. PAI1 was found to be associated with TA, insulin, CRP and HGF levels.

After a simple logistic regression analysis, the increment in 1 unit of PAI1, down to 5.2% (P: 0.010), the probability to have a good fitness. The increase of 1 ng/L of MCP1 is associated with a decrease in exercise practice (1%, P: 0.019).

Discussion

The results of the present study indicate that CRF and PA may influence plasma adipokine levels regardless of BMI, age and sex. Higher levels of leptin and lower levels of IL-6 were observed in children with low CRF as compared to children in good physical condition. The sedentary group also showed higher levels of MCP1 as compared to the group of physically active children.

At present, the potential anti-inflammatory role of CRF and PA in children is not yet clear. However, the role of risk factors during childhood on the development of CVDs later in adulthood should be further analyzed. According to the results of Martinez-Gómez (2010)[28], they seem to indicate that PA may have an indirect role through CRF and body fat, which are health determinants. Preliminary evidence suggests that achieving a healthy weight during adolescence might be the most effective strategy to prevent chronic low-grade inflammation and future CVDs and metabolic diseases. Whereas genetic and early programming features have been associated with low-grade inflammation in young people[27], an active lifestyle and a desirable CRF may attenuate its effects[28].

Adiponectin levels are negatively correlated with CVDs; therefore, this hormone should be expected to be positively correlated with CRF. After programs of exercise training with no loss of body mass, plasma adiponectin concentration did not change

253 while insulin action improved, suggesting that its action depends on changes in fat
254 mass[15,30-31]. Thus, highly trained adults present similar resting plasma adiponectin levels
255 after a prolonged training. Nevertheless, in trained subjects, adiponectin increase during
256 and after a high exercise while subjects with a low CRF show an increase in training
257 stress but accompanied by a decrease in postexercise adiponectin plasma values[16].

258 In healthy children, two previous studies found either no significant association[32]
259 or an inverse association between CRF and adiponectin[33], although it is important to
260 note that body fat was not controlled by statistical analysis in these studies[33]. In the
261 present study, no differences were observed in adiponectin levels between groups with
262 good and poor CRF, and between SG and PAG after adjustment for BMI.

263 Respect to PA, in cross-sectional studies performed in children and adolescents
264 found no significant associations between adiponectin levels and self-reported PA,
265 using subjective measurement tools[11,34]. However, using an accelerometer to evaluate
266 PA, an inverse association has been found between PA and adiponectin and leptin levels
267 in school-children after assessment of body fat[35-36]. Consequently, it has been suggested
268 that adiponectin secretion is inhibited in adolescents with appropriate insulin sensitivity,
269 potentially due to PA and fitness[36]. So, a limitation to this work could be that we did not
270 objectively measure PA using an accelerometer, and PA intensity could also affect the
271 results.

272 As regards the relationship between gender and adiponectin levels in young
273 people, previous studies have failed to demonstrate sex differences[37], but a study with a
274 large population-based sample (most of whom were prepubertal) showed that girls
275 presented greater mean adiponectin concentrations than boys of any age. Moreover,
276 high levels of self-reported intense PA have been positively associated with adiponectin
277 levels in adolescent girls regardless of their weight status[38]. The prepubertal girls of the

present study presented higher adiponectin levels than boys, but when data were adjusted for BMI, age, fitnesss and PA, these differences disappeared. It has been reported that the effects of age on adiponectin levels in girls could be explained by their BMI and total fat mass[39]. Hence, sex differences could be associated with fat mass even in the prepubertal stage, so further research studies adjusted for confounding factors – sex and pubertal status– should be performed.

Leptin has been reported to be independently and inversely related to PA and CRF. Nonetheless, inconsistent results have been obtained by previous studies in young people examining PA and leptin[40] with decreases after a prolonged intense exercise session[41]. Moreover, there is scarce literature regarding the association between CRF and leptin. CRF and PA might stimulate insulin sensitivity and induce a decrease in insulin release, which in turn might reduce leptin levels. Consequently, PA could decrease IL-6 and TNF-α levels and, ultimately, the production of CRP by reducing obesity and leptin levels and increasing adiponectin and insulin sensitivity[42]. On the other hand, leptin expression may also be reduced by catecholamines and other adipokines stimulated by PA[43]. In the present study and after statistical adjustment, the group with LF still presented higher leptin and IL-6 levels as compared to the HF group (Table 2).

IL-6 is structurally related to leptin and both are adipokines which are produced by the adipose tissue. Although adipocyte-derived IL-6 does not seemingly play a major role in inflammatory responses, it is probably involved in the regulation of energy expenditure and it might function as an anti-obesity cytokine[44]. However, increased sympathetic activity during PA could be responsible for the synthesis of IL-6 not only in adipose tissue, but also in muscles[45]. Although most literature suggests that proinflammatory cytokines such as IL-6 promote catabolism, at lower levels (as those

observed in the present study), they might promote muscle and blood vessel growth and act as a beneficial response to exercise[46]. In fact, increased IL-6 levels have been reported after exercise in healthy young people with different levels of fitness[47].

Little is known about the biological effects of proinflammatory cytokines on healthy subjects, probably due to the fact that IL-6 and TNF-α are only present at very low concentrations. However, inverse correlations have been found between circulating TNF-α and IL-6 levels[48]. It has been suggested that TNF-α and BMI are covariates in the etiology of insulin resistance in young people[48]. As muscle activation increases IL-6 levels[46], then physically fit children with low adiposity should have greater levels of IL-6 and normal levels of TNF-α[48]. In fact, in the present work, we found strong positive associations between leptin, insulin and IL-6 levels, and CRP, and weaker significant correlations between these adipokines and TNF-α levels (Table 4). A meta-analysis of cytokine responses to laboratory stressors has suggested that while IL-6 is responsive to acute psychological stressors, TNF-α is not[49]. In our study, we found that IL-6 levels – but not TNF-α levels– are also correlated with CRP, which is consistent with the fact that IL-6 is a major inducer of CRP production (Table 4).

Despite pro-inflammatory cytokines such as IL-6 or TNF-α may increase in response to each individual exercise bout, exercise's long-term anti-inflammatory effects. These acute processes may be accompanied by simultaneous elevations of anti-inflammatory cytokines by the translocation/migration of leukocytes in response to chemokines as MCP1[14]. In the sedentary group, MCP1 remained higher after adjustments as compared to the PA group. Moreover, Trøseid et al. (2004)[50] showed that an exercise program for healthy adults reduced plasma MCP1 levels and other study in children reported a reduction in MCP1, TNF-α and IL-8 plasma levels during

and 30 min after of exercise cessation[14]. These data are consistent with the results of this study, which shows that exercise reduces MCP1 levels regardless of BMI, age or sex.

An increase in fibrinolytic activity has been also associated with physical exercise, resulting in a decrease in PAI1[51]. Fibrinolytic activity appears to be influenced by exercise intensity in healthy adolescents and returns to normal levels after 24 h[52]. However, to our knowledge, no studies have assessed fibrinolytic activity after a training program, and the number of studies associating this biomarker with fitness is scarce as well. Gallistl et al. (2000)[53] showed that after adjustment for age and fat mass, fibrinolytic activity was significantly correlated with PAI1 levels in children, and independent of CRF. Our results are partially consistent with those, showing that PAI1 levels were higher in the poor fitness group but once adjustments were made, this difference disappeared when groups were compared by CRF and PA (Table 2). In fact, PAI1 was positively associated with other adipokines as leptin, IL-8 and HGF, and with other cardiovascular risk parameters as TA, insulin or CRP (Table 4).

Similarly, we did not found any changes in resistin levels associated with fitness or PA in healthy prepubertal children. In addition, low correlations were found between resistin levels and insulin, TG and CRP levels, and no correlation was found with other inflammatory biomarkers (results not shown). Resistin has been reported to be one of the links between obesity and insulin resistance[54]. Although the role of resistin in energy homeostasis is not completely defined, interest in this hormone remains high. To better understand how resistin levels are regulated, further studies should be conducted to measure the concentrations of this hormone in overweight people and understand how lifestyle choices –such as regular aerobic exercise– affect resistin concentrations[55]. There are inconclusive results as to whether long-term exercise training increases or not resistin concentrations, suggesting that training does not consistently induce favourable

changes in resistin concentrations[56]. The results of the present study suggest that resistin might be intimately associated with body fat and metabolic syndrome, and that it is probably not altered in normal weight individuals. Adolescents have been described to present a decrease in resistin concentrations during fasting associated with a decrease in percent body fat, which has been interpreted as a positive response to long-term exercise[55].

Conclusion: This study provides new information about the influence of level of fitness and physical activity in plasma adipokines in healthy prepubertal children, regardless of BMI, age and sex. Sedentary and low fitness groups show some changes in inflammatory cytokines comparing to the group of physically active children that may be further studied in childhood to establish a cardiovascular risk.

There is no conflict of interest.

References

1 Körner A, Kratzsch J, Gausche R, Schaab M, Erbs S, Kiess W. New predictors of the metabolic syndrome in children--role of adipocytokines. *Pediatr Res* 2007; **61**: 640-5.

2 Siegrist M, Hanssen H, Lammel C, Haller B, Halle M. A cluster randomised school-based lifestyle intervention programme for the prevention of childhood obesity and related early cardiovascular disease (JuvenTUM 3). *BMC Public Health* 2011; **11**: 258.

3 Kampert JB, Blair SN, Barlow CE, Kohl HW 3rd. Physical activity, physical fitness, and all-cause and cancer mortality: a prospective study of men and women. *Ann Epidemiol* 1996; **6**: 452-7.

4 Steele RM, Brage S, Corder K, Wareham NJ, Ekelund U. Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. *J Appl Physiol* 2008; **105**: 342-51.

5 Wärnberg J, Nova E, Romeo J, Moreno LA, Sjöström M, Marcos A. Lifestyle-related determinants of inflammation in adolescence. *Br J Nutr* 2007; **98**: S116-20.

6 Llorente-Cantarero FJ, Pérez-Navero JL, de Dios Benitez-Sillero J, Muñoz-Villanueva MC, Guillén-Del Castillo M, Gil-Campos M. Non-traditional markers of metabolic risk in prepubertal children with different levels of cardiorespiratory fitness. *Public Health Nutr* 2012; **16**: 1-8.

7 Thomas NE, Williams DR. Inflammatory factors, physical activity, and physical fitness in young people. *Scand J Med Sci Sports* 2008; **18**: 543-56.

8 Aronson D, Sheikh-Ahmad M, Avizohar O, Kerner A, Sella R, Bartha P *et al*. C-Reactive protein is inversely related to physical fitness in middle-aged subjects. *Atherosclerosis* 2004; **176**: 173-9.

9 Noland RC, Baker JT, Boudreau SR, Kobe RW, Tanner CJ, Hickner RC *et al*. Effect of intense training on plasma leptin in male and female swimmers. *Med Sci Sports Exerc* 2001; **33**: 227-31.

10 Von Känel R, Hong S, Pung MA, Mills PJ. Association of blood pressure and fitness with levels of atherosclerotic risk markers pre-exercise and post-exercise. *Am J Hypertens* 2007; **20**: 670-5.

11 Romon M, Lafay L, Bresson JL, Oppert JM, Borys JM, Kettaneh A *et al*. Relationships between physical activity and plasma leptin levels in healthy children:

400 the Fleurbaix-Laventie Ville Santé II Study. *Int J Obes Relat Metab Disord* 2004;
401 **28**: 1227-32.
402 12 Barbeau P, Gutin B, Litaker MS, Ramsey LT, Cannady WE, Allison J *et al*.
403 Influence of physical training on plasma leptin in obese youths. *Can J Appl Physiol*
404 2003; **28**: 382-96.
405 13 Jankord R, Jemiolo B. Influence of physical activity on serum IL-6 and IL-10 levels
406 in healthy older men. *Med Sci Sports Exerc* 2004; **36**: 960-4.
407 14 Rosa JS, Oliver SR, Flores RL, Graf SC, Pontello AM, Lbardolaza M *et al*. Kinetic
408 profiles of 18 systemic pro- and anti-inflammatory mediators during and following
409 exercise in children. *J Pediatr Endocrinol Metab* 2007; **20**: 1293-305.
410 15 Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA,
411 Yannakoulia M *et al*. Aerobic exercise training improves insulin sensitivity without
412 changes in body weight, body fat, adiponectin, and inflammatory markers in
413 overweight and obese girls. *Metabolism* 2005; **54**: 1472-9.
414 16 Jürimäe J, Purge P, Jürimäe T. Adiponectin and stress hormone responses to
415 maximal sculling after volume-extended training season in elite rowers. *Metabolism*
416 2006; **55**: 13-9.
417 17 Magnussen CG, Raitakari OT. Are we there yet? Pediatric screening for
418 inflammatory biomarkers and low cardiorespiratory fitness to identify youth at
419 increased risk of cardiovascular disease. *J Adolesc Health* 2010; **47**: 319-21.
420 18 Tanner, J.M. *Growth at adolescente. Oxford: Blackwell*; 1962.
421 19 Léger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 meter shuttle run
422 test for aerobic fitness. *J Sports Sci* 1988; **6**: 93-101.

20 Olds T, Tomkinson G, Léger L, Cazorla G. Worldwide variation in the performance of children and adolescents: an analysisof 109 studies of the 20-m shuttle run test in 37 countries. *Sports Sci* 2006; **24**: 1025-38.

21 García-Artero E, Ortega FB, Ruiz JR, Mesa JL, Delgado M, González-Gross M *et al*. Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study). *Rev Esp Cardiol* 2007; **60**: 581-8.

22 McGavock JM, Torrance BD, McGuire KA, Wozny PD, Lewanczuk RZ. Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. *Obesity* 2009; **17**: 1802-7.

23 Sobradillo B, Aguirre A, Aresti U, Bilbao A, Fernández-Ramos C, Lizárraga A. (2004) Curvas y tablas de crecimiento (estudios longitudinal y transversal). Madrid: Fundacio´n Faustino Orbegozo Eizaguirre.

24 Cole TJ, Bellizzi MC, Flegal KM Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; **320**: 1240-1243.

25 Pianta RC. National Institute of Child Health and Human Development. Developmental science and education: the NICHD study of early child care and youth development findings from elementary school. *Adv Child Dev Behav* 2007; **35**: 253-296.

26 Committee of Experts on Sports Research EUROFIT (1993) Handbook for the EUROFIT Tests of Physical Fitness. Strasburg: Council of Europe.

27 Kellar KL, Douglass JP. Multiplexed microsphere-based flow cytometric immunoassays for human cytokines. *J Immunol Methods* 2003; **279**: 277-85.

28 Martinez-Gomez D, Eisenmann JC, Wärnberg J, Gomez-Martinez S, Veses A, Veiga OL *et al.*; AFINOS Study Group. Associations of physical activity, cardiorespiratory

448 fitness and fatness with low-grade inflammation in adolescents: the AFINOS Study.
449 *Int J Obes (Lond)* 2010; **34**: 1501-7.
450 29 Labayen I, Ortega FB, Sjöström M, Ruiz JR. Early life origins of low-grade
451 inflammation and atherosclerosis risk in children and adolescents. *J Pediatr* 2009;
452 **155**: 673-7.
453 30 Hulver MW, Zheng D, Tanner CJ, Houmard JA, Kraus WE, Slentz CA *et al*.
454 Adiponectin is not altered with exercise training despite enhanced insulin action. *Am*
455 *J Physiol Endocrinol Metab* 2002; **283**: E861-5.
456 31 Yokoyama H, Emoto M, Araki T, Fujiwara S, Motoyama K, Morioka T *et al*. Effect
457 of aerobic exercise on plasma adiponectin levels and insulin resistance in type 2
458 diabetes. *Diabetes Care* 2004; **27**: 1756-8.
459 32 McVean JJ, Carrel AL, Eickhoff JC, Allen DB. Fitness level and body composition
460 are associated with inflammation in non-obese children. *J Pediatr Endocrinol Metab*
461 2009; **22**: 153-9.
462 33 Nemet D, Wang P, Funahashi T, Matsuzawa Y, Tanaka S, Engelman L *et al*.
463 Adipocytokines, body composition, and fitness in children. *Pediatr Res* 2003; **53**:
464 148-52.
465 34 Platat C, Wagner A, Klumpp T, Schweitzer B, Simon C. Relationships of physical
466 activity with metabolic syndrome features and low-grade inflammation in
467 adolescents. *Diabetologia* 2006; **49**: 2078-85.
468 35 Metcalf BS, Jeffery AN, Hosking J, Voss LD, Sattar N, Wilkin TJ. Objectively
469 measured physical activity and its association with adiponectin and other novel
470 metabolic markers: a longitudinal study in children (EarlyBird 38). *Diabetes Care*
471 2009; **32**: 468-73.

36 Martinez-Gomez D, Eisenmann JC, Gomez-Martinez S, Veses A, Romeo J, Veiga OL *et al.*; AFINOS Study Group. Associations of physical activity and fitness with adipocytokines in adolescents: the AFINOS Study. *Nutr Metab Cardiovasc Dis* 2012; **22**: 252-9.

37 Camhi SM, Katzmarzyk PT. Tracking of cardiometabolic risk factor clustering from childhood to adulthood. *Int J Pediatr Obes* 2010; **5**: 122-9.

38 Rubin DA, McMurray RG, Harrell JS, Thorpe DE, Hackney AC. Vigorous physical activity and cytokines in adolescents. *Eur J Appl Physiol* 2008; **103**: 495-500.

39 Punthakee Z, Delvin EE, O'loughlin J, Paradis G, Levy E, Platt RW *et al*. Adiponectin, adiposity, and insulin resistance in children and adolescents. *J Clin Endocrinol Metab* 2006; **91**: 2119-25.

40 Ischander M, Zaldivar F Jr, Eliakim A, Nussbaum E, Dunton G, Leu SY *et al*. Physical activity, growth, and inflammatory mediators in BMI-matched female adolescents. *Med Sci Sports Exerc* 2007; **39**: 1131-8.

41 Ruiz JR, Ortega FB, Wärnberg J, Moreno LA, Carrero JJ, Gonzalez-Gross M *et al*. Inflammatory proteins and muscle strength in adolescents: the Avena study. Arch Pediatr Adolesc Med. 2008;162:462-8.

42 Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE *et al*. Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation* 2004; **109**: 2181-5.

43 Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; **352**: 1685-95.

44 Wallenius V, Wallenius K, Ahrén B, Rudling M, Carlsten H, Dickson SL *et al*. Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med* 2002; **8**: 75-9.

45 Czarkowska-Paczek B, Bartlomiejczyk I, Gabrys T, Przybylski J, Nowak M, Paczek L. Lack of relationship between interleukin-6 and CRP levels in healthy male athletes. *Immunol Lett* 2005; **99**: 136-40.

46 Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N *et al*. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. *Pflugers Arch* 2003; **446**: 9-16.

47 Wardyn GG, Rennard SI, Brusnahan SK, McGuire TR, Carlson ML, Smith LM *et al*. Effects of exercise on hematological parameters, circulating side population cells, and cytokines. *Exp Hematol* 2008; **36**: 216-23.

48 Andersen LB, Müller K, Eiberg S, Froberg K, Andersen JF, Bugge A *et al*. Cytokines and clustered cardiovascular risk factors in children. *Metabolism* 2010; **59**: 561-6.

49 Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun* 2007; **21**: 901-12.

50 Trøseid M, Lappegård KT, Claudi T, Damås JK, Mørkrid L, Brendberg R *et al*. Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. *Eur Heart J* 2004; **25**: 349-55.

51 El-Sayed MS, Lin X, Rattu AJ. Blood coagulation and fibrinolysis at rest and in response to maximal exercise before and after a physical conditioning programme. *Blood Coagul Fibrinolysis* 1995; **6**: 747-52.

52 Ribeiro J, Almeida-Dias A, Ascensão A, Magalhães J, Oliveira AR, Carlson J *et al*. Hemostatic response to acute physical exercise in healthy adolescents. *J Sci Med Sport* 2007; **10**: 164-9.

520 53 Gallistl S, Sudi KM, Borkenstein M, Troebinger M, Weinhandl G, Muntean W.
521 Determinants of haemostatic risk factors for coronary heart disease in obese children
522 and adolescents. *Int J Obes Relat Metab Disord* 2000; **24**: 1459-64.
523 54 Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM *et al*. The
524 hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307-12.
525 55 Jones TE, Basilio JL, Brophy PM, McCammon MR, Hickner RC. Long-term
526 exercise training in overweight adolescents improves plasma peptide YY and
527 resistin. *Obesity (Silver Spring)* 2009; **17**: 1189-95.
528 56 Corpeleijn E, Feskens EJ, Jansen EH, Mensink M, Saris WH, Blaak EE. Lifestyle
529 intervention and adipokine levels in subjects at high risk for type 2 diabetes: the
530 Study on Lifestyle intervention and Impaired glucose tolerance Maastricht (SLIM).
531 *Diabetes Care* 2007; **30**: 3125-7.

532 **Table 1**. Demographic, anthropometric, blood pressure values and practice of physical
533 activity in prepubertal children with different levels of fitness
534

Variables	HF Group N: 70	LF Group N: 60	P*
Age (years)	9.62±1.06	9.60±1.23	0.846
Sex (boys/girls)	29/31	50/20	**0.012**
BMI (kg/m^2)	18.69±2.89	21.55±3.99	**<0.001**
PA	72%	42%	**0.007**
SBP (mmHg)	120.00±10.36	123.25±16.09	0.409
DBP (mmHg)	66.94±9.14	67.32±10.53	0.820

535
536 LF: Low Fitness Group. HF: Equal or Higher Fitness Group.
537 BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.
538 *Statistical significance after application of Student's T-test (or U of Mann-Whitney) to
539 data expressed as mean ± SD (or median ± intercuartile range), and chi-square for
540 comparison of proportions.
541

542 **Tabla 2**. Concentration of plasma adipokines in prepubertal children classified by
543 different levels of fitness and physical activity.

544

Variables	LF Group N: 70	HF Group N: 60	P CI 95%	SG N: 59	PPA N: 78	P CI 95%
Adiponectin	18.66±1.34	19.36±1.23	0.720	19.95±1.32	18.42±1.11	0.389
PAI1	17.00±1.29	1578±1.19	0.516	15.28±1.27	17.30±1.08	0.235
Resistin	11.90±0.85	13.66±0.78	0.156	11.68±0.83	13.67±0.70	0.073
HGF	1728.36±106.56	1989.01±98.40	0.095	1800.84±104.51	1912.52±88.98	0.426
IL8	3.14±0.40	4.14±0.37	0.087	3.11±0.39	4.02±0.33	0.083
Leptin	18373.10±1517.72	12044.00±1413.49	**0.005**	15522.13±1296.23	13822.05±1110.98	0.330
MCP1	154.24±11.94	161.73±11.02	0.667	178.10±11.54	145.72±9.83	**0.038**
NGF	12.20±0.84	10.24±0.081	0.121	11.00±0.87	11.22±0.73	0.848
TNF-α	7.08±0.38	7.12±0.35	0.937	6.70±0.37	7.30±0.31	0.226
IL6	0.95±0.22	1.67±0.21	**0.029**	1.07±0.22	1.53±0.19	0.124

545

546 LF: Low Fitness Group; HF: Equal or Higher Fitness Group; SG: Sedentary Group;
547 PPA: Practise Physical Activity Group.
548 HGF: Hepatocyte Growth Gactor; IL-6**:** Interleukin-6; IL-8: Interleukin-8; MCP-1**:**
549 Macrophage Chemoattractant Factor; NGF: Nerve Growth Factor; PAI 1: Plasminogen
550 Activator Inhibitor-1; TNF-α: Tumor Necrosis Factor Alpha.
551 *Statistical significance between groups of fitness (HF *vs* LF) and between groups of
552 physical activity (PPA *vs* SG), after application of ANCOVA (analysis of covariance)
553 after adjusting for age, BMI, fitness and physical activity. Data are expressed as mean
554 ±SEM.

555
556
557
558
559

560 **Tabla 3**. Plasma adipokine level in healthy prepubertal girls *vs* boys.

561

Variables	Girls N: 54	Boys N: 78	P CI 95%
Adiponectin	21.31±1.59	17.63±1.21	0.100
PAI1	16.46±1.50	15.90±1.16	0.790
Resistin	14.27±1.00	11.82±0.77	0.084
HGF	1993.70±125.56	1764.26±97.14	0.136
IL8	3.36±0.47	3.81±0.36	0.496
Leptin	14241.51±1522.78	14421.20±1186.61	0.934
MCP1	145.95±13.90	167.57±10.76	0.271
NGF	11.76±1.11	10.86±0.74	0.549
TNF-α	6.43±0.44	7.45±0.34	0.106
IL6	1.77±0.27	1.05±0.21	0.058

562

563 HGF: Hepatocyte Growth Gactor; IL-6**:** Interleukin-6; IL-8: Interleukin-8; MCP-1**:**
564 Macrophage Chemoattractant Factor; NGF: Nerve Growth Factor; PAI 1: Plasminogen
565 Activator Inhibitor-1; TNF-α: Tumor Necrosis Factor Alpha.

566 Statistical significance between groups of girls *vs* boys after application of ANCOVA
567 (analysis of covariance) after adjusting for age, BMI, fitness and physical activity. Data
568 are expressed as mean ±SEM.

569

570

571

572

573

574 **Tabla 4**. Correlations between plasma adipokines in healthy prepubertal children in
575 relation with fitness and physical activity.

576

Variables	R	P
PAI 1 // Leptin	0.527	<0.001
PAI 1 // HGF	0.437	<0.001
PAI 1 // IL 8	0.408	<0.001
PAI 1 // SBP	0.303	<0.001
PAI 1 // Insulin	0.503	<0.001
PAI 1 // PCR	0.246	0.005
IL 6 // PCR	0.390	<0.001
IL 6 // TNF- α	0.181	0.035
IL 6 // Leptin	0.293	0.001
TNF- α // IL 8	0.427	<0.001
TNF- α // Resistin	0.188	0.028
TNF- α // PAI 1	0.327	<0.001
TNF- α // Leptin	0.261	0.002
TNF- α // HGF	0.416	<0.001
Leptin // SBP	0.463	<0.001
Leptin // Insulin	0.570	<0.001
Leptin // PCR	0.364	<0.001

577

578 BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.
579 HGF: Hepatocyte Growth Gactor; IL-6**:** Interleukin-6; IL-8: Interleukin-8; MCP-1**:**
580 Macrophage Chemoattractant Factor; PAI 1: Plasminogen Activator Inhibitor-1; PCR:
581 Protein C-Reactive; TNF-α: Tumor Necrosis Factor Alpha.
582 r: Rho Spearman's correlation coefficient. P: probability.

583

584

EVALUATION OF SOLAR EXPOSURE, INTAKE AND PHYSICAL ACTIVITY IN RELATION WITH VITAMIN D SERUM STATUS IN SPANISH PREPUBERTAL GIRLS

Dianna Ramirez-Prada[1], Mª José de la Torre[1], Francisco Jesús Llorente-Cantarero[2], Juan Luis Pérez-Navero[1], Mercedes Gil-Campos[1]

Nutrición Hospitalaria 2012; VOL. 27, NO. 6.

VI

[1]Unidad de Metabolismo e Investigación Pediátrica, Hospital Universitario Reina Sofía, Córdoba, España. Instituto Maimónides de Investigación Biomédica, Córdoba (IMIBIC).
[2]Departamento de Expresión Corporal. Facultad de Educación. Universidad de Córdoba, IMIBIC.

Nutrición Hospitalaria

Nutr Hosp. 2012;27(6):1993-1998
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original

Evaluación de la exposición solar, ingesta y actividad física en relación con el estado sérico de vitamina D en niñas prepúberes españolas

D. Ramírez-Prada[1], M.ª J. de la Torre[1], F. J. Llorente-Cantarero[2], J. L. Pérez-Navero[1] y M. Gil-Campos[1]

[1]Unidad de Metabolismo e Investigación Pediátrica. Hospital Universitario Reina Sofía. Córdoba. España. Instituto Maimónides de Investigación Biomédica. Córdoba (IMIBIC). [2]Departamento de Expresión Corporal. Facultad de Educación. Universidad de Córdoba. Córdoba. España.

Resumen

Antecedentes: **Los niveles adecuados de vitamina D y calcio en la infancia determinan el desarrollo adecuado de la masa ósea. En la actualidad se ha detectado déficit de vitamina D en determinadas poblaciones infantojuveniles, como en lactantes y adolescentes pero existe información insuficiente respecto al estado de la vitamina D en niños de otras edades.**

Objetivo: **Determinar los niveles séricos de vitamina D en niñas prepuberales sanas de una ciudad del sur de Europa y estudiar el efecto de la exposición solar, la actividad física y la ingesta.**

Métodos: **Se estudiaron 56 niñas caucasianas, sanas y prepúberes entre 7-10 años. Se recogieron datos sobre ingesta nutricional y actividad física. Las muestras de sangre se obtuvieron en diciembre.**

Resultados: **La ingesta de vitamina D fue significativamente menor a las recomendaciones internacionales. Los niveles medios de calcidiol fueron de 40,07 ± 10,49 ng/ ml. Ninguna niña presentó un nivel inferior a 20 ng/ml; un 25% (14 niñas) tenían unos niveles entre 20-30 ng/ml, y un 75 % superiores a 30 ng/ml. No hemos encontrado diferencias en los niveles de vitamina D entre las niñas que realizaban actividad física, y las sedentarias.**

Conclusión: **Los niveles de vitamina D en niñas prepúberes que viven al sur de España al inicio del invierno son adecuados. No obstante, se debe asegurar una ingesta adecuada de vitamina D, así como una exposición solar suficiente y realizar seguimiento en estas edades para evitar deficiencias.**

(*Nutr Hosp.* 2012;27:1993-1998)

DOI:10.3305/nh.2012.27.6.6065

Palabras clave: *Vitamina D. Prepuberal. Actividad física. Nutrición.*

EVALUATION OF SOLAR EXPOSURE, INTAKE AND PHYSICAL ACTIVITY IN RELATION WITH VITAMIN D SERUM STATUS IN SPANISH PREPUBERTAL GIRLS

Abstract

Background: **Vitamin D and calcium play an important role in peak bone mass acquisition. Recent studies have suggested that vitamin D deficiency in children is widespread, mainly during infancy and adolescent years. However, the vitamin D status at others ages is unsufficiently investigated.**

Objectives: **To determine the vitamin D status in prepubertal, healthy South European girls, and to examine the relationship between serum vitamin D concentrations, sun exposure, physical activity and dietary intake.**

Methods: **A cross-sectional observational study was conducted on 56 Caucasian; healthy and pre-pubertal girls aged 7-10 years. Dietary information, amount of sunlight exposure and activity were estimated. Blood samples were extracted in the first week of December.**

Results: **Vitamin D intake was below the international recommended references. Mean serum vitamin D was 40.07 ± 10.49 ng/ml. No girl presented a level lower than 20 ng/ml; 25% had levels between 20-30 ng/ml and 75% above 30 ng/ml. We have not found differences in vitamin D levels from the girls who did sport and those who were sedentary.**

Conclusions: **Vitamin D status is suitable for prepubertal girls living in the South of Spain at the beginning of winter. However, it is necessary to follow-up girls and check and adequate vitamin D intake, as well as sufficient sun exposure.**

(*Nutr Hosp.* 2012;27:1993-1998)

DOI:10.3305/nh.2012.27.6.6065

Key words: *Vitamin D. Prepubertal. Physical activity. Nutrition.*

Correspondencia: Mercedes Gil Campos.
Unidad de Metabolismo e Investigación Pediátrica.
Hospital Universitario Reina Sofía.
Avda. Menéndez Pidal, s/n.
14004. Córdoba. España.
E-mail: mercedes_gil_campos@yahoo.es

Recibido: 18-VII-2012.
Aceptado: 23-VII-2012.

Introducción

Los niveles adecuados de vitamina D y calcio en la infancia determinan el desarrollo adecuado de la masa ósea y la prevención de enfermedades como la osteoporosis en etapas adultas. Estos niveles, entre otros factores, pueden estar condicionados por la ingesta del calcio y la vitamina D[1] pero también por la influencia de factores como la genética o la edad de la menarquía en las mujeres[2]. Por otra parte, los hábitos sociales y culturales actuales favorecen la vida sedentaria limitando la exposición al sol de los niños y la práctica de actividad física, pudiendo condicionar deficiencia de vitamina D y afectar la formación de la masa ósea[3]. De hecho, durante los periodos de crecimiento, este déficit puede tener una influencia negativa en el desarrollo óseo, causando no sólo raquitismo, que es el resultado final de la deficiencia severa de vitamina D, sino en la talla establecida genéticamente y en la masa ósea final[4].

Las mujeres son la población más afectada por enfermedad ósea, favorecida además, por los cambios hormonales propios de la menopausia[5]. Hasta el 90% de la masa ósea máxima se adquiere antes de los 18 años en las niñas, por lo que se considera la infancia un momento crítico para promover conductas que mejoren la salud ósea[1,6].

En la actualidad se ha detectado deficiencia o insuficiencia en los niveles de vitamina D en determinadas poblaciones infantojuveniles como resultado de múltiples factores[7-9]. Ello ha condicionado nuevas investigaciones en relación con la acción de esta vitamina sobre la mineralización ósea. Además, el déficit de este nutriente, se ha relacionado también con diversas patologías, como diabetes, esclerosis múltiple y cáncer[10-11].

Los estilos actuales de vida sedentarios junto a las recomendaciones de protección solar para reducir la incidencia de cáncer de piel, pueden condicionar unos niveles de vitamina D inadecuados. Igualmente, en la mayoría de las encuestas alimentarias realizadas en España y otros países, la ingesta de vitamina D en la infancia es menor a las recomendaciones internacionales establecidas[12-13].

Actualmente no hay consenso sobre cuáles deben ser las recomendaciones diarias en la ingesta, o si debe hacerse profilaxis con vitamina D, sobre todo en países en los que hay mayor exposición a la radiación solar. La mayoría de los trabajos sobre la evaluación del estado de la vitamina D en la infancia incluyen las dos etapas donde más aumentan las necesidades; la etapa de lactancia[14] y la de la adolescencia[15]. No obstante, aún existe información insuficiente respecto al estado de la vitamina D en niños de otras edades, y la influencia de determinados factores sobre sus niveles y funciones. Por ello, el objetivo de este trabajo es determinar los niveles séricos de vitamina D en niñas prepuberales sanas de una ciudad del sur de Europa (latitud 30º N), y estudiar el efecto de factores influyentes como la exposición solar, la actividad física, o la ingesta. Los resultados de este trabajo pueden aportar información para conocer las circunstancias que afectan el metabolismo de la vitamina D en niñas en edad temprana y la utilidad de diseñar proyectos dirigidos a la prevención de deficiencia de vitamina D antes de la etapa puberal y edad adulta.

Material y métodos

Sujetos

Se estudiaron 56 niñas caucasianas prepúberes con edades comprendidas entre 7-10 años. Se eligió al azar un colegio de Córdoba, al sur de España (latitud: 37.8º N), seleccionando a las 16 primeras niñas por orden de lista de varias clases de educación primaria. En el caso de que algún padre denegara el consentimiento para formar parte del estudio o la niña no cumpliera los criterios de inclusión, se le ofreció a la siguiente de la lista. Se incorporaron al estudio tras la aceptación del menor y la obtención del consentimiento informado del responsable legal. El estudio de investigación se llevó a cabo en la Unidad de Metabolismo e Investigación Pediátrica del Servicio de Pediatría del Hospital Universitario Reina Sofía de Córdoba, y fue aprobado por el Comité Local de Bioética e Investigación Clínica.

Los criterios de inclusión fueron: niñas sanas de 7-10 años con medidas antropométricas y de tensión arterial en el p50 ± 1DS, y en estadio puberal de Tanner I[16]. Se excluyeron las niñas que estuvieran en otro rango de edad al establecido, en percentiles de medidas antropométricas < P10 o > P90, con patología crónica o con signos clínicos o analíticos de desarrollo puberal.

Valoración antropométrica, hemodinámica, nutricional y de actividad física

Se realizó una historia clínica con una exploración física completa, valorando los antecedentes personales y familiares, así como la existencia de patología previa o tratamientos farmacológicos. Se determinaron parámetros antropométricos como la talla, el peso y el perímetro de la cintura; posteriormente se calculó el índice de masa corporal (IMC): peso (kg)/ talla^2 (m). Para la medida del peso y talla se utilizó una báscula y tallímetro SECA (SECA, Hamburg, Germany). El perímetro de cintura se midió con una cinta métrica siguiendo un método estandarizado. Se realizó una exploración física exhaustiva para valorar la etapa de desarrollo puberal según Tanner[16].

La valoración nutricional se realizó mediante una encuesta de frecuencia de alimentos, analizando el aporte nutricional estimado por día para energía, macronutrientes, calcio, fósforo, magnesio y vitamina D, según la tabla de composición de alimentos española[17] y el sistema equivalente de alimentos[18]. La ingesta media fue comparada con las de referencia internacionales (Dietary Reference Intakes: DRIs) para niñas de 7 a 10 años[19].

La actividad física practicada fuera del horario escolar se evaluó mediante cuestionarios diseñados de acuerdo con la "Lista de Actividad Ayer", validado para la población española[20]. Se registraron datos de horario y duración, vestimenta utilizada, y lugar (interior o exterior) en el que se realizaba la actividad. Las encuestas fueron realizadas a las propias niñas.

Toma de muestras y análisis en sangre

Las extracciones se realizaron en situación de reposo tras 12 h de ayuno, durante la primera semana de diciembre. Se realizó una hematimetría y un análisis bioquímico general. En el estudio hormonal básico se midieron: FSH (Hormona folículo estimulante) (mU/L), LH (Hormona luteinizante) (mU/L), estradiol (mU/L) y testosterona (mU/L). Además se midió calcio y calcio corregido con proteínas (mg/ dl), calcidiol (ng/ml) y calcitriol (pg/ml). Los niveles plasmáticos de vitamina D se evaluaron mediante radioinmunoanálisis (RIA) con el analizador contador gamma PACKARD Cobre II E 5005. Con este método se cuantifican los dos derivados (D2 y D3) de la vitamina D. Para evaluar los niveles de calcidiol se utilizaron los puntos de corte basados en varios estudios[21-23] que describen los valores adecuados como aquellos superiores a 30 ng/ ml (> 75 nmol/L); insuficientes entre 20-30 ng/ml (50-75 nmol/L), y deficientes por debajo de 20 ng/ ml (< 50 nmol/L).

Análisis estadístico

El análisis estadístico se realizó con el programa SPSS versión 18.0 de software. Se realizó un análisis descriptivo para variables cuantitativas mediante el cálculo de media (m) y desviación típica o standard (DS); y para las variables cualitativas mediante el cálculo de proporciones (%). La determinación de la bondad de ajuste a una distribución normal (datos normales) se hizo mediante la prueba de Shapiro-Wilk. Si las muestras seguían una distribución normal y las varianzas eran homogéneas se aplicaron test paramétricos, en caso contrario se utilizaron test no paramétricos. La comparación de los valores medios de las variables cuantitativas entre dos grupos (deporte sí/no), se realizó mediante pruebas t de Student para grupos independientes (prueba paramétrica); o pruebas U de Mann-Whitney (prueba no paramétrica).

Tabla I
Características demográficas y antropométricas del grupo de 56 niñas prepúberes

Variable	*Media*	*DE*
Edad (años)	8,8	0,9
Peso (kg)	36,8	9,2
Talla (cm)	138,6	6,2
IMC (kg/m²)	19	3,7
PC (mm)	63,8	8,6
TAS (mmHg)	115,3	13,2
TAD (mmHg)	63,8	10

IMC: Índice de masa corporal; PC: Perímetro de cintura; TAS: Tensión arterial sistólica; TAD: Tensión arterial diastólica. Los datos se expresan como media ± DE.

Tabla II
Valores medios de ingesta alimentaria vs DRI en niñas prepúberes de 7 a 10 años de edad

	Media ingesta/día	*DRIs*	*P*
Energía (kcal)	1.749,43 ± 572,15	2.000	< 0,002
Proteínas (g)	107,78 ± 44,33	34	< 0,001
Grasas (g)	55,29 ± 17,11	35	ns
Carbohidratos (g)	243,74 ± 92,97	100	< 0,001
Calcio (mg)	1.211,69 ± 346,82	1.300	0,062
Fósforo (mg)	1.922,43 ± 651,15	1.250	ns
Vitamina D (UI)	161,39 ± 64,70	200	< 0,001

DRIs: Dietary Reference Intakes[19]. Los datos se expresan como media ± DE. Significación: P ≤ 0,05; ns: no significativo.

Tabla III
Niveles séricos de calcio y vitamina D vs valores de referencia del laboratorio en 56 niñas prepúberes de 7 a 10 años de edad, y niveles de calcidiol por grupos de práctica de actividad física

Variable	*Media*	*Valor referencia*
Calcio (mg/ dl) (N = 56)	10,2 ± 0,3	8,4 ± 10,2
Calcio corregido (mg/dl) (N = 56)	10 ± 0,3	8,5 ± 10,5
Calcitriol (pg/ml) (N = 56)	70,89 ± 14,34	18-71
Calcidiol (ng/ml) (N = 56)	40,07 ± 10,49	20-100
Práctica deporte (N = 29)	41,48 ± 10,11	
No práctica deporte (N = 26)	37,8 ± 11,4	

Los datos se expresan como media ± DE. P = 0,098 al comparar los niveles séricos de calcidiol entre niñas con práctica deportiva o sin práctica de deporte.

Resultados

Todas las niñas del estudio presentaron una talla, peso e IMC así como valores de tensión arterial, en percentiles adecuados para su sexo y edad (tabla I).

Respecto a la valoración de nutrientes, se observó que la media de ingesta de calorías/día fue menor a la establecida en las DRIs[19] aunque la ingesta de macronutrientes fue mucho mayor (tabla II). En relación con la ingesta diaria de calcio no hubo diferencias significativas respecto a las recomendaciones pero la ingesta de vitamina D fue inferior.

En la tabla III se muestran los niveles medios de calcio sérico, calcio corregido con las proteínas totales y los de calcidiol y calcitriol, que se encontraron dentro del rango establecido como normal por el laboratorio.

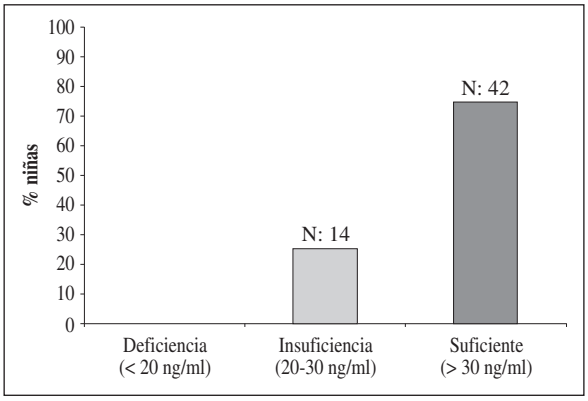


Fig. 1.—Porcentaje de niñas con diferentes niveles séricos de vitamina D.

En relación a los puntos de corte establecidos en la literatura para los niveles de vitamina D[7,15,21-24], ninguna niña presentó un nivel menor de 20 ng/ml (50 nmol/L), un 25% tenían unos niveles entre 20-30 ng/ml que se consideran como insuficientes y el 75 % restante superiores a 30 ng/ml que son niveles óptimos (fig. 1). Si se hubiera considerado el rango referido para adultos en 32 ng/ml[23,25] como insuficiente un 28% estaría dentro de estos límites.

La práctica de actividad física fuera del horario escolar se evaluó en 55 niñas, de las cuales el 52,7% (n = 29) sí realizaba deporte fuera del horario escolar, y el 47,3% (n = 26) tan sólo realizaba las 2 horas de deporte escolar obligatorio. La actividad deportiva extraescolar fue generalmente baloncesto, al aire libre durante 3 horas semanales, en horario de tarde. La actividad física escolar obligatoria fue de 2 horas a la semana en horario de mañana. Ambas se realizaban, si no llovía, al aire libre, utilizando normalmente una camiseta de manga corta y un pantalón corto. Entre ambos grupos, no existieron diferencias significativas de niveles de la vitamina D séricos (tabla III).

Discusión

Los resultados de este estudio indican que las niñas españolas prepúberes que viven en el sur no presentan deficiencia de vitamina D al inicio del invierno aunque la ingesta de este micronutriente fuera menor a la recomendada, sin encontrar tampoco diferencias en relación con la práctica deportiva.

En los últimos años se ha detectado un aumento en la prevalencia del déficit de vitamina D durante diferentes etapas de la infancia y adolescencia, surgiendo nuevos estudios que intentan clarificar la necesidad de uso de suplementos como profilaxis o tratamiento[23,26]. Durante los primeros años de vida es fundamental mantener unos niveles adecuados de vitamina D para conseguir un pico de masa ósea correcto, existiendo dos periodos críticos para la obtención de este pico, durante los tres primeros años de vida, y en la pubertad. Por eso, la mayoría de los artículos pediátricos se centran en estudiar el metabolismo óseo y de la vitamina D, y los factores que los condicionan en ambas etapas[15,27]. No obstante, es plausible pensar que las deficiencias que se detectan en la adolescencia procedan de etapas previas. Por ello, en este trabajo se ha abordado la etapa prepuberal, previa al inicio de un crecimiento acelerado, en la que aún existen escasos datos sobre el estado sérico de la vitamina D en niñas sanas.

Los niveles de vitamina D están influidos por numerosos factores entre los que destacan la ingesta, edad, sexo, tipo de piel, exposición solar, vestimenta, actividad física, y principalmente, la latitud y la estación del año[23]. En algunos estudios realizados se plantea una interpretación difícil al coexistir estos elementos de confusión u otros como la obesidad, la suplementación farmacológica, o la práctica de actividad física al aire libre o en ambientes cerrados[27]. Para tratar de limitar la interacción de estos factores, en este trabajo se ha estudiado una muestra homogénea seleccionando sólo niñas y en estado prepúber. Además, el sexo femenino tiene niveles más bajos de vitamina D[28] y estos valores, sobre todo en las deficiencias, condicionan la edad de la menarquia[29], siendo el sexo más afectado por la osteoporosis del adulto[5]. Por otra parte, todas ellas eran sanas con IMC normal, excluyendo a aquéllas con fallo de medro, desnutrición u obesidad, ya que en la mayoría de los estudios se ha establecido una relación inversa entre la adiposidad y los niveles de vitamina D[30]. Para que el efecto estacional estuviera controlado, todas las muestras se extrajeron durante la misma semana.

La comparación de los resultados entre los artículos se dificulta además por las diferencias metodológicas en las técnicas de medición de la vitamina D y en la falta de consenso para definir el punto de corte entre deficiencia y normalidad vitamínica en relación con la edad y el sexo[7,15,21-24]. Las diferentes pruebas de laboratorio difieren por su grado de precisión, siendo importante que se cuantifiquen los dos metabolitos de la vitamina D (D2 y D3), ya que se puede llegar a un diagnóstico incorrecto de deficiencia al despreciar los niveles de vitamina D2[23]. Aunque la espectroscopia en tándem de masas es considerada la prueba oro para la medición de vitamina D, los resultados obtenidos por RIA son satisfactoriamente comparables. Para la evaluación de los niveles séricos de vitamina D, se recomienda medir los niveles de 25-(OH)D ó calcidiol, metabolito que presenta una vida media de 30 días y que posteriormente se convierte a la forma activa 1,25 dihidroxi-colecalciferol, habiéndose demostrado además su correlación con el grado de mineralización ósea[31].

Por otra parte, existen diferencias para establecer el rango de normalidad sérico de la vitamina D. En niños se siguen considerando como suficientes unos niveles por encima de 20 ng/ml[24]. No obstante, en los últimos años también se ha destacado en los adultos que cuando existen valores de vitamina D por debajo de 32 ng/ml, se altera la absorción del calcio y la densidad mineral ósea baja por lo que se está considerando este valor como punto límite de la normalidad incluso en la infancia[25,32].

Considerando lo anteriormente expuesto, y tomando como rango de referencia el del laboratorio utilizado, no se ha detectado deficiencia de vitamina D3 en esta muestra de niñas prepúberes, aunque un 25% de ellas podrían presentar unos niveles insuficientes de vitamina D. No obstante estos resultados varían respecto a los de otros trabajos publicados en poblaciones similares. En un estudio realizado en Madrid (España) durante 1 año encontraron que un 51% de los niños presentaron niveles por debajo de 20 ng/ml en relación a un bajo consumo de vitamina D[33]. En el trabajo de Mansbach y cols.[7], realizado durante 5 años en USA observaron que en niñas entre los 6-11 años, hasta un 75% tenían niveles de vitamina D menores a 30 ng/ml. Otro estudio realizado en Reino Unido[34] con 7.500 niños con una edad media de 9,9 años, un 29% presentaban niveles de vitamina D3 menores a 20 ng/ml. Las diferencias en los niveles de vitamina D en todos estos estudios, pueden explicarse en relación a las razones expuestas anteriormente: poblaciones con rangos de edad, grupos étnicos, hábitos culturales, de ingesta, o de actividad física diferentes, o toma de muestras y evaluaciones durante distintas estaciones del año, y con diferencias en la exposición solar. Por ello, probablemente no se pueden establecer recomendaciones generales para la detección de deficiencias o para el uso de suplementos, sino que deberá hacerse una evaluación individual buscando aquéllos grupos de riesgo.

Al medir los niveles en sangre de calcidiol se evalúa no sólo la cantidad de vitamina D procedente de la dieta sino también la de la síntesis cutánea[21]. Esta última podría condicionar que existieran unos niveles séricos de vitamina D adecuados, a pesar de que la ingesta de esta vitamina fuera inferior a la recomendada. La síntesis cutánea depende de múltiples factores: exposición al sol, ubicación geográfica y época del año. La longitud de onda de los rayos ultravioletas B no está presente en la luz solar durante los meses de invierno. En múltiples estudios relacionan los niveles de vitamina D con la estación del año[9,30] presentando niveles más elevados en las muestras tomadas en los meses de Junio a Noviembre. Es importante recordar que las recomendaciones de ingesta de vitamina D de 600 UI al día para niños de 6 a 10 años se realizan asumiendo una mínima exposición solar[24]. Sin embargo, en zonas como la referida en este estudio, en el Sur de España, que está a una latitud de 37º N y con veranos calurosos donde las actividades al aire libre y la exposición solar son frecuentes, probablemente esta recomendación de ingesta estaría sobreestimada. En el trabajo de Rodriguez y cols.[33], indican que consumiendo un 67% de las DRI[19], se consiguen unos niveles adecuados en sangre de vitamina D en niños escolares.

En relación con la práctica de actividad física, no se han observado diferencias significativas en los niveles de vitamina D. Este resultado podría indicar por un lado, que la exposición por un tiempo más prolongado al aire libre no parece tener una gran influencia en que haya niveles más elevados de vitamina D, a diferencia de lo descrito en otras publicaciones[8,34] donde realizar una actividad al aire libre durante más de 1/2 hora es un factor protector de la deficiencia de vitamina D. Quizás la situación geográfica es la que dar explicación a estas diferencias. Así, en nuestra localización estarían justificadas las recomendaciones de una exposición moderada (en cara y extremidades) de 5-10 minutos, 2 ó 3 veces a la semana para mantener unos niveles adecuados de vitamina D[21] (y que cumplieron los dos grupos de niñas). Por otra parte, en este trabajo no se asocia la práctica de actividad física per se con mayores niveles de vitamina D, como se ha observado en estudios en adultos y adolescentes[28,35]. No obstante, no se ha monitorizado la intensidad del deporte y ello podría constituir una limitación a encontrar asociación.

Tras los resultados del presente estudio, se puede concluir que los niveles de vitamina D en niñas prepúberes que viven al sur de España al inicio del invierno son adecuados. No obstante, existe un grupo de riesgo en el que se debería realizar un seguimiento y asegurar una ingesta adecuada de vitamina D, así como una exposición solar suficiente. Probablemente en estos grupos en los que se puede asegurar la acción de factores protectores, no habría que plantear la administración de vitamina D en forma de suplemento. Aún así, sigue siendo necesario realizar más estudios en diferentes poblaciones para indicar cuáles son los niveles adecuados de vitamina D y seleccionar los grupos de riesgo que se beneficiarían de los suplementos de vitamina D sin intentar generalizar esta práctica a toda la población, ya que unos niveles elevados no estarían exentos de problemas de salud.

Referencias

1. Sharma SV, Hoelscher DM, Kelder SH, Diamond P, Day RS, Hergenroeder A. Psychosocial factors influencing calcium intake and bone quality in middle school girls. *J Am Diet Assoc* 2010; 110: 932-6.
2. Chevalley T, Bonjour JP, Ferrari S, Rizzoli R. The influence of pubertal timing on bone mass acquisition: a predetermined trajectory detectable five years before menarche. *J Clin Endocrinol Metab* 2009; 94: 3424-31.
3. Scharla S. Diagnosis of disorders of vitamin D-metabolism and osteomalacia. *Clin Lab* 2008; 54: 451-9.
4. Pettifor JM, Prentice A. The role of vitamin D in paediatric bone health. *Best Pract Res Clin Endocrinol Metab* 2011; 25: 573-84.
5. Schnatz PF. The 2010 North American Menopause Society position statement: Updates on screening, prevention and management of postmenopausal osteoporosis. *Conn Med* 2011; 75: 485-7.
6. Gruodyté R, Jürimäe J, Saar M, Maasalu M, Jürimäe T. Relationships between areal bone mineral density and jumping height in pubertal girls with different physical activity patterns. *J Sports Med Phys Fitness* 2009; 49: 474-9.
7. Mansbach JM, Ginde AA, Camargo CA Jr. Serum 25-hydroxyvitamin D levels among US children aged 1 to 11 years: do children need more vitamin D? *Pediatrics* 2009; 124: 1404-10.
8. Absoud M, Cummins C, Lim MJ, Wassmer E, Shaw N. Prevalence and predictors of vitamin D insufficiency in children: a Great Britain. *PLoS One* 2011; 6: e22179.
9. Whiting SJ, Langlois KA, Vatanparast H, Greene-Finestone LS. The vitamin D status of Canadians relative to the 2011

Dietary Reference Intakes: an examination in children and adults with and without supplement use. *Am J Clin Nutr* 2011; 94: 128-35.
10. Annweiler C, Fantino B, Schott AM, Krolak-Salmon P, Allali G, Beauchet O. Vitamin D insufficiency and mild cognitive impairment: cross-sectional association. *Eur J Neuro*l 2012. doi: 10.1111/j.1468-1331.
11. Moreno LA, Valtueña J, Pérez-López F, González-Gross M. Health effects related to low vitamin D concentrations: beyond bone metabolism. *Ann Nutr Metab* 2011; 59: 22-7.
12. Biró L, Regöly-Mérei A, Nagy K, Péter S, Arató G, Szabó C, et al. Dietary habits of school children: representative survey in metropolitan. *Ann Nutr Metab* 2007; 51: 454-60.
13. Suárez Cortina L, Moreno Villares JM, Martínez Suárez V, Aranceta Bartrina J, Dalmau Serra J, Gil Hernández A et al. Calcium intake and bone mineral density in a group of Spanish school-children. *An Pediatr (Barc)* 2011; 74: 3-9.
14. Wagner CL, Taylor SN, Johnson DD, Hollis BWP. The role of vitamin D in pregnancy and lactation: emerging concepts. *Womens Health (Lond Engl)* 2012; 8: 323-40.
15. González-Gross M, Valtueña J, Breidenassel C, Moreno LA, Ferrari M, Kersting M et al. HELENA Study Group. Vitamin D status among adolescents in Europe: the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr* 2012; 107: 755-64.
16. Tanner JM. Growth at adolescente. Oxford: Blackwell; 1962.
17. Farran A, Zamora R, Cervera P. Tabla de composición de los alimentos del CESNID. Barcelona: McGraw Hill; 2003.
18. Vásquez de Plata G, Gómez E. Sistemas de alimentos equivalentes. Publicaciones UIS; 2006.
19. Food and Nutrition Board (FNB), Institute of Medicine (IOM). Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and for Calcium and Related Nutrients. National Academy Express. Washington D.C., 2005
20. Delgado M, Tercedor P. Estrategias de intervención en Educación para la salud desde la Educación Física. Barcelona: Inde; 2002.
21. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 19; 357: 266-81.
22. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005; 16: 713-6.
23. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics* 2008; 122: 398-417.
24. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; 96: 53-8.
25. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005; 135: 317-22.
26. Pramyothin P, Holick MF. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. *Curr Opin Gastroenterol* 2012; 28: 139-50.
27. Dong Y, Pollock N, Stallmann-Jorgensen IS, Gutin B, Lan L, Chen TC et al. Low 25-hydroxyvitamin D levels in adolescents: race, season, adiposity, physical activity, and fitness. *Pediatrics* 2010; 125: 1104-11.
28. Andıran N, Çelik N, Akça H, Do an G. Vitamin D deficiency in children and adolescents. *J Clin Res Pediatr Endocrinol* 2012; 4: 25-9.
29. Villamor E, Marin C, Mora-Plazas M, Baylin A. Vitamin D deficiency and age at menarche: a prospective study. *Am J Clin Nutr* 2011; 94: 1020-5.
30. Gilbert-Diamond D, Baylin A, Mora-Plazas M, Marin C, Arsenault JE, Hughes MD, Willett WC, Villamor E. Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study. *Am J Clin Nutr* 2010; 92: 1446–51.
31. Binkley N, Krueger DC, Morgan S, Wiebe D. Current status of clinical 25-hydroxyvitamin D measurement: an assessment of between-laboratory agreement. *Clin Chim Acta* 2010; 411: 1976-82.
32. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. *Am J Med* 2004; 116: 634-9.
33. Rodríguez-Rodríguez E, Aparicio A, López-Sobaler AM, Ortega RM. Vitamin D status in a group of Spanish school-children. *Minerva Pediatr* 2011; 63: 11-18.
34. Tolppanen AM, Fraser A, Fraser WD, Lawlor DA. Risk factors for variation in 25-hydroxyvitamin D3 and D2 concentrations and vitamin d deficiency in children. *J Clin Endocrinol Metab* 2012; 97: 1202-10.
35. Scragg R, Camargo CA Jr. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition. *Am J Epidemiol* 2008; 168: 577-86.

CONCLUSIONES

V

Conclusión General:

Adquirir una adecuada condición física y realizar práctica de actividad física en la etapa prepuberal promueven un mejor estado metabólico, y una mayor protección ante procesos de oxidación e inflamación, disminuyendo las posibilidades de desarrollar patologías asociadas en la adolescencia y la edad adulta.

Conclusiones Específicas:

I. El perfil de salud metabólica en los niños prepúberes que presentan altos niveles de condición física se caracteriza por niveles bajos de triglicéridos, HOMA-IR, ácido úrico y proteína C-reactiva, y mayores niveles de HDL-colesterol y apolipoproteinas A1, en comparación con los niños con baja condición física.

II. El género, en relación a la condición física y la actividad física parece condicionar el estado metabólico afectando principalmente el perfil de lípidos en las niñas. Estos cambios se asocian con baja condición física y una actividad física deficiente.

III. El glutatión total, el glutatión oxidado y la ratio GSH/GSSG parecen ser marcadores fiables de estrés oxidativo en niños prepúberes sanos con baja condición física o hábitos sedentarios, en relación a niños con mejor condición física o que practican ejercicio. Las correlaciones encontradas podrían indicar que el sistema enzimático actuaría para compensar el estrés oxidativo y evitar un aumento de oxidantes, aunque son necesarios estudios posteriores en niños.

IV. Las niñas prepúberes presentan un mayor estrés oxidativo que los niños, aunque esta situación parece ser compensada con mayores niveles de superóxido dismutasa, independientemente de la edad, el índice de masa corporal, la condición física y la actividad física.

V. No parece haber grandes cambios en las adipocitoquinas y otros factores inflamatorios, relacionados con la condición física o el ejercicio en niños prepúberes sanos, aunque se han observado algunas diferencias que deberían confirmarse en futuros estudios.

VI. Los niveles de vitamina D en niñas prepúberes que viven al sur de España al inicio del invierno son adecuados. No obstante, existe un grupo de riesgo en el que se debería realizar un seguimiento y asegurar una ingesta adecuada de vitamina D, así como una exposición solar suficiente y práctica de actividad física. Probablemente no habría que plantear la administración de vitamina D en forma de suplemento.

V

General:

To acquire an adequate fitness and practice physical activity at prepubertal age promotes a metabolic health status, a greater protection from oxidation and inflammation, and reduce the development of associated diseases in adolescence and adulthood.

Specifics:

I. The metabolic health profile of prepubertal children displaying high levels of cardiorespiratory fitness is characterized by low triglycerides, HOMA-IR, uric acid and C-Reactive Protein levels, and higher levels of HDL-cholesterol and apolipoproteins apo-A1, compared with children with low cardiorespiratory fitness.

II. Gender in relation to cardiorespiratory fitness and physical activity seems to condition the metabolic status mainly affecting the lipid profile in girls. These changes are associated with low cardiorespiratory fitness and poor physical activity.

III. Total glutathione, oxidized glutathione and GSH/GSSG ratio seem to be reliable markers of oxidative stress in healthy prepubertal children with low cardiorespiratory fitness or sedentary habits compared with children with higher fitness or those that practice physical activity. The correlations found could indicate that the enzymatic system would act to compensate for oxidative stress to avoid an increase in oxidants, although further studies in children are necessary.

IV. Prepubertal girls presented higher oxidative stress than boys, a situation that was compensated with elevated levels of superoxide dismutase, independently of age, body max index, fitness and physical activity.

V. There seems no to be major changes in plasma adipocytokines and inflammatory factors related to physical fitness or exercise in healthy prepubertal children. However, there have been some differences that should be confirmed in future studies.

VI. Vitamin D levels in prepubertal girls living in southern Spain at the beginning of winter are adequate. However, there is a risk group which should monitor and ensure an adequate intake of vitamin D and enough sun exposure and physical activity practice. Probably, in these groups we should not advise the administration of vitamin D in supplement form.

BIBLIOGRAFIA

A

Antoncic-Svetina M, Sentija D, Cipak A, Milicic D, Meinitzer A, Tatzber F, Andrisic L, Zelzer S, Zarkovic N. Ergometry induces systemic oxidative stress in healthy human subjects. *Tohoku J Exp Med.* 2010; 221: 43-8.

B

Barbeau P, Litaker MS, Woods KF, Lemmon CR, Humphries MC, Owens S, Gutin B. Hemostatic and inflammatory markers in obese youths: effects of exercise and adiposity. *J Pediatr*. 2002; 141: 415-20.

Barbeau P, Gutin B, Litaker MS, Ramsey LT, Cannady WE, Allison J, Lemmon CR, Owens S. Influence of physical training on plasma leptin in obese youths. *Can J Appl Physiol*. 2003; 28: 382-96.

Benitez-Sillero JD, Perez-Navero JL, Tasset I, Guillen-Del Castillo M, Gil-Campos M, Tunez I. Cardiorespiratory fitness and oxidative stress: effect of acute maximal aerobic exercise in children and adolescents. *J. Sports Med. Phys. Fitness.* 2011; 51:204–210.

Bloomer RJ, Smith WA. Oxidative stress in response to aerobic and anaerobic power testing: influence of exercise training and carnitine supplementation. *Res Sports Med.* 2009; 17: 1-16.

Bouglé D, Zunquin G, Sesbouë B, Sabatier JP.Relationships of cardiorespiratory fitness with metabolic risk factors, inflammation, and liver transaminases in overweight youths. *Int J Pediatr*. 2010; 2010: 580897.

Bueno G, Bueno O, Moreno LA, García R, Tresaco B, Garagorri JM, Bueno M. Diversity of metabolic syndrome risk factors in obese children and adolescents. *J Physiol Biochem.* 2006; 62: 125-33.

C

Casado A, de la Torre R, López-Fernández ME. Copper/zinc superoxide dismutase activity in newborns & young people in Spain. *Indian J Med Res*. 2007; 125: 655-60.

Cavas L, Tarhan L. Effects of vitamin-mineral supplementation on cardiac marker and radical scavenging enzymes, and MDA levels in young swimmers. *Int J Sport Nutr Exerc Metab*. 2004; 14: 133-46.

Chevion S, Moran DS, Heled Y, Shani Y, Regev G, Abbou B, Berenshtein E, Stadtman ER, Epstein Y. Plasma antioxidant status and cell injury after severe physical exercise. *Proc Natl Acad Sci U S A*. 2003; 100: 5119-23.

Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000; 320: 1240-3.

Committee of Experts on Sports Research EUROFIT (1993). Handbook for the EUROFIT Tests of Physical Fitness. Strasburg: Council of Europe.

Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, Miller GJ, Strachan DP. C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. *Atherosclerosis*. 2000; 149: 139-50.

D

Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*. 2004; 25:4-7.

Devaraj S, Rosenson RS, Jialal I. Metabolic syndrome: an appraisal of the pro-inflammatory and procoagulant status. *Endocrinol Metab Clin North Am*. 2004; 33: 431-53.

E

Erdelmeier I, Gérard-Monnier D, Yadan JC, Chaudière J. Reactions of N-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Mechanistic aspects of the colorimetric assay of lipid peroxidation. *Chem Res Toxicol*. 1998; 11: 1184-94.

F

Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. *Dyn Med*. 2009; 8: 1.

Flohé L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol*. 1984; 105: 114-21.

G

García-Artero E, Ortega FB, Ruiz JR, Mesa JL, Delgado M, González-Gross M, García-Fuentes M, Vicente-Rodríguez G, Gutiérrez A, Castillo MJ. Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study). *Rev Esp Cardiol*. 2007; 60: 581-8.

Gil-Campos M, Aguilera CM, Cañete R, Gil A. Uric acid is associated with features of insulin resistance syndrome in obese children at prepubertal stage. *Nutr Hosp*. 2009; 24: 607-13.

Gil-Campos M, Ramírez-Tortosa MC, Aguilera CM, Cañete R, Gil A. Fasting and postprandial adiponectin alterations anticipate NEFA and TNF-α changes in prepubertal obese children. *Nutr Metab Cardiovasc Dis.* 2011; 21: 62-68.

Gonenc S, Acikgoz O, Semin I, Ozgonul H. The effect of moderate swimming exercise on antioxidant enzymes and lipid peroxidation levels in children. *Indian J Physiol Pharmacol*. 2000; 44: 340-4.

González-Gross M, Castillo MJ, Moreno L, Nova E, González-Lamuño D, Pérez-Llamas F, Gutiérrez A, Garaulet M, Joyanes M, Leiva A, Marcos A. Feeding and assessment of nutritional status of spanish adolescents (AVENA study). Evaluation of risks and interventional proposal. I.Methodology. *Nutr Hosp*. 2003; 18: 15-28.

Gougoura S, Nikolaidis MG, Kostaropoulos IA, Jamurtas AZ, Koukoulis G, Kouretas D. Increased oxidative stress indices in the blood of child swimmers. *Eur J Appl Physiol*. 2007; 100: 235-9.

Gutin B, Yin Z, Humphries MC, Barbeau P. Relations of moderate and vigorous physical activity to fitness and fatness in adolescents. *Am J Clin Nutr*. 2005; 81: 746-50.

Guillen del Castillo M, Benítez-Sillero JD, Morente Montero A, Rabadán de Cos I. Betería EUROFIT. Segovia J, López-Silvarrey FJ, Cesar Legido J. Coord. Manual de Valoración Funcional. *Elsevier*. 2007. 307 – 318.

I

Imperatore G, Cheng YJ, Williams DE, Fulton J, Gregg EW. Physical activity, cardiovascular fitness, and insulin sensitivity among U.S. adolescents: the National Health and Nutrition Examination Survey, 1999-2002. *Diabetes Care*. 2006; 29: 1567-72.

Isik A, Koca SS, Ustundag B, Selek S. Decreased total antioxidant response and increased oxidative stress in Behcet's disease. *Tohoku J Exp Med*. 2007; 212: 133-41.

J

Ji LL, Radak Z, Goto S. Hormesis and exercise: how the cell copes with oxidative stress. *Am. J. Pharmacol. Toxicol*. 2008; 3: 44–58.

K

Kelishadi R, Cook SR, Amra B, Adibi A. Factors associated with insulin resistance and non-alcoholic fatty liver disease among youths. *Atherosclerosis*. 2009; 204: 538-43.

Kellar KL, Douglass JP. Multiplexed microsphere-based flow cytometric immunoassays for human cytokines. *J Immunol Methods*. 2003; 279: 277-85.

Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Emotions, morbidity, and mortality: new perspectives from psychoneuroimmunology. *Annu Rev Psychol*. 2002; 53: 83-107.

Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin--the classical, resistin--the controversical, adiponectin--the promising, and more to come. *Best Pract Res Clin Endocrinol Metab*. 2005; 19: 525-46.

König D, Wagner KH, Elmadfa I, Berg A. Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress. *Exerc Immunol Rev*. 2001; 7: 108-33.

Körner A, Kratzsch J, Gausche R, Schaab M, Erbs S, Kiess W. New predictors of the metabolic syndrome in children--role of adipocytokines. *Pediatr Res*. 2007; 61: 640-5.

L

Léger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci*. 1988; 6: 93-101.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol*. 1990; 186: 464-78.

Luo S, Levine RL. Methionine in proteins defends against oxidative stress. *FASEB J*. 2009; 23: 464-72.

M

Mamiya T, Katsuoka F, Hirayama A, Nakajima O, Kobayashi A, Maher JM, Matsui H, Hyodo I, Yamamoto M, Hosoya T. Hepatocyte-specific deletion of heme oxygenase-1 disrupts redox homeostasis in basal and oxidative environments. *Tohoku J Exp Med*. 2008; 216: 331-9.

Martinez-Gomez D, Ortega FB, Ruiz JR, Vicente-Rodriguez G, Veiga OL, Widhalm K, Manios Y, Béghin L, Valtueña J, Kafatos A, Molnar D, Moreno LA, Marcos A, Castillo MJ, Sjöström M; HELENA study group. Excessive sedentary time and low cardiorespiratory fitness in European adolescents: the HELENA study. *Arch Dis Child*. 2011; 96: 240-6.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28: 412-9.

Mauras N, Delgiorno C, Kollman C, Bird K, Morgan M, Sweeten S, Balagopal P, Damaso L. Obesity without established comorbidities of the metabolic syndrome is associated with a proinflammatory and prothrombotic state, even before the onset of puberty in children. *J Clin Endocrinol Metab*. 2010; 95: 1060-8.

McGavock JM, Torrance BD, McGuire KA, Wozny PD, Lewanczuk RZ. Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. *Obesity (Silver Spring)*. 2009; 17: 1802-7.

McMurray RG, Zaldivar F, Galassetti P, Larson J, Eliakim A, Nemet D, Cooper DM. Cellular immunity and inflammatory mediator responses to intense exercise in overweight children and adolescents. *J Investig Med*. 2007; 55: 120-9.

Mesa JL, Ruiz JR, Ortega FB, Wärnberg J, González-Lamuño D, Moreno LA, Gutiérrez A, Castillo MJ. Aerobic physical fitness in relation to blood lipids and fasting glycaemia in adolescents: influence of weight status. *Nutr Metab Cardiovasc Dis*. 2006; 16: 285-93.

Moliner-Urdiales D, Ruiz JR, Ortega FB, Jiménez-Pavón D, Vicente-Rodriguez G, Rey-López JP, Martínez-Gómez D, Casajús JA, Mesana MI, Marcos A, Noriega-Borge MJ, Sjöström M, Castillo MJ, Moreno LA; AVENA and HELENA Study Groups. Secular trends in health-related physical fitness in Spanish adolescents: the AVENA and HELENA studies. *J Sci Med Sport*. 2010; 13: 584-8.

Moreno LA, Pineda I, Rodríguez G, Fleta J, Sarría A, Bueno M. Waist circumference for the screening of the metabolic syndrome in children. *Acta Paediatr*. 2002; 91: 1307-12.

N

Naylor PJ, McKay HA. Prevention in the first place: schools a setting for action on physical inactivity. *Br J Sports Med*. 2009; 43: 10-3.

O

Olds T, Tomkinson G, Léger L, Cazorla G. Worldwide variation in the performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle run test in 37 countries. *J Sports Sci*. 2006; 24: 1025-38.

Ortega FB, Artero EG, Ruiz JR, España-Romero V, Jiménez-Pavón D, Vicente-Rodriguez G, Moreno LA, Manios Y, Béghin L, Ottevaere C, Ciarapica D, Sarri K, Dietrich S, Blair SN, Kersting M, Molnar D, González-Gross M, Gutiérrez A, Sjöström M, Castillo MJ; HELENA study. Physical fitness levels among European adolescents: the HELENA study. *Br J Sports Med*. 2011; 45: 20-9.

Ortega FB, Tresaco B, Ruiz JR, Moreno LA, Martin-Matillas M, Mesa JL, Warnberg J, Bueno M, Tercedor P, Gutiérrez A, Castillo MJ; AVENA Study Group. Cardiorespiratory fitness and sedentary activities are associated with adiposity in adolescents. *Obesity (Silver Spring)*. 2007; 15: 1589-99.

Ortega FB, Ruiz JR, Castillo MJ, Moreno LA, González-Gross M, Wärnberg J, Gutiérrez A; Grupo AVENA. Low level of physical fitness in Spanish adolescents. Relevance for future cardiovascular health (AVENA study). *Rev Esp Cardiol*. 2005; 58: 898-909.

P

Pate RR, O'Neill JR. After-school interventions to increase physical activity among youth. *Br J Sports Med*. 2009; 43: 14-8.

Platat C, Wagner A, Klumpp T, Schweitzer B, Simon C. Relationships of physical activity with metabolic syndrome features and low-grade inflammation in adolescents. *Diabetologia*. 2006; 49: 2078-85.

Pianta RC; NICHD. Developmental science and education: the NICHD study of early child care and youth development findings from elementary school. *Adv Child Dev Behav*. 2007; 35: 253-96.

Price JA, Sanny CG, Shevlin D. Application of manual assessment of oxygen radical absorbent capacity (ORAC) for use in high throughput assay of "total" antioxidant activity of drugs and natural products. *J Pharmacol Toxicol Methods*. 2006; 54: 56-61.

R

Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*. 2006; 27: 24-31.

Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protoc*. 2006; 1: 3159-65.

Ricart-Jané D, Llobera M, López-Tejero MD. Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess method. *Nitric Oxide*. 2002; 6: 178-85.

Rietjens SJ, Beelen M, Koopman R, VAN Loon LJ, Bast A, Haenen GR. A single session of resistance exercise induces oxidative damage in untrained men. *Med Sci Sports Exerc*. 2007; 39: 2145-51.

Roberts CK, Chen AK, Barnard RJ. Effect of a short-term diet and exercise intervention in youth on atherosclerotic risk factors. *Atherosclerosis*. 2007; 191: 98-106.

Rubin DA, McMurray RG, Harrell JS, Hackney AC, Thorpe DE, Haqq AM. The association between insulin resistance and cytokines in adolescents: the role of weight status and exercise. *Metabolism*. 2008; 57: 683-90.

Ruiz JR, Ortega FB, Meusel D, Harro M, Oja P, Sjöstrom M. Cardiorespiratory fitness is associated with features of metabolic risk factors in children. Should

cardiorespiratory fitness be assessed in a European health monitoring system? The European Youth Heart Study. *J Public Health*. 2006; 14: 94-102.

S

Sacheck J. Pediatric obesity: an inflammatory condition? *JPEN J Parenter Enteral Nutr*. 2008; 32: 633-7.

Sellers EA, Singh GR, Sayers SM. Apo-B/AI ratio identifies cardiovascular risk in childhood: the Australian Aboriginal Birth Cohort study. *Diab Vasc Dis Res*. 2009; 6: 94-9.

Steele RM, Brage S, Corder K, Wareham NJ, Ekelund U. Physical activity, cardiorespiratory fitness, and the metabolic syndrome in youth. *J Appl Physiol*. 2008; 105: 342-51.

Sobradillo B, Aguirre A, Aresti U et al. (2004) Curvas y tablas de crecimiento (estudios longitudinal y transversal). Madrid: Fundación Faustino Orbegozo Eizaguirre.

T

Tailor AM, Peeters PH, Norat T, Vineis P, Romaguera D. An update on the prevalence of the metabolic syndrome in children and adolescents. *Int J Pediatr Obes*. 2010; 5: 202-13.

Tanner JM. Growth at adolescente. Oxford: Blackwell; 1962.

Tirakitsoontorn P, Nussbaum E, Moser C, Hill M, Cooper DM. Fitness, acute exercise, and anabolic and catabolic mediators in cystic fibrosis. *Am J Respir Crit Care Med*. 2001; 164: 1432-7.

Tsukahara H. Biomarkers for oxidative stress: clinical application in pediatric medicine. *Curr Med Chem*. 2007; 14: 339-51.

Twisk JW, Kemper HC, van Mechelen W. The relationship between physical fitness and physical activity during adolescence and cardiovascular disease risk factors at adult age. The Amsterdam Growth and Health Longitudinal Study. *Int J Sports Med*. 2002; 23 Suppl 1:S8-14.

U

Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*. 2003; 189: 41-54.

V

Valle M, Martos R, Gascón F, Cañete R, Zafra MA, Morales R. Low-grade systemic inflammation, hypoadiponectinemia and a high concentration of leptin are present in very young obese children, and correlate with metabolic syndrome. *Diabetes Metab*. 2005; 31: 55-62.

W

Wärnberg J, Moreno LA, Mesana MI, Marcos A; AVENA group. Inflammatory mediators in overweight and obese Spanish adolescents. The AVENA Study. *Int J Obes Relat Metab Disord*. 2004; 28 Suppl 3:S59-63.

Weiss R. Metabolic syndrome in childhood - causes and effects. *Endocr Dev*. 2010; 19: 62-72.

Z

Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, Wong G, Bennett P, Shaw J, Caprio S; IDF Consensus Group. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes*. 2007; 8: 299-306.