

# 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

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**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<sup>-1</sup> 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 through 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

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.

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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

## 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

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

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  $14.8 \pm 1.2$  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<sup>-1</sup> of MVPA) and active adolescents (≥60 min day<sup>-1</sup> 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<sup>-1</sup>, which is increased by 0.5 km h<sup>-1</sup> min<sup>-1</sup> (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<sup>2</sup>) 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 ( <i>n</i> = 373)	Non-active ( <i>n</i> = 234)	Active ( <i>n</i> = 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 <sup>-1</sup> )	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 ( <i>n</i> = 118)		125 ± 20*	132 ± 19
T2 ( <i>n</i> = 128)		151 ± 21*	167 ± 20
T3 ( <i>n</i> = 120)		179 ± 28*	194 ± 29
Hand grip (kg)	27.7 ± 8.0	26.5 ± 7*	30 ± 9
T1 ( <i>n</i> = 122)		20.7 ± 3.9	21.5 ± 3.7
T2 ( <i>n</i> = 122)		27.2 ± 5.2	29.1 ± 5.3
T3 ( <i>n</i> = 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 ( <i>n</i> = 118)		11.2 ± 0.7*	10.7 ± 0.6
T2 ( <i>n</i> = 118)		11.8 ± 0.6*	11.4 ± 0.5
T3 ( <i>n</i> = 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 ( <i>n</i> = 89)		5.8 ± 0.4	5.8 ± 0.5
T2 ( <i>n</i> = 95)		5.3 ± 0.3*	5.1 ± 0.3
T3 ( <i>n</i> = 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 ( <i>n</i> = 100)		3.4 ± 1.4*	4.4 ± 1.6
T2 ( <i>n</i> = 100)		5.7 ± 1.6*	6.9 ± 1.5
T3 ( <i>n</i> = 85)		7.5 ± 1.9*	9.5 ± 2.5
MVPA by accelerometer (min day <sup>-1</sup> )	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	
	Non-active	Active	Non-active	Active	Non-active	Active
<b>Physical fitness</b>						
<b>Strength</b>						
Standing broad jump (cm)						
T1	1,895.42 ± 23.97 <sup>b</sup>	1,990.24 ± 32.75	113.66 ± 1.58	120.49 ± 2.34	361.35 ± 4.81 <sup>b</sup>	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 <sup>ab</sup>	1,992.52 ± 35.06	105.94 ± 1.59 <sup>ab</sup>	117.26 ± 2.45	353.08 ± 5.20 <sup>ab</sup>	405.59 ± 7.24
T2	1,999.1 ± 24.41	2,035.78 ± 36.19	116.89 ± 1.51 <sup>c</sup>	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
<b>Speed/agility</b>						
4 × 10 m shuttle run (s)						
T1	1,906.65 ± 26.31 <sup>b</sup>	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 <sup>b</sup>	2,015.86 ± 46.5	110.82 ± 1.92 <sup>b</sup>	123.70 ± 3.30	352.83 ± 5.35 <sup>ab</sup>	398.60 ± 9.53
T2	2,001.24 ± 32.29	2,115.5 ± 34.55 <sup>c</sup>	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
<b>CRF</b>						
20 m shuttle run (stage)						
Males						
T1	2,046.02 ± 31.79 <sup>b</sup>	2,069 ± 28.58 <sup>b</sup>	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 <sup>b</sup>	108.92 ± 1.38	108.77 ± 2.86 <sup>b</sup>	336.48 ± 4.06	345.72 ± 7.03
T2	1,902.78 ± 32.5	1,913.65 ± 57.81 <sup>c</sup>	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 ( $p < 0.05$ ), <sup>a</sup> between T1–T2

Significant differences ( $p < 0.05$ ), <sup>b</sup> between T1–T3

Significant differences ( $p < 0.05$ ), <sup>c</sup> 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  $p < 0.05$ ).

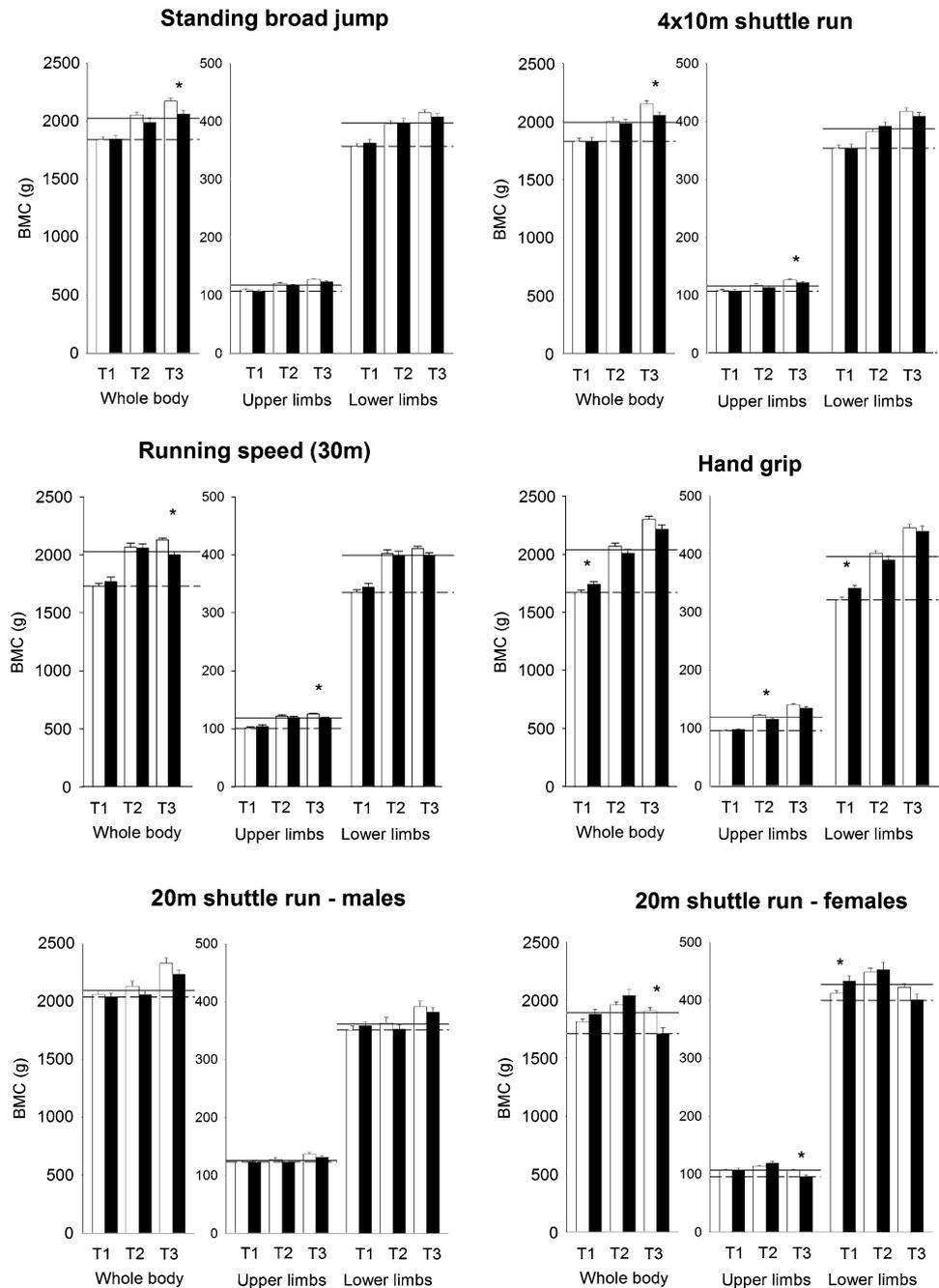
In active adolescents, those performing worse in: (a) speed/agility test (only 30 m running speed test) in T2 had higher whole body BMC ( $p < 0.05$ ); (b) CRF test had

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<sup>-1</sup> (data not shown). The results comparing adolescents doing <30 min day<sup>-1</sup> with those doing ≥90 min day<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> 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 (Willett 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 through 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).

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**Conflict of interest** None.

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