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Sex differences in the longitudinal associations between body composition and bone stiffness index in European children and adolescents

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Abbreviations

BMI: body mass index

FM: fat mass

FFM: fat free mass

BF: body fat

SI: stiffness index

QUS: quantitative ultrasound

BUA: broadband ultrasound attenuation

BMD: bone mineral density

DXA: dual-energy X-ray absorptiometry

pQCT: peripheral quantitative computed tomography

SOS: speed of sound

CV_{RMS}: root-mean-square coefficient of variation

BIA: bioelectrical impedance analysis

SES: family socioeconomic status

ISCED: International Standard Classification of Education

SB: sedentary behaviors

PA: physical activity

SD: standard deviation

CI: confidence interval

BMC: bone mineral content

CRP: C-reactive protein

Abstract

Fat mass (FM) and fat free mass (FFM) may influence bone health differentially. However, existing evidences on associations between FM, FFM and bone health are inconsistent and vary according to sex and maturity. The present study aims to evaluate longitudinal associations between FM, FFM and bone stiffness index (SI) among European children and adolescents with 6 years follow-up. A sample of 2468 children from the IDEFICS/I.Family was included, with repeated measurements of SI using calcaneal quantitative ultrasound, body composition using skinfold thickness, sedentary behaviors and physical activity using self-administrated questionnaires. Regression coefficients (β) and 99%-confidence intervals (99%CI) were calculated by sex-specified generalized linear mixed effects models to analyze the longitudinal associations between FM and FFM z-scores (zFM and zFFM) and SI percentiles, and to explore the possible interactions between zFM, zFFM and maturity. Baseline zFFM was observed to predict the change in SI percentiles in both boys (β= 4.57, 99%CI: 1.36, 7.78) and girls (β= 3.42, 99%CI: 0.05, 6.79) after 2 years. Moreover, baseline zFFM (β= 8.72, 99%CI: 3.18, 14.27 in boys and β= 5.89, 99%CI: 0.34, 11.44 in girls) and the change in zFFM (β= 6.58, 99%CI: 0.83, 12.34 in boys and β= 4.81, 99%CI: -0.41, 10.02 in girls) were positively associated with the change in SI percentiles after 6 years. In contrast, a negative association was observed between the change in zFM and SI percentiles in boys after 6 years (β= -3.70, 99%CI: -6.99, -0.42). Besides, an interaction was observed between the change in zFM and menarche on the change in SI percentiles in girls at 6 years follow-up (p= 0.009), suggesting a negative association before menarche while a positive association after menarche. Our findings support the existing evidences for a positive relationship between FFM and SI during growth. Furthermore, long-term FM gain was inversely associated with SI in boys, whereas opposing associations were observed across menarche in girls.

Keywords Pediatrics, body composition, bone stiffness index, sex differences, longitudinal study

1 Introduction

Effects of body composition on bone development are of increasing interest recently. In adulthood, adiposity serves as a protective factor against osteoporotic fractures [1, 2], whereas studies investigating the effect of adiposity on bone growth in children and adolescents still appear to be diverse [3]. In previous pediatric studies, the most widely used category of excess adiposity is body mass index (BMI). However, BMI cannot distinguish between fat mass (FM) and fat free mass (FFM), which are contributors to weight status and both of them have a mechanical loading on bone growth. Meanwhile FM may have both negative and positive effects on bone mass accrual mediated via endocrine pathways [4]. Childhood and adolescence present a particularly critical stage for bone growth, understanding the impact of body composition on bone health is important when planning prevention strategies towards fracture and osteoporosis in later life.

The effect of FM on bone strength is considered controversial, diverging results were reported in different sex, age and pubertal groups. For example, positive associations between FM and bone strength were observed in some studies among younger children, i.e. infants and pre-school children [5-7]. In contrast, Pollock et al. [8] found that percentage of body fat (BF, %) was inversely related to bone strength indexes in late adolescent females. However, Farr et al. [9] observed that total body FM was not cross-sectional associated with all bone strength parameters, while positively associated with 2 years changes of bone strength and density at weight-bearing site in 8 to 13 years old girls [10]. For now, the role of FM on bone strength during growth remains unclear.

The acquisition of bone strength is significantly influenced by the muscle function, and the positive associations between the muscle parameters (e.g. FFM, lean mass, skeletal muscle mass etc.) and bone strength have already been demonstrated in various cross-sectional [11, 12] and prospective studies [13]. In the IDEFICS study (Identification and prevention of dietary- and lifestyle induced health effects in children and infants) with children from 2 to 9 years old, muscular fitness and FFM were found to be positively associated with bone stiffness index (SI) measured using calcaneal

quantitative ultrasound (QUS) [14]. Another cross-sectional study among adolescents reported positive associations between lean mass and other QUS parameters such as broadband ultrasound attenuation (BUA) [15]. However, the associations between FFM and bone strength may also be sex-dependent during growth [16], and few studies have reported the association of FM on bone strength in children and adolescents while taking FFM into consideration. Some studies found that the associations among the FM and bone mass might be attenuated even reversed after adjusting for lean mass [9, 17, 18]. We also previously demonstrated in the IDEFICS study that primary school children with higher BF had higher calcaneal SI, but after adjusting for FFM, this relationship turned to be inverse [19]. Therefore, it is still important to clarify the independent effects of FFM and FM on bone strength development.

Some sophisticated methods such as dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) have been shown to be useful measurements for bone parameters. However, these methods appear to be not suitable for large-scale studies among healthy children and adolescents. In this context, QUS as an alternative method was applied in the IDEFICS/I.Family study, which is gaining popularity because of its quick, cheap and non-radiating characteristics [20-22]. Calcaneal QUS has shown good correlation with DXA measurements [22] and has been suggested as an important indicator in determining fracture risk in adults [21, 23]. Previous studies that compared calcaneal SI with bone mineral density (BMD) measured by DXA of whole body, lumbar spine and hip among children and adolescents also reported significant correlation coefficients range from 0.5 to 0.7 [24-26]. Besides, QUS measurements provided good precision for the risk of osteopenia in young patients [24]. To our best knowledge, there are no studies addressing the role of body composition on QUS measured bone parameters in children and adolescents age from 2 to 15 years old using a large longitudinal multi-country cohort. In order to extend the current understanding between body composition and bone strength during growth, we

aimed to conduct a prospective analysis to evaluate relationship of changes in FM, FFM and SI, and to estimate whether these associations differ from sex and maturity.

2 Materials and Methods

2.1 Study sample

Data for the present longitudinal investigation was obtained from the IDEFICS/I.Family studies. Briefly, the aim of the IDEFICS study was to investigate dietary and behavioral disorders in young children, mainly focusing on overweight and obesity. The baseline data were collected within 16229 children aged 2 to 9.9 years old between September 2007 and June 2008 in eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden). The first follow-up examinations were performed between September 2009 and June 2010. The further follow-up examinations were performed between January 2013 and June 2014 in context of the I.Family study including 7117 children from the original IDEFICS cohort, to further explore the familial characteristics related to children's health development. All the examinations were conducted according to the Declaration of Helsinki. Parents gave written informed consent prior to study participation and children gave oral or signed simplified consent prior to the examinations. All participating centres have obtained ethical approval from the regional committees. Other details regarding study design have been published previously [27, 28].

As an optional examination module, approximate 50% of children in the IDEFICS study participated in the calcaneal QUS examination, and 5 of 8 participating countries with approximate 30% of children and adolescents in the I.Family study participated. In the present longitudinal analyses, 3422 participants who had baseline and at least one follow-up QUS measurements were included. According to a previous reliability study which compared the SI measurements across QUS devices among a convenience sample (N=91), a significant discrepancy was observed between the devices for the absolute SI difference of the left and the right foot (unpublished data). Hence, we excluded the sample with absolute difference of SI value above 97th percentile (41 unit) between the right and

left foot to control the discrepancy (N=200). Moreover, parents were asked to report a health and medical history questionnaire, whose answers were used to exclude children from the analysis with a history of medical condition known to affect bone metabolism or limit physical exercise (N=43) [14]. Furthermore, 711 participants had to be excluded because of incomplete data of body composition or covariates, leaving a total of 2468 children for the final analysis. Given these restrictions, no children from Cyprus remained in the final analysis sample.

2.2 Bone SI

The calcaneal QUS was used in the IDEFICS/I.Family cohort with Achilles Lunar Insight TM (GE Healthcare, Milwaukee, WI, USA), which had previously been described in details [19]. The calcaneus, as a weight-bearing skeletal site and consists of 90% trabecular bone, is the most common used measuring site. Two parameters were measured by calcaneal QUS: broadband ultrasound attenuation (BUA, dB/MHz), which represents the spatial orientation of the bone trabeculae and increases with greater trabecular complexity; the speed of sound (SOS, m/s), which represents the velocity of sound traveling through the bone and increases with greater structures density [29]. SI was automatically calculated from BUA and SOS by the device and expressed as 'unit' according to the equation: SI = (0.67*BUA) + (0.28*SOS) – 420. Measurements were performed by trained nurses following standardized procedures, two adaptors were used for different foot size. Both of the left and right feet were measured once at each of three time points. According to a previous reliability study which examined the reproducibility of SI measurements among 60 children from the baseline survey of the IDEFICS study, the root-mean-square coefficient of variation (CV_{RMS}) on the left foot and right foot were 7.2% and 9.2%, respectively. Besides, no significant difference was observed of repeated SI measurements compared to the first measurement in children (unpublished data). In the present study, the mean of two SI for each foot was used for analysis. For each individual, the SI percentiles were calculated additionally according to age, sex and height based on the IDEFICS/I.Family reference population [30].

2.3 Body composition

FM (kg) was estimated by skinfold thickness based on Slaughter's equations, which are most commonly used for population-based studies in children and adolescents, and showed the reliable results for the assessment of BF [31, 32]. FFM (kg) was used as an indicator of skeletal muscle mass, which was calculated by the equation FFM = Body weight - FM. The Tanita scales (BC420 MA for children and BC418 MA for adolescents, TANITA Europe GmbH, Sindelfingen, Germany) were used to measure body weight (kg) to the nearest 0.1 kg in light clothes without shoes. Skinfold thickness (mm) was measured at subscapular and triceps according to the international standards for anthropometric assessment [33]. Subscapular was measured about 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body, and triceps was measured halfway between the acromion and the olecranon process at the back of the arm. Measurements were obtained twice at each site to the nearest 0.2 mm with a skinfold calliper (Holtain, Crosswell, UK; range 0–40 mm). The mean of the two measurements was calculated and used for later analyses. All the measurements were performed by well-trained field staffs, standard operation procedures were pre-tested in each participating centre for their feasibility and acceptability before the baseline survey [34]. The intra and inter- observer reliability of skinfold thickness was considered within an acceptable range in the IDEFICS [33] and I.Family validation studies (unpublished data). In the present analysis, FM and FFM age- and sex-specific z-scores (zFM and zFFM) were derived based on the IDEFICS/I.Family reference population [35].

In the exploratory phase of the study, the indicators of FM and FFM measured using the Tanita scales were also taken into consideration. However, according to the IDEFICS validation study, the explained variances of skinfold measurement were found to be slightly higher than the bioelectrical impedance analysis (BIA) [36, 37]. Besides, in a subsample of young obese children, skinfold estimate rather than BIA estimate was found to be positively correlated with BF(%) measured using

DXA and BodPod (unpublished results). Hence this part of results was not included in the final analyses.

2.4 Self-assessment maturational status

Menarche in girls and voice change in boys were used as indicators of maturation [38], which have been found to occur around Tanners pubertal stages 3 and 4 [39, 40]. Menarcheal age is widely used in epidemiological studies to provide sexual maturational information in female [41], and voice change as a proxy for male maturity has been related to anthropometric growth [42]. In the I.Family study, boys and girls above 8 years old were instructed either by the study nurse or physician to self-report their maturity using a sex-appropriate one-page questionnaire.

2.5 Confounding variables

The age and sex of children as well as family socioeconomic status (SES) were obtained by one of the parents from a self-administered proxy-questionnaire. SES was estimated by the maximum of parental education based on International Standard Classification of Education (ISCED), levels 0 to 2 were defined as low and level 3 to 4 were defined as medium while level 5 and 6 were defined as high [43]. In addition, parents of children up to 11 years and 12 to 15 years old adolescents completed a questionnaire to assess sedentary behaviors (SB) and physical activity (PA) of the child, by reporting the weekly duration of total screen time (including watching/TV/videos/DVDs and playing computer/game) and participating in sports clubs. Height (cm) was measured by stadiometer (SECA 225, Seca GmbH & KG, Birmingham, UK) to the nearest 0.1 cm without shoes, and age- and sex- specific z-scores of height were calculated using the LMS method by Cole [44]. Considering the sun exposure is associated with vitamin D synthesis and further may influence the bone mass accrual [45], we calculated mean daylight duration (±0.1 h) at baseline for each examination month in each location using astronomical tables [14], as a proxy for the child exposure to sunlight.

2.6 Statistical analyses

All statistical calculations were performed using SAS software (V9.3; SAS Institute Inc, Cary, North Carolina, USA). Simple descriptive statistics (means, standard deviations (SD), and frequencies) at baseline and twice follow-up were presented by cross-classified tables, stratified by sex. The changes of all dependent and independent variables were calculated as the within-individual difference between a follow-up measurement and the corresponding baseline measurement.

Sex-specific generalized linear mixed effects models were used to analyze the longitudinal relationship between body composition and SI percentiles, with country as a random effect (at the level of the intercept) to take into account the cluster sampling design. Meanwhile, age (continuous variable), SES, daylight duration, SI percentiles and height z-score at baseline as well as change in height z-score were included as fixed terms, while maturational status was only available at 6 years follow-up. The outcomes were changes in SI percentiles after 2 years and 6 years, and the exposures in terms of zFM and zFFM were considered as both baseline covariates and change covariates. In model 1 and model 2, zFM and zFFM were included in the models separately, and then were included simultaneously in model 3. In order to further explore whether the associations between body composition z-scores and SI percentiles were influenced by SB and PA, we additionally adjusted for the average duration of SB and PA in model 4, which were derived from the means of baseline and corresponding follow-up value. In all models, means of parameter estimates (β) and 99%-confidence intervals (99%CI) were calculated. To avoid that meaningless associations become statistically significant (just because of the large sample size), we carried out multiple tests of associations with choosing a more stringent criterion for statistical significance ($\alpha = 0.01$).

Moreover, interaction effects were analyzed between body composition and maturity based on model 3. Possible interactive effects were stratified by maturity when statistically significant.

3 Results

3.1 Baseline and follow-up descriptive characteristics

Among 2468 participants who were included in the study, 1274 (51.6%) were boys, and the average age in boys and girls were 6.23 and 6.39, respectively. Of these, 2144 individuals provided full information after 2 years and 833 individuals provided after 6 years. 42.5% of boys reported having voice change and 40.1% of girls reported having first menstrual period at 6 years follow-up (Table 1). We further conducted the attrition analysis regarding main demographic characteristics (i.e. sex, age and family SES) between the participants who were included in each follow-up analytic sample and the non-participants who took part in the QUS module at baseline but didn't provide follow-up and/or complete co-variate information (N=5071). Overall, there was no significant difference for sex and SES after 2 years, while the participants (6.44±1.69) were older than the non-participants (5.96±1.82, $p< 0.001$). Meanwhile, no significant difference was found for sex and age after 6 years, while more participants were defined as low (10.4%) and medium level SES (67.2%) than the non-participants (9.2% and 51.0%, respectively, $p< 0.001$).

At baseline, the mean SI was 78.09 ± 12.41 in boys and 77.24 ± 12.86 in girls. Boys had a slightly higher FFM (19.74 ± 4.74) while lower FM (4.14 ± 2.94) compared to girls (19.20 ± 4.53 and 4.73 ± 3.03, respectively). The mean height of boys (119.40 ± 12.55) and girls (119.40 ± 12.50) were similar. All the anthropometric measurements showed comparable increasing trends in both sexes over 2 years and 6 years periods (Table 1)

3.2 Longitudinal effects of body composition z-scores on changes in SI percentiles

As presented in table 2, the baseline zFFM positively predicted the change in SI percentiles in both boys and girls after 2 years (Model 1), these positive associations persisted after adjustments of baseline and change in zFM in model 3 (β= 4.57, 99%CI: 1.36, 7.78 in boys and β= 3.42, 99%CI: 0.05, 6.79 in girls, respectively). Meanwhile, the baseline zFM tended to be positively related to change in SI percentiles in both sexes (Model 2), these associations were reversed, however still not statistically significant, after taking zFFM into consideration in model 3 (β= -1.18, 99%CI: -3.02, 0.66 in boys and β= -0.14, 99%CI: -2.00, 1.72 in girls, respectively). Additional adjustments of SB

and PA in model 4 only resulted in a slight decrease in the effect sizes compared to model 3, whereas remained nearly unchanged.

In table 3, the baseline zFFM was also observed to positively predict the changes in SI percentiles in both sexes after 6 years (model 1). These results were also valid when additionally adjusted for zFM in model 3 (β= 8.72, 99%CI: 3.18, 14.27 in boys and β= 5.89, 99%CI: 0.34, 11.44 in girls). Besides, a positive association between changes in zFFM and SI percentiles were also observed in boys (β= 6.58, 99%CI: 0.83, 12.34), similar but not statistically significant association also can be seen in girls (β= 4.81, 99%CI: -0.41, 10.02) in model 3. Likewise, additionally adjusting for SB and PA in model 4 nearly did not change these associations. On the contrary, the positive effect of baseline zFM on change in SI percentiles in model 2 was attenuated in girls (β= 1.42, 99%CI: -1.72, 4.56) and reversed in boys (β= -0.20, 99%CI: -3.55, 3.14) after adjusting for zFFM in model 3. Moreover, a negative association between change in zFM and SI percentiles was observed in boys (β= -3.70, 99%CI: -6.99, -0.42), whereas the effect estimate decreased and became statistically insignificant in model 4.

3.3 Interactive effects between body composition z-scores and maturational status on changes in SI percentiles

Different estimates were found in girls when investigating possible interactions, resulting in a p-value of 0.009 for the interaction term of change in zFM and menarche. A negative association between change in zFM and SI percentiles was observed in girls before menarche (β= -1.81, 99%CI: -5.91, 2.30, p= 0.254), whereas a positive association was observed in girls after menarche (β= 3.46, 99%CI: -1.55, 8.47, p= 0.074), these associations however were not statistically significant (Fig. 1). Besides, tests for interaction between body composition and voice change among boys were not statistically significant.

4 Discussion

Overall, we investigated the 2 years and 6 years longitudinal associations between zFM, zFFM and SI percentiles in 2 to 15 years old children and adolescents. Our findings added to the existing evidences that FFM was a significant determinant of bone stiffness development in both sexes. Specifically, baseline zFFM was identified as a positive predictor of change in SI percentiles during 2 years and 6 years follow-up periods. Furthermore, change in zFFM was positively associated with change in SI percentiles after 6 years. These associations were more pronounced in boys compared to girls, and were independent of PA and SB level. In contrast, detrimental effect on bone stiffness accrual may occur with long-term FM increase in boys, whereas the association between the change in FM and bone stiffness in girls depends on maturational status, suggesting a negative association before menarche while a positive association after menarche.

The positive relationship between FFM and bone strength during growth has been well described, most of these bone-related indicators were measured by DXA or pQCT: A number of cross-sectional studies described positive associations between lean mass and weight-bearing bone mass, geometry and architecture in male and female children and adolescents [12, 18, 46]. Few longitudinal studies also suggested that lean mass was a significant predictor of bone strength, and change in lean mass was positively related to change in bone strength [47-49]. Apart from previous findings from the IDEFICS study, there is only a few cross-sectional studies reported the correlation between body composition and calcaneus QUS parameters. For example, in a population of Spanish school children aged 4 to 16 years, FFM were observed positively related to BUA in the calcaneus [50]. In another sample of Malaysian adolescents aged 15-17 years, lean mass was reported to be positively associated with calcaneus BUA [15]. The present study allows an extension to the relatively few longitudinal studies, and adds to the weak evidence that QUS measurements are meaningful for bone development in children and adolescents.

Exploring independent effects of FFM and FM on bone strength are important. There is a consensus that the stimulatory effect exerted by body weight is mainly explained by FFM rather than FM.

Findings from several cross-sectional studies supported this conclusion. For example, FM has been shown to be positively correlated to bone strength, while negative associations were observed when lean mass or body weight was included [8, 9, 17, 18]. One study investigated the role of FM and BF% simultaneously and observed an opposite direction of these two parameters in bivariate correlation of cortical bone parameters at the tibia and radius [8]. In a longitudinal pathway analysis, they found the positive association between BMI at 11 years old and whole body bone mineral content (BMC) and bone mineral density (BMD) at age 18 years old was largely mediated by FFM but not FM at age 18 in both female and male adolescents [51]. Our results were consistent with these studies, suggesting a robust and independent effect of FFM on bone stiffness, whereas the potentially predicting effect of FM was attenuated and even reversed after taking FFM into account.

Previous studies have shown that sex differences in body composition and bone are emerging during puberty [52]. On the one hand, we observed the estimate effect of FFM on SI was higher in boys compared to girls after 2 years, and this discrepancy was even more pronounced at 6 years follow-up, which about 40% of participants were considered as in maturity. Findings from a cross-sectional study among 10 to 17 years old healthy children also suggested that the contribution of lean mass to BMC variance was 6–12% in boys, which was larger than 4–10% in girls [53]. These sex differences may partially be explained by the greater FFM and bone size in boys than in girls [54], which may lead to a stronger impact of FFM on bone growth in boys. One the other hand, existing evidences reached contradictory conclusions in the relationship between fat and bone strength across sex groups. For example, Kim et al. [55] found FM was negatively related to total-body-less-head BMD in boys, but was positively associated with BMD of the lumbar spine and femur neck in girls (12 to 19 years old). On the contrary, Zulfarina et al. [15] found FM was negatively associated with SI in 15-17 years old female adolescents rather than male. Further, Sayers et al. [56] found positive associations between FM and BMC in cortical bone geometry, while these associations were considerably stronger in girls compared to boys, whom were defined as pubertal adolescents in

Tanner stages 4 or 5. For now, there is still no consensus in the association between FM and bone strength in male and female during growth, future work should continue to explore the potential mechanisms in sex differences to enhance our knowledge.

Several mechanisms could explain unfavourable changes in bone stiffness after long-term FM increase in boys in our results. Adipose tissue may regulate bone metabolism through exerting adipokines [57], and evidences suggested that adiponectin was inversely related to BMD in childhood and adolescence [58]. Meanwhile, leptin may stimulate osteoblast activity and inhibit osteoclast activity, resulting in increased bone formation and decreased bone resorption [59]. Moreover, adiposity was associated with inflammatory cytokines, and C-reactive protein (CRP) has been related to BMD in healthy adults [60]. However, we didn't find associations or modified effects of CRP in our subsample. Further studies are still needed to clarify the impact of various biological functions of adiposity on bone strength accrual.

Even though we didn't observe any association between FM and bone stiffness in girls, an interaction between menarche and FM gain were observed from our 6 years follow-up data. These results were similar with longitudinal findings from Wey et al. [49], who also found an interaction of FM gain with menarche in females, with the negative associations between FM and total BMC and BMD only existing before menarche. Clark et al. [61] also reported the altered effects of baseline FM on 2 years gain in bone mass and size across different pubertal status, suggested a positive association at Tanner stage 1, no association at stage 2, and a negative association at stage 3 in girls. Hence, it cannot be assumed that relationship between fat and bone strength remains constant over the pubertal status in females. A possible explanation may be attributed to the influence of the rising sexual hormones such as estrogen on bone mass acquisition during puberty, thereby modify the effect of FM on bone metabolism.

Some limitations must be acknowledged in the present study. The major weakness of the study was that body composition was not measured using DXA, which was not feasible in such large-scale

cohort among children and adolescents. Instead, skinfold thickness as the best alternatives was used in the present study. The Tanita scale was also used in our study to measure leg-to-leg bioelectrical impedance (ohm) and the Tyrrell formula was used to calculate the FFM (kg) and BF (%) [62]. In order to further clarify our findings, we performed sensitivity analyses with FFM and BF, and found similar results regardless of the technique used. Besides, we could not consider potential confounders such as calcium intake or vitamin D status. Instead, a proxy variable of consumption frequency of milk and dairy products was used for further adjustment, which did not influence the results. Hence we didn't consider this variable in our final analysis in order to not reduce the sample size. Furthermore, no information on maturity at 2 years follow-up was available, and the information at 6 years follow-up was measured by voice change for boys and first menstrual period for girls rather than the Tanner stages. Therefore the interaction effect between body composition and puberty on bone stiffness cannot be further evaluated in the present study. Finally, it is worth to mention the limitation of prospective cohort studies with decreasing sample size over a long follow-up period. In the present study, only 33.4% of the initial baseline cohort with QUS measurements provided follow-up data and complete co-variable information. The differences on some demographic characteristics may cause a possible selective bias. However, our results suggested robust associations of baseline as well as change in body composition with SI percentiles, and these longitudinal associations showed to be stable over 2 and 6 years periods.

5 Conclusions

Our findings highlight the importance of FFM for optimizing bone stiffness during growth. Furthermore, deleterious effect on bone stiffness may occur after relatively long-term exposures to FM gain in boys, while the effect of FM on bone stiffness seems to be opposing across menarche in girls. Future bone health intervention program in children and adolescents should focus on promoting body composition instead of weight status, particularly differences of sex and maturity also should be taken into consideration.

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Declarations of interest

None

Author contributions

The authors roles are as follow: L.C., W.A. and A.H. conceptualized and designed the study. L.C. conducted the initial analysis and wrote the original draft. H.P. and A.H. assisted with data analysis and interpretation. P.R., T.V., C.C., D.M., G.E., S.D.H., L.M., A.P. contributed to coordination and data collection. All authors revised and improved the manuscript, and approved the final manuscript.

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Table 1. Descriptive characteristics for participants at baseline, 2 years and 6 years follow-up, stratified by sex

	Baseline (N=2468)		2 years follow-up (N=2144)		6 years follow-up (N=833)	
	Boys	Girls	Boys	Girls	Boys	Girls
	N=1274	N=1194	N=1112	N=1032	N=402	N=431
Age (Mean, SD)	6.23(1.75)	6.39(1.75)	8.35(1.70)	8.50(1.68)	11.90(1.78)	12.00(1.79)
Socioeconomic status (N, %)						
Low	110(8.6)	119(10.0)	89(8.0)	101(9.8)	42(10.5)	45(10.4)
Medium	742(58.2)	639(53.5)	630(56.7)	530(51.4)	275(68.4)	285(66.1)
High	422(33.1)	436(36.5)	393(35.3)	401(38.9)	85(21.1)	101(23.4)
Maturational status (N, %)*						
Pre- or early mature	/	/	/	/	231(57.5)	258(59.9)
Mature	/	/	/	/	171(42.5)	173(40.1)
Country (N, %)						
Belgium	176(13.8)	139(11.6)	176(15.8)	139(13.5)	/	/
Estonia	222(17.4)	202(16.9)	154(13.9)	135(13.1)	126(31.3)	121(28.1)
Germany	356(27.9)	352(29.5)	298(26.8)	285(27.6)	143(35.6)	180(41.8)
Hungary	86(6.8)	78(6.5)	86(7.7)	78(7.6)	/	/
Italy	244(19.2)	231(19.4)	218(19.6)	212(20.5)	100(24.9)	96(22.3)
Spain	90(7.1)	85(7.1)	80(7.2)	76(7.4)	33(8.2)	34(7.9)
Sweden	100(7.9)	107(9.0)	100(9.0)	107(10.4)	/	/
Anthropometric measurements (Mean, SD)						

Bone stiffness index	78.09(12.41)	77.24(12.86)	82.24(13.46)	82.14(12.95)	88.88(14.56)	90.26(15.76)
Bone stiffness index percentiles	43.19(27.50)	42.05(26.80)	46.32(28.81)	47.21(28.04)	50.39(28.11)	53.34(28.21)
Fat free mass (kg)	19.74(4.74)	19.20(4.53)	24.63(5.23)	24.03(5.20)	36.61(8.91)	35.13(7.83)
Fat free mass z-score	0.23(1.25)	0.25(1.27)	0.25(1.19)	0.19(1.27)	0.18(1.09)	0.25(1.25)
Fat mass (kg)	4.14(2.94)	4.73(3.03)	6.19(5.07)	6.90(4.49)	10.78(7.64)	12.14(6.94)
Fat mass z-score	0.33(1.48)	0.48(1.58)	0.42(1.53)	0.56(1.66)	0.72(1.39)	0.69(1.56)
Height (cm)	119.40 (12.55)	119.40(12.50)	132.60(11.42)	132.60(11.36)	154.10(12.42)	153.00(11.48)
Height z-score	0.54(1.03)	0.45(1.04)	0.58(1.05)	0.49(1.00)	0.69(1.00)	0.58(1.11)
Reported healthy behaviors (Mean, SD)						
Duration of screen time (hours/week)	12.17(7.47)	10.62(6.38)	14.52(7.83)	12.60(6.96)	19.85(11.80)	13.98(8.56)
Duration of sports clubs (hours/week)	1.33(1.66)	1.39(1.77)	2.09(1.92)	2.10(2.25)	2.73(2.38)	2.41(2.72)

** Menarche in girls and voice change in boys were used as indicators of maturation*

Table 2. Associations between body composition z-scores and change in bone stiffness index percentiles after 2 years, stratified by sex

	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value
Boys (N=1112)								
Baseline fat free mass z-score	3.44(0.69,6.19)	0.001	/	/	4.57(1.36,7.78)	<0.001	4.35(1.14,7.56)	0.001
Change in fat free mass z-score	2.11(-2.05,6.28)	0.191	/	/	2.94(-1.35,7.24)	0.077	2.78(-1.52,7.07)	0.096
Baseline fat mass z-score	/	/	0.14(-1.43,1.72)	0.812	-1.18(-3.02,0.66)	0.097	-1.06(-2.90,0.77)	0.136
Change in fat mass z-score	/	/	-1.02(-3.63,1.58)	0.311	-2.00(-4.71,0.71)	0.057	-1.89(-4.60,0.82)	0.073
Average duration of screen time (hours/week)	/	/	/	/	/	/	0.01(-0.29,0.31)	0.954
Average duration of sports clubs (hours/week)	/	/	/	/	/	/	1.23(-0.12,2.59)	0.019
Girls (N=1032)								
Baseline fat free mass z-score	3.21(0.76,5.66)	0.001	/	/	3.42(0.05,6.79)	0.009	3.19(-0.17,6.56)	0.015
Change in fat free mass z-score	1.35(-2.58,5.27)	0.377	/	/	1.65(-2.47,5.76)	0.302	1.47(-2.63,5.58)	0.354
Baseline fat mass z-score	/	/	1.15(-0.21,2.51)	0.029	-0.14(-2.00,1.72)	0.847	0.05(-1.82,1.91)	0.950
Change in fat mass z-score	/	/	-0.15(-2.60,2.30)	0.875	-0.87(-3.44,1.70)	0.383	-0.60(-3.18,1.99)	0.552
Average duration of screen time (hours/week)	/	/	/	/	/	/	-0.14(-0.47,0.19)	0.283
Average duration of sports clubs (hours/week)	/	/	/	/	/	/	0.97(-0.10,2.05)	0.019

All the models were adjusted for age, socioeconomic status, daylight, bone stiffness index percentiles and height z-score at baseline as well as change in height z-score, country as a random effect

[a] Model 1 only included baseline and change in fat free mass z-score as exposures; [b] Model 2 only included baseline and change in fat mass z-score as exposures; [c] Model 3 included baseline and change in fat free mass z-score as well as fat mass z-score to test their independent associations with bone stiffness index percentiles; [d] Model 4 was Model 3 additionally adjusted for average duration of screen time and sports clubs

Table 3. Associations between body composition z-scores and change in bone stiffness index percentiles after 6 years, stratified by sex

	Model 1		Model 2		Model 3		Model 4	
	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value	β (99%CI)	p-value
Boys (N=402)								
Baseline fat free mass z-score	9.21(4.30,14.11)	<0.001	/	/	8.72(3.18,14.27)	<0.001	8.15(2.62,13.67)	<0.001
Change in fat free mass z-score	5.52(-0.24,11.28)	0.014	/	/	6.58(0.83,12.34)	0.003	5.93(0.21,11.66)	0.008
Baseline fat mass z-score	/	/	1.95(-0.99,4.90)	0.087	-0.20(-3.55,3.14)	0.874	0.11(-3.20,3.42)	0.932
Change in fat mass z-score	/	/	-2.93(-6.25,0.39)	0.023	-3.70(-6.99,-0.42)	0.004	-2.99(-6.29,0.31)	0.020
Average duration of screen time (hours/week)	/	/	/	/	/	/	-0.11(-0.56,0.34)	0.530
Average duration of sport clubs (hours/week)	/	/	/	/	/	/	2.56(0.43,4.69)	0.002
Girls (N=431)								
Baseline fat free mass z-score	7.61(3.67,11.54)	<0.001	/	/	5.89(0.34,11.44)	0.006	5.15(-0.36,10.67)	0.016
Change in fat free mass z-score	4.49(-0.28,9.26)	0.015	/	/	4.81(-0.41,10.02)	0.018	5.34(0.05,10.63)	0.009
Baseline fat mass z-score	/	/	3.62(1.38,5.86)	<0.001	1.42(-1.72,4.56)	0.243	1.99(-1.14,5.12)	0.100
Change in fat mass z-score	/	/	1.47(-1.39,4.33)	0.185	-0.30(-3.49,2.89)	0.809	0.02(-3.14,3.17)	0.988
Average duration of screen time (hours/week)	/	/	/	/	/	/	-0.23(-0.77,0.31)	0.268
Average duration of sport clubs (hours/week)	/	/	/	/	/	/	2.04(0.39,3.69)	0.002

All the models were adjusted for age, socioeconomic status, daylight, bone stiffness index percentiles and height z-score at baseline, as well as change in height z-score and maturity after 6 years, country as a random effect

[a] *Model 1 only included baseline and change in fat free mass z-score as exposures;* [b] *Model 2 only included baseline and change in fat mass z-score as exposures;* [c] *Model 3 included baseline and change in fat free mass z-score as well as fat mass z-score to test their independent associations with bone stiffness index percentiles;* [d] *Model 4 was Model 3 additionally adjusted for average duration of screen time and sports clubs*

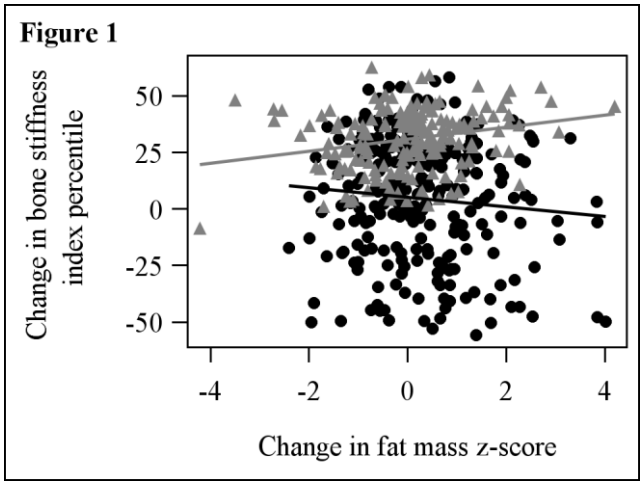

Figure 1 Interaction between change in fat mass z-score and menarche on bone stiffness index percentiles in girls. Accordingly, separate models were stratified by menarche, adjusted for age, socioeconomic status, daylight and bone stiffness index percentiles, height z-score at baseline and change in height z-score, country as a random effect. The black dots and regression line refer to before menarche (β= -1.81, 99%CI: -5.91, 2.30, p= 0.254), whereas the grey triangles and regression line refer to after menarche (β= 3.46, 99%CI: -1.55, 8.47, p= 0.074).