

MUSCLE BONE-INTERACTION DURING GROWTH: INFLUENCE OF PHYSICAL ACTIVITY

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ABSTRACT

To determine the independent effect of soft tissues on bone mineral content (BMC) and density (BMD), and how physical activity (PA) could affect these relationships, body composition by DXA, and the physical fitness (PF) were assessed longitudinally (3-yr) in 26 active and 16 non-active prepubertal boys. Significant advantage in the development of PF, muscle mass and total and lumbar spine BMC and total, lumbar spine and lower-limb BMD were found in the active compared with non-active boys. Results imply that during puberty, lean mass development is the best predictor of bone mass accrual; and physical activity promotes bone acquisition by increasing lean mass but also by lean mass-independent mechanisms.

Key words: skeletal health, body composition, development, exercise.

RESUMEN

Para determinar el efecto independiente de los tejidos blandos sobre el contenido mineral óseo (BMC) y la densidad (BMD), y como la actividad física (PA) podría afectar a ésta relación, evaluamos longitudinalmente (3 años) la composición corporal mediante DXA, y la condición física (PF) en 26 niños activos y 16 sedentarios, todos ellos prepúberes cuando comenzó el estudio. Los niños activos presentaban ventajas significativas en el desarrollo de la condición física y de la masa magra (masa muscular) comparados con los sedentarios. Igualmente, los niños activos acumularon más BMC en la región lumbar de la columna vertebral y en el total corporal y también más BMD en la región lumbar de la columna vertebral, en las extremidades inferiores y en el total corporal que sus compañeros sedentarios. Estos resultados sugieren que durante la pubertad, el desarrollo de la masa magra es el mejor predictor de la acumulación de masa ósea. Así mismo, la actividad física estimula la adquisición de masa ósea aumentando la masa muscular pero también mediante mecanismos independientes de la masa magra.

Palabras clave: salud del esqueleto, composición corporal, desarrollo, ejercicio.

INTRODUCTION

Several studies have demonstrated that physical exercise promotes bone mass accrual (Bailey, et al., 1999) and this effect seems to be greater when participation in physical activity starts before the pubertal spurt (Kannus, et al., 1995, Vicente-Rodríguez, et al., 2004). In fact, we have recently observed that participation in sport during prepubertal years is associated with enhanced lumbar spine and femoral areal bone mineral density (BMD) (Vicente-Rodríguez, et al., 2003), which is in accordance with the study of Bradney et al (Bradney, et al., 1998), the first controlled trial examining this relationship in boys. Additionally, physical exercise has an influence on muscle and fat mass (Hass, et al., 2001). Previous studies in adults document associations between total body and lean masses and bone mineral content and areal density (Ferretti, et al., 1998). In children a close relationship

between muscle and bone masses have been reported (Daly, et al., 2004, Rauch, et al., 2004, Vicente-Rodriguez, et al., 2004), but remarking that other loading-related factors, i.e. exercise, could have a greater influence on bone development (Daly, Saxon, Turner, Robling and Bass, 2004, Vicente-Rodriguez, Ara, Perez-Gomez, Dorado, Serrano-Sanchez and Calbet, 2004). The muscle-bone relationship is presumably explained by the mechanostat theory, which proposes that the bone strength is regulated by modelling and remodelling processes depending on the forces acting on bones (Rauch, Bailey, Baxter-Jones, Mirwald and Faulkner, 2004, Schoenau and Frost, 2002), as biggest muscles exert higher tensile forces on the bones they attach.

A weak relationship between fat mass and bone mass has also been reported in adults (Reid, et al., 1992), but it remains unknown if fat mass has “per se” any influence on bone mass acquisition during growth. In fact, very little is known about the influence that the changes in soft tissue body composition might have on bone mass acquisition during growth in children (Faulkner, et al., 1993, Ferretti, Capozza, Cointy, Garcia, Plotkin, Alvarez Filgueira and Zanchetta, 1998, Pietrobelli, et al., 2002). Although body weight has been identified as a determinant of BMD, some controversy exists over the independent effect of its major components, lean and fat masses (Lohman, et al., 1995). A cross-sectional study suggests that lean mass may be a better predictor of total BMD than body weight in children (Faulkner, Bailey, Drinkwater, Wilkinson, Houston and McKay, 1993). Recent longitudinal studies confirmed muscle-bone association (Forwood, et al., 2004, Rauch, Bailey, Baxter-Jones, Mirwald and Faulkner, 2004, Vicente-Rodriguez, et al., 2005, Vicente-Rodriguez, Ara, Perez-Gomez, Dorado, Serrano-Sanchez and Calbet, 2004) and revealed the increase in lean mass as the best predictor of femoral bone mass and density accumulation (Vicente-Rodriguez, Ara, Perez-Gomez, Dorado and Calbet, 2005). While fat mass has been associated with bone mass in children between 7 and 17 years (Pietrobelli, Faith, Wang, Brambilla, Chiumello and Heymsfield, 2002), but no relationship has been found between change in fat mass and bone accrual during prepubertal growth (Vicente-Rodriguez, Ara, Perez-Gomez, Dorado and Calbet, 2005, Vicente-Rodriguez, Ara, Perez-Gomez, Dorado, Serrano-Sanchez and Calbet, 2004). Participation in sport is another factor that could influence both bone mass and body composition. Despite of the findings of the latter studies, the influence that the changes in soft tissue body composition might have on whole body, or regional bone mass acquisition during growth in children are unclear. Similarly, how physical activity may modulate the relationship between the changes in soft tissues and bone mass in children who started their sport participation at a prepubertal age remain elusive.

Therefore, the aims of this study were: 1) to test if boys involved in out-of-school sport participation enhance their physical fitness more than their non-active counterparts and if this has any influence on bone mass accrual. 2) To determine to what extent bone mass accrual is determined by lean, fat or total body mass acquisition during growth in prepubertal boys. 3) To elucidate whether physical activity may modulate the soft tissue-bone relationship.

MATERIALS AND METHODS

Subjects

The subjects included in this study represent a subgroup of the Growth and Physical Activity Study of Gran Canaria, specific details on how the sample was selected are given elsewhere (Vicente-Rodriguez, Jimenez-Ramirez, Ara, Serrano-Sanchez, Dorado and Calbet, 2003).

One hundred and thirty two boys were prepuberal at the start of the study (Tanner 1-2), but only 42 maintained their initial physical activity pattern, active been active and non-active been non-active, reaching Tanner 3-4 at the end of the survey. The subjects were divided into two groups depending on the level of their physical activity, and all tests were carried out on two different occasions, being 3.3 years the mean time between tests. Twenty-six boys were assigned to the physically active group (active group) as they practiced extracurricular physical activities regularly: at the beginning of the study all of them had been footballers for at least one year (2.0 ± 0.25) and for at least 3 hours per week. At the end of the follow up one subject changed his sport from football to basketball and another two subjects changed from football to handball. The other 16, whose physical activities were limited to those programmed during the compulsory physical education curriculum (60-90 min per week) until the end of the follow-up, were assigned to the control group (non-active group). Boys answered a medical and physical activity questionnaire, and, if needed, their parents complemented the medical history. Information regarding physical activity, past injuries, medication, known diseases and daily consumption of dairy products to calculate calcium intake was obtained from every subject, as it has been previously reported (Vicente-Rodriguez, Jimenez-Ramirez, Ara, Serrano-Sanchez, Dorado and Calbet, 2003). Chronic diseases that might influence the results and any medication that might affect the skeleton were defined as exclusion criteria. None of the subjects was excluded from the study at any moment. Parents and children were informed about the aims and procedures of the investigation protocol, as well as the possible risks and benefits before they gave their written consent. The study was carried out according the Helsinki Declaration, and was approved by the ethical committee of the University of Las Palmas de Gran Canaria.

Pubertal status assessment

Tanner pubertal status was determined by auto-evaluation, a method of recognised validity and reliability ($r = 0.97$) (Faulkner, 1996).

Bone mass and body composition

Anthropometrical measurements were carried out on each subject. Height was measured in the upright position to the nearest millimetre (Atlántida, Año Sayol, Barcelona, Spain). Body mass was determined using a balance with 50g precision (Atlántida, Año Sayol, Barcelona, Spain) calibrated with M1 calibration masses (tolerance $< 0.005\%$ in mass). Thereafter, the total and regional bone, lean (body mass $-[\text{fat mass} + \text{bone mass}]$) and fat masses were measured using dual-energy X-ray absorptiometry (DXA) (QDR-1500, Hologic Corp., Software version 7.10, Waltham, MA). The dual-energy X-ray absorptiometer 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. 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. Areal bone mineral density ($\text{g}\cdot\text{cm}^{-2}$) was calculated using the formula $\text{BMD} = \text{BMC} \cdot \text{area}^{-2}$. The regional analysis was performed as described elsewhere (Calbet, et al., 2001). Lean mass of the limbs was assumed equivalent to the muscle mass.

An additional examination was conducted to estimate bone mass at the lumbar spine region. In this case, the values reported for the lumbar vertebrae L2-4 were obtained from an anteroposterior lumbar scan and expressed as the mean bone mineral content (BMC) and mean BMD of the three vertebra.

The laboratory precision error for the regional analysis of the whole body scan, as defined by the coefficient of variation (CV) for repeated measurements in young volunteers were 0.4; 0.7; 3.1 and 1.0 respectively for the BMC, BMD, fat mass and lean mass at the whole body; and 1.4; 2.4; 5.2 and 1.4 at the lower extremities.

Physical fitness

Dynamic and maximal isometric force. The forces generated during vertical jumps were measured with a force platform (Kistler, Winterthur, Switzerland). Each boy performed two kinds of maximal jumps. The Squat Jump (SJ), starting with knees bent at 90° and without previous counter movement, and the Counter Movement Jump (CMJ), starting from a standing position allowing for counter movement, with the intention of reaching knee bending angles of around 90° just before impulsion. The knee angle was measured with a digital goniometer (Lafayette Instrument Company, Lafayette Indiana). The jumping height (Hj), the peak force

(Fp, being Fp = maximal force-body mass), the positive impulse and the mean power (Mp) generated were determined in the best of three trials.

The maximal isometric force (MIF) was also measured with the same plate force, during leg extension in the upright position with the knees bent at a 90° angle, i.e., in the same position as for the squat jump. Subjects pulled maximally against a weightlifting bar placed over the shoulders and attached with two lateral chains to the floor where the force plate was also fixed. During 5 seconds subjects were encouraged to exert the highest strength in the lowest time. The best of three attempts, with 1 min rest period in between, was recorded.

Anaerobic capacity. A three hundred meter running test was used to estimate the anaerobic capacity because the anaerobic capacity is the first determinant of performance in maximal all-out efforts eliciting exhaustion between 30 and 60 seconds (Calbet, et al., 2003). The test was performed on a 400 m track, and running times were measured manually using a stopwatch. The boys were asked to run the 300 m as fast as possible.

Running speed test. The time needed to cover 30 meters (T_{30}) was measured with photoelectric cells (General ASDE, Valencia). The timer is automatically activated when the subject crosses the first cell, every 5 meters thereafter. The boys were motivated to run as fast as they could, and the best performance achieved in three trials separated by at least 1 min rest period was taken as the representative value of this test.

Aerobic maximal power. The maximal oxygen uptake (VO_{2max}) was estimated using a reliable maximal multistage 20-m shuttle run test as it is described elsewhere (Vicente-Rodriguez, Jimenez-Ramirez, Ara, Serrano-Sanchez, Dorado and Calbet, 2003). Subjects were required to run back and forth on a 20 m course and be on the 20 m line at the same time that a beep is emitted from a tape. The frequency of the sound signals increases in such a way that running speed starts at 8.5 $Km \cdot h^{-1}$ and is increased by 0.5 $Km \cdot h^{-1}$ each minute. The length of time the subjects were able to run for was recorded to calculate the VO_{2max} with the following equation: $VO_{2max} = 31.025 + (3.238 \cdot V) - (3.248 \cdot Age) + (0.1536 \cdot V \cdot Age)$, where V is the mean velocity reached during the test.

Statistical analysis

Mean, standard deviation (SD) and standard error of estimate (SEE, for the equations) are given as descriptive statistics. Differences between groups were established using Student's unpaired t-test. Chi-square test was applied to check the similarity of the Tanner stages distribution between groups. Analyses of covariance (ANCOVA) were performed to evaluate the influence of physical activity on bone and lean masses, entering the increment in height, body mass, age and the age at the

end of the study as covariates. Additionally, assessment of sexual maturation was used to control for differences in maturation among subjects. These covariates were used based on evidence identifying height, age and body mass as influential factors on the growing skeleton (Faulkner, et al., 1996). One factor (with two levels: physical activity and non-physical activity) ANCOVA with repeated measures (baseline and 3.3 years) and with lean mass increment as covariate was used to assess the influence of lean mass increment on bone mass and density changes. Additionally, bivariate correlation and linear stepwise multiple regression was applied to identify the relationship between physical fitness related variables and bone, also between the changes in lean mass, fat mass or total body mass and the changes in bone mass variables in the loaded and unloaded areas. To test the similarity of slopes and intercepts of these relationships between active and non-active boys, the corresponding t-test was applied for the model: $Y_{ij} = \alpha_i + \beta_i X_{ij} + \varepsilon_{ij}$ for $i=1,2$ (1 = actives, 2 = non-actives) and $j=1, \dots, n_i$ being ε_{ij} i.i.d. random variables following a distribution $N(0, \sigma_i)$. SPSS package (SPSS Inc, Chicago, USA) for Personal Computer was used for the statistical analysis. P-value less than 0.05 was considered significant.

RESULTS

Physical characteristics

The subjects' age and anthropometrical data were comparable at baseline and 3.3 years later (Table 1), as was maturation (Tanner) being 3-4 for active and non-active groups (Tanner 3: 61.5% Vs. 43.8% and Tanner 4: 38.5% Vs. 43.8%, $p = 0.2$) at the end of the survey. As expected, the body height, weight and maturation increased with age ($p < 0.05$), but not the percentage of body fat (%BF). No differences were observed between groups in calcium intake (data not shown).

TABLE 1

Subjects' age, daily calcium intake (Ca^{++}), Tanner stage and anthropometrics results (mean \pm SD).

	Baseline		Final	
	Active	Controls	Active	Controls
Age (years)	9.3 \pm 1.5	9.5 \pm 1.2	12.7 \pm 1.9	12.8 \pm 0.8
Height (cm)	136.5 \pm 10.7	136.8 \pm 6.0	156.6 \pm 14.0	158.6 \pm 9.2
Body mass (Kg)	31.9 \pm 7.6	32.3 \pm 6.4	47.1 \pm 12.7	49.3 \pm 10.8
Ca^{++} (mg)	1574.2 \pm 662.5	1457.9 \pm 538.0	806.8 \pm 365.5	830.6 \pm 278.1

Physical fitness

All physical fitness related variables, except the time needed to cover 30 m, improved over the study period in both groups (Table 2, $p < 0.05$). $VO_2\max$ was improved only in the physically active boys (Table 2). At the end of the survey the active group needed 6 second less to cover 300 m, and its $VO_2\max$ was 8% higher compared to the control group (both $p < 0.05$, Table 2). After accounting for differences in weigh, height and age, the 30m running time tended to worsen (8% more time) in the sedentary controls compared to the active boys, that kept constant their performance ($p = 0.08$). Only the increase in anaerobic capacity was significantly better for the active boys ($10\% \pm X$, $p < 0.05$).

TABLE 2

Subjects' physical fitness results (mean \pm SD). Thirty meters running speed test (T_{30}), anaerobic capacity (AC), maximal oxygen uptake in $ml \cdot kg^{-1} \cdot min^{-1}$ ($VO_2\max$), maximal isometrical force (MIF), height jumped (HJ), peak force (PF) and mean power (MP) during countermovement jump (CMJ) and squat jump (SJ).

Physical fitness	Baseline		Final	
	Active	Controls	Active	Controls
T_{30} (s)	5.29 \pm 0.29	5.11 \pm 0.37	5.32 \pm 0.31	5.32 \pm 0.40
AC (s)	77.3 \pm 11.0	78.5 \pm 11.4	61.3 ^{#*} \pm 7.2	67.0 [#] \pm 9.7
$VO_2\max$	50.3* \pm 3.4	47.5 \pm 3.7	55.2 ^{#*} \pm 3.8	48.4 \pm 3.5
MIF (kp)	76.0 \pm 17.3	82.3 \pm 18.8	102.9 [#] \pm 23.6	101.3 [#] \pm 19.3
CMJ				
HJ (cm)	18.8 \pm 4.0	18.6 \pm 4.1	21.8 [#] \pm 4.2	24.0 [#] \pm 8.2
PF (Kp)	41.1 \pm 12.2	43.4 \pm 12.4	61.3 [#] \pm 19.9	71.2 [#] \pm 23.5
MP (W)	229 \pm 84.9	226.4 \pm 71.2	407.7 [#] \pm 144.7	473.6 [#] \pm 163.0
SJ				
HJ (cm)	17.5 \pm 5.0	14.9 \pm 5.3	21.7 [#] \pm 5.5	21.1 [#] \pm 6.1
PF (Kp)	37.3 \pm 10.3	37.9 \pm 8.5	48.2 [#] \pm 18.7	49.4 [#] \pm 14.2
MP (W)	164.2 \pm 40.7	149.4 \pm 48.2	271.5 [#] \pm 95.9	281.3 [#] \pm 105.8

* $p < 0.05$ intergroup differences. [#] $p < 0.05$ intragroup differences between 1st and 2nd determinations.

Body composition

The change in total lean mass during the three-year follow-up was greater in the active than in the control group ($p < 0.05$; Table 3). Nevertheless, no differences were found in the lean mass increment of the lower-limb between groups.

ANCOVA for repeated measures adjusted for lean mass increment and maturational development showed that total BMC and BMD, as well as lower-limb and lumbar BMD increased more after 3.3 years follow up in the active than in the control boys (all $p < 0.05$).

TABLE 3
Percentage of changes of bone mineral content (BMC), density (BMD) and lean mass from the whole body, and lumbar scans (mean \pm SD).

Variables	Δ BMC (%)		Δ BMD (%)		Δ Lean mass (%)	
	Active	Controls	Active	Controls	Active	Controls
Whole body	59.4* \pm 9.2	52.7 \pm 9.2	14.5* \pm 4.6	9.2 \pm 4.8	50.9* \pm 6.6	44.1 \pm 6.8
Lower-limb	86.2 \pm 9.2	82.3 \pm 9.6	25.9* \pm 5.1	20.4 \pm 5.2	57.0 \pm 9.2	55.1 \pm 9.2
Lumbar spine	64.1* \pm 15.8	49.3 \pm 15.6	21.9* \pm 8.2	13.7 \pm 8.0		

* $p < 0.05$

At the beginning of the study, after accounting for weight, height and age, the active group presented 10% and 8.4% higher lower-limb and lumbar BMC, and 5% and 6% higher lower-limb and lumbar BMD than the control group (all $p < 0.05$; Table 4). However, the increments in whole body and lumbar BMC and BMD, and lower-limb BMD were greater in the active than in the non-active group during 3-yr growth ($p < 0.05$; Table 3). As a result, higher total, lower-limb and lumbar BMC (9%, 12% and 15.2% respectively) and BMD (5.4%, 7.2% and 11.5% respectively) were observed in the active than in the control group at the end of the follow-up (all $p < 0.05$; Table 4).

TABLE 4
Bone mineral content (BMC) and density (BMD) from the whole body, and lumbar scans (mean \pm SD).

BMC (g)	Baseline		Final	
	Active	Controls	Active	Controls
Whole body	1045.0 \pm 268.2	987.7 \pm 154.0	1654.1* Φ \pm 515.0	1570.6 \pm 206.4
Lower-limb	191.7* \pm 68.8	175.8 \pm 35.2	352.5* \pm 122.9	329.6 \pm 46.8
Lumbar	6.3* \pm 1.5	6.0 \pm 0.8	10.2* \pm 3.6	9.4 \pm 1.6
BMD (g \cdot cm ⁻²)				
Whole body	0.854 \pm 0.1	0.835 \pm 0.1	0.969* Φ \pm 0.1	0.927 \pm 0.1
Lower-limb	0.879* \pm 0.1	0.843 \pm 0.1	1.096* Φ \pm 0.2	1.041 \pm 0.1
Lumbar	0.657* \pm 0.1	0.623 \pm 0.0	0.787* Φ \pm 0.1	0.728 \pm 0.1

* $p < 0.05$ for ANCOVA using weight- age- and height as covariates; Φ $p < 0.05$ for ANOVA with repeated measures and lean mass increment as covariate.

When adjusted for lean mass increase, active boys experienced a 42.8% and 22.2% greater enhancement of total and lower-limb BMD per unit of lean mass development than the boys in the control group did.

Relationship between the increments in physical fitness and bone mass and density

The highest correlations were found between time needed to cover 30 m and bone mass related variables ($r = 0.40-0.64$, $p < 0.01$). The anaerobic capacity and jumped height in the CMJ and SJ as well as peak force and mean power in the SJ also correlated with BMC and BMD in all analyzed regions ($r = 0.30-0.54$, $p < 0.05$). However, multiple regression analysis showed that the jumped height in the CMJ and the peak force in the SJ explained respectively between (12-58 % and 17-56 %) of the total variance in BMC and areal density related variables.

Relationship between the increments in body mass, lean mass, bone mass and bone density

The strongest correlations were found between lean mass increment and the increment in bone mass variables ($r = 0.70-0.89$, $p < 0.001$), even after controlled for maturation. The slope of the relationship between lean mass and both lumbar BMC and BMD was steeper in the active group than in the non-active group ($p < 0.05$, figure 1). In addition, a strong relationship was detected between body mass increment and the increment in whole body and lower-limb BMC ($r = 0.75$, $p < 0.001$; $r = 0.79$, $p < 0.001$ respectively). However, body mass increment presented a weak correlation with the increment in the total and regional BMD ($r = 0.32-0.56$, $p < 0.05$).

Weak relationships were found between lean mass and the BMC of the head ($r = 0.45$, $p < 0.05$) and no correlation exists with head BMD at the end of the survey. A significant, although small, correlation was observed between the change in lean mass and the increase in the head BMC ($r = 0.38$, $p < 0.05$) but no relationship was found with the increase in the BMD of the head.

Multiple regression analysis showed that the change in whole body lean mass (Δ LM in %) had the highest predictive value for the BMC and BMD increments in the whole body, lower limbs and lumbar spine in growing boys, as reflected in the following equations:

$$\Delta \text{ whole body BMC} = 0.83 \cdot \Delta \text{ LM} + 16.591 \text{ (R} = 0.88, p < 0.001, \text{SEE} = 7.4)$$

$$\Delta \text{ whole body BMD} = 0.231 \cdot \Delta \text{ LM} + 1.475 \text{ (R} = 0.70, p < 0.001, \text{SEE} = 3.9)$$

$$\Delta \text{ lower-limb BMC} = 0.798 \cdot \Delta \text{ LM} + 45.712 \text{ (R} = 0.66, p < 0.001, \text{SEE} = 12.3)$$

$$\Delta \text{ lower-limb BMD} = 0.3 \cdot \Delta \text{ LM} + 9.296 \quad (R = 0.74, p < 0.001, \text{SEE} = 4.5)$$

$$\Delta \text{ lumbar BMC} = 1.392 \cdot \Delta \text{ LM} - 8.989 \quad (R = 0.89, p < 0.001, \text{SEE} = 12.1)$$

$$\Delta \text{ lumbar BMD} = 0.56 \cdot \Delta \text{ LM} - 8.219 \quad (R = 0.82, p < 0.001, \text{SEE} = 6.5)$$

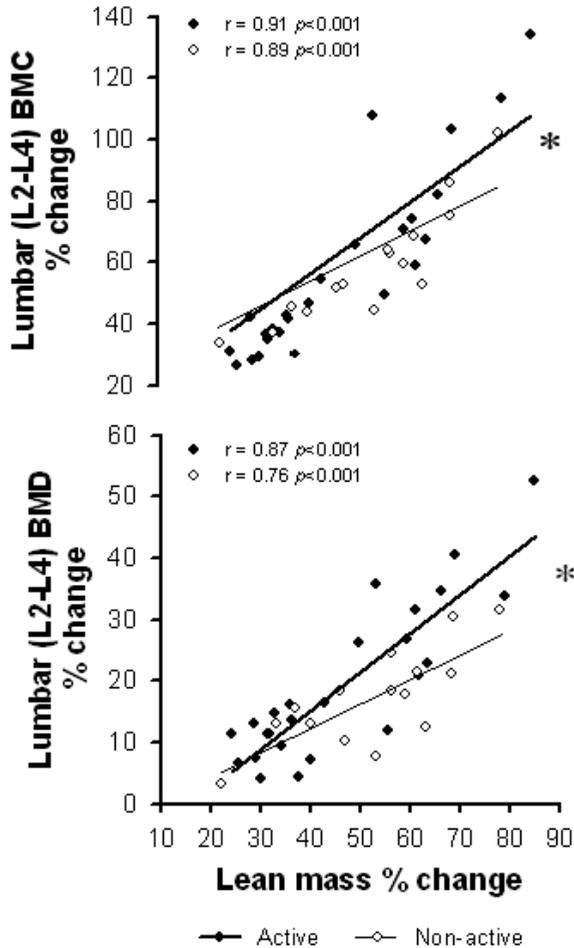


FIGURE 1. Relationship between the increase in percentage in lumbar spine (L₂-L₄) bone mineral content (BMC) and areal density (BMD) and the change in percentage in whole body lean mass. * The slope was significantly steeper in active than in non-active boys ($p < 0.05$).

DISCUSSION

Some major findings emerge from this study. 1) peripubertal boys, who started their activity at prepubertal age and maintained a constant participation in an extracurricular sport programme for three hours per week over a three-year period, increased their total and lumbar BMC and BMD compared to their non-physically active matched counterparts. 2) The active boys developed their aerobic power and anaerobic capacity more than the sedentary controls. 3) Lean mass development was shown to be the best predictor for total and regional BMC and BMD acquisition, while the increment in fat mass did not show any relationship with bone mass development. 4) Physical activity has been shown to be an important environmental factor affecting bone development.

Physical activity and bone mass acquisition during growth

Anaerobic capacity and maximal aerobic power ($VO_2\max$) were 9 and 8 % significantly higher at the end of the follow up in the active boys compared to the controls. When the study started all the subjects were able to run the 300 m in the same time, while 3 years later the active boys needed a mean 6 second less than the controls to run the same distance. In addition, the active group increased their $VO_2\max$ during growth while their non-active counterparts did not. The $55 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of $VO_2\max$ observed in the active group at the end of the follow up is 10% higher than that of the active adult males. This is particularly important since several studies have shown that physical fitness is associated with lower mortality and morbidity rates in the general population (Wei, et al., 1999). It should be noted that this improvement in anaerobic and aerobic fitness was achieved with a relatively low effort: three hours per week of extracurricular physical activity participation.

At the beginning of the present study, active boys already had enhanced lower-limb and lumbar BMC and BMD compared to their matched non-active counterparts. This was likely because these children had been participating regularly in sports for a mean of 2 years before the start of the follow up. This finding concurs with previous longitudinal (Bailey, McKay, Mirwald, Crocker and Faulkner, 1999, MacKelvie, et al., 2004) and cross-sectional studies (Haapasalo, et al., 1998, Vicente-Rodriguez, Jimenez-Ramirez, Ara, Serrano-Sanchez, Dorado and Calbet, 2003).

Our findings support those of several other studies. Bailey et al (Bailey, McKay, Mirwald, Crocker and Faulkner, 1999) found in a six-year longitudinal study, which controlled for maturational and size differences, a 9 to 17% higher whole body BMC for active boys and girls respectively compared to non-active counterparts. More recently, it has been shown that intensive participation in sports that involve jumps,

stops, and rapid changes in the direction of movement, elicit higher changes of BMC and BMD during growth in young postpubertal males (Gustavsson, et al., 2003). A similar relationship was reported by Vicente-Rodriguez et al (2004). Additionally, handball participation has recently been associated with enhanced bone mass in young postmenarcheal females (Vicente-Rodriguez, Dorado, Perez-Gomez, Gonzalez-Henriquez and Calbet, 2004).

In the present study, we have observed that boys who regularly participate in football, accumulate a third more BMC in the lumbar spine, 7% more BMC in the whole body and approximately a third more BMD in the whole body, lower-limb and lumbar spine. These increments are similar to those reported by Gustavsson et al, (Gustavsson, Thorsen and Nordstrom, 2003) in postpubertal boys after three years of sport. However, in a 2-year controlled trial of exercise, girls increased their femoral neck and lumbar BMC compared with controls (MacKelvie, et al., 2003), but significant changes in femoral BMC only were reported in boys (MacKelvie, Petit, Khan, Beck and McKay, 2004). Some animal and human studies found that the benefits of exercise on bone are greater when exercise is regularly practiced throughout the puberty, possibly due to the responsiveness of immature bone to mechanical stimulation (Bailey, McKay, Mirwald, Crocker and Faulkner, 1999). It has also been proposed that participation in physical exercise should start before the onset of puberty rather than during or after it in order to obtain the greatest stimulation for bone acquisition (Calbet, Dorado, Diaz-Herrera and Rodriguez-Rodriguez, 2001). The results of the present investigation support the latter since boys who started sports at prepubertal age, three years later have noticeably greater bone mass accumulation throughout the whole body, lower-limb and lumbar spine regions than their non-active counterparts. It has been reported that 26% of total body bone mineral in adults, is accrued during a two-years period of fast bone mineral accrual during growth, approximately between 13 to 15 years of age in boys (Bailey, et al., 1996). Our follow up terminated when the subjects were under 13 years of age. However, the boys who were involved in sports increased their bone mass and density more than the control group, who showed only the effect of development. The latter clearly suggests that, apart from pubertal development, the most significant positive influence on bone mass acquisition is participation in sports. So according to Bailey et al (1996) and Gustavsson et al (2003) what remains unanswered is the question of whether the subjects could further enhance their bone mass by remaining active after the puberty when their bone mass is already enhanced.

Part of the positive effect that participation in sport has for the acquisition of a greater peak bone mass at the end of the growth, may require an early introduction

of extracurricular physical activities during childhood. This is particularly meaningful since some researchers have hypothesized that increased peak bone mass during growth may provide a protection against osteoporosis in old age (Kelly, et al., 1990). However, more research is needed to determine whether this supposed protection against osteoporotic fractures is really effective later in life.

Lean mass growth and bone mass.

Body mass has been identified as the primary predictor of bone mass in both children (Faulkner, Bailey, Drinkwater, McKay, Arnold and Wilkinson, 1996, Lima, et al., 2001). During childhood BMD seems to be more influenced by lean mass than by fat mass (Young, et al., 1995), suggesting that lean mass development could play a relevant role in the acquisition of a higher bone mineral peak (Lima, De Falco, Baima, Carazzato and Pereira, 2001, Vicente-Rodriguez, Ara, Perez-Gomez, Dorado, Serrano-Sanchez and Calbet, 2004). The mechanostat theory postulates that forces elicited by muscles during growth could drive bone strength, meaning that muscular development should result in the increase in BMC. However, only few investigations have addressed longitudinally the relationship between body composition and bone mass development in children (Forwood, Bailey, Beck, Mirwald, Baxter-Jones and Uusi-Rasi, 2004, Rauch, Bailey, Baxter-Jones, Mirwald and Faulkner, 2004). Forwood et al (2004) observed that greater increase in lean body mass and the consequent related mechanical loadings that impose, accounted for the sexual dimorphism in bone structure. Additionally, Rauch et al (2004), have reported that the peak whole body BMC accrual occurred 0.36 years after the maximal increase in total lean mass, suggesting that the additional higher stimuli elicited by a bigger muscle mass contributed to this increase in peak BMC accrual in boys. Our results show that the increase in lean mass is the best predictor of the enhancement of whole body and regional bone mineral content and areal density with growth even after accounting for the effect of pubertal development. In fact, the enhancement of lean mass explains between 44 and 79% of the variability that the increments in whole body- and regional bone mineral content and areal density showed during the three-year follow-up. In a 10-year follow up study with adults Bakker et al (Bakker, et al., 2003) observed that the changes in lean mass are closely related with the changes in bone mass. Nevertheless, if we compare our findings with those of Bakker et al (Bakker, Twisk, Van Mechelen and Kemper, 2003) we could see that the changes in lean mass explain twice as much the variability in bone mass in boys than in adults. This could mean that muscle development is even more important during growth.

In addition, active boys had 42.8% and 22.2% higher increases in whole body and lower-limb BMD per unit of lean mass increase compared to the control group.

This combined with the fact that bone mass increased more in the active boys than in the controls, after accounting for lean mass changes, implies that additional factors apart from lean mass account for the differences encountered in BMC and BMD. The results of this study show that the active group improved their physical fitness more than the sedentary control group. Additionally, we observed that some physical fitness related-variables explained between 12 and 56 % of the total variance in total and regional BMC and areal density acquisition. This may support the notion that sports or a similar kind of regular physical activity are required to achieve the full potential in bone mass acquisition during growth. The positive effect of exercise is brought about by a direct stimulation of bone formation and indirectly through its effects on lean mass (muscle mass). Nevertheless, further studies are necessary to clarify which kind of sports are better suited to facilitate both bone mass and muscle mass acquisition in children.

Body mass, fat mass and bone mass

As expected, total body mass enhancement also correlated with bone mass development. In contrast with the close correlation between fat mass and bone mineral content reported by Pietrobelli et al in a cross-sectional study (Pietrobelli, Faith, Wang, Brambilla, Chiumello and Heymsfield, 2002), no relationship has been found between changes in fat and bone mass in the present investigation. This finding implies that the longitudinal changes in fat mass do not appear to contribute significantly to the gain in bone mass with increasing age.

Limitations

The DXA scanners are not able to measure the real bone mineral density or volumetric bone mineral density (vBMD, commonly expressed in $\text{g}\cdot\text{cm}^{-3}$), what they measure is areal bone mineral density ($\text{g}\cdot\text{cm}^{-2}$). To circumvent these limitations, the effect of body size on areal BMD determinations was taken into account by adjusting it for body mass, height, age and final age and sexual maturation. In addition, the effect of sports participation was determined by comparing the increments observed in areal BMD in the active group with those observed in the control group. Since both groups had similar heights and weights at the start and the end of the follow up, we can rule out any confounding effect of the increase in bone size on the effects of sports participation on areal BMD.

Self-selection could be another limitation of the study since the strongest or more developed boys with higher muscle mass and bone mass could chose to enrol in sport activities because their condition facilitates greater achievement and success, which may positively reinforce participation in sports. Similarly, it is

possible that genetic factors, which establish the potential for muscle and bone masses development, also accounted for some of the effect here reported. However, the longitudinal design of the study, the comparable maturational development and the fact that the BMC and BMD of the head (a non-loaded region) were similar and developed similarly in both groups (data not shown), while the loaded regions increased significantly more in the active than in the control boys, suggest that there exists a cause-effect relationship between exercise participation and muscle and bone development.

CONCLUSION

In summary, this study clearly shows that the human prepubertal skeleton is rather sensitive to mechanical stimulation. The increase of lean mass with increasing age is the most important predictor of bone mineral mass accumulation during pubertal growth in that group of boys. Since lean mass is composed primarily of skeletal muscle, participation in sports could have not only a direct osteogenic effect, but also an indirect effect by increasing muscle mass during peripubertal years. However, for a given enhancement of lean mass, physically active children accumulate additional bone mass during growth. Therefore, sport participation should be encouraged before puberty to improve the skeletal health of the population.

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