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ORIGINAL ARTICLE

Differences in the relation between bone mineral content and lean body mass according to gender and reproductive status by age ranges

Edgar Denova-Gutiérrez[1] · Patricia Clark[2,3] · Ricardo Francisco Capozza[4] · Laura Marcela Nocciolino[4] · Jose Luis Ferretti[4] · Rafael Velázquez-Cruz[5] · Berenice Rivera[6] · Gustavo Roberto Cointry[4] · Jorge Salmerón[6,7]

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Abstract

The present study aims: (1) to explore the influence of lean mass (LM) on bone mineral content (BMC), (2) to investigate the pubertal influences on the BMC–LM relation, and (3) to perform *Z*-score charts of BMC–LM relation, stratified by gender and reproductive status categorized by age ranges. A cross-sectional analysis was conducted using 4001 healthy subjects between 7 and 90 years participating in the Health Workers Cohort Study. Of these, 720 participants were ≤ 19 years, 2417 were women ≥ 20 years, and 864 were men ≥ 20 years. Using Dual X-ray absorptiometry (DXA), we measured BMC and LM. Participants' pubertal development was assessed according to Tanner's stage scale. To describe BMC–LM relation, simple correlation coefficients were computed. To produce best-fit equations, an ANOVA test was conducted. *Z*-score graphs for the BMC–LM relation were obtained. In general, the BMC–LM correlations were linear and highly significant. For boys, curves were virtually parallel, with similar intercepts and a progressive displacement of values toward the upper-right region of the graph, for each Tanner subgroup. For girls, curves for Tanner 1-2 and 4-5 stages were parallel; but, in girls Tanner 4-5, the intercepts were significantly higher by about +300–400 g of BMC ($P < 0.001$). For postmenopausal women, the curve was parallel to that for the premenopausal but showed a lower intercept ($P < 0.001$). We provide DXA reference data on a well-characterized cohort of 4001 healthy subjects. These reference curves provide a reference value for the assessment and monitoring of bone health in all age groups included in the present study.

Keywords Bone mineral content · Lean mass · Muscle–bone relation · Tanner stage

Introduction

Bone mass develops primarily in childhood and during adolescence, with peak bone mass (PBM) reached in early adulthood [1, 2]. Adequate PBM during this age range influences lifelong bone health and predicts osteoporosis risk at an older age [3]. Multiple factors; like sex, heredity, ethnicity, hormonal process, physical activity, diet, body weight, and muscle strength,

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✉ Gustavo Roberto Cointry
gcointry@gmail.com

[1] Centro de Investigación en Nutrición y Salud, Instituto Nacional de Salud Pública, Cuernavaca, Mexico

[2] Unidad de Investigación en Epidemiología Clínica, Hospital Infantil de México Federico Gómez, Mexico City, Mexico

[3] Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico

[4] Centro de Estudios de Metabolismo Fosfocálcico, Universidad Nacional de Rosario, Rosario, Argentina

[5] Laboratorio de Genómica del Metabolismo Óseo, Instituto Nacional de Medicina Genómica, Mexico City, Mexico

[6] Unidad Académica en Investigación Epidemiológica, Centro de Investigación en Políticas, Población y Salud, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico

[7] Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Mexico

have been related with bone mass accumulation [4]. Of these factors, muscle strength and body weight are suggested as the most important determinants of bone mass [4, 5]. However, it is important to take into account mechanical, anthropometric, and endocrine or metabolic aspects that may affect the skeletal structure or mass when evaluating muscle–bone interaction [6].

Dual-energy X-ray absorptiometry (DXA) constitutes the most extensively employed method for the measurement of bone mineral content (BMC), lean mass (LM), and fat mass (FM), which allows the evaluation of interesting relations within the same DXA variables, as well as with anthropometric, metabolic, and mechanical variables [7, 8]. Recently, the relation between BMC and LM has gained increasing recognition in medical practice with regard to the analysis of muscle–bone interactions and fracture risk [7, 9], independent of FRAX [7], especially in postmenopausal women [7, 10].

Over the past four decades, the relation between bone mass and muscle mass has been studied [11–19]. Currently, DXA surrogate measurements are used which yield similar correlations between BMC and LM. In addition, some studies [6, 7, 20] have developed reference curves and/or *Z*-score values of the corresponding BMC–LM relation in the whole body and limbs. According to BMC–LM *Z*-score values, in healthy subjects, there are two main uses: (1) to diminish the variability of BMC crude data, and (2) to evaluate the bone–muscle balance for diagnostic reasons [6, 20]. In this case, a normal *Z*-score value would suggest a natural congruence between bone and muscle mass development and status, as predicted by the mechanostat theory [17], with no interference of any systemic or metabolic factor. Also, in osteopenic individuals, BMC–LM *Z*-score values can be used as a parameter to evaluate the metabolic influences on bone loss determination; where, a low *Z*-score value (low bone/muscle mass proportionality) may reflect a metabolic interaction on the mechanical determination of bone mass by normal skeleton usage, which is referred to as a "true" or "metabolic" osteopenia [21, 22].

As previously suggested [6, 20], BMC–LM curves and *Z*-scores should be specific for gender and reproductive status and not just for age or other anthropometric indicators. Given this antecedent, the present study aims: (1) to evaluate the influence of lean mass (LM) on bone mineral content (BMC), (2) to investigate pubertal influences on the BMC-LM relation, and (3) to perform *Z*-score charts for BMC-LM relation, stratified by gender and reproductive status categorized by age ranges.

Materials and methods

Study design and participants

The health workers cohort study (HWCS) is an ongoing cohort study being conducted in Mexico to examine different health outcomes that are associated with specific lifestyle and genetic factors. Details of the study design, methodology, and participants' baseline characteristics have been previously described elsewhere [23–27]. In brief, the HWCS participants are administrative, academic, and medical employees (and their families) from three different institutions: the Mexican Social Security Institute (IMSS, by its acronym in Spanish), the National Institute of Public Health (INSP, by its acronyms in Spanish), which are both located in Cuernavaca, Morelos, and the Autonomous University of Mexico State (UAEM, by its acronym in Spanish) located in Toluca, Mexico. Throughout the baseline examination, taking place from March 2004 to April 2006, approximately 10,769 female and male participants were registered in the HWCS after providing informed consent. For the baseline assessment, all participants were invited to complete a self-administered questionnaire. After completing the questionnaire, the participants visited the Research Centers for numerous physical examinations and a fasting blood sample collection.

Our current analysis excluded participants with a diagnosis of type 2 diabetes mellitus, rheumatoid arthritis, cirrhosis, chronic kidney disease, asthma, degenerative arthritis, hip, or femur fracture, osteoporosis, and hepatitis, as well as excluding participants with a BMI ≥ 30 kg/m^2 and those with missing data regarding the main variables of interest (BMC, BMD, LM, FM, weight, height) ($n = 6768$). The remaining 4001 participants aged 7–90 years were included in our analysis. Of these, 2417 were adult women (1717 premenopausal and 700 postmenopausal) and 864 adult men (20–90 years). Additionally, 720 participants were younger than 19 years (410 girls and 310 boys). Within sex, participants were divided into 3 groups according to their tanner stages (Tanner 1–5, respectively).

The study protocol, questionnaires, procedures, and informed consent forms were approved by the corresponding Institutional Review Board of all participating institutions: the Mexican Social Security Institute (12CEI 09 006 14), the National Institute of Public Health (13CEI 17 007 36), and the Autonomous University of the Mexico State (1233008X0236). Signed written informed consent was previously obtained from all participants.

Body composition assessment

A Lunar DPX NT instrument was used to measure whole-body BMC, LM and FM. Using standardized protocols, well-trained technicians took all DXA measurements

throughout the study. Daily quality assurance scans were conducted using the phantom provided by the manufacturer; technicians ensured that the daily variation coefficient (VC) was within normal operational standards and in vivo VC was lower than 1.0–1.5% [23].

Anthropometric measurements

Body weight was measured with a calibrated electronic scale (model BC-533; Tanita) with participants wearing minimum clothing and no shoes. Height was measured using a conventional stadiometer (Seca), with barefoot participants standing with their shoulders in a normal position. Measurements were taken with the tape in a horizontal plane perpendicular to the vertical scale, touching the top of the head at the moment of inspiration. As we described in previous studies [23, 24], body mass index was obtained from standardized measurements of weight and height and was computed as a ratio of weight (kg) to height squared (m^2).

Trained nurses performed all measurements according to standardized procedures. We ensured reproducibility with concordance coefficients between 0.83 and 0.90 for anthropometric measurements.

Assessment of other variables

We evaluated demographic characteristics through self-administered questionnaires (e.g., sex, age, education, age at onset of menarche, etc.). Participants were asked about the amount of time they devoted to physical activities (PA). Our measurements of PA were based on the International Physical Activity Questionnaire (IPAQ) [23]. Individuals answered questions on the period of time they spent performing PA while at work, during leisure time, and while doing housework. PA was measured in metabolic equivalents (METs) and was categorized according to intensity and time allotted to distinct activities such as walking, dancing, swimming, and/or other types of exercise for at least 1 week within the previous year. They were also asked about the weight changes they had experienced within the previous year, which was categorized as the following: no weight change, weight lost, or weight gained ($<$ or $>$ 5 kg) [23]. Participants' pubertal development was assessed according to Tanner's stage scale, which uses the appearance of secondary sexual characteristics such as breast development and pubic hair for girls, and testicular development and pubic hair for boys [28]. These data were collected through self-administered questionnaires that included images of the secondary sexual characteristics of people at different Tanner stages; participants were asked to identify the image that most closely matched their current stage of sexual maturation. We also assessed other reproductive factors like age at menarche, menopausal status, and the time since the last menstrual period. Age at menarche was defined as the age of first menstrual bleeding. In the present study, women were considered postmenopausal if they self-reported natural menopause (cessation of menses), self-reported surgical menopause involving bilateral oophorectomy; or self-reported current use of hormone therapy. Women who did not meet these criteria were defined as premenopausal [23].

Statistical analysis

We conducted a descriptive analysis of the main characteristics of interest. Statistical analyses were performed separately for females (girls: Tanner 1–5; premenopausal women; and postmenopausal women) and males (boys: Tanner 1–5; adult men). To describe the relation among BMC, LM, FM, body weight and height, simple correlations coefficients were computed. To evaluate curve fitness and to produce best-fit equations, ANOVA test was conducted. Additionally, we used ANCOVA test to evaluate the differences between slopes and intercepts of the curves. For all groups, the *Z*-scores as per the SD values describing the dispersion of the data (BMC–LM relation) around each regression line and the corresponding, $+3$, $+2$, $+1$, 0, -1, -2, and -3 *Z*-score bands were indicated as normal references for comparative diagnosis of individual cases matching the inclusion/exclusion criteria of the study and analyzed with a similar DXA device. In other words, the *Z*-score is a standardized measurement of variation derived from dividing the difference by the individual value (x) and the population mean (μ) by the population SD (σ) [24].

$$Z = \frac{x - \mu}{\sigma}$$

All P values < 0.05 were considered as statistically significant. All statistical analyses were conducted using STATISTICA data analysis software system (version 8.0, 2008: StatSoft, inc., USA).

Results

Table 1 describes demographic and body composition characteristics of participants in the Health Workers Cohort Study in the different groups studied. For females, the groups were divided into Tanner 1–5, premenopausal, and postmenopausal women. Whereas males were stratified as follows: Tanner 1–5, adult men. After exclusion criteria, the final sample consisted of 4001 subjects (2827 females and 1174 males).

In general, the BMC–LM correlations were linear and highly significant. Among boys' subgroups (Fig. 1a), curves were virtually parallel, showing similar intercepts and a progressive displacement of values for each Tanner subgroup

Table 1 Demographic and body composition characteristics of participants in the health workers cohort study (HWCS)

n	Males				Females				
	Boys			Men	Girls			Premenopausal women	Postmenopausal women
	Tanner 1-2	Tanner 3	Tanner 4-5		Tanner 1-2	Tanner 3	Tanner 4-5		
	137	31	142	864	127	77	206	1717	700
Age, years[a]	9.8 (7.0–15.0)	13.3 (10.0—17.0)	15.8 (12.0–19.0)	45.4 (20.0–90.0)	9.2 (7.0–14.0)	13.0 (9.0–17.0)	15.4 (10.0–19.0)	35.6 (20.0–54.0)	61.9 (51.0–89.0)
Weigth, kg	33.4 (19.2–70.1)	48.6 (31.9–71.0)	59.6 (37.3–38.2)	72.6 (40.3–114.2)	33.8 (17.3–58.8)	46.6 (30.1–65.6)	53.3 (30.1–76.3)	60.0 (36.8–90.0)	60.1 (38.3–81.2)
Heigth, cm	137.0 (115.0–168.0)	157.0 (138.0–181.0)	169.0 (137.0–188.0)	169.0 (148.0–193.0)	136.0 (115.0–157.0)	154.0 (135.0–160.0)	157.0 (142.0–171.0)	157.0 (137.0–178.0)	153.0 (134.0–180.0)
BMI[1]	17.69 (12.2–26.3)	19.4 (15.3–28.6)	20.8 (14.7–29.0)	25.5 (15.3–30)	18.0 (12.1–28.6)	19.7 (15.5–28.6)	21.5 (15.0–29.6)	24.5 (15.2—30)	25.6 (16.2–30)
BMC[2], g	1198.0 (625.0–2206.0)	1954.8 (1322.0–3495.0)	2532.0 (1255.0—3623.0)	2813.0 (1832.0-4721.0)	1157.0 (512.0–1930.0)	1857.0 (764.0–2625.0)	2190.0 (1437.0–3211.0)	2338.0 (1442.0–3353.0)	1943.0 (1050.0–3296.0)
BMD[3], g/cm^2	0.88 (0.71–1.08)	1.02 (0.84–1.21)	1.19 (0.82–1.41)	1.21 (0.89–1.62)	0.87 (0.74–1.03)	1.01 (0.86–1.04)	1.10 (0.95–1.36)	1.16 (0.83–1.47)	1.03 (0.75–1.34)
LM[4], g	23954.0 (11454.0–40236.0)	36043.0 (21932.0–58326.0)	44221.0 (23634.0–59524.0)	48319.0 (31722.0–64789.0)	21736.0 (12752.0–35426.0)	28871.0 (15964.0–37965.0)	31283.0 (22231.0–47256.0)	33428.0 (22456.0–56239.0)	32611.0 (21468.0–42680.0)
FM[5], g	8704.0 (890.0–30521.0)	10567.0 (3724.0–21322.0)	12778.0 (1125.0–34210.0)	20997.0 (975.0–50235.0)	10669.0 (754.0–30254.0)	15283.0 (1268.0–29345.0)	19387.0 (1852.0–29789.0)	23632.0 (5412.0––51236.0)	25061.0 (8125.0–49541.0)

[a]Mean and (Q_{25}–Q_{75})

[1]Body mass index (BMI kg/m^2)

[2]Whole body bone mineral content (BMC)

[3]Whole body bone mineral density (BMD)

[4]Whole body lean mass (LM)

[5]Whole body fat mass (FM)

toward the upper-right region of the graph. The men's curve (Fig. 1b) exhibited a slightly lower slope than those generally observed for boys, with the data more dispersed. In contrast with boys, the curves for the girls (Fig. 1c) presented distinct patterns within the different Tanner subgroups. For example: Curves for Tanner 1-2 and 4-5 stages were virtually parallel, but the intercepts were significantly higher by about + 300 to + 400 g of BMC ($P < 0.001$) in girls of the Tanner group 4-5. In contrast, the Tanner 3 stage curve showed a strikingly higher slope ($P < 0.001$) 'connecting' the other two curves, representing a progressive trend from a lower steady state maintained during 1-2 stages to a higher steady state reached and maintained during the 4-5 stage. On the other hand, for premenopausal women (Fig. 1d), curves ranked at similar ordinate values to girls at Tanner 4-5 stage, with a slightly lower significance level ($P < 0.01$) and more dispersed data. In the same graph, the curve for postmenopausal women was parallel to the premenopausal ones, with a significantly lower intercept ($P < 0.001$), which virtually coincides with the intercept of the girls in Tanner 4-5 stage. Some gender-related differences were also observed. Curves for boys and girls were statistically significant amongst Tanner 1-2 subgroups; however, the Tanner 4-5 subgroup for girls showed higher significance ($P < 0.001$) than boys in Tanner 4-5 stage. Furthermore, in premenopausal women, the intercepts of the curves were significantly higher than those observed for adult men ($P < 0.001$ in both cases). While, as expected, curves in postmenopausal women were lower than those performed for adult men ($P < 0.001$).

Correlations between BMC and height were all linear and highly significant (Supplementary Fig. 1a–d). Among boys (Supplementary Fig. 1a), Tanner subgroups 3 and 4-5 showed parallel and coincident curves with a significantly higher slope when compared with Tanner 1-2 ($P < 0.001$). The relation between BMC and body weight (Supplementary Fig. 2a–d) reflected exponential increases with

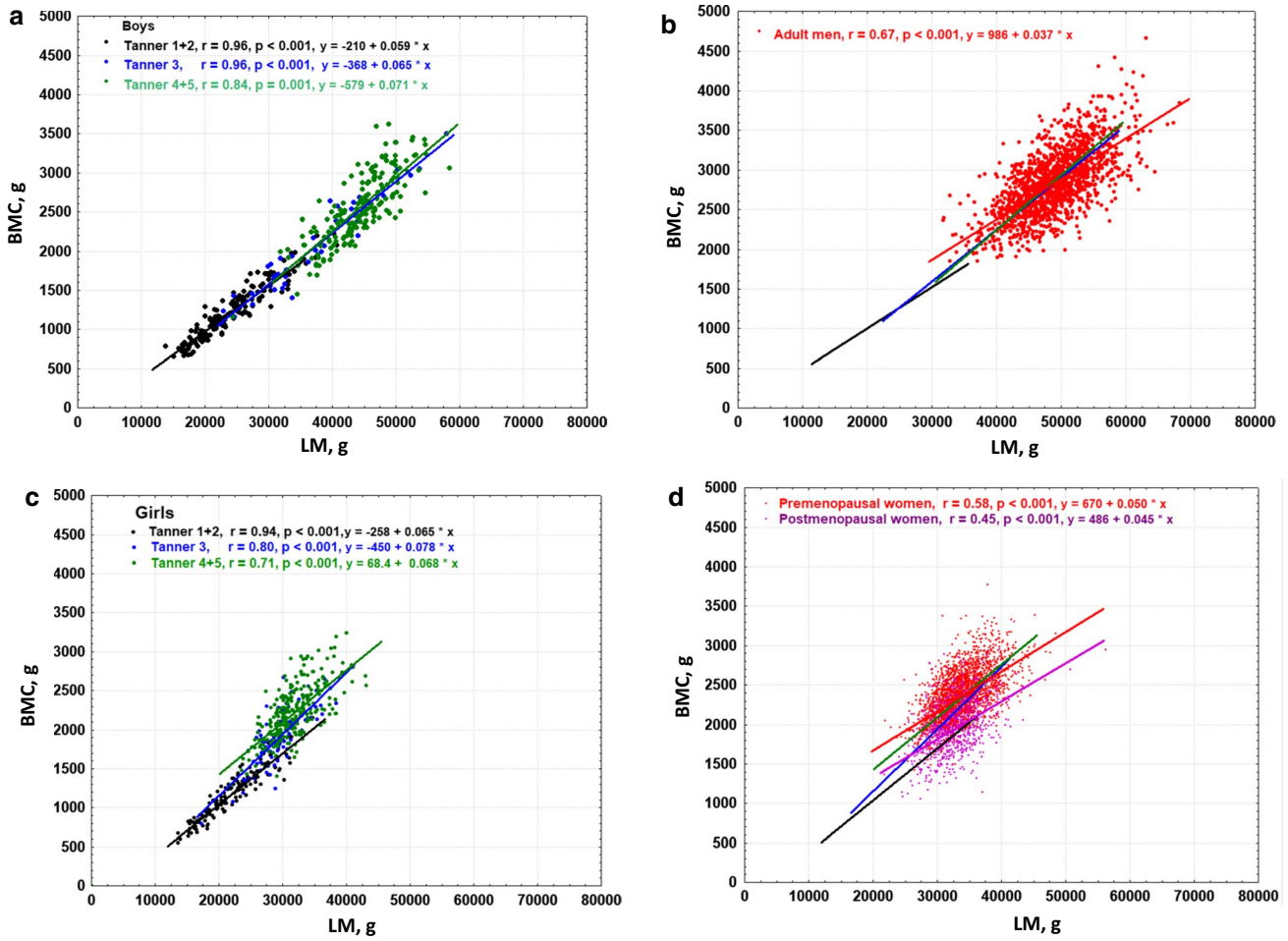

Fig. 1 Relation between the bone mineral content (BMC) and lean mass (LM) of all the studied groups and subgroups. **a** Boys at Tanner stages: [1-2 (black), 3 (blue), and 4-5 (green)]. **b** Adult men and boys (in the same color scheme as in **(a)**, and adult men (red). **c** Girls at Tanner stages [1-2 (black), 3 (blue), and 4-5 (green)]. **d** Girls, in the same color scheme as in **(c)**, premenopausal women (red) and postmenopausal women (magenta) (color figure online)

a trend towards saturation. Girls' curves (Supplementary Fig. 2c) showed significantly lower BMC values for Tanner 1-2 than for 3 and 4-5 subgroups, the latter two plotting quite close to each other ($P < 0.001$). Curves for men (Supplementary Fig. 2b) did not differ from those of Tanner 3–5 subgroups. The postmenopausal women's curve (Supplementary Fig. 2d) plotted at an intermediate region between those for premenopausal women and Tanner 1-2 sub-groups ($P < 0.001$). Additionally, correlations between BMC and FM are shown in Supplementary Fig. 3a–d. In this case, curves for men (Supplementary Fig. 3b) and premenopausal women (Supplementary Fig. 3d) are similar to those for Tanner subgroup 4-5. Additionally, postmenopausal women's curves (Supplementary Fig. 2d) were plotted at intermediate values between those for Tanner 1–3 ($P < 0.001$).

Correlations between BMC–LM ratio and age were computed. The female curve shows a relatively steep, age-related increase in BMC values from 7 to 34 years of age followed by a slight decrease thereafter. The ratio of BMC-LM was significantly higher in females than in males ($P < 0.001$) throughout the 7–65-year age range and less significant afterwards (Figure not shown).

The *Z*-score charts for the BMC and LM relations are shown for: boys in all Tanner stages together (Tanner 1–5 stages) in the same graph (Fig. 2a); and adult men (Fig. 2b). For girls, the *Z*-score charts for the BMC–LM relation were presented in separate graphs for Tanner 1-2 (Fig. 3a), Tanner 3 (Fig. 3b), and Tanner 4-5 (Fig. 3c). Finally, for adult women, premenopausal and postmenopausal charts were presented (Fig. 3d, e).

Discussion

Concerning the relation between BMC and LM, our results support the high determinant power of LM on BMC. In general, in almost all of the groups studied, we found a high ($r \geq 0.65$) and statistically significant ($P < 0.001$) correlation between BMC and LM; while, for premenopausal ($r = 0.585$ $P < 0.001$) and postmenopausal women, slightly lower correlations, but still statistically significant, were observed ($r = 0.585$ $P < 0.001$; and $r = 0.454$ $P < 0.001$, for premenopausal and postmenopausal women, respectively).

On the other hand, the inclusion of Tanner stage as a source of the growth-related increase in BMC allowed us to detect Tanner 3 as the main developmental period at which the intercept (not the slope) of the BMC–LM relation leaps from a 'child' to an 'adult' level in girls. Similarly, the slope of the BMC–LM curve for girls Tanner 3 stage were much different than those observed for all other Tanner groups (which were all virtually parallel), as much as to completely 'bridging' the span between the curves for Tanner (1-2) and adults. This important finding suggests that sex hormones or related factors can predict the occurrence of growth spurts. In this sense, multiple studies have reported anabolic properties of sex steroids found in bones, with evidence suggesting that for adolescent girls early bone mass accretion and accumulation is associated with the Tanner stage. This is also supported by the osteoregulatory effects that estrogens impose on mechanical loading. In other words, sex hormones can change bone setpoint mechanostat in girls [6, 21, 29]. This interpretation aligns with the recent observation that in Tanner stage 5, height growth has a more pronounced

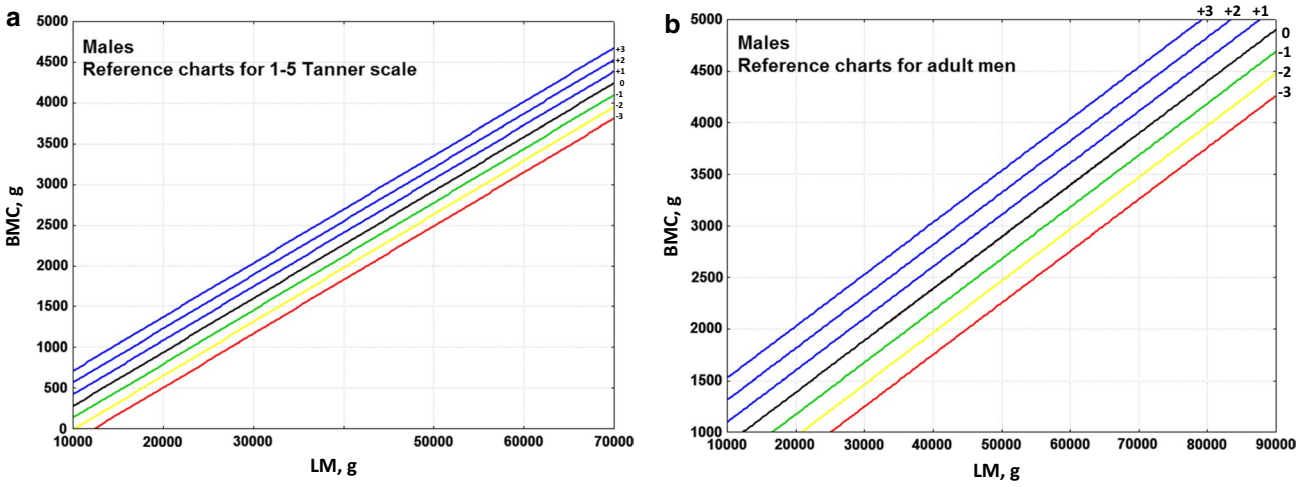

Fig. 2 *Z*-score charts of the BMC-LM relationships for all the male individuals studied calculated as per the corresponding SD's of the data shown in Fig. 1a, b. Graphs show the regression line (black) and the +1, +2, +3 (blue), −1 (green), −2 (yellow) and −3 (red) SD limits around the curve for graphic estimation of the corresponding *Z*-scores. Charts are given for all the studied boys at Tanner stages 1–5 together **(a)**, and for adult men **(b)** (color figure online)

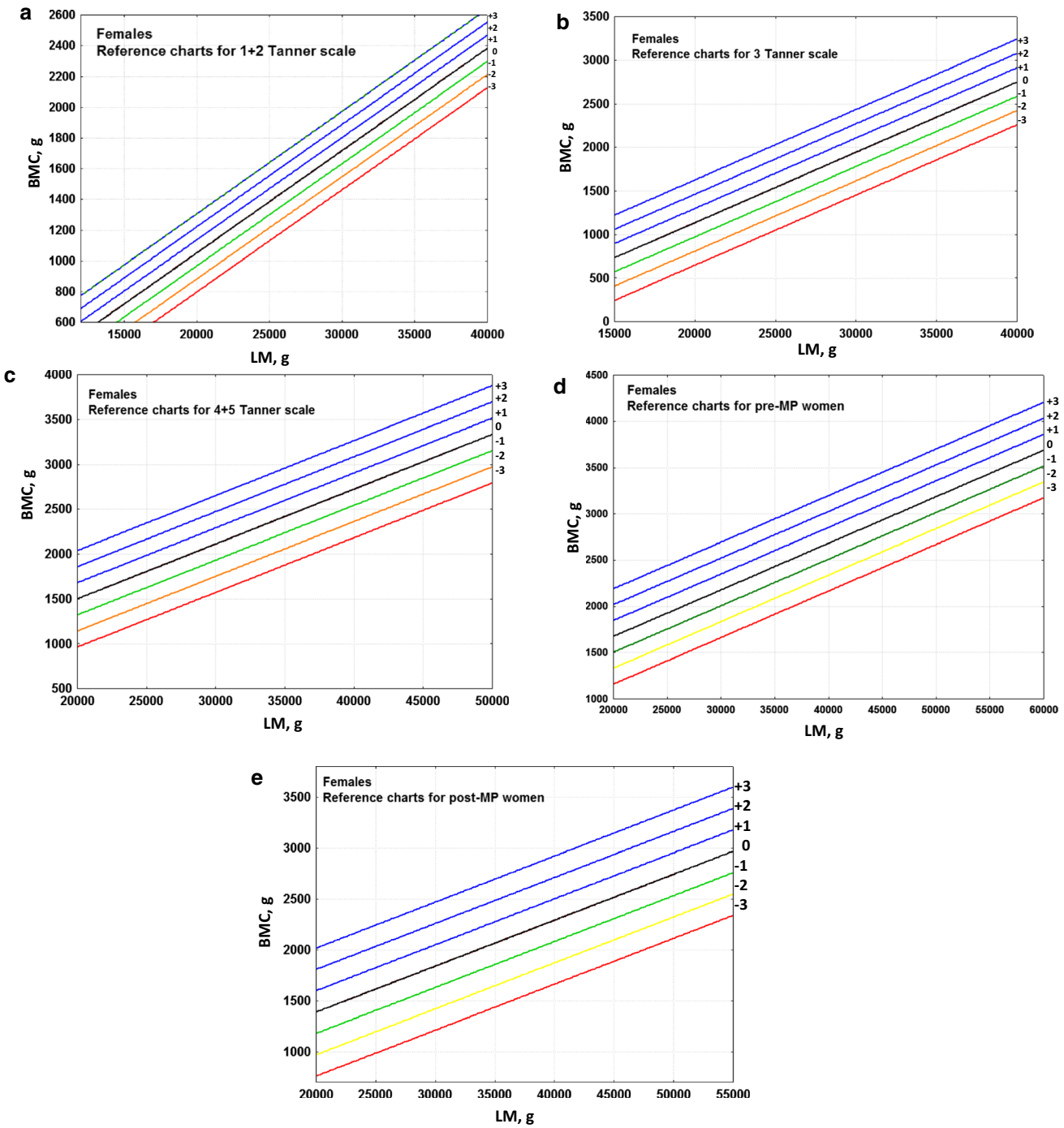

Fig. 3 *Z*-score charts of the BMC-LM relationships for all the female individuals studied calculated as per the corresponding SD's of the data shown in Fig. 1c. Graphs show the regression line (black) and the +1, +2, +3 (blue), −1 (green), −2 (yellow) and −3 (red) SD limits around the curve for graphic estimation of the corresponding *Z*-scores. Charts are given for the sub-groups of girls at Tanner stages 1-2 **(a)**, 3 **(b)** and 4-5 **(c)**, premenopausal (d) and postmenopausal women **(e)** (color figure online)

effect on bone accretion than at the beginning of puberty [30]. On the other hand, the slope for boys is similar for Tanner 1–5 and slightly different from adults. In this sense, Weeks [31] found that maturity has a greater influence on bone mass than physical activity in girls but not in boys, in whom physical activity has a larger influence than pubertal development. Additionally, Macdonald and colleagues [32] reported that geometric measure of the tibia in early pubertal children (aged 9 − 11) was significantly and positively correlated with physical activity in boys, but not girls; whereas in

girls, this measure was significantly and positively correlated with maturity, but not for boys. From a practical perspective, this study points out that Tanner stage influence varies in the BMC-LM ratio in girls but not in boys, which maintains the same relationships through puberty. This different behavior in girls and boys, coinciding with other studies [31, 32], could be important for the establishment of different reference charts for both genders, depending on Tanner stages in girls and independent of Tanner stages in boys.

The BMC–LM intercept was significantly higher for men than for boys Tanner 1-2 stage and even much higher for premenopausal women than for girls Tanner 1-2 stage. The slopes of the curves were similar for the men and boys Tanner 1-2 groups; whereas, for premenopausal women, they were significantly lower than for the girls Tanner 1-2 group. A similar pattern was previously observed [6] as derived from the well-known, estrogen-induced inhibition of negative-balance remodeling and possibly also by a shift in the "mechanostat" setpoint for strain sensing by osteocytes [29, 30] in girls and the skeletal impact of the androgen-induced enhancement of periosteal growth and muscle force development in boys [18].

The BMC–LM relation was observed to be fairly constant within the men's group. In contrast, in postmenopausal women, the intercept (not the slope) of the curve sloped down in a similar way as the curve of girls Tanner 1-2 stage. In agreement with all of the above, the age-related evolution of the BMC-LM ratio highly suggests that fertile women have a large reserve of bone mass per unit of muscle mass than men until menopause. Whether this fact reflects a selective condition of female skeletons to protect some bone mass from bone resorption (about 300–400 g of BMC as per this study, with similar values reported previously [6]), over the male bone–muscle relation to be eventually passed to the offspring is an open question [6, 33].

From a practical point of view, the relation observed between BMC and LM in this study, as well as the differences between slopes and intercepts of the curves amongst sexes, highlights the importance of standardized (*Z*-scored) charts of the corresponding BMC–LM correlations, to be able to evaluate individuals separately by sex and reproductive status. Such charts were performed separately for boys (disregarding Tanner stage) and for girls (separately for Tanner stages 1–5, according to the discussed results), men, and premenopausal and postmenopausal women. Individual *Z*-scores of the BMC–LM relation can be easily determined graphically from these charts. A more precise calculation of the *Z*-score can be achieved by the application of a computer-aided algorithm that can be easily introduced into software specifically designed for this function.

The BMC–height curves show similar patterns to those describing the BMC–LM relation in relation to the way Tanner 3 and 4-5 stages reflect the occurrence of pubertal growth spurts on mineral accumulation in bones among boys and girls in parallel with the development of the biomechanical muscular system. In this case, the higher curve slope for Tanner 3 and 4-5 groups in both sexes when compared to all other groups suggests that the faster the growth, the larger the proportion of cortical–trabecular bone accretion on the skeleton. This condition would also retake the normal proportions after completion of the pubertal development. The relation between BMC and body weight show slopes that are significantly steeper (exponential relationships) than those observed between the BMC and FM (logarithmic associations) with a quicker trend to saturation, already described in previous studies [6, 10]. Since we excluded obese individuals, we included both muscle and bone masses on our calculation of body mass. At any rate, there is a visible trend to reach very low BMC values at low values of either FM or body weight in this study similar to results reporting significant low values of body mass index as predictors of fracture risk in other studies with large adult samples.

It is recognized that cross-sectional studies contain inherent design limitations. Furthermore, for logistical reasons, it was necessary to rely on indirect measures of some variables, including Tanner stage. We assumed that LM is a reasonable surrogate for muscle mass but this is not without error [34]. We have assumed that ''muscle mass'' assessed as previously described is representative of the corresponding muscle strength. However, this is not always true, but it does not affect the interpretation of the observed relations in this study. The interpretation and derivations of our results can only be interpreted for non-obese individuals. We should also consider the characteristics of the population we evaluated including the many nutritional and metabolic factors that were shown to affect bone health in this population as well as the fact that we disregarded known neuronal factors which control both bone and muscle. Results may also depend on the type of DXA equipment employed. Our data could only be compared with samples obtained with Lunar-DPX-type devices.

In brief, this study describes the simple relation between BMC–LM among boys and girls (considering their Tanner stage of sexual development), men, premenopausal and postmenopausal women. Additionally, the study provides an original set of *Z*-scored charts of the DXA-assessed BMC–LM relation as normal references for boys and girls (dealing with the different Tanner stages of sexual development where applicable), men, and premenopausal and postmenopausal women. In general, these reference curves provide robust reference values for the assessment and monitoring of bone health and comparative estimations of the participation degree of the mechanical environment in the determination of any osteopenic condition individually.

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Author Contributions The authors' responsibilities were as follows: ED-G and JS designed the study and secured funding; ED-G, RV-C, BR, and JS conducted the research; RFC, LMN, JLF, and GRC performed the statistical analyses; EDG and GRC wrote the manuscript; EDG, PC, JLF, and GRC critically reviewed the manuscript. All authors reviewed and commented on the manuscript. All authors read and approved the final version of the paper.

Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest.

Statement of human and animal rights The present study was conducted according to the Declaration of Helsinki guidelines. The ethics and research committees of all participating institutions [Comité de Ética e Investigación, Instituto Mexicano del Seguro Social (No. 12CEI0900614); Comité de Ética e Investigación, Instituto Nacional de Salud Pública (No.13CEI1700736); Comité de Ética, Centro de Investigación en Ciencias Médicas (No.1233008X0236)] reviewed and approved the study protocol and informed consent forms.

Informed consent Signed written informed consent was previously obtained from all participants.

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