Departamento de Fisiología

Facultad de Medicina

Universidad de Granada

La Condición Física como Determinante de Salud en Personas Jóvenes

"Fitness as a Health Determinant in Young People"

Jonatan Ruiz Ruiz

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A mis padres y hermana

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Granada, 11 de Enero de 2007

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Proyectos de Investigación

El presente trabajo de investigación ha sido posible gracias a las subvenciones obtenidas en los siguientes proyectos de investigación:

- Beca de Formación de Profesorado Universitario del Ministerio de Educación, Cultura y Deporte (AP2003-2128). Departamento de Fisiología de la Facultad de Medicina de la Universidad de Granada.

- Estudio AVENA (Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles). Proyecto Nacional multicéntrico financiado por el Instituto de Salud Carlos III con Fondos de Investigación Sanitaria, Ministerio de Sanidad y Consumo (nº 00/0015), por el Consejo Superior de Deportes (05/UPB32/0, 109/UPB31/03 y 13/UPB20/04), por el Ministerio de Educación (AP2002-2920, AP2003-2128 y AP-2004-2745), y por varias empresas privadas: Panrico S.A., Madaus S.A., y Procter and Gamble S.A.

- Estudio B12: Detección precoz de la deficiencia de vitamina B12 en población de riesgo. Proyecto financiado por el Instituto de Salud Carlos III con Fondos de Investigación Sanitaria (FIS PI021830).

- Estudio EYHS (European Youth Heart Study). Proyecto Europeo realizado en Dinamarca, Estonia, Noruega, Portugal y Suecia. El doctorando sólo ha trabajado con los datos correspondientes a Estonia y Suecia. El estudio realizado en Estonia recibió financiación de Estonian Science Foundation No. 3277 and 5209, y el Estonian Centre of Behavioural and Health Sciences. El estudio realizado en Suecia recibió financiación por el Stockholm County Council.

- Estudio HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence). Estudio financiado por la Comunidad Europea: European Community Sixth RTD Framework Programme (Contract FOOD-CT-2005-007034).

- Ayudas a Grupos de Investigación de la Junta de Andalucía.

Resumen

Conocer la relación entre capacidad aeróbica, fuerza muscular y factores de riesgo de enfermedad cardiovascular en niños y adolescentes es de interés científico y sanitario. Además, para poder interpretar de una manera más precisa estas asociaciones es necesario disponer de una metodología sencilla y fiable. Esto puede ayudar a crear estrategias de prevención primaria desde las edades más tempranas.

El objetivo general de esta memoria de Tesis Doctoral es estudiar la relación entre condición física (especialmente capacidad aeróbica y fuerza muscular) y factores de riesgo de enfermedad cardiovascular en jóvenes, así como desarrollar nuevos métodos de estimación de la capacidad aeróbica y fuerza muscular en adolescentes.

Un total de 873 niños de 9 a 10 años y 971 adolescentes de 12 a 19 años conforman las poblaciones que han participado en los tres estudios de cohortes incluidos en la presente memoria de Tesis: El estudio AVENA (Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles), el EYHS (European Youth Heart Study), y el estudio HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence).

Los principales resultados de la memoria de Tesis sugieren que: a) La condición física se relaciona con parámetros de salud en niños y adolescentes. b) La capacidad aeróbica se asocia inversamente con factores tradicionales de enfermedad cardiovascular en niños de 9 a 10 años. c) La capacidad aeróbica se asocia con un factor novel de enfermedad cardiovascular tal como la homocisteína en niñas adolescentes, y esto tras ajustar por distintas variables de confusión incluido el genotipo MTHFR 677C>T. d) La fuerza muscular se asocia a proteínas de inflamación aguda tales como la proteína C reactiva en adolescentes. e) Se ha desarrollado y validado una nueva fórmula de estimación del consumo máximo de oxígeno a partir del resultado obtenido en el test de ida y vuelta de 20 metros, el sexo, la edad, el peso y la talla del adolescente. f) Hay un tamaño de agarre óptimo que debería ser ajustado en el dinamómetro cuando se evalúe la fuerza de prensión manual en adolescentes.

Los resultados de la presente memoria de Tesis muestran que la condición física en general y la capacidad aeróbica y la fuerza muscular en particular constituyen un importante marcador de salud en jóvenes, al igual que ya se había mostrado en adultos. Estos datos confirman la necesidad de incluir este tipo de mediciones en los sistemas educativos y de salud pública. El desarrollo de nuevos métodos de evaluación de la condición física para ser aplicados en estudios epidemiológicos ayudará a mejorar la calidad y el rigor de los mismos.

Summary

For public health strategies and preventive purposes, it is of interest to understand the associations between cardiorespiratory fitness, muscle strength and cardiovascular disease risk factors in children and adolescents. Development of new and more accurate methodology to assess cardiorespiratory fitness and muscle strength may help to better elucidate the links between fitness and health from on early ages.

The overall objective of this thesis was to examine the association between physical fitness (focused on cardiorespiratory fitness and muscle strength) and both traditional and novel cardiovascular disease risk factors in young populations, and to develop new methods to better estimate cardiorespiratory fitness and muscle strength in adolescents.

A total of 873 children (aged 9 to 10 years), and 971 adolescents (aged 12 to 19 years) from three different studies were involved in the present work: the AVENA study (Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles), the EYHS (European Youth Heart Study), and The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study.

The main outcomes were: a) Physical fitness is associated to a myriad of health parameters in young people. b) Cardiorespiratory fitness is inversely associated with traditional cardiovascular disease risk factors in children. c) Cardiorespiratory fitness is inversely associated with a novel cardiovascular disease risk factor, such as homocysteine levels in female adolescents after controlling for potential confounders including the MTHFR 677C>T genotype. d) Muscle strength is inversely associated with inflammatory proteins, such as C-reactive protein, in adolescents. e) A new equation to estimate maximum oxygen consumption from 20m shuttle run test performance (last half stage completed), sex, age, weight and height in adolescents has been developed and cross-validated. f) There is an optimal grip span to which the dynamometer should be adjusted when measuring handgrip strength in adolescents.

The results show that physical fitness, and especially cardiorespiratory fitness and muscle strength is an important health marker in also young people, as has been shown in adults. Health information systems should include monitoring of cardiorespiratory and muscle fitness among young individuals. Development of efficient methodology for large-scale collection of the cardiorespiratory and muscle fitness data may help to improve the quality and accuracy of the outcome.

Abreviaturas

ACSM	American College of Sports Medicine
ANCOVA	Analysis of covariance
ANN	Artificial neural network
ANOVA	Analysis of variance
Apo	Apolipoprotein
AVENA	Alimentación y Valoración del Estado Nutricional de los Adolescentes
BF	Body fat
BMI	Body mass index
BP	Blood pressure
CD	Compact disc
CRF	Cardiorespiratory fitness
CVF	Cardiovascular fitness
DNA	Desoxirribonucleic acid
EYHS	European Youth Heart Study
HDLc	High density lipoprotein cholesterol
HOMA	Homeostasis model assessment
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence
LDLc	Low density lipoprotein cholesterol
Ln	Natural logarithm
Lp(a)	Lipoprotein (a)
MET	Metabolic equivalent
MSE	Mean sum of squared errors
MTHFR	Methylenetetrahydrofolate reductase
PA	Physical activity
RMSE	Root mean sum of squared errors
RNA	Ribonucleic acid
SD	Standard deviation
SEE	Standard error of estimate
SPSS	Statistical Package for Social Sciences
SRT	Shuttle run test
SSE	Sum of squared errors
TC	Total cholesterol
TG	Triglycerides

VO2	Oxygen consumption
VO_{2max}	Maximum oxygen consumption
WC	Waist circumference
W/H	Waist to hip ratio

Introducción

En España, al igual que en el resto de los países occidentales, las enfermedades cardiovasculares constituyen la principal causa de muerte. Hay numerosos factores de riesgo para desarrollar enfermedad cardiovascular entre los que se incluyen una alteración del perfil lipídico, resistencia a la insulina, parámetros inflamatorios elevados, hipertensión, sobrepeso y obesidad. Tradicionalmente, la prevención y tratamiento de estos factores ha estado enfocada a la población adulta. No obstante, investigaciones recientes han puesto de manifiesto su incidencia en niños y adolescentes (Bao *et al.*, 1995; Katzmarzyk *et al.*, 2001; Srinivasan *et al.*, 2002; Raitakari *et al.*, 2003; Moreno *et al.*, 2005). De hecho, existen evidencias científicas que indican que el inicio de la enfermedad cardiovascular se da en la adolescencia o incluso en la infancia, aún cuando las manifestaciones clínicas de la misma aparecen y alcanzan máxima relevancia en la vida adulta tardía (Berenson *et al.*, 1998; Strong *et al.*, 1999).

Son diversos los factores que pueden contribuir al inicio precoz, aunque subclínico, de la enfermedad cardiovascular. Entre esos factores se encuentran los cambios en los patrones alimenticios, descenso de los niveles de actividad física, aumento de los patrones de sedentarismo y otros. Estos patrones de comportamiento y su repercusión fisiológica se fijan principalmente durante la etapa adolescente. La adolescencia es una etapa decisiva en el desarrollo humano por los múltiples cambios fisiológicos y psicológicos que en ella ocurren. Este periodo se caracteriza por un intenso crecimiento, hasta el punto que se llega casi a duplicar el peso corporal del niño. A esto contribuye también el desarrollo sexual, el cual va a desencadenar importantes cambios en la composición corporal del niño. Por otro lado, se producen importantes cambios psicológicos que tienden a afectar su imagen corporal, la forma de alimentarse y el modo de comportarse. Con frecuencia, los hábitos que comienzan en la adolescencia (tales como fumar, consumir alcohol, comer de manera saludable o hacer ejercicio) suelen persistir durante muchos años o incluso durante toda la vida.

Estimaciones recientes sugieren que tanto la falta de actividad física como una dieta no saludable son dos claros factores de riesgo no sólo para desarrollar enfermedad cardiovascular sino para desarrollar muchas otras enfermedades. Ambos factores se cree que son responsables de alrededor de 400.000 muertes por año en Estados Unidos (Mokdad *et al.*, 2004). Estas cifras están cerca de sobrepasar al tabaco como causa de mortalidad prevenible, y es previsible que la situación sea similar en el resto de los países occidentales.

Un factor íntimamente ligado al nivel de actividad física y/o ejercicio que se realiza es el estado de condición física que tiene la persona. La condición física se define como la capacidad que una persona tiene para realizar ejercicio. La condición física constituye una medida integrada de todas las funciones y estructuras que intervienen en la realización de activad física o ejercicio. Estas funciones son la músculo-esquelética, cardio-respiratoria, hemato-circulatoria, endocrino-metabólica y psico-neurológica. Un alto nivel de condición física implica una buena respuesta fisiológica de todas ellas. Por el contrario, tener una mala condición física podría indicar un malfuncionamiento de una o varias de esas funciones.

La condición física comprende un conjunto de cualidades físicas tales como la capacidad aeróbica, fuerza y resistencia muscular, movilidad articular, velocidad de desplazamiento, agilidad, coordinación, equilibrio, y composición corporal. La medición de estas cualidades físicas en estudios epidemiológicos es relativamente reciente, y su aplicación al ámbito de la salud ha originado el sobrenombre de condición física relacionada con la salud (en inglés *health-related fitness*).

Condición física y salud

De todas las cualidades que componen la condición física, la capacidad aeróbica, la fuerza muscular y la composición corporal han sido las que han adquirido una mayor relevancia científica en el ámbito sanitario. No obstante, la relación del resto de cualidades físicas con distintos parámetros de salud también ha sido reconocida en personas jóvenes y adultas (American College of Sports Medicine, 1998).

Condición física y salud: capacidad aeróbica

La capacidad aeróbica (en inglés *aerobic capacity, cardiorespiratory fitness, cardiovascular fitness*) es una de las cualidades más importantes de la condición física relacionada con la salud. La capacidad aeróbica representa una medida directa del estado general de salud y de manera específica del estado del sistema cardiovascular, respiratorio y metabólico.

Recientes investigaciones han puesto de manifiesto el interés que tiene conocer el nivel de capacidad aeróbica que posee una persona. Tener un nivel medio-alto de capacidad aeróbica disminuye el riesgo de desarrollar enfermedad cardiovascular y aumenta la esperanza de vida en adultos (Blair *et al.*, 1989; Lee *et al.*, 1999; Carnethon *et al.*, 2005; LaMonte *et al.*, 2005). Asimismo, una mejora de la capacidad aeróbica se asocia directamente con una mejora de la calidad de vida no sólo en personas sanas sino también en personas con cáncer (Herrero *et al.*, 2006). La capacidad aeróbica también se ha asociado inversamente con distintos parámetros de salud en jóvenes, tales como el perfil lipídico, la resistencia a la insulina, la masa grasa, parámetros relacionados con el síndrome metabólico y la resistencia arterial (Gonzalez-Gross *et al.*, 2003; Gutin *et al.*, 2004; Eisenmann *et al.*, 2005; Gutin *et al.*, 2005; Reed *et al.*, 2005; Mesa *et al.*, 2006; Ruiz *et al.*, 2006).

Condición física y salud: fuerza muscular

El papel de la fuerza muscular en la práctica de ejercicio y actividades de la vida diaria, así como en la prevención de diversas enfermedades está siendo objeto de creciente atención en los último años (Stump *et al.*, 2006; Wolfe, 2006). La fuerza muscular se puede mejorar mediante el entrenamiento contrarresistencia, ejercicio que está recomendado por importantes organizaciones relacionadas con la salud para mejorar la condición física y la salud tanto de personas sanas como de personas con alguna enfermedad (Pollock *et al.*, 2000; Kraemer *et al.*, 2002).

La fuerza muscular se ha asociado inversamente con distintos parámetros relacionados con el síndrome metabólico (i.e. triglicéridos, lipoproteínas de alta densidad, glucosa, tensión arterial, y circunferencia de cintura) en hombres (Jurca *et al.*, 2004), así como con proteínas de inflamación aguda en hombres y mujeres (Visser *et al.*, 2002; Schaap *et al.*, 2006). Además, resultados de estudios prospectivos han mostrado que aquellos hombres que tenían mejor fuerza muscular tenían también menor incidencia de síndrome metabólico, y esto tras ajustar por varios parámetros de confusión entre los que se incluía la capacidad aeróbica (Jurca *et al.*, 2005). Los resultados de un estudio longitudinal en los que se siguió durante 40 años a más de 1.000 hombres, mostraron que una baja fuerza de prensión manual se asociaba a un mayor índice de morbilidad y mortalidad por todas las causas independientemente del nivel de actividad física y de la masa muscular de los participantes (Metter *et al.*, 2002). Estos resultados muestran la importancia de mantener unos niveles de fuerza muscular relativamente altos para mantener una buena calidad de vida y reducir la incidencia de morbilidad.

Por todo ello, es de capital importancia desarrollar herramientas de diagnóstico y prevención a aplicar ya desde edades tempranas para identificar alteraciones en aquellos factores que puedan incrementar el riesgo de desarrollar alguna enfermedad cardiovascular durante estos años y en la vida adulta.

Con base en estos antecedentes, la presente memoria de Tesis fija los siguientes objetivos:

Objetivos

General:

El objetivo general de la Tesis Doctoral es estudiar la relación entre condición física (especialmente capacidad aeróbica y fuerza muscular) y factores de riesgo de enfermedad cardiovascular en jóvenes, así como desarrollar nuevos métodos de estimación de la capacidad aeróbica y fuerza muscular en adolescentes.

Específicos:

- **I.** Describir el estado de salud del adolescente español en lo que referente a los niveles de lípidos y lipoproteínas sanguíneas.
- **II.** Describir la influencia de la edad cronológica y el desarrollo madurativo durante la adolescencia sobre los niveles de lípidos y lipoproteínas sanguíneas, el índice de masa corporal y la circunferencia de la cintura.
- **III.** Analizar la relación existente entre condición física y estado de salud en jóvenes.
- **IV.** Estudiar la asociación entre la capacidad aeróbica y los factores tradicionales de riesgo de enfermedad cardiovascular en niños de 9 a 10 años.
- **V.** Estudiar la asociación entre la capacidad aeróbica y un factor novel de riesgo de enfermedad cardiovascular, la homocisteína.
- **VI.** Estudiar la relación entre la fuerza muscular y parámetros de inflamación, examinando si esta asociación se ve influenciada por el peso corporal en adolescentes.
- **VII.** Desarrollar una ecuación basada en el modelo de redes neuronales para mejorar la estimación de la capacidad aeróbica en estudios poblacionales en adolescentes.
- **VIII.** Determinar si el tamaño de la mano de los adolescentes influye sobre la media de la fuerza de prensión manual.
- **IX.** Discutir la relación entre dieta, actividad física, condición física y parámetros de riesgo cardiovascular en niños y adolescentes.

Aims

Overall:

The overall objective of this thesis was to examine in young populations the association between physical fitness (focused on cardiorespiratory fitness and muscle strength) and both traditional and novel cardiovascular disease risk factors, and to develop new methods to better estimate cardiorespiratory fitness and muscle strength in adolescents.

Specific:

- **I.** To provide current reference values for serum lipid and lipoprotein levels in Spanish adolescents according to age and sex.
- **II.** To describe the effects of chronological age and pubertal development on serum lipid and lipoprotein levels, body mass index and waist circumference in Spanish adolescents.
- **III.** To study the association between physical fitness and health in young people.
- **IV.** To examine the associations between cardiorespiratory fitness and metabolic risk factors in children aged 9 to 10 years.
- **V.** To examine the association between cardiorespiratory fitness and homocysteine levels in adolescents.
- **VI.** To analyse the associations between inflammatory proteins and muscle strength, and to determine whether these associations vary in overweight and non-overweight adolescents.
- **VII.** To develop an artificial neural network-based equation for estimating maximum oxygen consumption in adolescents.
- **VIII.** To determine if there is an optimal grip span for determining the maximum handgrip strength in adolescents.
- **IX.** To study the associations between physical activity, fitness and cardiovascular disease risk factors from on early ages.

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Material y Métodos

Se presenta una tabla resumen de la metodología utilizada en los artículos que componen la presente memoria de Tesis.

Proyecto	Artículo	Diseño	Sujetos	Variables estudiadas	Metodología
AVENA	**I.** Serum lipid and lipoprotein reference values of Spanish adolescents; The AVENA study	Transversal	299 niños 282 niñas Edad: 13-18.5	TG, TC, HDLc, LDLc, apo A-1, Apo B-100, Lp(a) y edad de menarquia	Analizador enzimático estándar e inmunonefelometría
AVENA	**II.** Serum lipids, body mass index and waist circumference during pubertal development in Spanish adolescents: The AVENA Study	Transversal	254 niños 272 niñas Edad: 13-18.5	TG, TC, HDLc, LDLc, TC, apo A-1, apo B-100, Lp(a), edad de menarquia, Tanner, BMI y WC	Analizador enzimático estándar, inmunonefelometría, cuestionarios, peso, talla y cinta métrica
AVENA EYHS HELENA	**III.** Health-related physical fitness assessment in childhood and adolescence; A European approach based on the AVENA, EYHS and HELENA Studies	Revisión	Niños y adolescentes	CA, flexibilidad, fuerza muscular, velocidad de movimiento, agilidad, y varios parámetros de salud	Revisión bibliográfica y contextualización de resultados propios
EYHS	**IV.** 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	Transversal	429 niños 444 niñas Edad: 9-10	TG, TC, HDLc, HOMA, TA, MG y CRF	Analizador enzimático estándar, pliegues cutáneos, tensiómetro automático, test máximo en cicloergómetro
AVENA B12	**V.** Cardiovascular fitness is negatively associated with homocysteine levels in female adolescents	Transversal	76 niños 80 niñas Edad: 13-18.5	CA, MG, tHcy, MTHR 677C>T, folato y vitamina B12 sérico, Tanner, peso al nacer, SES y cigarrillos	20mSRT, pliegues cutáneos, técnica de PCR, inmunoensayo, y cuestionarios
AVENA	**VI.** Inflammatory proteins are associated with muscle strength in adolescents; The AVENA Study	Transversal	230 niños 186 niñas Edad: 13-18.5	CRP, C3,C4, ceruloplasmina y transthyretina, CA, fuerza muscular, BMI, MG, Tanner y SES	Análisis estándar por inmunoturbodiometría, 20mSRT, dinamometría manual, salto de longitud a pies juntos, peso, talla y cuestionarios
HELENA	**VII.** Use of artificial neural network-based equation for estimating VO_{2max} in adolescents	Transversal Artículo metodológico	122 niños 71 niñas Edad: 13-19	CA, peso y talla	20mSRT, medidor de gases portátil
HELENA	**VIII.** Hand span influences optimal grip span in male and female teenagers	Experimental Artículo metodológico	101 niños 106 niñas Edad: 13-18	Fuerza de prensión manual y tamaño de la mano	Dinamómetro manual
AVENA EYHS HELENA	**IX.** A Mediterranean diet is not enough for health: physical fitness is an important additional contributor to health for the adults of tomorrow	Revisión	Niños y adolescentes	CA, fuerza muscular, actividad física, dieta y parámetros de salud cardiovascular	Revisión bibliográfica y contextualización de resultados propios

TG: triglicéridos, TC: colesterol total, HDLc: lipoproteínas de alta densidad, LDLc: lipoproteínas de alta densidad, Apo: apolipoproteínas, Lp: lipoproteína, HOMA: homeostasis model assessment, tHcy: homocisteína, TA: tensión arterial, CRP: proteína C reactiva, BMI: índice de masa corporal, SES: estatus socieconómico, MG: masa grasa, CA: capacidad aeróbica, 20mSRT: test de ida y vuelta de 20 metros, PCR: polimerase chaín reaction.

Resultados y Discusión

Los resultados y discusión se presentan a continuación en la forma en que han sido previamente publicados/sometidos en revistas científicas.

I

REFERENCE VALUES FOR SERUM LIPIDS AND LIPOPROTEIN IN SPANISH ADOLESCENTS THE AVENA STUDY

Jonatan R. Ruiz[1], Francisco B. Ortega[1], Luis A Moreno[2], Julia Wärnberg[3,4], Marcela Gonzalez-Gross[1,5], Maria D. Cano[6], Ángel Gutiérrez[1], Manuel J. Castillo[1], and the AVENA Study Group

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II

SERUM LIPIDS, BODY MASS INDEX AND WAIST CIRCUMFERENCE DURING PUBERTAL DEVELOPMENT IN SPANISH ADOLESCENTS; THE AVENA STUDY

Jonatan R. Ruiz[1,2], Francisco B. Ortega[1,2], Beatriz Tresaco[3], Julia Wärnberg[2,4], José L. Mesa[1], Marcela González-Gross[1,5], Luis A. Moreno[3], Ascensión Marcos[4], Ángel Gutiérrez[1], Manuel J. Castillo [1], and the AVENA study group

Horm Metab Res 2006; 38: 832-837

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III

HEALTH-RELATED FITNESS ASSESSMENT IN CHILDHOOD AND ADOLESCENCE: A EUROPEAN APPROACH BASED ON THE AVENA, EYHS AND HELENA STUDIES

Jonatan R. Ruiz[1,2], Francisco B. Ortega[1,2], Ángel Gutiérrez[1], Dirk Meusel[3], Michael Sjöström[2], Manuel J. Castillo [1]

J Public Health 2006; 14: 269-277

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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

IV

Jonatan R. Ruiz[1,2], Francisco B. Ortega[1,2], Dirk Meusel[3], Maarike Harro[4], Pekka Oja[1], Michael Sjöström[2]

J Public Health 2006; 14: 94-102

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CARDIOVASCULAR FITNESS IS NEGATIVELY ASSOCIATED WITH HOMOCYSTEINE LEVELS IN FEMALE ADOLESCENTS

Jonatan R. Ruiz[1,2], Ricardo Sola[1,3], Marcela Gonzalez-Gross[4], Francisco B. Ortega[1,2], German Vicente-Rodirguez[5], Miguel García-Fuentes[6], Ángel Gutiérrez[1], Michael Sjöström[2], Kalus Pietrzik[7], Manuel J. Castillo[1]

Arch Pediatr Adolesc Med *in press*

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INFLAMMATORY PROTEINS ARE ASSOCIATED WITH MUSCLE STRENGTH IN ADOLESCENTS

THE AVENA STUDY

Jonatan R. Ruiz[1,2], Francisco B. Ortega[1,2], Julia Wärnberg[2,3], Luis A. Moreno[4], Juan J. Carrero[5], Marcela González-Gross[6], Ascensión Marcos[3], Ángel Gutiérrez[1], Michael Sjöström[2]

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VI

USE OF ARTIFICIAL NEURAL NETWORK-BASED EQUATION FOR ESTIMATING VO_{2MAX} IN ADOLESCENTS

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VII

HAND SPAN INFLUENCES OPTIMAL GRIP SPAN IN MALE AND FEMALE TEENAGERS

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J Hand Surg [Am] 2006; 31: 1367-1372

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VIII

A MEDITERRANEAN DIET IS NOT ENOUGH FOR HEALTH: PHYSICAL FITNESS IS AN IMPORTANT ADDITIONAL CONTRIBUTOR TO HEALTH FOR THE ADULTS OF TOMORROW

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World Rev Nutr Diet 2007; 97: 114-138

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IX

Conclusiones

I. Se han descrito los valores de referencia para los niveles de lípidos y lipoproteínas sanguíneas en adolescentes españoles, hallando que un número elevado de los mismos presenta un perfil lipídico poco saludable.

II. El desarrollo madurativo en el que se encuentra el adolescente influencia el perfil lipídico así como la cantidad y distribución de la grasa corporal.

III. El nivel de condición física se relaciona con parámetros de salud de niños y adolescentes.

IV. La capacidad aeróbica en niños de 9 y 10 años se asocia inversamente con factores tradicionales de riesgo cardiovascular, tales como el perfil lipídico, resistencia a la insulina y masa grasa.

V. La capacidad aeróbica en niñas adolescentes se asocia inversamente con factores noveles de riesgo cardiovascular, tales como el nivel de homocisteína, y esto tras controlar por diversos factores de confusión incluido el genotipo MTHFR 677C>T.

VI. La fuerza muscular se relaciona inversamente con parámetros de inflamación. Los patrones de estas asociaciones son más relevantes en adolescentes son sobrepeso.

VII. Se ha desarrollado y validado una fórmula para estimar la capacidad aeróbica basada en los modelos de redes neuronales construida a partir de: test de ida y vuelta de 20 metros, la edad, el sexo, la talla y el peso del adolescente.

VIII. La fuerza de prensión manual en adolescentes está influenciada por el tamaño de la mano y el tamaño del agarre de dinamómetro.

IX. Los datos publicados en la literatura científica reclaman la necesidad de desarrollar, evaluar e implementar estrategias de prevención en Salud Pública haciendo especial hincapié en la mejora de la condición física.

Conclusión general:

Los resultados de la presente memoria de Tesis ponen de manifiesto la importancia y utilidad de la valoración de la condición física como un determinante de salud que puede ser utilizado en instituciones sanitarias y educativas como una estrategia más para la prevención de enfermedades cardiovasculares en la vida adulta.

Conclusions

I. The reference values regarding the distribution of serum lipid and lipoprotein levels of Spanish adolescents are presented. A high number of subjects had an unhealthy lipid profile.

II. The assessment of pubertal development may provide additional valuable information when interpreting serum lipid profile and body fat in adolescents.

III. Physical fitness is a key health marker in children and adolescents.

IV. Cardiorespiratory fitness is inversely associated with traditional cardiovascular disease risk factors, such as serum lipid profile, insulin resistance and body fat, in children aged 9 to 10 years.

V. Cardiorespiratory fitness is inversely associated with plasma homocysteine levels in female adolescents after controlling for potential cofounders including MTHFR 677C>T genotype.

VI. Low-grade inflammation is negatively associated with muscle strength in adolescents. The patterns of these associations seem more relevant in overweight adolescents.

VII. An artificial neural network-based equation to estimate VO_{2max} from 20m shuttle run test performance (last half stage completed), sex, age, weight and height in adolescents has been developed and cross-validated.

VIII. There is an optimal grip span to which the dynamometer should be adjusted when measuring handgrip strength in adolescents.

IX. Scientific data demonstrate that there is an urgent need for the development, testing and implementation of preventive strategies in Public Health with stronger emphasis on physical fitness.

Overall conclusion:

The results of the present work highlight the importance and usefulness of measuring physical fitness. Physical fitness should be measured in schools and included in the Health Monitoring Systems.

CV abreviado [Short CV]

Actividad académica

- Diplomado en Magisterio, especialidad Educación Física. Universidad de Granada, Facultad de Ciencias de la Educación (Junio 1999).
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- Estancia de investigación en el Departamento de Biosciences and Nutrition, Karolinska Institutet, Suecia (Julio 2005 a Diciembre 2005, Agosto 2006 a Diciembre 2006).
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32. **Ruiz JR**, Ortega FB, Rizzo NS, Villa L, Hurtig-Wennlöf A, Oja L, Sjostrom M. High cardiorespiratory fitness is associated with low metabolic risk score in children; The European Youth Heart Study **Pediatrics Res** *in press*.

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Premios recibidos

1. **Premio de Libros a los 200 Mejores Rendimientos Académicos**, Curso 1998/1999. Sección de Becas Propias de la Universidad de Granada. Universidad de Granada. 19 de Octubre de 1999.

2. **Premio de Libros a los 200 Mejores Rendimientos Académicos**, Curso 1999/2000. Sección de Becas Propias de la Universidad de Granada. Universidad de Granada. 22 de Enero de 2001.

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4. **Segundo premio en la modalidad de póster**. III Congreso Internacional de Educación Física e Interculturalidad. Universidad Politécnica de Cartagena, Cartagena. 14-17 de Noviembre de 2002. En busca de un índice de condición física fiable en adolescentes: estudio piloto de AVENA.

5. **3er Premio Nacional Fin de Carrera de Educación Universitaria.** Curso Académico 2001-2002. BOE 170 de 17 de Julio 2003. Ministerio de Educación, Cultura y Deporte. ORDEN ECD/2008/2003, de 30 de mayo, por la que se adjudican los Premios Nacionales de Fin de Carrera de Educación Universitaria correspondientes al curso académico 2001/2002.

6. **Mención Honorífica** por el Vicerrectorado de Planificación, Calidad y Evaluación Docente de la Universidad de Granada. Utilización del sistema de mensajes cortos (SMS) para mejorar la calidad del proceso enseñanza-aprendizaje en la universidad. 30 de Junio de 2003.

7. **Premio a los 10 mejores artículos** sometidos por investigadores jóvenes al 9th European Nutrition Conference, Rome, 1-4 de Octubre de 2003. Simple physical assessment for lipid disturbances screening in adolescents: The AVENA study" (reference number 438).

8. **Segundo accésit de los premios de la Sociedad Española de Cardiología** a los mejores artículos publicados en Revista Española de Cardiología al trabajo: “Bajo nivel de forma física en los adolescentes españoles. Implicaciones para la salud cardiovascular futura (Estudio AVENA). Rev Esp Cardiol 2005; 58: 898-909.

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Reference values for serum lipids and lipoproteins in Spanish adolescents: the AVENA study

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Summary

Objectives: To provide current reference values for serum lipid and lipoprotein levels in Spanish adolescents according to age and sex.

Methods: A cross sectional study conducted in five representative Spanish cities (Granada, Madrid, Murcia, Santander and Zaragoza) including a representative sample of 581 adolescents (299 male and 282 female), aged 13 to 18.5 years. Age- and sex-specific means, standard deviations and percentiles were determined for: Total (TC), high density lipoprotein (HDLc) and low density lipoprotein (LDLc) cholesterol, triglycerides, apolipoprotein A-1 and B-100, and lipoprotein(a).

Results: The 90th percentile for TC was 4.95 mmol/L for males and 5.19 mmol/L for females. HDLc levels were significantly higher in females of all age groups. LDLc levels ranged from 2.32 to 2.54 mmol/L in males and from 2.38 to 2.62 mmol/L in females, peaking at 13 years of age in both sexes. Triglyceride levels tended to increase gradually and to peak at 17 years of age for both sexes. Apolipoprotein A-1 and B-100 levels paralleled those of HDLc and LDLc values, respectively. The geometric mean for lipoprotein(a) levels ranged from 0.44 to 0.57 μmol/L in males and from 0.50 to 0.67 μmol/L in females.

Conclusions: The present study provides reference data on the distribution of lipid and lipoprotein levels of Spanish adolescents.

Keywords: Adolescents – Lipids – Lipoproteins – Cardiovascular disease – Percentiles

Coronary heart disease (CHD) is a leading cause of global mortality, accounting for almost 17 million deaths every year (Smith et al. 2004). Nearly 80 % of this mortality and disease burden occurs in the industrialized countries; the data for Spain reflect this picture (Instituto Nacional de Estadistica, 2001). Pathological data have shown that atherosclerosis begins in childhood (Berenson et al. 1998; Strong et al. 1999), and CHD is known to occur more frequently in adult members of families in which children's cholesterol levels are high. Aortic fatty streaks can be found in children, and fibrous plaques are often evident in adolescence (McGill et al. 1997). This finding, plus the alarming increase in the prevalence of obesity (Moreno et al. 2002; Moreno et al. 2005) and the reduction in physical activity among children and adolescents (Kimm et al. 2002; Moreno et al. 2002; Tercedor 2003), shows the need for improved health education in this age group (Gaziano et al. 1998). The relationship between serum lipids and the development of CHD in children and adolescents is well established (Berenson et al. 1998).

The meta-analysis performed by Plaza (1991) showed that the serum total cholesterol (TC) levels of Spanish children and adolescents increased throughout the 1980s. However, no current data on serum lipid or lipoprotein levels data are available. The AVENA Study was therefore designed to asses the health and nutritional status of a representative population of Spanish adolescents. This report describes the current serum lipid and lipoprotein profiles of Spanish adolescents living in urban areas, and compares the results with those obtained in other countries.

Materials and methods

Population and sample recruitment

The methodology used in this study has been described

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elsewhere (Gonzalez-Gross et al. 2003a, b; Moreno et al. 2005). Briefly, a multicenter study was performed involving a representative sample of Spanish adolescents aged from 13 to 18.5 years. The population was selected by multiple-step, simple random sampling – first taking into account location (Madrid, Murcia, Granada, Santander and Zaragoza) and then by random assignment of the school within each city. The cities were chosen according to the population rate (>100 000 inhabitants), geographical location in the country (north-south gradient, in order to be representative) and taking into account the main technical question, that is, the necessity of having a research group in the city. Sample size was stratified by age and sex. The socio-economic variable was considered to be associated to location within the city and type of school. As the selection of schools was done by random selection proportionally to the number of schools in each city district, guaranteeing the presence of almost one school per district, the socio-economic variable was also considered to be randomly assigned. After analysis of the data, this method has proven to be adequate, as the socio-economic status of our sample has a normal distribution according to the distribution in the Spanish society.

To calculate the number of adolescents to be included in the study in order to guarantee a representative sample of the whole country, we selected the variable with the greatest variance for this age group from the data published in the literature at the time the study was planned; that was body mass index (BMI) (Moreno et al. 1997). The sampling was determined for the distribution of this variable; the CI was established at 95 % with an error ±0.25 %. The minimum subject population was established at 1 750 for the complete study and at 500 for a subgroup from whose member's blood samples were required. A similar number of subjects was evaluated in each city, and proportionally distributed by sex and age group (13, 14, 15, 16, 17–18.5 years).

The sample was oversized in order to prevent loss of information and because technically it was necessary to perform fieldwork in complete classrooms. After finishing the fieldwork, the subjects who did not fulfill the inclusion criteria were excluded. Finally, the sample was adjusted by a weight factor in order to balance the sample in accordance to the distribution of the Spanish population and to guarantee the real representativeness of each of the groups, already defined by the previously mentioned factors (age and sex). The final number of subjects included in the AVENA Study was 2 859 adolescents, from which 581 (299 males and 282 females) had blood measurements, and were then included in this study.

In each school all the adolescents of one classroom were proposed to participate in the survey. A detailed verbal description of the nature and purpose of the study was given to both the children and their teachers. This information was also sent to parents by letter; written consent to be included was requested from both parents and children. The exclusion criteria were: no personal history of cardiovascular or metabolic disease; free of disease and medication at the time of the study; pregnancy. In order to avoid a selection bias, a family history record of metabolic and cardiovascular diseases was obtained for all subjects participating in the study.

The protocol for the complete multicenter study was approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

Blood measurements

Blood (20 ml) was collected from an antecubital vein between 8:00 and 9:00 a.m, after an overnight fast.

Measurement of serum lipids, lipoproteins and lipoprotein(a)

Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDLc) were measured by enzymatic assay using a Hitachi 911 Analyzer (Roche Diagnostics, Indianapolis, Ind, USA). HDLc was precipitated before analysis using the Boehringer Mannheim method. Low density lipoprotein cholesterol (LDLc) was calculated using the Friedewald et al. (1972) formula adjusted for serum TG levels (Morley et al. 1998). Apolipoprotein (apo) A-1, apo B-100 and lipoprotein(a) [Lp(a)] were measured by immunonephelometric assay using an Array 306 system (Beckman GMI, Inc., Albertville, Minnesota, USA). Quality control of the assays was assured by the Regional Health Authority. The coefficients of variation were less than 3 % and the intra-class coefficients were higher than 0.96 % for all blood variables. The following atherogenic indices were also calculated: TC/HDLc, TC-HDLc, (TC-HDLc)/HDLc, TG/HDLc, LDLc/HDLc, apo B-100/apo A-1, and apo B-100/LDLc.

Age at menarche was determined from the self-reported date of first menses based on administered questionnaire.

Statistical analysis

For data analysis, the studied population was divided into five age groups: 13–13.99, 14–14.99, 15–15.99, 16–16.99 and 17–18.5 years. Age- and sex-specific means, standard deviations (SD) and percentiles were determined. Kolmogorov-Smirnov test was used to check data distribution by both sex and age and only by sex. The studied variables were quasi-normal distributed, but the asymmetry and kurtosis levels were adequate for all, except for Lp(a) that was achieved after logarithmic transformation. Mean values were compared with one way analysis of variance (ANOVA), and post hoc Bonferroni test. The Mann-Whitney U test was used

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to determine any differences in BMI (the variable selected to calculate the number of subjects to be included in the study) between the subgroup from which blood samples were obtained (N = 581) and the remaining subjects (N = 2278) (for each age subgroup and sex). No differences were seen between any of the age and sex groups (Tab. 1). The error was fixed at 0.05.

Results

The means and SDs for lipid and lipoprotein levels, according to age and sex are shown in Table 2. The percentiles distributions for lipid and lipoprotein levels and atherogenic indices, according to age and sex are shown in tables 3–10. Comparison between the sexes shows both higher TC and HDLc levels in females than in males adolescents. Higher LDLc levels were only observed in females aged 15 years ($P < 0.05$). The differences in apo A-1 and apo B-100 levels between the sexes were entirely superimposable on those for HDLc and LDLc levels. Triglycerides levels were slightly lower in females although the differences failed to reach statistical significance, except for the 14 year-olds.

An 8.2 % decline in mean TC serum levels was observed in males between the ages of 13 and 15 years ($P < 0.05$). For males 13 years of age, the 90th percentile for TC (5.39 mmol/L) was the highest estimate for all age groups and both sexes. For females, mean serum TG levels were no different among age groups. For females aged 17–18.5 years, the 90th

Table 1 Comparisons of body mass index between sub-group in which blood sample was obtained (blood group) and group in which blood sample was not obtained (non blood group). Body mass index was calculated as body weight (kg) without shoes and with light clothing, divided by height (m) squared.

Sex	Age group (years)	Body Mass Index		P =
		Blood group (N = 581)	Non Blood group (N = 2278)	
Male	13	20.6 ± 3.3	20.6 ± 4.0	0.63
	14	22.1 ± 3.9	21.4 ± 3.6	0.19
	15	22.3 ± 4.0	21.9 ± 3.5	0.74
	16	21.7 ± 3.3	21.8 ± 3.1	0.86
	17–18.5	23.6 ± 4.2	22.7 ± 3.5	0.15
Female	13	21.0 ± 3.9	21.7 ± 3.6	0.08
	14	21.4 ± 4.2	21.2 ± 3.5	0.35
	15	21.3 ± 3.2	21.5 ± 3.0	0.67
	16	21.9 ± 3.2	21.6 ± 3.1	0.52
	17–18.5	21.8 ± 2.9	21.7 ± 3.3	0.68

Table 2 Lipids and lipoprotein mean and SD values in Spanish adolescents aged 13 to 18.5 years. Values are means ± SD. TC: total cholesterol; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; TG: triglycerides; Apo: apolipoprotein; Lp(a): lipoprotein a. [*]Geometric mean ± SD. [a]P < 0.05 for differences between sexes. [*]P < 0.05 (in comparison to males 15 years of age). [¶]P < 0.05 (in comparison to males 17 years of age). [#]P < 0.05 (in comparison to males 13, 14 and 15 years of age).

Age groups (years)	TC (mmol/L)	HDLc (mmol/L)	LDLc (mmol/L)	TG (mmol/L)	Apo A-1 (g/L)	Apo B (g/L)	Lp(a)[û] (µmol/L)
Males							
13	4.26 ± 0.80[*]	1.35 ± 0.29[a]	2.54 ± 0.66	0.82 ± 0.41	1.16 ± 0.17[a]	0.71 ± 0.18	0.44 ± 0.06
14	4.02 ± 0.59[a]	1.32 ± 0.27[a]	2.32 ± 0.54	0.84 ± 0.41[a]	1.10 ± 0.19	0.67 ± 0.15	0.49 ± 0.05
15	3.91 ± 0.60[a]	1.31 ± 0.23[a¶]	2.24 ± 0.54[a]	0.78 ± 0.28	1.12 ± 0.20[a]	0.65 ± 0.14[a]	0.49 ± 0.04
16	4.07 ± 0.64	1.41 ± 0.27[a]	2.30 ± 0.59	0.79 ± 0.33	1.26 ± 0.20[#]	0.68 ± 0.13	0.48 ± 0.06
17–18.5	4.01 ± 0.73[a]	1.23 ± 0.18[a]	2.39 ± 0.71	0.86 ± 0.37	1.20 ± 0.17[a]	0.70 ± 0.16	0.57 ± 0.06
Total (13–18.5)	4.05 ± 0.68	1.32 ± 0.25[a]	2.35 ± 0.62	0.82 ± 0.36	1.17 ± 0.19	0.68 ± 0.15	0.49 ± 0.05
Females							
13	4.51 ± 0.59	1.53 ± 0.27	2.62 ± 0.52	0.78 ± 0.24	1.24 ± 0.15	0.71 ± 0.11	0.59 ± 0.05
14	4.32 ± 0.70	1.53 ± 0.28	2.48 ± 0.67	0.69 ± 0.27	1.14 ± 0.26	0.72 ± 0.16	0.52 ± 0.05
15	4.38 ± 0.63	1.57 ± 0.33	2.48 ± 0.56	0.72 ± 0.23	1.28 ± 0.24	0.70 ± 0.13	0.55 ± 0.05
16	4.23 ± 0.69	1.53 ± 0.32	2.38 ± 0.58	0.69 ± 0.24	1.29 ± 0.23	0.68 ± 0.13	0.50 ± 0.05
17–18.5	4.40 ± 0.76	1.51 ± 0.28	2.51 ± 0.65	0.83 ± 0.64	1.34 ± 0.22	0.73 ± 0.15	0.67 ± 0.05
Total (13–18.5)	4.37 ± 0.68	1.53 ± 0.30	2.49 ± 0.60	0.74 ± 0.37	1.26 ± 0.23	0.71 ± 0.14	0.56 ± 0.05

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Table 3 Mean and percentile distributions for total cholesterol (mmol/L) according to age and sex group. To convert cholesterol values in mmol/L to mg/dL divided by 0.02586.

Age groups (years)			Total cholesterol				
	N	Mean	10^{th}	25^{th}	50^{th}	75^{th}	90^{th}
Males							
13	54	4.26	3.32	3.70	4.20	4.69	5.39
14	54	4.02	3.32	3.65	3.94	4.46	4.86
15	63	3.91	3.29	3.56	3.86	4.19	4.68
16	63	4.07	3.34	3.60	4.03	4.43	4.80
17–18.5	65	4.01	3.06	3.46	4.11	4.51	4.95
Total (13–18.5)	299	4.05	3.29	3.57	3.99	4.49	4.95
Females							
13	50	4.51	3.69	4.14	4.45	4.98	5.23
14	55	4.32	3.43	3.78	4.32	4.88	5.23
15	55	4.38	3.68	3.94	4.27	4.78	5.33
16	59	4.23	3.36	3.76	4.12	4.90	5.14
17–18.5	63	4.40	3.51	3.87	4.33	4.94	5.20
Total (13–18.5)	282	4.37	3.52	3.86	4.33	4.87	5.19

Table 4 Mean and percentile distributions of high density lipoprotein cholesterol (mmol/L) according to age and sex group. To convert cholesterol values in mmol/L to mg/dL divided by 0.02586.

Age groups (years)			High density lipoprotein cholesterol				
	N	Mean	10^{th}	25^{th}	50^{th}	75^{th}	90^{th}
Males							
13	54	1.35	0.93	1.11	1.40	1.50	1.68
14	54	1.32	0.91	1.14	1.32	1.53	1.65
15	63	1.31	1.04	1.17	1.32	1.47	1.60
16	63	1.41	1.03	1.24	1.40	1.58	1.79
17–18.5	65	1.23	1.04	1.06	1.22	1.37	1.48
Total (13–18.5)	299	1.32	1.02	1.14	1.32	1.49	1.66
Females							
13	50	1.53	1.16	1.36	1.55	1.68	1.93
14	55	1.53	1.11	1.35	1.50	1.79	1.94
15	55	1.57	1.18	1.37	1.52	1.71	1.99
16	59	1.53	1.09	1.26	1.53	1.72	2.05
17–18.5	63	1.51	1.18	1.30	1.45	1.71	1.90
Total (13–18.5)	282	1.53	1.14	1.32	1.53	1.71	1.95

percentile for TG (1.69 mmol/L) was the highest for all age groups and both sexes. For males, mean LDLc levels tended to decrease gradually from 13 to 15 years of age (11.6 %, $P = 0.07$). In males, the means and percentiles for serum apo A-1 showed distributions similar to those observed for HDLc. In females, apo A-1 levels increased significantly from 14 to 17–18.5 years of age. The percentile distributions for apolipoprotein B-100 were similar to those observed for LDLc, with no differences among age groups for either males or females. Differences were seen, however, between 15 year-old males and females ($P < 0.05$). No gender or age group differences were found in Lp(a) levels. For the atherogenic indices, the TG/HDLc index was significantly higher in males than in females at age 14 and 15 years. Among females, the apo B-100/apo A-1 ratio was significantly higher at 14 compared to 15 years of age.

The self-reported age of menarche ranged from 9 to 15 years of age. The age at first menses distribution was: 9 years (1.6 %), 10 years (2.9 %), 11 years (20.9 %), 12 years (34.7 %), 13 years (27.4 %), 14 years (11.3 %), and 15 years (1 %). No differences were observed in serum lipids variables within these groups.

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Age groups (years)			Low density lipoprotein cholesterol				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	2.54	1.73	2.07	2.48	2.86	3.39
14	54	2.32	1.67	1.97	2.27	2.61	3.04
15	63	2.24	1.54	1.95	2.19	2.53	2.88
16	63	2.30	1.69	1.92	2.25	2.56	2.98
17–18.5	65	2.39	1.35	1.88	2.37	3.04	3.37
Total (13–18.5)	299	2.35	1.66	1.95	2.31	2.72	3.20
Females							
13	50	2.62	1.87	2.32	2.59	2.94	3.30
14	55	2.48	1.64	1.96	2.45	2.97	3.33
15	55	2.48	1.79	2.09	2.46	2.82	3.27
16	59	2.38	1.67	2.02	2.36	2.72	3.35
17–18.5	63	2.51	1.60	2.02	2.51	2.98	3.27
Total (13–18.5)	282	2.49	1.74	2.07	2.46	2.92	3.30

Table 5 Mean and percentile distributions of low density lipoprotein cholesterol (mmol/L) according to age and sex group. To convert cholesterol values in mmol/L to mg/dL divided by 0.02586.

Age groups (years)			Apolipoprotein A-1				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	1.16	0.95	1.02	1.14	1.29	1.37
14	54	1.10	0.82	0.99	1.13	1.25	1.31
15	63	1.12	0.93	1.02	1.12	1.25	1.37
16	61	1.26	1.02	1.12	1.21	1.40	1.55
17–18.5	57	1.20	0.96	1.06	1.17	1.35	1.43
Total (13–18.5)	290	1.17	0.96	1.04	1.16	1.29	1.42
Females							
13	50	1.24	1.04	1.15	1.24	1.36	1.43
14	55	1.14	0.64	1.06	1.21	1.29	1.42
15	55	1.28	1.02	1.13	1.27	1.46	1.58
16	48	1.29	1.02	1.16	1.27	1.44	1.61
17–18.5	58	1.34	1.10	1.19	1.30	1.45	1.71
Total (13–18.5)	267	1.26	1.02	1.13	1.25	1.38	1.56

Table 6 Mean and percentile distributions for apolipoprotein A-1 (g/L) according to age and sex group. To convert apolipoprotein A-1 values in g/L to mg/dL divided by 0.01.

Discussion

This study provides national reference data for the serum lipid and lipoprotein levels of Spanish adolescents living in urban areas. The percentile distributions according to age and sex are also established. To our knowledge, this is the first report to record the entire serum lipid and lipoprotein profile of a representative sample of Spanish adolescents ranging from 13 to 18 years.

The mean TC, TG and LDLc levels of the present adolescents were similar or slightly lower than those observed in the meta-analysis of Plaza (1991), and then later by Garcés et al. (2004). HDLc levels were also slightly lower than those observed two decades ago (Plaza 1991), perhaps due to a loss of Mediterranean dietary patterns (Moreno et al. 2002; Serra-Majen et al. 1995; Zamora et al. 2003) or to the low level of physical activity recorded for the Spanish population (Moreno et al. 2002) and adolescents of the AVENA study (Tercedor 2003).

According to the NHANES III study, the mean serum TC levels of American children and adolescents aged 12–19 years were 4.09 mmol/L and 4.33 mmol/L for males and females respectively (Hickman et al. 1998). The present Spanish ado-

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Age groups (years)			Apolipoprotein B-100				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	0.71	0.49	0.60	0.70	0.79	0.96
14	54	0.67	0.46	0.56	0.64	0.75	0.91
15	63	0.65	0.49	0.57	0.64	0.72	0.87
16	61	0.68	0.51	0.57	0.68	0.75	0.84
17–18.5	57	0.70	0.49	0.60	0.72	0.82	0.91
Total (13–18.5)	290	0.68	0.49	0.58	0.67	0.77	0.88
Females							
13	50	0.71	0.58	0.64	0.71	0.79	0.85
14	55	0.72	0.51	0.60	0.73	0.82	0.93
15	55	0.70	0.52	0.61	0.69	0.78	0.91
16	48	0.68	0.49	0.57	0.69	0.75	0.88
17–18.5	58	0.73	0.54	0.61	0.75	0.83	0.88
Total (13–18.5)	267	0.71	0.53	0.61	0.71	0.80	0.88

Table 7 Mean and percentile distributions for apolipoprotein B-100 (g/L) according to age and sex group. To convert apolipoprotein B values in g/L to mg/dL divided by 0.01.

Age groups (years)			Triglycerides				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	0.82	0.36	0.49	0.73	1.11	1.41
14	54	0.84	0.46	0.59	0.70	1.02	1.43
15	63	0.78	0.47	0.57	0.71	0.90	1.16
16	63	0.79	0.42	0.55	0.77	0.94	1.23
17–18.5	65	0.86	0.52	0.64	0.77	1.00	1.36
Total (13–18.5)	299	0.82	0.44	0.58	0.75	0.96	1.31
Females							
13	50	0.78	0.45	0.58	0.76	0.94	1.11
14	55	0.69	0.38	0.50	0.63	0.83	1.00
15	55	0.72	0.45	0.57	0.68	0.86	1.16
16	59	0.69	0.47	0.53	0.62	0.82	0.94
17–18.5	63	0.83	0.40	0.51	0.68	0.81	1.69
Total (13–18.5)	282	0.74	0.44	0.54	0.68	0.84	1.09

Table 8 Mean and percentile distributions for triglycerides (mmol/L) according to age and sex group. To convert triglycerides values in mmol/L to mg/dL divided by 0.01125.

lescents (both males and females) show TC levels similar to those of their American counterparts. The age and sex specific trends for TC levels recorded in the present study were also similar to those reported in the NHANES III study (Hickman et al. 1998). Compared with data from Greece (Shulpis & Karikas 1998), another Mediterranean country, the Spanish mean serum TC levels were slightly higher. The NHANES III (Hickman et al. 1998) and LRC study (Kwiterovich 1991) reported higher TC levels in females than in males; this was also found in the present study. The NHANES III and LRC prevalence studies showed lower TC levels among males during puberty as a result of a decrease in HDLc levels (Hickman et al. 1998; Kwiterovich 1991). This agrees with that seen in the AVENA study. This reduction probably stems from hormonal changes experienced by males during puberty (Kwiterovich 1991). In the present adolescents, the HDLc levels were higher than those recorded for American adolescents (Hickman et al. 1998). This might be attributable to genetic factors, environmental factors and/or to the consumption of olive oil, a major component of the Mediterranean diet (Serra-Majem et al. 1993a, b; Moreno et al. 2002). Therefore, despite having a TC similar to that of American adolescents, the higher HDLc

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Table 9 Mean and percentile distributions for lipoprotein (a) (µmol/L) according to age and sex group. To convert lipoprotein (a) values in µmol/L to md/dL divided by 0.0357. *Geometric mean.

Age groups (years)			Lipoprotein (a)				
	N	Meanû	10th	25th	50th	75th	90th
Males							
13	54	0.44	0.04	0.17	0.54	1.46	3.19
14	54	0.49	0.04	0.21	0.54	1.59	2.83
15	61	0.49	0.07	0.21	0.44	1.02	2.55
16	51	0.48	0.05	0.14	0.40	1.70	3.58
17–18.5	63	0.57	0.10	0.27	0.55	2.45	3.25
Total (13–18.5)	284	0.49	0.05	0.21	0.48	1.50	3.00
Females							
13	50	0.59	0.08	0.28	0.80	1.58	3.71
14	55	0.52	0.05	0.23	0.61	1.74	2.91
15	55	0.55	0.07	0.18	0.49	1.37	3.35
16	55	0.50	0.07	0.21	0.37	1.14	3.00
17–18.5	63	0.67	0.04	0.22	0.57	1.38	3.55
Total (13–18.5)	279	0.56	0.07	0.22	0.55	1.37	3.06

Table 10 Atherogenic indices in Spanish adolescents aged 13 to 18 years. TC: total cholesterol; HDLc: high density lipoprotein cholesterol TG: triglycerides; LDLc: low density lipoprotein cholesterol; Apo: apolipoprotein. [a]P < 0.05 for differences between sexes. *P < 0.05 (in comparison to girls 15 years of age).

Age groups (Years)	TC/HDLc	TC-HDLc	(TC-HDLc)/ HDLc	TG/HDLc	LDLc/HDLc	Apo B-100 / Apo A-1	Apo B-100 / LDLc
Males							
13	3.15	2.91	2.15	0.60	1.88	0.61	0.28
14	3.04	2.70	2.04	0.63[a]	1.75	0.61	0.29
15	2.98	2.60	1.98	0.59[a]	1.71	0.58	0.29
16	2.90	2.66	1.90	0.56[a]	1.64	0.54	0.29[a]
17–18.5	3.26	2.78	2.26	0.70	1.94	0.59	0.29
Total (13–18.5)	3.06	2.73	2.06	0.62	1.78	0.58	0.29
Females							
13	2.94	2.98	1.94	0.51	1.71	0.57	0.27
14	2.82	2.79	1.82	0.45	1.61*	0.63	0.29
15	2.79	2.81	1.79	0.46	1.58	0.55	0.28
16	2.76	2.70	1.76	0.45	1.56	0.52	0.28
17–18.5	2.92	2.90	1.92	0.55	1.67	0.55	0.29
Total (13–18.5)	2.85	2.83	1.85	0.48	1.63	0.56	0.28

levels of the Spanish youngsters may renders them a healthier lipid profile. Their HDLc levels were, however, lower than those observed in Greek male adolescents aged 13 and 14 years (Shulpis & Karikas 1998). The HDLc levels recorded for females aged 13 and 14 years in the present study were the same as those of the Greek schoolchildren (Shulpis & Karikas 1998). The HDLc levels of Spanish females were higher than that of males; which agree with the results reported by other authors (Azizi et al. 2001). HDLc is a protective factor for females; it is estimated that for every 0.0259 mmol/L (1 mg/dL) increase in HDLc, the risk of a CHD event is reduced by at least by 3 % in females, and 2 % in men (Nicklas et al. 1997).

Low density lipoprotein cholesterol is the main carrier of cholesterol in the blood, and this compound plays a pivotal role in atherogenesis. The mean LDLc levels of Spanish adolescents were similar to those reported in American adolescents (Hickman et al. 1998) but much higher than in Greek adolescents (Shulpis & Karikas 1998). In contrast, Spanish adolescents had much lower TG values than those observed in either

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American or Greek adolescents. Comparison of the present serum lipid profiles with those obtained in 26 other countries (Brotons et al. 1998) showed no apparent differences. Since levels of physical activity are rapidly decreasing among Spanish adolescents (Moreno et al. 2002; Tercedor 2003) and the Mediterranean diet is losing its identity (Moreno et al. 2002; Serra-Majen et al. 1995; Zamora et al. 2003), increased obesity, and less favourable metabolic profile is expected to result (Moreno et al. 2002; Moreno et al. 2005). Nowadays, fruit and vegetable intake among Spanish children and adolescents is among the lowest in Europe (Yngve et al. 2005), and an increasing trend in fat consumption during the last decade has been observed (Moreno et al. 2000; Moreno et al. 2002). According to the well known relation between dietary fat, serum cholesterol and cardiovascular diseases (Ascherio et al. 1996), a significant increase in incidence and mortality from cardiovascular diseases should have been detected in Spain. However, this expected trend has not been observed in adults. This has been termed the 'Spanish paradox' (Serra-Majem et al. 1995). This paradox most likely stems from the interaction of multiple synergistic and antagonistic risk and protective factors for cardiovascular diseases.

Relatively little has been published on the apolipoprotein profiles of adolescents. It is therefore difficult to compare the results of the present study with those observed in other cross-sectional examinations. Reference values for apolipoproteins in children and adolescents are of interest since they have been established as new atherosclerosis risk factor (Glowinska et al. 2003). According to some authors, the concentrations of apo A-1 and apo B-100 show an even stronger correlation with atheroma development than their equivalent lipoproteins HDLc and LDLc (Gomez et al. 1996). The levels seen in children have been associated with the incidence of coronary heart disease in their parents (Srinivasan & Berenson 1995). As in adults, the distribution of Lp(a) values was highly skewed towards low values. The geometric means obtained for serum Lp(a) were similar to those reported in Spain in the 1990s (Gomez et al. 1996). However, when median Lp(a) serum concentrations are compared according to age and gender, the figures recorded in the present study are much higher than those reported by Gomez et al. (1996). Assessing new risk factors for atherosclerosis in children and adolescents may provide new insights into the mechanism of formation of atheromatous plaques, especially during the early stages when the process is entirely reversible (Libby 2000). In this regard, the reference values for several atherogenic indices has been provide.

The influence of age at the onset of menses on lipid and lipoprotein concentration is not clear. Associations between age at first menses and TG has been observed (Morrison et al. 1979), whereas either low correlations (Freedman et al. 1987) or no effect of age at first menses has been recently reported (Remsberg et al. 2005). Significant differences in lipid variables according to age of menarche were not observed in this study. This observation was consistent with the above mentioned studies.

The AVENA study included 2 859 adolescents, from which 581 had blood sample. The total number of adolescents to be included in the study was calculated taking into account the variance for BMI (Moreno et al. 1997), as mentioned above. Differences between BMI in the subgroup from which blood samples were obtained and the remaining subjects were not significant (Tab. 1). This suggests that the subgroup with blood data is representative of the whole population.

In conclusion, the serum lipid profile of Spanish adolescents suggests that special attention should be paid to lipid status in this crucial period of life. The present study provides reference data on the distribution of lipid and lipoprotein levels of Spanish adolescents, this information is crucial for planning interventions and education programs promoting the prevention of cardiovascular disease.

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Conflict of interest
No present or past conflict of interest exists for any of the authors or their institutions.

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Zusammenfassung

Referenzwerte für Serumlipide und Lipoprotein bei spanischen Jugendlichen: Die AVENA Studie

Ziel/Objekt: Bereitstellung aktueller Referenzwerte für Serumlipide und Lipoprotein spanischer Jugendlicher nach Alter und Geschlecht

Methode: Querschnittsanalyse durchgeführt in fünf repräsentativen spanischen Städten (Granada, Madrid, Murcia, Santander und Zaragoza); Studienpopulation von 581 Adoleszenten (299 Jungen und 282 Mädchen) im Alter von 13 bis 18,5 Jahren. Alters- und geschlechtsspezifische Mittelwerte, Standardabweichungen und Perzentile wurden bestimmt für: Gesamt (TC), Lipoprotein mit hoher Dichte (HDLc) und Lipoprotein mit niedriger Dichte (LDLc) Cholesterol, Triglyceride, Apolipoprotein A-1 und B-100 und Lipoprotein (a).

Ergebnisse: Die 90igste Perzentile für TC betrug 4,95 mmol/L in der Gruppe der Jungen und 5,19 mmol/L in der Gruppe der Mädchen. Die HDLc-Spiegel waren in allen Altersgruppen signifikant höher bei den Mädchen. Die LDLc-Werte bewegten sich zwischen 2,32 bis 2,54 mmol/L bei den Jungen und zwischen 2,38 bis 2,62 mmol/L bei den Mädchen und waren am höchsten bei den 13-Jährigen beider Geschlechter. Die Werte für Triglyceride wiesen eine steigende Tendenz auf und waren bei den 17-Jährigen beider Geschlechter am höchsten. Die Apolipoprotein A-1 und B-100- Spiegel entsprachen denen von HDLc und LDLc. Der geometrische Mittelwert für Lipoprotein(a) lag zwischen 0,44 und 0,57 μmol/L bei den Jungen und zwischen 0,50 und 0,67 μmol/L bei den Mädchen.

Fazit: Die AVENA Studie stellt Referenzmaterial von Lipiden und Lipoprotein-Spiegeln spanischer Adoleszenter zur Verfügung.

Resumé

Valeurs de référence pour les lipides et lipoprotéines sériques chez des adolescents espagnols. l'étude AVENA

Objectives: Apporter des valeurs de référence actualisées pour les taux sériques de lipides el lipoprotéines par rapport à l'age et au sex.

Méthodes: Une étude transversale fût réalise en 5 villes représentatives (Granada, Madrid, Murcie, Santander et Saragosse) incluant un échantillon représentatif de 581 adolescents (299 garçons et 282 filles), avec un age de 13 à 18.5 ans. Des moyennes spécifiques pour age et sexe, avec des écarts types et percentiles fûrent calculées pour: cholestérol total (TC), cholestérol des lipoprotéines de haute densité (HDLc), cholestérol des lipoprotéines de basse densité (LDLc), triglycérides, apolipoprotéines A-I et B, et lipoprotéine (a).

Résultats: Le percentile 90 pour TC était 4.95 mmol/L pour les garçons et 5.19 pour les filles. Les taux de HDLc étaient significativement plus élevés chez les filles des différentes groups d'age. Les niveaux de LDLc étaient compris entre 2.32 et 2.54 mmol/L chez les garçons, et entre 2.38 et 2.62 mmol/L chez les filles, avec des valeurs plus élevées à 13 ans dans les deux sexes. Les niveaux de triglycérides montraient une tendance à augmenter progressivement jusqu'à 17 ans dans les deux sexes. Les taux d'apolipoprotéines A-1 et B-100 étaient parallèles à ceux de HDLc et LDLc, respectivement. La moyenne géométrique pour les taux de lipoprotéine (a) était comprise entre 0.44 et 0.57 μmol/L chez les garçons et entre 0.50 et 0.67 μmol/L chez les filles.

Conclusions: Le présente étude apporte des valeurs de référence de la distribution des taux de lipides et lipoprotéines chez des adolescents espagnols.

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Serum Lipids, Body Mass Index and Waist Circumference during Pubertal Development in Spanish Adolescents: The AVENA Study

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Abstract

Aim: To describe the effects of chronological age and biological age (pubertal development) on serum lipid and lipoprotein levels, body mass index (BMI) and waist circumference in Spanish adolescents. **Methods:** A representative Spanish sample of 526 adolescents (254 males and 272 females), were studied. Total cholesterol (TC), high density lipoprotein cholesterol (HDLc), triglycerides, apolipoprotein A1 and B, and lipoprotein(a) were measured, and low density lipoprotein cholesterol (LDLc) was calculated. Additional measurements included BMI and waist circumference. Adolescents were classified according to chronological age, and pubertal development (also age of menarche in females). **Results:** In males, serum TC levels were lower at late puberty in comparison with early puberty, and serum LDLc levels were lower at late puberty in comparison with mid and early puberty. Serum HDLc levels were lower at mid puberty in comparison with early and late puberty. Serum TC and LDLc levels were not different when analyzed according to chronological age. In females, HDLc levels were lower at late puberty in comparison with early and mid puberty, but no differences were found when HDLc and the other studied lipid and lipoprotein variables were analyzed according to chronological age, or age of menarche. All the observed differences persisted after adjusting for BMI and waist circumference. In female adolescents, both BMI and waist circumference were higher at late puberty in comparison with early and mid puberty, while in males, BMI and waist circumference were different

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when analyzed according to chronological age. **Conclusion:** The results suggest that the assessment of pubertal development may provide additional valuable information when interpreting lipid profile and body fat in adolescents.

Key words
Adolescence · chronological age · biological age · cardiovascular risk factors

Introduction

The pattern of changes in lipids and lipoproteins during childhood and adolescence have encouraged the use of age- and gender-specific cut points for detecting children with increased, or decreased, blood lipid levels [1,2]. However, many factors make the screening difficult during this period. Some previous investigations have shown changes in serum lipids throughout adolescence [3–6]. Serum total cholesterol (TC), low density lipoprotein cholesterol (LDLc) and high density lipoprotein cholesterol (HDLc) levels seems to decrease throughout the adolescence period, which may be more readily explained by sexual maturation rather than by chronological age. Therefore, pediatricians should be aware of the influence of pubertal change on measurements of lipoproteins. In a randomized controlled trial, the observed lowering effect of a dietary intervention on LDLc in children with high cholesterol was confounded by the decrease associated with pubertal development [6]. This suggests that during puberty, chronological age may not be an adequate discriminating factor since pubertal development seems to vary between genders and individuals [7].

Other factors such as total body fat and abdominal adiposity have been shown to influence lipid and lipoprotein levels during the adolescence [8,9] and later in life [10]. We have previously shown that both body mass index (BMI) and abdominal adiposity (measured by waist circumference) are negatively associated with lipid and lipoprotein profile in Spanish adolescents [8,9]. The aim of this report was to describe the effects of chronological age and biological age (pubertal development) on serum lipid and lipoprotein levels, BMI and waist circumference in Spanish adolescents.

Material and Methods

Study population

The subjects were participants in the AVENA (Alimentación y Valoración del Estado Nutricional en Adolescentes, Food and Nutritional Status in Adolescents) study, a cross-sectional study designed to assess the nutritional status of a representative sample of Spanish adolescents. The complete methodology of the AVENA study has been described elsewhere [11–13]. The number of subjects included in the AVENA study was 2859 adolescents. Blood samples were randomly obtained from 581 of the subjects. From these, 526 adolescents (254 males and 272 females) had a complete set of Tanner stages and lipids measurements and were included in this study.

A verbal detailed description of the nature and purpose of the study was given to adolescents and school teachers. This information was also sent to parents or children supervisors by letter, and the written consents from parents and adolescents were requested. After receiving their written assent, the adolescents were considered for inclusion in the study. Exclusion criteria were: type 2 diabetes, pregnancy, alcohol or drug abuse, and non-directly related nutritional medical conditions. The study protocol was performed in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki (as revised in Hong-Kong in 1989 and in Edinburgh in 2000), and approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

Physical examination

Height and weight were measured by standardized procedures. BMI was calculated as weight/height squared (kg/m^2). Waist circumference was measured with an inelastic tape: the subject was in a standing position, and the tape was applied horizontally midway between the lowest rib margin and the iliac crest, at the end of gentle expiration [14]. Technical error of measurement was 0.95 cm, and reliability 98.0 %. The technical error of measurement was obtained by carrying out a number of repeated measurements on the same subject, by the same observer; the coefficient of reliability reveals what proportion of the between-subject variance in a measured population is free from measurement error [14].

Identification of pubertal stage (I–V) was assessed according to Tanner and Whitehouse [15]. The standard staging of pubertal maturity describes breast and pubic hair development in girls and genital and pubic hair development in boys. There were not any subject classified into Tanner stage I, and only 5.2 % (n = 13) of boys and 1.7 % (n = 4) of girls were classified into Tanner stage II. Therefore, the five established Tanner stages were re-grouped into Tanner stage II + III, IV, and V, here called early puberty, mid puberty and late puberty, respectively.

Age of menarche was determined from the self-reported age of first menses based on administered questionnaire to 208 female adolescents.

Blood sampling

Blood (20 ml) was collected from an antecubital vein between 8:00 and 9:00 AM, after an overnight fast. Serum concentrations of TC, HDLc, triglycerides (TG), apolipoprotein (apo) A1, apo B, and lipoprotein(a) [Lp(a)] were measured. LDLc was calculated with the Friedewald formula [16] adjusted for serum TG levels [17]. A detailed description of the blood analysis has been reported elsewhere [13].

Statistical analysis

Mean and standard deviation (SD) of all lipid and lipoprotein levels were calculated according to chronological age and biological age (pubertal development) for both male and female adolescents, and age of menarche only for female adolescents. Shapiro–Wilk test was used to check data distribution by gender

Table **1** Tanner stage distribution of study population by sex and age groups

Gender	*Tanner stage*		*Age group* 13	*14*	*15*	*16*	*17–18.5*
Males	III (early puberty)	N	22	14	7	2	5
		%	44.0	28.0	14.0	4.0	10.0
	IV (mid puberty)	N	25	21	12	23	23
		%	24.0	20.2	11.5	22.1	22.1
	V (late puberty)	N	7	17	38	20	18
		%	7.0	17.0	38.0	20.0	18.0
Females	III (early puberty)	N	6	14	0	2	2
		%	25.0	58.3	0.0	8.3	8.3
	IV (mid puberty)	N	32	25	28	38	25
		%	21.6	16.9	18.9	25.7	16.9
	V (late puberty)	N	8	12	23	41	16
		%	8.0	12.0	23.0	41.0	16.0

and age. The studied variables were quasi-normal distributed, and the asymmetry and kurtosis levels were adequate for all, except for Lp(a) that was achieved after logarithmic transformation.

Mean values were compared by one way analysis of covariance (ANCOVA), and pos hoc analysis were performed by Games–Howell test. Subsequent analyses were performed after adjusting for BMI (as an index of overall corpulence) and waist circumference (as an indicator of abdominal adiposity). BMI and waist circumference were entered as covariates, both separately and together. The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 14.0 for WINDOWS; SPSS Inc, Chicago), and the significance level was 5 %.

Results

Distributions of pubertal development of study population by age are shown in Table **1**. The chronological age range of adolescents within each stage of sexual maturation was large, for example males falling in the third stage (early puberty) of pubertal status could range from 13–18.5 years. The same is also valid in females.

In male adolescents, serum TC levels were significantly lower at late puberty in comparison with early puberty (Table **2**). Serum LDLc levels were significantly lower at late puberty in comparison with early and mid puberty. Serum TC and LDLc levels were not different when analysed according to chronological age (Table **3**). Serum HDLc levels were significantly lower at mid puberty in comparison with early and late puberty (Table **2**). Serum HDLc levels were significantly lower at 17–18.5 years of age in comparison with 15 years of age. Serum apo A1, apo B and Lp(a) levels were not different across puberty stages, and apo A1 levels were significantly higher in males aged 16 years compared with those aged 13, 14 and 15 years.

In female adolescents, serum HDLc levels were significantly lower at late puberty in comparison with early and mid puberty (Table **2**). No differences were found in lipid and lipoprotein levels according to chronological age, or age of menarche (data not shown). All previous observed differences did not change when the comparisons were controlled for BMI, or waist circumference separately, or when both variables were entered together as covariates.

In females, both BMI and waist circumference were significantly higher at late puberty in comparison with early and mid puberty, while no differences were observed when analyzed by chronological age or age of menarche. In males, BMI was significantly higher at 17–18.5 years of age in comparison with 13 an 16 years of age in male adolescents. Waist circumference was significantly higher at 17–18.5 years of age in comparison with 13 years of age in male adolescents.

The self-reported age of menarche in the present study ranged from 9 to 15 years of age, with the following distribution: 9 years (1.6 %), 10 years (2.9 %), 11 years (20.9 %), 12 years (34.7 %), 13 years (27.4 %), 14 years (11.3 %), or 15 years (1 %). Four (2 %) girls reported not to have menarche at the time of the study was performed.

Discussion

The present study describes the effects of chronological age and biological age (pubertal development) on serum lipid and lipoprotein levels, BMI and waist circumference in Spanish adolescents. The results suggest that the assessment of pubertal development may provide additional valuable information when interpreting lipid profile and body fat in adolescents.

Therefore, a measure of biological age should be included in epidemiologic studies dealing with serum lipid and lipoprotein and body fat measures among adolescents.

Our study supports previous results reporting significant effects of pubertal development on lipid and lipoprotein levels during adolescence [3–6,18–22]. Chronological age can be a simple discriminating factor because it is evidently associated with pubertal development; but, as the age of puberty onset and its velocity vary between genders and between individuals of the same gender [7], it represents an index not precise enough to establish normal ranges in adolescents. Results from the present study show that lipid distributions according to pubertal devel-

Table **2** Lipid and lipoprotein values, body mass index (BMI) and waist circumference (WC) in Spanish adolescents stratified by tanner stage

Outcome	***Males, Tanner stage*** III	***IV***	***V***	***p***
TC (mg/dl)	164.9 ± 23.5	158.0 ± 25.6	151.9 ± 28.1*	0.017
HDLc (mg/dl)	53.8 ± 9.5	48.3 ± 11.9**	52.6 ± 11.1	< 0.001
LDLc (mg/dl)	97.1 ± 21.3	94.5 ± 22.9	86.0 ± 23.9***	0.006
TG (mg/dl)	70.2 ± 27.6	76.0 ± 30.4	66.4 ± 39.8	0.168
Apo A1 (mg/dl)	118.1 ± 22.2	112.6 ± 23.3	113.8 ± 17.0	0.175
Apo B100 (mg/dl)	69.3 ± 13.0	68.4 ± 14.1	65.8 ± 14.6	0.289
Lp (a) (mg/dl)	13.0 ± 5.2	12.9 ± 4.4	15.0 ± 3.9	0.746
BMI (kg/m^2)	21.7 ± 4.5	22.7 ± 4.3	21.8 ± 3.3	0.180
WC (cm)	75.8 ± 10.9	78.1 ± 10.2	76.8 ± 8.4	0.334
Outcome	***Females, Tanner stage*** III	***IV***	***V***	***p***
TC (mg/dl)	176.8 ± 23.5	171.9 ± 25.6	168.0 ± 28.1	0.090
HDLc (mg/dl)	62.7 ± 9.5	60.8 ± 11.9	56.3 ± 11.1$^\sharp$	0.006
LDLc (mg/dl)	101.8 ± 21.3	97.7 ± 22.9	98.4 ± 23.9	0.687
TG (mg/dl)	61.3 ± 27.6	67.3 ± 30.4	67.0 ± 39.8	0.577
Apo A1 (mg/dl)	123.8 ± 22.2	125.6 ± 23.3	119.0 ± 17.0	0.262
Apo B100 (mg/dl)	71.6 ± 13.0	70.9 ± 14.1	71.7 ± 14.6	0.891
Lp (a) (mg/dl)	19.7 ± 2.9	14.9 ± 4.0	16.3 ± 4.4	0.650
BMI (kg/m^2)	19.9 ± 3.1	21.2 ± 3.0	22.6 ± 4.0^	0.001
WC (cm)	65.8 ± 7.2	69.7 ± 7.1	73.8 ± 8.3^^	< 0.001

Values are means ± SD. TC: total cholesterol; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; TG: triglycerides; Apo: apolipoprotein; Lp(a): lipoprotein a.Geometric mean ± SD. *p = 0.019 in comparison to Tanner stage III. **p = 0.019 and 0.018 in comparison to Tanner stage III and V, respectively. ***p = 0.013 and 0.015 in comparison to Tanner stage III and IV, respectively. $^\sharp$p = 0.013 and 0.019 in comparison to Tanner stage II and IV, respectively. ^p = 0.004 and 0.0035 in comparison to Tanner stage III and IV, respectively. ^^p < 0.001 and 0.002 in comparison to Tanner stage III and IV, respectively.

opment give valuable information especially in male adolescents. In female adolescents, only HDLc levels were different according to pubertal development. Subsequent analysis examining the potential effect of age at the onset of menses on lipid and lipoprotein levels did not reveal any further information. The influence of age at the onset of menses on lipid and lipoprotein concentration remains to be clarified. No significant effect of age of menarche on TC, TG, HDLc, or LDLc has been recently reported [23], whereas others found associations between age at first menses and TG levels [24].

Serum TC levels differed through pubertal stages in male adolescents, being higher in early puberty than in late puberty, similar to other reported studies [3–5, 18]. The reported TC decrease throughout the adolescence period is thought to be more related to pubertal development than to chronological age. In the present study, TC levels were not significantly different when analyzed according to chronological age. In females adolescents, the TC differences through pubertal stages seems to be absent [4, 20], or lower than in males [3, 18], which is in agreement with our results. In our study, TC levels were borderline significant, in female adolescents, however, no differences were found when analyzed according to chronological age.

The previously reported gender differences across pubertal stages may be due to changes in TC sub-fractions. The LDLc levels seem to decline with pubertal development in both genders [6]. Kwiterovich and co-workers [6] found lower LDLc levels associated with more advanced pubertal development in both boys and girls. In our study, LDLc levels were significantly different across pubertal development in male but not in female adolescents. The LDLc levels were not significantly different when analyzed according to chronological age in both male and female adolescents.

Serum HDLc levels seem to decrease with pubertal development [3, 4, 21, 22]. In male adolescents from the AVENA study, serum HDLc levels were lower at mid puberty in comparison with early and late puberty. The reported differences in HDLc through puberty seem attributable to an increase in testosterone levels [18, 19, 22]. Testosterone levels have been negatively associated with HDLc in adolescents [3, 4, 21, 22]. Results from the Bogalusa Heart Study [20] provided a negative relationship between testosterone and HDLc in young adolescents mainly distributed in Tanner stages I–II. However, a positive association between testosterone and HDLc levels, and between testosterone and apo A1 levels was found in older adolescents who were in advanced stages of pubertal development. These results suggest that after completion of pubertal development (Tanner stage V or late puberty) the impact of endogenous testosterone on lipoprotein levels may be minimal, perhaps because the levels may have exceeded a threshold. In female adolescents, the values of HDLc did not differ across puberty stages or age at menarche, which is in agreement with others [20, 21].

Previous studies have shown a relationship between pubertal development and TG levels [4, 5] while others did not [3]. In our

Table **3** Lipid and lipoprotein values, body mass index (BMI) and waist circumference (WC) in Spanish adolescents stratified by age group

Outcome	***Male, age group*** 13	14	15	16	17–18.5	***p***
TC (mg/dl)	164.5 ± 31.0	155.3 ± 22.9	151.0 ± 23.0	157.1 ± 24.8	155.0 ± 28.0	0.102
HDLc (mg/dl)	52.2 ± 11.0	51.0 ± 10.4	50.7 ± 8.9	54.3 ± 10.3	47.5 ± 6.9*	0.034
LDLc (mg/dl)	97.9 ± 25.3	89.5 ± 20.7	86.5 ± 21.0	88.8 ± 22.7	92.2 ± 27.5	0.133
TG (mg/dl)	72.2 ± 36.6	74.1 ± 36.0	68.9 ± 25.1	70.2 ± 29.2	76.5 ± 32.5	0.794
Apo A1 (mg/dl)	115.7 ± 17.0	110.3 ± 19.4	111.6 ± 19.5	126.1 ± 20.2**	119.6 ± 17.2	0.011
Apo B100 (mg/dl)	70.9 ± 18.4	66.8 ± 15.3	64.9 ± 14.1	67.5 ± 13.3	70.3 ± 15.8	0.335
Lp(a) (mg/dl)	12.3 ± 1.6	13.8 ± 1.4	13.7 ± 1.2	13.5 ± 1.6	15.9 ± 1.7	0.653
BMI (kg/m2)	20.6 ± 3.2	22.0 ± 3.9	22.2 ± 4.0	21.8 ± 3.5	24.2 ± 4.5***	< 0.001
WC (cm)	74.3 ± 9.4	77.4 ± 10.7	77.1 ± 9.2	76.2 ± 7.6	81.0 ± 10.3♯	0.001
Outcome	***Females, age group*** 13	14	15	16	17–18.5	***p***
TC (mg/dl)	174.3 ± 22.9	166.9 ± 27.2	169.2 ± 24.5	163.2 ± 26.8	170.0 ± 29.2	0.229
HDLc (mg/dl)	59.2 ± 10.5	59.2 ± 10.8	66.6 ± 12.7	59.0 ± 12.5	58.1 ± 10.8	0.258
LDLc (mg/dl)	101.3 ± 20.2	95.6 ± 25.9	95.8 ± 21.6	91.9 ± 22.5	97.1 ± 25.1	0.283
TG (mg/dl)	68.6 ± 21.1	60.6 ± 24.2	63.9 ± 20.2	61.4 ± 21.1	73.9 ± 56.4	0.108
Apo A1 (mg/dl)	124.4 ± 15.3	114.3 ± 25.8	127.6 ± 24.0	129.5 ± 22.8	133.8 ± ± 21.7	0.110
Apo B100 (mg/dl)	71.4 ± 11.2	71.6 ± 16.4	70.1 ± 13.4	67.6 ± 13.3	73.1 ± 15.1	0.316
Lp(a) (mg/dl)	16.6 ± 1.4	14.7 ± 1.5	15.4 ± 1.3	13.9 ± 1.5	18.77 ± 1.4	0.990
BMI (kg/m2)	21.0 ± 3.9	21.7 ± 4.2	21.3 ± 3.2	22.3 ± 3.0	21.7 ± 2.7	0.581
WC (cm)	70.3 ± 9.0	70.7 ± 10.7	70.8 ± 6.7	72.2 ± 7.7	70.1 ± 6.0	0.834

Values are means ± SD. TC: total cholesterol; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; TG: triglycerides; Apo: apolipoprotein; :p(a): lipoprotein a.Geometric mean ± SD. *p = 0.016 in comparison to 15 years of age. **p = 0.025, 0.013, 0.015 in comparison to 13, 14 and 15 years of age, respectively. ***p < 0.001 and 0.039 in comparison to 13 and 16 years of age, respectively. ♯p < 0.08 in comparison to 13 years of age.

study, TG levels did not differ across puberty stages neither in male nor in female adolescents (Table **2**). Although TG levels have been suggested to be better explained by chronological age than by pubertal development in males [3, 4], we did not find differences when analyzed according to chronological age (Table **3**).

Relatively little has been published about the apolipoprotein profiles in adolescents. Serum apolipoproteins and Lp(a) levels were not different when analyzed according to pubertal development, neither when analyzed according to chronological age, except for apo A1 levels, nor when analyzed according to chronological age in males (Table **3**). Serum levels of Lp(a) were not different either when analyzed according to chronological age or according to pubertal development, as it has been reported earlier [4]. This supports the contention that Lp(a) is predominantly genetically controlled.

One interesting finding is that apo B and A1 levels were not concordant with those for LDLc and HDLc, respectively. One possible explanation to the absence of concordance of LDLc and apo B levels may be because LDLc particles are losing cholesterol. When LDL particles lose cholesterol, the particle becomes smaller. Moreover, if the LDL particle loses cholesterol but not apo B which means that the LDL particle is becoming smaller and denser. The same apply to HDLc particle. According to several cross-sectional and prospective epidemiological studies, subjects with small, dense LDLc particles have a higher risk of coronary artery disease than subjects with large, buoyant LDLc particles [25]. Possible mechanisms mediating this increased atherogenicity of small LDLc particles include increased oxidation, diminished binding affinity to LDLc receptors [26], increased binding to arterial wall proteoglycans [27], and impaired *in vivo* endothelial function independent of HDLc, LDLc and TG concentrations. Reference values for the apo B and LDLc ratio in adolescents have recently been published [13].

All the above observed differences did not change when the comparisons were controlled for BMI and waist circumference, which may suggest that the associations between sexual maturation and lipid and lipoprotein profile are independent of body composition and body fat distribution at these ages.

The findings that both BMI and waist circumference were different across pubertal stages in females, but were not when analyzed according to chronological age or age of menarche are in concordance with others [28, 29]. Similarly, no association between BMI and age of menarche has been recently reported in a prospective study involving 124 healthy girls aged 8 to 18 years [28].

Taking together, these results suggest that pubertal development seems to have an influence on lipid and lipoprotein profile and body composition in adolescents. These findings may be related to disparate hormonal patterns that emerge during adolescence. Adolescence is highly sensitive to environmental factors which may influence the endogenous hormonal milieu. However, we did not measure sex hormones, which hamper a further study of hormone-lipoprotein relationships in the studied population.

The results should be interpreted with caution due to the limitations of the cross-sectional nature of the study. The absence of adolescents in Tanner stage I and II limits the possibility to make comparisons over the full spectrum of pubertal development. Longitudinal studies are needed in order to accurately examine the tracking of lipid and lipoprotein levels over adolescence, to accurately study the age- and pubertal development-related changes during this important period of life. The measurement of apolipoproteins and the fact that the present study sample is representative of the whole population [13] are strengths of the study.

In conclusion, results from this study suggest that the assessment of pubertal development may provide additional valuable information when interpreting lipid profile and body fat in adolescents.

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REVIEW ARTICLE

Health-related fitness assessment in childhood and adolescence: a European approach based on the AVENA, EYHS and HELENA studies

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Abstract Results from cross-sectional and longitudinal studies such as Alimentación y Valoración del Estado Nutricional en Adolescentes: Food and Assessment of the Nutritional Status of Spanish Adolescents (AVENA) and the European Youth Heart Study (EYHS) respectively, highlight physical fitness as a key health marker in childhood and adolescence. Moderate and vigourous levels of physical activity stimulate functional adaptation of all tissues and organs in the body (i.e. improve fitness), thereby also making them less vulnerable to lifestyle-related degenerative and chronic diseases. To identify children and adolescents at risk for these major public health diseases and to be able to evaluate the effects of alternative intervention strategies in European countries and internationally, comparable testing methodology across Europe has to be developed, tested, agreed upon and included in the health monitoring systems currently under development by the European Commission (EC): the Directorate General for Health and Consumer Affairs (DG SANCO); the Statistical Office of the European Communities (EUROSTAT), etc. The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study group plans, among other things, to describe the health-related fitness of adolescents in a number of European countries. Experiences from AVENA and EYHS will be taken advantage of. This review summarises results and experiences from the developmental work so far and suggests a set of health-related fitness tests for possible use in future health information systems.

Keywords Cardiorespiratory fitness · Muscular fitness · Physical activity · Non-communicable diseases · Young adults · Health-related fitness

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Introduction

The public health burden of lifestyle-related diseases in the European countries is high. The most common causes of morbidity and mortality are coronary heart disease, stroke, obesity, hypertension, type-2 diabetes, allergies and several cancers. A sedentary lifestyle is a major risk factor for these diseases and is close to overtaking tobacco as the leading cause of preventable death (Mokdad et al. 2004). The protective effect of intentional physical activity on the above mentioned non-communicable diseases has been widely reported in people of all ages (Strong et al. 2005; Jonker et al. 2006). Regular participation in moderate and vigorous levels of exercise increases physical fitness, which can lead to many health benefits (Ruiz et al. 2006a).

Physical fitness is also determined by constitutional factors, and it has been suggested that up to ~40% of variation in fitness may be attributable to genetic factors (Bouchard 1986). In adults, low physical fitness (mainly low cardiorespiratory fitness and low muscular strength) seems to be a stronger predictor of both cardiovascular and all-cause mortality than any other well established risk factors (Myers et al. 2002). In Spanish adolescents, results from the Alimentación y Valoración del Estado Nutritional en Adolescentes: Food and Assessment of the Nutritional Status of Spanish Adolescents (AVENA) study; (http://www.estudioavena.com), suggest significant associations between cardiorespiratory fitness and plasma lipid profile (Mesa et al. 2006a) inflammatory status (Wärnberg 2006) and abdominal adiposity (Ortega et al. in press). Similar results have been achieved in Swedish and Estonian children aged 9–10 years from the European Youth Hearth Study (EYHS), as well as in other cross-sectional and longitudinal studies across Europe (Ruiz et al. 2006a,b). Taken together, these results may have important implications for public-health-oriented lifestyle intervention programs.

Physical fitness refers to the full range of physical qualities, i.e. cardiorespiratory fitness, muscular strength, speed of movement, agility, coordination, and flexibility. It can be understood as an integrated measurement of all functions (skeletomuscular, cardiorespiratory, haematocirculatory, psychoneurological and endocrine–metabolic) and structures involved in the performance of physical activity and/or physical exercise (Castillo Garzon et al. 2005). There are several well-known, health-related fitness batteries to assess fitness in all its dimensions in young people. A good example in Europe is the EUROFIT battery (Committee of Experts on Sports Research EUROFIT, 1993) and in the USA is the FITNESSGRAM battery (Cooper Institute for Aerobics Research 1999). A number of studies have followed most of the indications given in these and other fitness batteries. Some of the suggested health-related fitness tests have been performed in American (Baquet et al. 2006), Finnish (Mikkelsson et al. 2006), Russian (Izaak and Panasiuk 2005), Greek (Koutedakis and Bouziotas 2003), Flemish (Deforche et al. 2003), African (Monyeki et al. 2005), Spanish (Ortega et al. 2005), Dutch (Kemper et al. 2000) and Swedish and Estonian (Ruiz et al. 2006a,b) adolescents. However, in most studies, an adaptation of the tests has been made according to local/national social, cultural or environmental considerations and instrument or budget issues at the time the study was done.

To identify children and adolescents at risk for the major public health diseases and to be able to evaluate effects of alternative intervention strategies in European countries and internationally, comparable testing methodology across Europe has to be developed, tested, agreed upon and included in the health monitoring systems currently under development by the European Commission (EC) (DG SANCO; EUROSTAT, etc.). In this work, experiences from previous projects across Europe (AVENA and EYHS) will be taken advantage of. The Healthy Lifestyle by Nutrition in Adolescence (HELENA) study; (http://www.helenastudy.com) is a European-Union (EU)-funded project on lifestyle and obesity among European adolescents. The HELENA study will provide, for the first time in Europe, harmonised and comparable data about health-related fitness and other health-related outcomes among male and female adolescents from ten European countries (Athens in Greece, Dortmund in Germany, Gent in Belgium, Heraklion in Crete, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria and Zaragoza in Spain). The health-related fitness test battery suggested for the HELENA study is summarised in Table 1. Methods for

Table 1 Summary of health-related fitness tests included in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study

Fitness dimensions	Fitness quality	Test	Included in the EUROFIT battery	Included in the FITNESSGRAM battery
Cardiorespiratory fitness	Aerobic capacity	20-m shuttle run	✓	✓
Flexibility	Flexibility	Back-saver sit and reach		✓
Muscular fitness	Maximal isometric muscle strength	Handgrip strength	✓	
	Muscular endurance	Curl up		✓
	Explosive strength	Standing broad jump	✓	✓
	Explosive strength, elastic energy, coordination	Squat jump, counter movement jump, Abalakov		
	Muscular endurance	Bent-arm hang	✓	✓
Speed of movement–agility	Speed, agility and coordination[a]	Shuttle run 4×10-m	✓	

[a] Modified from the EUROFIT battery

health-related fitness assessment have already been tested for feasibility and reliability.

This review summarises results and experiences from the developmental work so far in AVENA, EYHS and HELENA studies and suggests a set of health-related fitness tests for possible use in future health information systems.

Assessment of cardiorespiratory fitness

What is cardiorespiratory fitness?

Cardiorespiratory fitness is one of the most important components of health-related fitness. Cardiorespiratory fitness reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged strenuous exercise. Hence, cardiorespiratory fitness has been considered a direct measure of the physiological status of the person. Cardiorespiratory fitness, cardiovascular fitness, cardiorespiratory endurance, aerobic fitness, aerobic capacity, aerobic power, maximal aerobic power, aerobic work capacity, physical work capacity and maximal oxygen consumption (VO_{2max}) all refer to the same concept and are used interchangeably in the literature. In this manuscript, only the term cardiorespiratory fitness is used.

Why is cardiorespiratory fitness important in the young population?

High cardiorespiratory fitness during childhood and adolescence has been associated with a healthier cardiovascular profile during these years (Mesa et al. 2006a,b) and later in life (for review see Ruiz et al. 2006a,b). Results from the Swedish and Estonian part of the EYHS revealed negative associations between cardiorespiratory fitness and body fat (expressed as the sum of five skin folds) (Ruiz et al. 2006a). The same relationship was noted between cardiorespiratory fitness and other features of the metabolic syndrome [insulin resistance, raised triglycerides and total cholesterol to high-density lipoprotein (HDL) cholesterol ratio] in children (Ruiz et al. 2006b). Similar results have been found in Spanish counterparts from the AVENA study (Gonzalez-Gross et al. 2003; Mesa et al. 2006a) (Fig. 1). In the same study, we have shown associations between increased cardiorespiratory fitness and a favourable metabolic profile in both overweight and non-overweight adolescents [normal-weight category was categorised fol-

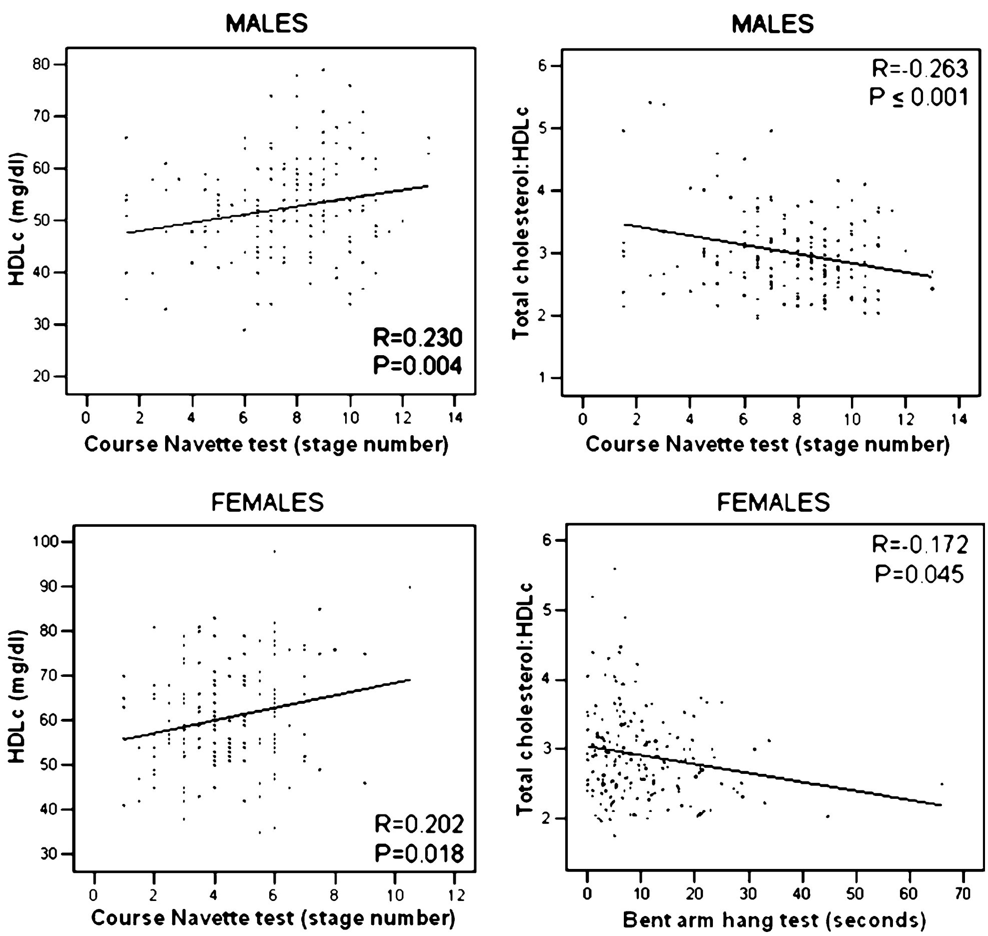

Fig. 1 Physical fitness variables associated with cardiovascular risk factors among normal-weight Spanish adolescents. Normal-weight category was categorised following the International Obesity Task Force (IOTF)-proposed gender- and age-adjusted body mass index (BMI) cutoff points

lowing the International Obesity Task Force (IOTF)-proposed gender- and age-adjusted body mass index (BMI) cutoff points (Cole et al. 2000)], and the main outcome was that cardiorespiratory fitness was an indicator of a favourable metabolic profile in male adolescents (Mesa et al. 2006a). Results are similar in other European children and adolescents (Klasson-Heggebo et al. 2006).

A number of longitudinal studies have suggested that low cardiorespiratory fitness during childhood and adolescence is associated with later cardiovascular risk factors, such as hyperlipidemia, hypertension and obesity (for review, see Ruiz et al. 2006b).

Cardiorespiratory fitness test methodology in young people

One of the most widely used tests to assess cardiorespiratory fitness among children and adolescents is the 20-m shuttle run test, also called “Course Navette” test (Léger et al. 1984). The initial speed is 8.5 km/h, which is increased by 0.5 km/h per min (1 min equal to one stage). Subjects are instructed to run in a straight line, to pivot upon completing a shuttle, and to pace themselves in accordance with audio signals given. The test is finished when the subject failed to reach the end lines concurrent with the audio signals on two consecutive occasions. A more detailed methodology and reference values of ~3,000 Spanish adolescents participating in the AVENA study can be found elsewhere (Ortega et al. 2005). The equations of Leger et al. (1984) are used to estimate the VO_{2max} from the result of the 20-m shuttle run test: VO_{2max}=31.025+ 3.238S−3.248A+0.1536SA, where A is the age and S the final speed (S=8+0.5 x last stage completed). Reliability and validity of this test for determining the VO_{2max} in children and adolescents has been widely documented. The test has many advantages as a fitness test because a large number of subjects can be tested at the same time, which enhances participant motivation and, because of its objectivity, standardisation, reliability, validity and availability of reference data. The 20-m shuttle run test has been included in several fitness batteries, such as the EUROFIT (Committee of Experts on Sports Research EUROFIT 1993), the Australian Coaching Council (Australian Sports Commission 1999), the British National Coaching Foundation (Brewer et al. 1988), the American Progressive Aerobic Cardiovascular Endurance Run (Cooper Institute for Aerobics Research 1999), and the Queen’s University (Riddoch 1990), among others.

Previous cross-sectional and longitudinal European studies (e.g. EYHS) have used a maximum cycle ergometer test (Hansen et al. 1989). This test is probably one of the most objective, reliable and valid indicator of cardiorespiratory fitness, but it is demanding on resources, especially when large groups of subjects are tested. Moreover, a major limitation to cycle ergometer testing is the discomfort and fatigue of the muscle quadriceps. In inexperienced subjects, leg fatigue may cause him/her to stop before reaching a true VO_{2max}. There are some studies showing that VO_{2max}, the ventilatory threshold, and minute ventilation are generally 10–20% higher with treadmill testing (Working Group on Cardiac Rehabilitation and Exercise Physiology 2001).

Assessment of flexibility

What is flexibility?

Flexibility is the ability of a specific muscle or muscle group to move freely through a full range of motion. It is of importance in a variety of athletic performances but also in the capacity to carry out the activities of daily living, which is very important from a public health perspective.

“Back-saver sit-and-reach”

What is “back-saver sit-and-reach?”

Back-saver sit and reach assesses flexibility by means of reaching forward as far as possible from a seated position with one leg bent at knee. The test requires a standardised box with a ruler, which has to be pushed by the subject.

Why is performing “back-saver sit-and-reach” important in the young population?

There is growing evidence about the associated benefits of flexibility, including range of motion and function, improved athletic performance, reduced injury risk, prevention or reduction of postexercise soreness and improved coordination (Pope et al. 2000). Some studies have shown that decreased hamstring flexibility is a risk factor for the development of patella tendinopathy and patellofemoral pain (Witvrouw et al. 2000, 2001), hamstring strain injury (Witvrouw et al. 2001) and symptoms of muscle damage following eccentric exercise (McHugh et al. 1999). Similarly, poor flexibility and subsequent injury has been established in several musculotendinous units, including the Achilles tendon (Leach et al. 1981) and plantar fascia (Kibler et al. 1991). Results from a recent longitudinal Finnish study suggest that hamstring flexibility (measured by the sit-and-reach test) was one of the best explanatory factors for adult health-related fitness for men (Mikkelsson et al. 2006).

Back-saver sit-and-reach test methodology in the young

One of the tests to assess lower body flexibility is the back-saver sit-and-reach test. The back-saver sit-and-reach test is part of the FITNESSGRAM battery (Cooper Institute for Aerobics Research 1999), and is a modification of the more traditional sit-and-reach test included in the EUROFIT battery (Committee of Experts on Sports Research EUROFIT 1993). The back-saver sit-and-reach test differs from the sit-and-reach test in that the subject performs the test with one leg bent at the knee; therefore, it may be safer on the back by restricting flexion. The traditional sit-and-reach test (both legs are stretched simultaneously) may result in overstretching of the lower back, especially in terms of excessive disc compression and posterior ligament and erector spinae muscle strain. It also involves a forward rotation of the pelvis and sacrum which elongates the hamstrings. The back-saver sit-and-reach allows the legs to be evaluated separately and therefore also the determination of symmetry (or asymmetry) in hamstring flexibility. In addition, testing one leg at a time eliminates the possibility of hyperextension of both knees. The reliability and validity of the back-saver sit-and-reach tests has been widely reported (Cooper Institute for Aerobics Research 1999). The sit-and-reach test has been usually performed in the background of school physical education classes, suggesting its feasibility and applicability in this context. Therefore, the possibility of preforming the back-saver sit-and-reach test instead of sit-and-reach test would not be a problem.

Assessment of muscular fitness

Balanced, healthy functioning of the musculoskeletal system requires that a specific muscle or muscle group be able to generate force or torque (measured as strength), resist repeated contractions over time or maintain a maximal voluntary contraction for a prolonged period of time (measured as muscular endurance) and to carry out a maximal, dynamic contraction of a muscle or muscle group (measured as explosive strength).

Handgrip strength

What is handgrip strength?

Handgrip strength refers to the maximal isometric force that can be mainly generated by the hand and forehand muscles involved in the handgrip performance.

Why is handgrip strength important in the young population?

The handgrip strength test is a simple and economical test that gives practical information on muscle, nerve, bone or joint disorders. In adults, handgrip strength has been proposed as a possible predictor of mortality and the expectancy of being able to live independently (Metter et al. 2002). Results from the AVENA study revealed a negative association between handgrip strength and total cholesterol/HDL cholesterol lipoprotein-related risk factors (Ortega et al. 2004).

Handgrip strength test methodology in young people

The handgrip strength test is a widely used test in experimental and epidemiological studies. The measure of handgrip strength is influenced by several factors, including age, gender, different angle of shoulder, elbow, forearm, and wrist (Richards et al. 1996), posture (Watanabe et al. 2005) and grip span (Ruiz-Ruiz et al. 2002). Another important factor affecting handgrip strength is hand size (Ruiz-Ruiz et al. 2002; Ruiz et al. in press). The handgrip test was measured in ~3,000 Spanish adolescents in the framework of the AVENA study. Detailed test methodology and reference values have been properly described elsewhere (Ortega et al. 2005; Ruiz et al. in press). Briefly, subjects performed the test in a standard bipedal position and with the arm in complete extension without touching any part of the body with the dynamometer except the hand being measured.

We made an attempt to find the optimal grip span that resulted in maximum handgrip strength and that increased reliable and reproducible handgrip strength in adult population (Ruiz-Ruiz et al. 2002). Recently, we have shown a standard procedure to evaluate the maximum handgrip strength in adolescents (Ruiz et al. in press). The results of our study suggest that there is an optimal grip span to which the dynamometer should be adjusted when measuring handgrip strength in young subjects. For males, the optimal grip span can be derived from the equation $y=x/7.2+3.1$ cm and for females $y=x/4+1.1$ cm, where y is optimal grip span and x is hand size measured from the tip of the thumb to the tip of the little finger with the hand open widely. These equations may improve the validity and accuracy of results and may guide clinicians and researchers in selecting the optimal grip span on the hand dynamometer when measuring handgrip strength in young, healthy subjects.

"Curl-up"

What is the "curl-up" test?

The curl-up test assesses trunk strength, i.e. abdominal muscular endurance. Muscular endurance is the ability of a muscle group to execute repeated contractions over time or to maintain a maximal voluntary contraction for a prolonged period of time.

Why is performing curl-up important in the young population?

The strength of abdominal muscles has been shown to have a significant association with lower back pain in adults

(Nourbakhsh and Arab 2002). Improvements in abdominal muscle strength have been shown to not only reduce low back pain but also to prevent injury recurrence in athletes (Trainor and Trainor 2004), and young adults (Arokoski et al. 2001). Low back pain is a common and costly complaint in society. Its multifactorial aetiology is not well understood, but it is assumed to involve biomechanical loading of the spine and psychosocial influences (Keyserling 2000). Also, overweight (Leboeuf-Yde 2000), smoking (Goldberg et al. 2000) and lack of physical exercise (Hildebrandt et al. 2000) may contribute to low back pain. To prospectively evaluate the influence of low abdominal strength in young people with the likelihood of developing low back pain later in life would be of special interest from a public health perspective.

"Curl-up" test methodology in young people

The cadence-based curl-up test is the recommended test for abdominal strength/endurance testing in the FITNESSGRAM battery (Cooper Institute for Aerobics Research 1999). The curl-up test is a modification of the traditional sit-up test included in the EUROFIT battery (Committee of Experts on Sports Research EUROFIT 1993). The differences between the former and the full sit up are arm placement, leg position and range of motion of movement. Moreover, the reduced action of the psoas iliac muscle in the curl-up test may prevent back pain when performing the test. The use of a cadence (25 reps per minute) with the curl up also seems to eliminate many concerns about the ballistic nature of 30-s (or 1-min) all-out speed tests. In addition, the use of a cadence allows students to focus on their own performance and avoid competitive speeding up.

Standing broad jump and Bosco jumps

What are standing broad jumps and Bosco jumps?

The standing broad jump assesses lower-limb explosive strength. Explosive strength is the ability to carry out a maximal, dynamic contraction of a muscle or muscle group. It is the maximum rate of working of a muscle or muscle group. In the HELENA study, a more detailed assessment of muscle performance of the lower limbs has been proposed. Different jump tests will be measured according to the Bosco protocol. The Bosco jump protocol includes, among other things, the following type of jumps: squat jump, countermovement jump and Abalakov jump. Performance in squat jump indicates explosive strength of the lower limbs; the countermovement jump assesses explosive strength plus the use of elastic energy; the Abalakov jump assesses explosive strength, plus the use of elastic energy, plus the coordinative capacity using trunk and upper limbs. These are usually performed by young subjects (Vicente-Rodriguez et al. 2003, 2004a).

Why is standing broad jump important in the young population?

Jump performance together with speed has been shown to be highly strongly correlated with mean hip and lumbar bone mass accretion (Vicente-Rodriguez et al. 2003, 2004a). Results from the AVENA study revealed a negative association between standing broad jump and total cholesterol in overweight/obese male adolescents (Fig. 2) (Ortega et al. 2004).

From a public health perspective, these observations are of greater interest mainly because the standing broad jump test is an easy and feasible test to be used in schools; in fact, it is preformed as a part of the curriculum in many European countries.

Standing broad jump test methodology in young people

The standing broad jump test is a simple and cost- and time-effective test and is part of the EUROFIT battery (Committee of Experts on Sports Research EUROFIT 1993). The subject is instructed to push off vigorously and jump as far as possible trying to land with both feet together. The score is the distance from the take-off line to the point where the back of the heel nearest to the take-off line lands on the mat. Reference values of a population

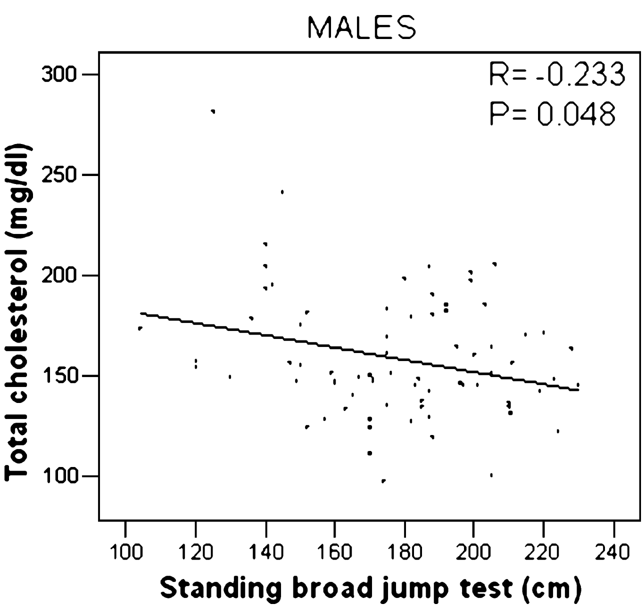

Fig. 2 Associations between standing broad jump and total cholesterol in overweight/obese Spanish adolescents. Overweight/obese category was categorised following the International Obesity Task Force (IOTF)-proposed gender- and age-adjusted body mass index (BMI) cutoff points

sample of Spanish adolescents participating in the AVENA study and a detailed description of the test can be found elsewhere (Ortega et al. 2005).

Bosco jump protocol

A more detailed and accurate information about muscle performance of the lower limbs can be obtained by use of the Bosco system (ERGOJUMP Plus, BOSCO SYSTEM, Byomedic, S.C.P., Barcelona, Spain). Briefly, the Ergojump Bosco system measures flight time during the vertical jump. This apparatus consists of a digital timer (±0.001 s) connected by a cable to two infrared bars. The timer is triggered by the feet of the subject at the moment of release from the platform and stops at the moment of contact coming down. As mentioned, the Bosco jump protocol includes three types of jumps (squat, countermovement and Abalakov) measuring different muscle characteristics. Briefly, the tests are performed as follows: in the squat jump, the subject performs a vertical jump starting from a half-squat position, with trunk straight and both hands on hips and without doing a previous countermovement; the countermovement jump is similar to the previous one, but the legs are extended in the start position, and a flexion–extension of the legs must be performed as fast as possible; finally, the Abalakov jump is a natural vertical jump. The results from these tests allow the calculation of relevant muscle-strength-related indexes, such as the elasticity index [measures elastic energy = ({counter movement jump − squat jump}/counter movement jump)x100] and the upper limbs coordination index [({Abalakov − countermovement jump})/Abalakov)×100]. Moreover, the software allows estimation of the percentage of fast-twitch fibres (Bosco et al. 1983).

"Bent-arm hang"

What is the "bent-arm hang" test?

The bent-arm hang assess upper-limb endurance strength. This test evaluates the ability to maintain a maximal voluntary contraction (hanging from a bar) for a prolonged period of time, i.e. assesses mainly the arm, shoulder and dorsal muscular endurance. It is proposed as a marker of functional strength.

Why is performing "bent-arm" hang important in the young population?

Results from the AVENA study suggest that the bent-arm hang test is positively associated with HDL cholesterol and with total cholesterol to HDL cholesterol ratio (Fig. 1), as well as with body fat, expressed as the sum of six skinfolds, and/or percentage of body fat estimated by the Slaughter equation (FB Ortega, JR Ruiz, MJ Castillo, A Gutierrez, unpublished data, 2006). Deforche et al. (2003) showed that obese subjects had significantly lower performances on bent-arm hang and other weight-bearing tasks compared with their non-obese counterparts; however, the obese had better results in handgrip strength test. These results support findings from the AVENA study. The bent-arm hang test has been shown to be a significant explanatory factor for adult health-related fitness in Finnish female pupils studied from 9 to 21 years of age (Mikkelsson et al. 2006).

"Bent-arm hang" test methodology in young people

The bent-arm hang test (also called flexed arm hang) is one of the recommended tests for upper-limb endurance strength in both the FITNESSGRAM battery (Cooper Institute for Aerobics Research 1999) and the EUROFIT battery (Committee of Experts on Sports Research EUROFIT 1993). Reference values of a population sample of Spanish adolescents participating in the AVENA study and detailed methodology of the test can be found elsewhere (Ortega et al. 2005).

Speed of movement/agility

This is the ability of a specific muscle or muscle group be able to move as quickly as possible over a distance.

Shuttle run (4×10-m)

What is the shuttle run (4×10-m)?

The shuttle run test (4×10-m) assesses the subjects' speed of movement, agility and coordination in an integrated fashion.

Why is performing shuttle run (4×10-m) important in the young population?

Preliminary results from the AVENA study have shown a strong independent relationship between speed (assessed by means of 4×10-m shuttle-run test) and bone mineral content in both male and female adolescents, regardless of the stage of maturation (G Vicente-Rodriguez, MI Mesana, LA Moreno, JR Ruiz, FB Ortega, M Bueno, unpublished data, 2006). Recently, it has been shown that some physical-fitness-related variables, specifically those related with speed and dynamic strength, had a high predictive value for both bone mineral content and density and also for the accumulation of bone mass during early puberty (Vicente-Rodriguez et al. 2003, 2004a,b).

Shuttle run test (4x10-m) methodology in young people

The shuttle run (4×10-m) test is a modification of the shuttle run (10×5-m) test included in the EUROFIT battery

(Committee of Experts on Sports Research EUROFIT 1993). The present test also includes four sponges that are carried one by one to the different lines. The subjects run back and forth four times along a 10-m track at the highest speed possible. At the end of each track section, the subjects deposit or pick up a sponge from a line on the floor. Therefore, it allows measurement not only speed of displacement but also agility and coordination. Validation studies have been done in our university, and results will soon be published. Detailed methodology and reference values from the AVENA study have been reported elsewhere (Ortega et al. 2005).

Concluding comment

Results and experiences obtained from pan-European research suggest that physical fitness is a key health marker in children and adolescents. The fitness tests to be included in the assessment of health-related fitness in the HELENA study seem to give relevant information regarding the health status of the young people and are feasible and objective. Validation studies of most tests are already done (Ruiz et al. in press) and others are under the validation process. Future health information systems should include monitoring of health-related fitness among adults as well as among young individuals, and results and experiences from recent and ongoing research projects on young people across Europe, such as AVENA, EYHS and HELENA studies, should be taken advantage of. Some of these experiences have been summarised in this review. Relevant methodology seems to be available. Development of efficient systems for large-scale collection of health-related fitness data and transfer of data to centrally located databases will be the next step. The working party “Lifestyle” within the Health Information Strand of the Public Health Programme 2003–2008 of the EC (DG SANCO) has developed an implementation and dissemination strategy to put into operation and ensure rapid transfer of data and experiences to the units within the commission, national health authorities and other stakeholders involved in the development and implementation of health information systems.

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ORIGINAL ARTICLE

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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

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Abstract The question as to whether fitness should be assessed in a European health monitoring system, perhaps from the early stages of life onwards, remains to be answered. We aimed to examine the associations between cardiorespiratory fitness and metabolic risk factors in children. A total of 873 healthy children from Sweden and Estonia aged 9–10 years (444 girls and 429 boys) were randomly selected. A maximal ergometer bike test was used to estimate cardiorespiratory fitness. Additional cardiovascular risk factors were assessed. Significant differences among cardiorespiratory fitness quartiles for the sum of five skinfolds, insulin resistance, triglycerides, and total cholesterol (TC) and high-density lipoprotein cholesterol (HDLc) ratio were shown in girls whereas in boys, the sum of five skinfolds and insulin resistance were significantly different. The lowest sum of five skinfolds and insulin resistance was shown in the highest cardiorespiratory fitness quartile in girls and boys, and the lowest values of triglyceride and TC/HDLc values in the highest cardiorespiratory fitness quartile was observed only in girls. Cardiorespiratory fitness was negatively associated with a clustering of metabolic risk factors in girls and boys. The results add supportive evidence to the body of knowledge suggesting that cardiorespiratory fitness in children is an important health marker and thus should be considered to be included in a pan-European health monitoring system.

Keywords Cardiorespiratory fitness · Children · Metabolic syndrome · Cardiovascular diseases

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Introduction

Low cardiorespiratory fitness seems to be an important health problem (Lee et al. 1999; Carnethon et al. 2003; Mora et al. 2003; Myers et al. 2002). It has been recently shown that low cardiorespiratory fitness is a strong and independent predictor of incident metabolic syndrome (i.e. hypertension, dyslipidemia, impaired glycemic control and obesity) in men and women (LaMonte et al. 2005), which could be one of the mechanism of overall cardiovascular disease. Moreover, cardiorespiratory fitness seems to prevent premature mortality regardless of body-weight status or the presence of metabolic syndrome in adult men (Katzmarzyk et al. 2004, 2005).

High cardiorespiratory fitness during childhood and adolescence has been associated not only with healthier cardiovascular profile during these years but also later in life (Twisk et al. 2002). However, the association between cardiorespiratory fitness and cardiovascular risk factors in children is uncertain, probably because of low research priority. Furthermore, most children are asymptomatic for cardiovascular disease. Cardiorespiratory fitness has been suggested to be included in the European Health Monitoring System for the adult population (Sjöström et al. 2005), but the question as to whether fitness should be assessed in European health monitoring systems from the early stages of life remains to be answered. Understanding the association between a low cardiorespiratory fitness and cardiovascular-disease-related outcomes in children would support the question as to whether cardiorespiratory fitness might or might not be proposed as a health marker at these ages. Therefore, the aim of the present report was to examine the associations of cardiorespiratory fitness to health-related variables in a wide cohort of children aged 9–10 years and to relate the findings with corresponding results from recent cross-sectional and prospective cohort studies.

Research design and methods

The present cross-sectional study involved 873 children aged 9–10 years (444 girls, 429 boys). The subjects comprised Estonian and Swedish children who were part of the European Youth Heart Study (EYHS) (Poortvliet et al. 2003). The pooling of data was assumed to be possible because of the use of common protocols in both countries (Poortvliet et al. 2003; Wennlof et al. 2003). Study design, selection criteria and sample calculations have been reported elsewhere (Riddoch et al. 2005).

In Estonia, the city of Tartu and its surrounding rural area was the geographical sampling area. In Sweden, seven municipalities in the Stockholm area and one in Örebro were chosen for data collection. The local ethical committees approved the study (University of Tartu no. 49/30-1997, University Hospital no. 474/98 Huddinge, and Örebro City Council no. 690/98). The study procedures were explained verbally and in written text to all parents and children. One parent or legal guardian provided written informed consent, and all children gave verbal consent.

Data collection

Physical examination

Height and weight were measured by standardized procedures. Body mass index was calculated as weight/height squared (kg/m^2). Skinfold thicknesses were measured with a Harpenden caliper at the biceps, triceps, subscapular, suprailiac and triceps surae areas on the left side of the body. These measures have been shown to highly correlate with dual-energy X-ray absorptiometry-measured body fat percentage in children of similar ages (Gutin et al. 1996). All measurements were taken twice and in rotation, and the mean was calculated. If the difference between the two measurements differed by >2 mm, a third measurement was taken, and the two closest measurements were averaged. The sum of five skinfold thicknesses was used as an indicator of body fat.

Blood pressure

The systolic and diastolic blood pressures were measured with an automatic oscillometric method (Dinamap model XL Critikron, Inc., Tampa, Florida.). The equipment has been validated in children (Park and Menard 1987). An appropriate cuff size was chosen according to the manufacturer's recommendation after checking the arm circumference. The subject was in a sitting, relaxed position, and recordings were made every second minute for 10 min with the aim of obtaining a set of systolic recordings not varying by more than 5 mmHg. The mean value of the last three recordings was used as the resting systolic and diastolic blood pressure in millimeters of mercury (mmHg).

Blood samples

With the subject in the supine position, blood samples were taken by venipuncture after an overnight fast, using vacuum tubes (Vacuette, Greiner Lab Technologies Inc). The fasting state was verbally confirmed by the subject before blood sampling. Blood was centrifuged for 10 min at 2,000 g, serum was separated within 30–60 min, and the samples were stored at −80°C. Serum concentrations of triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), and glucose were measured on an Olympus AU600 autoanalyser (Olympus Diagnostica GmbH, Hamburg, Germany). The insulin for the Estonian subjects was analyzed with an enzyme immunoassay (DAKO Diagnostics Ltd., Ely, England). All analyses were performed at Bristol Royal Infirmary, UK, with the exception of insulin for the Swedish subjects, which was performed at Huddinge University Hospital, Sweden (Elecsys, Roche Diagnostics GmbH, Mannheim, Germany). A more detailed description of the blood analysis has been reported elsewhere (Wennlof et al. 2005). Insulin resistance was estimated from fasting glucose and insulin according to the homeostasis model assessment (HOMA) (Matthews et al. 1985), and the ratio TC/HDLc was also calculated.

Cardiorespiratory fitness test

Cardiorespiratory fitness was determined by a maximum cycle-ergometer test, as described elsewhere (Hansen et al. 1989). Briefly, the workload was preprogrammed on a computerized cycle ergometer (Monark 829E Ergomedic, Vansbro, Sweden) to increase every third minute until exhaustion. Heart rate was registered continuously by telemetry (Polar Sport Tester, Kempele, Finland). Criteria for exhaustion were a heart rate ≥185 beats per minute, failure to maintain a pedaling frequency of at least 30 revolutions per minute, and a subjective judgment by the observer that the child could no longer keep up, even after vocal encouragement. The power output was calculated as $=W_1+(W_2 \cdot t/180)$, where W_1 is a work rate at fully

Table 1 Baseline characteristics of 873 children (444 girls, 429 boys)

	Girls		Boys	
	Mean	95% CI	Mean	95% CI
Age (years)	9.54	9.50–9.58	9.58	9.54–9.63
Height (m)	1.28	1.37–1.39	1.38	1.38–1.39
Weight (kg)	32.03	31.45–32.60	32.11	31.60–32–63
Body mass index (kg/m^2)	16.73	16.52–16.94	16.76	16.57–16.94
Sum of five skinfolds (mm)	44.65	42.96–46.35	37.67	34.32–37.01
Insulin (mU/L)	6.44	6.11–6.77	5.47	5.17–5.77
Glucose (mg/dl)	87.98	87.39–88.58	91.26	90.67–91.85
Insulin resistance	1.42	1.34–1.49	1.25	1.17–1.32
High density lipoprotein cholesterol (mg/dl)	55.22	54.16–56.27	57.61	56.54–58.69
Total cholesterol (mg/dl)	176.76	173.70–179.42	170.41	167.87–167.87
Triglycerides (mg/dl)	68.83	66.47–71.19	60.35	57.80–62.89
Systolic blood pressure (mmHg)	101.92	101.10–102.74	103.08	102.21–103.95
Diastolic blood pressure (mmHg)	60.65	60.00–61.29	60.10	59.41–60.79
Metabolic risk score	0.03	−0.01–0.08	−0.03	−0.08–0.01
Cardiorespiratory fitness (ml/kg/min)	37.16	36.69–37.63	43.06	42.48–43.63

completed stage, W_2 is the work rate increment at final incomplete stage, and t is time in second at final incomplete stage. The "Hansen formula" for calculated maximum oxygen consumption (VO_{2max}) in ml/min was = 12 x calculated power output + 5 x body weight in kg (Hansen et al. 1989). Cardiorespiratory fitness was expressed as VO_{2max} per kilogram of body mass.

Metabolic risk score

The metabolic risk score was computed from the following six variables: insulin, glucose, HDLc, triglycerides, the sum of five skindfolds, and blood pressure (systolic and diastolic blood pressure). Each of these variables was standardized as follow: standardized value = (value − mean)/SD. The HDLc standardized value was multiplied by −1 to indicate higher cardiovascular risk with increasing value. The standardized values of systolic and diastolic blood pressure were averaged. The metabolic risk score was compiled by the sum of the six standardized scores divided by six. The resulting risk score is a continuous variable with a mean of zero by definition, with lower scores denominating a more favorable profile.

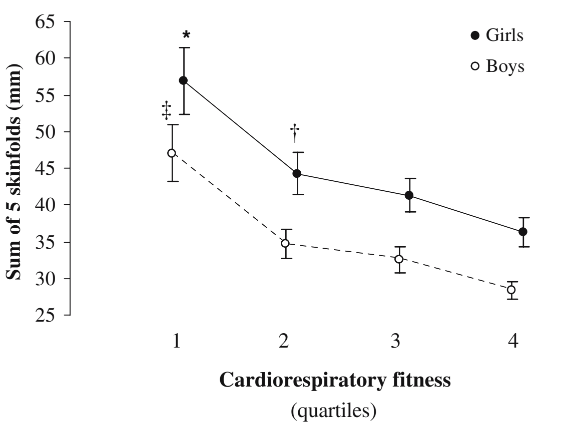

Fig. 1 Associations between sum of five skinfolds and cardiorespiratory fitness quartiles in girls and boys. Data shown as mean and 95% confidence interval (CI). Girls in the first quartile (*) had a higher sum of five skinfolds than in superior quartiles (P<0.001), and girls in the second quartile (†) had a higher sum of five skinfolds than in the fourth quartile (P=0.004). Boys in the first quartile (‡) had a higher sum of five skinfolds than in superior quartiles. (P=0.007)

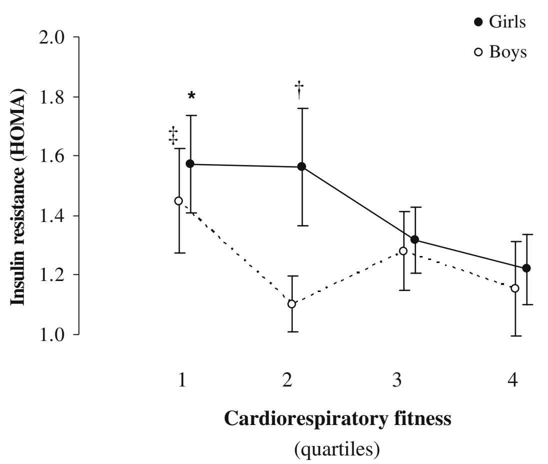

Fig. 2 Associations between insulin resistance estimated from the homeostasis model assessment (HOMA) equation and cardiorespiratory fitness quartiles in girls and boys. Data shown as mean and 95% confidence interval (CI). Girls in the first quartile (*) had a higher HOMA than in the fourth quartile (P<0.001), and girls in the second quartile (†) had a higher sum of five skinfolds than in the fourth quartile (P<0.001). Boys in the first quartile (‡) had a higher HOMA than in the fourth quartile (P=0.007)

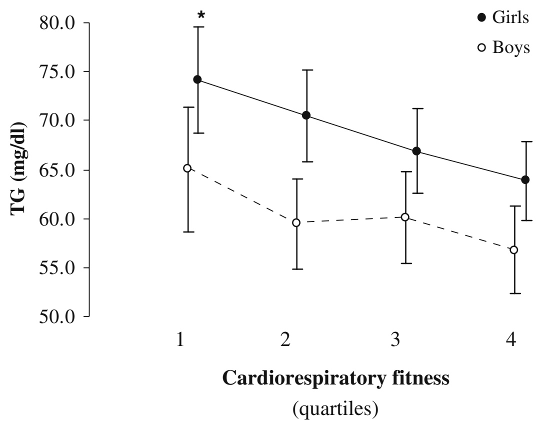

Fig. 3 Associations between triglycerides (TG) and cardiorespiratory fitness quartiles in girls and boys. Data shown as mean and 95% confidence interval (CI). Girls in the first quartile (*) had a higher TG values than in the fourth quartile ($P<0.001$)

Statistical analysis

All variables were checked for normality of distribution before the analysis, and appropriate transformations were applied when necessary. Sum of five skinfolds, triglycerides, low-density lipoprotein cholesterol (LDLc), TC, and TC/HDLc were logarithmically transformed, and HOMA was transformed by taking it by the power of (1/3). Differences between metabolic syndrome individual variables and cardiorespiratory fitness quartiles, and metabolic syndrome risk score and cardiorespiratory fitness quartiles were assessed by analysis of variance (ANOVA). Differences of metabolic syndrome individual variables among cardiorespiratory fitness quartiles were assessed by Tukey's test. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 13.0 for WINDOWS; SPSS Inc, Chicago, IL,USA), and the level of significance was set at $P<0.05$.

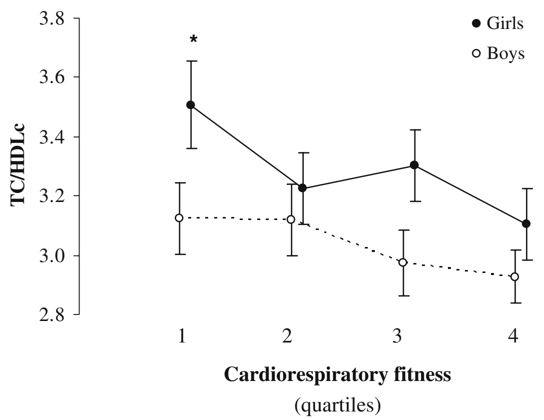

Fig. 4 Associations between total cholesterol (TC) and high-density lipoprotein cholesterol (HDLc) ratio and cardiorespiratory fitness quartiles in girls and boys. Data shown as mean and 95% confidence interval (CI). Girls in the first quartile (*) had a higher TC/HDLc ratio than in the second and fourth quartiles ($P<0.001$)

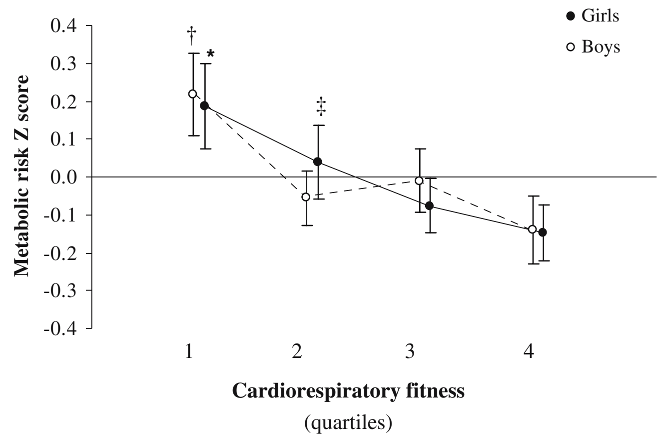

Fig. 5 Associations between metabolic risk score and cardiorespiratory fitness quartiles in girls and boys. Data shown as mean and 95% confidence interval (CI). Girls in the first quartile (*) had a higher risk score than in the second, third and fourth quartiles ($P<0.007$), and girls in the second quartile (‡) had a higher risk score than in the fourth quartile ($P<0.02$). Boys in the first quartile (†) had a higher risk score than in the second, third and fourth quartiles ($P=0.007$)

Results

The descriptive characteristics of the study sample are shown in Table 1. All subjects in this study were within the normal healthy ranges for all studied variables. The ANOVA showed significant differences among cardiorespiratory fitness quartiles for sum of five skinfolds, insulin resistance, triglycerides and TC/HDLc in girls whereas in boys, only sum of five skinfolds and insulin resistance were significantly different. Significant differences among cardiorespiratory fitness quartiles were also observed for metabolic risk score in girls and boys.

The Tukey's test showed that the sum of five skinfolds was significantly higher in the first cardiorespiratory fitness quartile compared with the second, third and fourth cardiorespiratory fitness quartiles in girls and boys (Fig. 1). Moreover, sum of five skinfolds was significantly higher in the second cardiorespiratory fitness quartile compared with the fourth cardiorespiratory fitness quartile in girls. In boys, the sum of five skinfolds was significantly lower in the fourth cardiorespiratory fitness quartile compared with the first, second and third cardiorespiratory fitness quartiles (Fig. 1).

Insulin resistance was significantly higher in the first cardiorespiratory fitness quartile compared with the fourth cardiorespiratory fitness quartile in both girls and boys. Moreover, insulin resistance was significantly higher in the second cardiorespiratory fitness quartile compared with the fourth cardiorespiratory fitness quartile in girls (Fig. 2).

Triglyceride values were significantly higher in the first cardiorespiratory fitness quartile compared with the fourth cardiorespiratory fitness quartile in girls (Fig. 3). The ratio of TC/HDLc was significantly higher in the first cardiorespiratory fitness quartile compared with the second and fourth cardiorespiratory fitness quartiles in girls (Fig. 4).

Table 2 Summary of recent cross-sectional studies examining the associations between cardiorespiratory fitness and health-related variables in children and adolescents

Author/study	Subjects	Age (years)	Outcome variables	Results
Boreham et al. 2001 The Northern Ireland Young Hearts Project	Boys = 251 Girls = 258	12	TC, HDLc, systolic BP, diastolic BP, sum of four skinfolds	Boys and girls CRF was inversely associated with TC, TC/HDLc, and systolic BP, but was not independent of fatness
	Boys = 252 Girls = 254	15		
Nielsen and Andersen 2003	Boys = 5,464 Girls = 8,093	15–20	Blood pressure	Boys The OR of hypertension in the lowest CRF quintile compared to the highest CRF quintile was 1.3 ($P<0.04$), after adjust for age and BMI Girls The OR of hypertension in the lowest CRF quintile compared with the highest CRF quintile was 1.5 ($P<0.001$), after adjust for age and BMI
Brage et al. 2004 European Youth Heart Study (Denmark)	Boys = 279 Girls = 380	8–10	TG, HDLc, sum of four skinfolds, insulin, glucose, systolic BP, and diastolic BP Metabolic syndrome Z score	Boys and girls CRF was inversely associated with insulin, TG, systolic BP, and skinfold thicknesses ($P \leq 0.033$) CRF was inversely associated with metabolic syndrome Z score ($P \leq 0.031$) CRF was positively associated with HDLc ($P=0.002$)
Gutin et al. 2004	Boys = 116 Girls = 166	14–18	Insulin, glucose	Boys and girls CRF was inversely associated with insulin concentrations, and the adverse impact of low CRF was greater in boys than in girls
Reed et al. 2005	Boys = 55 Girls = 44	9–11	BP, %BF, arterial compliance (large and small)	Boys and girls CRF accounted for 37% of the variance in large artery compliance. Highest CRF quartile had greater compliance than children in the two lowest CRF quartiles by as much as 34%
Eisenmann et al. 2005a Quebec family study	Boys = 416 Girls = 345	9–18	TG, TC, HDLc, LDLc, glucose, BP, BMI	Boys and girls CRF and BMI showed an independent association with cardiovascular risk factors
Gutin et al. 2005	Boys = 187 Girls = 211	14–18	TG, TC, HDLc, LDLc, LDLsz, Lp(a), BMI, WC, %BF	Boys and girls Higher CRF and lower fatness were associated with favorable lipid profile. For most variables, fatness was slightly greater than the influence of CRF

TC total cholesterol, *HDLc* high-density lipoprotein cholesterol, *LDLc* low-density lipoprotein cholesterol, *LDLsz* LDL particle size, *TG* triglycerides, *BP* blood pressure *Lp(a)* lipoprotein (a), *apo* apolipoprotein, *CRF* cardiorespiratory fitness, *BMI* body mass index, *OR* odds ratio, *%BF* percentage of body fat

Table 3 Summary of recent prospective cohort studies examining the associations between cardiorespiratory fitness and health-related variables in children and adolescents

Author/study	Years of follow-up	Subjects	Age (years)	Outcome variables	Results
Boreham et al. 2002 The Northern Ireland Young Hearts Project	10 1989/90 – 1992/93 – 1997/99	Boys = 229 Girls = 230	12 and 15 to 22.5	TC, HCLc, systolic BP, diastolic BP, sum of four skinfolds	Boys CRF changes were modestly associated with TC, HDLc, and systolic BP (P>0.5) Girls CRF changes were modestly associated with TC, HDLc, and skinfold thicknesses (P>0.17), and significantly associated with diastolic BP (P=0.03)
Hasselstrøm et al. 2002 Danish youth and sports study	8 1983–1991	Boys = 133 Girls = 132 Boys = 45 Girls = 57	15–19 to 23–27	TG, HDLc, systolic BP, diastolic BP, %BF Risk score	Boys CRF changes between 1983–1991 were inversely correlated with the changes in TC, TG, HDLc/TC (P<0.01) Girls CRF changes between 1983–1991 were inversely correlated with the changes in TG, systolic BP, %BF, and risk score (P<0.05)
Janz et al. 2002 The Muscatine Study	5	Boys = 63 Girls = 62	10.5 to 15	TC, HCLc, LDLc, sum of 6 skinfolds, WC	Boys and Girls CRF changes between year 1 to 5 were inversely correlated with the changes in sum of six skinfolds and WC (P<0.05)
Twisk et al. 2002 The Amsterdam Growth and Health Longitudinal Study	20	Boys = 132 Girls = 145	13 to 32	TC, HDLc, systolic BP, diastolic BP, sum of four skinfolds, W/H	Boys and girls The relationship between CRF during the adolescence was inversely associated with TC, sum of four skinfolds, and W/H (P<0.05)
Ferreira et al. 2003	24 with 9 repeated measurements	Boys = 75 Girls = 79	13.1 to 36	Carotid intima–media thickness and stiffness of the carotid, femoral, and brachial arteries	Boys and girls CRF changes were not associated with carotid intima–media thickness CRF changes were associated with large artery stiffness (P<0.05)
Andersen et al. 2004 Eight years follow–up in the Danish Youth and Sport Study	8	Boys = 133 Girls = 172	16–19 to 24–27	TG, TC/HDLc, systolic BP, %BF	Boys and girls CRF was associated with cardiovascular disease risk factors. The probability for “a case” at the first examination to be “a case” at the second was 6.0
Boreham et al. 2004 The Northern Ireland Young Hearts Project	8	Boys = 251 Girls = 203	12–15 to 20–25	Arterial stiffness	Boys and girls CRF was inversely associated with arterial stiffness
Eisenmann et al. 2005b The Aerobics Center Longitudinal Study	~ 11	Boys = 36 Girls = 12	15.9 to 27.2	TG, TC, HDLc, glucose, systolic BP, diastolic BP, BMI, WC, %BF	Boys and girls Adolescents’ CRF is related only to adult BMI, WC and %BF (P<0.05)
Ferreira et al. 2005 The Amsterdam Growth and Health Longitudinal Study	23	Boys = 175 Girls = 189	13 to 36	Prevalence of the metabolic syndrome	Boys and girls CRF changes were inversely associated with prevalence of metabolic syndrome

TC total cholesterol, *HDLc* high density lipoprotein cholesterol, *LDLc* low density lipoprotein cholesterol, *TG* triglycerides, *BP* blood pressure, *Lp(a)* lipoprotein (a), *apo* apolipoprotein, *CRF* cardiorespiratory fitness, *BMI* body mass index, *WC* waist circumference, *W/H* waist to hip ratio, *%BF* percentage of body fat

Metabolic risk score was significantly higher in the first cardiorespiratory fitness quartile than in the second, third and fourth cardiorespiratory fitness quartiles in girls and boys (Fig. 5). Significant differences were also found between metabolic risk score in the second and fourth cardiorespiratory fitness quartiles in girls (Fig. 5).

Discussion

The association between cardiorespiratory fitness and features of metabolic syndrome was investigated in a population sample of Swedish and Estonian children aged 9–10 years. Cardiorespiratory fitness was negatively associated with a clustering of metabolic risk factors in girls and boys, and the lowest values of sum of five skinfolds, insulin resistance, triglyceride and TC/HDLc were in the highest cardiorespiratory fitness quartile.

Theses results may suggests that cardiorespiratory fitness should be proposed as a health marker in children. In fact, it is biologically plausible that a high cardiorespiratory fitness provides more health protection than low cardiorespiratory fitness, even in healthy children as well as it has been found in adults (Balady 2002; Myers et al. 2002; Carnethon et al. 2003; Gulati et al. 2003; Kurl et al. 2003; Mora et al. 2003; Church et al. 2005; Katzmarzyk et al. 2004, 2005; LaMonte el al. 2005). Risk-factor levels are lower in children than in adults, but similar patterns have been seen in children. Previous cross-sectional studies in children have shown significant associations between cardiorespiratory fitness and plasma lipids and between cardiorespiratory fitness and clustering of metabolic syndrome risk factors (Table 2). In our study, triglyceride and TC/HDLc values differed among cardiorespiratory fitness quartiles (Fig. 3). Moreover, negative associations between increased cardiorespiratory fitness and clustering of metabolic syndrome risk factors in both girls and boys have been shown here (Fig. 5). Cardiorespiratory fitness has recently been associated with arterial compliance in children aged 9–11 years, which may support the concept that fitness may exert a protective effect on the cardiovascular system (Reed et al. 2005).

Associations between cardiorespiratory fitness and cardiovascular risk factors have also been found in adolescents (Table 2). Gutin et al. (2004) found inverse associations between cardiorespiratory fitness and insulin concentrations. Furthermore, inverse associations between cardiorespiratory fitness and the likelihood of having hypertension were shown in 15- to 20-year-old subjects (Nielsen and Andersen 2003). In the present study, insulin resistance was significantly lower in the fourth cardiorespiratory fitness quartile compared with the first cardiorespiratory fitness quartile in both girls and boys (Fig. 2). However, no differences were found in systolic or diastolic blood pressure among cardiorespiratory fitness quartiles (data not shown).

A summary of recent prospective cohort studies examining the associations between cardiorespiratory fitness and health-related variables in children and adolescents is shown in Table 3. A number of longitudinal studies have suggested that a low cardiorespiratory fitness during childhood and adolescence is associated with later cardiovascular risk factors, such as hyperlipidemia, hypertension and obesity (Boreham et al. 2001, 2002; Hasselstrøm et al. 2002; Janz et al. 2002; Twisk et al. 2002; Ferreira et al. 2005). In an 8-year follow-up study, fitness during adolescence was not associated to risk factors of cardiovascular disease in adulthood, but changes in fitness from adolescence to adulthood were related to risk in adulthood. Moreover, subjects who decreased their fitness levels also changed to a worse risk factor profile (Hasselstrøm et al. 2002). Changes in cardiorespiratory fitness from adolescence to adulthood were also inversely and significantly associated with large arterial stiffness (a major risk factor for cardiovascular disease) (Ferreira et al. 2003; Boreham et al. 2004). Taken together, these results seem to support the existence of a strong association between cardiorespiratory fitness and health-related outcomes in the young population, which may suggest the importance of including cardiorespiratory fitness tests in the monitoring system.

The test used to calculate cardiorespiratory fitness in this study was objectively and accurately measured, and it has been previously validated in children of the same age (Riddoch et al. 2005). However, laboratory tests present some disadvantages, as necessity of sophisticated instruments, qualified technicians and cost and time constraints, and it may cause problems for the subjects to go to the laboratory, etc. Therefore, in some circumstances, field tests may be a better option because a large number of subjects can be tested at the same time, as the tests are simple, safe and often the only feasible methods.

The cross-sectional nature of this study limits the ability to determine any causality in the results. We also do not know if an extrapolation of the association may be made for overweight and obese children or those with subclinical manifestations of cardiovascular pathologies. Nevertheless, with regular reports of increasing childhood obesity and related disease prevalence world wide, the results of this study are noteworthy. The ideal study to answer the question as to whether high levels of cardiorespiratory fitness during childhood lower the risk of developing cardiovascular diseases later in life is a randomized controlled trial with a lifetime follow-up, in which a large number of children is assigned to either an active or a sedentary life style.

In conclusion, the present study shows negative associations between cardiorespiratory fitness and features of metabolic syndrome in children aged 9–10 years. The results suggest that cardiorespiratory fitness in children, as has been shown in adults, is potentially an important health marker and should be considered to be included in a pan-European health monitoring system.

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Cardiovascular fitness is negatively associated with homocysteine levels in female adolescents

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ABSTRACT

Objective: To examine the association between CVF and homocysteine levels in adolescents.

Design: Cross-sectional study.

Setting: Spanish adolescents.

Participants: A total of 156 Spanish adolescents (76 males, and 80 females), aged 14.8 ± 1.4 years old were studied.

Main exposures: Cardiovascular fitness was measured by the 20 m shuttle run test. Pubertal status, birth weight, smoking, socioeconomic status, sum of six skinfolds, serum folate and vitamin B_{12} were also measured. Methylenetetrahydrofolate reductase (MTHFR 677C>T) polymorphism was done by DNA sequencing.

Main outcome measures: Fasting homocysteine levels.

Results: Mean values of homocysteine were significantly higher in the MTHFR 677CT and TT subgroups compared to the CC subgroup in males, while in females, mean values of homocysteine were significantly higher in the TT subgroup compared to CC and CT subgroup. Multiple regressions analyses showed that CVF was significantly associated with homocysteine levels in female adolescents after controlling for potential confounders including MTHFR 677C>T genotype (β = -0.395, semipartial correlation = -0.351, P = .007). No associations were found between CVF and homocysteine levels in male adolescents (β = 0.119, semipartial correlation = 0.080, P = .511).

Conclusions: The results suggest that CVF is negatively associated with homocysteine levels in female adolescents after controlling for potential cofounders including MTHFR 677C>T genotype.

INTRODUCTION

Homocysteine has been suggested to be an independent risk factor for several multi-system diseases,[1] including coronary heart disease,[2,3] stroke,[4] dementia and Alzheimer's disease,[5] risk of hip fracture,[6] and pregnancy complications.[7] Moreover, elevated homocysteine levels have been associated with increased oxidative stress and endothelial damage,[8,9] although the mechanisms are yet clarified. In children, it has been shown that elevated homocysteine levels are positively associated with cardiovascular disease in their parents,[10,11] grandparents,[2,13] and relatives.[14]

Homocysteine levels are influenced by modifiable and non-modifiable factors. Among the latter, age and gender seem to play a specific role. Levels of homocysteine are higher in boys than in girls, and this gender effect seems to be enhanced during and after puberty.[15] Genetic factors also seem to affect homocysteine levels.[16-19] Elevated levels of homocysteine can be due to mutations in enzymes involved in homocysteine metabolism, which give dysfunctional enzymes, for instance the single-nucleotide polymorphism at position 677 in the methylenetetrahydrofolate reductase (MTHFR) gene for MTHFR.[17] MTHFR is a key enzyme in homocysteine metabolism. The common polymorphism 677C>T gives a thermolabile form of the enzyme. Subjects homozygous for this mutation (or TT genotype) have elevated levels of homocysteine than individuals with CC or CT genotypes.[18,19]

Deficient serum levels of both folate and vitamin B_{12} have been associated with elevated homocysteine levels in children,[18] adults,[20] and in the elderly.[21] Lifestyles factors such as smoking, lack of physical activity, excessive alcohol intake and obesity have been associated with elevated levels of homocysteine in adults.[20,22-24]

Poor cardiovascular fitness (CVF) is another important risk factor for cardiovascular disease and it has also been shown to be a predictor of morbidity and all cause mortality.[25,26] Kuo and colleagues[27] have recently described a significant negative association between CVF and homocysteine levels in adult women. Cardiovascular fitness has been negatively associated with features of the metabolic syndrome in children and adolescents,[28,29] and with plasma lipid profile in both overweight and non-overweight adolescents.[30] However, studies examining the association between CVF and homocysteine levels in adolescents are lacking. Accordingly, we hypothesized that there would be a negative correlation between CVF and homocysteine levels in adolescents. For public health strategies and preventive purposes it is of interest to understand the relative influence of modifiable factors on homocysteine levels from an early age.

METHODS

Participants

The studied adolescents were a subsample of the AVENA study (Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles: Food and Assessment of the Nutritional Status of Spanish Adolescents). The AVENA Study was designed to assess the health and nutritional status of adolescents. Data collection took place from 2000 to 2002 in five Spanish cities (Madrid, Murcia, Granada, Santander and Zaragoza). The present report is based on the adolescents in which both homocysteine and MTHFR genotypes were measured (n = 156, 76 males, and 80 females). The AVENA study design has been reported in detail elsewhere.[31-33]

A comprehensive verbal description of the nature and purpose of the study was given to both the adolescents and their teachers. Written consent to participate was requested from both parents and adolescents. Adolescents with personal history of cardiovascular disease, under medication at the time of the study, or those who were pregnant, were excluded. The study protocol was performed in accordance with the ethical standards laid down in the 1961 Declaration of Helsinki (as revised in Hong-Kong in 1989, and in Edinburgh in 2000), and approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

Before any testing was performed, the parents completed a questionnaire part of which addressed the child's previous and current health status. Socioeconomic status was also assessed via the questionnaire, and was defined by the educational level and occupation of the father. According to this information, and following the recommendation of the Spanish Society for Epidemiology,[34] the adolescents were classified into five categories: low, medium-low, medium, medium-high and high socioeconomic status.

Smoking habits at the time of the study were reported via questionnaire by the adolescents, and categorized as smoking, non-smoking, and partial smoker (smoke once a week).

Physical examination

Anthropometric measurements were obtained as described elsewhere[35-37]. Briefly, skinfold thicknesses were measured at the biceps, triceps, subscapular, suprailiac, thigh and calf on the left side of the body to the nearest 0.2 mm. The sum of the six skinfold thicknesses was used as an indicator of body fat. These measures have been shown to correlate highly with dual-energy X-ray absorptiometry-measured body fat percentage in adolescents of similar ages.[38] Identification of pubertal development was assessed according to Tanner & Whitehouse.[39] Self-reported breast development in girls, and genital development in boys, was used for pubertal stage classification.

Measurement of cardiovascular fitness

Cardiovascular fitness was assessed by the 20 m shuttle run test as previously described.[40] Briefly, participants were required to run between two lines 20 m apart, while keeping pace. Running pace was determined by audio signals emitted from a pre-recorded cassette tape. The initial speed was 8.5 km·h^{-1}, which was increased by 0.5 km·h^{-1} per minute (one minute equal to one stage). The tape used was calibrated over one minute. Subjects were instructed to run in a straight line, to pivot upon completing a shuttle, and to pace themselves in accordance with the audio signals. The test was finished when the subject failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. All measurements were carried out under standardized conditions on an indoor rubber floored gymnasium. Constant vocal encouragement was given to participants throughout the test. All participants were very well familiarized with the test, since the 20 m shuttle run test is one of the fitness tests included in the physical education curriculum in our country. Adolescents were instructed to abstain from strenuous exercise in the 48 hours preceding the test.

Cardiovascular fitness was considered as the number of stages completed (precision of 0.5 steps) for being the most direct measure obtained. Moreover, for the purpose of comparing the results with previous publications, maximal oxygen consumption (VO_{2max}, $mL\cdot kg^{-1}\cdot min^{-1}$) was estimated by the Leger's equation:[40] VO_{2max} = 31.025+3.238S-3.248A+0.1536SA, where A is the age, and S the final speed (S = 8+0.5 x number of stages completed). The reliability and validity of this test has been shown in young people.[41,42]

Homocysteine, serum folate and vitamin B_{12} assays

With the subject in the supine position, blood samples were taken by venipuncture after an overnight fast, using vacuum tubes (Vacutainer) and placed on ice immediately. The fasting state was verbally confirmed by the subject before blood sampling. All samples were processed within 1 h by centrifugation, divided aliquots, and portions were stored at -80°C until withdrawn for analysis.

Homocysteine in acidified citrated plasma[43] was assayed using a fluorescence polarization immunoassay on a IMx® unit (Abbott Laboratories, IL, USA). Serum folate and vitamin B_{12} levels were measured using the flourometric method with an Abbot IMx® autoanalyser (Abbot Laboratory, Chicago, USA).

MTHFR genotyping

Total blood DNA was extracted and purified from 500 µL of whole blood anticoagulated with EDTA, using the Quiagen procedure described by Higuchi.[44] Genotyping of the 677C>T variant in the human MTHFR gene was performed by means of polimerase chain reaction and allele-specific restriction digestion of the amplified products with the restriction enzyme Hinf I (GE Healthcare), as previously described by Frosst et al.[17]

Statistical analysis

The data are presented as means ± SDs, unless otherwise stated. After serum folate and vitamin B_{12} were normalized by natural logarithm transformation, all the residuals showed a satisfactory pattern.

The impact on homocysteine of gender and MTHFR 677C>T were analyzed by one-way analysis of variance (ANOVA) since there was a significant interaction between gender and MTHFR 677C>T, and the subgroup means were compared by Tukey's test.

Following a bivariate correlation analysis, multiple regressions were used to study the relation between homocysteine and CVF after controlling for potential confounders. We used an extended-model approach: Model 1 examined the influence of CVF on homocysteine levels after controlling for age, puberty, birth weight, smoking, socioeconomic status, and sum of six skinfolds; Model 2 examined the influence of CVF on homocysteine levels after controlling for the confounders included in the Model 1 plus serum folate and vitamin B_{12}; Model 3 examined the influence of CVF on homocysteine levels after controlling for the cofounders included in the Model 1 and Model 2 plus the MTHFR 677C>T genotype. Semipartial correlation (*sr*) was used as a measure of the relationship between CVF and homocysteine after controlling for the effect that one or more additional variables (*e.g.* age, birth weigth, etc.) had on one of those variables. The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 14.0 for WINDOWS; SPSS Inc, Chicago), and the level of significance was set to = .05.

RESULTS

Data completeness and baseline characteristics

Both homocysteine and MTHFR genotype were measured in156 adolescents (76 males, and 80 females). Of these, 23% of the adolescents refused of continuing the 20 m shuttle run test because of discomfort or distress, so they were not included in the final data sample. The observed power for the sample size was 0.40. Pubertal development were obtained from 96%, and 94% had skinfolds thickness data. Birth weight, socioeconomic status, and smoking habit information were available for 93, 87 and 71% of the subjects, respectively.

The descriptive characteristics of the study sample are shown in Table 1. Males were significantly heavier and taller than females, and females had significantly higher skinfolds thicknesses. Males had significantly higher levels of homocysteine, lower levels of serum vitamin B_{12}, and significantly higher CVF (Table 1).

Means values of homocysteine were significantly higher in the CT and TT subgroups compared to the CC subgroup in males (CC: 8.3±1.4 µmol/L, CT: 11.1±5.4 µmol/L, TT: 12.8±5.5 µmol/L, CT vs CC, P = .01; TT vs CC, P = .003), while in females, mean values of homocysteine were significantly higher in the TT subgroup compared to the CC and CT subgroups (CC: 7.5±2.3 µmol/L, CT: 8.3±1.7 µmol/L, TT: 10.2±2.2 µmol/L, P = .001).

Bivariate correlations between homocysteine and the studied independent variables are shown in Table 2.

Relations, between homocysteine and cardiovascular fitness controlling for different confounders and separated by gender

The results of the regression models using homocysteine as the outcome variable are shown in Table 3. Variation in homocysteine levels was significantly explained by CVF (expressed as number of stages completed) in female adolescents after controlling for age, puberty, birth weight, smoking, socioeconomic status, and sum of six skinfolds (Model 1). Additional adjustments for serum folate and B_{12} (Model 2), and MTFHR 677C>T (Model 3) further strengthened the association between homocysteine and CVF in females. No significant association was found between homocysteine and CVF in male adolescents. The results did not change when the analyses were performed with CVF expressed as VO_{2max}, or speed (data not shown).

DISCUSSION

The results of this study suggest that CVF is negatively associated with homocysteine levels in female adolescents, but not associated with homocysteine levels in male adolescents. The results also suggest that homocysteine levels are higher in males than in females, that serum folate and vitamin B_{12} are negatively associated with homocysteine levels, and that MTHFR 677C>T plays and important role in homocysteine levels. There exist no other available data on the association of homocysteine levels with CVF in adolescents.

Cardiovascular fitness is a direct marker of physiological status and reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged strenuous exercise.[45] Theoretically, disturbances in the peripheral tissues and related vasculature, or in the coronary arteries and the heart may decrease CVF. High CVF during childhood and adolescence has been associated with a healthier metabolic profile during these years.[29,30] Moreover, CVF has recently been associated with arterial compliance in children aged 9-11 years, which supports the concept that CVF may exert a protective effect on the cardiovascular system from an early age.[46] It is biologically plausible that high CVF provides more health protection than low CVF, even in healthy adolescents as has been found in adults.[25,26]

Homocysteine is metabolized to homocysteine-thiolactone by methionyl-tRNA synthetase. Homocysteine-thiolactone acylates lysine residues of proteins, a process called protein homocysteinylation.[47] Protein homocysteinylation is a possible mechanism of homocysteine-related protein damage, which in conjunction with the increased oxidative stress and endothelial damage seen in subjects with elevated homocysteine levels may result in impaired CVF.[9] However, this can not explain why the association between homocysteine and CVF exists only in female adolescents. Our findings support a previous study examining the relationship between homocysteine and CVF in adults.[27] Kuo et al.[27] showed that high homocysteine levels were negatively associated with estimated CVF in adult women. However, they did not find any association in men, which is in concordance with our results. These results suggest sex hormones may play a role in mediating the CVF- homocysteine association, exerting different effects in females than in males; however further studies to determine whether this is the case are needed. One longitudinal study followed 499 independent community-dwelling elderly for 3 years and found that people with elevated homocysteine levels were at an increased risk of decline in physical function.[48] However, CVF data were not provided and a gender comparison was not performed.

None of the previous studies included MTHFR 677C>T genotype, which has been shown to affect homocysteine levels.[16-19] Balasa et al.[19] found that the MTHFR 677C>T polymorphism was an independent determinant of homocysteine in 197 healthy U.S. children aged 6 months to 16 years. Similarly, Papoutsakis et al.[18] reported in a healthy sample of Greek children that the TT genotype was associated with homocysteine concentrations. Homocysteine levels in our study sample were significantly higher in the MTHFR 677CT and TT subgroups compared to the CC subgroup in males, while in females, mean values of homocysteine were significantly higher in the TT subgroup compared to CC and CT subgroup.

In the present study, CVF was objectively measured by the 20 m shuttle run test. We did not have a direct measure of maximal oxygen uptake, the most valid method of measuring CVF. However, from a practical point of view, field tests may be a better option than laboratory testing (especially in epidemiological studies) because a large number of subjects can be tested at the same time, which enhances the motivation of the participants, and the tests are simple, safer, and often the only feasible choice, especially in schools settings. The 20 m shuttle run test has been shown to meet these criteria. As mentioned in the methods section, CVF was considered as the number of stages completed in the 20 m shuttle run test. However, CVF estimated from Leger's equation (VO_{2max}, $mL \cdot kg^{-1} \cdot min^{-1}$) was also provided for the purpose of making comparisons with other studies possible. When the analyses were performed using VO_{2max}, or speed (km/h) instead of the number of stages as the measurement of CVF, similar results were obtained.

Results from cross-sectional studies have shown associations between homocysteine and lifestyle-related factors[20,22-24]. However, findings are different when analysed prospectively.[49,50] Duncan et al.[50] found that six months of exercise increased homocysteine levels in sedentary adults, whereas Randeva et al.[51] showed that six months of sustained brisk walking for 20-60 min 3 days/week significantly decreased homocysteine levels and increased CVF in young overweight and obese women with polycystic ovary syndrome, a group at increased risk of premature atherosclerosis. Similarly, a weight reduction programme including physical activities had a positive effect on the homocysteine of obese children.[52] Taken together, these results suggest that modifications in lifestyle-related factors may influence homocysteine levels in a different manner in young people than in adults.

The results from the present study should be interpreted with caution due to the limitations of the cross-sectional design, i.e., direction of causality can not be determined. Elevated homocysteine levels may be simply a marker of an unhealthy lifestyle that is associated with poor exercise capacity. The relationship between levels of homocysteine and CVF should be studied prospectively. It must be borne in mind is that the subjects involved in this study were healthy adolescents with no previously diagnose cardiovascular pathologies. Also, our study included a moderate number of participants. In fact, the observed power for the sample size was low (0.40) which may have masked the "association" between CVF and homocysteine levels in males. This warrants further investigation. However, we believe that covariates that may confound the measures of association in our study were appropriately considered and controlled.

In conclusion, the results of this study suggest that CVF is negatively associated with homocysteine levels in female adolescents after controlling for potential cofounders including MTHFR 677C>T genotype. These results should stimulate a debate on whether the metabolism of homocysteine could be one way in which the benefits of high CVF are exerted.

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Table 1. Descriptive characteristics of the subjects[a]

	All (n = 156)	Males (n = 76)	Females (n = 80)
Age (y)	14.8 ± 1.4	15.1 ± 1.4	14.6 ± 1.4
Tanner (1/2/3/4/5) (%)	0/2/14/42/42	0/2/22/27/49	0/3/6/56/35
Weight (kg)	58.6 ± 12.8	61.8 ± 14.0	55.3 ± 10.5[b]
Height (cm)	165.4 ± 8.5	170.1 ± 7.5	160.8 ± 6.6[c]
Body mass index (kg/m^2)	21.3 ± 3.6	21.2 ± 3.7	21.3 ± 3.5
Sum of six skinfolds (mm)	82.1 ± 32.8	71.8 ± 29.5	92.9 ± 32.8[c]
Birth weight (kg)	3.3 ± 0.5	3.3 ± 0.6	3.3 ± 0.5
Homocysteine ($\mu mol \cdot L^{-1}$)[d]	9.2 ± 3.4	10.0 ± 4.1	8.4 ± 2.2[b]
Serum vitamin B_{12} ($pmol \cdot L^{-1}$)[d]	612.9 ± 208.8	558.0 ± 143.1	667.7 ± 247.2[b]
Serum folate ($ng \cdot mL^{-1}$)	5.4 ± 1.7	5.3 ± 1.7	5.4 ± 1.6
Cardiovascular fitness (steps)	5.8 ± 2.8	7.5 ± 2.3	3.8 ± 1.8[b]
Cardiovascular fitness ($km \cdot h^{-1}$)	10.9 ± 1.4	11.8 ± 1.2	9.9 ± 0.9[b]
Cardiovascular fitness ($mL \cdot kg^{-1} \cdot min^{-1}$)[e]	42.6 ± 7.9	47.5 ± 6.5	37.1 ± 5.4[b]
MTHFR 677C>T genotype n (%)			
CC	61 (39)	35 (46)	26 (33)
CT	72 (46)	31 (41)	41 (51)
TT	23 (15)	10 (13)	13 (16)
Smoking (%)			
No	83	80	87
Yes	10	12	7
Partial smoker	7	8	6
Socioeconomic status (%)			
Low	3	5	0
Medium-low	26	28	25
Medium	46	49	44
Medium-high	18	16	21
High	7	2	10

[a] Unless otherwise indicated, data are expressed as mean ± SD.

[b,c] Sex differences were conducted by one way analyses of variance. [b]*P < .01*, [c]*P < .001.*

[d] Natural log-transformed values were used in the analysis, but nontransformed values are presented in the table.

[e] Estimated from Leger's equation.[40]

MTHFR indicates methylenetetrahydrofolate reductase.

Table 2. Bivariate correlations between homocysteine[a] and independent variables.

	Males	Females
Age	0.124	0.077
Tanner	-0.134	-0.043
Birth weight	-0.264	-0.199
Body fat	0.022	-0.308
Serum folate	-0.617 [d]	-0.680 [d]
Serum vitamin B_{12}[a]	-0.177	-0.401[c]
Cardiovascular fitness	0.051	-0.383[b]
Socioeconomic status	0.026	-0.136

[a] Natural log-transformed values were used in the analysis.

[b] $P = .022$

[c] $P = .017$

[d] $P < .001$

Table 3. Standardized multiple regression coefficients (β), confidence interval (95% CI), standardized coefficient of determination (R^2), and semipartial correlation (*sr*) examining the association of cardiovascular fitness (expressed as number of stages completed) with homocysteine[a].

		Homocysteine[b]				
Model	β	95% CI		*P* value	R^2	*sr*
Males						
Model 1	0.127	-0.055	0.088	.638	0.100	0.086
Model 2	0.135	-0.035	0.070	.498	0.406	0.091
Model 3	0.119	-0.032	0.063	.511	0.507	0.080
Females						
Model 1	-0.404	-0.131	0.001	.050	0.160	-0.360
Model 2	-0.402	-0.108	-0.021	.006	0.637	-0.358
Model 3	-0.395	-0.107	-0.020	.007	0.651	-0.351

[a] Controlled confounders: Model 1 = age, puberty, birth weight, smoking, socioeconomic status, and sum of six skinfolds; Model 2 = model 1 plus serum folate, serum vitamin B_{12}[b]; Model 3 = model 2 plus MTHFR 677C>T genotype.

[b] Homocysteine and serum vitamin B_{12} values were natural log-transformed.

Inflammatory Proteins are Associated with Muscle Strength in Adolescents; The AVENA Study

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Running head: Muscle strength in adolescents.

ABSTRACT

Background: Low-grade inflammation seems to be negatively associated with cardiorespiratory fitness in overweight and non-overweight young person and adults. Whether low-grade inflammation is associated with muscle strength in adolescents is unknown.

Objective: The aim of this study was to examine the associations between inflammatory proteins and muscle strength, and to determine whether this association varies between overweight and non-overweight adolescents.

Design: C-reactive protein, complement factors C3 and C4, ceruloplasmin and transthyretin were measured in 416 Spanish adolescents (230 boys and 186 girls) aged 13 to 18.5 y. Muscle strength score was computed as the mean of the handgrip and standing broad jump standardized values, and cardiorespiratory fitness was measured by the 20 m shuttle run test. A muscle strength score was computed as the mean of the handgrip and standing broad jump standardized values. The adolescents were categorized as overweight and non-overweight according to body mass index.

Results: The analysis of covariance showed that C-reactive protein, C4 and ceruloplasmin were negatively associated with muscle strength. C-reactive protein and transthyretin were negatively associated with muscle strength in overweight adolescents after adjusting for sex, age, pubertal status, socioeconomic status, cardiorespiratory fitness and body fat.

Conclusions: Low-grade inflammation is negatively associated with muscle strength in adolescents. The fact that some inflammatory proteins were associated with muscle strength in overweight adolescents after adjusting for body fat indicate that muscle mass may be involved in this mechanism. Intervention studies examining the impact of strength training on inflammatory markers in adolescents are warranted.

Key Words: Inflammation, physical fitness, exercise, pediatrics, obesity.

INTRODUCTION

Low-grade inflammation seems to play a role in the development of cardiovascular disease from on early age (1,2). It is negatively associated with cardiorespiratory fitness and positively associated with body fat in young persons and adults (3-7). Recent findings show a higher prevalence of having high C-reactive protein levels in Spanish overweight unfit adolescents compared with their overweight fit counterparts (8). Inflammatory proteins have also been negatively associated with muscle strength in adults (9-11).

The role of muscle strength in the performance of exercise and activities of daily living, as well as in preventing disease has become increasingly recognized (12,13). Resistance exercise training increased muscle strength, and it is currently prescribed by the major health organizations for improving health and fitness (14,15). Cardiovascular disease risk factors have also been associated with aerobic exercise and cardiorespiratory fitness not only in adults but also young persons (15-17).

Whether low-grade inflammation is associated with muscle strength in adolescents is unknown. Therefore, the aim of the present study was to examine the associations between inflammatory proteins and muscle strength in adolescents, and to determine whether these associations vary in overweight and non-overweight adolescents.

SUBJECTS AND METHODS

Subjects

The study participants were adolescents aged 13 to 18.5 y from the AVENA study (Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles [Food and Assessment of the Nutritional Status of Spanish Adolescents]), which was designed to assess the health and nutritional status of adolescents. The AVENA study design and sampling procedure have been reported in detail elsewhere (18-10). Data were collected from 2000 to 2002 in five Spanish cities, including Granada, Madrid, Murcia, Santander and Zaragoza. After exclusion of nine adolescents with concentrations of C-reactive protein >10mg/L, the present article includes 416 adolescents (230 boys and 186 girls) whom had a complete set of inflammatory proteins, muscle strength and cardiorespiratory fitness measurements. A comprehensive verbal description of the nature and purpose of the study was given to both the adolescents and their teachers. Written consent to participate was requested from parents and adolescents, and all adolescents gave verbal assent. Adolescents with a personal history of cardiovascular disease, taking medication at the time of the study, or were pregnant, were excluded after completion of the field work. The study protocol was performed in accordance with the ethical standards establised in the 1961 Declaration of Helsinki (as revised in Hong-Kong in 1989, and in Edinburgh in 2000), and was approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

The parents completed a questionnaire, which addressed the adolescents' previous and current health status. Socioeconomic status was also assessed in the questionnaire, and was defined by the educational achievement and occupation of the father. According to this information, and following the recommendation of the Spanish Society for Epidemiology, the adolescents were classified into five socioeconomic categories: low, medium-low, medium, medium-high and high socioeconomic status.

Physical Examination

Anthropometric measurements were obtained as described elsewhere (19,21,22). Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Skinfold thickness was measured at the biceps, triceps, subscapular, suprailiac, thigh and calf on the left side of the body to the nearest 0.2 mm using a Holtain skinfold caliper. The sum of the six skinfold thicknesses was used as an indicator of total body fat (22).

BMI categories (non-overweight and overweight including obesity) were computed according the proposed gender- and age-adjusted BMI cut-off points derived from adult values associated with health risk (23). Overweight prevalence and anthropometric body fat composition values in the complete AVENA study have been described by Moreno et al (21,22).

Identification of pubertal status (I-V) was assessed according to Tanner and Whitehouse (24). The standard staging of pubertal maturity describes breast and pubic hair development in girls and genital and pubic hair development in boys.

Blood Sampling

After overnight fasting, blood samples were collected between 8:00 and 9:30 a.m. by venipuncture. Highly sensitive C-reactive protein, complement factors C3 and C4, and ceruloplasmin were measured by immunoturbidimetry (AU2700 biochemistry analyzer; Olympus, Rungis, France). Transthyretin was measured by immunoturbidimetry (Roche/Hitachi 912). Quality control of the assays was assured by the Regional Health Authority. A detailed description of the blood analysis has been already reported (20,25).

Muscle Strength

Upper body strength was assessed by handgrip strength test, and lower body strength was assessed by the standing broad jump test. The handgrip strength test was performed on both hands with a hand dynamometer (Takei T.K.K. 5101 Grip-D; Takey, Tokyo, Japan) standing, and with the arm completely extended. The dynamometer was in contact with the hand being measured only, and no other part of the body. The standing broad jump was performed in an indoor rubber floored gymnasium. The subjects were instructed to push off vigorously and jump as far forward as possible, trying to land on both feet. The score was the distance from the take-off line to the point where the back of the heel closest to the take-off line lands on the floor.

A muscle strength score was computed by combining the standardized values of handgrip strength and standing broad jump. Each of these variables was standardized as follows: standardized value = (value - mean)/ standard deviation. The standardized values of the handgrip strength obtained with the right and the left hand were averaged. The muscle strength score was calculated as the mean of the two standardized scores (handgrip strength and standing broad jump). The score was calculated separately for boys and girls and for each age group (13, 14, 15, 16, 17-18.5 y).

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed by the 20 m shuttle run test as previously described (26). In brief, participants were required to run between two lines 20 m apart. The initial speed was 8.5 km/hr, which was increased by 0.5 km/hr per minute (one minute equal to one stage). The test was finished when the subject failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. Cardiorespiratory fitness was considered as the number of stages completed.

The adolescents were instructed to abstain from strenuous exercise for the 48 hours preceding the fitness tests. The tests are part of the EUROFIT test battery, and have been validated and standardized by the Council of Europe (27). Detailed methodology and reference values of fitness tests performed in the AVENA Study have been reported by Ortega et al (28).

Data Analysis

The data are presented as means ± SDs, unless otherwise indicated. All the residuals showed a satisfactory pattern after skinfold thickness, C-reactive protein, C3, C4, ceruloplasmin and transthyretin were normalized by natural logarithm transformation. Gender differences were analyzed by one-way analysis of variance (ANOVA), and adjusted for mass significance as described by Holm (29). Nominal data (overweight/non-overweight, pubertal status and socioeconomic status) were analyzed using Chi-square tests. Partial correlations were used to examine bivariate relations between cardiorespiratory fitness and muscle strength after controlling for sex.

The association between inflammatory proteins and muscle strength was tested by one-way analysis of covariance (ANCOVA). Muscle strength was recoded into tertiles to be entered into the models. All the analyses were adjusted for age, pubertal status, weight, height, socioeconomic status and cardiorespiratory fitness. Since no interaction effects between sex and muscle strength was found, all the analyses were performed for boys and girls together. The association between inflammatory proteins and BMI was tested by one-way ANCOVA after adjustment for age, pubertal status, socioeconomic status and cardiorespiratory fitness. BMI was entered into the models as overweight and non-overweight.

To determine whether the association between inflammatory proteins and muscle strength varies between BMI categories (overweight and non-overweight), the analyses were performed by one-way ANCOVA separately in overweight and non-overweight adolescents after adjusting for sex, age, pubertal status, socioeconomic status and cardiorespiratory fitness. Because BMI does not discriminate between muscle and fat mass, all the analyses were repeated with an additional adjustment made for skinfold thickness (as an indicator of total body fat). The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 14.0 for WINDOWS; SPSS Inc, Chicago), and the level of significance was set to 0.05.

RESULTS

Data Completeness and Baseline Characteristics

All subjects (n = 416) had complete data for all variables measured, with the exception of except for pubertal status and socioeconomic status data, which were not available in 37 (9%) and 83 (20%) adolescents, respectively. The descriptive characteristics of the study sample are shown in Table 1. Adolescent boys had higher values of ceruloplasmin than adolescent girls, as well as higher values of cardiorespiratory fitness, handgrip strength and standing broad jump. Cardiorespiratory fitness was significantly associated with both handgrip strength and standing broad jump ($r = 0.148$, $P < 0.01$ and $r = 0.746$, $P < 0.001$, respectively) as well as with muscle strength score ($r = 0.339$, $P < 0.001$) after controlling for sex.

Inflammatory Proteins and Muscle Strength

The results of the associations between inflammatory proteins and muscle strength are shown in Table 2. C-reactive protein and ceruloplasmin were negatively associated with muscle strength after adjusting for sex, age, pubertal status, weight, height, socioeconomic status, and cardiorespiratory fitness. C4 was not statistically significantly associated with muscle strength (*P* for trend = 0.071). The results were similar when additional adjustment was made for body fat (expressed as sum of six skinfold thicknesses), except for ceruloplasmin, which was not significantly associated with muscle strength.

Inflammatory Proteins and BMI

The associations between inflammatory proteins and BMI are shown in Table 3. C-reactive protein, C3 and C4 were significantly associated with BMI after adjusting for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness. No inflammatory protein was associated with BMI once the analysis was additionally adjusted for total body fat, except C4, which remained significantly associated with BMI.

Inflammatory Proteins and Muscle Strength by BMI Categories

The associations between inflammatory proteins and muscle strength by BMI categories are shown in Figure 1. C-reactive protein and transthyretin were negatively associated with muscle strength in overweight adolescents after adjusting for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness. Ceruloplasmin was not statistically significantly associated with muscle strength in overweight adolescents (*P* for trend = 0.058). C3 and C4 were not significantly associated with muscle strength either in either overweight or in non-overweight adolescents (*P* for trend > 0.1). The associations between C-reactive protein, transthyretin and muscle strength remained significant (*P* for trend = 0.05 and 0.013, respectively) after the analysis were additionally adjusted for total body fat. Ceruloplasmin was not significantly (*P* for trend > 0.1) associated with muscle strength once total body fat was entered in the model.

DISCUSSION

The primary findings of this study show that 1) C-reactive protein, C4 and ceruloplasmin are negatively associated with muscle strength in adolescence; 2) C-reactive protein and transthyretin are associated with muscle strength in overweight adolescents after adjusting for different confounders including cardiorespiratory fitness and body fat. Moreover, it also shows that the increased low-grade inflammation found in overweight adolescents is mediated by body fat, which confirms previous findings (3,4,25). To the best of our knowledge, this is the first population based study showing that low-grade inflammation is associated with muscle strength in adolescents.

Inflammatory Proteins and Muscle Strength

The association between inflammatory proteins and muscle strength has been examined in a few studies in adults. Two cross-sectional studies have shown a negative association of C-reactive protein, interleukin-6 and tumor-necrosis factor-α with muscle strength (9,10). Additionally, one prospective study found that higher levels of interleukin-6 and C-reactive protein were associated with loss of muscle strength in older persons (11). Features of the metabolic syndrome have also been negatively associated with muscle strength in adult men (30). Findings from a prospective study in adult men suggested that muscle strength may exert additive protection against the incident of metabolic syndrome beyond that attributed to cardiorespiratory fitness, and that overweight men may obtain more benefits than non-overweight men (31).

The health benefits of cardiorespiratory fitness among young persons and adults are well established (7,15-17,32). A number of studies on young persons suggest that inflammatory proteins are negatively associated with cardiorespiratory fitness (3-6). Similar findings have also been obtained in Spanish adolescents from the AVENA study (8). The results of the current investigation suggest that the development of muscle strength may confer additional benefits beyond those attributed to cardiorespiratory fitness. Therefore, the results of the present study add supportive evidence to the body of knowledge suggesting that physical fitness in young persons is an important health marker.

High concentration of C-reactive protein is considered a major cardiovascular risk factor (1,2,33). Increasing evidence supports the link between abnormal C3 and C4 concentrations and vascular disease (34). Body fat is known to promote a state of low-grade inflammation (35), which lends credibility to the results obtained in the present study. Furthermore, higher concentrations of inflammatory proteins have been hypothesized to play a role in the functional decline of older persons (9,36,37). The causal pathway leading from inflammation to disability has not been fully explained, but it has been suggested that low-grade inflammation may cause a decline of physical functioning through its catabolic effects on skeletal muscle (38). Collectively, these mechanisms may give explanation to the observed association between C-reactive protein and muscle strength in overweight adolescents.

Transthyretin, also known as pre-albumin, is a negative acute-phase protein that declines in response to inflammation (39). Other factors such as starvation and decreased skeletal muscle function are also known to affect transthyretin concentrations (40). Transthyretin concentation has been shown to increase with increasing protein and calorie intake and to decrease when protein intake is inadequate (41). Therefore, the associations observed in our study between transthyretin levels and muscle strength in overweight adolescents could be explained by the putative association to muscular weakness, but also enhanced by the state of increased low-grade inflammation seen in the overweight adolescents as mentioned previously.

Inflammatory Proteins, Muscle Strength, BMI and Body Composition

It is noteworthy that the observed associations between C-reactive protein, transthyretin, and muscle strength in overweight adolescents remained significant after adjusting for body fat. This may indicate that other mechanisms beyond body fat are involved in these associations. The key role of muscle mass in a number of metabolic processes has been recently highlighted, as well as in the prevention of many common pathologic conditions and chronic diseases (12,13). Measures of fat free mass are not available in the adolescents of the AVENA study. However, its has been recently shown that obese children are heavier not only due to an excess of fat mass but also due to the higher levels of fat free mass (measured by dual X-ray absorptiometry) compared with non-obese children (42). Therefore, the theoretical increased muscle mass in overweight adolescents may partially explain why those with high levels of muscle strength (third tertile) are those with the lowest levels of C-reactive protein.

Collectively, these findings indicate that special efforts should be focused on sub-groups of adolescents at increased risk of early cardiovascular disease, such as the overweight/obese. As a first step, promotion of regular participation in strength activities may be of help, since this mode of exercise may be easier and better tolerated for overweight/obese youth than aerobic training (43). A limitation of weight-bearing activities at the start of an intervention for overweight/obese adolescent is recommended, and a bigger focus on non-weight-bearing activities and activities relying on muscle strength is suggested (43,44). Interventions that are not tailored to the fitness level of obese participants can be counterproductive, and may contribute to discouragement of future participation in physical activity.

Cardiovascular Health and Muscle Strength

Strength training may have a number of beneficial effects for overweight individuals, including increased muscle mass and decreased total and central fat mass (14,15). A recent study has shown that a 16 week resistance training program (2 days/week) significantly increased insulin sensitivity in overweight adolescent boys, independent of changes in body composition, suggesting that mechanisms other than alterations in body composition were operative for the enhanced insulin sensitivity (46). Resistance exercise has been also successful in improving brachial artery endothelial function in women (47), improving insulin sensitivity and fasting glycaemia, and decreasing abdominal fat in both adult men and women with type 2 diabetes (48,49). Nevertheless, further studies are required in order to show whether resistance exercise can effectively attenuate the moderately increased resting levels of inflammatory proteins as well as reduce the fat mass in overweight adolescents. Increases in muscle strength may also influence positively the levels of cardiorespiratory fitness, since both variables are significantly associated.

Study Limitations

The observations of the current study are limited by the cross-sectional design. Prospective and intervention studies are required in order to draw more robust conclusions on the determining effect of inflammatory proteins on muscle strength. We used a single blood measurement of inflammation that may not accurately reflect long-term inflammatory status. Although no subject with a known underlying cause of infection was included, we can not be sure that elevated concentrations were not due to the onset of an infection. However, to attempt to minimize the confounding effect of an ongoing infection, adolescents with high concentrations of C-reactive protein (>10mg/L) were not included in this study. The fitness tests included in the present study have been shown to be reliable, as well as simple, safe and feasible, especially in the schools setting, and are included in several fitness batteries to be performed in school-based epidemiological studies (27,50).

Conclusions

The results presented in this study suggest that low-grade inflammation is negatively associated with muscle strength in adolescents. The patterns of these associations seem more relevant in overweight adolescents. The fact that some inflammatory proteins were associated with muscle strength in overweight adolescents after adjusting for body fat may indicate that muscle mass may be involved in this mechanism. More studies are needed in order to elucidate the role of muscle mass and muscle strength on low-grade inflammation. Intervention studies examining the impact of strength training on muscle mass and inflammatory markers in adolescents are warranted.

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JRR conceived the hypothesis and conducted the statistical analyses for this manuscript. JRR drafted the manuscript. FOP, LAM, JW, JJC, MGG, ACM, AG and MS, contributed to the interpretation and discussion of the results. AM, MGG and AGS contributed to the concept and design of the AVENA study. All the authors critically revised the drafted manuscript. None of the authors had any conflict of interest.

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FIGURE 1. Association between inflammatory proteins and muscle strength tertiles (first, second and third tertile equals to low, middle and high) in overweight (grey columns) and non-overweight (white columns) adolescents. Columns are estimated means. Data were analysed by one-way analysis of covariance separately in overweight and non-overweight adolescents after adjusting for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness. Absence of *P* values indicates no statistically significant association. Ln indicates logarithmic transformation.

Table 1. Physical characteristics of the subjects[1]

	All (n = 416)	Boys (n= 232)	Girls (n= 186)
Age (y)	15.4 ± 1.4	15.4 ± 1.4	15.4 ± 1.4
Weight (kg)	61.3 ± 12.8	64.9 ± 13.4	56.8 ± 10.5[4]
Height (cm)	166.6 ± 8.7	171.0 ± 8.1	161.4 ± 6.2[4]
Body mass index (kg/m^2)	22.0 ± 3.7	22.1 ± 3.9	21.8 ± 3.6
Overweight (including obesity) (%)	26	31	20[†]
Pubertal status I/II/III/IV/V (%)	0/3/12/47/38	0/4/15/41/40	0/1/9/55/35[2]
Sum of six skinfolds (mm)	44.5 ± 5.8	43.0 ± 6.1	44.1 ± 5.3
C-reactive protein (mg/L)[5]	1.44 ± 3.14	1.56 ± 2.61	1.28 ± 3.69
C3 (g/L)[5]	1.35 ± 0.24	1.36 ± 0.24	1.33 ± 0.22
C4 (g/L)[5]	0.27 ± 0.10	0.27 ± 0.09	0.27 ± 0.10
Ceruloplasmin (g/L)[5]	0.21 ± 0.05	0.20 ± 0.04	0.22 ± 0.05[3]
Transthyretin (mg/dL)[5]	23.76 ± 6.56	24.30 ± 6.90	23.02 ± 6.02
Cardiorespiratory fitness (stages)	5.8 ± 2.8	7.1 ± 2.6	4.1 ± 1.9[4]
Handgrip strength (kg)	31.8 ± 8.0	35.5 ± 7.6	25.4 ± 4.0[4]
Standing broad jump, cm	173.9 ± 32.5	191.0 ± 29.1	152.7 ± 22.2[4]
Socioeconomic status (%)[6]	5/26/40/23/6	4/27/43/21/5	6/25/37/25/7

[1]Values are mean ± SD, unless stated.

[2]$P < 0.05$, [3]$P < 0.01$, [4]$P < 0.001$ for gender comparisons adjusted for mass significance (29).

[5]Analyses were performed on log-transformed data, but non transformed data are presented in the table. [6]Five categories: low, medium-low, medium, medium-high and high socioeconomic status.

Table 2. Associations between inflammatory proteins and muscle strength.

	Muscle strength				
Dependent variable[1]	Estimated mean differences 1st tertile - 3rd tertile	*P*	Estimated mean differences 2nd tertile - 3rd tertile	*P*	*P for trend*
C-reactive protein	0.351	0.015	0.292	0.032	0.030
C3	0.038	0.103	-0.008	0.713	0.103
C4	0.085	0.117	0.111	0.029	0.071
Ceruloplasmin	0.070	0.029	0.009	0.759	0.052
Tansthyretin	-0.173	0.292	-0.087	0.567	0.253

Data were analyzed by one-way analysis of covariance after adjusting for sex, age, pubertal status, weight, height, socioeconomic status, and cardiorespiratory fitness.

[1]All analyses were performed on log-transformed data.

Table 3. Associations between inflammatory proteins and body mass index.

Dependent variable3	Body mass index			
	Estimated mean differences[1] N-OVER - OVER	*P*	Estimated mean differences[2] N-OVER - OVER	*P*
C-reactive protein	-0.432	0.001	-0.236	0.225
C3	-0.095	<0.001	0.025	0.416
C4	-0.221	<0.001	-0.146	0.043
Ceruloplasmin	-0.049	0.099	0.027	0.519
Transthyretin	-0.067	0.653	-0.196	0.375

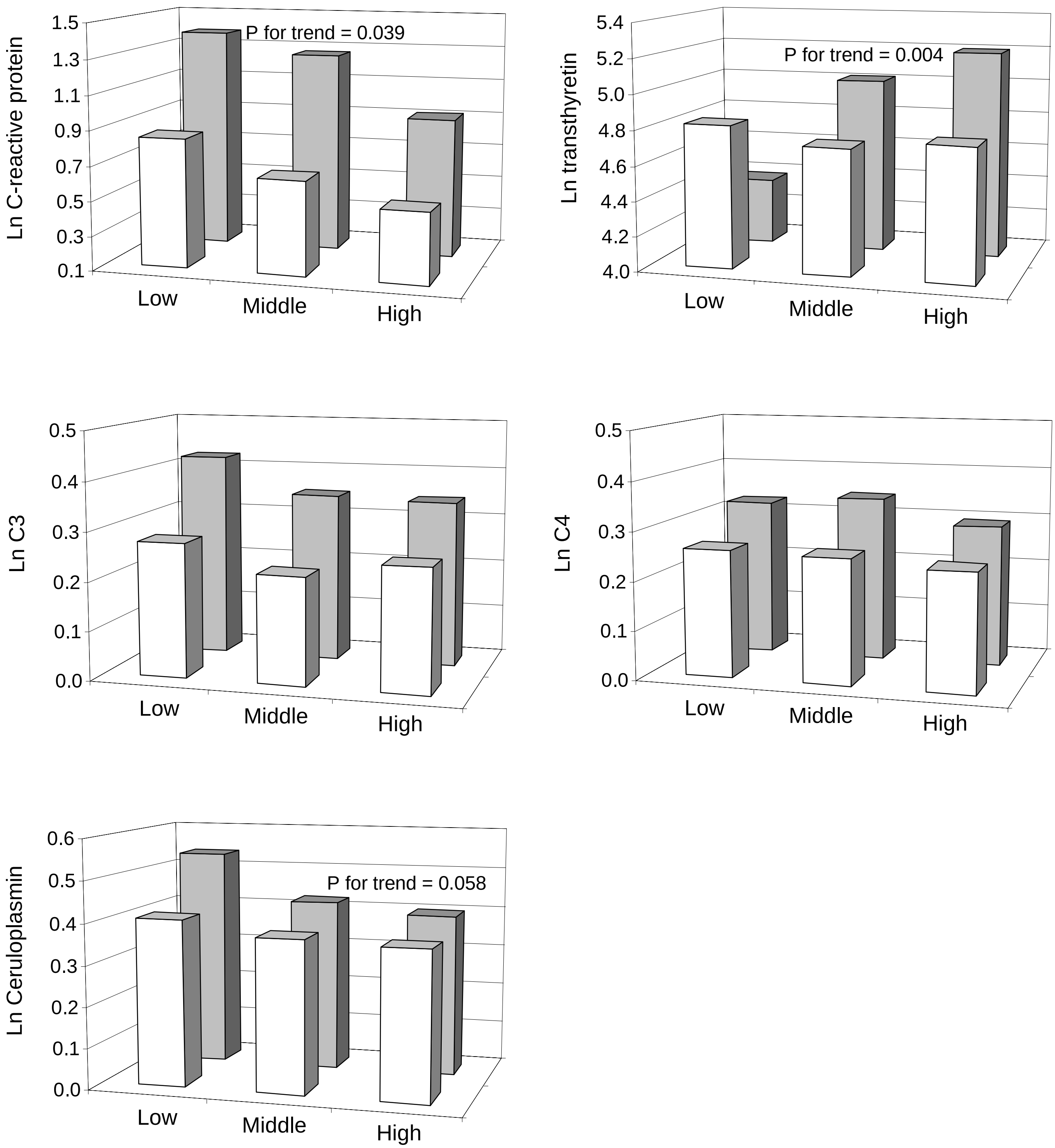
[1]Data were analyzed by one-way analysis of covariance after adjusting for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness, and [2]with an additional adjustment made for skinfold thickness.

N-OVER indicates non-overweight.

[3]All analyses were performed on log-transformed data.

FIGURE 1.

Inflammatory proteins and muscle strength tertiles by body mass index categories



Use of Artificial Neural Network-Based Equation for Estimating VO_{2max} in Adolescents

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Running title: Neural network-equation for estimating VO_{2max}

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ABSTRACT

Purpose: To develop an artificial neural network (ANN)-equation to estimate maximal oxygen uptake (VO_{2max}) from 20m shuttle run test (20mSRT) performance (stage), sex, age, weight and height in young persons.

Methods: The 20mSRT was performed by 193 (122 boys and 71 girls) adolescents aged 13-19 years. All the adolescents wore a portable gas analyzer to measure VO_2 and heart rate during the test. The equation was developed and cross-validated following the ANN mathematical model. The neural net performance was assessed through several error measures. Agreement between the measured VO_{2max} and estimated VO_{2max} from Léger's and ANN equations were analysed following the Bland and Altman method.

Results: The percentage error was 17.13 and 7.38 for Léger and ANN-equation, respectively, and the standard error of the estimate obtained with Léger's equation was 4.27 ml/kg/min, while for the ANN equation was 2.84 ml/kg/min. A Bland-Altman plot for the measured VO_{2max} and Léger-VO_{2max} showed a mean difference of 4.9 ml/kg/min (P < 0.001), while the Bland-Altman plot for the measured VO_{2max} and ANN-VO_{2max} showed a mean difference of 0.5 ml/kg/min (P = 0.654).

Conclusions: In this study, an ANN-based equation to estimate VO_{2max} from 20mSRT performance (stage), sex, age, weight and height in adolescents was developed and cross-validated. The newly developed equation was shown to be more accurate than Léger's equation in the sample of adolescents studied. The proposed model has been coded in a user friendly spread sheet.

Key words: Cardiorespiratory fitness; maximal oxygen uptake; aerobic capacity test; exercise field test.

INTRODUCTION

Paragraph Number 1

The maximal rate of oxygen uptake (VO_{2max}) is considered as a gold standard for measurement of cardiorespiratory fitness. Cardiorespiratory fitness is a direct marker of physiological status and reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged exercise (35). In addition, recent reports suggest that cardiorespiratory fitness is also an important health marker in young persons. High cardiorespiratory fitness during childhood and adolescence has been associated with a favourable plasma lipid profile in both overweight and non-overweight adolescents (18), with total body fat (29), features of the metabolic syndrome (4, 28), novel cardiovascular disease risk factors (30), and with arterial compliance (26) in young people. These findings support the concept that cardiorespiratory fitness may exert a protective effect on the cardiovascular system from an early age.

Paragraph Number 2

Cardiorespiratory fitness is one of the main health-related physical fitness components used in schools, sports centres and health institutions. One of the most widely used field tests for estimating cardiorespiratory fitness among adolescents is the 20m shuttle run test (20mSRT) also called the "Course Navette" test (12, 37). The 20mSRT or a slight modification of it, has been included in several fitness batteries, such as the EUROFIT (5), and the American Progressive Aerobic Cardiovascular Endurance Run and the FITNESSGRAM battery (36) among others. The 20mSRT is a feasible fitness test, since a large number of subjects can be tested at the same time, which enhances the motivation of the participants. It can be conducted indoors or outdoors in a relatively small area, and on different surfaces (slippery and rubber floors).

Several equations have been developed to estimate VO_{2max} from maximal speed attained during the 20mSRT (Table 1). Léger et al. (13) developed an equation based on a sample of 188 boys and girls aged 8-19 years to estimate the VO_{2max} from maximal speed attained during the 20mSRT, age and the speed and age interaction. However, Léger's equation has some limitations. Sex is not included in the model, yet it is well known that physical performance is highly different in boys and girls of all ages. Moreover, the estimates of VO_{2max} for low scores were based on extrapolated data from the study since the original study population did not have data for these points. The accuracy of the Léger's (13) prediction model has been examined by several researchers (1, 6, 14, 15, 24, 33, 34, 38), but no attempts have been made to develop a more accurate model in a wide age range.

Paragraph Number 3

It seems viable to develop a more accurate VO_{2max} equation for the adolescent period, while taking those variables which have been shown to have an impact on the level of cardiorespiratory fitness into account. Published equations for VO_{2max} have the shape of a linear or quasi-linear expression on different input variables (sex, age, body weight, and stage) (Table 1). Researchers have used these type of models mainly because of their simplicity, easy of use, and familiarity. A way forward in obtaining an improved model could be done by exploring the feasibility of some new methods. Recently, there has been a growing interest in artificial neural networks (ANN). ANNs have some theoretical advantages over more traditional regression methods (7). Predictive models based on ANNs have been studied extensively in many areas of medicine (e.g. breast cancer diagnosis, mortality assessment in intensive care units, diagnostic scoring, renal function evaluation).

The aim of this study was to develop an ANN-equation to better estimate VO_{2max} from 20mSRT performance (stage), sex, age, weight and height in adolescents.

Paragraph Number 4

METHODS

Subjects

A total of 203 adolescents (127 boys and 76 girls) aged 13-19 years volunteered to participate in the study after receiving a detailed explanation about the aim and the clinical implications of the investigation. A comprehensive verbal description of the nature and purpose of the study was also given to the teachers. Written informed consent was obtained from parents, and verbal assent was obtained from participants. The criteria for inxclusion were: smoking, no personal history of cardiovascular or metabolic disease, free of disease, any muscular or skeletal injuries, medication at the time of the study and pregnancy. The experimental protocol was approved by the Review Committee for Research Involving Human Subjects at the University of Granada, Spain.

Paragraph Number 5

A few adolescents (n = 5) discontinued the test because of discomfort or distress. A small number of technical problems (n = 5) also occurred, which probably yielded inaccurate VO_{2max} measurements as a result. Therefore, the final sample was confined to 193 (122 boys and 71 girls) adolescents with reliable measures of VO_{2max}.

Paragraph Number 6

Procedure

All participants performed the 20mSRT as previously described by Léger et al. (12). Participants were required to run between two lines 20m apart, while keeping the pace with audio signals emitted from a pre-recorded CD. The initial speed was 8.5 km/h, which was increased by 0.5 km/h per minute (one minute equal one stage). The CD used was calibrated over one minute of duration. Participants were instructed to run in a straight line, to pivot on completing a shuttle, and to pace themselves in accordance with the audio signals. The test was finished when the participant failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. All measurements were carried out under standardized conditions on an indoor rubber floored gymnasium. The participants were encourage to keep running as long as possible throughout the course of the test.

Paragraph Number 7

All participants were familiar with the test, because the 20mSRT is one of the fitness tests included in the curriculum of Physical Education in Spain. However, one week prior the test, participants received a comprehensive instruction after which they also practiced the test. Subjects were instructed to abstain from strenuous exercises 48 hours prior to the test. All the tests were conducted by the same investigators and at the same time for each subject (between 10:00 to 13:00 hrs).

Paragraph Number 8

Physiological measurements

Heart rate was recorded every 5 seconds throughout the 20mSRT using a Polar telemetry system (Polar 610i). Moreover, participants wore a portable gas analyzer (K4b^2, Cosmed, Rome, Italy), the purpose of which was to measure the VO_2 during the 20mSRT. Respiratory parameters were recorded breath-by-breath, which in turn were averaged over a 10 second period. VO_{2max} was the main parameter determined using the open circuit method. Exhaustion was confirmed when: 1) maximal heart rate was greater than 185 beats per minute, 2) respiratory exchange ratio was greater than 1.1, and/or 3) a detection of a plateau in the VO_2 curve, defined as an increase of VO_2 less than 2 ml/kg/min with a concomitant increase in stage.

Paragraph Number 9

The weight of the Cosmed K4b^2 equipment is 1.5 kg including the battery and a specially designed harness. It has been proven to be a valid device when compared with the Douglas bag method (17). Wearing the portable gas analyzer during the 20mSRT do not significantly alter the subjects' energy demands, as it has been reported (6).

Paragraph Number 10

Before each test was conducted, the oxygen and carbon dioxide analyzers were calibrated according to the manufacturer's instructions. This consisted of performing a room air calibration and a reference gas calibration using 15.93 % oxygen and 4.92 % carbon dioxide. The flow turbine was then calibrated using a 3-liter syringe (Hans-Rudolph). Finally, a delay calibration was performed to adjust for the lag time that occurs between the expiratory flow measurement and the gas analyzers. During each test, a gel seal was used to help prevent air leaks from the face mask.

Paragraph Number 11

The total time (in seconds) and the last half-stage completed (here called "stage") were recorded. The measured VO_{2max} was obtained directly from the K4b^2 data. Estimated VO_{2max} was calculated by the Léger's equation (13) (Table 1). Height and

Body weight was measured to 0.1 kg using a standard beam balance, and body height was measured to the 0.1 cm using a transportable stadiometer, with the participants clad only in their underwear. These measures were taken prior the test.

Paragraph Number 12

Statistical analyses

The mathematical model used to build a new equation to estimate VO_{2max} from 20mSRT performance (stage), sex, age, weight and height in adolescents was an ANN. An ANN is a mathematical model that emulates some of the observed properties of biological nervous systems and draw on the analogies of adaptive biological learning. The ANN modelling procedure is described in detail elsewhere (9). Briefly, to solve a problem using ANN, a number of steps must be taken:

Paragraph Number 13

1. Select the type of neural net for the type of regression problem that is to be map, i.e. identification of a VO_{2max} estimator. One of the best options for that purpose is to use a multilayered perceptron.

Paragraph Number 14

2. Data preprocessing. The data gathered for this study consists of a set of 193 instances, each instance being composed of six variables. All variables were originally expressed in their original units, i.e. sex (boys/girls), age (years), weight (kilograms), height (centimetres), stage (last-half stage completed), and VO_{2max} (ml/kg/min). The sample data was afterwards normalized to the [0.1, 0.9] interval, which simplified the learning of the ANN regression model.

Paragraph Number 15

3. Network design. The ANN architecture, i.e. the number of input and output variables is set by the problem. There are plenty of different models of neural networks to chose from, each one having its specific properties and advantages for its particular application. One of the most successful and most popular is the feed-forward multilayered perceptron. In this network, the computing units are arranged into three layers, which are conveniently ordered. The information flows forward from the five neurons of the input layer to the three connecting neurons of the hidden layer and finally, to the single neuron of the output layer using no backward connection. The first layer (the input layer) corresponds to the independent variables (sex, age, weight, height and stage), while the third layer (the output layer) corresponds to the dependent variable score (VO_{2max}). The intermediate layer, which is a

hidden layer, consisting of all possible connections between the input and the output layer, allows for a combined impact of a multiple set of independent variables on the output layer. This would be the same as testing all possible interactions in a regression model, but without adding any extra degrees of freedom. The neurons in each layer serve the purpose of optimally transforming each quantitative variable in a curvilinear fashion, similar to adding a spline function for each of the independent variables of a regression model. The architecture of the network used in this study is a multilayered perceptron (5-3-1), which is shown in Figure 1.

Paragraph Number 16

4. Find learning algorithm parameters. In order to obtain the synaptic weights of the ANN, we used the well-known backpropagation algorithm (31). The value for the algorithm parameters are 0.2 for the learning rate, and 0.5 for the momentum term. The training of the network is stopped when the sum of squared errors (SSE) falls below 0.00001 or when 1,500 training epochs have been performed.

Paragraph Number 17

5. Training of the network. The ANN-model is identified by means of a data-driven process, where a fraction of the available data set is used for designing the model and it is referred to as the *training set*. The remaining set of data is not used in the design of the model as such but rather for evaluating its validity once it is ready. This particular data set is called the *test set.*

Paragraph Number 18

6. Validation of the model. In order to validate the feasibility of the ANN-model for this problem, a cross-validation technique was applied (19). It means that the total dataset (composed of 193 samples) was randomly split into k parts with the same number of samples, except one of them (C = $c_1, \ldots, c_k$). The process consists of building k different neural networks. For the model i, with $i = 1,\ldots,k$ the part c_i is used as the test set, and the remainder

(all but c_i) are used as the training set. In our experiments, the value we have used for *k* is the total number of samples in the data set (n = 193). Thus each of the nets are built with different training sets, and evaluated on different and independent test sets. The overall evaluation of the methodology is measured as the average of the performance on the test sets.

Paragraph Number 19

The neural net performance is assessed through an error measure. Suppose that *N* cases are available to evaluate the model, where *y* is the actual output (the measured VO_{2max}) and $\hat{y}$ is the output computed by the net (estimated VO_{2max} from the ANN-equation). Then, a common measure is the SSE defined as:

$$SSE = \sum_{i=1}^{N} (y_i - \hat{y}_i)^2$$

An easier way of understanding the expression for the error is to use the percentage error, which can be computed as follows: First, the SSE is averaged over the number of cases, rendering the mean sum of squared errors (MSE):

$$MSE = \frac{1}{N} \sum_{i=1}^{N} (y_i - \hat{y}_i)^2$$

MSE is then converted into domain units by taking the root square and yielding the root mean sum of squared errors (RMSE):

$$RMSE = \sqrt{MSE}$$

The percentage error should intuitively serve as a good indicator of the performance of a given model:

$$\%Error = \frac{RMSE}{\text{domain width}} \times 100$$

The standard error of estimate (SEE), is another way to illustrate the performance of the ANN-model, which also serves for comparative purpose:

$$SEE = SD_y \sqrt{(1 - R^2_{y\hat{y}})}$$

where SD is the standard deviation of the estimated VO_{2max} from the ANN-model, and R^2 is the correlation between the measured the measured VO_{2max} and the estimated VO_{2max} from the ANN-model.

Paragraph Number 20

The SSE difference between the Léger's equation and the ANN-model was examined by paired *t* test. A second ANN-model was built with the same procedure and variables as the previous one, but instead of the last half-stage completed, the last stage completed was used. Sex differences were analysed by one-way analysis of variance (ANOVA), and adjusted for mass significance as described by Holm (10). Bivariate correlation analysis was done in order to examine the relationship between the measured VO_{2max} and the input variables (age, weight, height and stage) in boys and girls. The relationship between the measured VO_{2max} and a similar estimated VO_{2max} from Léger's equation and the ANN-model was also examined. The overall differences between the measured VO_{2max} and the similar estimated value from Léger's equation and ANN-model was calculated by means of paired *t* test. The agreement between the measured VO_{2max} and the similar value as estimated from Léger's and the ANN equation was assessed according to the method by Bland and Altman (2, 3).

Paragraph Number 21

RESULTS

Physical characteristics and the 20mSRT performance of the participants are presented in Table 2. Boys and girls were similar in age, but boys were significantly taller and heavier than girls. Moreover, boys had significantly higher values in all the 20mSRT performance-related variables. A bivariate correlation analysis between the measured VO_{2max}, age, weight, height and stage in boys and girls is presented in Table 3. VO_{2max} was significantly associated with age, weight and stage in both sexes. A borderline significant association was found between VO_{2max} and height in both boys and girls. Figure 2 shows the relationship between the measured VO_{2max} and the estimated VO_{2max} from the Léger's equation, and Figure 3 shows the relationship between the measured VO_{2max} and the estimated VO_{2max} from the ANN-equation. Estimated VO_{2max} from both the Léger's and the ANN-equation were significantly correlated with the measured VO_{2max} (r = 0.90 and 0.96, respectively, both $P < 0.001$).

Paragraph Number 22

The evaluation of the error of the VO_{2max} measurements obtained from Léger's and the ANN-equation is presented in Table 4. The SSE was significantly higher in Léger's equation than in the ANN-equation ($P < 0.001$), and the percentage error was 17.13 for the former and 7.38 for the latter. The SEE obtained with the Léger's equation was 4.27 ml/kg/min, while for the ANN equation was 2.84 ml/kg/min. The SSE obtained from the ANN-model built with the last stage completed was significantly higher than the SSE obtain from the ANN-model built with the last half-stage completed (1699.48 vs 1600.91 vs, respectively, $P = 0.002$).

Paragraph Number 23

A Bland-Altman plot for the measured VO_{2max} and VO_{2max} estimated from Léger equation showed a mean difference of 4.9 ml/kg/min (Figure 4). The 95% limits of agreement ranged from -4.3 to 14.1 ml/kg/min. There was a statistically significant difference between measured VO_{2max} and Léger equation (47.7 vs 43.0 ml/kg/min, $P < 0.001$). A Bland-Altman plot for the measured VO_{2max} and the ANN-equation showed a mean difference of 0.5 ml/kg/min (Figure 5). The 95% limits of agreement ranged from -5.1 to 6.1 ml/kg/min. There was no statistical significance difference between measured VO_{2max} and ANN-equation (47.7 vs 47.2 ml/kg/min, $P = 0.654$).

Paragraph Number 24

DISCUSSION

In this study, an ANN-based equation to estimate VO_{2max} from 20mSRT performance (stage), sex, age, weight and height in a sample of 193 adolescents aged 13-19 years was developed and cross-validated. The equation is based on: 1) direct VO_2 data collected while the adolescents performed the 20mSRT; 2) the use of a numerical procedure to build the ANN-equation; 3) a fairly large amount of adolescents participating in the test; 4) using variables, which have been previously shown to have an influence on the VO_{2max} for the particular age group being tested. All variables included in the equation are measured in field studies and no specific equipment is required to collect the data. All the technical and environmental variables that may have an influence on the results were carefully controlled in order to obtain highly reliable VO_2 measures; and 5) the use of a precise method for assessing agreement between two methods. The most frequently used summary statistics to assess overall agreement between the measurements of different methods were correlation coefficient. However, correlation is a measure of the strength of association between two variables but not necessarily a measure of agreement (27).

Paragraph Number 25

The ANN-based equation proved to be more accurate for a prediction of the VO_{2max} value than Léger's equation for the particular sample of adolescents studied here. Léger's equation had an error of 17.13%, while the ANN-equation had an error of 7.38%. The SEE calculated from Léger's equation was almost twice as high as that obtained with the ANN-equation (4.27 vs 2.84 ml/kg/min, respectively). Moreover, Léger's equation significantly underestimated VO_{2max} by 4.9 ml/kg/min when compared with the measured VO_{2max} ($P < 0.001$), while the ANN-equation slightly underestimated VO_{2max} by 0.5 ml/kg/min ($P = 0.654$). These results of this study are in alignment with previous research, which has shown a systematic underestimation of the VO_{2max} value calculated from Léger's equation (32, 33).

Paragraph Number 26

Differences between the results obtained from the ANN-equation and those obtained from Léger's equation may be partly explained by the test protocols and the gas analysis procedures used for the tests. Léger et al. recorded VO_{2max} by using the backward extrapolation technique (13). This technique has been extensively validated, but it can only be considered as an estimate of the actual VO_{2max}. The present method seems to be a more sensitive method, since data were averaged every 10 seconds, which allowed for the detection of a plateau in the VO_2 over the final workloads.

Paragraph Number 27

The ANN-equation has other advantages over Léger's equation, and also on more recently published regression equations (Table 1). The reason why sex, age, weight, height and stage were used as predictive input variables for estimating VO_{2max} in the ANN-equation is reviewed below.

Paragraph Number 28

Sex. As it could be expected, there was a significant difference between boys and girls in the measured VO_{2max} value. This result is also consistent with normative data showing lower levels of VO_{2max} for girls than for boys (23). However, Léger's equation does not account for sex. Factors explaining the lower VO_{2max} values for girls may be partially explained by the fact that girls usually have a lower development of muscular mass and higher fraction of body fat (20). Moreover, it has been suggested that women may be more prone to pulmonary limitations during heavy exercise (and perhaps submaximal intensities) than men, which is supposedly due to the influence of the reproductive hormones (estrogen and progesterone) in combination with a reduced pulmonary capacity (8). A greater ventilatory work associated with an increased expiratory flow limitation during the exercise and gas exchange impairments seems to be of primary importance. The influence of sex on VO_{2max} has also been taken into account in two recently published equations (15, 33). Sticklan et al. (33) developed two sex-specific equations with similar slopes for both men and women aged 18-38 years. They found a slightly lower Y-intercept value for women, which is in agreement with our findings. Mahar et al. (15) developed an equation based on a sample consisting of 61 boys and 74 girls in the age group between 12-14 years in which sex, number of laps completed, and body weight were included as independent variables (Table 1).

Paragraph Number 29

Age. Léger et al. (12) included age as one of the independent variables in their model, which was not the case in other published equations (6, 15, 33). The age range of the adolescents involved in the present study was similar to the study made by Léger et al. (12). However, the youngest adolescent in our study was 13 years old, while the youngest person in Léger's study was 8 years old. Findings from cross-sectional and longitudinal studies have shown that age is associated with VO_{2max} in both adolescents and adults (23).

Paragraph Number 30

Adolescence represents a period of life where changes such as growth and physiological development occur. Therefore, age might be an important factor to check for in order to understand the contribution of those age dependent factors. It has been suggested that rather than using the chronological age as a measure for this variable, sexual maturation (i.e. biological age) may be a more accurate marker of the physiological status of the person in this period of her/his life (25). However, findings from cross-sectional studies examining the influence of sexual maturation on VO_{2max} have shown that sexual maturation may account for only a small proportion of the variance in the measured VO_{2max} value (21), and that weight and height are primarily responsible for variation in VO_{2max} throughout maturation (11). One of the main reasons why sexual maturation was not included in the equation was due to the suspected inaccuracy in self-reporting tanner stage in some circumstances, and the need for a paediatrician or trained physician to make an objective measurement, which in most setting is not feasible.

Paragraph Number 31

Body size: weight and height. The increases in VO_{2max} are influenced by changes in body weight and height. Controlling the effect of a body size changes in growing adolescents is critical in order to understand the relative contributions of other factors influencing changes in the value of VO_{2max}, such as sex, maturation, habitual physical activity, and functional cardiorespiratory improvements. The conventional (ratio) approach for controlling or, "normalizing" VO_{2max} for body size has been to divide the VO_{2max} value by kilogram of body weight. However, in walking/running activities, height also has been shown to have an impact on the performance, and specially in those activities incorporating shuttle running such as the 20mSRT (6, 16). Body weight has usually been used as a measure of body size, but it has also been suggested that height could be used when scaling body size to account for possible disproportionate changes in muscle mass with increasing body size (40). This study shows that both body weight and height are significantly correlated with the 20mSRT performance (Table 3). Body weight was negatively correlated with VO_{2max} in both sex (r = -0.517, P < 0.001; r = -0.241, P < 0.043 for boys and girls, respectively), while the correlation between height and VO_{2max} was less evident for both sex (r = -0.170, P = 0.079; r = 0.219, P = 0.066 for boys and girls, respectively). It is worth noting that height is negatively correlated with VO_{2max} for boys, while the opposite is true for girls. Girls had significantly lower height than boys (161.0 vs 172.5 cm, respectively, P < 0.001), which may indicate that height has a positive contribution on the 20mSRT performance up to a certain level after which it has a negative impact. It is tenable that various biomechanical complexities of shuttle running may account for this. Other approaches have recommended the use of allometric scaling exponents (39) or accounting for fat free mass (22) in order to allow for a more appropriate study on the impact of body size differences on VO_{2max}. However, the allometric scaling exponents has not been universally reported (40), and the use of fat free mass needs either expensive instrumentation or trained evaluators (when derived from anthropometric measurements) which is not often a feasible choice, especially in schools settings.

Paragraph Number 32

To our knowledge there is only one model that includes body size measurements in the model (15). The equation developed by Mahar et al. (15) includes both weight and height as single variables and as a ratio [body weight in kilogram divided by height in meters squared (BMI)] (Table 1). They also developed another equation where only weight is used as a predictive variable in the model. A SEE of 6.38 and 6.35 ml/kg/min was reported for the first and second equation, respectively. These results are slightly higher than the SEE obtained in the present study by means of both the Léger's and the ANN-equation (4.27 and 2.84 ml/kg/min, respectively). Some aspects of the used methods could probably explain the observed differences in the SEE values. Mahar et al. (15) used a multiple regression model to predict the measured VO_{2max} from the number of laps completed on the 20mSRT. The following variables were included: sex and body mass or BMI. The dependent variable in the regression model was measured VO_{2max}, which was collected while running until exhaustion on the treadmill. Energy demands during shuttle running have been reported to be higher when compared with treadmill running (6), which can be attributed to the mode of exercise, technique, and musculature employed in the two conditions. This may confound the reliability of the equations built with VO_{2max} values collected from treadmill-based protocols (6, 15, 33).

Paragraph Number 33

Stage. In the ANN-equation, the maximal 20mSRT performance attained is calculated from the last half-stage completed, so it allows credit for 30 second when participants fall short of completing a full stage. This increased precision should help in detecting changes in fitness in interventional studies, follow up studies, in athletes before and after a period of training, etc. The Léger's equation used maximum speed calculated from the last stage completed, and therefore, subjects falling just short of completing a full one-minute stage would be ascribed to the previously completed stage. Consequently, the ANN-equation may allow for a greater sensitivity in the estimation of VO_{2max} when compared with Léger's equation. Stickland et al. (33) also used the last half-stage completed as the measure of the 20mSRT performance to build a prediction model for adults, and it allowed for a higher degree of accuracy when compared with Léger's equation.

Paragraph Number 34

Constraints. It is important to acknowledge that the 20mSRT is a test requiring maximal effort. Special attention has to be paid during the course of the test as such, since today there are at least three major variants of the test available. Special attention should also be on the cassette or CDs to be used. Methodological variations in these cassettes (e.g. calling the stage number at the start versus the finish of each stage; using only full minutes versus both full minutes and half minutes to indicate completed stages) will mean that identical performances are reported in different ways.

Paragraph Number 35

The main limitation of the ANN is its complexity and its "black box" nature. The complexity of the ANN-equation may become rather inconvenient when applied in the field. However, even when using Léger's equation in a relatively big sample of subjects, a programmable device (spreadsheet) is required. Similarly, the estimation of VO_{2max} using the proposed ANN-equation can be done by means of a spreadsheet.

Paragraph Number 36

Some of the advantages of using an ANN-model will need some special attention: 1) Its capability of producing a nonlinear input-output mapping. A neural network computes a function, which maps its inputs variables with its output. A nonlinear relationship could exist between the input and the output variable. However, ANNs are especially suitable for modelling highly nonlinear maps; 2) its learning ability (adaptivity). A neural network can be trained to perform a specific task, for example, reproducing an unknown input-output mapping. There is always a neural network which will match your input variables as closely as possible with your output for a given set of data. In other words, you can approximate a given input-output map with a network as precise as you need; and 3) the ability to generalize. An ANN-model can be set up to be trained to produce a correct output for a given set of input data. The applications from the present investigation would be further increased by performing validation studies in specific populations and in different countries.

Paragraph Number 37

In conclusion, in this study an ANN-equation to estimate VO_{2max} from 20mSRT performance (stage), sex, age, weight and height in adolescents aged 13-19 years was developed and cross-validated. The newly developed equation was shown to be more accurate than Léger’s equation in the sample of adolescents studied. All variables included in the equation are usually measured in field studies, no specific equipment is required to collect the data, and is not time-consuming. The proposed model has been coded in a user friendly spread sheet.

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Figure legends

FIGURE 1. Neural network architecture. *Last half-stage completed.

FIGURE 2. Relationship between estimated VO_{2max} from Léger's equation and measured VO_{2max}. Crossed line represents the line of equality.

FIGURE 3. Relationship between estimated VO_{2max} from artificial neural network (ANN)-equation and measured VO_{2max}. Crossed line represents the line of equality.

FIGURE 4. Bland-Altman plot for the measured VO_{2max} and estimated VO_{2max} from Léger's equation. Central line represent the mean difference between equations (4.9 ml/kg/min) and broken lines represent upper and lower limits of agreement (± 95 Confident Intervals: -4.3 to 14.1 ml/kg/min).

FIGURE 5. Bland-Altman plot for the measured VO_{2max} and estimated VO_{2max} from artificial neural network (ANN)-equation. Central line represent the mean difference between equations (0.5 ml/kg/min) and broken lines represent upper and lower limits of agreement (± 95 Confident Intervals: -5.1 to 6.1 ml/kg/min).

Hand Span Influences Optimal Grip Span in Male and Female Teenagers

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Purpose: To determine if there is an optimal grip span for determining the maximum handgrip strength in male and female teenagers, and if the optimal grip span was related to hand span. If they are related then the second aim was to derive a mathematic equation relating hand span and optimal grip span.

Methods: One hundred healthy teenage boys (15.1 ± 1.1 y) and 106 girls (15.4 ± 1.3 y) were evaluated (age range, 13–18 y). Each hand was randomly tested on 10 occasions using 5 different grip spans, allowing a 1-minute rest between attempts. The hand span was measured from the tip of the thumb to the tip of the small finger with the hand opened as wide as possible.

Results: The results showed that an optimal grip span to determine the maximum handgrip strength was identified for both genders, and the optimal grip span and hand span correlated in both genders.

Conclusions: The results suggest that there is an optimal grip span to which the dynamometer should be adjusted when measuring handgrip strength in teenagers. The optimal grip span was influenced by hand span in both genders. For males the optimal grip span can be derived from the equation $y = x/7.2 + 3.1$ cm, and for females from the equation $y = x/4 + 1.1$ cm. where y is the optimal grip span and x is the hand-span. These equations may improve the reliability and accuracy of the results and may guide clinicians and researchers in selecting the optimal grip span on the hand dynamometer when measuring handgrip strength in teenagers. (J Hand Surg 2006;31A:1367–1372. Copyright © 2006 by the American Society for Surgery of the Hand.)

Key words: Dynamometry, handgrip strength, reliability, standardization, young subjects.

The handgrip strength test is a simple and economic test that gives practical information about muscle, nerve, bone, or joint disorders.[1–5] In adults, handgrip strength has been proposed as a possible predictor of mortality and the expectancy of being able to live independently.[6,7]

The measure of handgrip strength is influenced by several factors including age; gender; different angle of shoulder, elbow, forearm, and wrist[8–10]; posture[9,11]; and grip span.[9,11–15]

Another important factor affecting handgrip strength is hand span.[14,15] Several attempts have been made to find the optimal grip span that results in maximum handgrip strength and that increases reliable and reproducible handgrip strength in adult and elderly populations. Härkönen et al[14] showed that handgrip strength varied with handgrip position and was slightly affected by hand span. We have shown that there is an optimal grip span at which the maximum handgrip strength is obtained in adults.[15] Moreover, the optimal grip span has been shown to be influenced by individual hand span in adult women, but not in men. This can be in relation to the smaller hand span and/or less grip strength in women compared with men. Teenagers also present a smaller hand span and less handgrip strength than adults. Handgrip strength is a widely used test in experimental and epidemiologic studies.

The first aim of the present study was to determine if there is an optimal grip span for determining the maximum handgrip strength in male and female teenagers, and if that grip span is related to hand span. If these are related than the second aim was to derive a mathematic equation relating hand span and optimal grip span.

Materials and Methods

Subjects

One hundred boys (15.1 ± 1.1 y) and 106 girls (15.4 ± 1.3 y), with an age range of 13 to 18 years, volunteered to participate in the study after receiving information about the aim and clinical implications of the investigation. The study was conducted in 3 schools located in 3 different geographic areas of Spain. All of the teenagers included in the present study were in good health and free of any lesion or impairments in the upper limbs. The subjects were encouraged to do their best when performing the tests. The study was approved by the Review Committee for Research Involving Human Subjects at our University.

Methods

Measurement of hand span. Hand span was measured in both hands from the tip of the thumb to the tip of the small finger with the hand opened as wide as possible (Fig. 1). The precision of the measure was 0.5 cm, but the results of the hand span measurement were rounded to the nearest whole centimeter.

Measurement of handgrip strength. Handgrip strength was measured using a digital dynamometer (T.K.K. 5101 Grip-D; Takey, Tokyo, Japan), and the scores were recorded in kilograms. The reported precision of the dynamometer was 0.1 kg. When performing the measurement, subjects were instructed to maintain the standard bipedal position during the entire test with the arm in complete extension and not to touch any part of the body with the dynamometer except the hand being measured. Each subject performed (alternately with both hands) the test twice using different grip spans in random order, allowing a 1-minute rest between the measurements.[11] For each measure, the hand to be tested first was chosen randomly. The grip spans used were 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 cm. If the hand span was less than 20 cm then the highest grip span was rejected; if the hand span was more than 20 cm then the lowest grip span was rejected. For each hand the best result for each grip span was retained. For the hand dynamometer (Jamar; Fit Systems Inc., Calgary, Canada) the grip span equivalence for the different positions are as follows: position 1, 3.5 cm; position 2, 4.8 cm; position 3, 6.0 cm; position 4, 7.3 cm; and position 5, 8.6 cm.

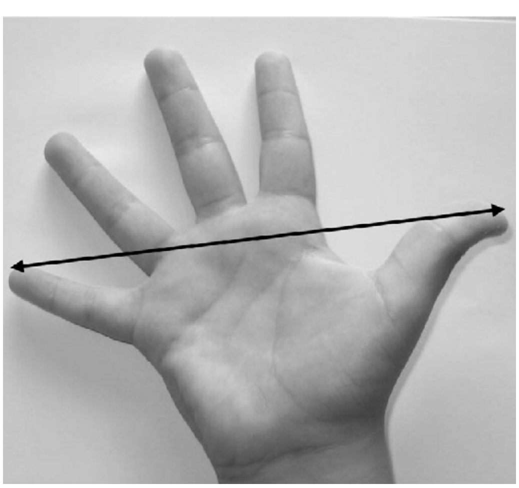

Figure 1. Measure of hand span (0.5-cm precision).

Determination of optimal grip span. The optimal grip span is the grip span at which the maximum handgrip strength is obtained. To determine the individual optimal grip span for each hand of each individual we first established the kind of association relating grip span and handgrip strength (ie, the results of handgrip strength obtained at the different grip spans). For that purpose, statistical software (SPSS v.14.0; SPSS Inc., Chicago, IL) was used. The association could be lineal, logarithmic, potential, exponential, or polynomial. In all subjects (except for 6) the association was statistically significant. All functions were considered, and the most relevant one was retained. The mathematic function of the relation was individually determined through the least-square fit and graphically represented (Fig. 2). In 190 of the patients it was quadratic and parabolic (corresponding to a second-degree polynomial equation). Once we defined the equation, the optimal grip span was calculated as $x/f'(x) = 0$, where x equals the optimal grip span (cm) and f(x) equals the handgrip strength (kg). In graphic terms, this corresponded to the maximum of the curves (Fig. 2). For nonpolynomial equations (n = 16), the optimal grip span was graphically determined and this corresponded to one of the extreme grip spans used for that particular subject. In those subjects in whom there was no association

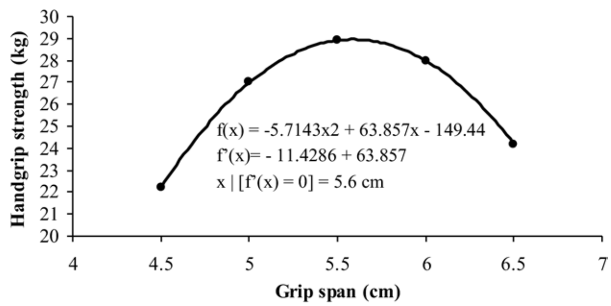

Figure 2. Association of handgrip strength and grip span in 1 subject. The maximum of the second-degree polynomial regression equation relating handgrip strength and grip span [f′(x)] was the optimal grip span for each hand of each individual. $f(x) = -5.7143x2 + 63.857x - 149.44$; $f'(x) = -11.4286 + 63.857$; $x \mid [f'(x) = 0] = 5.6$ cm.

between handgrip strength and grip span ($n = 6$), the average of the chosen grip spans was retained.

Determination of the optimal grip span for a given hand span.

By using statistical software (SPSS package v.14.0), we studied whether optimal grip spans were significantly related to hand spans ($p < .05$). In case of a significant relationship, we used the least-square fit to calculate the mathematic function relating both variables. This equation allows the establishment of the optimal grip span for a given hand span. In case of a nonsignificant relationship, the conclusion is that optimal grip spans are not related to hand spans.

Usefulness and reliability of the optimal grip span.

To confirm the usefulness of using the optimal grip span when measuring handgrip strength, an additional group of 21 teenagers (13 males, 8 females) ages 14 to 17 years volunteered to perform the handgrip strength test at 3 grip spans: optimal grip span, 1 cm below the optimal grip span, and 1 cm above the optimal grip span. Each subject performed (alternately with both hands) the test twice using different grip spans in a random order, allowing a 1-minute rest between the measurements.[11] For each measure, the hand to be tested first was chosen randomly. For each hand the best result at each grip span was retained.

To confirm the reliability of measurements of handgrip strength at the optimal grip span, 17 (13 males, 4 females) of the previous 21 teenagers less than 18 years of age performed the test at the optimal grip span 3 hours later. The subjects were advised not to perform strenuous exercise during the 3 hours preceding the second test.

Statistical Analysis

The normality of the distribution of the measured variables was ascertained by the Shapiro-Wilk test. The hand span, handgrip strength, and the optimal grip span obtained for each hand span was compared by 1-way analysis of variance (ANOVA). Bivariate correlation analysis was performed to examine the relationship between optimal grip span and hand span for each hand and gender. In case of an association, the mathematic function defining the association was calculated through the least-square fit.

For confirming the usefulness of measuring handgrip strength at the optimal grip span, 1 cm below the optimal grip span, and 1 cm above the optimal grip span, a 1-way ANOVA was used. The reliability coefficient of handgrip strength measured at the optimal grip span on 2 different occasions was calculated; values were compared through 1-way ANOVA and correlated through parametric bivariate correlation analysis. The α error was fixed at .05.

Results

All subjects completed the tests satisfactorily. The mean ± SD measured hand span was 21.0 ± 1.3 cm for males ($n = 100$) and 18.7 ± 1.1 cm for females ($n = 106$) ($p < .001$). Males obtained higher values of handgrip strength at each grip span than females ($p < .01$) (data not shown). In both genders, and for both hands, an optimal grip span was obtained. The optimal grip span for each hand span for males and females is presented in Tables 1 and 2, respectively. No significant differences were obtained between both hands for each hand span ($p > .70$). Because the optimal grip span was not different between the right and left hands, the mean value was retained and used for subsequent analysis.

Table 1. Optimal Grip Span Determined in Females (n = 106) for Each Hand Span

Hand Span, cm	Optimal Grip Span for Right Hand, cm	Optimal Grip Span for Left Hand, cm	Optimal Grip Span, cm*
16	5.0 ± 0.7	4.9 ± 0.5	5.0
17	5.6 ± 0.7	5.6 ± 0.6	5.6
18	5.5 ± 0.7	5.5 ± 0.6	5.5
19	5.8 ± 0.6	5.8 ± 0.5	5.8
20	5.8 ± 0.5	6.4 ± 0.6	6.1

The precision of the hand-span measurement was 0.5 cm and was rounded to the nearest whole centimeter. No significant differences were obtained between both hands for each hand span ($p > .70$).

*Optimal grip span obtained from the mean of the right- and left-hand optimal grip spans.

Table 2. Optimal Grip Span Determined in Males (n = 100) for Each Hand Span

Hand Span, cm	Optimal Grip Span for Right Hand, cm	Optimal Grip Span for Left Hand, cm	Optimal Grip Span, cm*
18	5.3 ± 0.7	5.6 ± 0.9	5.5
19	5.9 ± 0.5	5.7 ± 0.9	5.8
20	6.1 ± 0.6	6.0 ± 0.6	6.1
21	6.0 ± 0.6	6.0 ± 0.7	6.0
22	6.0 ± 0.6	6.2 ± 0.7	6.1
23	6.2 ± 0.8	6.3 ± 0.6	6.3

The precision of the hand-span measurement was 0.5 cm and was rounded to the nearest whole centimeter.

No significant differences were obtained between both hands for each hand span ($p > .70$).

*Optimal grip span obtained from the mean of the right- and left-hand optimal grip spans.

Table 3. Optimal Grip Span for Each Hand Span Calculated From the Equations Provided

Hand Span, cm	Optimal Male and Female Grip Span, cm	Optimal Male Grip Span, cm	Optimal Female Grip Span, cm
16.0	5.2	5.3	5.1
16.5	5.3	5.4	5.2
17.0	5.4	5.5	5.4
17.5	5.5	5.5	5.5
18.0	5.5	5.6	5.6
18.5	5.6	5.7	5.7
19.0	5.7	5.7	5.9
19.5	5.8	5.8	6.0
20.0	5.9	5.9	6.1
20.5	5.9	5.9	6.2
21.0	6.0	6.0	6.4
21.5	6.1	6.1	6.5
22.0	6.2	6.1	6.6
22.5	6.3	6.2	6.7
23.0	6.3	6.3	6.9

For males and females: $y = 0.16x + 2.66$ ($r = .92$, $p = .001$); males: $y = x/7.2 + 3.1$ ($r = .92$, $p = .01$); females: $y = x/4 + 1.1$ ($r = .93$, $p = .02$), where x is the hand span (maximal width between the thumb and small finger, with 0.5-cm precision), and y is the optimal grip span in cm.

In teenagers, hand span and optimal grip span showed a significant linear association ($y = 0.16x + 2.66$; $r = .92$, $p = .001$) where x is the hand span, and y is the optimal grip span at which the dynamometer should be adjusted before the test. The equation relating grip span as a function of hand span in males is formulated as $y = 0.1386x + 3.101$ ($r = .92$, $p = .01$). A simplification of this algorithm would be the following: $y = x/7.2 + 3.1$ (Fig. 3). The equation relating grip span as a function of hand span in females is formulated as $y = 0.25x + 1.09$ ($r = .93$, $p = .02$). A simplification of this algorithm would be the following: $y = x/4 + 1.1$ (Fig. 3). Table 3 shows the optimal grip span calculated from the equations provided, for each hand span in males and females.

The handgrip strength obtained at the optimal grip span was significantly higher ($p < 0.006$) than the strength obtained when the grip was set 1 cm below or 1 cm above the optimal grip span, in both hands and genders (Fig. 4).

Seventeen adolescents (13 males, 4 females) from the previous 21 repeated the test 3 hours later at the optimal grip span. The results showed a reliability coefficient of 0.98 and 0.96 for the right and left hands, respectively. Moreover, the 1-way ANOVA did not show a statistical difference between the test and retest results ($p = .45$ and $.53$ for the right and left hands, respectively). A significant correlation between the test and retest results was obtained for right ($r = .96$, $p < .001$) and left ($r = .92$, $p < .001$) hands at the optimal grip span.

Discussion

This study suggests that there is an optimal grip span to which the standard dynamometer should be ad-

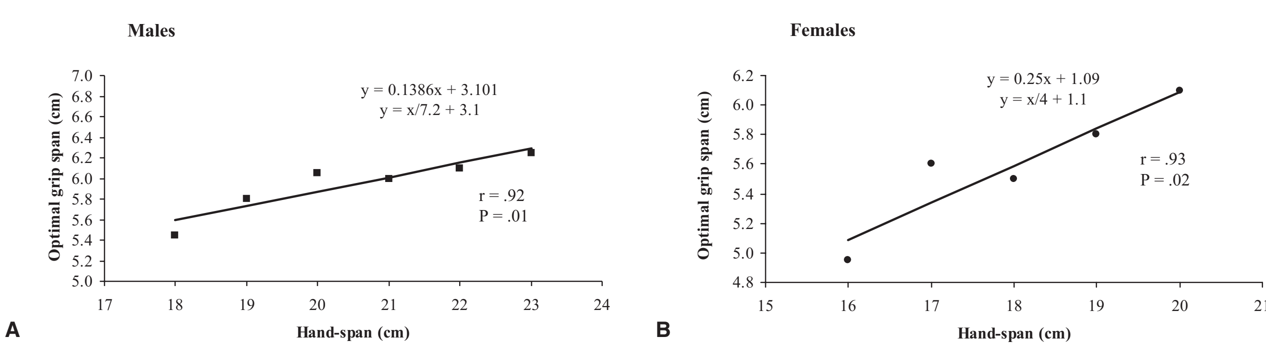

Figure 3. Association between hand span and optimal grip span in (A) males (n = 100) and (B) females (n = 106). (A) $y = 0.1386x + 3.101$; $y = x/7.2 + 3.1$; $r = 0.92$; $p = .01$. (B) $y = 0.25x + 1.09$; $y = x/4 + 1.1$; $r = 0.93$; $p = .02$.

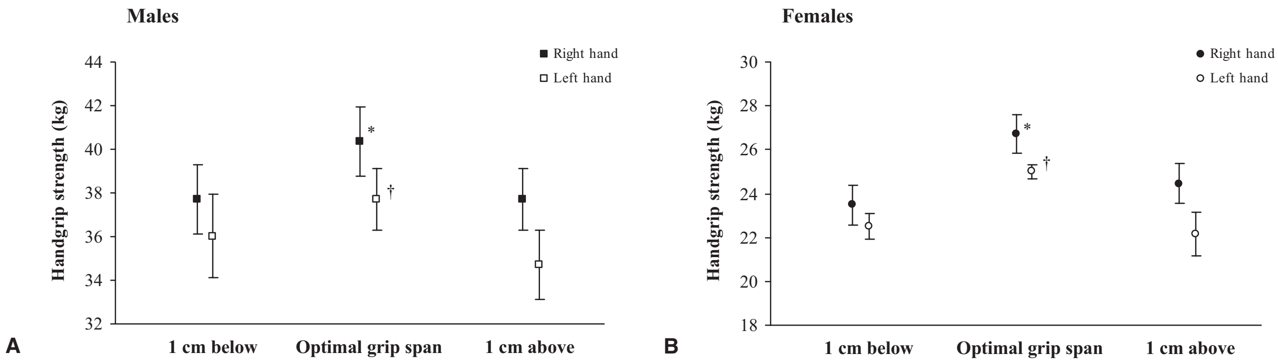

Figure 4. Handgrip strength measured for the right and left hands at the optimal grip span, 1 cm below the optimal grip span, and 1 cm above the optimal grip span in (A) males (n = 13) and (B) females (n = 8) (age range, 14–17 y). The values are mean ± standard error of the mean. *p < .005 compared with 1 cm below and 1 cm above the optimal grip span. †p < .006 compared with 1 cm below and 1 cm above the optimal grip span. (A) ■, right hand; □, left hand; (B) ●, right hand; ○, left hand.

justed when measuring handgrip strength in both males and females ages 13 to 18 years. In both genders the optimal grip span is influenced by hand span, which implies the need for adjustment of the grip span of the dynamometer to the hand span. For that purpose gender-specific equations are proposed, and are valid for both hands. Handgrip strength is a widely used test in experimental and epidemiologic studies in young people.

We have previously shown similar results in adult men and women.[12] In women the optimal grip span was influenced by hand span, and an equation to calculate the optimal grip span from the measure of the hand span was proposed ($y = x/5 + 1.5$). In men there was an optimal grip span for determining the maximum handgrip strength, but that optimal grip span was not hand-span dependent; therefore a fixed optimal grip span was proposed (5.5 cm). Teenagers have smaller hand spans and less handgrip strength compared with adults. Because of these differences one would expect that teenagers may need a different optimal grip span when measuring handgrip strength compared with adults. In the present study, the optimal grip span was influenced by hand span in both male and female teenagers, similar to what we found previously in adult women, but not in adult men. Adult men, usually already part of the workforce (mostly manual workers), might compensate for the hand-span effect with higher muscle mass and muscle strength in their forearm. This could partially explain the lack of association between the hand span and the optimal grip span in adult men.

Other studies also have shown a specific grip span at which the maximum handgrip strength is obtained.[11–13,16,17] Middle grip spans seem to favor greater forces than smaller or larger grips.[16] Oh and Radwin[17] reported that hand span affected maximal and submaximal handgrip strengths. They found that hand span affected grip strength, grip force, and exertion level. In another study,[13] the optimal grip span was suggested to be 5.0 to 6.0 cm for women and 5.5 to 6.5 cm for men. Similar values have been found recently in a larger study[11] in which the subjects performed the handgrip test at 3 different grip spans: one grip span, called the *standard grip span*, was calculated from the half distance between the index fingertip and the metacarpophalangeal joint flexion crease at the base of the thumb (men, 5.8 cm; women, 5.4 cm), the other grip spans were at −10% and +10% of the standard grip span. It was concluded that the grip span that achieves maximum handgrip strength is somewhere between the standard grip span and a 10% increase of that distance. The age and the number of participants in the earlier-mentioned studies make comparisons difficult.

Different measures of handgrip strength are currently used worldwide. There are some international physical fitness test batteries specifically designed for the young population that include a handgrip strength test (eg, EUROFIT test battery[18]). From a public health perspective it is important to standardize the procedure and increase the reliability because otherwise the measurement error may be too large to detect actual changes in strength; however, different kinds of dynamometers and postures might change the results. We do not know whether these findings can be directly transferred to measurements with other dynamometers.

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A Mediterranean Diet Is Not Enough for Health: Physical Fitness Is an Important Additional Contributor to Health for the Adults of Tomorrow

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Is It Only Diet?

Cardiovascular diseases are the major cause of death in Western Societies. Nevertheless, important differences exist among different populations and regions. In Europe, for instance, large differences exist in mortality from coronary heart disease and stroke. These diseases show a clear West-East and South-North gradient with high rates in Eastern and Northern Europe and lower rates in most Mediterranean countries [1]. Interestingly, large regional differences in ischemic heart disease and prevalence of cardiovascular risk factors occur within the same country and even within the same region. These differences are present both in countries with high and low incidence of cardiovascular disease [2].

Classical risk factors for cardiovascular disease include age, sex, hypertension, smoking, diabetes, elevated plasma low-density lipoprotein (LDL)-cholesterol, and low high-density lipoprotein (HDL)-cholesterol, lack of exercise and increased body fat. Nevertheless, the contribution of changes in these factors to trends in coronary event rates can only explain half of the cases [3]. Emerging independent risk factors include abdominal adiposity, high plasma levels of triglycerides, lipoprotein(a), modified LDL-cholesterol particles, homocysteine, several markers of inflammation, and thrombotic risk factors. It is quite possible that even taking all these factors into account, differences in coronary heart disease rates could not be fully explained.

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Table 1. Beneficial effects on health of practising regular physical exercise

Reduction in the risk of developing ischemic heart disease and other cardiovascular diseases
Reduction in the risk of developing obesity and diabetes
Reduction in the risk of developing (and control of) high blood pressure and dyslipidemia
Reduction in the risk of developing breast and colon cancer
Helps in the control of body weight and improves 'body image'
Tonifies muscles and preserves or increases muscular mass
Strengthens bones and joints
Increases coordination and neuromotor responses; reduces the risk of falls
Improves immune system activity
Reduces depression and anxiety
Promotes wellbeing and social integration

Growing evidence demonstrates that the Mediterranean life-style is beneficial to health. The evidence is stronger for coronary heart disease, but it also applies to stroke and some forms of cancer [4–6]. Diet is one outstanding component of the Mediterranean life-style. Large life-style and dietary variations occur in different regions and countries. In many of them, a progressive departure from the traditional Mediterranean life-style and diet is being observed [7]. In this departure, more affluent economies and younger subjects are, probably, more easily influenced.

In addition to diet, a sedentary life-style is a major risk factor for noncommunicable diseases (e.g. coronary heart disease, stroke, obesity, hypertension, type 2 diabetes, allergies and several types of cancers) and is close to overtaking tobacco as the leading cause of preventable death [8]. The protective effect of regular physical activity on the above mentioned diseases has been widely reported in young people, in adults and in the elderly. It is now well known that regular participation in moderate and vigorous levels of exercise can lead to many health benefits (table 1).

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The Spanish-Mediterranean Life-Style (and Diet)

The Spanish-Mediterranean life-style (and diet) is that usually followed by the inhabitants of Spain. Nevertheless, geographical, economic and social differences result in many different dietary practices and physical activity patterns. This, obviously, precludes a single definition of the Spanish-Mediterranean life-style. Nonetheless, regarding diet, there is a dietary pattern that is common in the different diets in the country. This traditional dietary pattern is composed of a cluster of basic foods that have been easily available in the region during centuries. This determines a diet that is high in fruits, green and root vegetables, bread, other forms of cereals, beans, nuts and seeds of different types. A common and outstanding characteristic of the Spanish Mediterranean diet is the use of olive oil which represents the more important source of fat in the diet. Animal products intake includes eggs, dairy products and poultry. There are significant variations in the intakes of fish, red meat (pork, beef, lamb) and meat derived products. Red wine and beer have been traditionally consumed. A main difference between the Spanish-Mediterranean diet and other Mediterranean diets is the lower intake of pasta and potatoes, and the higher intake of bread, legumes and fish [9, 10]. Many of these components may have an effect on cardiovascular risk factors, particularly by influencing the plasma lipid profile.

One specific characteristic of the traditional Spanish-Mediterranean life-style is the time spent outdoor which is favored by the favorable weather conditions. This may determine higher levels of physical activity. In Spain, the timetable for meals is different from other countries. The main meal of the day is usually taken in early afternoon (around 2–3 p.m.) and the dinner is late in the evening (around 10 p.m.) and rather light. There is a widely spread culture of eating outside the home in an informal way and usually standing up. It is the typical 'tapas' eating. These 'tapas' are taken between meals and occasionally represent an alternative to a more formal meal.

Diet and Physical Activity Interaction

Diet and physical activity interact in the development and prevention of ischemic heart disease and several other health conditions. Both factors affect the plasma lipid profile and body composition, and probably influence other risk factors. In fact, a physiological means of influencing diet-induced modifications of the plasma lipid profile and body fat content is physical activity [11]. Physical activity favorably influences all three components of the atherogenic lipoprotein phenotype: the HDL-cholesterol concentration may increase, LDL-cholesterol may decrease, and serum triglycerides can also be reduced [12, 13]. In addition, physical activity precludes body fat accumulation. Complex interactions between diet, physical activity, life-style, lipoprotein metabolism and other factors determine the development of atherosclerosis and its complications. These interactions may start early in life. In this way, adolescence is a critical period because it is at that time when the individual takes control of his/her own life-style and diet.

We have studied a representative sample of Spanish adolescents aged 13–18.5 years participating in the 'Alimentación y Valoración del Estado Nutricional de los Adolescentes' (AVENA) study (www.estudioavena.com). The

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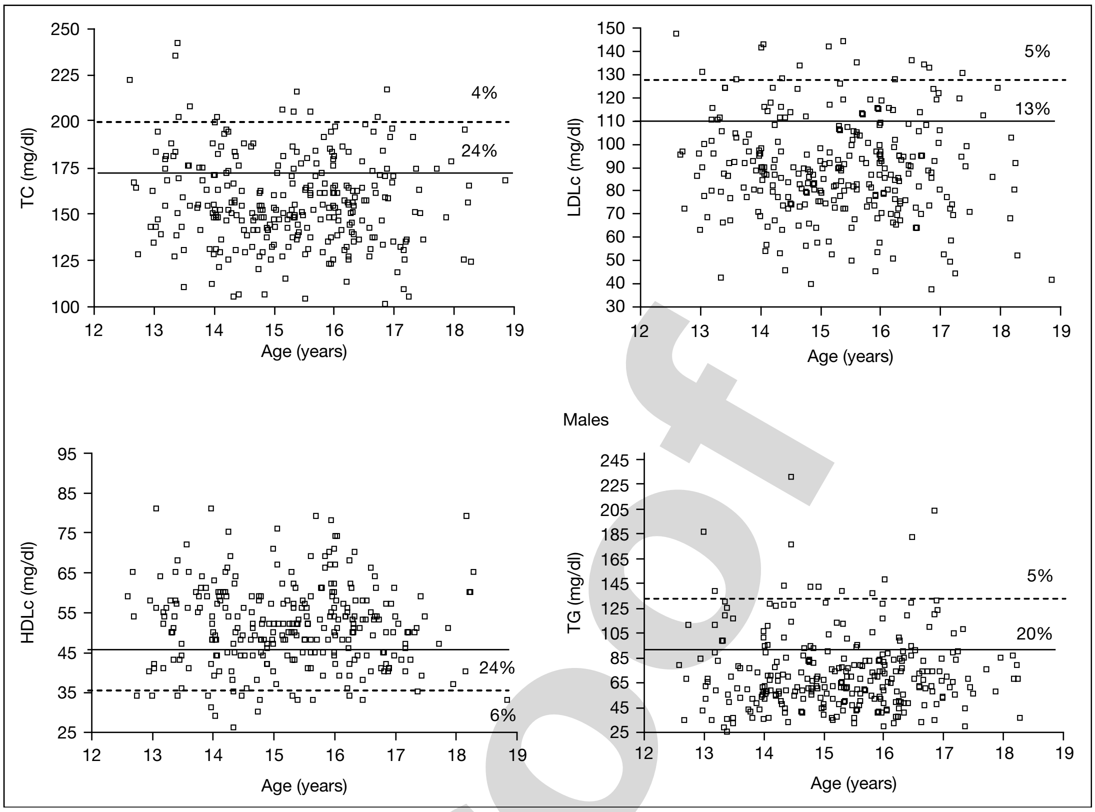

Fig. 1. Serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc) and triglycerides (TG) in male Spanish adolescents. Solid lines represent the limit level considered as healthy. Broken lines represent the limit level considered as unhealthy. Subjects between both lines can be considered as borderline.

AVENA study is a population-based cross-sectional survey conducted in five different geographic areas of Spain (Madrid, Murcia, Granada, Santander and Zaragoza), addressing genetic and environmental factors in relation to metabolic traits during adolescence [14]. Some interesting data regarding cardiovascular risk factors are being obtained from this study. Interestingly, we have observed a high prevalence of an unfavorable plasma lipid profile, both in boys (fig. 1) and girls (fig. 2) [15]. Similarly, it is well known the high prevalence of obesity in Mediterranean children and adolescents (fig. 3) [16]. These results underline the importance of implementing effective measures for preventing the deleterious consequences that these conditions are going to have in the health of tomorrow's

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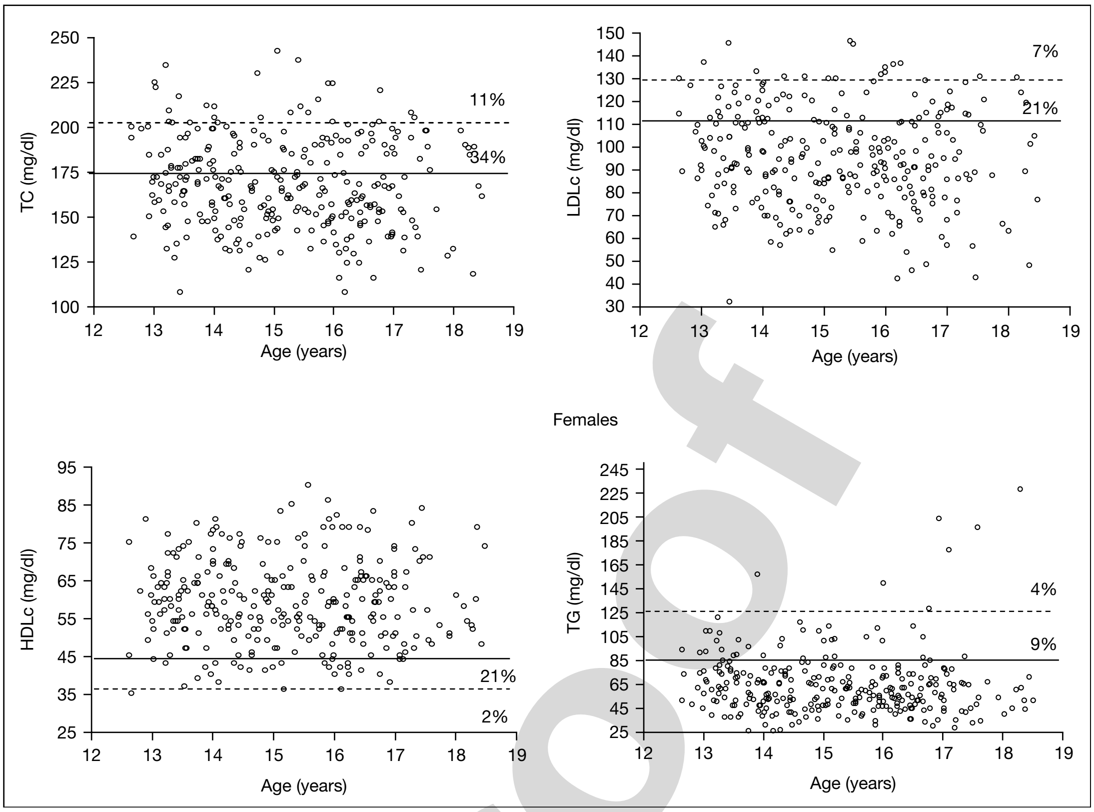

Fig. 2. Serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc) and triglycerides (TG) in female Spanish adolescents. Solid lines represent the limit level considered as healthy. Broken lines represent the limit levels considered as unhealthy. Subjects between both lines can be considered as borderline.

adults. One positive measure is the return to the traditional Spanish-Mediterranean diet; the other is to increase the levels of physical activity. In other words, the return to the traditional Spanish-Mediterranean life-style.

Physical Activity, Physical Exercise and Physical Fitness

Regular physical activity stimulates functional adaptation of all tissues and organs in the body, thereby also making them less vulnerable to lifestyle-related degenerative and chronic diseases. Physical activity refers to any body movement produced by muscle action that increases energy expenditure. Physical exercise refers to planned, structured, repetitive and purposeful physical activity.

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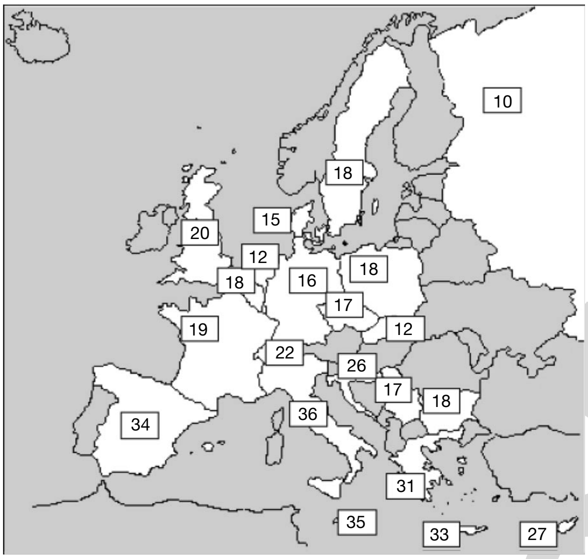

Fig. 3. Prevalence (%) of children (7–10 years old) with overweight in Europe [16].

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Physical fitness is the capacity to perform physical exercise. Physical fitness makes reference to the full range of physical qualities, e.g. aerobic capacity, muscle strength, speed, agility, coordination and flexibility. It can be understood as an integrated measurement of most, if not all, the body structures and functions (skeletomuscular, cardiorespiratory, hematocirculatory, psychoneurological and endocrine-metabolic) involved in the performance of physical activity and/or physical exercise [17]. Thus, being physically fit implies that the response of these functions and structures will be adequate. A person cannot be more physically fit than that allowed by the function or structure in lowest condition. Health-oriented physical fitness includes those components of physical fitness more associated with aspects of good health and/or disease prevention [17].

Physical Fitness as a Health Determinant

Aerobic capacity or cardiorespiratory fitness is one of the key components of physical fitness. Maximum aerobic capacity is expressed in terms of maximum oxygen consumption (VO_{2max}). The VO_{2max} can be expressed with respect to subject weight (ml/kg/min), in absolute terms (l/min) or in metabolic equivalents (METs). One MET is the energy expenditure at rest (~3.5 ml/kg/min). Thus, if a subject has a VO_{2max} of 42 ml/kg/min, he/she also

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has an energy expenditure of 12 METS (i.e. he/she is able to increase his/her resting energy expenditure 12-fold).

A number of important prospective studies have shown that VO_{2max} is the most important predictor of all-cause mortality, and in particular of cardiovascular death. This is true for both healthy persons and for people with cardiovascular disease [18], and for both men [19–21] and women [22, 23] of different ages [24]. An almost linear reduction in mortality is seen as the cardiorespiratory fitness increases [23, 24]. For each increase of 1 MET, there is a 12% increase in the life expectancy of men [24] and a 17% increase in women [22]. This is even more evident if cardiovascular mortality is considered alone, and again is true for both men [18, 21] and women [22, 23]. An inverse relationship has also been found between cardiorespiratory fitness and mortality due to cancer independently of age, alcohol intake, diabetes mellitus and tobacco [25–28]. Similarly, it has been shown that VO_{2max} is associated with insulin sensitivity [29]; low VO_{2max} levels are also associated with metabolic syndrome (abdominal obesity, glucose intolerance, type II diabetes, hypertension, hyperlipidemia and insulin resistance) [30, 31]. High levels of cardiorespiratory fitness reduce the neuronal losses associated with aging [32] and protects against cognitive dysfunction [33].

Handgrip strength, assessed by the manual dynamometer test, is currently considered to be a reliable marker of health and well-being and a potent predictor of mortality and the expectancy of being able to live independently [34, 35]. Efforts are made to reduce the errors associated with its measurement in adolescents [36] and adults [37].

Physical Fitness and Cardiovascular Risk Factors in Mediterranean Adolescents

Cardiorespiratory Fitness and Traditional Cardiovascular Risk Factors

Cardiorespiratory fitness is a direct marker of physiological status and reflects the overall capacity of the cardiovascular and respiratory systems. Results from several cross-sectional studies have clearly shown strong negative associations between cardiorespiratory fitness and cardiovascular risk factors not only in adults but also in children and adolescents (table 2). In addition, results from prospective studies suggest that high cardiorespiratory fitness during childhood seems to provide more health protection in adulthood.

Associations between increased cardiorespiratory fitness and several cardiovascular risk factors have been repeatedly found. As it is known, elevated level of triglycerides is strongly associated with an increased risk of coronary artery disease. In Spanish adolescents (aged 13–18.5 years) it was found a

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negative correlation between cardiorespiratory fitness and triglycerides, especially in males (fig. 4). In females, a trend toward lower levels of triglycerides with increasing fitness was also observed. These findings concur with the results obtained in children and adolescents from other European countries (table 2). Indeed, a negative correlation between cardiorespiratory fitness and triglycerides has been found in Danish, Swedish and Estonian children from the European Youth Heart Study, which is also in agreement with findings from their American peers (table 2).

Similar associations were also observed between cardiorespiratory fitness and LDL-cholesterol. There was a trend indicating lower levels of LDL-cholesterol with higher levels of fitness in both male and females (fig. 5). These findings are noteworthy since it is known that LDL-cholesterol and their oxidized derivatives initiate and promote the atherosclerotic process, leading to the development of coronary artery disease.

Plasma HDL-cholesterol has anti-atherogenic proprieties with its concentration inversely related to risk of coronary artery disease. It is estimated that for every 1 mg/dl (0.026 mmol/l) increase in HDL-cholesterol, the risk for a coronary heart disease event is reduced by 2% in men and at least 3% in women. Cardiorespiratory fitness has been shown to be negatively correlated with HDL-cholesterol in children, adolescents and adult population. Figure 6 clearly shows the associations between fitness and HDL-cholesterol. This is also the case for fitness and apolipoprotein (apo) A-1 (fig. 7). Apo A-1 is the most abundant protein of HDL-cholesterol. An increase in the apo A-1 can lead to an increase of HDL-cholesterol. Alternatively, increased catabolism or removal of apo A-1 will lead to a reduction in plasma HDL-cholesterol levels.

A more favorable metabolic profile (computed with age and gender specific standardized values of triglycerides, LDL-cholesterol, HDL-cholesterol and fasting glycemia) with increased levels of cardiorespiratory fitness has also been shown in Spanish adolescents [13]. Figure 8 shows the association of cardiorespiratory fitness and metabolic profile in non-overweight and overweight adolescents. These results suggest that both fitness and weight management are necessary for the prevention of lipid-related cardiovascular risk in adolescents. In fact, the odds ratio for having an unfavorable lipid profile is increased in subjects with low cardiorespiratory fitness even after adjusting for age and waist circumference (fig. 9).

Cardiorespiratory Fitness and Emerging Cardiovascular Risk Factors

Cardiorespiratory fitness has also been associated with recently recognized cardiovascular risk factors such as low grade inflammation markers (e.g. C-reactive protein, fibrinogen, ceruloplasmin, complement factor C3 and C4) and homocysteine. Findings from the AVENA study suggest that cardiorespiratory

Table 2. Summary of recent studies examining the associations between cardiorespiratory fitness and health-related variables in children and adolescents

Study	Type of study	Subjects	Age	Outcome
Gutin et al. [38]	cross-sectional	boys = 116 girls = 166	14–18 years	boys and girls CRF was inversely associated with insulin concentrations, and the adverse impact of low CRF was greater in boys than in girls
Brage et al. [39]	cross-sectional	boys = 279 girls = 380	8–10 years	boys and girls CRF was inversely associated with insulin, TG, systolic BP, and skinfold thicknesses ($p \leq 0.033$). CRF was inversely associated with metabolic syndrome Z score ($p \leq 0.031$). CRF was positively associated with HDLc ($p = 0.002$)
Reed et al. [40]	cross-sectional	boys = 55 girls = 44	9–11 years	boys and girls CRF accounted for 37% of the variance in large artery compliance. Highest CRF quartile had greater compliance than children in the two lowest CRF quartiles, by as much as 34%
Eisenmann et al. [41]	cross-sectional	Boys = 416 Girls = 345	9–18 years	CRF and BMI showed an independent association with cardiovascular risk factors
Gutin et al. [42]	cross-sectional	boys = 187 girls = 211	14–18 years	higher CRF and lower fatness were associated with favorable lipid profile; for most of the variables, fatness was slightly greater than the influence of CRF

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Ruiz et al. [43]	cross- sectional	boys = 429 girls = 444	9–10 years	boys and girls CRF was inversely associated with insulin resistance, and skinfold thicknesses ($p < 0.001$). CRF was inversely associated with metabolic syndrome Z score ($p \leq 0.02$) CRF was negatively associated with TG in girls ($p = 0.026$)
Andersen et al. [44]	prospective	boys = 133 girls = 172	16–19 years to 24–27 years	boys and girls CRF was associated with cardiovascular disease risk factors; the probability for ‘a case’ at the first examination to be ‘a case’ at the second was 6.0
Boreham et al. [45]	prospective	boys = 251 girls = 203	12–15 – 20–25 years	boys and girls CRF was inversely associated with arterial stiffness
Eisenmann et al. [46]	prospective	boys = 36 girls = 12	15.9 –27.2 years	boys and girls adolescent CRF is related only to adult BMI, WC and %BF ($p < 0.05$).
Ferreira et al. [47]	prospective	boys = 175 girls = 189	13–36 years	boys and girls CRF changes were inversely associated with prevalence of metabolic syndrome

apo = Apolipoprotein; %BF = percentage of body fat; BMI = body mass index; BP = blood pressure; CRF = cardiorespiratory fitness; HDLc = high-density lipoprotein cholesterol; LDLc = low-density lipoprotein cholesterol; Lp(a) = lipoprotein (a); TC = total cholesterol; TG = triglycerides; WC = waist circumference; W/H = waist to hip ratio.

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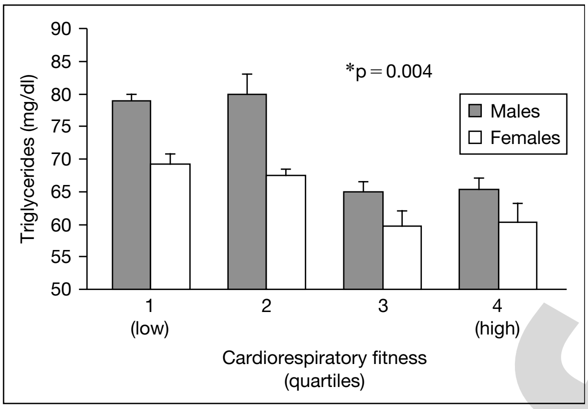

Fig. 4. Associations between triglycerides levels and cardiorespiratory fitness quartiles in male and female Spanish adolescents. Data shown as mean and SEM. * p for trend for males [13].

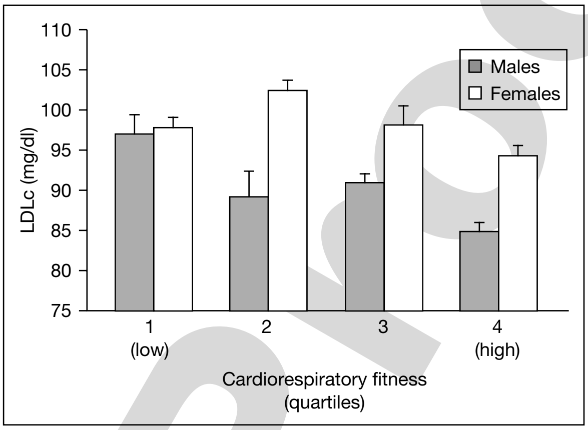

Fig. 5. Associations between low-density lipoprotein cholesterol (LDLc) levels and cardiorespiratory fitness quartiles in male and female Spanish adolescents. Data shown as mean and SEM [13].

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fitness is negatively associated with homocysteine levels in female adolescents after controlling for age, puberty, birth weight, smoking, socioeconomic status, sum of six skinfolds and methylenetetrahydrofolate reductase 677C>T genotype [48]. These findings support a previous study examining the relationship between homocysteine and cardiorespiratory fitness in adults [49]. Kuo et al.

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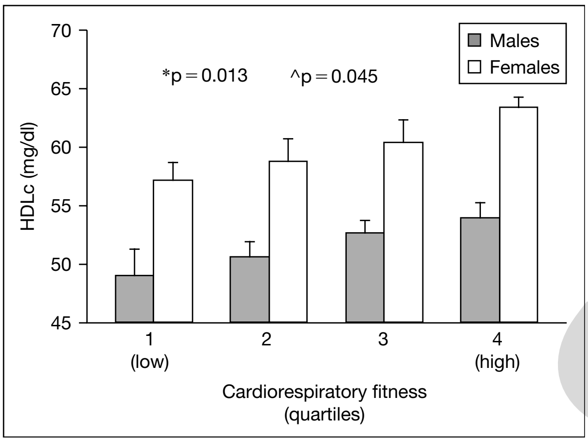

Fig. 6. Associations between high-density lipoprotein cholesterol (HDLc) levels and cardiorespiratory fitness quartiles in male and female Spanish adolescents aged. Data shown as mean and SEM. * p for trend for males; ^ p for trend for females [13].

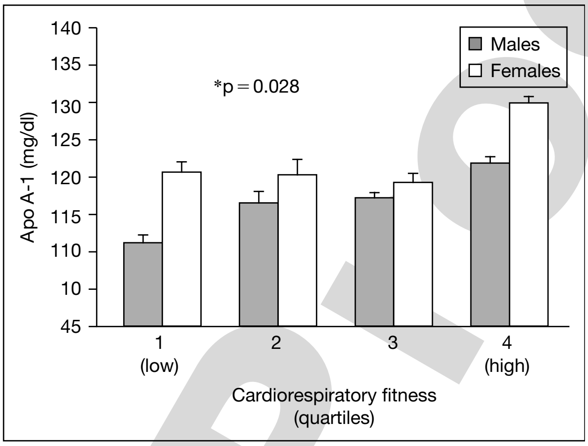

Fig. 7. Associations between apolipoprotein (apo) A-1 levels and cardiorespiratory fitness quartiles in male and female Spanish adolescents aged 13–18.5 years. Data shown as mean and SEM. * p for trend for males [13].

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[49] showed that high homocysteine levels were negatively associated with estimated cardiorespiratory fitness in adult women. Moreover, one longitudinal study followed 499 independent community-dwelling elderly for 3 years and found that people with elevated homocysteine levels were at an increased risk of decline in physical function [50]. However, cardiorespiratory fitness data

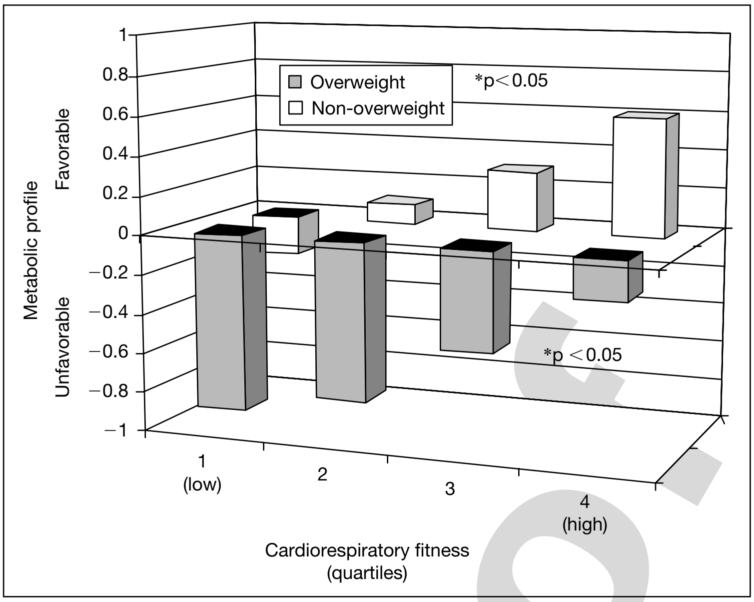

Fig. 8. Association between metabolic profile (computed with age- and gender-specific standardized values of triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol and fasting glycemia) and cardiorespiratory fitness quartiles in non-overweight and overweight Spanish adolescents. The higher is the metabolic profile the healthier. Weight categories were constructed following the International Obesity Task Force-proposed gender- and age-adjusted BMI cutoff points. Data shown as mean and SEM. *p for trend in both overweight and non-overweight categories [13].

were not provided and a gender comparison was not performed. These results should stimulate a debate on whether the metabolism of homocysteine could be one way in which the benefits of high cardiorespiratory fitness are exerted.

Cardiorespiratory fitness has also been shown to be associated with C-reactive protein and C3 in Spanish adolescents [51]. Similarly, Halle et al. [52] showed that cardiorespiratory fitness was negatively associated with low-grade inflammation in normal weight and overweight children aged 12 years. They reported that interleukin 6 levels were as low for obese and fit as for lean and unfit children, while the higher interleukin 6 levels were found in the obese and unfit group. In contrast, they also showed that tumor-necrosis factor-α seemed to be primarily dependent on cardiorespiratory fitness but not obesity since similar levels were found for non-obese as well as for obese children with low cardiorespiratory fitness.

Despite the evidence on the association between cardiorespiratory fitness and emerging and traditional cardiovascular risk factors in young and adult

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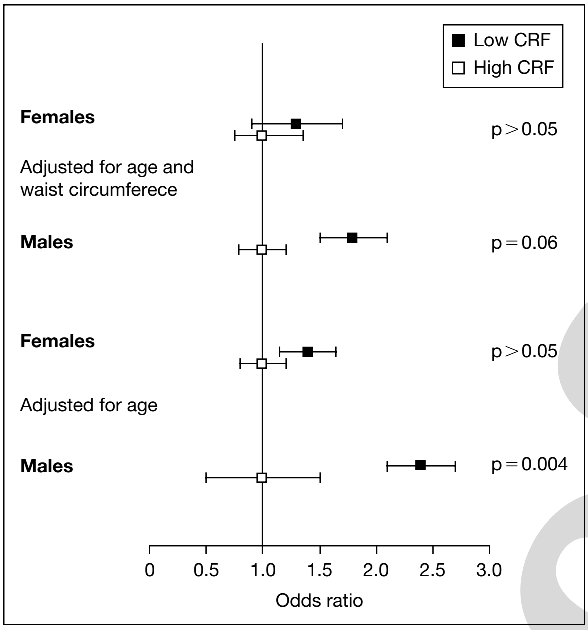

Fig. 9. Odds ratio for having an unfavorable lipid profile (triglycerides, high-density lipoprotein cholesterol, apolipoprotein A-1, apolipoprotein B-100, total cholesterol and high-density lipoprotein cholesterol ratio) in male and female Spanish adolescents.

populations, it is still uncertain whether health criterion values for cardiorespiratory fitness can be identified and the implications of these from the public health perspective. In this respect, several health-related threshold values of cardiorespiratory fitness have been suggested by world-wide recognized organizations [53, 54]. Based on expert judgment, the European Group of Pediatric Work Physiology considered a VO_{2max} of ≥35 ml/kg/min for girls and ≥40 ml/kg/min for boys as a 'Health Indicator' [53]. The Cooper Institute for Aerobics Research suggested ≥38 and ≥42 ml/kg/min for girls and boys respectively as a criterion standard for the 'Healthy Fitness Zone' [54]. The cut-off points proposed by the Cooper Institute for adolescents were extrapolated from the adults established thresholds.

Muscle Strength and Cardiovascular Risk Factors

Muscle strength refers to a balanced, healthy functioning of the musculoskeletal system and requires that a specific muscle or muscle group be able to generate force or torque. Muscle strength can also be a surrogate measure of both muscular endurance (that is the capacity to resist repeated contractions

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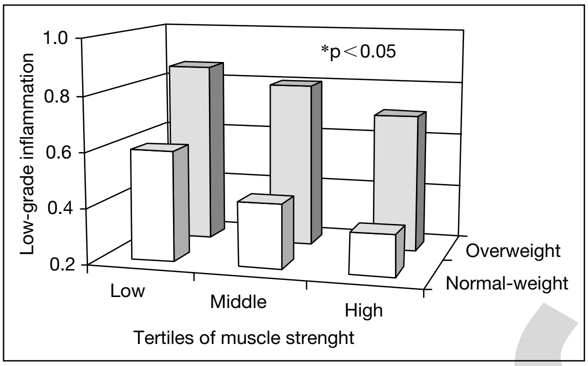

Fig. 10. Associations between tertiles of muscle strength and low-grade inflammation (estimated as a compound index of C-reactive protein and C3). These results are presented according to weight categories in Spanish adolescents. Weight categories were constructed following the International Obesity Task Force-proposed gender- and age-adjusted body mass index cut-off points. *p value from the regression analyses for the overweight category.

over time or maintain a maximal voluntary contraction for a prolonged period of time), and explosive strength (that is the capacity to carry out a maximal, dynamic contraction of a muscle or muscle group).

The importance of resistance exercise in promoting health and preventing disease has become increasingly recognized. Resistance exercise improves skeletal muscle strength and power, but also contributes to the prevention and management of atherosclerotic coronary heart disease, hypertension, diabetes, and overweight and obesity in adults. Muscle strength has been suggested to be inversely associated with all-cause mortality in men and women, independent of cardiorespiratory fitness levels [55]. However, little is known whether the health benefits of resistance exercise are independent of, or additive to, those already established for large muscle dynamic aerobic activity. Results from the AVENA study revealed significant associations between muscle strength and low-grade inflammation. It is known that low-grade inflammation seems to play a role in the pathogenesis of atherosclerosis from early ages, suggesting that preventive measures should start early in life. Figure 10 shows the associations between muscle strength and a compound index of low-grade inflammation integrated by C-reactive protein and C3, according to weight categories. Regression analysis was performed on muscle strength and the logarithmic of this index as continuous variables separately for non-overweight and overweight; however, in figure 10 they are broken into tertiles to illustrate the nature of the association. C-reactive protein has been recognized as cardiovascular risk factors, and nowadays there is increasing evidence about the link between C3 and cardiovascular disease.

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Taken together, these findings support the concept that cardiorespiratory fitness and muscle strength may exert a protective effect on the cardiovascular system from an early age [56]. In fact, it is biologically plausible that high fitness levels provides more health protection than low fitness, even in healthy adolescents as has been found in adults. Prospective studies are needed to examine the independent and joint effects of cardiorespiratory fitness and muscle strength in preventing the development of cardiovascular risk factors among young people and adults. For public health strategies and preventive purposes it is of interest to understand the associations between diet, cardiorespiratory fitness, muscle fitness and cardiovascular risk factors from early ages on.

Body Composition and Cardiovascular Risk Factors in Mediterranean Adolescence

Childhood overweight or obesity is associated with a variety of adverse consequences both at that early age and later in life. Since childhood obesity is now recognized as a worldwide epidemic [16] it seems relevant to study, in children and adolescents, the association between total body fatness and physical activity and physical fitness, particularly in regions which have been traditionally protected given their favorable diet and life-style. It is known that the amount of fatness is associated with a poor health status, but it is also important how the fat depots are distributed in the body. In fact, central body fatness is associated with coronary heart disease morbidity and mortality and coronary heart disease risk factors including dyslipidemia, insulin resistance and hypertension [57]. Most disturbances related to abdominal obesity have been established to show their onset during childhood [58]. Therefore, in this section both total and central/abdominal adiposity and their relationships with physical activity and cardiorespiratory fitness in children and adolescents are presented.

Physical Activity, Fitness and Total Body Fat

Total Body Fat in Young Populations

Defining obesity or overweight for children and adolescents is difficult, and there is no generally accepted definition of overweight or obesity for youths. However, body mass index (BMI) is a widely used tool to identify overweight and obese children and adolescents [59]. Indeed, we have observed elevated overweight and obesity prevalence in Spanish adolescents [60], similar to those observed in other European countries (including Mediterranean diet's countries) (fig. 3). Factors, such as socioeconomic status, seem to be inversely related to the overweight + obesity prevalence. Of note is that the rate of change in overweight prevalence in Spanish adolescents seems to be

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increasing [60]. Particularly in Mediterranean countries, there is an urge to establish preventive measures to fight against this alarming increasing in the childhood obesity epidemic. Measures to improve fitness could play a key role not only in obesity prevention but also in improving the health of the adults of tomorrow.

Although the BMI criterion is the most frequently used, an important number of adolescents classified as overweight or obese do not have really high adiposity (32.1% of females and 42% of males) [61]. Therefore, whenever possible, the anthropometric assessment of body composition should include a body fat estimation (i.e. from skinfold thickness). In this context, body fat reference data from Spanish adolescents have been recently reported, helping us to classify adolescents in comparison with a well-established reference population, and to estimate the proportion of adolescents with high or low adiposity amounts [62].

Associations of Total Body Fat with Physical Activity and Fitness

A sedentary lifestyle and a significant reduction in daily physical activity are one of the key factors of the obesity epidemic in the children and adolescents. By contrast, high cardiorespiratory fitness during childhood and adolescence has been associated not only with a healthier cardiovascular profile during these years but also later in life (table 2). For preventive purposes, it is of interest to understand the relative importance of the amount and intensity of physical activity not only on total body fat but also in cardiorespiratory fitness levels. New data have shown positive associations between physical activity, especially vigorous physical activity (>6 METs) and cardiorespiratory fitness (fig. 11) [63], as well as negative associations between vigorous physical activity and fatness in children and adolescents (fig. 12) [63, 64]. These results suggest that a certain level of physical activity needs to be achieved in order to improve the fitness and fatness status. Vigorous physical activity seems to be more relevant in increasing fitness and reducing body fat in young people. From a public health perspective these findings are particularly relevant.

Physical Activity, Fitness and Body Fat Distribution

Body Fat Distribution in Young Populations

The study of fat distribution among children and adolescents is complex because there are marked changes in circumferences and skinfold thickness during growth and development [65]. The two types of fat depots are abdominal and truncal fat. In population studies, the best anthropometric marker of abdominal obesity is waist circumference. Waist circumference correlates well with intra-abdominal and subcutaneous fat measured by magnetic resonance

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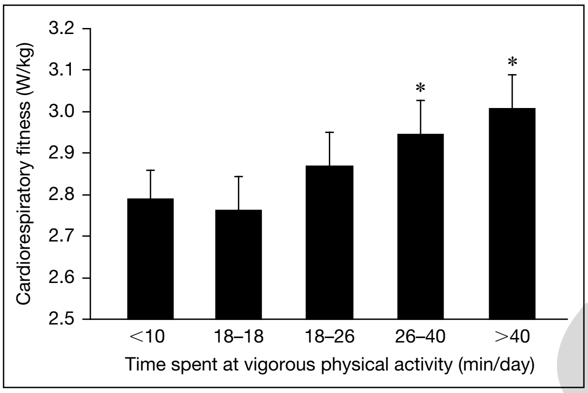

Fig. 11. Mean cardiorespiratory fitness stratified by time spent at vigorous physical activity in Swedish and Estonian children. Error bars represent 95% CIs. * A significant difference was observed between those who accumulated >40 min/day of vigorous physical activity and those who accumulated <18 min/day at this intensity level. ^ A significant difference was also observed between children who accumulated 26–40 min/day of vigorous physical activity compared to those who accumulated 10–18 min/day at this level of intensity [63].

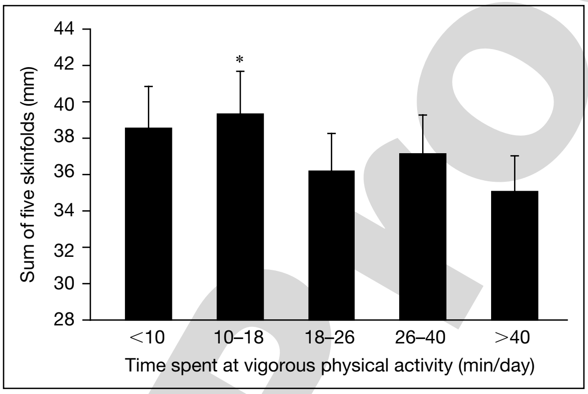

Fig. 12. Mean sum of five skinfolds (body fat) stratified by time spent at vigorous physical activity in Swedish and Estonian children. Errors bars represent 95% CIs. * A significant difference was observed between those who accumulated >40 min/day of vigorous physical activity and those who accumulated 10–18 min/day at this intensity level [63].

imaging in children and adolescents [66]. Waist circumference is a good tool for the screening of total body fat and the metabolic syndrome. That is why waist circumference is also a central feature of the metabolic syndrome and several diagnostic criteria of the condition include this marker in the definition

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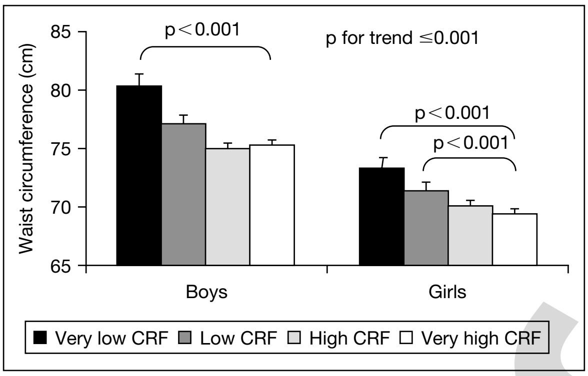

Fig. 13. Waist circumference (means ± standard error of the mean) according to cardiorespiratory fitness (CRF) quartiles in Spanish adolescents [69].

[67]. In the absence of a recognized definition of increased central adiposity in young people, the terms 'overweight' and 'obesity' referred to central adiposity are currently being arbitrarily defined. Therefore, they have been recently reported reference data for waist circumference and other fat patterning indices from a large sample of Spanish adolescents [Moreno et al., unpubl. data]. These data, together with data from other countries, will help to establish international central obesity criteria for adolescents, giving the possibility to estimate the proportion of adolescents with high or low regional adiposity.

Associations of Body Fat Distribution with Physical Activity and Fitness

It has been reported that, even within a given BMI category, children and adolescents with a large waist circumference are more likely to have abnormal cardiovascular disease risk factors compared to those with a small waist circumference [68]. Consequently, waist circumference could be a useful tool for studying obesity in adolescents. In adults, several studies have reported that individuals with better cardiorespiratory fitness have less abdominal fat and/or smaller waist circumferences for a given BMI [69]. However, the results obtained so far on the relationship between physical activity and central obesity in children and adolescents are also inconsistent.

Recent results from Spanish adolescents [70] suggest that moderate to high levels of cardiorespiratory fitness, but not self-reported physical activity, are associated with lower abdominal adiposity, as measured by waist circumference (fig. 13). However, given that the questionnaire used in that study does not provide either the intensity level of physical activity or the frequency of

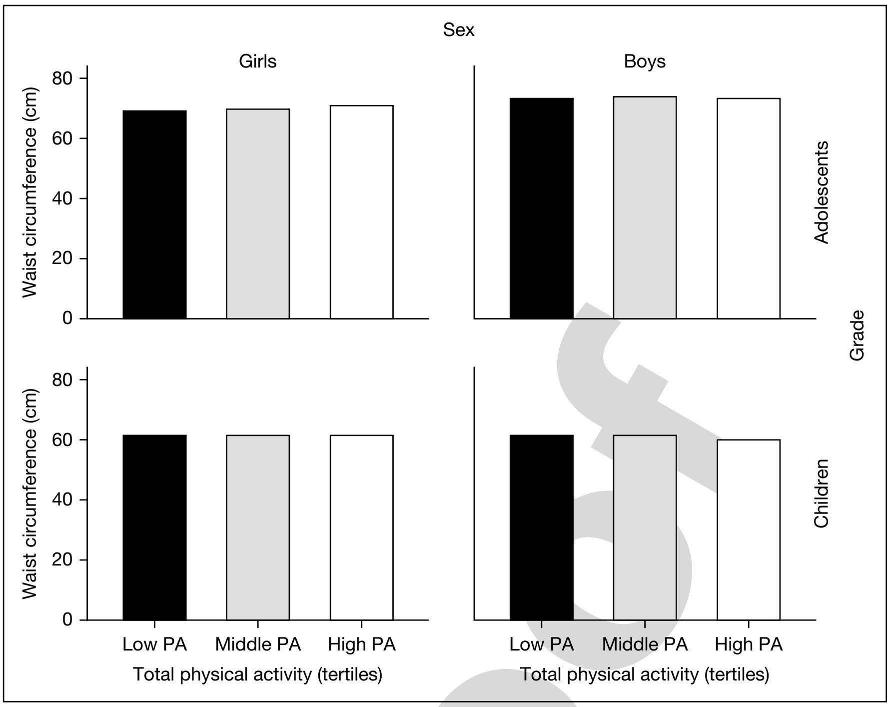

Fig. 14. Waist circumference (means) according to total physical activity (PA) in Swedish children and adolescents. Data were adjusted for age group and height. Total PA was not associated with waist circumference. No relationship was found between the PA intensities levels (moderate, vigorous, or moderate plus vigorous) and waist circumference.

physical activity, it is necessary to be cautious with the physical activity-related conclusions from that study. Research with objective methods for measuring physical activity, such as accelerometry, will provide accurate information about physical activity patterns (intensity, frequency and duration), helping to clarify this issue. In this context, data obtained from the Swedish part of the European Youth Heart Study using physical activity objectively measured, has recently obtained the same conclusion [Ortega et al., unpubl data]. Both in children and adolescents, physical activity (either total, moderate or vigorous) is not associated with abdominal adiposity, as measured by waist circumference (fig. 14). This is not the case with cardiorespiratory fitness. These results suggest that the beneficial effects of physical activity on abdominal adiposity may be explained by its association with cardiorespiratory fitness in children and adolescents.

Conclusion

Growing evidence demonstrates that the Mediterranean life-style is beneficial to health, especially for coronary heart disease, stroke and some forms of cancer. Diet is one outstanding component of the Mediterranean life-style. But it is not only diet – physical activity is another critical component. Diet and physical activity interact in the development (or prevention) of coronary heart disease and several other health conditions. These interactions may start early in life. In this way, adolescence is a critical period because it is at that time when the individual takes control of his/her own life-style and diet. Large life-style and dietary variations occur in different regions and countries. In many of them, a progressive departure from the traditional Mediterranean life-style and diet is being observed. In Spain, a high prevalence of an unfavorable plasma lipid profile has been observed both in boys and girls. Similarly, the high prevalence of obesity in Mediterranean children and adolescents is well known. But it is not only physical activity. Physical fitness (especially cardiorespiratory fitness and muscle strength) is strongly associated with cardiovascular risk factors. For public health strategies and preventive purposes it is of interest to understand the associations of diet, physical activity and fitness on cardiovascular risk factors from early ages on. It is important to implement measures for preventing the deleterious consequences that these conditions are going to have in the health of tomorrow's adults [71]. One positive measure is the return to the traditional Mediterranean diet; the other is to increase the levels of physical activity in order to improve physical fitness. Measures to improve fitness could play a key role in improving the health of the adults of tomorrow.

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