TESIS DE LA UNIVERSIDAD DE ZARAGOZA

2011

33

Luis A. Gracia Marco

Masa ósea y su relación con la actividad física, condición física y marcadores del metabolismo óseo en adolescentes

Departamento

Fisiatría y Enfermería

Director/es

Ortega Porcel, Francisco B.

Moreno Aznar, Luis

Vicente Rodríguez, Germán

ISSN 2254-7606

Prensas de la Universidad Universidad Zaragoza

Universidad Zaragoza
1542

Tesis Doctoral

MASA ÓSEA Y SU RELACIÓN CON LA ACTIVIDAD FÍSICA, CONDICIÓN FÍSICA Y MARCADORES DEL METABOLISMO ÓSEO EN ADOLESCENTES

Autor

Luis A. Gracia Marco

Director/es

Ortega Porcel, Francisco B.
Moreno Aznar, Luis
Vicente Rodríguez, Germán

UNIVERSIDAD DE ZARAGOZA

Fisiatría y Enfermería

2011

Tésis Doctoral Europea [European PhD Thesis]
2011

DEPARTAMENTO DE FISIATRÍA Y ENFERMERÍA

ESCUELA UNIVERSITARIA DE CIENCIAS DE LA SALUD
UNIVERSIDAD DE ZARAGOZA

ZARAGOZA, ESPAÑA

MASA ÓSEA Y SU RELACIÓN CON LA ACTIVIDAD FÍSICA, CONDICIÓN FÍSICA Y MARCADORES DEL METABOLISMO ÓSEO EN ADOLESCENTES

BONE MASS AND ITS ASSOCIATION WITH PHYSICAL ACTIVITY, PHYSICAL FITNESS AND BONE METABOLISM MARKERS IN ADOLESCENTS

LUIS A. GRACIA MARCO

ZARAGOZA, JUNIO DE 2011

“A todos aquellos que han creído en este trabajo.

Especialmente a Yoli”

MASA ÓSEA Y SU RELACIÓN CON LA ACTIVIDAD FÍSICA, CONDICIÓN FÍSICA Y MARCADORES DEL METABOLISMO ÓSEO EN ADOLESCENTES

BONE MASS AND ITS ASSOCIATION WITH PHYSICAL ACTIVITY, PHYSICAL FITNESS AND BONE METABOLISM MARKERS IN ADOLESCENTS

DIRECTORES DE TESIS

Dr. Luis A. Moreno Aznar	**Dr. Germán Vicente Rodríguez**	**Dr. Francisco B. Ortega Porcel**
Escuela Universitaria de Ciencias de la Salud	Facultad de Ciencias de la Actividad Física y el Deporte	Department of Bioscience and Nutrition / Department of Physiology
Universidad de Zaragoza, España	Universidad de Zaragoza, España	Karolinska Institute, Sweden / Universidad de Granada, España
MD, PhD	PhD	PhD

MIEMBROS DEL TRIBUNAL

Presidente	***Secretario***	
Dr. Jesús Mº Garagorri Otero	**Dr. Ignacio Ara Royo**	
Facultad de Medicina	Facultad de Ciencias de la Actividad Física y el Deporte	
Universidad de Zaragoza, España	Universidad de Castilla la Mancha, España	
MD, PhD	PhD	
Vocal 1	***Vocal 2***	***Vocal 3***
Dr. Alejandro Legaz Arrese	**Dra. Cecilia Dorado García**	**Dr. John Reilly**
Facultad de Ciencias de la Actividad Física y el Deporte	Facultad de Ciencias de la Actividad Física y el Deporte	Medical Faculty
Universidad de Zaragoza, España	Universidad de Las Palmas de Gran Canaria, España	University of Glasgow, Scotland
PhD	PhD	MD, PhD

Zaragoza, junio de 2011

LISTA DE PUBLICACIONES [LIST OF PUBLICATIONS]

La presente Tesis Doctoral es un compendio de trabajos previamente publicados. Las referencias de los artículos que componen el cuerpo de la Tesis Doctoral se detallan a continuación:

I. **Gracia-Marco L**; Tomás C; Vicente-Rodríguez G; Jiménez-Pavón D; Rey-López JP; Ortega FB; Lanza-Saiz R and Moreno LA. *Extra-curricular participation in sports and socio-demographic factors in Spanish adolescents. The AVENA study.* **J Sports Sci** 2010 Nov;28(13):1383-9.

II. **Gracia-Marco L**; Vicente-Rodríguez G; Valtueña, J Rey-López JP; Díaz Martínez AE; Mesana MI; Widhalm K; Ruiz JR; González-Gross M; Castillo MJ and Moreno LA. *Bone mass and bone metabolism markers during adolescence; The HELENA study*. **Horm Res Paediatr** 2010;74(5):339-50.

III. **Gracia-Marco L**; Ortega FB; Jiménez-Pavón D; Rodríguez G; Castillo MJ; Vicente-Rodriguez G and Moreno, LA. *Adiposity and bone health in Spanish adolescents. The HELENA study*. **Osteoporos Int** 2011 [Epub ahead of print]. *DOI 10.1007/s00198-011-1649-3.*

IV. **Gracia-Marco L**; Vicente-Rodríguez G; Casajús JA; Molnar D; Castillo MJ and Moreno LA. *Effect of fitness and physical activity on bone mass in adolescents. The HELENA study.* **Eur J Appl Phys** 2011 [Epub ahead of print*]*. *DOI 10.1007/s00421-011-1897-0.*

V. **Gracia-Marco L**; Moreno LA; Ortega FB; León F; Sioen I; Kafatos A; Martínez D; Widhalm K; Castillo MJ and Vicente-Rodríguez G. *Levels of physical activity that predict optimal bone mass in adolescents. The HELENA study*. **Am J Prev Med** 2011; Jun;40(6):599-607.

Prof. Dr. Luis A. MORENO AZNAR

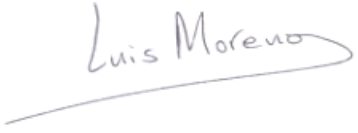
Prof. Titular de Universidad

Departamento de Fisiatría y Enfermería
E.U. Ciencias de la Salud
Universidad de Zaragoza

LUIS A. MORENO AZNAR, PROFESOR TITULAR DE LA UNIVERSIDAD DE ZARAGOZA

CERFIFICA:

Que la Tesis Doctoral titulada "Masa ósea y su relación con la actividad física, condición física y marcadores del metabolismo óseo en adolescentes" que presenta D. **LUIS A. GRACIA MARCO** al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2008-2011, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.



Fdo. Luis A. Moreno Aznar

En Zaragoza, a 13 de junio de 2011

Prof. Dr. Germán VICENTE RODRÍGUEZ

Prof. Titular de Universidad

Departamento de Fisiatría y Enfermería

E.U. Ciencias de la Salud

Universidad de Zaragoza

GERMÁN VICENTE RODRÍGUEZ, PROFESOR TITULAR DE LA UNIVERSIDAD DE ZARAGOZA

CERFIFICA:

Que la Tesis Doctoral titulada "Masa ósea y su relación con la actividad física, condición física y marcadores del metabolismo óseo en adolescentes" que presenta D. **LUIS A. GRACIA MARCO** al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2008-2011, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. Germán Vicente Rodríguez

En Zaragoza, a 13 de junio de 2011

Dr. Francisco B. ORTEGA PORCEL
Investigador Post-Doctoral

Department of Bioscience and Nutrition
Unit for Preventive Nutrition
Karolinska Institute (Sweden)

FRANCISCO B. ORTEGA PORCEL, INVESTIGADOR POST-DOCTORAL DEL MINISTERIO DE EDUCACIÓN Y CIENCIA EN EL INSTITULO KAROLISKA

CERFIFICA:

Que la Tesis Doctoral titulada "Masa ósea y su relación con la actividad física, condición física y marcadores del metabolismo óseo en adolescentes" que presenta D. **LUIS A. GRACIA MARCO** al superior juicio del Tribunal que designe la Universidad de Zaragoza, ha sido realizada bajo mi dirección durante los años 2008-2011, siendo expresión de la capacidad técnica e interpretativa de su autor en condiciones tan aventajadas que le hacen merecedor del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

Fdo. Francisco B. Ortega Porcel

En Estocolmo, a 13 de junio de 2011

CONTENIDOS [CONTENTS]

PROYECTOS DE INVESTIGACIÓN [RESEARCH PROJECTS]

El trabajo que se desarrolla a continuación, así como los artículos que forman parte de esta investigación, están basados en los siguientes proyectos de investigación:

1- **Estudio AVENA** *(Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles)*. Proyecto Nacional multicéntrico financiado por el Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo (nº 00/0015), y por varias empresas privadas: Panrico S.A., Madaus S.A., y Procter and Gamble S.A.

Página web: www.estudioavena.es

Coordinador/a: Ascensión Marcos

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

Página web: www.helenastudy.com

Coordinador: Luis A. Moreno

LISTADO DE ABREVIATURAS [LIST OF ABBREVIATIONS*]

AF	Actividad física
AFMI	Actividad física moderada-intensa
AFI	Actividad física intensa
ANCOVA	Análisis de la covarianza
ANOVA	Análisis de la varianza
AVENA	Alimentación y Valoración del Estado Nutricional de los Adolescentes
CEICA	Comité Ético de Investigación Clínica de Aragón
CF	Condición física
CMO	Contenido mineral óseo
COR	Característica operativa del receptor (curva)
CPM	"Counts" por minuto
DE	Desviación estándar
DXA	Absorciometría fotónica dual de rayos X
DMO	Densidad mineral ósea
HELENA-CSS	Healthy Lifestyle in Europe by Nutrition in Adolescence - Cross-sectional study
PINP	Propéptido aminoterminal del procolágeno de tipo I
SPSS	Paquete estadístico para ciencias sociales
β-CTX	Isómero β del telopéptido carboxiterminal del colágeno tipo I

* Abbreviations in English language are shown in the scientific papers included in the present Doctoral Thesis.

RESUMEN

La adolescencia representa una etapa crucial para el desarrollo de la masa ósea. En una sociedad con índices elevados de fracturas debido a la presencia de osteoporosis, es de gran importancia analizar los factores que permitan un óptimo desarrollo óseo, especialmente durante la pubertad, con el fin de prevenir dicha enfermedad en el futuro. A nivel general, los objetivos de la presente Tesis Doctoral son: 1) describir la situación actual de la práctica deportiva extra-curricular y su asociación con factores socio-demográficos y de composición corporal y 2) analizar el rol de la actividad física, condición física, marcadores del metabolismo óseo y tejidos blandos (masa grasa y magra) y su asociación con el contenido y densidad mineral ósea en distintas regiones corporales. Para el *objetivo 1* se obtuvieron mediciones en 2165 adolescentes españoles (13-18.5 años) que participaron en el Estudio AVENA (Alimentación y Valoración del Estado Nutricional de los Adolescentes). Para el *objetivo 2* se obtuvieron mediciones en 390 adolescentes españoles (12.5-17.5 años) que participaron en el Estudio HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence).

Los principales resultados y conclusiones son: I) El género, la edad y el nivel de educación del padre están asociados con la práctica deportiva extra-curricular. II) Nuestros resultados sugieren un dimorfismo sexual óseo específico de cada región corporal. Además, los varones presentan un mayor y duradero proceso de remodelado óseo durante la adolescencia. III) Los adolescentes con mayores niveles de adiposidad tienen una mayor masa ósea, no como resultado de su mayor masa grasa, sino de su mayor masa magra. IV) Un peor rendimiento en pruebas de condición física está asociado con un menor contenido mineral óseo, especialmente en adolescentes no activos. La asociación actividad física, condición física y masa ósea puede estar

influenciada entre otros, por factores como la genética y el tipo de deporte practicado. V) La cantidad e intensidad de actividad física recomendada por los organismos oficiales (≥60 minutos de actividad física moderada-intensa) parecen no garantizar un correcto desarrollo óseo. Sin embargo, unos pocos minutos al día de actividad física intensa permiten obtener adaptaciones óseas en regiones de relevancia clínica en el diagnóstico de la osteoporosis.

En resumen, existe una fuerte evidencia de que la práctica deportiva extra-curricular está asociada con factores socio-demográficos. Además, la actividad física (especialmente intensa), condición física y la masa magra están asociadas positivamente con la masa ósea.

ABSTRACT

Adolescence is key period for bone mass development. High incidence rates of fractures related to osteoporosis have been reached in the society. Therefore, to analyze the factors associated with an optimum bone development, especially during puberty, are of great importance in order to prevent this disease. In general, the aims of the present Doctoral Thesis are: 1) to describe the current status of extra-curricular participation in sports and its association with socio-demographic and body composition factors, and 2) to analyze physical activity, physical fitness, bone metabolism markers and soft tissues (fat and lean masses) and their association with bone mineral content and density in different body regions. For the aim 1, measurements were obtained in 2165 Spanish adolescents (13-18.5 years) that participated in the AVENA (Feeding and assessment of nutritional status of adolescents) Study. For the aim 2, measurements were obtained in 390 Spanish adolescents (12.5-17.5 years) that participated in the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study.

The main findings and conclusions are: I) Gender, age and father's education were linked to extra-curricular participation in sports. II) Our results support the evidence of dimorphic site-specific bone accretion between sexes. In addition, our results show that males present an increased and longer-lasting bone turnover compared to females. III) Adolescents with high levels of adiposity have an increased bone mass, but this is not the result of their high fat mass but of their high lean mass. IV) Lower levels of fitness were associated with lower bone mineral content, especially in non-active adolescents. The association among physical activity, physical fitness and bone mass could be influenced by several factors such as genetics or type of exercise or sport, among others. V) The recommended amount and intensity of physical activity (≥60 minutes of moderate-vigorous physical activity) seem to be insufficient stimulus to guarantee

increased bone mass. However, with some minutes per day of vigorous physical activity, bone adaptations could be obtained at regions with clinical relevance to osteoporosis.

In summary, there is strong evidence that extra-curricular participation in sports is associated with socio-demographic factors. In addition, physical activity (especially vigorous), physical fitness and lean mass are positively associated with bone mass.

1. INTRODUCCIÓN [INTRODUCTION]

1.1. Masa ósea

La osteoporosis es una enfermedad caracterizada por una disminución de la masa ósea y deterioro del tejido óseo [1], que a su vez está relacionada con el pico de masa ósea alcanzado antes de los 20 años de edad [2]. Se trata de un importante problema de salud en todo el mundo [3], que implica elevados costes para la sanidad y para la calidad de vida de las personas que la sufren. De hecho, cada año, alrededor de 2.7 millones de hombres y mujeres europeos sufren una fractura debido a la osteoporosis, estando además asociada con altos índices de morbilidad y mortalidad [4]. El coste económico de la osteoporosis en Europa es mayor al que supone cualquier tipo de cáncer (excepto el pulmonar) o enfermedades cardiorrespiratorias crónicas [4, 5], alcanzando los 36 mil millones de euros anuales [6].

Adquirir una elevada masa ósea durante la infancia y adolescencia determina en gran medida, la salud ósea adulta [7], lo que podría disminuir el riesgo de sufrir fracturas relacionadas con la osteoporosis en un 50% [8, 9]. El pico de masa ósea se alcanza en torno a los 25-30 años de edad, pero durante la adolescencia se observan los mayores incrementos en la masa ósea, especialmente entre los 11 y 14 años en chicas y entre los 14 y 16 años en chicos [10], pudiendo alcanzar el 51% del pico de masa ósea en este periodo de desarrollo puberal [11, 12] y el 37% de la densidad mineral ósea (DMO) de los adultos [13].

El percentil de masa ósea de una persona tiende a mantenerse constante a lo largo de toda su vida [14]. Esto significa que una persona con niveles bajos de contenido mineral óseo (CMO) y/o DMO en una determinada etapa, por ejemplo durante la adolescencia, es un serio candidato a padecer una osteopenia temprana y finalmente, desarrollar

osteoporosis. Por este motivo resulta crucial identificar aquellos niños y adolescentes con niveles reducidos de CMO y DMO, así como las causas que lo producen. Éste es sin duda el paso previo para poder actuar en la mejora de los niveles de masa ósea y sentar las bases de una prevención temprana de osteoporosis, que actualmente, debido a la reducida eficacia en el tratamiento de esta enfermedad, es la mejor solución.

Tal y como se ha comentado anteriormente, la osteoporosis está asociada a altos índices de morbilidad y mortalidad, es por ello que hoy en día, la mineralización ósea está considerada como un grave problema de salud [15, 16].

Los niveles de CMO y DMO presentan un alto componente hereditario [17]. Algunos estudios muestran que un 70% de la masa ósea está determinada genéticamente [18]. Sin embargo, los factores ambientales y el estilo de vida también presentan importantes implicaciones osteogénicas. Algunos ejemplos son: la actividad física (AF) [19], la condición física (CF) [20], que puede ser un reflejo de la AF realizada a lo largo de la vida, y la nutrición (principalmente la ingesta de calcio) [21]. De lo anterior se desprende que es posible actuar para aumentar el CMO, pero también es cierto que, una vez adquirido, se puede perder [22]. Sin embargo, algunos estudios demuestran que es posible mantener los niveles de masa ósea si persisten las demandas de carga mecánica en el hueso [23], por ejemplo a través de ejercicio físico, el cual se ha relacionado positivamente con mayor contenido y adquisición de mineral óseo [24]. La práctica de ejercicio físico desde edades tempranas está debidamente justificada, no sólo a nivel de salud general sino también a nivel de salud ósea. En concreto, se ha observado que el esqueleto humano responde mejor al ejercicio durante el crecimiento [25]. Todo lo anteriormente descrito justifica la importancia del estudio de la masa ósea durante la adolescencia.

1.2. Asociación entre la masa ósea y los tejidos blandos

Son diversos los autores que han estudiado el efecto de la masa grasa y/o magra en el desarrollo de la masa ósea. La masa magra se considera un factor predictivo importante del CMO así como del incremento óseo que tiene lugar durante la pubertad [26, 27]. Durante esta etapa de la vida, los incrementos en la masa magra están asociados con incrementos en la masa ósea. Según postula la Teoría del Mecanostato: *los músculos más grandes ejercen fuerzas mayores de tracción/tensión en los huesos en los que se insertan* [28, 29]. Tal y como se describe más adelante (apartado 1.4), tanto la AF como la práctica deportiva tienen un efecto directo en la ganancia de masa ósea, pero también indirecto a través del incremento de la masa magra debido al ejercicio [30, 31].

Mientras que el rol de la masa magra en el desarrollo de la masa ósea está claro, la relación entre la masa grasa y la masa ósea, presenta cierta controversia. Algunos estudios sugieren que la masa grasa está positivamente relacionada con la masa ósea en chicas [32, 33], lo cual se ha corroborado longitudinalmente [34, 35]. Sin embargo, otros estudios defienden que la masa grasa está negativamente asociada con la DMO en chicos [32, 36].

Diversos estudios han mostrado que los niños y adolescentes con sobrepeso y obesidad tienen mayores niveles de CMO y DMO [37-40], mientras que otros estudios muestran lo contrario [41, 42]. Muchos de estos estudios no han tenido en cuenta en sus análisis la presencia de algunas variables de confusión como puede ser la AF, la ingesta de calcio y la masa magra, entre otras.

En este contexto, la presente Tesis Doctoral aborda el tema teniendo en cuenta un conjunto de variables de confusión, considerando la siguiente hipótesis: Los adolescentes con sobrepeso y/o obesidad presentan un exceso de masa grasa que tienen

que soportar en las actividades cotidianas y del día a día. Dicha sobrecarga se asocia al desarrollo de la masa magra [43], que es la que finalmente tienen una incidencia directa en el desarrollo de la masa ósea. Es decir, la masa grasa incrementaría de forma indirecta la masa ósea mediante el incremento de masa magra. Esta hipótesis es especialmente importante en chicas debido a sus bajos niveles de AF [44] y masa magra, mayor masa grasa y a su mayor riesgo de sufrir osteoporosis en la edad adulta, en comparación con los chicos [45].

1.3. Marcadores del metabolismo del hueso

El desarrollo óseo depende de su actividad metabólica, que incluye tanto formación como resorción ósea (Tabla 1). Como consecuencia de estos dos procesos, aparece un fenómeno conocido como remodelado óseo, que podría definirse como un *proceso continuo mediante el cual el esqueleto es "renovado" a través de la resorción del hueso existente y la formación de hueso nuevo*. Este proceso se encuentra influenciado por diversas hormonas y micronutrientes.

Analizar el metabolismo del hueso durante el crecimiento puede darnos información temprana de las diferencias del desarrollo del hueso entre chicos y chicas. Se han descrito varios marcadores bioquímicos de actividad metabólica del hueso [46]. Algunos ejemplos de marcadores de formación ósea son la osteocalcina y el propéptido aminoterminal del procolágeno de tipo I (PINP), ambos medidos en suero y, como marcador de resorción ósea, el isómero β del telopéptido carboxiterminal del colágeno tipo I (β-CTX), medido tanto en suero como en orina.

Tabla 1. Marcadores bioquímicos del metabolismo del hueso

Marcadores bioquímicos del metabolismo del hueso	**Muestra analítica**	**Especificidad ósea**
Formación ósea		
Fosfatasa alcalina total (total ALP)	Suero	*
Fosfatasa alcalina específica del hueso (bone BALP)	Suero	***
Osteocalcina	Suero	**
Propéptido C-terminal del procolágeno de tipo I (PICP)	Suero	**
Propeptido aminoterminal del procolágeno de tipo I (PINP)	Suero	**
Resorción ósea		
Hidroxiprolina, total y dializable (Hyp)	Orina	*
Piridolina (Pyd)	Orina, suero	*
Deoxipiridolina (DPD)	Orina, suero	**
Telopéptido carboxilo terminal del colágeno tipo I (CTX-MMP)	Suero	**
Telopéptido amino terminal del colágeno tipo I (NTX-I)	Orina, suero	-
Telopéptido carboxilo terminal del colágeno tipo I (Isomerización) (CTX-I)	Orina, suero	-
Hidroxilisina glicosilada	Orina	*
Sialoproteína ósea	Suero	**
Fosfatasa ácida tartrato-resistente (TRACP)	Suero plasmático	**

Especificidad ósea: ***sólo encontrado en hueso; **encontrado en hueso y uno o dos tejidos más, * encontrado en hueso y tres o cuatro tejidos más, - encontrado en todos los tejidos.

Para más detalles: Seibel et al 2007 [47]

La osteocalcina es comúnmente utilizada como marcador de formación ósea y es además la mayor proteína no colagenosa de la matriz de hueso [46], siendo producida específicamente por los osteoblastos. Existen estudios que han asociado concentraciones elevadas de osteocalcina con altos niveles de formación e incluso remodelado óseo [48]. El PINP es un indicador específico de la deposición de colágeno tipo I, y por lo tanto puede ser definido como un marcador específico de formación ósea. Más del 90% del colágeno tipo I se encuentra en la matriz orgánica del hueso [49]. Durante la rotura del colágeno, se liberan fragmentos de diferentes tamaños del telopéptido C-terminal a la circulación. Se ha observado que las moléculas de colágeno tipo I pueden sufrir una β-isomerización del residuo del ácido aspártico dentro de sus telopéptidos [50, 51]. Como consecuencia de esta isomerización, dichas moléculas pueden encontrase en la matriz del hueso como linear (α) o C-telopépidos β-isomerizados (β-CTX) [52].

La nutrición y el estilo de vida son factores de gran importancia en el desarrollo y mantenimiento de una masa y remodelado óseo correcto [47]. Sin embargo, los estudios se muestran contradictorios en la asociación entre la práctica de AF y las concentraciones de estos marcadores [53]. En la actualidad, existe poca información sobre marcadores de formación y resorción ósea, así como valores de referencia en adolescentes españoles. La aplicación de lo anteriormente expuesto es especialmente importante en las chicas, ya que presentan un mayor riesgo que los chicos de desarrollar osteoporosis en la vida adulta [45].

1.4. Actividad física

La actividad física (AF) se define como *cualquier movimiento corporal producido por los músculos esqueléticos que resulta en un gasto de energía adicional al basal* [54]. La práctica regular de AF durante la adolescencia implica una serie de beneficios a nivel físico, mental y social, disminuyendo no sólo el riesgo de enfermedades cardiovasculares sino también el riesgo de diferentes tipos de cáncer [55], diabetes tipo 2 [56] y obesidad [57], estando además relacionada con el alivio de la depresión y ansiedad [58]. Varios estudios han mostrado una asociación positiva entre la práctica de AF (valorada subjetivamente mediante el uso de cuestionarios y entrevistas) y el desarrollo de la masa ósea [59-63]. Sin embargo, este tipo de métodos están asociados con casos de infra y/o sobreestimación de la práctica de AF [64-66]. Actualmente, hay muy pocos estudios que hayan mostrado la relación entre la AF valorada objetivamente (por ejemplo, mediante el uso de acelerometría) y el desarrollo de la masa ósea [67, 68], mostrando una asociación positiva. A pesar de todos los beneficios que en líneas previas se han descrito, el periodo de la adolescencia se caracteriza por un descenso en la práctica de AF [69, 70].

En la literatura científica se han descrito más de 30 métodos diferentes para valorar la AF, así como sus ventajas, desventajas y su aplicación en estudios con niños y adolescentes [71]. Dichos métodos se pueden clasificar en tres categorías: técnicas de referencia, técnicas objetivas y técnicas subjetivas.

1) Las **técnicas de referencia** más usadas son la observación directa, la evaluación del gasto energético total mediante el método de agua doblemente marcada y la calorimetría indirecta. Brevemente, la *observación directa* está reconocida como un método muy

práctico y apropiado [71], con un gran porcentaje de fiabilidad inter-observador en mediciones al mismo sujeto (84-99%). Sin embargo, resulta muy complicado hacer un seguimiento de un día completo. El método del *agua doblemente marcada*, es conocido como el método de referencia o "gold standard" para valorar el gasto energético total de forma directa [72], sin embargo, su elevado coste presenta una limitación. Finalmente, la *calorimetría indirecta* representa un método válido y preciso para evaluar el gasto energético a corto plazo [73]. Sin embargo, su elevado coste, las baterías de corta duración (2-3 horas) de los analizadores de gases portátiles actuales suponen una limitación. Además, no es válida para medir AF habitual, debido a la incomodidad que supondría hacer uso de la máscara y el analizador de gases. Existen otros métodos, como la calorimetría directa [74] que dado su coste económico y el tiempo que se invierte en realizarla, son menos usados.

2) Las **técnicas objetivas** están siendo cada vez más usadas. Entre ellas encontramos los monitores de frecuencia cardíaca, los acelerómetros y los podómetros. Brevemente, los *monitores de frecuencia cardíaca* presentan una interesante opción debido a su bajo coste y la posibilidad de emplearlos en estudios epidemiológicos [75]. Sin embargo, la frecuencia cardíaca está influenciada no sólo por la AF sino también por otros factores como la cafeína o medicamentos, ansiedad, estrés emocional, fatiga, composición corporal, el grupo muscular activado, entrenamiento, estado de hidratación, temperatura ambiente y humedad [71, 72]. Los *acelerómetros* constituyen la mejor opción como método objetivo de valoración de la AF. Tienen un coste reducido, son fáciles de usar y aportan una medición precisa de la cantidad e intensidad de la AF. Dependiendo del número de ejes en los que son capaces de registrar movimiento, se clasifican en 3 grupos: uniaxiales (eje longitudinal), biaxiales (ejes longitudinal y transversal) y triaxiales (ejes longitudinal, transversal y antero-posterior). La principal limitación que

tienen es que no es posible saber el tipo de AF que se está realizando (fútbol, saltos, tenis, etc). El *Actigraph GT1M* (uniaxial) es el instrumento más utilizado, ya que ha sido validado en adultos [76] y niños y adolescentes [77]. Como limitaciones de este modelo de acelerómetro encontramos que no son capaces de registrar movimiento en un eje que no sea el vertical (por lo que no registra movimiento en situaciones de bicicleta) y que no es sumergible, lo cual no permite registrar actividades en medio acuático, como la natación [78]. Recientemente se ha diseñado y comercializado una evolución del citado acelerómetro llamado *Actigraph GT3X+* que es triaxial y sumergible, con el cual se eliminarían algunas de las limitaciones anteriormente descritas.

En la actualidad existen pocos estudios que hayan evaluado la asociación entre la AF valorada objetivamente (acelerometría) y la masa ósea [67, 68].

3) El uso de **técnicas subjetivas** para la valoración de la AF está asociado con una elevada proporción de infra y/o sobreestimación [64-66]. Tal y como se ha mostrado anteriormente, son numerosos los estudios que han analizado el efecto de la AF en la masa ósea [19, 25, 59-63, 67, 68] y la mayoría, a pesar de los inconvenientes que ello conlleva, han usado cuestionarios y entrevistas para la valoración de la AF, concluyendo que existe una relación directa entre la cantidad de AF y masa ósea [59-63]. Dicha relación ha sido confirmada en estudios longitudinales [28, 30, 60].

Tres factores son imprescindibles a la hora de describir y analizar la AF: frecuencia, duración e intensidad. Las recomendaciones actuales de AF para niños y adolescentes, recomiendan una hora o más de AF de intensidad moderada-intensa (AFMI) al día [79]. En España, un estudio reciente mostró que sólo el 48% de los niños y adolescentes entre 6 y 18 años hacían al menos 60 minutos de AF diariamente [70], mientras que en Finlandia sólo el 23% de los adolescentes varones y el 10% de las chicas cumplían con

dichas recomendaciones [80] (en ambos estudios la AF se estimó mediante el uso de cuestionarios). Un reciente estudio en Estados Unidos usando acelerometría mostró que sólo el 42% de los niños y un 6-8% de los adolescentes hacían 60 minutos de AFMI al día [81], en el cual se aprecia el drástico descenso de práctica de AF entre estas dos etapas de la vida. Centrando la atención en las diferencias de práctica de AF entre sexos se observa que la balanza se inclina a favor de los chicos. Estas diferencias entre sexos han sido también observadas en diversos estudios [82-85].

Los niños y adolescentes pasan una gran parte del tiempo en la escuela o instituto, lo cual puede suponer un obstáculo para la práctica de AF. El currículum de educación física se convierte en el medio más importante para la promoción de AF en centros escolares [86], pero la actual estructuración no ayuda a la consecución de las recomendaciones [87, 88]. Es por ello que la práctica de AF o deporte extra-curricular se convierte en el complemento necesario para una buena salud, tanto cardiovascular como ósea, teniendo en cuenta además, que la práctica deportiva extra-curricular puede ser considerada como la principal fuente de AF (al menos de intensidad moderada) [85].

Existe suficiente evidencia científica que demuestra que el ejercicio está fuerte y positivamente asociado al desarrollo de masa magra y como consecuencia, al aumento de la masa ósea durante la infancia y adolescencia [25], principalmente cuando se dan situaciones de AF de alto impacto y que impliquen la propia carga corporal. Por lo tanto, el ejercicio puede incrementar de forma indirecta la masa ósea incrementando la masa magra. Las recomendaciones actuales de AF, principalmente orientadas hacia la salud cardiovascular, se rigen principalmente en base a los parámetros de duración e intensidad. Si bien, se especifica pero de forma muy general, que al menos 3 días por semana la AF realizada debe de incluir actividades para la mejora de la salud ósea y la fuerza muscular [79]. Actualmente, la literatura al respecto basa sus hipótesis en los

parámetros de duración e intensidad, pero no en relación al tipo de AF, el cual no se puede de determinar mediante el uso de acelerometría.

1.5. Condición Física

La condición física (CF) se define como un *conjunto de atributos relacionados con la habilidad de un sujeto para desarrollar actividades físicas que requieren condición aeróbica, resistencia, fuerza o flexibilidad y que está determinada por una combinación entre actividad regular y habilidad adquirida genéticamente* [54].

Condición física, ejercicio físico y AF son conceptos relacionados pero no intercambiables. La AF y la CF ya han sido definidas previamente. El concepto de ejercicio físico se define como un *subconjunto de AF planeada, estructurada y sistemática* [54].

La CF es evaluada frecuentemente en adolescentes [89, 90] y, recientemente, ha tomado un papel muy importante como marcador de salud actual pero también de salud futura (etapa adulta) [91]. Algunos tests adaptados de la Batería Eurofit se han usado con este propósito [89], entre los que se encuentran tests de fuerza, velocidad/agilidad y capacidad aeróbica, los cuales ya han sido descritos en la literatura científica [92]. La fiabilidad de estos tests en población adolescente ha sido demostrada y publicada [89, 93].

El ejercicio físico está relacionado con el desarrollo de la masa ósea debido al impacto que provoca en el hueso, cuyo hecho favorece el desarrollo [10, 30, 94], mantenimiento [95] y la fuerza del mismo [96]. Además, la CF está también relacionada con la masa ósea y con la acumulación de masa ósea durante el crecimiento [25], especialmente la

fuerza muscular [93]. Tal y como se ha comentado anteriormente, la CF es frecuentemente evaluada en adolescentes [89, 90], pero con el fin de conocer a grandes rasgos su salud general. Sin embargo, es posible evaluar no sólo la salud general del adolescente, sino también su salud cardiovascular [91, 97] y ósea [93, 98-101].

Desde el punto de vista científico, se puede pensar que una mayor AF durante el crecimiento resulte en una mejor CF [102] y, como consecuencia, en una mayor masa ósea. La masa ósea de un sujeto en un determinado momento de su vida es un reflejo de, entre otras cosas, el ejercicio realizado en el pasado, del mismo modo que la CF actual de una persona puede ser un reflejo de sus niveles de AF en el pasado. Por lo tanto, la relación entre la CF y masa ósea puede estar influenciada por la AF al menos de dos formas diferentes: 1) el efecto de la AF en la CF (efecto entrenamiento) y 2) el efecto de la AF en la masa muscular, la cual está fuerte y positivamente relacionada con el desarrollo de la masa ósea [93]. Además, tal y como se ha mostrado previamente, no es sólo importante la cantidad e intensidad, sino también el tipo de AF, siendo más osteogénicas aquellas actividades que impliquen fuertes impactos, la propia carga corporal y aquellas dirigidas a la mejora de la fuerza y/o masa muscular [25]. La Teoría del Mecanostato [28, 29] previamente descrita explica las asunciones previas.

Los artículos que forman parte de la Tesis Doctoral comparten una misma unidad temática, reflejándose en el título de la misma: **Masa ósea y su relación con la actividad física, condición física y marcadores del metabolismo óseo en adolescentes.**

2. OBJETIVOS

El objetivo general de la presente Tesis Doctoral ha sido mejorar el conocimiento sobre la relación entre la AF, CF, marcadores del metabolismo óseo y tejidos blandos con la masa ósea, en una muestra española de adolescentes españoles.

Los objetivos específicos de los cinco artículos (I-V) que componen esta Tesis Doctoral son los siguientes:

Artículo I. Describir las actividades deportivas extra-curriculares más practicadas por los adolescentes españoles y analizar la asociación de dicha práctica con factores socio-demográficos y masa grasa.

Artículo II. Describir la masa ósea y los marcadores del metabolismo óseo a lo largo de la adolescencia en función del sexo, rangos de edad y maduración sexual.

Artículo III. Valorar la asociación entre los tejidos blandos (masa grasa y magra) y la masa ósea y estudiar las diferencias de masa ósea entre grupos con diferente índice de masa corporal, todo ello controlando por un conjunto de variables de confusión.

Artículo IV. Analizar la asociación entre la CF y la masa ósea para un determinado nivel de AF y, entre la AF y la masa ósea para un determinado nivel de CF.

Artículo V. Analizar la asociación entre AFMI y AFI y la masa ósea en diferentes regiones (cuerpo entero, pelvis, espina lumbar y cadera total) y sub-regiones (trocánter, intertrocánter y cuello femoral).

2. AIMS

The overall aim of this Thesis was to increase the knowledge on the associations among physical activity, physical fitness, bone metabolism markers and soft tissues with bone mass in Spanish adolescents.

The specific aims of the five scientific papers (I-V) in which the present Doctoral Thesis is based are:

Paper I. To highlight the sports that are practiced most as extra-curricular activities and to determine its association with body fat and socio-demographic factors in Spanish adolescents.

Paper II. To describe bone mass and its metabolism through adolescence, according to age and Tanner stages in male and female adolescents.

Paper III. To assess the association of fat mass and lean mass on bone mass in adolescents, after adjusting for each other, physical activity and calcium intake. In addition, to assess the differences in bone mass by weight status in adolescents, after controlling for relevant confounders, such as physical activity, calcium intake and lean mass.

Paper IV. To analyze the effect of physical fitness performance on bone mass on a specific group of physical activity and, physical activity on a specific physical fitness performance group.

Paper V. To analyze the relationships between moderate to vigorous physical activity and vigorous physical activity and bone mass in different regions (whole body, pelvis, lumbar spine and total hip) and sub-regions (trochanter, intertrochanter and femoral neck).

3. MATERIAL Y MÉTODOS [MATERIAL AND METHODS]

La presente Tesis Doctoral está basada en datos de los estudios AVENA **(Artículo I)** y HELENA **(Artículos II, III, IV y V)**. En el caso del estudio HELENA, se valoró sólo la muestra de Zaragoza, ya que era la única en la cual se pudo valorar la masa ósea y el metabolismo óseo. A continuación se adjunta una copia del resumen del artículo descriptivo-metodológico de cada estudio:

- Alimentación y valoración del estado nutricional de los adolescentes españoles (estudio AVENA). Evaluación de riesgos y propuesta de intervención. I. Descripción metodológica del proyecto. [Feeding and assessment of nutritional status of Spanish adolescents (AVENA study). Evaluation of risks and interventional proposal. I. Methodology].

González-Gross M, Castillo MJ, Moreno L, Nova E, González-Lamuño D, Pérez-Llamas F, Gutiérrez A, Garaulet M, Joyanes M, Leiva A, Marcos A. *Nutr Hosp* 2003; 18(1): 15-28.

BACKGROUND: Adolescence is a decisive period in human life due to the multiple physiological and psychological changes that take place. These changes will condition both nutritional requirements and eating/physical activity behavior. It has been demonstrated that these "adolescence" factors are of significant influence in health status during adult life. Due to its importance and adequate development the project has been granted by the Fondo de Investigacion Sanitaria of the Institute of Health Carlos III.

OBJECTIVE: To develop a methodology to evaluate the health and nutritional status of a representative population of Spanish adolescents. Specific attention is paid to three specific health problems: obesity, anorexia nervosa/bulimia, dislipidemia.

METHODOLOGY: The following magnitudes will be studied: 1) dietary intake, food habits and nutrition knowledge; 2) daily physical activity and personal approach; 3) physical condition; 4) anthropometry and body composition; 5) hematobiochemical study: plasma lipid phenotypic and metabolic profile, blood cell counts; 6) genotipic profile of cardiovascular risk lipid factors; 7) immune function profile related to nutritional status; 8) psychological profile.

CONCLUSION: This project includes the co-ordinate activity of five Spanish centers of five different cities (Granada, Madrid, Murcia, Santander, Zaragoza). Each center is specialized in a specific area and will be responsible for the corresponding part of the study. From the data obtained, we will elaborate a specific intervention program in order to improve nutrition and neutralize the risk for nutritional related problems in adolescence. By this, we will contribute to improve the health status of the Spanish population in the new millennium.

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

LA Moreno, S De Henauw, M González-Gross, M Kersting, D Molnár, F Gottrand, L Barrios, M Sjöström, Y Manios, CC Gilbert, C Leclercq, K Widhalm, A Kafatos and A Marcos, on behalf of the HELENA Study Group. *Int J Obes (Lond)* 2008; 32 Suppl 5: S4-11.

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

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

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

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

3.1. Comités de ética

Estudio AVENA (Artículo I)

El protocolo del estudio se desarrolló según la normativa española y siguiendo las consignas éticas establecidas en la Declaración de Helsinki en 1975 (revisada en Hong Kong en 1989 y Edimburgo en 2000). Dicho protocolo fue aprobado por los comités de ética del Consejo Superior de Investigaciones Científicas (CSIC) y del Hospital Universitario Marqués de Valdecilla (Santander, España). Además, se concertó una reunión con los adolescentes para explicarles la naturaleza y propósitos del estudio. Finalmente, tanto adolescentes como padres entregaron un consentimiento firmado para participar en el estudio.

Estudio HELENA (Artículos II, III, IV y V)

El protocolo del estudio se desarrolló según la normativa española y siguiendo las consignas éticas establecidas en la Declaración de Helsinki en 1975 (revisión de Edimburgo en 2000). Dicho protocolo fue aprobado por el Comité Ético de Investigación Clínica de Aragón (CEICA). Finalmente, tanto adolescentes como padres entregaron un consentimiento firmado para participar en el estudio.

3.2. Muestra y diseño del estudio

Estudio AVENA (Artículo I)

El estudio AVENA es un estudio transversal y multicéntrico cuya toma de datos se llevó a cabo entre los años 2000-2002. La muestra comprendió 2856 adolescentes con edades comprendidas entre los 13 y 18.5 años, procedentes de Granada, Madrid, Santander, Zaragoza y Murcia. Con objeto de conseguir una muestra de la población lo más heterogénea posible, el estudio se realizó tanto en centros públicos como privados de enseñanza secundaria y/o formación profesional.

Tras la exclusión de 692 adolescentes debido a valores perdidos en variables de AF y estatus socio-económico, la muestra que se presenta en el **Artículo I** comprende 2164 adolescentes (1124 varones y 1040 mujeres).

La metodología completa del estudio ya ha sido previamente publicada en detalle [103].

Estudio HELENA (Artículos II, III, IV y V)

El estudio HELENA es un estudio transversal y multicéntrico cuya toma de datos se llevó a cabo entre los años 2006-2007. La muestra comprendió 3892 adolescentes con edades comprendidas entre los 12.5 y 17.5 años, procedentes de 10 ciudades europeas: Dortmund (Alemania), Viena (Austria), Gante (Bélgica), Lille (Francia), Atenas y Heraklion (Grecia), Pécs (Hungría), Roma (Italia), Estocolmo (Suecia) y Zaragoza (España). En la presente Tesis Doctoral se presentan datos de la ciudad de Zaragoza, ya que fue la única en la que se disponía de "Absorciometría Fotónica Dual de Rayos-X" (DXA) para la valoración de la composición corporal, en la que se incluye la masa ósea.

La muestra en la que se basan los **artículos II-V** es de 390 adolescentes (si bien puede existir alguna variación debido a la presencia de valores perdidos en alguna de las variables de análisis y/o criterios de inclusión establecidos para cada artículo).

En el protocolo del estudio HELENA, se estableció que se recogerían muestras de sangre en 1/3 de la muestra de estudio. Tras la obtención de dichas muestras, se analizaron marcadores del metabolismo óseo. Finalmente, se obtuvieron las siguientes muestras: Osteocalcina (n=97), Propeptido Aminoterminal del Procolágeno tipo I (PINP; n=92), C-telopépidos β-isomerizados (β-CTX; n=65). Este último también se analizó en muestra de orina (n=237).

Las características generales del estudio ya han sido previamente publicadas con detalle [104].

3.3. Métodos de medida

Estudio AVENA (Artículo I)

Composición corporal

Dos antropometristas en cada ciudad llevaron a cabo todas las mediciones. Uno midió el peso, altura y circunferencias, mientras que el otro midió los pliegues cutáneos. El peso y la altura se midieron siguiendo procedimientos estandarizados. Los pliegues cutáneos se midieron utilizando un *lipómetro Holtain* (0 - 40mm) en el lado izquierdo del cuerpo, siguiendo el procedimiento descrito por Lohman et al. [105]. Todas las mediciones se realizaron dos veces y en rotación. La media de ambas mediciones se tomo como resultado final. En los casos en que la diferencia entre ambas mediciones difería en más de 2 milímetros, se realizaba una tercera medición y se tomaba como valor final la media de los dos valores más próximos. El porcentaje de grasa corporal se calculó a partir de los pliegues cutáneos (tríceps y sub-escapular) mediante la fórmula de Slaughter [106], ya que es la que presenta un mayor correlación con la estimación de grasa corporal mediante DXA, tal y como se vio en un estudio con adolescentes de Zaragoza que participaron en el estudio AVENA [107]. Para el análisis, se clasificó a los adolescentes en cuatro grupos en función del percentil de grasa corporal (percentil <15, 15-85, 85-95 and >95), representando bajo peso, normo peso, sobrepeso y obesidad, respectivamente. Dichos percentiles se establecieron tomando como referencia la literatura al respecto [108, 109].

Actividad Física

Se diseñó un *cuestionario* específicamente para el estudio AVENA, el cual se entregó a los adolescentes para ser cumplimentado. Para la obtención de la variable de AF que se utiliza en el Artículo I de la presente Tesis Doctoral, se utilizó la siguiente pregunta del cuestionario: "¿Realizas alguna actividad físico-deportiva después del colegio/instituto? Además de esta pregunta, los adolescentes también indicaron cuáles eran los deportes que más practicaban.

Factores socio-demográficos

Para el análisis de los factores socio-demográficos se recogieron datos de: *edad* (13, 14, 15, 16 y 17-18.5 años), *género* (varones y mujeres), *educación de los padres* (graduado escolar, bachillerato, diplomatura y licenciatura) y *trabajo de los padres* (director de empresa, personal cualificado y personal no cualificado).

Estudio HELENA

Composición corporal (Artículos II, III, IV y V)

Para la valoración de la composición corporal se utilizó un escáner *DXA* (versión pediátrica del software QDR-Explorer, Hologic Corp., software versión 12.4, Bedford, MA, USA). Se realizaron mediciones a nivel de cuerpo entero, cadera y espina lumbar. Se registraron datos de masa ósea, grasa y magra [peso corporal – (masa grasa + masa ósea)]. El DXA se calibró usando un fantoma de espina lumbar siguiendo las recomendaciones del fabricante. Para el análisis de cuerpo entero, los adolescentes se tumbaron en decúbito supino y el escáner se realizó a alta resolución [100]. La masa magra (g), grasa (g), área total (cm^2) y la densidad mineral ósea (DMO; g/cm^2) se calcularon a partir del análisis total y regional del cuerpo entero. El contenido mineral óseo (CMO) se calculó utilizando la fórmula CMO = DMO · area. El análisis regional de las extremidades superiores e inferiores así como el de la región pélvica ha sido descrito y publicado anteriormente [110]. Además, se llevaron a cabo dos escáneres adicionales y necesarios para estimar la masa ósea en la espina lumbar (calculada como la media de las vértebras lumbares, L1-L4) y la masa ósea de las sub-regiones de la cadera (trocánter, intertrocánter y cuello femoral). La metodología más detallada ya ha sido publicada previamente [26].

Marcadores del metabolismo óseo (Artículo II)

Las concentraciones de osteocalcina, propéptido aminoterminal del procolágeno de tipo I (PINP), ambos medidos en suero y, del isómero β del telopéptido carboxiterminal del colágeno tipo I (β-CTX), éste último medido suero y orina, se determinaron mediante

inmunoensayo de electroquimioluminiscencia (ECLIA) usando un analizador *Elecsys 2010*. Para el control de calidad se usó *"Elecsys PreciControl Bone 1, 2, y 3".* Los controles con diferentes intervalos de concentración se realizaron con el test en determinaciones simples (1 vez cada 24 horas), con cada kit de reactivos y después de cada calibración.

Osteocalcina en suero. El test *Elecsys N-MID Osteocalcin* emplea dos anticuerpos monoclonales dirigidos específicamente contra los epítopes del fragmento N-MID y del fragmento N-terminal, detectando con ello tanto el fragmento N-MID estable como la osteocalcina (todavía) intacta. En el suero recogido se evitó la hemólisis, porque los eritrocitos contienen proteasa, la cual degrada la osteocalcina. Se usó la *Técnica Sándwich* con una duración total de 18 minutos cada ensayo. El intervalo de medición fue 0.500-300 ng/mL (definido por el límite de detección y el máximo de la curva principal). Los valores inferiores al límite de detección se indican como < 0.500 ng/mL. Los valores por encima del intervalo de medición fueron diluidos. La sensibilidad analítica (límite inferior de detección) fue < 0,50 ng/mL.

PINP total en suero. Se usó el *test inmunológico in vitro* para la determinación cuantitativa del PINP total en suero y plasma humanos. Este test determina el extremo aminoterminal, el así llamado PINP - propéptido aminoterminal del procolágeno de tipo 1. El test *Elecsys PINP* detecta ambas fracciones presentes en la sangre, razón por la cual se lo considera un análisis del PINP total. Se usó la *Técnica Sándwich* con una duración total de 18 minutos cada ensayo. El intervalo de medición fue 5-1200 ng/mL (definido por el límite de detección y el máximo de la curva principal). Los valores

inferiores al límite de detección se indican como < 5 ng/mL. Los valores por encima del intervalo de medición fueron diluidos. La sensibilidad analítica (límite inferior de detección) fue < 5 ng/mL.

β-CTX en suero y orina. El test *Elecsys β-CrossLaps/serum* está destinado específicamente para determinar los fragmentos reticulados isomerizados de colágeno de tipo I independientemente de la naturaleza de la reticulación. La especificidad del test se garantizó por la aplicación de dos anticuerpos monoclonales que reconocen los octapéptidos lineares β-8AA (EKAHD-β-GGR). El test *Elecsys β-CrossLaps/serum* cuantificó así todos los fragmentos de la degradación del colágeno de tipo I que contienen el octapéptido β-8AA isomerizado (β-CTX) por partida doble. Se usó la *Técnica Sándwich* con una duración total de 18 minutos cada ensayo. El intervalo de medición fue 0.010-6.00 ng/mL (definido por el límite de detección y el máximo de la curva principal). La hemólisis (Hb > 0,5 g/dL) produce concentraciones disminuidas falsas de β-CTX. La sensibilidad analítica (límite inferior de detección) fue 0.01 ng/mL La sensibilidad funcional fue 0.07 ng/mL.

Ingesta de calcio (Artículos II, III, IV y V)

La ingesta media de calcio se estimó a partir de *dos recuentos de 24 horas no consecutivos* usando el software *HELENA-DIAT (HELENA - dietary assessment tool)* [111]. Para la evaluación de la ingesta de calcio, se usaron las tablas de composición de alimentos publicadas por Farrán et al. [112].

Antropometría (Artículos II, III, IV y V)

Se aplicaron las pautas Internacionales de antropometría en adolescentes [105, 106, 113, 114]. Se pesó y talló a los sujetos en ropa interior y descalzos. El peso (kg) y altura (cm) fueron medidos con una báscula electrónica (marca *SECA 861*), precisión 100g, rango 0-150 kg y un estadiómetro (marca *SECA 225*), precisión 0.1cm, rango 70-200 cm., respectivamente.

Maduración sexual (Artículos II, III, IV y V)

Una médico designada para tal propósito realizó un *examen físico* para clasificar a los adolescentes en uno de los cinco estadíos de maduración sexual definidos por Tanner and Whitehouse [115].

Actividad Física (Artículos III-V)

Para la medición objetiva de la AF, se utilizaron *acelerómetros uni-axiales* (Actigraph GT1M, Manufacturing Technology Inc. Pensacola, FL, USA). Los adolescentes llevaron el acelerómetro debajo de la ropa, en la zona lumbar, sujetado mediante una cinta elástica que lo mantuvo estable durante 7 días consecutivos. Se comunicó a los adolescentes que debían quitarse el acelerómetro para dormir y en el caso de actividades que implicasen contacto con el agua (por ejemplo, natación). Con el fin de evitar perder datos de AF, junto con el acelerómetro se entregó un diario en el que los adolescentes debían de anotar las horas a la que se ponían o quitaban el acelerómetro, así como el motivo. Al menos 3 días de registro con un mínimo de 8 horas diarias se fijó como

criterio de inclusión, con el fin de obtener datos válidos de AF. Todos los acelerómetros se configuraron para registrar datos cada 15 segundos (epoch). El tiempo invertido en AF de intensidad moderada [3-6 equivalente metabólicos (METs)] se calculó tomando como valor el punto de corte de 2000-3999 "counts" por minuto (cpm) [116]. El tiempo invertido en AF intensa (AFI) (>6 METs) se calculó en función del punto de corte de 4000 cpm. Finalmente, el tiempo invertido en AF de intensidad moderada-intensa (AFMI) (>3METs) se calculó sumando ambos valores. Estos puntos de corte son similares a los empleados en estudios previos con niños y adolescentes [117]. En los **artículos IV y V**, se establecieron dos grupos con diferentes niveles de AF: <60 minutos de AFMI (adolescentes no activos) y ≥60 minutos de AFMI (adolescentes activos). Dicha clasificación se basó en las recomendaciones actuales de AF para niños y adolescentes, publicadas por el Departamento Estadounidense de Salud y Servicios Humanos y otras instituciones médicas [79, 118].

Condición Física (Artículo IV)

Todos los investigadores que desarrollaron el trabajo de campo leyeron un extenso y detallado manual diseñado para dicho cometido. Además, en enero de 2006, se llevó a cabo un "taller" en Zaragoza, con el objetivo de estandarizar la medición de cada uno de los test de CF. Todos los test de CF se realizaron en el mismo orden en todos los centros involucrados en el estudio, con el objetivo de minimizar al máximo posible la variabilidad que pudiese surgir y se dieron exactamente las mismas instrucciones en todos ellos para la realización de los test.

Los test de CF (fuerza, velocidad/agilidad y capacidad aeróbica) que se han utilizado en la presente Tesis Doctoral, han sido descritos en detalle [92], y están adaptados de la

Batería Eurofit. La fiabilidad de estos test en población adolescente ha sido demostrada y publicada [89, 93]. De forma breve, todos los test se realizaron dos veces y la mejor marca fue registrada, excepto en el caso del *20m shuttle run test* (conocido también como Course Navette), el cual sólo se realizó una vez al tratarse de una prueba de esfuerzo máximo. La fuerza de las extremidades superiores se evaluó mediante el *handgrip test* (Kg, test de prensión manual) usando un *dinamómetro digital* [Takei TKK 5101 digital dynamometer (range, 5–100 kg; precision, 0.1 kg)]. En el caso de las extremidades inferiores se realizó el *standing broad jump test* (cm, test de salto horizontal). La velocidad/agilidad se evaluó mediante el *4x10m shuttle run test* (seg) y el *30m running speed test* (seg, test de velocidad). Finalmente, la capacidad aeróbica fue evaluada mediante el *20m shuttle run test* (palier).

Análisis estadístico: Consideraciones generales

De forma general, las características descriptivas de los sujetos se presentan en forma de porcentajes para variables nominales y, como media ± desviación estándar (DE) para variables continuas, excepto en casos de variables que carecen de distribución normal, en los que se muestra la mediana y los intervalos intercuartiles (percentiles 25 y 75). Las diferencias entre sexos y grupos de edad de variables continuas se analizaron mediante *análisis de varianza* (ANOVA, con el sexo y la edad como factores fijos) o *test de muestras independientes* (t de Student). En aquellas variables que no mostraron una distribución normal, se usó el *test de Kruskal-Wallis* para determinar diferencias entre sexos. Las variables nominales se analizaron mediante el test *Chi-cuadrado*.

El *análisis de regresión logística binaria* se usó **(Artículo I)** para analizar la asociación entre la práctica de deporte extra-curricular con las variables independientes (edad, educación de los padres, trabajo de los padres y la grasa corporal).

El *análisis de covarianza* (ANCOVA) junto con el *test de Bonferroni* se usó **(Artículos II, III, IV y V)** para analizar diferencias entre variables óseas en función de grupos de edad, maduración sexual, índice de masa corporal, AF y CF, controlando los análisis por una serie de covariables.

La *regresión lineal* **(Artículo III)** se usó para analizar la asociación entre la masa grasa y/o magra con la masa ósea, estableciendo diferentes modelos de covariables que permitiesen establecer asociaciones entre ellas.

El análisis (curva) *Característica Operativa del Receptor* (COR) **(Artículo V)** se aplicó para calcular la cantidad necesaria de AFMI y AFI que permita clasificar a los adolescentes en grupos de masa ósea reducida o aumentada.

Los factores de interacción, como por ejemplo el sexo o los grupos de AFMI, fueron analizados previamente a la realización de los test anteriormente descritos. En aquellos casos en los que se encontraron interacciones significativas, los análisis se llevaron a cabo separadamente por sexo y/o grupos de AFMI. En los casos en los que esta condición no estuvo presente, todos los sujetos se analizaron juntos.

Todos los análisis se realizaron usando las versiones 14.0 y 15.0 de SPSS. Como norma general el nivel de significancia se fijó al 5%. Una información más detallada acerca del proceso estadístico empleado aparece descrita en detalle en cada uno de los artículos que componen la presente Tesis Doctoral.

4. REFERENCIAS [REFERENCES]

1. Ferrari SL. Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. *World Rev Nutr Diet*. 2005;95:35-51.

2. Bailey DA, Faulkner RA, McKay HA. Growth, physical activity, and bone mineral acquisition. *Exerc Sport Sci Rev*. 1996;24:233-66.

3. Hellekson KL. NIH releases statement on osteoporosis prevention, diagnosis, and therapy. *Am Fam Physician*. 2002;66:161-2.

4. Kanis JA, Burlet N, Cooper C, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int*. 2008;19:399-428.

5. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos Int*. 2006;17:1726-33.

6. Kanis JA, Johnell O. Requirements for DXA for the management of osteoporosis in Europe. *Osteoporos Int*. 2005;16:229-38.

7. Rizzoli R, Bianchi ML, Garabedian M, et al. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. *Bone*. 2010;46:294-305.

8. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. *Lancet*. 1993;341:72-5.

9. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *Bmj*. 1996;312:1254-9.

10. Bailey DA, McKay HA, Mirwald RL, et al. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the

university of Saskatchewan bone mineral accrual study. *J Bone Miner Res*. 1999;14:1672-9.

11. Gordon CL, Halton JM, Atkinson SA, et al. The contributions of growth and puberty to peak bone mass. *Growth Dev Aging*. 1991;55:257-62.

12. MacKelvie KJ, Khan KM, McKay HA. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? a systematic review. *Br J Sports Med*. 2002;36:250-7.

13. Derman O, Cinemre A, Kanbur N, et al. Effect of swimming on bone metabolism in adolescents. *Turk J Pediatr*. 2008;50:149-54.

14. Loro ML, Sayre J, Roe TF, et al. Early identification of children predisposed to low peak bone mass and osteoporosis later in life. *J Clin Endocrinol Metab*. 2000;85:3908-18.

15. Moreno LA, Gonzalez-Gross M, Kersting M, et al. Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutr*. 2008;11:288-99.

16. De Henauw S, Gottrand F, De Bourdeaudhuij I, et al. Nutritional status and lifestyles of adolescents in a public health perspective. The HELENA Project - Healthy Lifestyle in Europe by Nutrition in Adolescence. *J Publ Health*. 2007;15:187-97.

17. Kelly PJ, Eisman JA, Sambrook PN. Interaction of genetic and environmental influences on peak bone density. *Osteoporos Int*. 1990;1:56-60.

18. Cheng S, Volgyi E, Tylavsky FA, et al. Trait-specific tracking and determinants of body composition: a 7-year follow-up study of pubertal growth in girls. *BMC Med*. 2009;7:5.

19. Branca F, Valtuena S. Calcium, physical activity and bone health--building bones for a stronger future. *Public Health Nutr*. 2001;4:117-23.

20. Mattila VM, Tallroth K, Marttinen M, et al. Physical fitness and performance. Body composition by DEXA and its association with physical fitness in 140 conscripts. *Med Sci Sports Exerc*. 2007;39:2242-7.

21. Vicente-Rodriguez G, Ezquerra J, Mesana MI, et al. Independent and combined effect of nutrition and exercise on bone mass development. *J Bone Miner Metab*. 2008;26:416-24.

22. Bonjour JP, Chevalley T, Rizzoli R, et al. Gene-environment interactions in the skeletal response to nutrition and exercise during growth. *Med Sport Sci*. 2007;51:64-80.

23. Sanchis-Moysi J, Dorado C, Vicente-Rodriguez G, et al. Inter-arm asymmetry in bone mineral content and bone area in postmenopausal recreational tennis players. *Maturitas*. 2004;48:289-98.

24. Zanker CL, Osborne C, Cooke CB, et al. Bone density, body composition and menstrual history of sedentary female former gymnasts, aged 20-32 years. *Osteoporos Int*. 2004;15:145-54.

25. Vicente-Rodriguez G. How does exercise affect bone development during growth? *Sports Med*. 2006;36:561-9.

26. Vicente-Rodriguez G, Ara I, Perez-Gomez J, et al. Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. *Br J Sports Med*. 2005;39:611-6.

27. Courteix D, Lespessailles E, Loiseau-Peres S, et al. Lean tissue mass is a better predictor of bone mineral content and density than body weight in prepubertal girls. *Rev Rhum Engl Ed*. 1998;65:328-36.

28. Rauch F, Bailey DA, Baxter-Jones A, et al. The 'muscle-bone unit' during the pubertal growth spurt. *Bone*. 2004;34:771-5.

29. Schoenau E, Frost HM. The "muscle-bone unit" in children and adolescents. *Calcif Tissue Int*. 2002;70:405-7.

30. Vicente-Rodriguez G, Ara I, Perez-Gomez J, et al. High femoral bone mineral density accretion in prepubertal soccer players. *Med Sci Sports Exerc*. 2004;36:1789-95.

31. Daly RM, Saxon L, Turner CH, et al. The relationship between muscle size and bone geometry during growth and in response to exercise. *Bone*. 2004;34:281-7.

32. El Hage RP, Courteix D, Benhamou CL, et al. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. *Eur J Appl Physiol*. 2009;105:759-64.

33. Pietrobelli A, Faith MS, Wang J, et al. Association of lean tissue and fat mass with bone mineral content in children and adolescents. *Obes Res*. 2002;10:56-60.

34. Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. *J Clin Endocrinol Metab*. 2010;95:699-706.

35. Young D, Hopper JL, Macinnis RJ, et al. Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. *Osteoporos Int*. 2001;12:506-15.

36. El Hage R, Moussa E, El Hage Z, et al. Influence of age and morphological characteristics on whole body, lumbar spine, femoral neck and 1/3 radius bone mineral apparent density in a group of Lebanese adolescent boys. *J Bone Miner Metab* [Epub ahead of print].

37. Reid IR. Relationships among body mass, its components, and bone. *Bone*. 2002;31:547-55.

38. Cobayashi F, Lopes LA, Taddei JA. [Bone mineral density in overweight and obese adolescents]. *J Pediatr (Rio J)*. 2005;81:337-42.

39. Ellis KJ, Shypailo RJ, Wong WW, et al. Bone mineral mass in overweight and obese children: diminished or enhanced? *Acta Diabetol*. 2003;40 Suppl 1:S274-7.

40. Leonard MB, Shults J, Wilson BA, et al. Obesity during childhood and adolescence augments bone mass and bone dimensions. *Am J Clin Nutr*. 2004;80:514-23.

41. Goulding A, Taylor RW, Jones IE, et al. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int*. 2002;13:835-40.

42. Rocher E, Chappard C, Jaffre C, et al. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. *J Bone Miner Metab*. 2008;26:73-8.

43. Wells JC, Fewtrell MS, Williams JE, et al. Body composition in normal weight, overweight and obese children: matched case-control analyses of total and regional tissue masses, and body composition trends in relation to relative weight. *Int J Obes (Lond)*. 2006;30:1506-13.

44. Gracia-Marco L, Tomas C, Vicente-Rodriguez G, et al. Extra-curricular participation in sports and socio-demographic factors in Spanish adolescents: the AVENA study. *J Sports Sci*. 2010;28:1383-9.

45. Campion JM, Maricic MJ. Osteoporosis in men. *Am Fam Physician*. 2003;67:1521-6.

46. Lehtonen-Veromaa M, Mottonen T, Irjala K, et al. A 1-year prospective study on the relationship between physical activity, markers of bone metabolism, and bone acquisition in peripubertal girls. *J Clin Endocrinol Metab*. 2000;85:3726-32.

47. Seibel MJ. Bone turnover in nutrition-related disorders. *Wien Med Wochenschr*. 2007;157:582-8.

48. Delmas PD. Biochemical markers of bone turnover. *Acta Orthop Scand Suppl*. 1995;266:176-82.

49. Burgeson RE. New collagens, new concepts. *Annu Rev Cell Biol*. 1988;4:551-77.

50. Fledelius C, Johnsen AH, Cloos PA, et al. Characterization of urinary degradation products derived from type I collagen. Identification of a beta-isomerized Asp-Gly sequence within the C-terminal telopeptide (alpha1) region. *J Biol Chem*. 1997;272:9755-63.

51. Garnero P, Fledelius C, Gineyts E, et al. Decreased beta-isomerization of the C-terminal telopeptide of type I collagen alpha 1 chain in Paget's disease of bone. *J Bone Miner Res*. 1997;12:1407-15.

52. Kraenzlin ME, Seibel MJ. Measurement of Biochemical Markers of Bone Resorption. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of Bone and Cartilage Metabolism. San Diego, California (USA): Academic Press; 1999. p. 411-26.

53. Maimoun L, Sultan C. Effects of physical activity on bone remodeling. *Metabolism*. 2011;60:373-88.

54. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep*. 1985;100:126-31.

55. Slattery ML, Edwards SL, Ma KN, et al. Physical activity and colon cancer: a public health perspective. *Ann Epidemiol*. 1997;7:137-45.

56. Steanovv TS, Vekova AM, Kurktschiev DP, et al. Relationship of physical activity and eating behaviour with obesity and type 2 diabetes mellitus: Sofia Lifestyle (SLS) study. *Folia Med (Plovdiv)*. 2011;53:11-8.

57. Blair SN. Evidence for success of exercise in weight loss and control. *Ann Intern Med*. 1993;119:702-6.

58. Lavie CJ, Milani RV, O'Keefe JH, et al. Impact of exercise training on psychological risk factors. *Prog Cardiovasc Dis*. 2011;53:464-70.

59. Burrows M, Baxter-Jones A, Mirwald R, et al. Bone mineral accrual across growth in a mixed-ethnic group of children: are Asian children disadvantaged from an early age? *Calcif Tissue Int*. 2009;84:366-78.

60. Delvaux K, Lefevre J, Philippaerts R, et al. Bone mass and lifetime physical activity in Flemish males: a 27-year follow-up study. *Med Sci Sports Exerc*. 2001;33:1868-75.

61. Kemper HC, Twisk JW, van Mechelen W, et al. A fifteen-year longitudinal study in young adults on the relation of physical activity and fitness with the development of the bone mass: The Amsterdam Growth And Health Longitudinal Study. *Bone*. 2000;27:847-53.

62. McVeigh JA, Norris SA, Cameron N, et al. Associations between physical activity and bone mass in black and white South African children at age 9 yr. *J Appl Physiol*. 2004;97:1006-12.

63. Sundberg M, Gardsell P, Johnell O, et al. Physical activity increases bone size in prepubertal boys and bone mass in prepubertal girls: a combined cross-sectional and 3-year longitudinal study. *Calcif Tissue Int*. 2002;71:406-15.

64. Westerterp KR. Assessment of physical activity: a critical appraisal. *Eur J Appl Physiol*. 2009;105:823-8.

65. Bassett DR, Jr. Validity and reliability issues in objective monitoring of physical activity. *Res Q Exerc Sport*. 2000;71:S30-6.

66. Hagstromer M, Bergman P, De Bourdeaudhuij I, et al. Concurrent validity of a modified version of the International Physical Activity Questionnaire (IPAQ-A) in European adolescents: The HELENA Study. *Int J Obes (Lond)*. 2008;32 Suppl 5:S42-8.

67. Kriemler S, Zahner L, Puder JJ, et al. Weight-bearing bones are more sensitive to physical exercise in boys than in girls during pre- and early puberty: a cross-sectional study. *Osteoporos Int*. 2008;19:1749-58.

68. Tobias JH, Steer CD, Mattocks CG, et al. Habitual levels of physical activity influence bone mass in 11-year-old children from the United Kingdom: findings from a large population-based cohort. *J Bone Miner Res*. 2007;22:101-9.

69. Brodersen NH, Steptoe A, Boniface DR, et al. Trends in physical activity and sedentary behaviour in adolescence: ethnic and socioeconomic differences. *Br J Sports Med*. 2007;41:140-4.

70. Roman B, Serra-Majem L, Ribas-Barba L, et al. How many children and adolescents in Spain comply with the recommendations on physical activity? *J Sports Med Phys Fitness*. 2008;48:380-7.

71. Sirard JR, Pate RR. Physical activity assessment in children and adolescents. *Sports Med*. 2001;31:439-54.

72. Armstrong N, Welsman JR. The physical activity patterns of European youth with reference to methods of assessment. *Sports Med*. 2006;36:1067-86.

73. Gracia-Marco L, Vicente-Rodríguez G, Rey-López J, et al. Energy metabolism and childhood obesity development. In: Overton LT, Ewente MR, editors. Child Nutrition Physiology. New York: Nova Biomedical; 2009. p. 245-55.

74. Jung CM, Melanson EL, Frydendall EJ, et al. Energy expenditure during sleep, sleep deprivation and sleep following sleep deprivation in adult humans. *J Physiol*. 2011;589:235-44.

75. Strath SJ, Bassett DR, Jr., Swartz AM, et al. Simultaneous heart rate-motion sensor technique to estimate energy expenditure. *Med Sci Sports Exerc*. 2001;33:2118-23.

76. Abel MG, Hannon JC, Sell K, et al. Validation of the Kenz Lifecorder EX and ActiGraph GT1M accelerometers for walking and running in adults. *Appl Physiol Nutr Metab*. 2008;33:1155-64.

77. Plasqui G, Westerterp KR. Physical activity assessment with accelerometers: an evaluation against doubly labeled water. *Obesity (Silver Spring)*. 2007;15:2371-9.

78. Trost SG, McIver KL, Pate RR. Conducting accelerometer-based activity assessments in field-based research. *Med Sci Sports Exerc*. 2005;37:S531-43.

79. U.S. Department of Health and Human Services. Key Guidelines for Children and Adolescents. 2008; Available from: http://www.health.gov/PAGuidelines/. [Accessed 2011 January]

80. Tammelin T, Ekelund U, Remes J, et al. Physical activity and sedentary behaviors among Finnish youth. *Med Sci Sports Exerc*. 2007;39:1067-74.

81. Troiano RP, Berrigan D, Dodd KW, et al. Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc*. 2008;40:181-8.

82. Aaron DJ, Kriska AM, Dearwater SR, et al. The epidemiology of leisure physical activity in an adolescent population. *Med Sci Sports Exerc*. 1993;25:847-53.

83. Sallis JF, Zakarian JM, Hovell MF, et al. Ethnic, socioeconomic, and sex differences in physical activity among adolescents. *J Clin Epidemiol*. 1996;49:125-34.

84. Kristjansdottir G, Vilhjalmsson R. Sociodemographic differences in patterns of sedentary and physically active behavior in older children and adolescents. *Acta Paediatr*. 2001;90:429-35.

85. Seabra AF, Mendonca DM, Thomis MA, et al. Sports participation among Portuguese youth 10 to 18 years. *J Phys Act Health*. 2007;4:370-80.

86. Biddle S, Mutrie N. Psychology of physical activity: Determinants, well-being and interventions. London: Routledge; 2001.

87. Simons-Morton BG, Taylor WC, Snider SA, et al. Observed levels of elementary and middle school children's physical activity during physical education classes. *Prev Med*. 1994;23:437-41.

88. Sallis JF, McKenzie TL, Alcaraz JE, et al. The effects of a 2-year physical education program (SPARK) on physical activity and fitness in elementary school students. Sports, Play and Active Recreation for Kids. *Am J Public Health*. 1997;87:1328-34.

89. Ortega FB, Artero EG, Ruiz JR, et al. Reliability of health-related physical fitness tests in European adolescents. The HELENA Study. *Int J Obes (Lond)*. 2008;32 Suppl 5:S49-57.

90. Przeweda R, Dobosz J. Growth and physical fitness of Polish youths in two successive decades. *J Sports Med Phys Fitness*. 2003;43:465-74.

91. Ortega FB, Ruiz JR, Castillo MJ, et al. Physical fitness in childhood and adolescence: a powerful marker of health. *Int J Obes (Lond)*. 2008;32:1-11.

92. Ortega FB, Artero EG, Ruiz JR, et al. Physical fitness levels among European adolescents: The HELENA study. *Br J Sports Med*. 2009.

93. Vicente-Rodriguez G, Urzanqui A, Mesana MI, et al. Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone

mass through adolescence: a cross-sectional study. *J Bone Miner Metab*. 2008;26:288-94.

94. Gustavsson A, Thorsen K, Nordstrom P. A 3-year longitudinal study of the effect of physical activity on the accrual of bone mineral density in healthy adolescent males. *Calcif Tissue Int*. 2003;73:108-14.

95. Uzunca K, Birtane M, Durmus-Altun G, et al. High bone mineral density in loaded skeletal regions of former professional football (soccer) players: what is the effect of time after active career? *Br J Sports Med*. 2005;39:154-7.

96. Bradney M, Pearce G, Naughton G, et al. Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. *J Bone Miner Res*. 1998;13:1814-21.

97. Ortega FB, Ruiz JR, Castillo MJ, et al. Low level of physical fitness in Spanish adolescents. Relevance for future cardiovascular health (AVENA study). *Rev Esp Cardiol*. 2005;58:898-909.

98. Fonseca RM, de Franca NM, Van Praagh E. Relationship between indicators of fitness and bone density in adolescent Brazilian children. *Pediatr Exerc Sci*. 2008;20:40-9.

99. Vicente-Rodriguez G, Dorado C, Perez-Gomez J, et al. Enhanced bone mass and physical fitness in young female handball players. *Bone*. 2004;35:1208-15.

100. Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, et al. Enhanced bone mass and physical fitness in prepubescent footballers. *Bone*. 2003;33:853-9.

101. Wang Q, Alen M, Nicholson P, et al. Weight-bearing, muscle loading and bone mineral accrual in pubertal girls--a 2-year longitudinal study. *Bone*. 2007;40:1196-202.

102. Martinez-Gomez D, Ruiz JR, Ortega FB, et al. Recommended levels and intensities of physical activity to avoid low-cardiorespiratory fitness in European adolescents: The HELENA study. *Am J Hum Biol*. 2010;22:750-6.

103. Gonzalez-Gross M, Castillo MJ, Moreno L, et al. [Feeding and assessment of nutritional status of spanish adolescents (AVENA study). Evaluation of risks and interventional proposal. I.Methodology]. *Nutr Hosp*. 2003;18:15-28.

104. Moreno LA, De Henauw S, Gonzalez-Gross M, et al. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. *Int J Obes (Lond)*. 2008;32 Suppl 5:S4-11.

105. Lohman TM. Assessment of body composition in children. *Pediatr Exerc Sci*. 1989;1:19-30.

106. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol*. 1988;60:709-23.

107. Rodriguez G, Moreno LA, Blay MG, et al. Body fat measurement in adolescents: comparison of skinfold thickness equations with dual-energy X-ray absorptiometry. *Eur J Clin Nutr*. 2005;59:1158-66.

108. Moreno LA, Blay MG, Rodriguez G, et al. Screening performances of the International Obesity Task Force body mass index cut-off values in adolescents. *J Am Coll Nutr*. 2006;25:403-8.

109. Moreno LA, Mesana MI, Gonzalez-Gross M, et al. Body fat distribution reference standards in Spanish adolescents: the AVENA Study. *Int J Obes (Lond)*. 2007;31:1798-805.

110. Calbet JA, Dorado C, Diaz-Herrera P, et al. High femoral bone mineral content and density in male football (soccer) players. *Med Sci Sports Exerc*. 2001;33:1682-7.

111. Vereecken CA, Covents M, Sichert-Hellert W, et al. Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. *Int J Obes (Lond)*. 2008;32 Suppl 5:S26-34.

112. Farrán A, Zamora R, Cervera P. Tablas de Composición de Alimentos del Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID). Barcelona; 2004.

113. Moreno LA, Rodriguez G, Guillen J, et al. Anthropometric measurements in both sides of the body in the assessment of nutritional status in prepubertal children. *Eur J Clin Nutr*. 2002;56:1208-15.

114. Moreno LA, Joyanes M, Mesana MI, et al. Harmonization of anthropometric measurements for a multicenter nutrition survey in Spanish adolescents. *Nutrition*. 2003;19:481-6.

115. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child*. 1976;51:170-9.

116. Andersen LB, Harro M, Sardinha LB, et al. Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *Lancet*. 2006;368:299-304.

117. Ekelund U, Anderssen SA, Froberg K, et al. Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. *Diabetologia*. 2007;50:1832-40.

118. Strong WB, Malina RM, Blimkie CJ, et al. Evidence based physical activity for school-age youth. *J Pediatr*. 2005;146:732-7.

5. RESULTADOS Y DISCUSIÓN [RESULTS AND DISCUSSION]

Los resultados y discusión de la presente Tesis Doctoral se muestran a continuación en forma de artículos científicos, siguiendo el formato en que han sido publicados.

Journal of Sports Sciences, November 2010; 28(13): 1383–1389

Routledge
Taylor & Francis Group

Extra-curricular participation in sports and socio-demographic factors in Spanish adolescents: The AVENA Study

LUIS GRACIA-MARCO[1], CONCEPCIÓN TOMÀS[1], GERMÁN VICENTE-RODRÍGUEZ[2], DAVID JIMÉNEZ-PAVÓN[3], JUAN P. REY-LÓPEZ[1], FRANCISCO B. ORTEGA[2], RICARDO LANZA-SAIZ[4], & LUIS A. MORENO[5]

[1]*GENUD (Growth, Exercise, Nutrition and Development) Research Group, University of Zaragoza, Zaragoza, Spain,* [2]*Unit for Preventive Nutrition, Department of Bioscience and Nutrition, Karolinska Institute, Huddinge, Sweden,* [3]*Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, Polytechnic University of Madrid, Madrid, Spain,* [4]*Unit of Metabolism, Genetics and Nutrition (IFIMAV), University of Cantabria, Santander, Spain and* [5]*Physiotherapy and Nursing, University of Zaragoza, Zaragoza, Spain*

(Accepted 21 July 2010)

Abstract
The aims of this study were to identify differences between the sexes in extra-curricular participation in sports and to determine its association with body fat and socio-demographic factors in Spanish adolescents. A total of 2165 adolescents (1124 males and 1041 females) aged 13.0–18.5 years from the AVENA Study participated. Participants filled in an ad hoc questionnaire for extra-curricular participation in sports, which was the dependent variable. Independent variables were: age, percent body fat, and father's and mother's educational level and occupation. Chi-square tests and logistic regression were applied. Bivariate analysis showed for male adolescents that age and father's occupation were related to extra-curricular participation in sports. In addition, body fat and mother's education and occupation (all $P < 0.05$) were related to extra-curricular participation of in sports for female adolescents. Logistic regression analysis showed that the likelihood of involvement in extra-curricular participation in sports was 5.3-fold (3.86–7.38) higher for males than females. Age and father's education in both males and females were independently associated with extra-curricular participation in sports. In summary, Spanish male adolescents were shown to engage in more extra-curricular sports than females. In addition, age and father's education (in both sexes) were associated with the participation of their offspring in extra-curricular sports during adolescence.

Keywords: *Physical activity, parents' occupations, parents' education, body fat, exercise*

Introduction

Regular participation in physical activity provides adolescents with very important physical, mental, and social health benefits, decreasing the risk of cardiovascular diseases, cancer, type 2 diabetes (Slattery, Edwards, Ma, Friedman, & Potter, 1997), and obesity (Blair, 1993), as well as relieving depression and anxiety (Weyerer, 1993), and improving bone mass (Vicente-Rodriguez, Dorado, Perez-Gomez, Gonzalez-Henriquez, & Calbet, 2004).

Children and adolescents spend a lot of their time at school. Therefore, the physical education curriculum is commonly recognized as the most important vehicle for the promotion of physical activity in schools (Biddle & Mutrie, 2001). However, current recommendations (one hour or more of moderate-to-vigorous physical activity per day) for children and adolescents (Strong et al., 2005) cannot be met through physical education alone (Sallis et al., 1997; Simons-Morton, Taylor, Snider, Huang, & Fulton, 1994). In Spain, only 48% of individuals aged 6–18 years were shown to engage in at least 60 min of physical activity a day (Roman, Serra-Majem, Ribas-Barba, Perez-Rodrigo, & Aranceta, 2008). In the USA, only 42% of children and an alarming 6–8% of adolescents achieved the recommended levels (Troiano et al., 2008), and in Finland only 23% of boys and 10% of girls did so (Tammelin, Ekelund, Remes, & Nayha, 2007). Much research shows that boys are more active than girls (Aaron et al., 1993; Kristjansdottir & Vilhjalmsson, 2001; Sallis, Zakarian, Hovell, & Hofstetter, 1996; Seabra, Mendonca, Thomis, Malina, & Maia,

Correspondence: L. Gracia-Marco, GENUD, Escuela Universitaria de Ciencias de la Salud, Universidad de Zaragoza, 50009 Zaragoza, Spain.
E-mail: lgracia@unizar.es

ISSN 0264-0414 print/ISSN 1466-447X online © 2010 Taylor & Francis
DOI: 10.1080/02640414.2010.510846

2007). The period of adolescence is characterized by a marked decrease in physical activity (Brodersen, Steptoe, Boniface, & Wardle, 2007; Roman et al., 2008), which could be due to a reduction in sports participation; however, further investigation is needed.

Extra-curricular sports participation is possibly the best way to attain the recommendations for physical activity (Seabra et al., 2007), and may even be the only way to attain a vigorous level of exercise. In Spain, physical activity is performed systematically in schools and sporting clubs. This usually involves 3 h per week plus weekend competition. Therefore, it is important to investigate which sports are practised most, the different participation of males and females, and the factors that affect their participation.

To date, only one study has assessed the physical activity levels and socio-economic variables in 6- to 18-year-old Spanish children and adolescents (Roman et al., 2008); thus, information about socio-demographics and extra-curricular participation in sports in Spanish adolescents is limited. The aims of the present study were to highlight the sports that are practised most as extra-curricular activities, identify differences between the sexes in extra-curricular participation in sports, and determine its association with body fat and socio-demographic factors in Spanish adolescents.

Materials and methods

Participants

The complete methodology of the study is described elsewhere (Gonzalez-Gross et al., 2003). The study was conducted in the context of the multi-centre AVENA Study (Alimentación y Valoración del Estado Nutricional en Adolescentes / Food and Assessment of the Nutritional Status of Adolescents). The AVENA Study is cross-sectional in nature and designed to evaluate the nutritional status of Spanish adolescents. Briefly, 2851 Spanish adolescents (1354 males and 1497 females) aged 13.0–18.5 years were selected by means of a multiple-step, simple random sampling, taking account of the location (Madrid, Murcia, Granada, Santander, and Zaragoza) and random assignment of the schools within each city.

In each selected school, the study was presented to all the adolescents of one class. Adolescents' parents and tutors were informed about the nature and aims of the study through a letter. Once informed consent had been received, the participants were considered to be appropriate as long as they fulfilled the following inclusion criteria: (1) no alcohol, drug or steroid abuse; (2) no type 2 diabetes; (3) not pregnant; and (4) no directly related nutritional medical conditions. Adolescents were effective excluded once the field study had ended, so as to avoid discrimination. Of the 2851 adolescents who participated in the study, only 2165 met the inclusion criteria (1124 males and 1041 females).

The protocol received approval from the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

Measures

Extra-curricular participation in sports. This was determined from each adolescent's answers to a question designed specifically for the AVENA Study (Gonzalez-Gross et al., 2003): "Do you undertake any physical sporting activity after school?" The answer was classified as no extra-curricular participation in sports ("not active") and extra-curricular participation in one or more sports (active). The participants were also asked which sports they practised.

Socio-demographic factors. Age (13, 14, 15, 16, and 17–18.5 years), gender (males and females), parents' education (elementary school, high school, first university degree, and second university degree), and parents' occupation (company director, qualified staff, and non-qualified staff) were the socio-demographic factors.

Anthropometry

Two anthropometrists in each city performed all the measurements; one measured weight, height, and circumferences, while the other assessed skinfolds (Gonzalez-Gross et al., 2003). As part of the analysis, percent body fat was classified into four categories (percentiles <15, 15–85, 85–95, and >95). We chose the 85th and 95th percentiles because they are widely used to assess overweight and obesity (Moreno et al., 2006, 2007). As a mirror, we also used the 15th percentile. We assume children under the 15th percentile are underweight. Body fat percentage was calculated by the equations described by Slaughter et al. (1988). These equations showed the best agreement with total body fat percentage as assessed by dual-energy X-ray absorptiometry (DXA) in a subsample including the adolescents from Zaragoza (Spain) in the AVENA Study (Rodriguez et al., 2005): females (bias 1.64%; confidence intervals [CI] $\pm$ 0.56; $P < 0.01$) and males (bias -0.77%; CI $\pm$ 0.67; $P < 0.02$).

Statistical analysis

Percentages and odds ratio (OR) with 95% confidence intervals are reported as descriptive

values, unless otherwise stated. Bivariate analysis between dependent (extra-curricular participation in sports) and independent (age, body fat, parents' occupations, and parents' education) variables was carried out using the chi-square test of independence. Those variables showing significant associations were included in the logistic regression model, which was used to assess the association between extra-curricular participation in sports and the independent variables (age, parents' education, parents' occupations, and body fat). Since an interaction between sex and the studied variables was observed (all $P \leq 0.001$), all analyses were performed separately for male and female participants. All statistical analysis was carried out using SPSS v.14.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

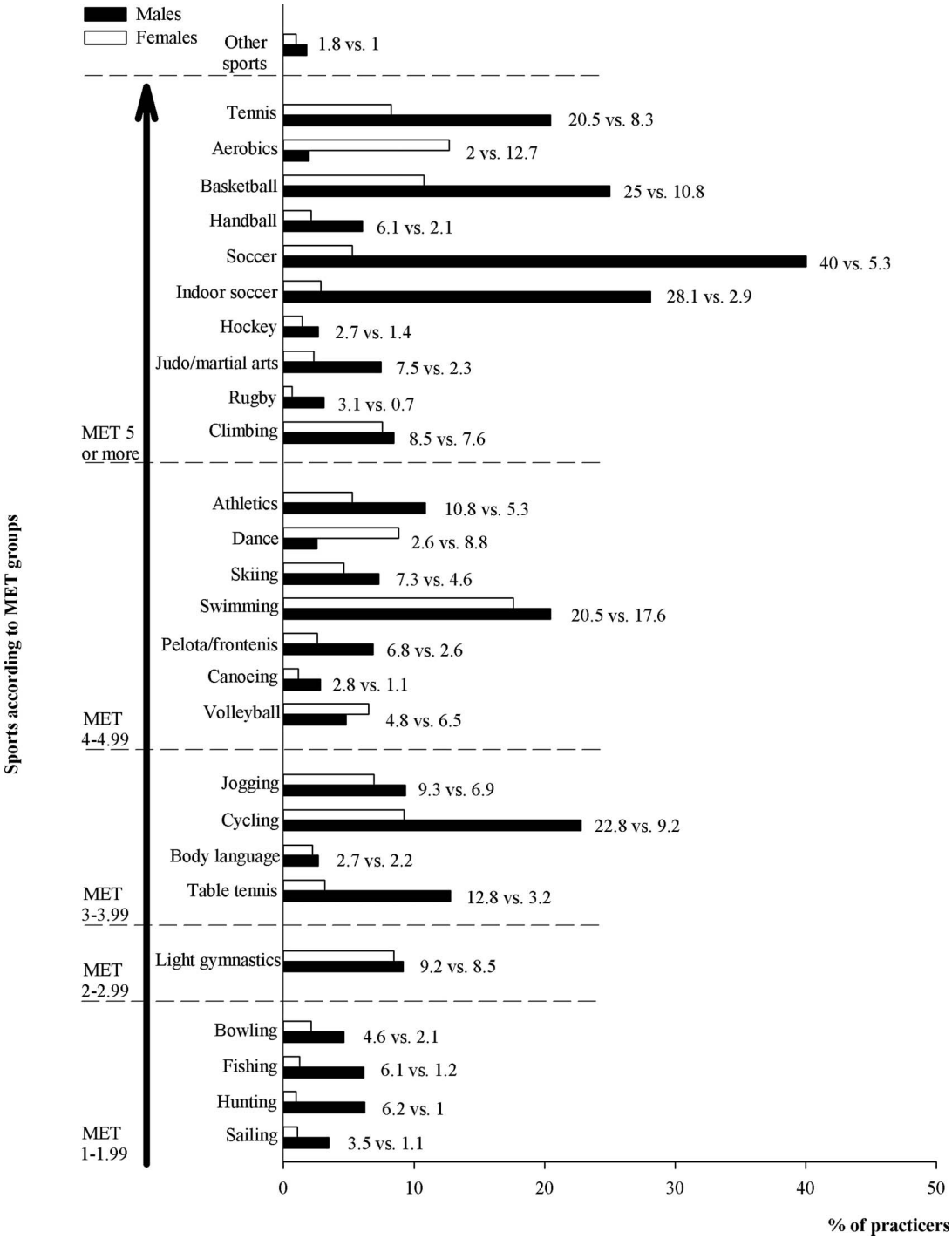


Figure 1. Prevalence of sports practised as extracurricular physical activities by Spanish adolescents. They have been grouped by metabolic equivalents, as proposed by Willmore and Costill (2004).

Table I. Chi-square test of independence for extra-curricular participation in sports (ECPS) and socio-demographic factors in Spanish adolescents.

	Males					Females				
	No ECPS		ECPS in at least one sport			No ECPS		ECPS in at least one sport		
Variable	*n*	%	*n*	%	*P*	*n*	%	*n*	%	*P*
Age										
13 years	30	14	203	22.4	**0.001**	84	16.6	123	23	**0.011**
14 years	42	19.5	192	21.1		101	20	118	22.1	
15 years	53	24.7	208	22.9		118	23.3	130	24.3	
16 years	43	20	194	21.4		100	19.8	86	16.1	
17–18.5 years	47	21.9	111	12.2		103	20.4	78	14.6	
Body fat										
Percentile < 15	60	36.1	357	46.3	0.062	51	13.3	84	20.8	**0.032**
Percentile 15–85	39	23.5	165	21.4		192	50.1	172	42.7	
Percentile 85–95	23	13.9	103	13.4		109	28.5	115	28.5	
Percentile > 95	44	26.5	146	18.9		31	8.1	32	7.9	
Father's occupation										
Company director	48	38.4	270	43.6	0.362	127	37.4	164	44	**0.005**
Qualified staff	71	56.8	331	53.5		195	57.4	204	51.1	
Non-qualified staff	6	4.8	18	2.9		18	5.3	5	1.3	
Homemaker	0	0	0	0		0	0	0	0	
Mother's occupation										
Company director	25	18.8	197	29.7	0.068	83	21.5	107	24.4	0.141
Qualified staff	34	25.6	154	23.2		86	22.3	111	25.3	
Non-qualified staff	4	3	24	3.6		18	4.7	10	2.3	
Homemaker	70	52.6	289	43.5		199	51.6	211	48.1	
Father's education										
Elementary school	60	40	207	29.3	**0.013**	146	35.6	111	24.8	**0.001**
High school	29	19.3	116	16.4		81	19.8	80	17.9	
First university degree	26	17.3	133	18.8		80	19.5	120	26.8	
Second university degree	35	23.3	251	35.5		103	25.1	136	30.4	
Mother's education										
Elementary school	59	38.6	233	32.5	0.282	173	41.1	161	34.9	**0.028**
High school	29	19	140	19.6		83	19.7	77	16.7	
First university degree	36	23.5	159	22.2		90	21.4	136	29.5	
Second university degree	29	19	184	25.7		75	17.8	87	18.9	

Results

When the 686 participants were effectively excluded once the field study had ended, 2165 adolescents aged 15.2 ± 1.5 years remained who met the inclusion criteria (1124 males and 1041 females).

Of the active adolescents, males engaged in more extra-curricular sporting activity than females ($P < 0.05$). The five most prevalent sports were soccer, indoor soccer, basketball, cycling, swimming or tennis for males; and swimming, aerobics, basketball, cycling, and light gymnastics for females (Figure 1).

Bivariate analysis showed that age and father's occupation were related to extra-curricular participation in sports by male adolescents (all $P < 0.05$; Table I), while age, body fat, father's occupation and education, and mother's education were related to extra-curricular participation in sports by female adolescents (all $P < 0.05$; Table I).

Logistic regression analysis (Table II) showed that the likelihood of engagement in extra-curricular sports was 5.3-fold (3.86–7.38) greater for male than for female adolescents. The likelihood of such involvement was 45% (0.31–0.98) lower for males aged 15 and 70% (0.16–0.55) lower for males aged 17.0–18.5, compared with 13-year-old boys; and 2.1-fold (1.32–3.33) greater for the highest level of education of the fathers compared with the lowest. In females, the likelihood of involvement in extra-curricular sports was 50% (0.27–0.9) lower in those aged 17.0–18.5 compared with 13-year-old girls, and 1.9-fold (1.1–3.18) greater for females whose fathers reported holding a first university degree compared with those reporting elementary school education.

Discussion

The main findings of the present study are that male adolescents are five times more likely than their female

counterparts to engage in extra-curricular sports. Age and father's education are socio-demographic factors associated with both boys' and girls' extra-curricular participation in sports.

Extra-curricular participation in sports can be considered the main source of at least moderate physical activity (Seabra et al., 2007). In the present study, soccer and swimming were the sports practised most by male and female adolescents, respectively. Our results are consistent with those of a study of Portuguese adolescents (Seabra et al., 2007). Cycling (both males and females) and aerobics (only females) were also widely practised sports, as found in San Diego adolescents (Sallis et al., 1996). It is important to know which sports are most prevalent, because different sports have different intensities and place different demands on energy expenditure, and exercise of higher intensities is known to be associated with health (Baba, Koketsu, Nagashima, & Inasaka, 2009). The choice of sports most widely practised is conditioned by the culture and customs of countries and the proximity between them. This would help to explain the differences between studies; for example, soccer is the sport most widely practised by males in Spain and Portugal, whereas soccer is not in the top five sports practised in San Diego or North Carolina in the USA.

Spanish male adolescents are more active than their female counterparts (Garcia-Artero et al., 2007). Similar results have been reported among adolescents from other countries (Kristjansdottir & Vilhjalmsson, 2001; Sallis et al., 1996; Shi, Lien, Kumar, & Holmboe-Ottesen, 2006). In Spain at least, this is because Spanish male adolescents engage in more extra-curricular sporting activity than female adolescents, as the present study shows. Therefore, encouraging adolescents to engage in extra-curricular sporting activity could be a useful strategy to increase their physical activity so as to attain the recommended levels (Strong et al., 2005). Several studies with Spanish pre-puberal children (Ara et al., 2004, 2006) have shown an association between extra-curricular participation in sports, obesity, and fitness. Although similar trends may be expected in adolescents, several confounders may influence extra-curricular participation in sports. The age of adolescents is one of the most important factors determining extra-curricular participation in sports. As in other studies focused on overall physical activity (Aaron et al., 1993; Brodersen et al., 2007; Roman et al., 2008), we found an inverse association between age and extra-curricular participation in sports. In males, the likelihood of participating in extra-curricular sports is 70% lower than in 13-year-old males. This supports the hypothesis that the decrease in physical activity with age is due to a decrease in sports participation. Efforts should therefore be made to increase extra-curricular participation in sports during adolescence.

Of the socio-demographic variables assessed, father's education for males, and father's education and occupation and mother's education for females, appear to influence participation in extra-curricular sports. The logistic regression confirmed the independent association between father's education with extra-curricular participation in sports; that is, those adolescents whose father's education level was a second university degree were more likely to engage in extra-curricular sports than those whose fathers had the lowest educative level (elementary school). Similar results were reported for Italian (La Torre et al., 2006) and Hungarian adolescents (Piko & Keresztes, 2008). In addition, father's education was inversely associated with inactivity in Chinese adolescents (Li, Dibley, Sibbritt, & Yan, 2006). The latter suggests that strategies to increase an adolescent's participation in sport should also take into account the influence of the father. However, the reason that the education of the father, rather than that of the mother, is independently associated with adolescents' extra-curricular participation in sports could be because, in most cultures, the education of the father determine the socio-economic status of a family more than the education of the mother. This requires further investigation.

Strength and limitations

It should be noted that the present cross-sectional study only provides associations between variables. However, it is unlikely that the extra-curricular participation in sports by adolescents could influence the occupation or education of their parents. The use of questionnaires could introduce some under- or over-reporting. Nevertheless, the relatively large sample of adolescents from five cities in Spain provides a general picture of the association between extra-curricular participation in sports and socio-demographic factors in Spain's adolescent population.

Conclusions

Spanish male adolescents engaged more in extra-curricular sporting activity than their female counterparts. They were also involved in more intense sports. Sex, age, and father's education were linked to extra-curricular participation in sports. Gender-specific strategies may be needed to increase the participation of females in sport. Extra-curricular participation in sports seems to decrease with age; this could explain the decrease in physical activity during adolescence.

Table II. Independent associations between extra-curricular participation in sports and socio-demographic factors.

	Extra-curricular participation in sports	
	OR	95%CI
Entire group		
Gender		
Female	1.00	
Male	5.34	3.86–7.38
Males		
Age		
13	1.00	
14	0.86	0.49–1.60
15	0.55	0.31–0.98
16	0.77	0.42–1.42
17–18.5	0.30	0.16–0.55
Father's education		
Elementary school	1.00	
High school	1.17	0.70–1.95
First university degree	1.38	0.82–2.32
Second university degree	2.10	1.32–3.33
Females		
Age		
13	1.00	
14	0.96	0.56–1.63
15	0.92	0.54–1.57
16	0.88	0.48–1.58
17–18.5	0.50	0.27–0.9
Body fat		
Percentile > 95	1.00	
Percentile < 15	1.42	0.63–3.19
Percentile 15–85	1.00	0.49–2.03
Percentile 85–95	1.13	0.53–2.34
Father's occupation		
Non-qualified staff	1.00	
Company director	4.76	0.96–23.58
Qualified staff	4.35	0.91–20.74
Father's education		
Elementary school	1.00	
High school	1.08	0.64–1.82
First university degree	1.87	1.10–3.18
Second university degree	1.60	0.86–2.98
Mother's education		
Elementary school	1.00	
High school	0.74	0.43–1.27
First university degree	1.25	0.76–2.07
Second university degree	0.82	0.44–1.53

Note: Significant differences are highlighted by tints.

References

Aaron, D. J., Kriska, A. M., Dearwater, S. R., Anderson, R. L., Olsen, T. L., Cauley, J. A. et al. (1993). The epidemiology of leisure physical activity in an adolescent population. *Medicine and Science in Sports and Exercise*, *25*, 847–853.

Ara, I., Vicente-Rodriguez, G., Jimenez-Ramirez, J., Dorado, C., Serrano-Sanchez, J. A., & Calbet, J. A. (2004). Regular participation in sports is associated with enhanced physical fitness and lower fat mass in prepubertal boys. *International Journal of Obesity and Related Metabolic Disorders*, *28*, 1585–1593.

Ara, I., Vicente-Rodriguez, G., Perez-Gomez, J., Jimenez-Ramirez, J., Serrano-Sanchez, J. A., Dorado, C. et al. (2006). Influence of extracurricular sport activities on body composition and physical fitness in boys: A 3-year longitudinal study. *International Journal of Obesity (London)*, *30*, 1062–1071.

Baba, R., Koketsu, M., Nagashima, M., & Inasaka, H. (2009). Role of exercise in the prevention of obesity and hemodynamic abnormalities in adolescents. *Pediatrics International*, *51*, 359–363.

Biddle, S., & Mutrie, N. (2001). *Psychology of physical activity: Determinants, well-being and interventions.* London: Routledge.

Blair, S. N. (1993). Evidence for success of exercise in weight loss and control. *Annals of Internal Medicine*, *119*, 702–706.

Brodersen, N. H., Steptoe, A., Boniface, D. R., & Wardle, J. (2007). Trends in physical activity and sedentary behaviour in adolescence: Ethnic and socioeconomic differences. *British Journal of Sports Medicine*, *41*, 140–144.

Garcia-Artero, E., Ortega, F. B., Ruiz, J. R., Mesa, J. L., Delgado, M., Gonzalez-Gross, M. et al. (2007). Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA Study). *Revista Española de Cardiología*, *60*, 581–588 (in Spanish).

Gonzalez-Gross, M., Castillo, M. J., Moreno, L., Nova, E., Gonzalez-Lamuno, D., Perez-Llamas, F. et al. (2003). Feeding and assessment of nutritional status of Spanish adolescents (AVENA Study): Evaluation of risks and interventional proposal. I. Methodology. *Nutrición Hospitalaria*, *18*, 15–28 (in Spanish).

Kristjansdottir, G., & Vilhjalmsson, R. (2001). Sociodemographic differences in patterns of sedentary and physically active behavior in older children and adolescents. *Acta Paediatrica*, *90*, 429–435.

La Torre, G., Masala, D., De Vito, E., Langiano, E., Capelli, G., & Ricciardi, W. (2006). Extra-curricular physical activity and socioeconomic status in Italian adolescents. *BMC Public Health*, *6*, 22.

Li, M., Dibley, M. J., Sibbritt, D., & Yan, H. (2006). Factors associated with adolescents' physical inactivity in Xi'an City, China. *Medicine and Science in Sports and Exercise*, *38*, 2075–2085.

Moreno, L. A., Blay, M. G., Rodriguez, G., Blay, V. A., Mesana, M. I., Olivares, J. L. et al. (2006). Screening performances of the International Obesity Task Force body mass index cut-off values in adolescents. *Journal of the American College of Nutrition*, *25*, 403–408.

Moreno, L. A., Mesana, M. I., Gonzalez-Gross, M., Gil, C. M., Ortega, F. B., Fleta, J. et al. (2007). Body fat distribution reference standards in Spanish adolescents: The AVENA Study. *International Journal of Obesity (London)*, *31*, 1798–1805.

Piko, B. F., & Keresztes, N. (2008). Sociodemographic and socioeconomic variations in leisure time physical activity in a sample of Hungarian youth. *International Journal of Public Health*, *53*, 306–310.

Rodriguez, G., Moreno, L. A., Blay, M. G., Blay, V. A., Fleta, J., Sarria, A. et al. (2005). Body fat measurement in adolescents: Comparison of skinfold thickness equations with dual-energy X-ray absorptiometry. *European Journal of Clinical Nutrition*, *59*, 1158–1166.

Roman, B., Serra-Majem, L., Ribas-Barba, L., Perez-Rodrigo, C., & Aranceta, J. (2008). How many children and adolescents in Spain comply with the recommendations on physical activity? *Journal of Sports Medicine and Physical Fitness*, *48*, 380–387.

Sallis, J. F., McKenzie, T. L., Alcaraz, J. E., Kolody, B., Faucette, N., & Hovell, M. F. (1997). The effects of a 2-year physical education program (SPARK) on physical activity and fitness in elementary school students: Sports, Play and Active Recreation for Kids. *American Journal of Public Health*, *87*, 1328–1334.

Sallis, J. F., Zakarian, J. M., Hovell, M. F., & Hofstetter, C. R. (1996). Ethnic, socioeconomic, and sex differences in physical activity among adolescents. *Journal of Clinical Epidemiology*, *49*, 125–134.

Seabra, A. F., Mendonca, D. M., Thomis, M. A., Malina, R. M., & Maia, J. A. (2007). Sports participation among Portuguese youth 10 to 18 years. *Journal of Physical Activity and Health*, *4*, 370–380.

Shi, Z., Lien, N., Kumar, B. N., & Holmboe-Ottesen, G. (2006). Physical activity and associated socio-demographic factors among school adolescents in Jiangsu Province, China. *Preventive Medicine*, *43*, 218–221.

Simons-Morton, B. G., Taylor, W. C., Snider, S. A., Huang, I. W., & Fulton, J. E. (1994). Observed levels of elementary and middle school children's physical activity during physical education classes. *Preventive Medicine*, *23*, 437–441.

Slattery, M. L., Edwards, S. L., Ma, K. N., Friedman, G. D., & Potter, J. D. (1997). Physical activity and colon cancer: A public health perspective. *Annals of Epidemiology*, *7*, 137–145.

Slaughter, M. H., Lohman, T. G., Boileau, R. A., Horswill, C. A., Stillman, R. J., Van Loan, M. D. et al. (1988). Skinfold equations for estimation of body fatness in children and youth. *Human Biology*, *60*, 709–723.

Strong, W. B., Malina, R. M., Blimkie, C. J., Daniels, S. R., Dishman, R. K., Gutin, B. et al. (2005). Evidence based physical activity for school-age youth. *Journal of Pediatrics*, *146*, 732–737.

Tammelin, T., Ekelund, U., Remes, J., & Nayha, S. (2007). Physical activity and sedentary behaviors among Finnish youth. *Medicine and Science in Sports and Exercise*, *39*, 1067–1074.

Troiano, R. P., Berrigan, D., Dodd, K. W., Masse, L. C., Tilert, T., & McDowell, M. (2008). Physical activity in the United States measured by accelerometer. *Medicine and Science in Sports and Exercise*, *40*, 181–188.

Vicente-Rodriguez, G., Dorado, C., Perez-Gomez, J., Gonzalez-Henriquez, J. J., & Calbet, J. A. (2004). Enhanced bone mass and physical fitness in young female handball players. *Bone*, *35*, 1208–1215.

Weyerer, S. (1993). Effects of physical inactivity on all-cause mortality risk in Upper Bavaria. *Perceptual and Motor Skills*, *77*, 499–505.

Wilmore, J. H., & Costill, D. L. (2004). *Physiology of sport and exercise.* Champaign, IL: Human Kinetics.

Original Paper

HORMONE RESEARCH IN PÆDIATRICS

Horm Res Paediatr
DOI: 10.1159/000314965

Received: December 18, 2009
Accepted: May 3, 2010
Published online: July 21, 2010

Bone Mass and Bone Metabolism Markers during Adolescence: The HELENA Study

L. Gracia-Marco[a, b] G. Vicente-Rodríguez[a, c] J. Valtueña[d] J.P. Rey-López[a, b] A.E. Díaz Martínez[e] M.I. Mesana[a, b] K. Widhalm[f] J.R. Ruiz[g] M. González-Gross[d, h] M.J. Castillo[i] L.A. Moreno[a, b] on behalf of the HELENA Study Group

[a]GENUD (Growth, Exercise, NUtrition and Development) Research Group and [b]School of Health Science (EUCS), Department of Physiotherapy and Nursing, University of Zaragoza, Zaragoza, [c]Faculty of Health and Sport Science (FCSD), Department of Physiotherapy and Nursing, University of Zaragoza, Huesca, [d]Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences-INEF, Technical University of Madrid, and [e]Clinical Laboratory, Sport Medicine Center, Consejo Superior de Deportes, Madrid, Spain; [f]Division of Clinical Nutrition and Prevention, Department of Pediatrics, Medical University of Vienna, Vienna, Austria; [g]Unit for Preventive Nutrition, Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; [h]Institut für Ernährungs- und Lebensmittelwissenschaften, Humanernährung, Rheinische Friedrich-Wilhelms Universität, Bonn, Germany; [i]Department of Physiology, School of Medicine, University of Granada, Granada, Spain

Key Words
Adolescents · Growth · Osteocalcin · Propeptide of type I procollagen · β-Isomerized C-telopeptide

Abstract
Background/Aims: The assessment of bone mineral content (BMC) and density (BMD) status in children and adolescents is important for health and the prevention of diseases. Bone metabolic activity could provide early information on bone mass development. Our aim was to describe bone mass and metabolism markers according to age and Tanner stage in adolescents. ***Methods:*** Spanish adolescents (n = 345; 168 males and 177 females) aged 12.5–17.5 years participated in this cross-sectional study. Body composition variables were measured by dual-energy X-ray absorptiometry. Serum osteocalcin (n = 101), aminoterminal propeptide of type I procollagen (n = 92) and β-isomerized C-telopeptides (β-CTX, n = 65) and urine samples (β-CTX; n = 237) were analyzed by electrochemiluminescence immunoassay. ***Results:*** Analysis of covariance showed that females had higher values for BMC and BMD in most of the regions. Both males and females had a significant decrease in bone markers while sexual maturation increases (all $p < 0.05$). Males had an increased bone turnover compared to females (all $p < 0.05$, except for urine β-CTX in Tanner ≤ IV). ***Conclusion:*** Our results support the evidence of dimorphic site-specific bone accretion between sexes and show an increased bone turnover in males, suggesting higher metabolic activity.

Introduction

Acquiring a high bone mass during childhood and adolescence is a key determinant of adult skeletal health [1], and is known to contribute to more than half to the vari-

For information regarding the HELENA Study Members, please see Appendix.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1663–2818/10/0000–0000$26.00/0

Accessible online at:
www.karger.com/hrp

Luis Gracia-Marco
GENUD (Growth, Exercise, NUtrition and Development) Research Group
Department of Physiotherapy and Nursing, School of Health Sciences
University of Zaragoza, Avd. Domingo Miral s/n, ES–50009 Zaragoza (Spain)
Tel. +34 976 40 03 38, ext. 301, Fax +34 976 40 03 40, E-Mail lgracia@unizar.es

ability of bone mass with age [2]. The most rapid gains in bone mass are observed during adolescence, especially between 11 and 14 years in girls and between 14 and 16 years in boys [3], with as much as 51% of peak bone mass accumulating during pubertal growth [4, 5] and reaching 37% of the bone mineral density (BMD) of adults [6]. Osteopenia and osteoporosis are health concerns that have their origin in adolescents, mainly affected by nutritional and physical activity habits [7, 8]. Osteoporosis is a disease characterized by decreased bone mass and deterioration of bone tissue [9] and is affected by the peak bone mass attained before the age of 20 years [10]. Therefore, assessment of bone mineral content (BMC) and BMD status in children and adolescents is important for health and the prevention of diseases. Bone development depends on its metabolic activity, which includes bone formation, resorption and, as a consequence, bone turnover that is mediated by several hormones and micronutrients. To assess bone metabolism during growth may provide early information of impaired bone mass development.

Several biochemical markers of bone metabolism have been described [11]: osteocalcin, aminoterminal propeptide of type I procollagen (PINP) as markers of bone formation and serum or urine β-isomer of the carboxiterminal telopeptide of type I collagen (β-CTX), as marker of bone resorption. Osteocalcin is an established and extensively used biochemical marker of bone formation because it is the major non-collagenous protein of bone matrix [11] and it is specifically produced by osteoblasts in the bone. High levels of osteocalcin are associated with both high bone formation and high bone turnover [12]. Type I collagen accounts for more than 90% of the organic matrix of the bone [13]. PINP is a specific indicator of type I collagen deposition and thus may be defined as a specific bone formation marker. This PINP is released into the intracellular space and eventually into the blood stream during type I collagen formation, and it is present in the circulation before the collagen molecules are assembled into fibers [12]. During collagen breakdown, C-terminal telopeptide fragments of various sizes are released into the circulation. It has been observed that type I collagen molecules can undergo β-isomerization of the aspartic acid residue within its telopeptides [14, 15]. Consequently, type I collagen molecules may be present in bone matrix as either linear (α) or β-isomerized C-telopeptides (β-CTX) [16]. By determining bone formation and resorption markers, bone turnover activity can be estimated.

To our knowledge, little information exists about bone formation and resorption markers during adolescence. This is especially important in girls, because they are at a higher risk than males of developing osteoporosis in adulthood [17]. Therefore, the aim of this report is to describe bone mass and metabolism (measuring formation and resorption markers – osteocalcin, PINP and β-CTX) through adolescence, according to age and Tanner stage in male and female adolescents.

Subjects and Methods

The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) is a European Union-funded project [7, 8] which includes a cross-sectional multicenter study (CSS) that was performed in adolescents aged 12.5–17.5 years from 10 European cities. The general characteristics of the HELENA-CSS have been described in detail elsewhere [18]. For this study, we considered only Spanish adolescents from Zaragoza, because dual-energy X-ray absorptiometry (DXA) was only performed at this study center. From a total sample of 390 adolescents recruited in the schools of Zaragoza, a subsample of 345 (168 males and 177 females, mean age 14.78 ± 1.19 years) had valid data on DXA and were then included in this study. For the analysis, subjects were classified into groups according to their age based on their visit to the laboratory (12.5–14.99 and 15–17.5 years) and also according to their Tanner stage (≤IV and V). In the HELENA-CSS protocol, it was established that blood samples were obtained in one third of the population sample. Analysis of bone markers was carried out on serum (osteocalcin, n = 101; PINP, n = 92, and β-CTX, n = 65) and urine samples β-CTX, n = 237). Written informed consent was obtained from parents and adolescents [19]. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000). The study protocol was approved by the Ethics Committee for Clinical Research from the Government of Aragón (CEICA, Spain). A complete description of ethical issues and good clinical practice within the HELENA-CSS can be found elsewhere [19].

The general HELENA inclusion criteria were: not to participate simultaneously in another clinical trial; to be free of any acute infection lasting until <1 week before inclusion, and to have valid data for age, sex and body mass index. In addition, medical history of diseases or medications affecting bone metabolism were established as specific exclusion criteria for this report. Finally, 45 of 390 adolescents were excluded in Zaragoza.

Anthropometric Measurements

International guidelines for anthropometry in adolescents [20–23] were applied. Barefoot and clad in light indoor clothing, body weight (kg) and height (cm) were measured with an electronic scale (Type SECA 861; precision 100 g, range 0–150 kg) and a stadiometer (Type Seca 225; precision 0.1 cm, range 70–200 cm).

Tanner Stage

Physical examination was performed by a physician aiming to classify the adolescents into 1 of the 5 stages of pubertal maturity defined by Tanner and Whitehouse [24]. As previously described, subjects were classified into 2 groups depending on sexual maturation: Tanner ≤IV and V.

Bone, Lean and Fat Mass

Adolescents were scanned in order to obtain bone measurements of the whole body, pelvis, hip, lumbar spine and average of arms and legs. The bone mass and lean mass [body mass – (fat mass + bone mass)] were measured using DXA (pediatric version of the software QDR-Explorer, Hologic Corp., software version 12.4, Waltham, Mass., USA). DXA equipment was calibrated using a lumbar spine phantom as recommended by the manufacturer. Subjects were scanned in the supine position and the scans were performed at high resolution [25]. Lean mass (g), fat mass (g), total area (cm^2) and BMC (g) were calculated from total and regional analysis of the whole body scan. BMD ($g \cdot cm^{-2}$) was calculated using the formula BMD = BMC·area^{-2}. The regional analysis (upper and lower extremities and pelvic region) was performed as described elsewhere [25]. Additional examinations were conducted to estimate bone mass at the lumbar spine (L1–L4) and hip regions as previously described [26].

Calcium Intake

Mean daily calcium intake was estimated from two non-consecutive 24-hour recalls using the HELENA-DIAT (Dietary Assessment Tool) software [27]. For the assessment of calcium intake, the food composition tables published by Farrán et al. [28] were used for Spanish adolescents. The calcium intake/lean mass ratio (mg/kg) was also calculated.

Specimen Blood Collection and Biochemical Analyses

Fasting blood samples (24.3 ml) were collected by venipuncture at school between 8 and 10 o'clock in the morning after a 10-hour overnight fast. Centrifugation was performed at room temperature. For the measurement of bone parameters, blood was collected in heparinized tubes, immediately placed on ice and centrifuged within 30 min (3,500 r.p.m. for 15 min) to avoid hemolysis. Immediately after centrifugation, the samples were stored and transported at 4–7°C (during a maximum of 14 h) to the central laboratory in Bonn and stored there at –80°C until assayed. Then at the Universidad Politécnica in Madrid, Spain, bone marker (osteocalcin, PINP and serum and urine β-CTX) concentrations were determined by electrochemiluminescence immunoassay using an Elecsys 2010 analyzer from Roche Diagnostics GmbH (Germany). The kits used were also purchased from Roche Diagnostics GmbH.

The measuring range of serum osteocalcin was 0.50–300 μg/l (defined by the lower detection limit and the maximum of the calibration curve). Values below the detection limit were reported as <0.50 μg/l. Values above the measuring range were diluted by Elecsys diluent universal at a concentration of >60 μg/l. Osteocalcin presented coefficients of variation of 4.0 and 6.5% at 15.5 μg/l and 1.4 and 1.8% at 68.3 μg/l. Total PINP in serum had a measuring range of 5–1,200 μg/l. Intra- and inter-assay coefficients of variation were 1.8 and 2.3% at 274 μg/l and 2.9 and 3.7% at 799 μg/l. Values below the detection limit were reported as <5 μg/l. Values above the measuring range of 1,200 μg/l were diluted by Elecsys diluent universal at a recommended concentration of >100 μg/l. Analytical sensitivity (lower detection limit) was <5 μg/l. Serum and urine β-CTX had intra- and inter-assay coefficients of variation of 1.0 and 1.6% at 3.59 μg/l and 4.6 and 4.7% at 0.08 μg/l. Measuring range was 0.010–6.00 μg/l; analytical sensitivity (lower detection limit) was 0.01 μg/l, and functional sensitivity was 0.07 μg/l.

Statistics

As descriptive statistics, mean and standard deviation (SD) are given for raw data bone mass-related variables and mean and standard error for bone mass-adjusted results. Since residuals did not show satisfactory patterns, bone markers are presented as median and interquartile intervals.

For the analysis by age and pubertal status, we used the independent samples t test and the Kruskal-Wallis H to determine sex differences for bone mass- and bone marker-related variables, respectively. To determine differences between age groups or Tanner stages in bone mass- and bone marker-related variables, one-way ANOVA, with Bonferroni post hoc or Kruskal-Wallis H, was applied. For adjusted results, one-way analysis of covariance (ANCOVA) with Bonferroni post hoc was used, including as covariates: height; whole body lean mass (arm lean mass for the upper limbs and leg lean mass for the lower limbs); percentage of fat mass, and pubertal development (only when grouping by age). Effect size statistics is a measure of the magnitude of effect and in this study was assessed using Cohen's d (standardized mean difference) and 95% confidence interval [29]. Taking into account the cutoff established by Cohen, the effect size (Cohen's d) can be small (~0.2), medium (~0.5) or large (~0.8).

SPSS version 14.0 was used for analysis. The probability value for the significance level was fixed at 0.05.

Results

Table 1 shows a descriptive analysis (mean ± SD) of the total sample categorized by age groups in male and female adolescents. Males had higher whole body BMC and lean mass in both groups (all $p < 0.05$) compared with females. In females, the percentage of fat mass was higher compared to males in both groups (all $p < 0.05$). However, no differences were found in the ratio calcium/lean mass (mg/kg) between sexes.

Bone Mass in Male and Female Adolescents

In general, males showed higher BMC and BMD in most of the regions in both age groups ($p < 0.05$; table 2) and Tanner groups ($p < 0.05$; table 3).

After adjusting for differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs), percentage of fat mass and sexual maturation (only for age groups; tables 4, 5), we observed that females had higher BMC and BMD in most of the regions in the age groups and especially in Tanner V ($p < 0.05$). In the whole group, females presented higher values for BMC and BMD in most of the regions except for hip BMC ($p = 0.692$) and BMD ($p = 0.237$) and lower limb BMD ($p = 0.724$). We observed medium–large effect sizes for all bone mass-related variables. Additional analyses were made after further including calcium intake as a covariate, but the results did not change (data not shown).

Table 1. Characteristics of the sample by age groups (mean ± SD)

	12.5–14.99 years	15–17.5 years	Whole group
Males	90	78	168
Age, years	13.81 ± 0.68	15.94 ± 0.6	14.8 ± 1.24
Height, cm	164.14 ± 19.72*	172.9 ± 13.83*	168.2 ± 17.74*
Body mass, kg	58.97 ± 13.39*	66.81 ± 16.21*	62.61 ± 15.24*
BMC, g	1,946.78 ± 460.98*	2,368.97 ± 373.21*	2,142.8 ± 471.24*
Lean mass, kg	42.57 ± 8.71*	49.71 ± 6.06*	45.89 ± 8.38*
Fat mass, %	22.84 ± 7.2*	19.01 ± 6.19*	21.06 ± 7*
Calcium/lean, mg/kg	20.4 ± 7.76	18.5 ± 8.06	19.6 ± 7.93
Females	95	82	177
Age, years	13.89 ± 0.7	15.77 ± 0.56	14.76 ± 1.13
Height, cm	159.33 ± 7.24	159.53 ± 19.01	159.42 ± 13.95
Body mass, kg	53.79 ± 9.99	55.67 ± 7.64	54.66 ± 9
BMC, g	1,789.28 ± 324.93	1,972.47 ± 274.18	1,874.15 ± 315.24
Lean mass, kg	34.65 ± 4.92	36.58 ± 4.13	35.54 ± 4.66
Fat mass, %	30.54 ± 5.51	29.51 ± 4.99	30.06 ± 5.29
Calcium/lean, mg/kg	20.7 ± 9.2	19.2 ± 9.96	20 ± 9.56

* $p < 0.05$ for sex differences.

Table 2. Bone mineral content (BMC) and density (BMD) in males and females by age groups (mean ± SD)

	12.5–14.99 years	15–17.5 years	Whole group	p
Males	90	78	168	
BMC, g				
Whole body	1,946.78 ± 460.98*	2,368.97 ± 373.21*	2,141.82 ± 470.01*	0.000
Pelvis	219.41 ± 70.83*	286.44 ± 69.79*	250.24 ± 77.6*	0.000
Hip	33.51 ± 8.51*	41.07 ± 8.77*	37.33 ± 10.35*	0.000
Lumbar spine	45.41 ± 12.99	60.80 ± 12.12*	52.51 ± 14.69	0.000
Upper limbs	114.64 ± 33.91*	143.82 ± 25.30*	128.12 ± 33.39*	0.000
Lower limbs	405.23 ± 109.33*	485.14 ± 78.54*	442.2 ± 103.68*	0.000
BMD, g·cm^{-2}				
Whole body	1.021 ± 0.108	1.127 ± 0.103*	1.07 ± 0.118*	0.000
Pelvis	1.031 ± 0.167	1.150 ± 0.144*	1.086 ± 0.167	0.000
Hip	0.962 ± 0.140*	1.051 ± 0.138*	1.004 ± 0.146*	0.000
Lumbar spine	0.819 ± 0.127*	0.963 ± 0.119	0.886 ± 0.142*	0.000
Upper limbs	0.670 ± 0.061*	0.733 ± 0.061*	0.699 ± 0.068*	0.000
Lower limbs	1.143 ± 0.144*	1.265 ± 0.123*	1.199 ± 0.148*	0.000
Females	95	82	177	
BMC, g				
Whole body	1,789.28 ± 324.93	1,972.47 ± 274.18	1,874.15 ± 315.24	0.000
Pelvis	195.93 ± 48.02	216.38 ± 42.03	205.4 ± 46.36	0.000
Hip	25.53 ± 4.94	27.02 ± 5.10	26.22 ± 5.06	0.000
Lumbar spine	48.21 ± 10.45	52.06 ± 10.43	50.01 ± 10.58	0.000
Upper limbs	103.97 ± 20.06	114.09 ± 17.86	108.66 ± 19.68	0.000
Lower limbs	328.29 ± 60.75	354.70 ± 55.18	340.52 ± 59.55	0.000
BMD, g·cm^{-2}				
Whole body	1.014 ± 0.105	1.074 ± 0.086	1.042 ± 0.101	0.000
Pelvis	1.042 ± 0.142	1.077 ± 0.115	1.059 ± 0.131	0.000
Hip	0.886 ± 0.109	0.916 ± 0.110	0.9 ± 0.11	0.000
Lumbar spine	0.919 ± 0.132	0.953 ± 0.103	0.935 ± 0.12	0.000
Upper limbs	0.632 ± 0.048	0.658 ± 0.045	0.644 ± 0.048	0.000
Lower limbs	1.045 ± 0.101	1.088 ± 0.091	1.065 ± 0.098	0.000

* $p < 0.05$ for sex differences.

Bone Metabolism Markers in Male and Female Adolescents

Figure 1 describes the osteocalcin, PINP and serum β-CTX and urine β-CTX concentrations in male and female adolescents by age or sexual maturation groups. Compared to females, males presented higher levels of all formation and resorption biochemical markers in all age and Tanner groups (all $p < 0.05$), except urine β-CTX in Tanner ≤4 ($p = 0.081$).

Males had a significantly lower concentration in PINP in the 15–17.5 years group compared to the younger group ($p < 0.05$) and females also had lower concentrations in the 15–17.5 years group in bone formation markers (all $p < 0.05$) and urine β-CTX ($p < 0.05$) compared to the younger group. Both males and females had a significantly lower concentration in bone formation and resorption biochemical markers in Tanner V compared to Tanner ≤IV (all $p < 0.05$).

In the whole group, compared to females, males presented higher concentrations in both formation and resorption markers (all $p < 0.05$). Therefore, males showed increased bone turnover compared to females. Medium–large effect sizes were found for all bone markers except for serum β-CTX and osteocalcin (in females), both with small effect sizes.

Table 3. Bone mineral content (BMC) and density (BMD) in males and females by Tanner stage (mean ± SD)

	Tanner ≤IV	Tanner V	p
Males	52	116	
BMC, g			
Whole body	1,791.83 ± 373.17*	2,300.13 ± 424.34*	0.000
Pelvis	196.65 ± 58.51*	274.68 ± 73.20*	0.000
Hip	31.45 ± 8.18*	39.50 ± 8.85*	0.000
Lumbar spine	41.11 ± 10.55	57.69 ± 13.41*	0.000
Upper limbs	102.08 ± 26.39*	139.90 ± 29.54*	0.000
Lower limbs	367.65 ± 88.69*	475.81 ± 92.52*	0.000
BMD, g·cm^{-2}			
Whole body	0.994 ± 0.091*	1.105 ± 0.112*	0.000
Pelvis	0.981 ± 0.143*	1.133 ± 0.156*	0.000
Hip	0.927 ± 0.141*	1.037 ± 0.136*	0.000
Lumbar spine	0.781 ± 0.117	0.933 ± 0.127	0.000
Upper limbs	0.651 ± 0.050*	0.721 ± 0.064*	0.000
Lower limbs	1.098 ± 0.124*	1.245 ± 0.135*	0.000
Females	19	158	
BMC, g			
Whole body	1,429.81 ± 280.37	1,927.58 ± 275.00	0.000
Pelvis	142.68 ± 37.06	212.95 ± 41.45	0.000
Hip	22.30 ± 5.98	26.69 ± 4.74	0.006
Lumbar spine	36.90 ± 10.47	51.51 ± 9.53	0.000
Upper limbs	84.32 ± 16.47	111.59 ± 17.97	0.000
Lower limbs	275.75 ± 59.96	348.31 ± 54.73	0.005
BMD, g·cm^{-2}			
Whole body	0.897 ± 0.089	1.059 ± 0.088	0.000
Pelvis	0.882 ± 0.103	1.080 ± 0.117	0.006
Hip	0.785 ± 0.101	0.914 ± 0.103	0.013
Lumbar spine	0.764 ± 0.128	0.954 ± 0.103	0.000
Upper limbs	0.592 ± 0.055	0.650 ± 0.044	0.000
Lower limbs	0.939 ± 0.097	1.080 ± 0.087	0.002

* $p < 0.05$ for sex differences.

Discussion

The main results of this study are: (1) males presented higher values of BMC and BMD when classified by age and sexual maturation, but after adjusting for differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs), percentage of fat mass and sexual maturation (only for age groups), results showed that females had higher values of BMC and BMD in most regions; (2) males showed an increased bone turnover compared to females across adolescence, and (3) bone formation markers were lower in both sexes in advanced age and puberty groups when compared with the early age and puberty group.

Bone Mass through Adolescence

The period of puberty is characterized by sex differences in bone size and bone strength, and those are the result of the greater endocortical and periosteal expansion during prepubertal years and the minimal endocortical contraction in males compared with the high endocortical contraction and the inhibition of periosteal apposition in females after the pubertal growth spurt [30, 31]. Although genetics may explain up to 70% of the variance in bone mass [32], environmental and lifestyle factors are likely to contribute to the development of a strong skeleton during childhood and adolescence. This will help to prevent future osteopenia and osteoporosis. Studies assessing bone mass in children and adolescents presented different results according to the body region assessed and depending on the confounders used for the adjustment. In our study, adolescent males were taller, heavier, with higher lean mass and lower fat mass percentage, factors that potentially could account for higher bone mass. Fat accumulation has been shown to be a protective value for bone health, although it has been shown that during growth it is more important to increase lean mass than fat mass to promote at least femoral bone mass

Table 4. Bone mineral content (BMC) and density (BMD) in males and females by age groups adjusted by differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs), percentage of fat mass and sexual maturation (mean ± SE)

	12.5–14.99 years	15–17.5 years	Whole group	p
Males	90	78	168	
BMC, g				
Whole body	1,772.45 ± 28.41*	1,960.28 ± 38.56*	1,867.05 ± 22.86*	0.179
Pelvis	191.73 ± 5.04*	220.22 ± 8.06*	206.41 ± 4.34*	0.439
Hip	29.8 ± 0.66	33.59 ± 1.07	31.56 ± 0.57	0.137
Lumbar spine	40.56 ± 0.98*	48 ± 1.62*	44.1 ± 0.87*	0.005
Upper limbs	101.59 ± 1.82*	116.51 ± 2.37*	108.4 ± 1.46*	0.026
Lower limbs	361.88 ± 6.41	397.34 ± 7.82*	378.92 ± 4.88*	0.526
BMD, g·cm^{-2}				
Whole body	0.988 ± 0.011*	1.037 ± 0.015*	1.012 ± 0.009*	0.014
Pelvis	0.982 ± 0.015*	1.058 ± 0.021*	1.018 ± 0.012*	0.892
Hip	0.919 ± 0.013	0.95 ± 0.02	0.937 ± 0.011	0.765
Lumbar spine	0.796 ± 0.013*	0.88 ± 0.019*	0.833 ± 0.011*	0.007
Upper limbs	0.645 ± 0.005	0.676 ± 0.008*	0.658 ± 0.005*	0.021
Lower limbs	1.1 ± 0.012	1.155 ± 0.016	1.127 ± 0.01	0.083
Females	95	82	177	
BMC, g				
Whole body	1,954.44 ± 27.31	2,361.23 ± 37.04	2,135.88 ± 21.93	0.025
Pelvis	222.15 ± 4.84	279.37 ± 7.74	247.28 ± 4.18	0.454
Hip	29.04 ± 0.63	34.04 ± 1.02	31.35 ± 0.55	0.708
Lumbar spine	52.9 ± 0.95	64.24 ± 1.56	58.13 ± 0.84	0.774
Upper limbs	116.33 ± 1.75	140.07 ± 2.27	127.45 ± 1.41	0.002
Lower limbs	369.36 ± 6.16	438.21 ± 7.51	400.71 ± 4.7	0.211
BMD, g·cm^{-2}				
Whole body	1.045 ± 0.011	1.159 ± 0.014	1.097 ± 0.009	0.021
Pelvis	1.088 ± 0.014	1.164 ± 0.02	1.123 ± 0.012	0.772
Hip	0.926 ± 0.013	1.011 ± 0.019	0.962 ± 0.01	0.862
Lumbar spine	0.942 ± 0.013	1.032 ± 0.018	0.986 ± 0.01	0.934
Upper limbs	0.655 ± 0.005	0.712 ± 0.007	0.683 ± 0.004	0.005
Lower limbs	1.086 ± 0.012	1.193 ± 0.016	1.134 ± 0.009	0.203

* $p < 0.05$ for sex differences.

acquisition [26]. DXA infra estimates bone mass when measuring subjects with a higher amount of fat mass. Because we found big differences between the sexes in percentage of fat mass (21.06 vs. 30.06%, males and females), the percentage of body fat was also used as covariate.

After considering the differences in height, lean mass, percentage of fat mass and sexual maturation we found that in most body regions females had significantly higher values of BMC and BMD than males in the whole group. Most of the descriptive studies published do not adjust for differences in these factors. Differences between sexes were not found after adjusting by weight, height and age in any pubertal stage for lumbar spine and whole body in 11- to 15-year-old children [33]. This could be due to the age range in which the study was performed, during the pubertal growth spurt of girls [10]. The latter study [33] showed that whole body BMD increased until pubertal stage IV, but an increase in bone mass was not detected after this stage in boys. Our data show increases until Tanner stage V. The discrepancies could be explained by the differences in sample size between studies and because lean mass was not previously taken into account, even when it has been observed to have a great influence on bone development [26, 34, 35]. Since most of the studies do not show adjusted data, the present results add new evidence of bone sex dimorphism accounting for differences in height, percentage of fat mass and lean mass. It should be acknowledged that the differences be-

Table 5. Bone mineral content (BMC) and density (BMD) in males and females by Tanner Stage adjusted by differences in height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for the lower limbs) and percentage of fat mass (mean ± SE)

	Tanner ≤IV	Tanner V	p
Males	52	116	
BMC, g			
Whole body	1,690.07 ± 22.7	1,856.72 ± 30.49*	0.538
Pelvis	180.54 ± 4.31	205.08 ± 5.98*	0.539
Hip	29.5 ± 0.76	31.86 ± 0.76	0.409
Lumbar spine	38.09 ± 0.79*	44.05 ± 1.21*	0.564
Upper limbs	94.93 ± 1.63*	109.34 ± 1.95*	0.033
Lower limbs	343.81 ± 4.99	380.55 ± 6.69*	0.729
BMD, g·cm^{-2}			
Whole body	0.97 ± 0.01	1.003 ± 0.012*	0.775
Pelvis	0.945 ± 0.012	1.02 ± 0.016*	0.887
Hip	0.894 ± 0.013	0.938 ± 0.015*	0.35
Lumbar spine	0.75 ± 0.012*	0.841 ± 0.014*	0.208
Upper limbs	0.636 ± 0.006	0.658 ± 0.006*	0.236
Lower limbs	1.066 ± 0.011	1.128 ± 0.013	0.28
Females	19	158	
BMC, g			
Whole body	1,708.31 ± 40.77	2,253.12 ± 23.98	0.000
Pelvis	186.75 ± 7.74	264.05 ± 4.7	0.000
Hip	27.62 ± 1.37	32.25 ± 0.59	0.408
Lumbar spine	45.61 ± 1.43	61.59 ± 0.95	0.000
Upper limbs	103.88 ± 2.98	134.02 ± 1.53	0.000
Lower limbs	340.99 ± 8.95	418.25 ± 5.25	0.000
BMD, g·cm^{-2}			
Whole body	0.962 ± 0.017	1.134 ± 0.009	0.000
Pelvis	0.979 ± 0.022	1.163 ± 0.013	0.000
Hip	0.877 ± 0.024	0.986 ± 0.012	0.002
Lumbar spine	0.852 ± 0.022	1.022 ± 0.011	0.000
Upper limbs	0.632 ± 0.01	0.696 ± 0.005	0.000
Lower limbs	1.028 ± 0.02	1.166 ± 0.01	0.000

* $p < 0.05$ between genders.

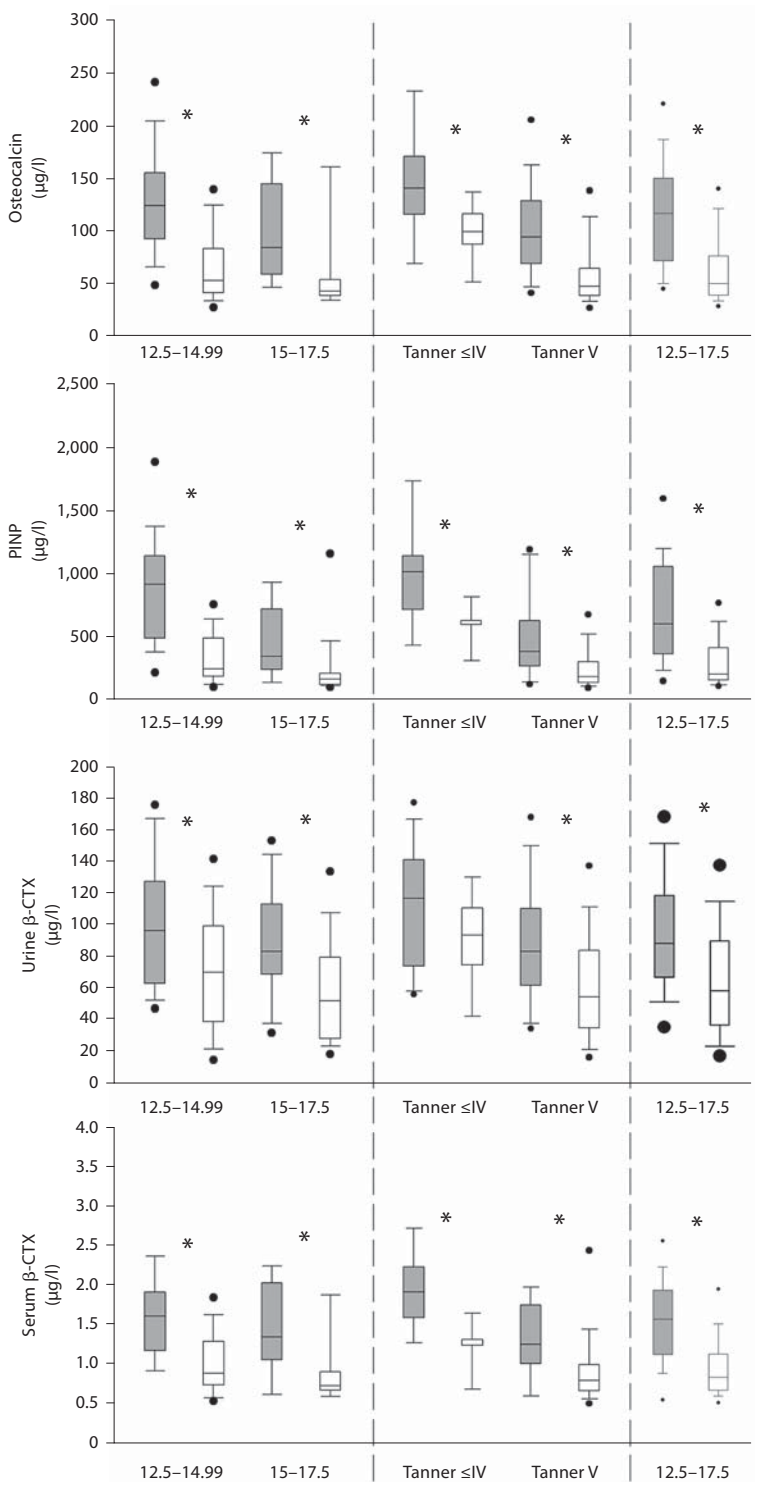
tween males and females might also be influenced by the fact that skeletal age may be more advanced in female adolescents; we tried to minimize this effect by adjusting for maturation. However, it could be interesting for future research to study this confounder in more depth.

Several studies have observed that the most important increases in BMD (lumbar spine, femoral neck, radius and hip) in both sexes occur at Tanner stages IV–V [36–41]. Our adjusted data show similar results for females in all measured regions except for the hip, and also our crude results change significantly from Tanner ≤IV to Tanner V in all regions in both sexes. However, Slemenda et al. [40] found that pubertal development has varying effects on skeletal mineral deposition depending on the skeletal site considered. Other factors such as physical activity and normal growth have also been positively associated with skeletal mineralization [34, 42, 43], and also depending on skeletal site and sexual maturation. Our analyses were grouped into 2 categories (Tanner ≤IV and Tanner V) in order to obtain a higher sample in the first group, and were then not comparable with those of Slemenda et al. [40]. Additional analyses were made to classify the subjects into 3 Tanner stages (III, IV and V; data not shown) and these were in agreement with Slemenda et al. [40], showing the most important BMD increase in all regions between Tanner stages III and IV in males, while in females differences were found depending on the region: whole body, pelvis and average arms at Tanner stage IV–V, and hip, lumbar spine and average legs at Tanner stage III–IV. The latter results reinforce the evidence of dimorphic site-specific bone accretion between sexes.

Bone Markers through Adolescence

The use of bone turnover markers deals with three important difficulties: (1) the variability of nearly all bone markers makes it difficult to apply the results derived from large studies to individual patients; (2) many countries lack pivotal quality control programs for bone turnover markers, and (3) the lack of valid reference ranges makes it difficult for clinicians to interpret a given result [44]. Thus, our study tries to enlarge the knowledge in this field by providing data for Spanish adolescents.

The period of puberty is associated with high bone turnover [45–47]. Our results show that the formation (osteocalcin and PINP) and resorption (serum and urine β-CTX) markers were lower in both sexes in advanced puberty and age groups when compared with the early groups. Those results are consistent with Caucasian girls aged 9–15 years [11] for bone formation (osteocalcin, serum bone-specific alkaline phosphatase and PINP) and bone resorption (serum CTX). In Dutch children aged 8–15 years, Van Coeverden et al. [48] showed that, from the peak values to the end of puberty, markers of formation (serum alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin and PINP) and resorption (serum carboxyterminal telopeptide and urine deoxypyridoline) significantly decreased in girls, except for deoxypyridoline/creatinine; but significant decreases were not found in boys, except for carboxyterminal telopeptide. We cannot discuss the peak concentration as we have 2 groups for both age and sexual maturation, thus we refer to maximum concentration. Significantly lower concentrations were found for PINP in both sexes and osteocal-



	Males	Females
p between age groups	0.113	**0.048**
p between Tanner groups	**0.005**	**0.005**

	Males	Females
p between age groups	**0.001**	**0.007**
p between Tanner groups	**0.001**	**0.002**

	Males	Females
p between age groups	0.229	**0.04**
p between Tanner groups	**0.01**	**0.002**

	Males	Females
p between age groups	0.626	**0.094**
p between Tanner groups	**0.001**	**0.004**

Fig. 1. Osteocalcin, PINP and serum and urine β-CTX in males and females by age groups and Tanner Stage. Grey boxes = Males; white boxes = females. * $p \leq 0.05$ between sexes. All values are medians and interquartile intervals.

cin and urine β-CTX in females in the 15–17.5 years age group compared to the younger group suggesting lower bone metabolic activity, especially in females. Further analyses were performed to classify the sample into Tanner III, IV and V showing that the maximum concentration of osteocalcin, serum β-CTX and urine β-CTX occurred in Tanner IV in both sexes, except for serum β-CTX in females, which occurred in Tanner stage III, while the maximum concentration of PINP occurred in Tanner stage III in both sexes (data not shown). At least in males for osteocalcin, these results are consistent with those of Van Coeverden et al. [48]. However, in the study by Yilmaz et al. [33], the peak of osteocalcin was reached in Tanner III in both sexes, which is not consistent with our results. They also observed that osteocalcin did not change significantly in pubertal stages. On the other hand, we found significantly lower osteocalcin concentrations in Tanner V compared to Tanner III in males and between Tanner stages III–V and IV–V in females (data not shown). The latter suggests that osteocalcin seems to vary depending on sexual maturation as well as on increasing age, again especially in females.

In the whole age range (12.5–17.5 years) and also in all age and Tanner groups, we found sex differences in all bone markers, showing higher concentrations in males compared to females, except for urine β-CTX in Tanner ≤IV. These suggest higher metabolic activity in males and, as a consequence, an increased bone turnover.

The differences between studies could be due to differences in methodology, the analyzer, sample size, diurnal and seasonal differences, and menarche in girls. These factors might influence the concentration of each bone marker and should be taken into account in future research.

In summary, our results indicate that males had higher values of BMC and BMD in most regions in advanced puberty or age groups when compared with early puberty or younger groups, possibly because they are taller and have higher lean mass, but perhaps also for the influence of several other confounders such as fat mass and sexual maturation. However, after taking into account differences in these confounders, females showed higher BMC and BMD. Our results support the evidence of dimorphic site-specific bone accretion between sexes.

For bone markers, we describe sex-specific data for bone formation (osteocalcin and PINP) and bone resorption markers (serum and urine β-CTX). Our results show that males present an increased and longer-lasting bone turnover compared to females, suggesting higher bone metabolic activity in males during adolescence.

Strengths and Limitations

The main strength of the study was that the estimation of BMC and BMD was performed in the same adolescents in whom we measured bone turnover, but we need to consider that DXA scanners are not able to measure real BMD or volumetric BMD (usually expressed in g/cm^3); what they measure is an areal BMD (g/cm^2). However, DXA scanners are commonly used for children and adolescents. In addition, our study does not include adolescents of all pubertal stages, thus our results are limited to Tanner stages III–V. The use of potential confounders such as height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for lower limbs), percentage of fat mass, and sexual maturation (when grouping by age) are also strengths of our study.

The biochemical markers used in our study have an important degree of variability which could be due to the analytical performance characteristics of the method, but also to the biological variability of the markers, as well as the influence of pre-analytical conditions. The factors that confound measurement of the markers we used to a variable degree could be circadian rhythm, diet, age, sex, menstrual cycle, liver function, kidney clearance, as well as thermal stability, storage, and repeated freeze-thaw cycles.

With the aim of minimizing drawbacks, in the present study all adolescents were measured under the same conditions. Blood draws and urine samples were collected and stored at the same time in the morning. All adolescents were measured from October 2006 to June 2007 at stable temperature. It could be interesting for future research to adjust by the remaining factors that could influence bone marker concentrations.

Acknowledgements

The HELENA Study took place with the financial support of the European Community 6th RTD Framework Program (Contract FOOD-CT-2005-007034). The content of this article reflects only the authors' views, and the European Community is not liable for any use that may be made of the information contained therein.

This study was also supported by a grant from the Spanish Ministry of Health: Maternal, Child Health and Development Network (No. RD08/0072), the Spanish Ministry of Education (EX-2007-1124, AGL2007-29784-E/ALI; AP-2005-3827), the Swedish Council for Working Life and Social Research (FAS), and the Universidad Politécnica de Madrid (CH/018/2008).

This study was also supported (L.G.-M., G.V.-R., J.P.R.-L., M.I.M., L.M.A.) by a grant from Fundación MAPFRE (Spain).

Appendix

Coordinator: Luis A. Moreno.

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

Steering Committee: Anthony Kafatos (President), Luis A. Moreno, Christian Libersa, Stefaan De Henauw, Jackie Sánchez, Fréderic Gottrand, Mathilde Kersting, Michael Sjöstrom, Dénes Molnár, Marcela González-Gross, Jean Dallongeville, Chantal Gilbert, Gunnar Hall, Lea Maes, Luca Scalfi.

Project Manager: Pilar Meléndez.

Universidad de Zaragoza (Spain): Luis A. Moreno, Jesús Fleta, José A. Casajús, Gerardo Rodríguez, Concepción Tomás, María I. Mesana, Germán Vicente-Rodríguez, Adoración Villarroya, Carlos M. Gil, Ignacio Ara, Juan Revenga, Carmen Lachen, Juan Fernández Alvira, Gloria Bueno, Aurora Lázaro, Olga Bueno, Juan F. León, Jesús Mª Garagorri, Manuel Bueno, Juan Pablo Rey López, Iris Iglesia, Paula Velasco, Silvia Bel, Luis A. Garcia Marco.

Consejo Superior de Investigaciones Científicas (Spain): Ascensión Marcos, Julia Wärnberg, Esther Nova, Sonia Gómez, Esperanza Ligia Díaz, Javier Romeo, Ana Veses, Mari Angeles Puertollano, Belén Zapatera, Tamara Pozo.

Université de Lille 2 (France): Laurent Beghin, Christian Libersa, Frédéric Gottrand, Catalina Iliescu, Juliana Von Berlepsch.

Research Institute of Child Nutrition Dortmund, Rheinische Friedrich-Wilhelms Universität Bonn (Germany): Mathilde Kersting, Wolfgang Sichert-Hellert, Ellen Koeppen.

Pécsi Tudományegyetem (University of Pécs, Hungary): Dénes Molnar, Eva Erhardt, Katalin Csernus, Katalin Török, Szilvia Bokor, Mrs. Angster, Enikö Nagy, Orsolya Kovács, Judit Répasi.

University of Crete School of Medicine (Greece): Anthony Kafatos, Caroline Codrington, María Plada, Angeliki Papadaki, Katerina Sarri, Anna Viskadourou, Christos Hatzis, Michael Kiriakakis, George Tsibinos, Constantine Vardavas, Manolis Sbokos, Eva Protoyeraki, Maria Fasoulaki.

Institut für Ernährungs- und Lebensmittelwissenschaften – Ernährungphysiologie, Rheinische Friedrich-Wilhelms Universität (Germany): Peter Stehle, Klaus Pietrzik, Marcela González-Gross, Christina Breidenassel, Andre Spinneker, Jasmin Al-Tahan, Miriam Segoviano, Anke Berchtold, Christine Bierschbach, Erika Blatzheim, Adelheid Schuch, Petra Pickert.

University of Granada (Spain): Manuel J. Castillo Garzón, Ángel Gutiérrez Sáinz, Francisco B. Ortega Porcel, Jonatan Ruiz Ruiz, Enrique García Artero, Vanesa España Romero, David Jiménez Pavón, Cristóbal Sánchez Muñoz, Victor Soto, Palma Chillón, Jose M. Heredia, Virginia Aparicio, Pedro Baena, Claudia M. Cardia, Ana Carbonell.

Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Italy): Davide Arcella, Giovina Catasta, Laura Censi, Donatella Ciarapica, Marika Ferrari, Cinzia Le Donne, Catherine Leclerq, Luciana Magrì, Giuseppe Maiani, Rafaela Piccinelli, Angela Polito, Raffaela Spada, Elisabetta Toti.

University of Naples 'Federico II', Department of Food Science (Italy): Luca Scalfi, Paola Vitaglione, Concetta Montagnese.

Ghent University (Belgium): Ilse De Bourdeaudhuij, Stefaan De Henauw, Tineke De Vriendt, Lea Maes, Christophe Matthys, Carine Vereecken, Mieke de Maeyer, Charlene Ottevaere, Inge Huybrechts.

Medical University of Vienna (Austria): Kurt Widhalm, Katharina Phillipp, Sabine Dietrich, Birgit Kubelka, Marion Boriss-Riedl.

Harokopio University (Greece): Yannis Manios, Eva Grammatikaki, Zoi Bouloubasi, Tina Louisa Cook, Sofia Eleutheriou, Orsalia Consta, George Moschonis, Ioanna Katsaroli, George Kraniou, Stalo Papoutsou, Despoina Keke, Ioanna Petraki, Elena Bellou, Sofia Tanagra, Kostalenia Kallianoti, Dionysia Argyropoulou, Katerina Kondaki, Stamatoula Tsikrika, Christos Karaiskos.

Institut Pasteur de Lille (France): Jean Dallongeville, Aline Meirhaeghe.

Karolinska Institutet (Sweden): Michael Sjöstrom, Patrick Bergman, María Hagströmer, Lena Hallström, Mårten Hallberg, Eric Poortvliet, Julia Wärnberg, Nico Rizzo, Linda Beckman, Anita Hurtig Wennlöf, Emma Patterson, Lydia Kwak, Lars Cernerud, Per Tillgren, Stefaan Sörensen.

Asociación de Investigación de la Industria Agroalimentaria (Spain): Jackie Sánchez-Molero, Elena Picó, Maite Navarro, Blanca Viadel, José Enrique Carreres, Gema Merino, Rosa Sanjuán, María Lorente, María José Sánchez, Sara Castelló.

Campden BRI (UK): Chantal Gilbert, Sarah Thomas, Elaine Allchurch, Peter Burguess.

SIK – Institutet foer Livsmedel och Bioteknik (Sweden): Gunnar Hall, Annika Astrom, Anna Sverkén, Agneta Broberg.

Meurice Recherche and Development asbl (Belgium): Annick Masson, Claire Lehoux, Pascal Brabant, Philippe Pate, Laurence Fontaine.

Campden and Chorleywood Food Development Institute (Hungary): Andras Sebok, Tunde Kuti, Adrienn Hegyi.

Productos Aditivos SA (Spain): Cristina Maldonado, Ana Llorente.

Cárnicas Serrano SL (Spain): Emilio García.

Cederroth International AB (Sweden): Holger von Fircks, Marianne Lilja Hallberg, Maria Messerer.

Lantmännen Food R&D (Sweden): Mats Larsson, Helena Fredriksson, Viola Adamsson, Ingmar Börjesson.

European Food Information Council (Belgium): Laura Fernández, Laura Smillie, Josephine Wills.

Universidad Politécnica de Madrid (Spain): Marcela González-Gross, Agustín Meléndez, Pedro J. Benito, Javier Calderón, David Jiménez-Pavón, Jara Valtueña, Paloma Navarro, Alejandro Urzanqui, Ulrike Albers, Raquel Pedrero, Juan José Gómez Lorente.

References

1 Rizzoli R, Bonjour JP: Determinants of peak bone mass and mechanisms of bone loss. Osteoporos Int 1999;9(suppl 2):S17–S23.
2 Hui SL, Slemenda CW, Johnston CC Jr: The contribution of bone loss to postmenopausal osteoporosis. Osteoporos Int 1990;1:30–34.
3 Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA: A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study. J Bone Miner Res 1999;14: 1672–1679.
4 Gordon CL, Halton JM, Atkinson SA, Webber CE: The contributions of growth and puberty to peak bone mass. Growth Dev Aging 1991;55:257–262.
5 MacKelvie KJ, Khan KM, McKay HA: Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. Br J Sports Med 2002;36:250–257.
6 Derman O, Cinemre A, Kanbur N, Dogan M, Kilic M, Karaduman E: Effect of swimming on bone metabolism in adolescents. Turk J Pediatr 2008;50:149–154.
7 Moreno LA, Gonzalez-Gross M, Kersting M, Molnar D, de Henauw S, Beghin L, Sjostrom M, Hagstromer M, Manios Y, Gilbert CC, Ortega FB, Dallongeville J, Arcella D, Warnberg J, Hallberg M, Fredriksson H, Maes L, Widhalm K, Kafatos AG, Marcos A: Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 2008;11:288–299.
8 De Henauw S, Gottrand F, De Bourdeaudhuij I, Gonzalez-Gross M, Leclercq C, Kafatos A, Molnar D, Marcos A, Castillo M, Dallongeville J, Gilbert C, Bergman P, Widhalm K, Manios Y, Breidenassel C, Kersting M, Moreno Lobot HSG: Nutritional status and lifestyles of adolescents in a public health perspective. The HELENA Project – Healthy Lifestyle in Europe by Nutrition in Adolescence. J Publ Health 2007;15:187–197.
9 Ferrari SL: Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. World Rev Nutr Diet 2005;95:35–51.
10 Bailey DA, Faulkner RA, McKay HA: Growth, physical activity, and bone mineral acquisition. Exerc Sport Sci Rev 1996;24: 233–266.
11 Lehtonen-Veromaa M, Mottonen T, Irjala K, Nuotio I, Leino A, Viikari J: A 1-year prospective study on the relationship between physical activity, markers of bone metabolism, and bone acquisition in peripubertal girls. J Clin Endocrinol Metab 2000;85: 3726–3732.
12 Delmas PD: Biochemical markers of bone turnover. Acta Orthop Scand Suppl 1995; 266:176–182.
13 Burgeson RE: New collagens, new concepts. Annu Rev Cell Biol 1988;4:551–577.
14 Fledelius C, Johnsen AH, Cloos PA, Bonde M, Qvist P: Characterization of urinary degradation products derived from type I collagen. Identification of a beta-isomerized Asp-Gly sequence within the C-terminal telopeptide (alpha1) region. J Biol Chem 1997;272:9755–9763.
15 Garnero P, Fledelius C, Gineyts E, Serre CM, Vignot E, Delmas PD: Decreased beta-isomerization of the C-terminal telopeptide of type I collagen alpha 1 chain in Paget's disease of bone. J Bone Miner Res 1997;12:1407–1415.
16 Kraenzlin ME, Seibel MJ: Measurement of biochemical markers of bone resorption; in Seibel MJ, Robins SP, Bilezikian JP (eds): Dynamics of Bone and Cartilage Metabolism. San Diego, Academic Press, 1999, pp 411–426.
17 Campion JM, Maricic MJ: Osteoporosis in men. Am Fam Physician 2003;67:1521–1526.
18 Moreno LA, De Henauw S, Gonzalez-Gross M, Kersting M, Molnar D, Gottrand F, Barrios L, Sjostrom M, Manios Y, Gilbert CC, Leclercq C, Widhalm K, Kafatos A, Marcos A: Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes (Lond) 2008;32(suppl 5):S4–S11.
19 Beghin L, Castera M, Manios Y, Gilbert CC, Kersting M, De Henauw S, Kafatos A, Gottrand F, Molnar D, Sjostrom M, Leclercq C, Widhalm K, Mesana MI, Moreno LA, Libersa C: Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. Int J Obes (Lond) 2008;32(suppl 5): S12–S18.
20 Lohman TM: Assessment of body composition in children. Pediatr Exerc Sci 1989;1: 19–30.
21 Moreno LA, Rodriguez G, Guillen J, Rabanaque MJ, Leon JF, Arino A: Anthropometric measurements in both sides of the body in the assessment of nutritional status in prepubertal children. Eur J Clin Nutr 2002;56:1208–1215.
22 Moreno LA, Joyanes M, Mesana MI, Gonzalez-Gross M, Gil CM, Sarria A, Gutierrez A, Garaulet M, Perez-Prieto R, Bueno M, Marcos A: Harmonization of anthropometric measurements for a multicenter nutrition survey in Spanish adolescents. Nutrition 2003;19:481–486.
23 Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, Bemben DA: Skinfold equations for estimation of body fatness in children and youth. Hum Biol 1988;60:709–723.
24 Tanner JM, Whitehouse RH: Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170–179.
25 Calbet JA, Dorado C, Diaz-Herrera P, Rodriguez-Rodriguez LP: High femoral bone mineral content and density in male football (soccer) players. Med Sci Sports Exerc 2001; 33:1682–1687.
26 Vicente-Rodriguez G, Ara I, Perez-Gomez J, Dorado C, Calbet JA: Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. Br J Sports Med 2005;39:611–616.
27 Vereecken CA, Covents M, Sichert-Hellert W, Alvira JM, Le Donne C, De Henauw S, De Vriendt T, Phillipp MK, Beghin L, Manios Y, Hallstrom L, Poortvliet E, Matthys C, Plada M, Nagy E, Moreno LA: Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. Int J Obes (Lond) 2008; 32(suppl 5):S26–S34.
28 Farrán A, Zamora R, Cervera P: Tablas de Composición de Alimentos del Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID). Barcelona, 2004.
29 Nakagawa S, Cuthill IC: Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev Camb Philos Soc 2007;82:591–605.
30 Seeman E: Clinical review 137: sexual dimorphism in skeletal size, density, and strength. J Clin Endocrinol Metab 2001;86: 4576–4584.
31 Zhang XZ, Kalu DN, Erbas B, Hopper JL, Seeman E: The effects of gonadectomy on bone size, mass, and volumetric density in growing rats are gender-, site-, and growth hormone-specific. J Bone Miner Res 1999;14: 802–809.
32 Kelly PJ, Eisman JA, Sambrook PN: Interaction of genetic and environmental influences on peak bone density. Osteoporos Int 1990;1: 56–60.
33 Yilmaz D, Ersoy B, Bilgin E, Gumuser G, Onur E, Pinar ED: Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters. J Bone Miner Metab 2005;23:476–482.
34 Vicente-Rodriguez G: How does exercise affect bone development during growth? Sports Med 2006;36:561–569.
35 Lima F, De Falco V, Baima J, Carazzato JG, Pereira RM: Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. Med Sci Sports Exerc 2001;33:1318–1323.
36 De Schepper J, Derde MP, Van den Broeck M, Piepsz A, Jonckheer MH: Normative data for lumbar spine bone mineral content in children: influence of age, height, weight, and pubertal stage. J Nucl Med 1991;32:216–220.

37 del Rio L, Carrascosa A, Pons F, Gusinye M, Yeste D, Domenech FM: Bone mineral density of the lumbar spine in white Mediterranean Spanish children and adolescents: changes related to age, sex, and puberty. Pediatr Res 1994;35:362–366.
38 Goslings WR, Cole TJ, Prentice A, Bishop NJ: Rate of radial bone mineral accretion in healthy children. Acta Paediatr 1995;84: 383–387.
39 Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R, Dalsky G: Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. J Pediatr 1993;123:863–870.
40 Slemenda CW, Reister TK, Hui SL, Miller JZ, Christian JC, Johnston CC Jr: Influences on skeletal mineralization in children and adolescents: evidence for varying effects of sexual maturation and physical activity. J Pediatr 1994;125:201–207.
41 Hasanoglu A, Tumer L, Ezgu FS: Vertebra and femur neck bone mineral density values in healthy Turkish children. Turk J Pediatr 2004;46:298–302.
42 Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez JA, Dorado C, Calbet JA: Enhanced bone mass and physical fitness in prepubescent footballers. Bone 2003;33: 853–859.
43 Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA: High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc 2004;36:1789–1795.
44 Meier C, Seibel MJ, Kraenzlin ME: Use of bone turnover markers in the real world: are we there yet? J Bone Miner Res 2009;24:386–388.
45 Blumsohn A, Hannon RA, Wrate R, Barton J, al-Dehaimi AW, Colwell A, Eastell R: Biochemical markers of bone turnover in girls during puberty. Clin Endocrinol (Oxf) 1994; 40:663–670.
46 Mora S, Prinster C, Proverbio MC, Bellini A, de Poli SC, Weber G, Abbiati G, Chiumello G: Urinary markers of bone turnover in healthy children and adolescents: age-related changes and effect of puberty. Calcif Tissue Int 1998;63:369–374.
47 Mora S, Pitukcheewanont P, Kaufman FR, Nelson JC, Gilsanz V: Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. J Bone Miner Res 1999;14:1664–1671.
48 van Coeverden SC, Netelenbos JC, de Ridder CM, Roos JC, Popp-Snijders C, Delemarre-van de Waal HA: Bone metabolism markers and bone mass in healthy pubertal boys and girls. Clin Endocrinol (Oxf) 2002;57:107–116.

Osteoporos Int
DOI 10.1007/s00198-011-1649-3

ORIGINAL ARTICLE

Adiposity and bone health in Spanish adolescents. The HELENA study

L. Gracia-Marco · F. B. Ortega · D. Jiménez-Pavón · G. Rodríguez · M. J. Castillo · G. Vicente-Rodríguez · L. A. Moreno

Received: 28 February 2011 / Accepted: 18 April 2011

Abstract
Summary While the association of lean mass (LM) with bone mass is well understood, the association of fat mass (FM) with bone mass is controversial. Our results support that adolescents with higher levels of adiposity have greater bone mass, but this association is fully explained by their higher levels of LM.
Introduction We aimed (1) to study the independent association of FM and LM with bone mass and (2) to study the differences in bone mass by weight status in adolescents, after controlling for relevant confounders, such as physical activity (PA), calcium intake, and LM.
Methods Participants were 330 adolescents (167 boys, 12.5–17.5 years) from the HELENA study. The relationships of FM (DXA, n=330; BodPod, n=282) and LM (DXA, n=330) with different bone variables (whole body, total hip, lumbar spine, and femoral neck) were analyzed by linear regression, and differences between weight status were analyzed by ANCOVA.
Results Fat mass (DXA) was positively associated with bone variables in both sexes, after adjustment for height, calcium intake, and sexual maturation. Additional adjustment by PA slightly increases the associations. However, adjustment for LM inverted these associations. Similar results were obtained using BodPod instead of DXA for assessing FM. Overweight/obese adolescents had higher BMC than their non-overweight peers in most of regions studied. Additional adjustment for PA slightly increased the differences between weight status groups, while adjusting for LM inverted the associations. LM was strong and positively associated with all bone variables in both sexes. Additional adjustment for PA or FM did not change the results.
Conclusions Adolescents with higher levels of adiposity have greater bone mass, but this association is explained by their higher levels of LM.

Keywords Adolescents · BMI · Bone health · Soft tissues

L. Gracia-Marco · D. Jiménez-Pavón · G. Rodríguez · G. Vicente-Rodríguez · L. A. Moreno
GENUD (Growth, Exercise, Nutrition and Development) Research Group, University of Zaragoza,
Avd. Domingo Miral s/n,
50009 Zaragoza, Spain

L. Gracia-Marco (✉) · L. A. Moreno
Department of Physiatry and Nursing, School of Health Sciences, University of Zaragoza,
Avd. Domingo Miral s/n,
50009 Zaragoza, Spain
e-mail: lgracia@unizar.es

F. B. Ortega
Unit for Preventive Nutrition,
Department of Bioscience and Nutrition, Karolinska Institute,
Huddinge, Sweden

F. B. Ortega · D. Jiménez-Pavón · M. J. Castillo
Department of Physiology, School of Medicine,
University of Granada,
Granada, Spain

G. Rodríguez
Department of Pediatrics, Faculty of Medicine,
University of Zaragoza,
Zaragoza, Spain

G. Rodríguez
Instituto Aragonés de Ciencias de la Salud,
Aragón, Spain

G. Vicente-Rodríguez
Faculty of Health and Sport Science (FCSD),
Department of Physiatry and Nursing, University of Zaragoza,
Ronda Misericordia 5,
22001-Huesca Zaragoza, Spain

Published online: 12 May 2011

Introduction

Osteoporosis is a disease characterized by decreased bone mass and bone tissue deterioration [1]. Acquiring a high bone mass during childhood and adolescence is a key determinant of adult skeletal health [2] and it may decrease the risk of osteoporotic fractures by 50% [3, 4]. Although genetics plays an important role on bone mass, environmental and lifestyle factors such as physical activity (PA) [5] and nutrition, i.e., calcium intake [6] have important osteogenic effects.

Lean mass (LM) is a major predictor for bone mineral content (BMC) and for bone accumulation during puberty [7, 8]. During this period of life, increases in LM contribute to increase bone mass, as explained by the mechanostat theory (bigger muscles exert higher tensile forces on the bones they attach) [9, 10]. Therefore, PA and sport participation could indirectly increase bone mass via increasing LM. In addition, sport participation has a direct influence on bone mass because of the extra load that some sport activities have on the bone [11, 12].

While the role of LM in bone formation is well understood, much less is known about fat mass (FM) and bone mass. Some studies have suggested that FM is positively related to bone mass in girls [13, 14], which has been longitudinally confirmed [15, 16]; whereas, others have observed that FM is negatively associated with bone mineral density (BMD) in boys [13, 17].

Whether overweight and obese people have a better or worse bone health is unknown. Hypothetically, both a positive and negative association could be reasonable. Overweight and obese people are known to have both greater body weight and LM, involving an extra load on the skeleton and higher tensile forces on bones. As a consequence of this greater LM, they could have higher bone mass. On the other hand, overweight and obese people are known to be less active than their normal peers [18], and because of these low activity levels, they could be at a higher risk for low bone mass. However, the literature on this topic is contradictory. Therefore, it could be hypothesized that, independently of their PA levels, overweight and obese people could have greater bone mass, result of the higher LM developed as consequence of their higher FM, which is a new point of view on this topic.

Several studies have shown that overweight and obese children and adolescents have higher levels of BMC and BMD [19-22], while others have observed the opposite [23, 24]. Most of previous studies have not properly adjusted for relevant confounders, such as PA, calcium intake, or LM. Since adolescence is a key period of life for bone development, to analyze the role of FM and LM on bone mass as well as to know whether this relationships depends on weight status are of high interest. This is especially important in girls, as they are less active [25], with higher FM, lower LM, and at higher risk for developing osteoporosis in adulthood than boys [26].

Therefore, we aimed (1) to study the association of FM and LM on bone mass in adolescents after adjusting for each other, PA and calcium intake, and (2) to study the differences in bone mass by weight status in adolescents, after controlling for relevant confounders, such as PA, calcium intake, and LM.

Methods

Subjects

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study is a European Union-funded project that included a cross-sectional, multicentre study (HELENA-CSS) conducted on adolescents aged 12.5–17.5 yrs from 10 European cities in 2006–2007 [27]. The general characteristics of the HELENA-CSS have been described in detail elsewhere [28]. In this report, we focus on the sample from Zaragoza, one of the 10 centres involved in the HELENA study, where BMC and BMD were measured by dual-energy X-ray absorptiometry (DXA). After exclusion of 60 subjects since they did not fulfill all the inclusion criteria for our study, a total of 167 boys and 163 girls with complete data on DXA (bone, FM, and LM), height, objective PA, calcium intake, and sexual maturation assessment were included in the analyses. Fat mass was also measured by air displacement plethysmography using the BodPod device in a subsample of 282 adolescents (48 missing values). The participants included in the study did not differ from those excluded in weight, height, and body mass index (BMI, $p>0.3$). Signed informed consent was obtained from parents and adolescents, and the protocol was approved by the ethics committee of clinical research from the government of Aragón (CEICA, Spain) [29].

Anthropometric measurements

International guidelines for anthropometry in adolescents were used in the HELENA study [30]. While the participants were barefoot and clad in light indoor clothing, body weight (in kilograms) and height (in centimeters) were measured with an electronic scale (Type SECA 861), precision 100 g, range 0–150 kg, and a stadiometer (Type Seca 225), precision 0.1 cm, range 70–200 cm, respectively.

Definition of weight status

Body mass index was calculated as body mass (in kilograms) divided by height (in meters) squared. Participants were

categorized according to the international gender and age-specific BMI (in kilograms per square meter) cutoff points [31, 32]. The cutoff points were equivalent to those internationally accepted for adult population, i.e., underweight (BMI <18.5 kg/m^2), normal weight (BMI=18.5–24.9 kg/m^2), overweight (BMI=25–29.9 kg/m^2), and obesity (BMI ≥30 kg/m^2). Weight status was recoded as a dichotomic variable: non-overweight (underweight+normal weight) and overweight/obese (overweight+obese), because of the low sample size in the underweight and obese groups.

Pubertal development

Physical examination was performed by a physician aiming to classify the adolescents in one of the five stages of pubertal maturity defined by Tanner and Whitehouse [33].

Bone, lean and fat mass (DXA)

Adolescents were scanned with DXA (Hologic Explorer scanner, using a pediatric version of the software QDR-Explorer; Hologic Corp., software version 12.4, Bedford, MA, USA). DXA equipment was calibrated using a lumbar spine phantom as recommended by the manufacturer. For the whole body measurement, subjects were scanned in supine position and the scans were performed at high resolution [34]. The BMD (in grams per square centimeter), area (in square centimeters), FM (in grams), and LM (in grams) [body mass−(FM+bone mass)] were determined for each individual from total and regional analysis of the whole body scan. Bone mineral content (in grams) was calculated using the formula BMC=BMD×area. Two additional examinations were conducted to estimate bone mass at the lumbar spine (mean L1–L4) and proximal region of the femur (total hip and femoral neck) as previously described [7].

We have previously examined the test–retest (with repositioning) precision error for regional analysis of the complete body scan, using coefficients of variation (CV) in 49 adolescents. The CV were: BMC=2.3%, BMD=1.3%, bone area=2.6%, and fat-free lean mass=1.9% [35].

Fat mass (BodPod)

Body volume was measured by BodPod (Body Composition System; Life Measurement Instruments, Concord, CA) using standardized procedures [36]. The BodPod was calibrated daily according to the manufacturer's guidelines. Subjects wore clothing according to the manufacturer's recommendation (a swimsuit and a swim cap) to rule out air trapped in clothes and hair. Adolescents were weighted on the BodPod calibrated digital scale and then entered the BodPod chamber. Body volume was measured two times by the machine to ensure measurement reliability. If the first two readings for body volume differed by more than 150 ml, a third measurement was taken. If additional readings were needed, the BodPod was recalibrated and the measurements were repeated for that subject [37]. Percentage of whole body FM was calculated using the equation reported by Siri [38, 39]. Thoracic gas volume was measured following the manufacturer's recommendations [36]. This value was integrated into the calculation of body volume. Whole body FM was calculated as percentage of whole body FM multiplied by body mass (in kilograms) and then divided by 100.

Calcium intake

Mean daily calcium intake was estimated from a two non-consecutive, 24-h recalls using the HELENA-DIAT (Dietary Assessment Tool) software [40]. For the assessment of calcium intake, the food composition tables published by Farrán et al. [41] were used for the Spanish adolescents. The calcium intake/LM ratio (in milligrams per kilogram) was also calculated.

Physical activity

A uniaxial accelerometer (Actigraph GT1M; Manufacturing Technology Inc. Pensacola, FL, USA) was used to assess PA. Adolescents were instructed to place the monitor underneath the clothing, at the lower back, using an elastic waist band and wear it for seven consecutive days. They were also instructed to wear the accelerometer during all times awake and only to remove it during water-based activities. At least 3 days of recording with a minimum of 8 h registration per day was set as an inclusion criterion. The time sampling interval (epoch) was set at 15 s. Average PA was calculated as the total number of counts per epoch divided by total daily registered time (counts per minute) [37].

Statistics

All the residuals showed a satisfactory pattern (normal distribution). Data are presented as mean values±standard deviation unless otherwise stated. Gender differences were assessed by one-way analysis of variance (ANOVA). Relationships of FM (measured with DXA and BodPod) with different bone mass related variables (i.e., whole body, total hip, lumbar spine, and femoral neck) were analyzed using multiple linear regression models, including height, calcium intake, and sexual maturation as covariates (model 0). Model 1 included model 0+average PA to test the role of PA in this

association. Model 2 included model 1+LM to test the role of LM in this association. The same procedure was used to analyze the relationships of LM with bone mass, but with adjustment for FM instead of LM (model 2).

In order to analyze the role of body composition in the association between bone mass related variables and weight status, one-way analysis of covariance (ANCOVA) was performed. The categorized variable of BMI was entered as a fixed factor, bone mass related variables were entered as dependent variables, and, in addition to the previous confounders (i.e., height, calcium intake, and sexual maturation), the average PA and LM were gradually entered as confounders.

All the analyses were performed using the Statistical Package for Social Sciences software (SPSS, v. 15.0 for WINDOWS; SPSS Inc., Chicago, IL, USA), and values of $p<0.05$ were considered statistically significant.

Results

Table 1 shows descriptive characteristics (mean±SD) of the study sample. The ANOVA showed that there were no differences between boys and girls in the mean age, BMD, BMI, and calcium intake/lean mass ratio; however, most traits differed by gender.

Table 2 shows the association between FM (DXA and BodPod) and bone mass after adjusting by potential sets of confounders. Fat mass (DXA) was positively associated with whole body and femoral neck BMC in boys [semi-partial correlation (semip corr), 0.200 and 0.130; $p=0.001$ and 0.05, respectively]. Additional adjustment by PA slightly increases the positive association between FM and whole body BMC, femoral neck BMC, and BMD (semip corr, 0.139 to 0.213; $p<0.001$ to 0.049). Finally, the inclusion of LM as a covariate inverted the associations between FM and most of bone mass related variables (semip corr, −0.112 to −0.270; $p<0.001$ to 0.012). Similarly, FM was positively associated with most of bone mass related variables in girls (semip corr, 0.155 to 0.295; $p<0.001$ to 0.018), except for total hip and lumbar spine BMC ($p=0.057$ to 0.339). Additional adjustment for PA slightly increase the positive association between FM and all bone mass related variables (semip corr, 0.163 to 0.302; $p<0.001$ to 0.014), except for the lumbar spine BMC ($p=0.071$). Finally, additional adjustment for LM inverted the associations between FM and total hip and lumbar spine BMC (semip corr, −0.130 to−0.156; $p=0.006$ to 0.025). Similar results were obtained when using BodPod instead of DXA for assessing FM.

Table 3 shows the association between LM and bone mass after adjusting by potential sets of confounders. Lean mass was strong and positively associated with all bone mass related variables in boys (semip corr, 0.426 to 0.632; all $p<0.001$). Additional adjustment for PA (semip corr, 0.409 to 0.615; all $p<0.001$) as well as FM (semip corr, 0.395 to 0.587; all $p<0.001$) did not change the results. Slightly weaker associations were obtained between LM and bone mass in girls (semip corr, 0.358 to 0.626; all $p<0.001$). Additional adjustment for PA (semip corr, 0.339 to 0.601; all $p<0.001$) as well as FM (semip corr, 0.247 to 0.534; all $p<0.001$) did not change the results.

Table 1 Descriptive characteristics of the studied adolescents ($n=330$) by gender

	Boys ($n=167$)	Girls ($n=163$)	p value
Age (years)	14.7±1.3	14.7±1.1	0.631
Sexual maturation (I/II/III/IV/V), (%)	(0/5/11/20/64)	(0/1/4/7/88)	**<0.001**
Body mass (kg)	61.2±14.9	54.5±9	**<0.001**
Height (cm)[a]	169.3 (160.7–175.3)	159.9 (155.0–165.2)	**0.001**
BMI (kg/m^2)	21.2±3.2	21.2±3.2	0.897
Fat mass (DXA), (kg)[a]	11.4 (8.2–17.2)	15.7 (12.7–19.6)	**<0.001**
Fat mass (BodPod, $n=282$), (kg)[a]	10.6 (6.9–16.6)	14.0 (11.1–18.3)	**<0.001**
Lean mass (kg)	44.8±8.8	35.4±4.6	**<0.001**
BMC (g)	2,087.63±474.53	1,867.13±320.55	**<0.001**
BMD (g/cm^2)	1.058±0.119	1.038±0.103	0.107
Calcium intake (mg/day)[a]	811.4 (648.9–1,105.8)	650.1 (492.3–822.2)	**<0.001**
Calcium intake/lean mass ratio (mg/g)	0.020±0.009	0.020±0.009	0.614
Average PA (counts/min)	477.33±154.77	368.57±117.41	**<0.001**

Normally distributed variables are showed as mean±SD (ANOVA), proportions are noted for categorical variables.Gender differences are in bold

BMI body mass index, *BMC* bone mineral content, *BMD* bone mineral density, *PA* physical activity

[a] Non-normally distributed variables are showed as median and interquartile intervals (25th and 75th, *U* Mann–Whitney)

Table 2 Multiple linear regression analysis of BMC (in grams) and BMD (in grams per square centimeter) in Spanish adolescents participating in the HELENA study as regards to fat mass

				FAT MASS (DXA)					
Dependent variables	Model 0[a]			Model 1[b]			Model 2[c]		
Boys (n=167)									
BMC (g)	B[d]	Semip corr	p value	B[d]	Semip corr	p value	B[d]	Semip corr	p value
Whole body	0.200	0.200	**0.001**	0.216	0.213	**<0.001**	−0.130	−0.112	**<0.001**
Total hip	0.060	0.060	0.386	0.084	0.083	0.226	−0.212	−0.182	**0.001**
Lumbar spine	−0.012	−0.011	0.844	0.000	0.000	0.996	−0.314	−0.270	**<0.001**
Femoral neck	0.130	0.130	0.052	0.155	0.153	**0.020**	−0.150	−0.129	**0.011**
BMD (g/cm^2)									
Whole body	0.018	0.018	0.783	0.028	0.028	0.668	−0.288	−0.248	**<0.001**
Total hip	0.098	0.098	0.152	0.118	0.117	0.085	−0.164	−0.141	**0.012**
Lumbar spine	0.014	0.014	0.819	0.020	0.019	0.753	−0.255	−0.219	**<0.001**
Femoral neck	0.117	0.116	0.102	0.140	0.139	**0.049**	−0.095	−0.081	0.192
Girls (n=163)									
BMC (g)									
Whole body	0.311	0.295	**<0.001**	0.329	0.302	**<0.001**	−0.051	−0.040	0.371
Total hip	0.194	0.184	0.339	0.215	0.197	**0.007**	−0.168	−0.130	**0.025**
Lumbar spine	0.139	0.132	0.057	0.135	0.124	0.071	−0.200	−0.156	**0.006**
Femoral neck	0.316	0.300	**<0.001**	0.352	0.323	**0.000**	0.070	0.054	0.358
BMD (g/cm^2)									
Whole body	0.164	0.155	**0.018**	0.177	0.163	**0.014**	−0.068	−0.053	0.371
Total hip	0.213	0.202	**0.003**	0.248	0.228	**0.001**	0.021	0.016	0.796
Lumbar spine	0.273	0.260	**<0.001**	0.263	0.241	**<0.001**	0.064	0.049	0.404
Femoral neck	0.228	0.217	**0.002**	0.265	0.243	**0.001**	0.079	0.061	0.353
				FAT MASS (BODPOD)					
Dependent variables	Model 0[a]			Model 1[b]			Model 2[c]		
Boys (n=134)									
BMC (g)	B[d]	Semip corr	p value	B[d]	Semip corr	p value	B[d]	Semip corr	p value
Whole body	0.167	0.166	**0.011**	0.176	0.174	**0.008**	−0.127	−0.113	**0.002**
Total hip	0.072	0.071	0.361	0.084	0.083	0.286	−0.181	−0.161	**0.010**
Lumbar spine	−0.046	−0.046	0.475	−0.042	−0.042	0.517	−0.308	−0.273	**<0.001**
Femoral neck	0.099	0.098	0.197	0.113	0.112	0.137	−0.157	−0.139	**0.017**
BMD (g/cm^2)									
Whole body	−0.021	−0.021	0.770	−0.017	−0.016	0.820	−0.278	−0.247	**<0.001**
Total hip	0.053	0.052	0.503	0.065	0.065	0.404	−0.192	−0.162	**0.016**
Lumbar spine	−0.018	−0.018	0.795	−0.017	−0.017	0.801	−0.237	−0.211	**<0.001**
Femoral neck	0.065	0.065	0.423	0.078	0.077	0.334	−0.121	−0.108	0.137
Girls (n=148)									
BMC (g)									
Whole body	0.157	0.151	0.892	0.183	0.168	**0.017**	−0.140	−0.116	**0.014**
Total hip	0.063	0.060	0.440	0.096	0.088	0.259	−0.218	−0.180	**0.003**
Lumbar spine	−0.013	−0.013	0.860	−0.010	−0.009	0.901	−0.291	−0.241	**<0.001**
Femoral neck	0.178	0.171	**0.024**	0.228	0.209	**0.005**	−0.029	−0.024	0.691
BMD (g/cm^2)									
Whole body	0.032	0.030	0.667	0.059	0.054	0.445	−0.145	−0.121	0.056
Total hip	0.103	0.098	0.189	0.154	0.141	0.056	−0.036	−0.030	0.659
Lumbar spine	0.125	0.120	0.086	0.123	0.113	0.108	−0.058	−0.048	0.453
Femoral neck	0.122	0.117	0.122	0.170	0.156	**0.037**	0.012	0.010	0.885

Significant results are in bold

Semip corr semi-partial correlation

BMC bone mineral content, *BMD* bone mineral density

[a] In addition to whole body fat mass (FM) (in kilograms), the following independent variables were entered into the model: height (in centimeters), calcium intake (in milligrams per day), and sexual maturation (model 0)

[b] Model 0+average physical activity (PA) (counts per minute)

[c] Model 1+whole body lean mass (LM) (in kilograms)

[d] B is the estimated standardized regression coefficient

Table 3 Multiple linear regression analysis of BMC (in grams) and BMD (in grams per square centimeter) in Spanish adolescents participating in the HELENA study as regards to lean mass (n=330)

LEAN MASS (DXA)									
Dependent variables	Model 0[a]			Model 1[b]			Model 2[c]		
Boys (n=167)									
BMC (g)	B[d]	Semip corr	p value	B[d]	Semip corr	p value	B[d]	Semip corr	p value
Whole body	0.907	0.632	**<0.001**	0.885	0.615	**<0.001**	0.976	0.587	**<0.001**
Total hip	0.718	0.501	**<0.001**	0.689	0.479	**<0.001**	0.841	0.505	**<0.001**
Lumbar spine	0.688	0.480	**<0.001**	0.665	0.462	**<0.001**	0.888	0.534	**<0.001**
Femoral neck	0.788	0.550	**<0.001**	0.756	0.525	**<0.001**	0.863	0.518	**<0.001**
BMD (g/cm^2)									
Whole body	0.711	0.496	**<0.001**	0.681	0.473	**<0.001**	0.882	0.530	**<0.001**
Total hip	0.708	0.494	**<0.001**	0.676	0.470	**<0.001**	0.790	0.475	**<0.001**
Lumbar spine	0.611	0.426	**<0.001**	0.589	0.409	**<0.001**	0.768	0.461	**<0.001**
Femoral neck	0.626	0.436	**<0.001**	0.592	0.411	**<0.001**	0.657	0.395	**<0.001**
Girls (n=163)									
BMC (g)									
Whole body	0.715	0.626	**<0.001**	0.683	0.601	**<0.001**	0.701	0.521	**<0.001**
Total hip	0.656	0.574	**<0.001**	0.632	0.556	**<0.001**	0.719	0.534	**<0.001**
Lumbar spine	0.544	0.479	**<0.001**	0.516	0.457	**<0.001**	0.618	0.463	**<0.001**
Femoral neck	0.593	0.519	**<0.001**	0.570	0.502	**<0.001**	0.525	0.390	**<0.001**
BMD (g/cm^2)									
Whole body	0.442	0.387	**<0.001**	0.407	0.359	**<0.001**	0.434	0.322	**<0.001**
Total hip	0.457	0.400	**<0.001**	0.430	0.379	**<0.001**	0.410	0.304	**<0.001**
Lumbar spine	0.424	0.373	**<0.001**	0.398	0.353	**<0.001**	0.354	0.265	**<0.001**
Femoral neck	0.409	0.358	**<0.001**	0.385	0.339	**<0.001**	0.333	0.247	**<0.001**

Significant results are in bold

Semip corr semi-partial correlation

BMC bone mineral content, *BMD* bone mineral density

[a] In addition to whole body lean mass (LM) (in kilograms), the following independent variables were entered into the model: height (in centimeters), calcium intake (in milligrams per day), and sexual maturation (model 0)

[b] Model 0+average physical activity (PA) (counts per minute)

[c] Model 1+whole body fat mass (FM) (in kilograms)

[d] B is the estimated standardized regression coefficient

Figure 1a shows the differences in bone-related variables by weight status in boys. Overweight/obese boys (n=42) had higher BMC at the whole body than their non-overweight peers (n=125). Additional adjustment for PA showed slightly higher differences between weight status groups. However, when adjusting for LM, the association between weight status and most of bone-related variables (both BMC and BMD) was inverted (p<0.05).

Figure 1b shows that overweight/obese girls (n=30) had higher BMC and BMD than their non-overweight peers (n=133) in most of the regions analyzed. Additional adjustment for PA resulted in slightly higher differences between weight status groups, while adjusting for LM, inverted the associations between weight status and BMC (p<0.05) and eliminated the association with BMD (p>0.1).

Discussion

The findings of the present study indicate that adolescents with higher levels of adiposity have greater bone mass, yet this association is fully explained by their higher LM. In fact, once LM is accounted for, the association between FM and bone mass become negative in most of the bone variables studied. This finding is consistent when using two accurate methods to assess FM, i.e., DXA and BodPod. Physical activity does not seem to have an important confounding role in these associations.

Some studies have analyzed the association of FM on bone mass, obtaining contradictory results [13-17, 42], probably due to adjustment or not for key confounders. Some studies have reported positive associations between FM and bone mass in children and adolescents, after controlling for age,

sex, and height [14, 15]. However, the study of Weiler et al. [42] showed a negative relationship between FM and BMC and BMD in 10–19-year girls, after controlling for age, weight, and height. Others have reported a positive association between FM and whole body BMD in girls and negative associations with whole body and lumbar spine BMD in boys [13], after adjusting for LM. In our study, the positive associations between FM and bone mass became

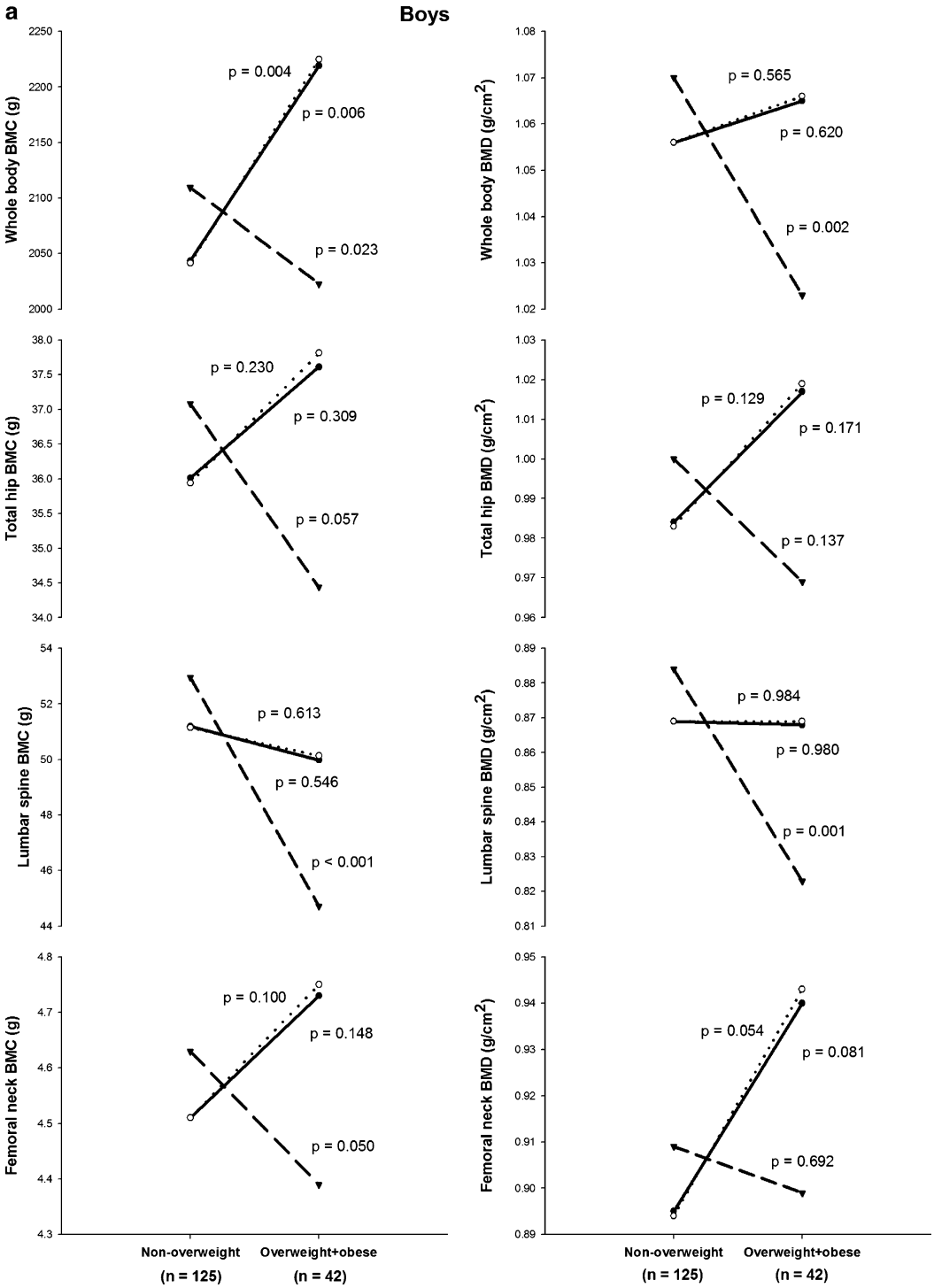


Fig. 1 Bone mineral content and density in relation to weight status in boys (**a**) and girls (**b**). Whole body, total hip, lumbar spine, and femoral neck scans. ——●—— adjusted by confounders (height, calcium intake, and sexual maturation), ·····○····· adjusted by confounders+average PA, – – –▼– – – adjusted by confounders+average PA+lean mass. Lines between points are shown only to facilitate the interpretation of the figure, i.e., which means (*points*) belong to a same model, and how the difference between means is modified after adjustment for several confounders

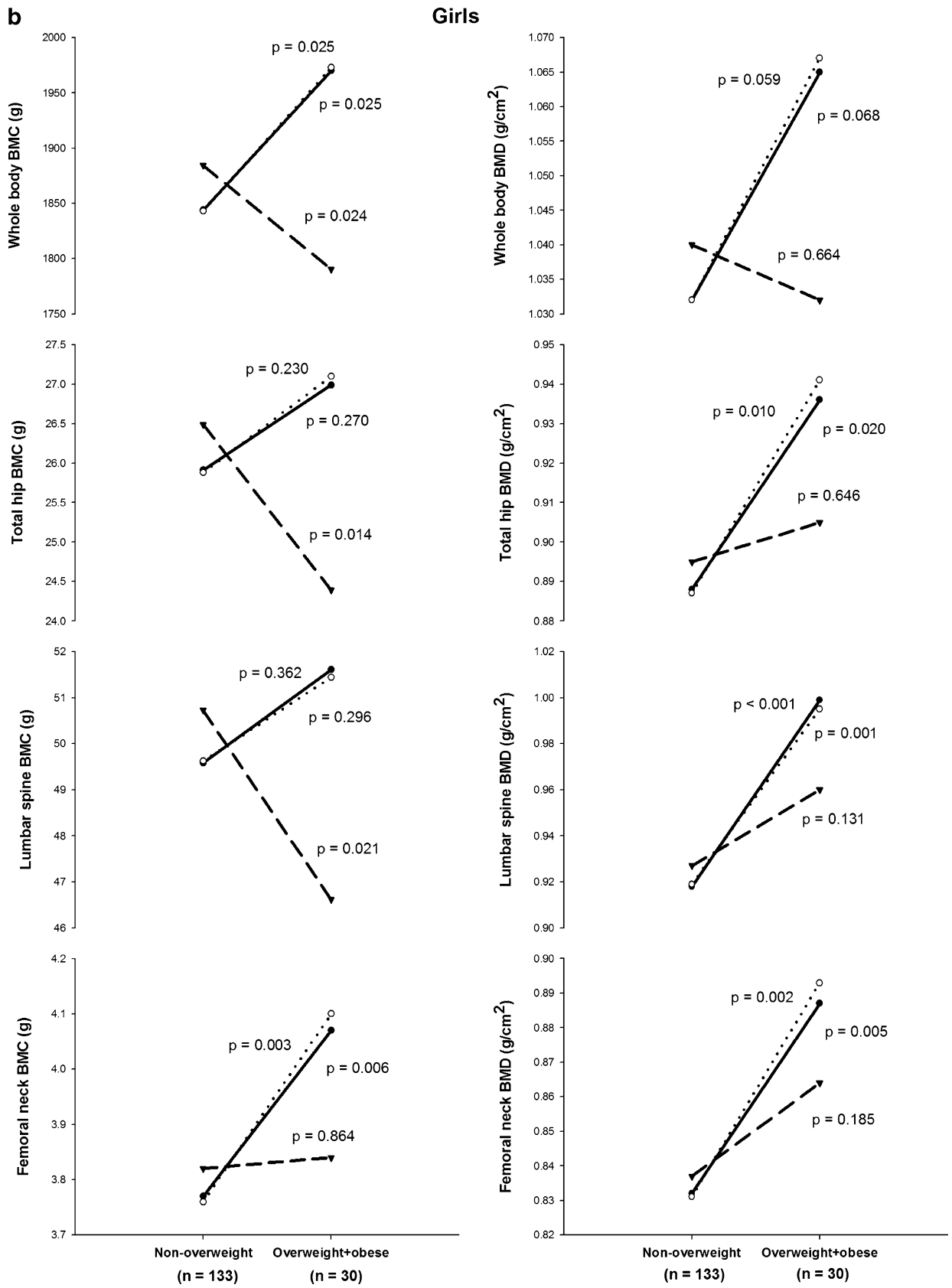


Fig. 1 (continued)

inverse after including LM as a confounder, both in boys and girls. The relationship between bone mass and weight status has been widely discussed. Some studies have shown that BMC and BMD are higher in overweight or obese children and adolescents [19-22]. In contrast, opposite results have been found after controlling for some potential confounders such as sexual maturation [23] or LM [24]. The present study took into account a set of potential confounders that have been shown to be associated with bone mass, such as height, calcium intake, sexual maturation, average PA and LM. Further ANCOVA's were performed including LM as a covariate (in addition to the previous confounders), in order to analyze the influence of LM in the association bone–weight status.

The results of the present study show that overweight/obese boys had higher BMC at the whole body than their non-overweight peers, and overweight/obese girls had higher BMC and BMD in most of analyzed regions than their non-overweight peers. Additional adjustment for PA (counts per minute) did not result in remarkable changes. Therefore, in spite that overweight and obese adolescents have shown to be less active than their normal peers [18], these differences in activity levels do not seem to be enough to have an adverse effect on bone mass. After controlling for LM, the association between weight status and bone was inverted in most of analyzed regions, indicating that the higher levels of bone mass in overweight/obese adolescents were explained by their higher LM. Our results support previous studies that used LM as a confounder [24, 43].

Two main mechanisms might explain the observed associations: (1) obese people had larger muscles and larger muscles determine higher bone mass and (2) the bones of heavier people could be more stimulated than those from normal weight people, due to the extra load that a high weight has in everyday life activities, like walking, jumping, etc. With our data we can mainly support the first mechanism but we could not confirm or reject the second one. This suggests that FM could indirectly increase bone mass via LM. However, once LM is controlled, most of the associations between FM and bone were inverted and became negative, indicating that FM per se has no beneficial effect on bone mass.

The role of LM as a major predictor of bone mass during puberty is well understood [7, 8]. The present study confirms the strong and positive association between LM and bone mass previously reported [14, 15], even after controlling for PA, calcium intake, and FM. The association was stronger in boys than in girls, also in agreement with previous studies [14, 16].

Limitations and strength

Some limitations of this study deserve comment. Although we controlled for several potential confounders, we cannot be certain that other unmeasured confounders such as dietary intake or genetic variation have not influenced our observations. Cross-sectional studies only can provide suggestive evidence concerning causal relationships. However, in this specific case, it seems reasonable to think that FM or LM can influence BMC or BMD, whereas it is not so clear the mechanisms by which bone mass could determine higher or lower levels of FM or LM.

The use of sophisticated methods, such as DXA to assess body composition, BodPod to assess FM (recognized as the "gold standard" method), and the use of accelerometers to assess PA are strengths of the study. This study includes a rather complete set of confounders, i.e., height, sexual maturation, calcium intake, average PA, FM and LM, which is crucial to examine the current research question.

Conclusions

Adolescents with higher levels of adiposity have greater bone mass, but this is not the result of their higher FM. Our results suggest that this association is fully explained by their higher levels of LM. In fact, after controlling for LM, the association between bone mass and FM is inverse, indicating a proportional lower BMD and BMC for a same body LM unit in those overweight/obese adolescents.

Acknowledgments The HELENA study takes place with the financial support of the European Community Sixth RTD Framework Programme (Contract FOOD-CT-2005-007034). The writing group takes sole responsibility for the content of this article. This study was also supported by a grant from the Spanish Ministry of Health: Maternal, Child Health and Development Network (number RD08/0072), grants from the Spanish Ministry of Science and Innovation (EX-2008-0641; JCI-2010-07055), and the Swedish Heart-Lung Foundation (20090635). Finally, this study was also supported (LGM, GVR, LAM) by a grant from Mutua de la Agrupación de Propietarios de Fincas Rústicas de España (Fundación MAPFRE), Spain. We gratefully acknowledge all participating adolescents and their parents for their collaboration.

Conflicts of interest None.

Appendix

http://www.helenastudy.com/research.php

Research groups from Zaragoza and Granada were involved in the preparation of this paper.

References

1. Ferrari SL (2005) Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. World Rev Nutr Diet 95:35–51

2. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA (2010) Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone 46:294–305
3. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM (1993) Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet 341:72–75
4. Marshall D, Johnell O, Wedel H (1996) Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ 312:1254–1259
5. Branca F, Valtuena S (2001) Calcium, physical activity and bone health—building bones for a stronger future. Public Health Nutr 4:117–123
6. Vicente-Rodriguez G, Ezquerra J, Mesana MI, Fernandez-Alvira JM, Rey-Lopez JP, Casajus JA, Moreno LA (2008) Independent and combined effect of nutrition and exercise on bone mass development. J Bone Miner Metab 26:416–424
7. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Dorado C, Calbet JA (2005) Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. Br J Sports Med 39:611–616
8. Courteix D, Lespessailles E, Loiseau-Peres S, Obert P, Ferry B, Benhamou CL (1998) Lean tissue mass is a better predictor of bone mineral content and density than body weight in prepubertal girls. Rev Rhum Engl Ed 65:328–336
9. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R (2004) The 'muscle-bone unit' during the pubertal growth spurt. Bone 34:771–775
10. Schoenau E, Frost HM (2002) The "muscle-bone unit" in children and adolescents. Calcif Tissue Int 70:405–407
11. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA (2004) High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc 36:1789–1795
12. Daly RM, Saxon L, Turner CH, Robling AG, Bass SL (2004) The relationship between muscle size and bone geometry during growth and in response to exercise. Bone 34:281–287
13. El Hage RP, Courteix D, Benhamou CL, Jacob C, Jaffre C (2009) Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. Eur J Appl Physiol 105:759–764
14. Pietrobelli A, Faith MS, Wang J, Brambilla P, Chiumello G, Heymsfield SB (2002) Association of lean tissue and fat mass with bone mineral content in children and adolescents. Obes Res 10:56–60
15. Sayers A, Tobias JH (2010) Fat mass exerts a greater effect on cortical bone mass in girls than boys. J Clin Endocrinol Metab 95:699–706
16. Young D, Hopper JL, Macinnis RJ, Nowson CA, Hoang NH, Wark JD (2001) Changes in body composition as determinants of longitudinal changes in bone mineral measures in 8 to 26-year-old female twins. Osteoporos Int 12:506–515
17. El Hage R, Moussa E, El Hage Z, Theunynck D, Jacob C (2010) Influence of age and morphological characteristics on whole body, lumbar spine, femoral neck and 1/3 radius bone mineral apparent density in a group of Lebanese adolescent boys. J Bone Miner Metab. doi:10.1007/s00774-010-0246-4
18. Martinez-Gomez D, Ruiz JR, Ortega FB et al (2010) Recommended levels of physical activity to avoid an excess of body fat in European adolescents: the HELENA Study. Am J Prev Med 39:203–211
19. Reid IR (2002) Relationships among body mass, its components, and bone. Bone 31:547–555
20. Cobayashi F, Lopes LA, Taddei JA (2005) Bone mineral density in overweight and obese adolescents. J Pediatr (Rio J) 81:337–342
21. Ellis KJ, Shypailo RJ, Wong WW, Abrams SA (2003) Bone mineral mass in overweight and obese children: diminished or enhanced? Acta Diabetol 40(Suppl 1):S274–S277
22. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS (2004) Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 80:514–523
23. Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM (2002) Spinal overload: a concern for obese children and adolescents? Osteoporos Int 13:835–840
24. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D (2008) Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 26:73–78
25. Gracia-Marco L, Tomas C, Vicente-Rodriguez G, Jimenez-Pavon D, Rey-Lopez JP, Ortega FB, Lanza-Saiz R, Moreno LA (2010) Extra-curricular participation in sports and socio-demographic factors in Spanish adolescents: the AVENA study. J Sports Sci 28:1383–1389
26. Campion JM, Maricic MJ (2003) Osteoporosis in men. Am Fam Physician 67:1521–1526
27. Moreno LA, Gonzalez-Gross M, Kersting M et al (2008) Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 11:288–299
28. Moreno LA, De Henauw S, Gonzalez-Gross M et al (2008) Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes (Lond) 32(Suppl 5):S4–S11
29. Beghin L, Castera M, Manios Y et al (2008) Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. Int J Obes (Lond) 32(Suppl 5):S12–S18
30. Nagy E, Vicente-Rodriguez G, Manios Y et al (2008) Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. Int J Obes (Lond) 32(Suppl 5):S58–S65
31. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320:1240–1243
32. Cole TJ, Flegal KM, Nicholls D, Jackson AA (2007) Body mass index cut offs to define thinness in children and adolescents: international survey. BMJ 335:194
33. Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170–179
34. Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez JA, Dorado C, Calbet JA (2003) Enhanced bone mass and physical fitness in prepubescent footballers. Bone 33:853–859
35. Gracia-Marco L, Vicente-Rodriguez G, Casajus JA, Molnar D, Castillo MJ, Moreno LA (2011) Effect of fitness and physical activity on bone mass in adolescents: the HELENA Study. Eur J Appl Physiol (in press)
36. McCrory MA, Gomez TD, Bernauer EM, Mole PA (1995) Evaluation of a new air displacement plethysmograph for measuring human body composition. Med Sci Sports Exerc 27:1686–1691
37. Moliner-Urdiales D, Ruiz JR, Ortega FB, Rey-Lopez JP, Vicente-Rodriguez G, Espana-Romero V, Munguia-Izquierdo D, Castillo MJ, Sjostrom M, Moreno LA (2009) Association of objectively assessed physical activity with total and central body fat in Spanish adolescents: the HELENA Study. Int J Obes (Lond) 33:1126–1135
38. Siri WE (1961) Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Henschel A (eds) Techniques

for measuring body composition. National Academy of Sciences, National Research Council, Washington, pp 223–234
39. Siri WE (1961) Body composition from fluid spaces and density: analysis of methods. Nutrition 9:480–491, discussion 480, 492
40. Vereecken CA, Covents M, Sichert-Hellert W et al (2008) Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. Int J Obes (Lond) 32(Suppl 5):S26–S34
41. Farrán A, Zamora R, Cervera P (2004) Tablas de Composición de Alimentos del Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID). Barcelona
42. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC (2000) Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 27:203–207
43. Janicka A, Wren TA, Sanchez MM, Dorey F, Kim PS, Mittelman SD, Gilsanz V (2007) Fat mass is not beneficial to bone in adolescents and young adults. J Clin Endocrinol Metab 92:143–147

Eur J Appl Physiol
DOI 10.1007/s00421-011-1897-0

ORIGINAL ARTICLE

Effect of fitness and physical activity on bone mass in adolescents: the HELENA Study

L. Gracia-Marco · G. Vicente-Rodríguez · J. A. Casajús · D. Molnar · M. J. Castillo · L. A. Moreno

Received: 7 September 2010 / Accepted: 26 February 2011

Abstract Our aim is to analyse the effect on bone mass of: (1) physical fitness performance on a specific group of physical activity (PA) and, (2) PA on a specific physical fitness performance group. Bone mineral content (BMC) by dual energy X-ray absorptiometry (DXA) and PA by accelerometers was assessed in 373 Spanish adolescents (182 males). Adolescents were classified as: active and non-active ($\geq$60 or <60 min day^{-1} of moderate-vigorous PA). Fitness was assessed through speed/agility, strength and cardiorespiratory tests. Adolescents were classified by tertiles (T1, T2 and T3). ANCOVA was used for the analysis with sex, height, lean mass, calcium intake and pubertal status as covariates. Adolescents with lower strength, speed/agility and cardiorespiratory fitness (CRF) showed lower BMC in the whole body and extremities compared with adolescents with better results in these tests, mainly those non-active adolescents. Non-active adolescents with high fitness levels showed higher BMC (whole body and upper limbs) than active ones. The conclusions included: (1) within the non-active group, lower levels of fitness were associated with lower BMC; this might be through PA or trough an effect of PA on muscle mass. (2) Non-active adolescents with high level of fitness in most fitness tests showed higher BMC than their active peers, in spite of their lower PA levels. These unexpected results could be influenced by several factors such as genetics, nutrition, type of exercise or sport, hormones and skeletal age.

Keywords Physical activity recommendations · Fitness testing · Accelerometers · Bone health

Introduction

Osteoporosis is a disease characterized by decreased bone mass and deterioration of bone tissue (Ferrari 2005). Acquiring a high bone mass during childhood and adolescence is a key determinant of adult skeletal health (Rizzoli

On behalf of the HELENA Study Group: for complete information regarding Helena Study Members please see the Electronic Supplementary Material. The writing group takes sole responsibility for the content of this article.

Electronic supplementary material The online version of this article (doi:10.1007/s00421-011-1897-0) contains supplementary material, which is available to authorized users.

L. Gracia-Marco (✉) · G. Vicente-Rodríguez · J. A. Casajús · L. A. Moreno
Department of Physiatry and Nursing,
School of Health Sciences, GENUD "Growth, Exercise, Nutrition and Development" Research Group,
University of Zaragoza, Avd. Domingo Miral s/n,
50009 Zaragoza, Spain
e-mail: lgracia@unizar.es

L. Gracia-Marco · L. A. Moreno
Department of Physiatry and Nursing,
School of Health Science (EUCS), University of Zaragoza,
C/Domingo Miral s/n, 50009 Zaragoza, Spain

G. Vicente-Rodríguez · J. A. Casajús
Department of Physiatry and Nursing,
Faculty of Health and Sport Science (FCSD),
University of Zaragoza, Ronda Misericordia 5,
22001 Huesca, Spain

D. Molnar
Paediatrics, Medical Faculty, Pécs university, Pécs, Hungary

M. J. Castillo
Department of Physiology, School of Medicine,
University of Granada, Granada, Spain

Published online: 11 March 2011

et al. 2010). It may contribute to more than half of the variability of bone mass with age with as much as 51% of peak bone mass accumulated during pubertal growth (Rizzoli et al. 2010). In general, male adolescents have a significant higher bone mineral content (BMC) and density (BMD) than female adolescents in most of regions, in both age and sexual maturation groups (Gracia-Marco et al. 2010). This should be considered taking into account that girls are at a higher risk than males of developing osteoporosis in adulthood (Campion and Maricic 2003). Environmental and lifestyles factors such as physical activity (PA) (Branca and Valtuena 2001) and nutrition, i.e., calcium intake (Vicente-Rodriguez et al. 2008a) have important osteogenic effects.

Exercise has been largely suggested as one of the most important factors because of its impact on bone development (Bailey et al. 1999; Gustavsson et al. 2003; Vicente-Rodriguez et al. 2004a), maintenance (Uzunca et al. 2005) and strength (Bradney et al. 1998). Current Physical Activity Guidelines for children and adolescents, recommend 1 h or more of moderate-vigorous PA (MVPA) (US Department of Health and Human Services 2008) per day. In Spain, only 48% of individuals aged between 6 and 18 did at least 60 min of PA daily (Roman et al. 2008). Physical fitness is also related to bone mass and bone accumulation during growth (Vicente-Rodriguez 2006), especially muscle strength (Vicente-Rodriguez et al. 2008b). Fitness is frequently evaluated in adolescents (Ortega et al. 2008a; Przeweda and Dobosz 2003) and it has been recently revealed as a powerful marker for actual (youth) and future (adult) health (Ortega et al. 2008b). Some tests adapted from the Eurofit Battery have been used for this proposal (Ortega et al. 2008a).

The relationship between fitness and PA (Ischander et al. 2007), and also the association between fitness and bone mass are well known (Fonseca et al. 2008; Vicente-Rodriguez et al. 2004b; Vicente-Rodriguez et al. 2003; Vicente-Rodriguez et al. 2008b; Wang et al. 2007). However, studies evaluating the association of objectively measured PA and bone mass are limited in adolescents. In one study, PA levels (US Department of Health and Human Services 2008) were related with increased bone mass in 6- to 13-year-old males, but not in females (Kriemler et al. 2008). However, another study with 11-year-old children found an association in both sexes (Tobias et al. 2007). Whether the association between either fitness or PA and bone mass in adolescents depends on each other is also unknown. Therefore, it could be hypothesized that a higher PA during growth results in higher physical fitness (Martinez-Gomez et al. 2010), and as a consequence, in higher bone mass. Skeletal mass is a reflection of what happened in the past, as well as physical fitness could be a reflection of PA in the past. Thus, the relationship between physical fitness and bone mass may be mediated by PA at least in two different ways: (1) the effect of PA on fitness (training effect), and (2) the effect of PA on muscle mass, which has been strong and positively related with bone mass (Vicente-Rodriguez et al. 2008b). In addition, it has been showed that it is not only important the amount and intensity of PA but also the type being more osteogenic and those activities including high impact loads or increasing strength and/or muscle mass (Vicente-Rodriguez 2006). To clarify the association of PA and physical fitness with bone mass, we aimed to analyse the effect on bone mass of: (1) physical fitness performance on a specific group of PA and, (2) PA on a specific physical fitness performance group.

Materials and methods

Subjects

HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) is a European Union-funded project including a cross-sectional multi-centre study (HELENA-CSS) that was performed in adolescents aged 12.5–17.5 years from 10 European cities (De Henauw et al. 2007; Moreno et al. 2008b). The general characteristics of the HELENA-CSS have been described in detail elsewhere (Moreno et al. 2008a). In this paper, we only include adolescents from Zaragoza (Spain), because this was the only city in which dual energy X-ray absorptiometry (DXA) was available to measure bone mass. To make maximum use of the data, all valid data on physical fitness tests were included in this study. Consequently, sample sizes vary for the different physical fitness tests.

From a total sample of 390 adolescents recruited in 2006–2007, 373 (182 males and 191 females, mean age 14.8 ± 1.2 year) had valid data on DXA and at least in one fitness test, and were then included in this study. All the data for each subject was taken within a week and all body composition assessments were carried out on the same day. Written informed consent was obtained from parents and adolescents (Beghin et al. 2008). The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000). The protocol study was approved by the Ethics Committee of Clinical Research from the Government of Aragón (CEICA; Spain).

Anthropometric measurements

International guidelines for anthropometry in adolescents were applied. Barefoot and wearing light indoor clothing body weight (kg) and height (cm) were measured with an electronic scale (Type SECA 861), precision 100 g, range 0–150 kg and a stadiometer (Type Seca 225), precision 0.1 cm, range 70–200 cm, respectively. These measurements

were carried out between 8 and 11 o'clock in the morning after a 10 h overnight fast.

Tanner stage

Physical examination was performed by a physician aiming to classify the adolescents in one of the five stages of pubertal maturity defined by Tanner and Whitehouse (Tanner and Whitehouse 1976).

Calcium intake

Mean daily calcium intake was estimated from a two non-consecutive 24 h recalls using the HELENA-DIAT (Dietary Assessment Tool) software (Vereecken et al. 2008). For the assessment of calcium intake, the food composition tables published by Farrán et al. (Farrán et al. 2004) were used for the Spanish adolescents.

Physical activity

A uni-axial accelerometer (Actigraph GT1 M, Manufacturing Technology Inc., Pensacola, FL, USA) was used to assess PA. Adolescents were instructed to place the monitor underneath the clothing, at the lower back, using an elastic waist band and wear it for seven consecutive days. They were also instructed to wear the accelerometer during all time awake and only to remove it during water-based activities. At least 3 days of recording with a minimum of 8 h registration per day was set as an inclusion criterion. The time sampling interval (epoch) was set at 15 s. The time engaged at moderate PA [3–6 metabolic equivalents (METs)] was calculated based upon a cut-off of 2,000–3,999 counts per minute (Andersen et al. 2006). The time engaged at vigorous PA (>6 METs) was calculated based upon a cut-off of 4,000 cpm. Also, the time spent in at least moderate intensity level (>3 METs) was calculated as the sum of time spent in moderate and vigorous PA (MVPA). The cut-offs to define the intensity categories are similar to those used in the previous studies (Ekelund et al. 2007). Subjects were classified as non-active adolescents (<60 min day^{-1} of MVPA) and active adolescents ($\geq$60 min day^{-1} of MVPA) according to the recent guidelines launched by the US Department of Health and Human Services and other medical institutions (Strong et al. 2005; US Department of Health and Human Services 2008).

Physical fitness testing protocols

An extended and detailed manual of operations was designed for and thoroughly read by every researcher involved in field work before the data collection started.

The physical fitness components, i.e., muscular fitness, speed/agility and aerobic capacity (also called CRF), were assessed by the physical fitness tests previously described in detail (Ortega et al. 2009). The scientific rationale for the selection of all of these tests, as well as their reliability in young people, was previously published (Ortega et al. 2008a; Vicente-Rodriguez et al. 2008b). In brief, all the tests were performed twice and the best score was retained, except the 20 m shuttle run test, which was performed only once. Upper-body muscular strength was assessed with the handgrip test (kg). Lower-body muscular strength was assessed with the standing broad jump test (cm). Speed-agility was assessed with the 4 × 10 m shuttle run test (s) and the 30 m running speed test (s). CRF was assessed with the 20 m shuttle run test (stage). A stage is the period of time in which the speed maintains constant. In this test, the initial speed is 8.5 km h^{-1}, which is increased by 0.5 km h^{-1} min^{-1} (1 min equals one stage).

Subjects were classified by tertiles (T1, T2 and T3) according to their results in the fitness tests, males and females, separately. T1 includes the worst and T3 the best fitness condition. Adjusted data showed that the level of fitness for the same tertile when it was equivalent for non-active and active adolescents (data not shown).

Bone and lean mass

The bone and lean mass [body mass—(fat mass + bone mass)] of the whole body, upper limbs and lower limbs were measured using DXA (Hologic Explorer scanner, using a paediatric version of the software QDR-Explorer, Hologic Corp., Software version 12.4, Waltham, MA, USA). DXA equipment was calibrated using a lumbar spine phantom as recommended by the manufacturer. Subjects were scanned in supine position and the scans were performed at high resolution (Vicente-Rodriguez et al. 2004a). Lean mass (g), fat mass (g), total area (cm^2) and BMC (g) were calculated from total and regional analysis of the whole body scan. The regional analysis (upper and lower limbs) was performed as described elsewhere (Vicente-Rodriguez et al. 2003).

Statistics

For bone mass related variables, mean and standard error are given as descriptive statistics, unless otherwise stated. All the bone, PA and fitness variables showed a normal distribution pattern and the residuals showed a satisfactory pattern. Interaction between sexes was only observed in the 20 m shuttle run test.

Differences in bone mass related variables (i.e., whole body, upper and lower limbs BMC) according to performance (tertiles 1, 2 and 3) in each fitness test were analysed

Table 1 Descriptive characteristics of Spanish adolescents

	All (n = 373)	Non-active (n = 234)	Active (n = 139)
Age (years)	14.8 ± 1.2	14.8 ± 1.2	14.7 ± 1.3
Sexual maturation (I/II/III/IV/V) (% of adolescents)	(0/3/7/12/78)	(0/5/10/18/67)	(0/1/4/6/89)
Height (cm)	163.2 ± 18.1	162.1 ± 14.8	165 ± 18.9
Body mass (kg)	58.3 ± 13.2	57.9 ± 11.4	58.8 ± 15.6
Lean mass by DXA (kg)	40.4 ± 8.6	39.1 ± 7.8*	42.5 ± 9.4
BMC by DXA (g)	1,993.74 ± 427.07	1,968.29 ± 405.54	2,034.25 ± 457.79
Calcium intake (mg day^{-1})	788.3 ± 354.7	738.3 ± 309.9*	869.7 ± 405.7
Calcium intake/lean mass ratio (mg/kg)	0.02 ± 0.009	0.019 ± 0.009	0.021 ± 0.009
Standing broad jump (cm)	156 ± 34	149 ± 32*	168 ± 34
T1 (n = 118)		125 ± 20*	132 ± 19
T2 (n = 128)		151 ± 21*	167 ± 20
T3 (n = 120)		179 ± 28*	194 ± 29
Hand grip (kg)	27.7 ± 8.0	26.5 ± 7*	30 ± 9
T1 (n = 122)		20.7 ± 3.9	21.5 ± 3.7
T2 (n = 122)		27.2 ± 5.2	29.1 ± 5.3
T3 (n = 124)		31.7 ± 6.7*	38.8 ± 7.2
4 × 10 m shuttle run test (s)	11.78 ± 1.02	11.9 ± 1*	11.5 ± 1.1
T1 (n = 118)		11.2 ± 0.7*	10.7 ± 0.6
T2 (n = 118)		11.8 ± 0.6*	11.4 ± 0.5
T3 (n = 117)		12.8 ± 0.8	12.6 ± 0.9
30 m running speed test (s)	5.28 ± 0.56	5.3 ± 0.5*	5.1 ± 0.6
T1 (n = 89)		5.8 ± 0.4	5.8 ± 0.5
T2 (n = 95)		5.3 ± 0.3*	5.1 ± 0.3
T3 (n = 97)		4.9 ± 0.3*	4.7 ± 0.3
20 m shuttle run test (stage)	6.0 ± 2.7	5.2 ± 2.3*	7.2 ± 2.8
T1 (n = 100)		3.4 ± 1.4*	4.4 ± 1.6
T2 (n = 100)		5.7 ± 1.6*	6.9 ± 1.5
T3 (n = 85)		7.5 ± 1.9*	9.5 ± 2.5
MVPA by accelerometer (min day^{-1})	57.74 ± 24.17	41.75 ± 11.31*	80.71 ± 18.54

All values are mean ± standard deviation (SD)

I/II/III/IV/V stages of pubertal maturity, *BMC* bone mineral content, *MVPA* moderate-vigorous physical activity

* Significant differences ($p < 0.05$) between non-active and active adolescents

by one-way analysis of covariance (ANCOVA) and Bonferroni post hoc. Each fitness test was entered as a fixed factor, bone mass related variables were entered as dependent variables and sex (except for 20 m shuttle run test), height, lean mass (whole body, upper or lower limbs lean mass depending on the dependent variable), calcium intake and pubertal status were entered as covariates.

The same statistic was performed to analyse the differences in bone mass related variables according to time spent at MVPA (non-active vs. active adolescents). MVPA was entered as a fixed factor instead of fitness tests. Dependent variables and covariates remained unchanged.

SPSS version 14.0 was used for the analysis. The probability value for the significance level was fixed at 0.05.

Results

Table 1 shows descriptive characteristics (mean ± standard deviation) of the study sample. In the whole sample, active adolescents showed better results in all fitness tests as well as higher calcium intake, lean mass and the amount of minutes spent at least at moderate intensity (all $p < 0.05$). In addition, active adolescents showed better results in most of tertiles of each fitness test ($p < 0.05$) than non-active ones. However, after adjustment by sex, calcium intake, lean mass, height and sexual maturation, these differences disappeared and the level of fitness for the same tertile was equivalent for non-active and active adolescents.

Two-hundred and twenty-seven adolescents out of 373 (60.9%) provided a second recall on calcium intake.

Table 2 Differences in BMC (g) according to fitness performance in active and non-active adolescents adjusted for sex (except for 20 m shuttle run test), height, lean mass, calcium intake and pubertal status

BMC (g)	Whole body		Upper limbs		Lower limbs	
Physical fitness	Non-active	Active	Non-active	Active	Non-active	Active
Strength						
Standing broad jump (cm)						
T1	1,895.42 ± 23.97[b]	1,990.24 ± 32.75	113.66 ± 1.58	120.49 ± 2.34	361.35 ± 4.81[b]	397.58 ± 6.70
T2	1,978.74 ± 25.68	2,044.05 ± 32.87	114.33 ± 1.69	120.49 ± 2.33	376.37 ± 5.13	415.79 ± 6.80
T3	2,040.05 ± 28.47	2,061.86 ± 33.11	117.85 ± 1.88	120.79 ± 2.36	386.54 ± 5.71	414.81 ± 6.82
Hand grip (kg)						
T1	1,839.6 ± 25.8[ab]	1,992.52 ± 35.06	105.94 ± 1.59[ab]	117.26 ± 2.45	353.08 ± 5.20[ab]	405.59 ± 7.24
T2	1,999.1 ± 24.41	2,035.78 ± 36.19	116.89 ± 1.51[c]	118.98 ± 2.48	381.75 ± 4.95	407.24 ± 7.42
T3	2,063.89 ± 26.07	2,082.57 ± 38.13	123.34 ± 1.61	126.57 ± 2.67	387.63 ± 5.27	416.97 ± 7.92
Speed/agility						
4 × 10 m shuttle run (s)						
T1	1,906.65 ± 26.31[b]	2,016.79 ± 35.89	113.77 ± 1.72	123.53 ± 2.54	365.97 ± 5.26	399.84 ± 7.33
T2	1,983.81 ± 27.59	2,070.06 ± 34.23	116.20 ± 1.80	120.75 ± 2.41	375.44 ± 5.53	419.62 ± 7.07
T3	1,996.43 ± 24.59	2,013.2 ± 29.69	115.24 ± 1.60	118.46 ± 2.11	378.04 ± 4.93	407.91 ± 6.10
30 m speed (s)						
T1	1,854.12 ± 27.97[b]	2,015.86 ± 46.5	110.82 ± 1.92[b]	123.70 ± 3.30	352.83 ± 5.35[ab]	398.60 ± 9.53
T2	2,001.24 ± 32.29	2,115.5 ± 34.55[c]	116.85 ± 2.21	124.21 ± 2.46	384.16 ± 6.21	422.12 ± 7.28
T3	2,020.81 ± 19.82	1,985.4 ± 27.35	117.02 ± 1.36	117.16 ± 1.95	383.12 ± 3.81	402.39 ± 5.74
CRF						
20 m shuttle run (stage)						
Males						
T1	2,046.02 ± 31.79[b]	2,069 ± 28.58[b]	124.04 ± 1.83	126.02 ± 1.94	417.06 ± 7.79	433.17 ± 7.20
T2	2,135.80 ± 52.54	2,105.59 ± 41.14	126.60 ± 3.03	125.57 ± 2.79	446.45 ± 12.93	435.58 ± 10.31
T3	2,254.13 ± 54.91	2,198.47 ± 40.12	129.82 ± 3.15	127.44 ± 2.73	452.62 ± 13.48	449.29 ± 10.06
Females						
T1	1,854.01 ± 21.73	1,874.26 ± 41.98[b]	108.92 ± 1.38	108.77 ± 2.86[b]	336.48 ± 4.06	345.72 ± 7.03
T2	1,902.78 ± 32.5	1,913.65 ± 57.81[c]	109.92 ± 2.06	113.00 ± 3.89	348.85 ± 6.09	337.78 ± 9.59
T3	1,931.34 ± 38.99	1,665.42 ± 57.11	107.13 ± 2.47	95.46 ± 3.84	340.37 ± 7.29	320.46 ± 9.55

All values are mean ± standard error (SE)

Significant differences ($p < 0.05$), [a] between T1–T2

Significant differences ($p < 0.05$), [b] between T1–T3

Significant differences ($p < 0.05$), [c] between T2–T3

Effect of fitness on bone mass according to PA level

BMC according to fitness levels in both active and non-active adolescents is shown in Table 2.

In non-active adolescents, those performing worse in: (a) strength tests had lower BMC in all bone mass related variables, except for standing broad jump test for upper limbs BMC; (b) speed/agility tests had lower BMC in all bone mass related variables, except for 4 × 10 m shuttle run test for upper limbs BMC; (c) CRF test had lower whole body BMC (all $p < 0.05$).

In active adolescents, those performing worse in: (a) speed/agility test (only 30 m running speed test) in T2 had higher whole body BMC ($p < 0.05$); (b) CRF test had lower whole body BMC (in males) ($p < 0.05$). By contrast, females performing worse in CRF test had higher whole body and upper limbs BMC ($p < 0.05$).

Effect of physical activity according to fitness level

In general, non-active adolescents with better fitness levels (tertiles 2 and 3) showed higher BMC ($p < 0.05$; Fig. 1) than active ones in these tertiles. Those active adolescents with the worst results in handgrip showed higher BMC in the whole body and lower limbs than non-active ones, and only those females with the worst results in the 20 m shuttle run showed higher BMC in the lower limbs (all $p < 0.05$; Fig. 1) than non-active ones.

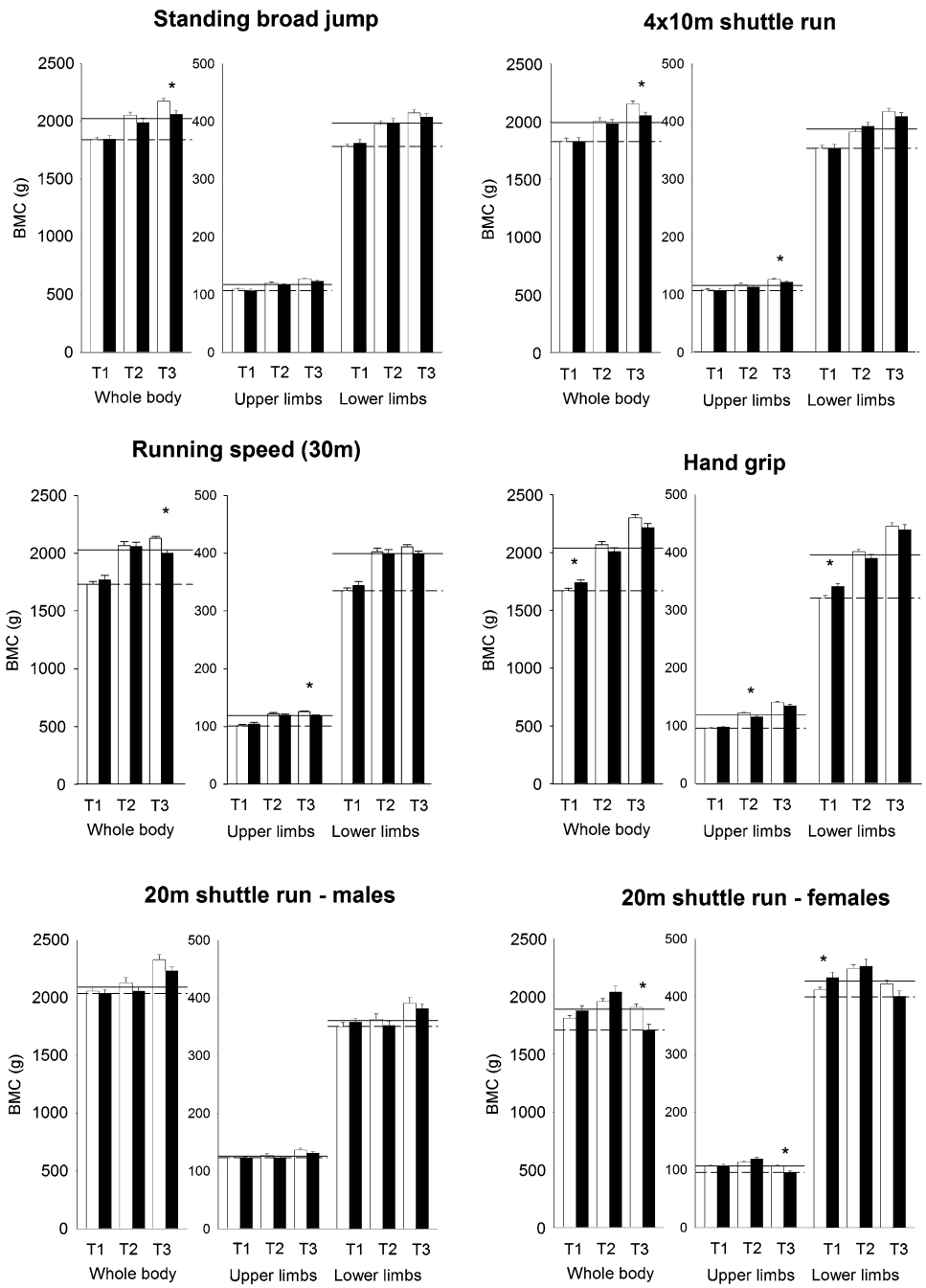


Fig. 1 Differences in BMC (g) according to physical activity level for each fitness level adjusted for sex (except for 20 m shuttle run test), height, lean mass, calcium intake and pubertal status. *White bar* represents non-active, *filled bar* represents active. *Continuous line* represents the median of the sample by fitness test and region. *Dotted line* represents the fifth centil of the sample by fitness test and region. $*p < 0.05$ between MVPA groups

Discussion

A major finding of the study is that the lower the fitness performance, the lower the bone mass of adolescents. This finding confirms that reported in a few previous studies (Vicente-Rodriguez et al. 2004a; Vicente-Rodriguez et al. 2004b; Vicente-Rodriguez et al. 2003). In addition, for the highest level of strength, speed/agility and CRF (tertile 3), non-active adolescents showed higher BMC compared with the active ones. Those active adolescents with the lowest fitness level of hand grip strength showed higher BMC than non-active ones.

Effect of fitness

A few studies have assessed the effect of fitness in relation to bone mass (Fonseca et al. 2008; Vicente-Rodriguez et al. 2004b; Vicente-Rodriguez et al. 2003; Vicente-Rodriguez et al. 2008b; Wang et al. 2007). In addition, the present study do so having into account the amount and intensity of objectively measured PA. In fact, adolescents were classified using the recent guidelines launched by the US Department of Health and Human Services and other medical institutions (Strong et al. 2005; US Department of Health and Human Services 2008). It has been shown that some

physical fitness related variables have a great predictive value for BMC and also for the accumulation of bone mass during early puberty (Vicente-Rodriguez et al. 2004a; Vicente-Rodriguez et al. 2004b; Vicente-Rodriguez et al. 2003). The results of the present investigation confirm a relationship between fitness performance and BMC, mainly in non-active adolescents. Several factors could be behind this phenomenon, such as the mechanical stress that bone must support, which depends on both the intensity and the type of exercise more than the amount of PA. In this regard, actions in sport that involves tensile, compressive, shear, bending and torsion stresses that can elicit mechanostat-related mechanisms during growth have an osteogenic potential (Heinonen 2001). In addition, other factors such as lean mass have been closely related with bone mass acquisition (Daly et al. 2004).

In relation to strength, lower performance in the standing broad jump test revealed decreased BMC in the whole body and lower limbs. The study of Mattila et al. revealed no association between lower limbs BMD and the distance of horizontal jump. However, they found an association between whole body bone mass and muscle strength (determined summing the scored items of five different muscle strength tests) (Mattila et al. 2007).

Lower performance in the hand grip test was related to decreased bone mass in the whole body, upper and lower limbs. Our results confirm the established relevance of strength, which was the strongest fitness variable correlated with BMC (Vicente-Rodriguez et al. 2008b), as we already observed.

As regard speed/agility, lower performance by adolescents in the 4 × 10 m shuttle run test was directly related to decreased whole body BMC. In addition, lower performance in the 30 m running speed test was also associated with decreased bone mass in the upper and lower limbs. Our results support a previous study about the performance in the 30 m running speed test, in which muscle power was associated with BMC (Vicente-Rodriguez et al. 2003).

In males, lower CRF (20 m shuttle run test) is directly related to whole body BMC. Active adolescents showed lower whole body BMC making for a worse performance in the 20 m shuttle run test for males. Surprisingly, in females worse results are related to higher whole body and upper limbs BMC, suggesting that the 20 m shuttle run test bears no relation to higher bone mass in females. Performance in the 20 m shuttle run test could possibly be gender-dependent, and there may be a different association with bone mass in males and females, but this hypothesis needs further research. It seems therefore, that exercise trying to improve speed/agility, maximal aerobic power and strength performance should be encouraged in adolescents.

Effect of physical activity

Several studies have assessed the effect of PA on bone mass (Branca and Valtuena 2001; Burrows et al. 2009; Delvaux et al. 2001; Kemper et al. 2000; Kriemler et al. 2008; McVeigh et al. 2004; Sundberg et al. 2002; Tobias et al. 2007; Vicente-Rodriguez 2006). As novelty, the present study do so having into account the level of different components of fitness and assessing PA objectively. Most of the previous researches have used questionnaires and interviews to assess the amount of PA, asserting that more the PA the more bone accretion (Burrows et al. 2009; Delvaux et al. 2001; Kemper et al. 2000; McVeigh et al. 2004; Sundberg et al. 2002). However, the use of questionnaires could introduce some cases of under or over-reporting (Westerterp 2009). Bone mineral content and density reference values were published in this population group (Gracia-Marco et al. 2010), showing that females had a significantly lower amount of BMC and BMD than males in most of analysed regions. For example, hip BMD, which has a great importance due to its clinical relevance regarding osteoporosis. The relationship between PA and BMC has been previously described and longitudinally confirmed (Delvaux et al. 2001; Rauch et al. 2004; Vicente-Rodriguez et al. 2004a). However, studies that objectively assess PA (i.e., using accelerometers) in association with BMC in children and young adolescents, showed different results (Kriemler et al. 2008; Tobias et al. 2007). In addition, it is important not only to assess the amount and intensity of PA (which is provided by the accelerometers), but also the type of PA, e.g., weight-bearing PA. In our study we found.

In our sample the level of fitness for the same tertile was equivalent for non-active and active adolescents (data not shown). Perhaps these fitness measures are highly dependent on skill and/or genetics. Studies have also shown that the amount of PA determines the fitness level (Ischander et al. 2007; Vicente-Rodriguez et al. 2004a; Vicente-Rodriguez et al. 2003). However, adolescents in the best fitness tertile with a lower amount of MVPA unexpectedly had higher levels of bone mass than those with higher amount of MVPA. In addition, some differences were found in regional BMC that might be influenced by the modality of practiced sport, which was not measured. Further analyses were made changing the MVPA cut-off points to <30 and ≥90 min day^{-1} (data not shown). The results comparing adolescents doing <30 min·day^{-1} with those doing ≥90 min day^{-1} showed similar fitness performance in all the fitness tests and similar BMC independently of the fitness test. The latter suggests that the type of PA or sport participation may be more important than the amount of general MVPA. Other factors, such as genetics, vitamin D intake, protein intake, hormonal changes, skeletal age, type of exercise, etc. might be related to the fact that those

adolescents doing less than 60 min day^{-1} of MVPA presented higher BMC. In fact, 30% of variation in bone density depends on the phenotype, in which we can intervene mainly by physical activity, and/or nutrition (which includes vitamin D, calcium and proteins intake), inducing physiological responses that permit high levels of bone mass to be reached (Vicente-Rodriguez et al. 2008a).

It is suppressive that active adolescents with the lowest hand grip and 20 m shuttle run (in females) levels have higher whole body and lower limbs BMC, respectively, than non-active adolescents. Therefore, to be engaged in $\geq$60 min day^{-1} of MVPA when both strength and CRF level (in females) are low could be associated with improvement of the BMC of these adolescents. Further studies are needed to investigate the effect of MVPA and/or physical fitness tests on bone mass, considering the importance of multiple confounders including genetic factors.

Strengths and limitations

Measuring performance in addition to PA (with a short sampling interval of 15 s) in a relatively large sample of adolescents are strengths of our study. The set of physical fitness tests used in this study has proved reliable in these populations (Ortega et al. 2008a). All analyses were adjusted by sex (except for the 20 m shuttle run test), height, whole body lean mass, arm lean mass (for the upper limbs), leg lean mass (for lower limbs), calcium intake and sexual maturation.

It is also noteworthy as a limitation that the present cross-sectional study only provides suggestive evidence concerning causal relations between fitness or physical activity variables and bone mineral content and density. Assessing the diets of younger age groups consider to be challenging because their diets are highly variable from day-to-day, and their food habits can change rapidly. Although adolescents are more able to report than younger children, may be less interested in giving accurate reports (Thompson and Subar 2008). Therefore, limitations in relation to the use of recalls as a method of assessment should be considered. For instance, recall intakes are prone to under reporting, rely on memory, require many days to capture individual's usual intake, are affected by within-person variation and provide imprecise estimation of servings (Willet 1998). In addition, response rate might be an issue as observed in our study, especially in such populations. Out of the 373 participants who provided complete dietary information, 146 did not provide a second recall.

Conclusions

We can conclude that (1) within the non-active group, lower levels of fitness were associated with lower BMC; this might be through PA or trough an effect of PA on muscle mass and; (2) non-active adolescents with high level of fitness in most fitness tests showed higher BMC than their active peers, in spite of their lower PA levels. These unexpected results could be influenced by several factors such as genetics, nutrition, type of exercise or sport, hormones and skeletal age.

The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000). The protocol study was approved by the Ethics Committee of Clinical Research from the Government of Aragón (CEICA; Spain).

Acknowledgments The HELENA Study takes place with the financial support of the European Community Sixth RTD Framework Programme (Contract FOOD-CT-2005-007034). This study was also supported by a grant from the Spanish Ministry of Health: Maternal, Child Health and Development Network (number RD08/0072), and the Spanish Ministry of Education (EX-2007-1124). Finally, this study was also supported (LGM, GVR, JPR, MMG, LMA) by a grant from Fundación MAPFRE (Spain). We gratefully acknowledge all participating adolescents and their parents for their collaboration.

Conflict of interest None.

References

Andersen LB, Harro M, Sardinha LB, Froberg K, Ekelund U, Brage S, Anderssen SA (2006) Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). Lancet 368:299–304

Bailey DA, McKay HA, Mirwald RL, Crocker PR, Faulkner RA (1999) A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. J Bone Miner Res 14:1672–1679

Beghin L, Castera M, Manios Y, Gilbert CC, Kersting M, De Henauw S, Kafatos A, Gottrand F, Molnar D, Sjostrom M, Leclercq C, Widhalm K, Mesana MI, Moreno LA, Libersa C (2008) Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA cross-sectional study. Int J Obes (Lond) 32(Suppl 5):S12–S18

Bradney M, Pearce G, Naughton G, Sullivan C, Bass S, Beck T, Carlson J, Seeman E (1998) Moderate exercise during growth in prepubertal boys: changes in bone mass, size, volumetric density, and bone strength: a controlled prospective study. J Bone Miner Res 13:1814–1821

Branca F, Valtuena S (2001) Calcium, physical activity and bone health–building bones for a stronger future. Public Health Nutr 4:117–123

Burrows M, Baxter-Jones A, Mirwald R, Macdonald H, McKay H (2009) Bone mineral accrual across growth in a mixed-ethnic group of children: are Asian children disadvantaged from an early age? Calcif Tissue Int 84:366–378

Campion JM, Maricic MJ (2003) Osteoporosis in men. Am Fam Physician 67:1521–1526

Daly RM, Saxon L, Turner CH, Robling AG, Bass SL (2004) The relationship between muscle size and bone geometry during growth and in response to exercise. Bone 34:281–287

De Henauw S, Gottrand F, De Bourdeaudhuij I, Gonzalez-Gross M, Leclercq C, Kafatos A, Molnar D, Marcos A, Castillo M,

Dallongeville J, Gilbert C, Bergman P, Widhalm K, Manios Y, Breidenassel C, Kersting M, LobotHSG Moreno (2007) Nutritional status and lifestyles of adolescents in a public health perspective. The HELENA project—healthy lifestyle in europe by nutrition in adolescence. J Publ Health 15:187–197

Delvaux K, Lefevre J, Philippaerts R, Dequeker J, Thomis M, Vanreusel B, Claessens A, Eynde BV, Beunen G, Lysens R (2001) Bone mass and lifetime physical activity in Flemish males: a 27-year follow-up study. Med Sci Sports Exerc 33:1868–1875

Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S (2007) Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. Diabetologia 50:1832–1840

Farrán A, Zamora R, Cervera P (2004) Tablas de Composición de Alimentos del Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID), Barcelona

Ferrari SL (2005) Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. World Rev Nutr Diet 95:35–51

Fonseca RM, de Franca NM, Van Praagh E (2008) Relationship between indicators of fitness and bone density in adolescent Brazilian children. Pediatr Exerc Sci 20:40–49

Gracia-Marco L, Vicente-Rodriguez G, Valtuena J, Rey-Lopez JP, Diaz Martinez AE, Mesana MI, Widhalm K, Ruiz JR, Gonzalez-Gross M, Castillo MJ, Moreno LA (2010) Bone mass and bone metabolism markers during adolescence: The HELENA Study. Horm Res Paediatr 74:339–350

Gustavsson A, Thorsen K, Nordstrom P (2003) A 3-year longitudinal study of the effect of physical activity on the accrual of bone mineral density in healthy adolescent males. Calcif Tissue Int 73:108–114

Heinonen A (2001) Biomechanics. In: Khan K, McKay H, Kannus P et al. (eds) Physical activity and bone health. Human kinetics. Champaign, Illinois, pp 23–34

Ischander M, Zaldivar F Jr, Eliakim A, Nussbaum E, Dunton G, Leu SY, Cooper DM, Schneider M (2007) Physical activity, growth, and inflammatory mediators in BMI-matched female adolescents. Med Sci Sports Exerc 39:1131–1138

Kemper HC, Twisk JW, van Mechelen W, Post GB, Roos JC, Lips P (2000) A fifteen-year longitudinal study in young adults on the relation of physical activity and fitness with the development of the bone mass: the Amsterdam growth and health longitudinal study. Bone 27:847–853

Kriemler S, Zahner L, Puder JJ, Braun-Fahrlander C, Schindler C, Farpour-Lambert NJ, Kranzlin M, Rizzoli R (2008) Weight-bearing bones are more sensitive to physical exercise in boys than in girls during pre- and early puberty: a cross-sectional study. Osteoporos Int 19:1749–1758

Martinez-Gomez D, Ruiz JR, Ortega FB, Casajus JA, Veiga OL, Widhalm K, Manios Y, Beghin L, Gonzalez-Gross M, Kafatos A, Espana-Romero V, Molnar D, Moreno LA, Marcos A, Castillo MJ, Sjostrom M (2010) Recommended levels and intensities of physical activity to avoid low-cardiorespiratory fitness in European adolescents: the HELENA study. Am J Hum Biol 22:750–756

Mattila VM, Tallroth K, Marttinen M, Pihlajamaki H (2007) Physical fitness and performance. Body composition by DEXA and its association with physical fitness in 140 conscripts. Med Sci Sports Exerc 39:2242–2247

McVeigh JA, Norris SA, Cameron N, Pettifor JM (2004) Associations between physical activity and bone mass in black and white South African children at age 9 year. J Appl Physiol 97:1006–1012

Moreno LA, De Henauw S, Gonzalez-Gross M, Kersting M, Molnar D, Gottrand F, Barrios L, Sjostrom M, Manios Y, Gilbert CC, Leclercq C, Widhalm K, Kafatos A, Marcos A (2008a) Design and implementation of the healthy lifestyle in Europe by nutrition in adolescence cross-sectional study. Int J Obes (Lond) 32(Suppl 5):S4–S11

Moreno LA, Gonzalez-Gross M, Kersting M, Molnar D, de Henauw S, Beghin L, Sjostrom M, Hagstromer M, Manios Y, Gilbert CC, Ortega FB, Dallongeville J, Arcella D, Warnberg J, Hallberg M, Fredriksson H, Maes L, Widhalm K, Kafatos AG, Marcos A (2008b) Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 11:288–299

Ortega FB, Artero EG, Ruiz JR, Vicente-Rodriguez G, Bergman P, Hagstromer M, Ottevaere C, Nagy E, Konsta O, Rey-Lopez JP, Polito A, Dietrich S, Plada M, Beghin L, Manios Y, Sjostrom M, Castillo MJ (2008a) Reliability of health-related physical fitness tests in European adolescents. The HELENA study. Int J Obes (Lond) 32(Suppl 5):S49–S57

Ortega FB, Ruiz JR, Castillo MJ, Sjostrom M (2008b) Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes (Lond) 32:1–11

Ortega FB, Artero EG, Ruiz JR, Espana-Romero V, Jimenez-Pavon D, Vicente-Rodriguez G, Moreno LA, Manios Y, Beghin L, Ottevaere C, Ciarapica D, Sarri K, Dietrich S, Blair SN, Kersting M, Molnar D, Gonzalez-Gross M, Gutierrez A, Sjostrom M, Castillo MJ (2009) Physical fitness levels among European adolescents: the HELENA study. Br J Sports Med 45:20–29

Przeweda R, Dobosz J (2003) Growth and physical fitness of polish youths in two successive decades. J Sports Med Phys Fitness 43:465–474

Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R (2004) The 'muscle-bone unit' during the pubertal growth spurt. Bone 34:771–775

Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA (2010) Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone 46:294–305

Roman B, Serra-Majem L, Ribas-Barba L, Perez-Rodrigo C, Aranceta J (2008) How many children and adolescents in Spain comply with the recommendations on physical activity? J Sports Med Phys Fitness 48:380–387

Strong WB, Malina RM, Blimkie CJ, Daniels SR, Dishman RK, Gutin B, Hergenroeder AC, Must A, Nixon PA, Pivarnik JM, Rowland T, Trost S, Trudeau F (2005) Evidence based physical activity for school-age youth. J Pediatr 146:732–737

Sundberg M, Gardsell P, Johnell O, Karlsson MK, Ornstein E, Sandstedt B, Sernbo I (2002) Physical activity increases bone size in prepubertal boys and bone mass in prepubertal girls: a combined cross-sectional and 3-year longitudinal study. Calcif Tissue Int 71:406–415

Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170–179

Thompson F, Subar A (2008) Dietary assessment methodology. In: Coulston A, Boushey C (eds) Nutrition in the prevention and treatment of disease. Elsevier, Burlington, pp 3–39

Tobias JH, Steer CD, Mattocks CG, Riddoch C, Ness AR (2007) Habitual levels of physical activity influence bone mass in 11-year-old children from the United Kingdom: findings from a large population-based cohort. J Bone Miner Res 22:101–109

US Department of Health and Human Services (2008) Key guidelines for children and adolescents

Uzunca K, Birtane M, Durmus-Altun G, Ustun F (2005) High bone mineral density in loaded skeletal regions of former professional football (soccer) players: what is the effect of time after active career? Br J Sports Med 39:154–157 discussion 154-157

Vereecken CA, Covents M, Sichert-Hellert W, Alvira JM, Le Donne C, De Henauw S, De Vriendt T, Phillipp MK, Beghin L, Manios Y, Hallstrom L, Poortvliet E, Matthys C, Plada M, Nagy E, Moreno LA (2008) Development and evaluation of a self-administered

computerized 24-h dietary recall method for adolescents in Europe. Int J Obes (Lond) 32(Suppl 5):S26–S34
Vicente-Rodriguez G (2006) How does exercise affect bone development during growth? Sports Med 36:561–569
Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez JA, Dorado C, Calbet JA (2003) Enhanced bone mass and physical fitness in prepubescent footballers. Bone 33:853–859
Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA (2004a) High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc 36:1789–1795
Vicente-Rodriguez G, Dorado C, Perez-Gomez J, Gonzalez-Henriquez JJ, Calbet JA (2004b) Enhanced bone mass and physical fitness in young female handball players. Bone 35:1208–1215
Vicente-Rodriguez G, Ezquerra J, Mesana MI, Fernandez-Alvira JM, Rey-Lopez JP, Casajus JA, Moreno LA (2008a) Independent and combined effect of nutrition and exercise on bone mass development. J Bone Miner Metab 26:416–424
Vicente-Rodriguez G, Urzanqui A, Mesana MI, Ortega FB, Ruiz JR, Ezquerra J, Casajus JA, Blay G, Blay VA, Gonzalez-Gross M, Moreno LA (2008b) Physical fitness effect on bone mass is mediated by the independent association between lean mass and bone mass through adolescence: a cross-sectional study. J Bone Miner Metab 26:288–294
Wang Q, Alen M, Nicholson P, Suominen H, Koistinen A, Kroger H, Cheng S (2007) Weight-bearing, muscle loading and bone mineral accrual in pubertal girls—a 2-year longitudinal study. Bone 40:1196–1202
Westerterp KR (2009) Assessment of physical activity: a critical appraisal. Eur J Appl Physiol 105:823–828
Willet W (1998) Nutritional epidemiology. Oxford, New York

Levels of Physical Activity That Predict Optimal Bone Mass in Adolescents
The HELENA Study

Luis Gracia-Marco, BSc, Luis A. Moreno, PhD, MD, Francisco B. Ortega, PhD, Francisco León, MD, Isabelle Sioen, PhD, Anthony Kafatos, PhD, MD, David Martinez-Gomez, MSc, Kurt Widhalm, PhD, MD, Manuel J. Castillo, PhD, MD, Germán Vicente-Rodríguez, PhD, on behalf of the HELENA Study Group*

Background: Physical activity is necessary for bone mass development in adolescence. There are few studies quantifying the associations between physical activity and bone mass in adolescents.

Purpose: To assess the relationship between moderate-to-vigorous physical activity (MVPA) and vigorous physical activity (VPA) and bone mass in adolescents.

Methods: Bone mass was measured by dual-energy X-ray absorptiometry and physical activity by accelerometers in 380 healthy Spanish adolescents (189 boys, aged 12.5–17.5 years) from the HELENA–CSS (2006–2007). Subjects were classified according to the recommended amount of MVPA (<60 minutes or ≥60 minutes of MVPA/day). Receiver operating characteristic curve analysis was applied to calculate the relationship between physical activity and bone mass.

Results: Less than 41 and 45 minutes of MVPA/day are associated with reduced bone mass at the trochanter and femoral neck. More than 78 minutes of MVPA/day is associated with increased bone mineral density (BMD) at the femoral neck. Regarding VPA, more than 28 minutes/day for the hip and intertrochanter and more than 32 minutes/day for the femoral neck are associated with increased BMD.

Conclusions: The recommended amount of physical activity (minutes/day) seems insufficient to guarantee increased bone mass. With some minutes of VPA/day, bone adaptations could be obtained at different bone sites.

(Am J Prev Med 2011;40(6):599–607) © 2011 American Journal of Preventive Medicine

Introduction

Osteoporosis is a common health problem. In fact, about 2.7 million of European men and women suffer an osteoporotic fracture every year,[1] which is associated with high morbidity and mortality rates.[2] The economic burden of osteoporosis in Europe is higher than any kind of cancer (except lung cancer) or chronic cardiorespiratory diseases[2,3] and represents a direct annual cost of $48 billion.[1] To improve the outcome for osteoporosis sufferers, prevention remains the most important action in public health.

Acquiring a high bone mass during childhood and adolescence is a key determinant of adult skeletal health[4]

From the GENUD: "Growth, Exercise, NUtrition and Development" Research Group (Gracia-Marco, Moreno, León, Vicente-Rodríguez), Department of Physiatry and Nursing (Gracia-Marco, Moreno, León), School of Health Science (EUCS); Department of Physiatry and Nursing (Vicente-Rodriguez), Faculty of Health and Sport Science (FCSD), University of Zaragoza, Zaragoza; Department of Physiology (Ortega, Castillo), School of Medicine, University of Granada, Granada; Immunonutrition Research Group, Department of Metabolism and Nutrition (Martinez-Gomez), Instituto del Frío, Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain; Department of Biosciences and Nutrition, Unit for Preventive Nutrition, Karolinska Institutet (Ortega), Huddinge, Sweden; Department of Public Health, Ghent University (Sioen), Ghent, Belgium; FWO, Research Foundation Flanders (Sioen), Brussels, Belgium; Preventive Medicine and Nutrition Unit, University of Crete School of Medicine (Kafatos), Heraklion, Crete, Greece; and Division of Clinical Nutrition and Prevention, Department of Pediatrics, Medical University of Vienna (Widhalm), Vienna, Austria

Address correspondence to: Luis Gracia-Marco, GENUD: Growth, Exercise, NUtrition and Development Research Group, Department of Psychiatry and Nursing, School of Health Sciences, University of Zaragoza, Avd. Domingo Miral s/n. CP: 50009, Zaragoza, Spain. E-mail: lgracia@unizar.es.

*See Appendix A (available online at www.ajpmonline.org) for list of members.

0749-3797/$17.00
doi: 10.1016/j.amepre.2011.03.001

and it may decrease the risk of osteoporotic fractures by 50%.[5,6] Exercise has been associated with bone accretion showing an important osteogenic effect, mainly when high-impact and weight-bearing physical activity occur.[7] Muscle mass is also a determinant of bone development.[8] Intensive physical activity, for example, participation in sport, is associated with increased development of muscle mass during growth.[8,9] Therefore, exercise may indirectly increase bone mass by increasing lean mass. In terms of bone health, it is not only the amount of physical activity that is important but also the type of physical activity.

Physical Activity Guidelines for children and adolescents recommend (1) that young people should accumulate at least 60 minutes (up to several hours) of moderate-to-vigorous physical activity (MVPA) per day; and (2) at least 3 days per week this should include activities to improve bone health and muscle strength.[10] To date, most studies assessed physical activity subjectively (i.e., using questionnaires), even when it has been shown that participants could under- or over-report physical activity in this population group,[11,12] which is an important issue. However, few studies have evaluated the association of objectively assessed physical activity and bone mass in adolescents. One study[13] showed a positive association between total hip BMC and the time spent (minutes/day) in vigorous and total physical activity in Swiss boys aged 6–13 years; although another study[14] of boys and girls aged 11 years from the United Kingdom showed a positive association between lower limbs' BMD and the time spent (minutes/day) in MVPA.

It is relevant to know whether current physical activity recommendations for adolescents are sufficient for healthy bone mass development, and this has not been studied yet. Therefore, the aim of this report is to analyze the relationships between MVPA and vigorous physical activity (VPA) and bone mass in different regions (whole body, pelvis, lumbar spine, and total hip) and subregions (trochanter, intertrochanter, and femoral neck).

Methods

Subjects

The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) project is funded by the European Union and includes a cross-sectional multicenter study (HELENA–CSS) that was performed in adolescents aged 12.5–17.5 years from ten European cities[15] in 2006–2007. The general characteristics of the HELENA–CSS have been described in detail elsewhere.[16] In this report, the only sample included is from the only city (Zaragoza) where bone mineral content (BMC) and bone mineral density (BMD) were measured by dual-energy X-ray absorptiometry (DXA). The total sample of adolescents with valid data (DXA and objectively measured physical activity) was 380 (189 boys and 191 girls). Ten subjects were excluded because they did not wear the accelerometer. Signed informed consent was obtained from parents and adolescents, and the protocol was approved by the Ethics Committee of Clinical Research from the Government of Aragón (CEICA, Spain).

Anthropometric Measurements

International guidelines for anthropometry in adolescents were applied.[17] Body weight (kg) and height (cm) were measured with an electronic scale (Type SECA 861, precision=100 g, range=0–150 kg) and a stadiometer (Type Seca 225, precision=0.1 cm, range=70–200 cm), respectively, while barefoot and wearing light indoor clothing.

Pubertal Development

Physical examination was performed by a physician aiming to classify the adolescents in one of the five stages of pubertal maturity defined by Tanner and Whitehouse.[18]

Bone, Lean, and Fat Mass

Adolescents were scanned using DXA (Hologic Explorer scanner, using a pediatric version of the software QDR-Explorer, version 12.4). Measurements were obtained from whole body, hip, and lumbar spine. The bone mass, fat mass, and lean mass [body mass – (fat mass + bone mass)] were measured. The DXA equipment was calibrated using a lumbar spine phantom as recommended by the manufacturer. For the whole body measurement, subjects were scanned in supine position and the scans were performed at a high resolution.[19] Lean mass (g); fat mass (g); total area (cm^2); and BMC (g) were calculated from total and regional analysis of the whole body scan. BMD (g/cm^2) was calculated using the formula BMD=BMC/area. Two additional examinations were conducted to estimate bone mass at the lumbar spine (mean L1–L4) and hip subregions (trochanter, intertrochanter, and femoral neck) as previously described.[20] Laboratory precision errors for regional analysis of the complete body scan, defined by the coefficient of variation (CV) for repeated measurements estimated in adolescent volunteers (n=49) with repositioning, were as follows: BMC=2.3%; BMD=1.3%; bone area=2.6%; and fat-free lean mass=1.9%.

Calcium Intake

Mean daily calcium intake was estimated from two nonconsecutive 24-hour recalls using the HELENA-DIAT (Dietary Assessment Tool) software.[21] For the assessment of calcium intake, the food composition tables published earlier[22] were used for the Spanish adolescents.

Physical Activity

A uniaxial accelerometer (Actigraph GT1M) was used to assess physical activity for 7 days, as described previously.[23] At least 3 days of recording with a minimum of 8 hours' registration per day was set as an inclusion criterion.

In this study, the interval of time (epoch) was set at 15 seconds. The time spent (minutes/day) at moderate physical activity (MPA; 3–6 METs) was calculated based on a cut-off of 2000–3999 counts per minute (cpm), which is approximately equivalent to an intensity of a brisk walk (4.5 km/h).[24] The time spent (minutes/day) at VPA (>6 METs) was calculated based on a cut-off of 4000 cpm. Further, MVPA (>3 METs) was calculated as the sum of moderate

and vigorous physical activity. The cut-offs to define the intensity categories are similar to those used in previous studies.[25]

Subjects were classified as non-active adolescents (<60 minutes/day of MVPA) and active adolescents (≥60 minutes/day of MVPA) according to the recent guidelines launched by the DHHS and other medical institutions.[10]

Statistics

All the variables showed normal distribution and the residuals showed a satisfactory pattern. Results are given separately by gender. Differences in bone mass–related variables by the time spent (minutes/day) in MVPA were established by one-way ANCOVA and Bonferroni post hoc. The dichotomized MVPA variable was entered as fixed factor, bone mass–related variables were entered as dependent variables, and height, pubertal status, lean mass, percentage of fat mass, and calcium intake were entered as covariates. Receiver operating characteristic (ROC) curve analysis was applied to calculate the relationship between MVPA and VPA and bone mass. BMC and BMD z-scores were calculated using a reference standard obtained by age and gender.[26] Once obtained, subjects were classified into four groups: less than M – 1 SD, less than M – 2 SD, more than M + 1 SD and more than M + 2 SD and considering each of them, they were entered in each model as a dichotomized variable (value of zero belongs to the group and value of one does not belong).

An ROC curve provides the whole spectrum of specificity/sensitivity values for all the possible cut-offs. The area under the curve (AUC) is determined from plotting sensitivity versus 1 – specificity of a test as the threshold varies over its entire range. Taking into account the suggested cut-off points, the test can be non-informative/test equal to chance (AUC=0.5); less accurate (0.5<AUC≤0.7); moderately accurate (0.7<AUC≤0.9); highly accurate (0.9<AUC≤ 1.0); and perfect discriminatory tests (AUC=1.0).[27] Cut-off points were selected for those scores optimizing sensibility–specificity relationship. In addition, ROC curve indexes of each cut-off point were calculated through the determination of positive and negative predictive values, overall misclassification rate, positive and negative likelihood ratios, and Youden Index.[28] Those indexes were calculated with EPIDAT software, version 3.1. SPSS, version 15.0, was used for the analysis. The probability value for the significance level was fixed at 0.05.

Results

Table 1 shows descriptive characteristics (M±SD) of the study sample. For boys, active adolescents had a significantly higher calcium intake and calcium intake/lean mass ratio and they spent more minutes on MVPA and VPA than non-active ones (all $p<0.05$). For girls, active adolescents were significantly taller and spent more minutes on MVPA and VPA, and they had significantly lower body mass and BMI than non-active ones (all $p<0.05$). Except for lumbar spine BMD in girls ($p<0.05$; Table 2), adjusted results showed no differences in BMC and BMD between MVPA groups.

As ROC curves showed only significant results for M – 1 SD and M + 2 SD, they appear throughout the paper as reduced and increased bone mass groups, respectively. ROC curves showed less-accurate specific thresholds ($p<0.05$) of MVPA (sensitivity range=0.797–0.880,

Table 1. Descriptive characteristics of the studied adolescents by MVPA recommended levels

	Boys		Girls	
	MVPA (minutes/day)		MVPA (minutes/day)	
	<60 minutes (n=85)	≥60 minutes (n=104)	<60 minutes (n=148)	≥60 minutes (n=43)
Age (years)	14.9 ± 1.2	14.6 ± 1.3	14.7 ± 1.1	15.1 ± 1.2
Sexual maturation (I/II/III/IV/V) (%)	(0/4/8/18/70)	(0/3/12/22/63)	(0/1/6/4/89)	(0/0/3/12/85)
Height (cm)	166.5 ± 21.2	166.2 ± 26.3	153.8 ± 33.5*	159.9 ± 6.6
Body mass (kg)	62.7 ± 12.7	61.6 ± 17.3	55.2 ± 9.6*	51.9 ± 6.2
Lean mass (kg)	45.3 ± 8	45.5 ± 9.4	35.5 ± 4.9	35.2 ± 3.9
Fat mass (%)	23.4 ± 6.4	25.7 ± 7.7	26.4 ± 7.7	27.7 ± 8.3
BMI	22.6 ± 3.8	22.3 ± 3.2	23.3 ± 3.4*	20.3 ± 2.2
Calcium intake (mg/day)	803.9 ± 310*	949.9 ± 420.1	702 ± 304.8	663.2 ± 277.4
Calcium intake/lean mass ratio (mg/kg)	0.018 ± 0.007*	0.021 ± 0.01	0.02 ± 0.01	0.019 ± 0.008
MVPA minutes	45 ± 11*	82 ± 20	40 ± 11*	77 ± 15
VPA minutes	14 ± 7*	33 ± 13	10 ± 6*	27 ± 12

Note: Results are given as M±SD.
*$p<0.05$ (between MVPA groups)
MVPA, moderate-to-vigorous physical activity; VPA, vigorous physical activity

Table 2. BMC and BMD by MVPA recommended levels adjusted for height, pubertal status, lean mass, percentage of fat mass, and calcium intake

	Boys		Girls	
	MVPA (minutes/day)		MVPA (minutes/day)	
	<60 minutes (n=85)	≥60 minutes (n=104)	<60 minutes (n=148)	≥60 minutes (n=43)
BMC (g)				
Whole body	2145.86 ± 23.66	2112.68 ± 21.41	1881.24 ± 16.21	1842.96 ± 31.56
Hip	36.52 ± 0.84	37.09 ± 0.75	26.25 ± 0.31	26.33 ± 0.60
Lumbar spine	52.60 ± 0.96	50.95 ± 0.87	50.23 ± 0.65	48.12 ± 1.26
Hip scan				
Trochanter	8.88 ± 0.21	8.64 ± 0.19	6.41 ± 0.11	6.48 ± 0.22
Intertrochanter	22.98 ± 0.80	23.85 ± 0.72	16.00 ± 0.22	16.04 ± 0.44
Femoral neck	4.66 ± 0.08	4.59 ± 0.07	3.83 ± 0.04	3.82 ± 0.07
BMD (g/cm^2)				
Whole body	1.073 ± 0.009	1.059 ± 0.008	1.044 ± 0.007	1.029 ± 0.014
Hip	10001 ± 0.013	0.993 ± 0.012	0.901 ± 0.008	0.892 ± 0.015
Lumbar spine	0.892 ± 0.011	0.865 ± 0.010	0.942 ± 0.009*	0.900 ± 0.016
Hip scan				
Trochanter	0.798 ± 0.015	0.794 ± 0.013	0.707 ± 0.007	0.704 ± 0.013
Intertrochanter	1.135 ± 0.015	1.113 ± 0.013	1.032 ± 0.010	1.018 ± 0.019
Femoral neck	0.916 ± 0.015	0.908 ± 0.013	0.843 ± 0.008	0.839 ± 0.015

Note: Results are given as M±SE.
*p<0.05 (between MVPA groups)
BMC, bone mineral content; BMD, bone mineral density; MVPA, moderate-to-vigorous physical activity; VPA, vigorous physical activity

specificity range=0.712–0.820) or VPA (sensitivity range=0.716–0.878, specificity range=0.730–0.927) for reduced bone mass groups in the femoral neck and trochanter subregion (Table 3) and less-accurate to moderately accurate specific thresholds (p<0.05) of MVPA (sensitivity=0.643, specificity=0.586) or VPA (sensitivity range=0.556–0.667, specificity range=0.608–0.846) for increased bone mass groups in the hip and intertrochanter and femoral neck subregions (Table 4), as the latter is of great importance because of its clinical relevance to osteoporosis. For all the significant cut-off points, the ROC curve indexes showed satisfactory results (Tables 3 and 4).

Discussion

The findings of the present study indicate that (1) there are no BMC and BMD differences in most body regions among adolescents regardless of whether they meet the current physical activity recommendations or not, and (2) specific thresholds of physical activity are associated with reduced or increased bone mass groups.

This is the first study analyzing, in adolescents, whether meeting the current physical activity recommendations (60 minutes/day of MVPA) or not has any effect on BMC and BMD at different body regions and subregions. In addition, there are no studies quantifying the amount of MVPA and VPA necessary to predict bone mass in such a critical period as adolescence.

Results showed no differences between active/nonactive adolescents (in both genders) in all analyzed body regions, except for the lumbar spine BMD in girls. Further analyses were made changing the MVPA cut-offs to tertiles. Results comparing adolescents in the different tertiles of MVPA showed no differences in most of the analyzed regions, except for trochanter BMC in boys (tertil 1 – tertil 2; p<0.05; data not shown). It has been reported that the effect of physical activity on bone mass could be mediated more by the kind of physical activity than by the total amount.[7] In a recent study[29] with prepubertal tennis players, the authors showed that the human skeleton has a great potential to adapt in response to

Table 3. Time of MVPA and VPA to predict low (–1 SD) BMC and BMD

	Minutes/day	AUC (CI)	Sensitivity	Specificity	OMR (%)[a]	PPV (%)[a]	NPV (%)[a]	PLR[a]	NLR[a]	A[a]
MVPA										
BMC										
Whole body	41	0.512 (0.428, 0.596)	0.736	0.344	—	—	—	—	—	—
Hip	46	0.538 (0.459, 0.618)	0.648	0.468	—	—	—	—	—	—
Lumbar spine	32	0.550 (0.406, 0.693)	0.879	0.294	—	—	—	—	—	—
Hip scan										
Trochanter	**41**	**0.579* (0.500, 0.659)**	**0.880**	**0.712**	**82.31**	**85.66**	**75.20**	**3.06**	**0.17**	**0.59**
Intertrochanter	32	0.534 (0.451, 0.616)	0.885	0.793	—	—	—	—	—	—
Femoral neck	**45**	**0.618** (0.540, 0.695)**	**0.797**	**0.820**	**82.05**	**87.66**	**73.55**	**4.11**	**0.21**	**0.63**
BMD										
Whole body	41	0.498 (0.416, 0.579)	0.742	0.323	—	—	—	—	—	—
Hip	46	0.536 (0.451, 0.621)	0.649	0.483	—	—	—	—	—	—
Lumbar spine	32	0.548 (0.429, 0.667)	0.879	0.261	—	—	—	—	—	—
Hip scan										
Trochanter	41	0.550 (0.464, 0.636)	0.761	0.397	—	—	—	—	—	—
Intertrochanter	47	0.534 (0.456, 0.612)	0.649	0.478	—	—	—	—	—	—
Femoral neck	41	0.568 (0.484, 0.652)	0.756	0.400	—	—	—	—	—	—
VPA										
BMC										
Whole body	15	0.516 (0.437, 0.596)	0.538	0.547	—	—	—	—	—	—
Hip	9	0.524 (0.444, 0.605)	0.757	0.323	—	—	—	—	—	—
Lumbar spine	7	0.557 (0.453, 0.614)	0.843	0.254	—	—	—	—	—	—
Hip scan										
Trochanter	**10**	**0.590* (0.513, 0.666)**	**0.878**	**0.730**	**82.43**	**85.04**	**77.44**	**0.17**	**3.26**	**0.61**
Intertrochanter	8	0.533 (0.451, 0.616)	0.793	0.293	—	—	—	—	—	—
Femoral neck	**19**	**0.624** (0.551, 0.697)**	**0.716**	**0.927**	**82.05**	**90.97**	**76.17**	**9.87**	**0.31**	**0.64**
BMD										
Whole body	15	0.511 (0.430, 0.591)	0.537	0.539	—	—	—	—	—	—
Hip	21	0.541 (0.460, 0.622)	0.423	0.741	—	—	—	—	—	—
Lumbar spine	6	0.560 (0.428, 0.692)	0.847	0.348	—	—	—	—	—	—
Hip scan										
Trochanter	21	0.552 (0.469, 0.634)	0.400	0.742	—	—	—	—	—	—
Intertrochanter	20	0.540 (0.464, 0.617)	0.419	0.702	—	—	—	—	—	—
Femoral neck	**20**	**0.581* (0.503, 0.660)**	**0.722**	**0.923**	**82.99**	**89.04**	**79.34**	**9.39**	**0.30**	**0.65**

Note: Boldface indicates significance.
[a]Only significant results are shown.
*$p<0.05$, **$p\leq0.01$
A, Youden index; AUC, area under the curve (ROC analysis); BMC, bone mineral content; BMD, bone mineral density; MVPA, moderate-to-vigorous physical activity; NLR, negative likelihood ratio; NPV, negative predictive value; OMR, overall misclassification rate; PLR, positive likelihood ratio; PPV, positive predictive value; VPA, vigorous physical activity

Table 4. Time of MVPA and VPA to predict high (+2 SD) BMC and BMD

	Minutes/ day	AUC (CI)	Sensitivity	Specificity	OMR (%)[a]	PPV (%)[a]	NPV (%)[a]	PLR[a]	NLR[a]	A[a]
MVPA										
BMC										
Whole body	41	0.562 (0.419, 0.705)	0.909	0.270	—	—	—	—	—	—
Hip	57	0.643 (0.501, 0.786)	0.778	0.537	—	—	—	—	—	—
Lumbar spine	73	0.581 (0.392, 0.770)	0.545	0.759	—	—	—	—	—	—
Hip scan										
Trochanter	41	0.591 (0.45, 0.732)	0.909	0.290	—	—	—	—	—	—
Intertrochanter	57	0.541 (0.369, 0.714)	0.667	0.534	—	—	—	—	—	—
Femoral neck	78	0.544 (0.340, 0.747)	0.444	0.825	—	—	—	—	—	—
BMD										
Whole body	44	0.566 (0.373, 0.76)	0.889	0.331	—	—	—	—	—	—
Hip	78	0.673 (0.497, 0.848)	0.500	0.824	—	—	—	—	—	—
Lumbar spine	82	0.442 (0.168, 0.717)	0.333	0.849	—	—	—	—	—	—
Hip scan										
Trochanter	53	0.475 (0.293, 0.657)	0.600	0.479	—	—	—	—	—	—
Intertrochanter	46	0.664 (0.509, 0.818)	0.889	0.263	—	—	—	—	—	—
Femoral neck	**78**	**0.835** (0.735, 0.936)**	**0.643**	**0.586**	**63.33**	**87.66**	**26.45**	**1.55**	**0.61**	**0.23**
VPA										
BMC										
Whole body	23	0.609 (0.447, 0.771)	0.545	0.668	—	—	—	—	—	—
Hip	**19**	**0.692* (0.557, 0.828)**	**0.583**	**0.608**	**60.77**	**4.52**	**97.87**	**1.49**	**0.68**	**0.19**
Lumbar spine	22	0.632 (0.435, 0.829)	0.727	0.648	—	—	—	—	—	—
Hip scan										
Trochanter	28	0.665 (0.513, 0.818)	0.545	0.778	—	—	—	—	—	—
Intertrochanter	19	0.576 (0.411, 0.741)	0.777	0.574	—	—	—	—	—	—
Femoral neck	27	0.608 (0.408, 0.809)	0.555	0.757	—	—	—	—	—	—
BMD										
Whole body	38	0.567 (0.349, 0.786)	0.333	0.898	—	—	—	—	—	—
Hip	**28**	**0.802** (0.666, 0.937)**	**0.667**	**0.794**	**79.23**	**4.82**	**99.35**	**3.24**	**0.42**	**0.46**
Lumbar spine	38	0.477 (0.188, 0.765)	0.333	0.896	—	—	—	—	—	—
Hip scan										
Trochanter	24	0.514 (0.309, 0.719)	0.500	0.683	—	—	—	—	—	—
Intertrochanter	**28**	**0.741** (0.578, 0.908)**	**0.556**	**0.795**	**78.97**	**6.02**	**98.70**	**2.71**	**0.56**	**0.35**
Femoral neck	**32**	**0.889** (0.813, 0.964)**	**0.667**	**0.846**	**84.36**	**6.35**	**99.39**	**4.34**	**0.39**	**0.51**

Note: Boldface indicates significance.
[a]Only significant results are shown.
*$p<0.05$; **$p\leq0.01$
A, Youden index; AUC, area under the curve (ROC analysis); BMC, bone mineral content; BMD, bone mineral density; MVPA, moderate-to-vigorous physical activity; NLR, negative likelihood ratio; NPV, negative predictive value; OMR; overall misclassification rate; PLR, positive likelihood ratio; PPV, positive predictive value; VPA, vigorous physical activity

mechanical loading, even in tennis players who trained only 2 days/week. Tennis participation before puberty is associated with increased lean mass and bone mass in the playing arm.[30]

It is necessary to take into account the mechanical stress that bone must support, which depends on both the intensity and the type of exercise more than the amount of physical activity. Therefore, actions in sport that involves tensile, compressive, shear, bending, and torsion stresses that can elicit mechanostat-related mechanisms during growth have an osteogenic potential.[31] Although the use of accelerometers presents an interesting option for measuring physical activity, mainly because of their objective measurement, it is not possible to know the kind of physical activity that was accumulated during the suggested 60 minutes (e.g., bone-strengthening activities). Direct observation of physical activity behavior would be needed. In addition, some activities without impact (e.g., cycling or swimming), known to be associated with lower bone mass, are not registered with the Actigraph MTI (model GT1M) accelerometer. Similarly, bouts of short time but high-intensity weight-bearing activities, such as jumps, are also not registered.

It is known that adolescence is a key period for bone acquisition and also that it is determinant for future skeletal health.[4] In this regard, all the efforts focusing on physical activity and exercise, mainly intense, high-impact and weight-bearing activities, may be positively related to the development of bone mass during adolescence.[7] Although genetics plays the most important role, physical activity and exercise should be also taken into account in order to prevent the development of osteopenia, or at least to delay the appearance of any bone fragility–related problem as long as possible. This is especially important in girls, as they are at higher risk than boys of developing osteoporosis in adulthood.[32] Therefore, it is of considerable interest that the adequate MVPA thresholds that permit the identification of adolescents within reduced or increased bone mass groups be found. With this aim, ROC curves were used. Additional analyses were also made using VPA because adolescents have been shown to be engaged in high-intensity extracurricular sports activities.[33]

In order to define those physical activity levels that could prevent the development of osteopenia, it was observed that periods of less than 41 and 45 minutes/day of MVPA were associated with reduced BMC at the trochanter and femoral neck, and less than 20 minutes/day of VPA were also associated with reduced BMD at the femoral neck. These recommendations should be considered taking into account that accelerometers measure the amount of physical activity but not the type. Therefore, the minutes suggested might change if adolescents were doing osteogenic activities (i.e., jumps), which it is not possible to register with accelerometers. It could be useful for future studies to determine not only the amount of physical activity but also the type.

In order to guarantee optimal bone health, it was found that more than 78 minutes/day of MVPA was associated with increased BMD at the femoral neck, more than 19 minutes/day of VPA with increased BMC at the hip and more than 28 and 32 minutes/day of VPA with increased BMD at the intertrochanter and femoral neck. At the opposite end, as has been mentioned, about 20 minutes/day of VPA is needed to ensure at least normal bone mass in the femoral neck, which is one of the most important regions in terms of clinical relevance.

Taking into account that an important number of regions and subregions have been analyzed, it might be thought that these tests are capitalizing on chance. However, even being stricter with the level of significance ($p \leq 0.01$), significant associations were found in an important number of bone sites, especially at the femoral neck subregion. The minutes proposed as cut-offs have shown satisfactory ROC curve indexes, which suggests a good classification of the subjects into the groups of reduced or increased bone mass. Vigorous physical activity includes activities with intensities greater than 6 METs, most of which are sports similar to those previously associated with increased bone mass (e.g., football, handball, artistic gymnastics, and hockey).[7,8,34] One study[35] also showed that participation in high-impact activities for 1 hour or more a day was associated with greater BMC, especially at the hip. Those results are consistent with those of the current study, which might be explained by the relationship between VPA and sports.

Although 33% of Spanish adolescents aged 12.5–18.5 years asserted that they did not play any extracurricular sport,[33] the sports that are most practiced among the rest of the adolescents are considered high-intensity sports.[36] Participation in extracurricular sports can be considered the main source of at least moderate physical activity.[37] Thus, a useful strategy to increase their physical activity (moderate–vigorous or vigorous) should be oriented toward encouraging adolescents to engage in extracurricular sporting activities. In addition, a few minutes of VPA has been found to be associated with increased bone mass at the total hip and subregions (trochanter, intertrochanter, and femoral neck), which are precisely those considered as regions/subregions of clinical relevance to osteoporosis. Participation in sport and VPA practice should be encouraged because of its important role in developing healthy bones.

Strengths and Limitations

The use of sophisticated methods, such as DXA, to assess bone mass and the use of accelerometers with a short

epoch (15 seconds) to assess physical activity in a relatively large sample of adolescents are the strengths of the current study. Although with the use of accelerometers, an objective measurement of adolescents' physical activity can be obtained, it is not possible to know the kind of activity the subjects are engaged in, which would be of great interest for analyzing if the current physical activity recommendations are good enough for bone mass–recommended physical sports activities.

It could be interesting to analyze these data according to sexual maturation because of its association with physical activity levels. However, with the present sample it was not possible to check if the effect of physical activity on bone mass is different at prepubertal ages because of a too limited number of individuals in some categories. To minimize this limitation, sexual maturation was used as a confounder in the analyses. It is also noteworthy as a limitation that the present cross-sectional study provides only suggestive evidence concerning causal relationships between physical activity variables and bone mineral content and density. In this specific case, it is feasible that the amount of physical activity (or even the sports practiced) has an effect on BMC and BMD and it does not seem feasible that bone mass determines the amount of physical activity.

Conclusion

The recommended levels of physical activity seem to be insufficient stimulus to guarantee increased bone mass. With some minutes/day of VPA, bone adaptations could be obtained at the hip. Specifically, BMD adaptations are obtained with just 32 minutes/day of VPA at the femoral neck, which is of great importance because of its clinical relevance to osteoporosis. It could be of interest if future studies aim to measure not only the amount of physical activity but also the type and, therefore, get a clearer picture of the current status of adolescents' physical activity and its influence on bone mass.

The HELENA Study takes place with the financial support of the European Community Sixth RTD Framework Programme (Contract FOOD-CT-2005-007034). This study was also supported by a grant from the Spanish Ministry of Health: Maternal, Child Health and Development Network (number RD08/0072), the Spanish Ministry of Education (EX-2008-0641, AP2006-02464), and the Swedish Heart-Lung Foundation (20090635). Finally, this study was also supported (LGM, GVR, LMA) by a grant from Fundación MAPFRE (Spain).

No financial disclosures were reported by the authors of this paper.

References

1. Kanis JA, Johnell O. Requirements for DXA for the management of osteoporosis in Europe. Osteoporos Int 2005;16(3):229–38.
2. Kanis JA, Burlet N, Cooper C, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int 2008;19(4):399–428.
3. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 2006;17(12):1726–33.
4. Rizzoli R, Bianchi ML, Garabedian M, McKay HA, Moreno LA. Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. Bone 2010;46(2):294–305.
5. Cummings SR, Black DM, Nevitt MC, et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet 1993;341(8837):72–5.
6. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ 1996;312(7041):1254–9.
7. Vicente-Rodriguez G. How does exercise affect bone development during growth? Sports Med 2006;36(7):561–9.
8. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Serrano-Sanchez JA, Dorado C, Calbet JA. High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc 2004;36(10):1789–95.
9. Vicente-Rodriguez G, Dorado C, Ara I, et al. Artistic versus rhythmic gymnastics: effects on bone and muscle mass in young girls. Int J Sports Med 2007;28(5):386–93.
10. DHHS. Key guidelines for children and adolescents. www.health.gov/PAGuidelines/.
11. Hagstromer M, Bergman P, De Bourdeaudhuij I, et al. Concurrent validity of a modified version of the International Physical Activity Questionnaire (IPAQ-A) in European adolescents: the HELENA Study. Int J Obes (Lond) 2008;32(5S):S42–8.
12. Bassett DR Jr. Validity and reliability issues in objective monitoring of physical activity. Res Q Exerc Sport 2000;71(2S):S30–6.
13. Kriemler S, Zahner L, Puder JJ, et al. Weight-bearing bones are more sensitive to physical exercise in boys than in girls during pre- and early puberty: a cross-sectional study. Osteoporos Int 2008;19(12):1749–58.
14. Tobias JH, Steer CD, Mattocks CG, Riddoch C, Ness AR. Habitual levels of physical activity influence bone mass in 11-year-old children from the United Kingdom: findings from a large population-based cohort. J Bone Miner Res 2007;22(1):101–9.
15. Moreno LA, Gonzalez-Gross M, Kersting M, et al. Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 2008;11(3):288–99.
16. Moreno LA, De Henauw S, Gonzalez-Gross M, et al. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes (Lond) 2008;32(5S):S4–11.
17. Nagy E, Vicente-Rodriguez G, Manios Y, et al. Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. Int J Obes (Lond) 2008;32(5S):S58–65.
18. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51(3):170–9.
19. Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez JA, Dorado C, Calbet JA. Enhanced bone mass and physical fitness in prepubescent footballers. Bone 2003;33(5):853–9.
20. Vicente-Rodriguez G, Ara I, Perez-Gomez J, Dorado C, Calbet JA. Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. Br J Sports Med 2005;39(9):611–6.
21. Vereecken CA, Covents M, Sichert-Hellert W, et al. Development and evaluation of a self-administered computerized 24-h dietary

recall method for adolescents in Europe. Int J Obes (Lond) 2008; 32(5S):S26–34.
22. Farrán A, Zamora R, Cervera P. Tablas de Composición de Alimentos del Centre d'Ensenyament Superior de Nutrició i Dietètica (CESNID). Barcelona, 2004.
23. Moliner-Urdiales D, Ruiz JR, Ortega FB, et al. Association of objectively assessed physical activity with total and central body fat in Spanish adolescents; the HELENA Study. Int J Obes (Lond) 2009;33(10):1126–35.
24. Ekelund U, Brage S, Froberg K, et al. TV viewing and physical activity are independently associated with metabolic risk in children: the European Youth Heart Study. PLoS Med 2006;3(12):e488.
25. Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S. Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: the European youth heart study. Diabetologia 2007;50(9):1832–40.
26. Gracia-Marco L, Vicente-Rodríguez G, Valtueña J, et al. Bone mass and bone metabolism markers during adolescence: the HELENA study. Horm Res Pediatr 2010;74(5):339–50.
27. Swets JA. Measuring the accuracy of diagnostic systems. Science 1988;240(4857):1285–93.
28. Jaeschke R, Guyatt G, Lijmer J. Diagnostic tests. In: Guyatt G, Rennie D, eds. Users' guides to the medical literature. Chicago: AMA Press, 2002:121–40.
29. Sanchis-Moysi J, Dorado C, Olmedillas H, Serrano-Sanchez JA, Calbet JA. Bone and lean mass inter-arm asymmetries in young male tennis players depend on training frequency. Eur J Appl Physiol 2010;110(1):83–90.
30. Sanchis-Moysi J, Dorado C, Olmedillas H, Serrano-Sanchez JA, Calbet JA. Bone mass in prepubertal tennis players. Int J Sports Med 2010;31(6):416–20.
31. Heinonen A. Biomechanics. In: Khan K, McKay H, Kannus P, Bailey D, Wark J, Bennell K, eds. Physical activity and bone health. 1st ed. Champaign IL: Human Kinetics, 2001:23–34.
32. Campion JM, Maricic MJ. Osteoporosis in men. Am Fam Physician 2003;67(7):1521–6.
33. Gracia-Marco L, Tomás C, Vicente-Rodríguez G, et al. Extracurricular participation in sports and socio-demographic factors in Spanish adolescents: the AVENA study. J Sports Sci 2010; 28(13):1383–9.
34. Vicente-Rodriguez G, Dorado C, Perez-Gomez J, Gonzalez-Henriquez JJ, Calbet JA. Enhanced bone mass and physical fitness in young female handball players. Bone 2004;35(5):1208–15.
35. Ginty F, Rennie KL, Mills L, Stear S, Jones S, Prentice A. Positive, site-specific associations between bone mineral status, fitness, and time spent at high-impact activities in 16- to 18-year-old boys. Bone 2005;36(1):101–10.
36. Wilmore JH, Costill DL. Physiology of sport and exercise. 3rd ed. Champaign IL: Human Kinetics, 2004.
37. Seabra AF, Mendonca DM, Thomis MA, Malina RM, Maia JA. Sports participation among Portuguese youth 10 to 18 years. J Phys Act Health 2007;4(4):370–80.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.amepre.2011.03.001.

6. APORTACIONES DE LA TESIS DOCTORAL

Las aportaciones de esta Tesis Doctoral se detallan a continuación:

Artículo I. Se ha observado una asociación entre la práctica deportiva extra-curricular y características socio-demográficas en una muestra representativa de adolescentes españoles, en la cual, el nivel de estudios del padre juega un papel fundamental en la práctica deportiva de los adolescentes. Se han observado diferencias por género.

Artículo II. Valores de referencia de masa ósea (CMO y DMO) así como marcadores del metabolismo óseo (Osteocalcina, PINP y β-CTX) en adolescentes españoles. Diferencias entre sexos.

Artículo III. Una nueva perspectiva que propone que la masa magra desarrolla la masa ósea, y no la masa grasa.

Artículo IV. Se han observado asociaciones entre los niveles de AF y CF en relación con la masa ósea.

Artículo V. Datos que muestran que las recomendaciones actuales de AF para niños y adolescentes, dada su generalidad a la hora de recomendar actividades de carácter osteogénico, no son válidas para el desarrollo de la masa ósea. Además, se aportan puntos de corte (minutos) de AF necesarios para un óptimo desarrollo de la masa ósea en regiones y sub-regiones de especial relevancia clínica en el diagnóstico de la osteoporosis.

6. CONTRIBUTIONS OF THIS DOCTORAL THESIS

The contributions of this Doctoral Thesis are shown in the following lines:

Paper I. Associations were observed between extra-curricular participation in sports and socio-demographic characteristics in a representative sample of Spanish adolescents, showing the important role of father education. Gender differences were observed.

Paper II. Reference values for bone mass (bone mineral content and density) and bone metabolism markers (Osteocalcin, PINP and β-CTX) in Spanish adolescents. Sex differences.

Paper III. A new point of view that shows that lean mass increases bone mass, but not fat mass.

Paper IV. Associations among physical activity, physical fitness and bone mass.

Paper V. Current physical activity recommendations for children and adolescents, which are quite general when recommending osteogenic activities, are not valid for bone mass development. In addition, physical activity cut-off points are showed for obtaining an optimal bone mass development in regions and sub-regions of clinical relevance in the diagnosis of osteoporosis.

7. CONCLUSIONES

Artículo I. Los chicos adolescentes practican más actividades físicas extra-curriculares que las chicas, siendo además de mayor intensidad. Además, la edad y el nivel de educación del padre también están asociados con la práctica deportiva extra-curricular en ambos sexos.

Artículo II. Nuestros resultados apoyan la evidencia de un dimorfismo sexual óseo específico de cada región corporal. Además, hemos observado un mayor y duradero proceso de remodelado óseo en los chicos durante la adolescencia.

Artículo III. Los adolescentes con mayores niveles de adiposidad tienen una mayor masa ósea que no es el resultado de su mayor masa grasa, sino que esta asociación está completamente explicada por su mayor masa magra. De hecho, tras ajustar los análisis por la masa magra, la asociación entre la masa ósea y grasa se vuelve inversa.

Artículo IV. En adolescentes no activos, bajos niveles de CF están asociados con menor CMO; que podría ser el resultado de la cantidad de AF o del efecto de la AF en la masa magra. Por otro lado, los adolescentes no activos pero con niveles altos de CF mostraron un mayor CMO que aquellos que eran activos. Estos resultados inesperados pueden estar influenciados por varios factores como la genética, nutrición, tipo de deporte practicado, factores hormonales y edad ósea.

Artículo V. Los minutos recomendados de AF diaria parecen no ser un estímulo suficiente para garantizar una correcta masa ósea. Con unos pocos minutos de AFI se pueden obtener adaptaciones óseas en la cadera. Concretamente, 32 minutos/día de AFI están asociados con una mayor DMO en el cuello femoral, sub-región de la cadera de gran importancia debido a su relevancia clínica en el diagnóstico de la osteoporosis.

7. CONCLUSIONS

Paper I. Spanish male adolescents engage more in extracurricular sporting activity than their female counterparts. They are also involved in more intense sports. Moreover, age and father's education are linked to extra-curricular participation in sports.

Paper II. Our results support the evidence of dimorphic site-specific bone accretion between sexes. In addition, our results show that males present an increased and longer-lasting bone turnover compared to females, suggesting higher bone metabolic activity in males during adolescence.

Paper III. Adolescents with higher levels of adiposity have greater bone mass, but this is not the result of their higher fat mass. Our results suggest that this association is fully explained by their higher levels of lean mass. In fact, after controlling for lean mass, the association between bone mass and fat mass is inverse.

Paper IV. Within the non-active group, lower levels of fitness are associated with lower bone mineral content; this might be through physical activity or trough an effect of physical activity on muscle mass. In addition, non-active adolescents with high level of fitness in most fitness tests show higher bone mineral content than their active peers, in spite of their lower physical activity levels. These unexpected results could be influenced by several factors such as genetics, nutrition, type of exercise or sport, hormones and skeletal age.

Paper V. The recommended levels of physical activity seem to be insufficient stimulus to guarantee increased bone mass. With some minutes per day of vigorous physical activity, bone adaptations could be obtained at the hip. Specifically, bone mineral density adaptations are obtained with just 32 minutes per day of vigorous physical

activity at the femoral neck, which is of great importance because of its clinical relevance to osteoporosis.

AGRADECIMIENTOS [ACKNOWLEDGEMENTS]

- A mis **padres**, por darme esa libertad a la hora de estudiar y escoger lo que me gusta. Espero que esta Tesis sirva para mostrar lo que he estado haciendo estos últimos años.

- A mis hermanos, **Eduardo y Óscar**, por vuestros consejos y buenos momentos pasados. Dicen que la experiencia es un grado. Y mis cuñadas, **(Ana-s)**, por ser como son.

- A mis sobrinos, **Daniel, Javier y Lilian** (por orden de nacimiento), por todas las horas de estudio que me habéis quitado a cambio de grandes momentos.

- Al **resto de la familia** (abuelos, tíos y primos) por vuestro apoyo. Con mención especial a los que ya no están.

- A mis Directores de Tesis, responsables de este trabajo. **Luis Moreno (LM**) por tu objetividad y su esfuerzo para sacar GENUD adelante. **Germán Vicente Rodríguez (GVR)** porque siempre estás allí para dar buenos consejos. **Francisco B. Ortega (FBO)**, porque haces todo tan fácil que parece que sea sencillo, cuando no lo es.

- A **Manuel J. Castillo**, mi "cuarto" Director de Tesis. Porque siempre es importante que alguien con tu experiencia y conocimiento colabore en el desarrollo de esta Tesis. Muchas gracias por tus consejos.

- Al resto de miembros del Grupo de Investigación GENUD (**Pilar M, Maribel, Paula, Iris, Silvia, Alba S, Esther, Pilar de M, Alba G, Mª Luisa, Theodora, Fernanda, Casajús, Gerardo, Nacho**, **Juan, Pablo, Alex, Diego, Guillén, David…**), por todas las horas y proyectos que hemos estado juntos. A Concepción Tomás **(Cuca)**, por guiarme durante el desarrollo del Diploma de Estudios Avanzados.

- A los compañeros de la Universidad Politécnica de Madrid, especialmente a **Marcela y Jara**, por el trabajo que juntos hemos desarrollado durante este tiempo, y el que nos queda por hacer.

- To **Michael Sjöström and Lars-Olov Bygren**, thanks for giving me the opportunity to stay at the Unit in Karolinska Institute, for your support and kind words during my stay.

- A todos los amigos que hice en Estocolmo: **Vicente**, por tus sabios consejos de "veterano". **Mairena**, porque hay poca gente con tanto entusiasmo como tú para transmitir lo que sabes. **Dani**, porque contigo no hubo primer día, parecía que te conocía de toda la vida. **Virginia**, porque dos días fueron suficientes para ver el entusiasmo que tienes en tu trabajo. **Mohammed**, because you are a great and hard-worker man doing an amazing work! **Signe**, the future of biology. Your always kind words since the first day helped me during my stay in Stockholm (I do not forget your advices about genetics, 7% of error!). Thanks to all for the nice moments we lived in Stockholm!

- A todos los **investigadores** que han colaborado en los proyectos AVENA y HELENA, así como todos los **adolescentes** que han participado. Ellos han sido la base para desarrollar ésta y otras muchas Tesis Doctorales que han sido, son y serán defendidas como merecen.

- Una histórica frase dice que *los últimos serán los primeros*. Así que en último (o primer lugar), quiero mostrar mi agradecimiento a la persona que quiero y ha estado a mi lado desde el primer día, **Yoli**. Siempre has creído que esto era posible, incluso más que yo mismo. Gracias.

APÉNDICE [APPENDIX]

Factor de impacto de las revistas y ranking en 2009 en ISI Journal Citation Reports (JCR) dentro de sus áreas temáticas correspondientes.

[Impact factor of the journals and ranking in 2009 ISI Journal Citation Reports (JCR) within their subject categories].

	Journal	***Factor de impacto***
Artículo I.	Journal of Sports Sciences	1.619
	Ranking in 2009 ISI JCR: 25/73 (Sport Sciences)	
Artículo II.	Hormone Research in Paediatrics	1.730
	Ranking in 2009 ISI JCR: 79/105 (Endocrinology and Metabolism)	
Artículo III.	Osteoporosis International	4.997
	Ranking in 2009 ISI JCR: 19/105 (Endocrinology and Metabolism)	
Artículo IV.	European Journal of Applied Physiology	2.047
	Ranking in 2009 ISI JCR: 16/73 (Sport Sciences)	
Artículo V.	American Journal of Preventive Medicine	4.235
	Ranking in 2009 ISI JCR: 11/122 (Public, environmental and occupational health)	