Body fat mass, lean body mass and associated biomarkers as determinants of bone mineral density in children 6–8 years of age – The Physical Activity and Nutrition in Children (PANIC) Study

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Abstract

Lean body mass (LM) has been positively associated with bone mineral density (BMD) in children and adolescents, but the relationship between body fat mass (FM) and BMD remains controversial. Several biomarkers secreted by adipose tissue, skeletal muscle, or bone may affect bone metabolism and BMD. We investigated the associations of LM, FM, and such biomarkers with BMD in children.

We studied a population sample of 472 prepubertal Finnish children (227 girls, 245 boys) aged 6-8 years. We assessed BMD, LM, and FM using whole-body dual-energy x-ray absorptiometry and analysed several biomarkers from fasting blood samples. We studied the associations of LM, FM, and the biomarkers with BMD of the whole body excluding the head using linear regression analysis.

LM (standardized regression coefficient $\beta=0.708$, $p<0.001$), FM ($\beta=0.358$, $p<0.001$), and irisin ($\beta=0.079$, $p=0.048$) were positive correlates for BMD adjusted for age, sex, and height in all children. These associations remained statistically significant after further adjustment for LM or FM. The positive associations of dehydroepiandrosterone sulphate (DHEAS), insulin, homeostatic model assessment for insulin resistance (HOMA-IR), leptin, free leptin index, and high-sensitivity C-reactive protein and the negative association of leptin receptor with BMD were explained by FM. The positive associations of DHEAS and HOMA-IR with BMD were also explained by LM. Serum 25-hydroxyvitamin D was a positive correlate for BMD adjusted for age, sex, and height and after further adjustment for FM but not for LM. LM and FM were positive correlates for BMD also in girls and boys separately. In girls, insulin, HOMA-IR, leptin, and free leptin index were positively and leptin receptor was negatively associated with BMD adjusted for age, height, and LM. After adjustment for age, height, and FM, none of the biomarkers was associated with BMD. In boys, leptin and free leptin index were positively and leptin receptor was negatively associated with BMD adjusted for age, height, and LM. After adjustment for age, height and FM, 25(OH)D was positively and IGF-1 and leptin were negatively associated with BMD. FM strongly modified the association between leptin and BMD.

LM but also FM were strong, independent positive correlates for BMD in all children, girls, and boys. Irisin was positively and independently associated with BMD in all children. The associations of other biomarkers with BMD were explained by LM or FM.

Keywords: bone mineral density; lean body mass; body fat mass; DXA; child; cytokine

Abbreviations

BF%, body fat percentage
BMC, bone mineral content
BMD, bone mineral density
BMI, body mass index
DHEAS, dehydroepiandrosterone sulphate
DXA, dual-energy x-ray absorptiometry
FM, body fat mass
HOMA-IR, the homeostatic model assessment for insulin resistance
hs-CRP, high-sensitivity C-reactive protein
IGF-1, insulin-like growth factor 1
IL-6, interleukin 6
LM, lean body mass
SD, standard deviation
SDS, standard deviation score
TNF-α, tumor necrosis factor α
25(OH)D, 25-hydroxyvitamin D

1. Introduction

Early childhood and puberty are the periods of rapid growth and bone accretion, and the majority of bone mass is gained during adolescence and early adulthood [1–3]. Bone mineral accrual during growth is dependent on multiple factors such as genetic background, sex, race, nutrition, physical activity, and hormone metabolism [2,3]. Higher lean body mass (LM) has been associated with higher bone mineral density (BMD) and bone mineral content (BMC) in children and adolescents [4–7], but the relationship of body fat mass (FM) with BMD or BMC remains controversial [5,6,8–10]. FM has been positively associated with BMD independent of LM in prepubertal children [6]. However, there is some evidence that higher FM is detrimental to bone accrual during and after puberty [5,8,9] and that overweight children and adolescents are at an increased risk of forearm fractures [10].

Mechanical loading increases bone formation, and weight-bearing exercise improves bone mineral accrual [11]. The classical Wolff's law and later the Frost's mechanostat theory propose that bone strength is regulated by modeling and remodeling processes which depend on the forces acting on the bones [12]. The mechanical load to bone is increased not only because of physical activity and increased muscle mass but also due to increased FM and particularly obesity [3].

In addition to the mechanical load, adipose tissue may influence bone metabolism through adipokines, other cytokines, and hormones [13–15]. Adipose tissue may stimulate bone formation by producing estrogens from steroid precursors and by increasing circulating leptin and insulin levels [13–15]. However, adipose tissue produces adiponectin and inflammation-related cytokines, such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), which may have deleterious effects on bone [13–15]. Vitamin D is a prohormone converted in the liver to 25-hydroxyvitamin D (25[OH]D) and then in the kidney to 1,25-dihydroxyvitamin D (1,25[OH]^{2}D), the active metabolite which regulates calcium, phosphorus, and bone metabolism [16]. Obesity has been associated with lower serum levels of 25(OH)D [17], that could therefore be one of the links between obesity and BMD.

More recently, also skeletal muscle and bone have been recognized as endocrine organs [18,19]. Skeletal muscle produces myokines, such as myostatin, insulin-like growth factor I (IGF-1), irisin, and IL-6, which may be important mediators in the interaction between skeletal muscle and bone [18,19]. IGF-1 may be one of the factors that mediate the response of bone and skeletal muscle to mechanical loading [19,20]. Osteocytes also secrete IL-6, IGF-1, and other hormone-like factors,

such as osteocalcin and fibroblast growth factor 23, which have been suggested to play a role in the association between skeletal muscle and bone metabolism [18,19].

Low BMD in childhood tends to persist until young adulthood [21], and bone mass attained during childhood and adolescence is one of the most important determinants of lifelong skeletal health [22]. Pediatric obesity is a growing global health problem [23], and it is therefore important to know how adiposity and associated increase in LM affects BMD among children. There is no consensus on the associations of FM and LM with BMD or the underlying mechanisms. We therefore studied the associations of LM, FM, and associated biomarkers, including adipokines, myokines, inflammation-related biomarkers, growth factors, and 25(OH)D, with BMD assessed by dual-energy x-ray absorptiometry (DXA) in a population sample of children 6-8 years of age.

2. Methods

2.1 Study design and participants

The present analyses are based on the baseline data of the Physical Activity and Nutrition in Children (PANIC) Study, which is an ongoing physical activity and dietary intervention study in a population sample of children 6–8 years of age from the city of Kuopio, Finland (ClinicalTrials.gov registration number NCT01803776). Altogether 736 children from the primary schools of Kuopio were invited to participate in the baseline examinations in 2007—2009. Of the invited children, 512 (70%) participated in the baseline examinations. The participants did not differ in age, sex distribution, or body mass index standard deviation score (BMI-SDS) from all children who started the 1st grade in the city of Kuopio in 2007–2009 based on data from the standard school health examinations. From the present analyses, we excluded children who had chronic diseases or medications that could affect BMD, such as juvenile arthritis demanding long-term treatment with oral corticosteroids. We also excluded 12 children who had entered puberty to avoid associated confounding. Complete data on the main variables used in the present analyses were available for 472 children (227 girls, 245 boys). The study was conducted according to the ethical guidelines laid down in the Declaration of Helsinki. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. Both children and their parents gave their written informed consent.

2.2 Assessment of bone mineral density and body composition

LM, FM, body fat percentage (BF %), and BMD of the whole body excluding the head were assessed using the Lunar Prodigy Advance® DXA device (GE Medical Systems, Madison, WI, USA) and the Encore® software, Version 10.51.006 (GE Company, Madison, WI, USA), according to the manufacturer's instructions using standardized protocols. The same DXA device and software were used in all measurements. Body weight was measured twice the children having fasted for 12 hours, emptied the bladder, and standing in light underwear by the InBody® 720 bioelectrical impedance device (Biospace, Seoul, Korea) to accuracy of 0.1 kg. The mean of these two values was used in the analyses. Body height was measured three times the children standing in the Frankfurt plane without shoes using a wall-mounted stadiometer to accuracy of 0.1 cm. The mean of the nearest two values was used in the analyses. BMI-SDS was calculated using national reference values [24]. Waist circumference was measured three times after expiration at mid-distance between the bottom of the rib cage and the top of the iliac crest with an unstretchable measuring tape to accuracy of 0.1 cm. The mean of the nearest two values was used in the analyses. Intraclass correlation coefficients for body weight and height and waist circumference were >0.99.

2.3 Biochemical analyses

Venous blood samples were taken the children having fasted for 12 hours. Blood was immediately centrifuged and stored at a temperature of -75ºC until biochemical analyses, except for glucose that was measured from non-frozen plasma samples. Serum 25(OH)D concentration was analysed by a chemiluminescence immunoassay called the LIAISON® 25 OH Vitamin D TOTAL Assay (DiaSorin Inc., Stillwater, USA) as described earlier [25,26]. Serum dehydroepiandrosterone sulphate (DHEAS) concentration was used as a marker of biochemical adrenarche and was determined using an enzyme linked immunosorbent assay (ELISA) kit (Alpha Diagnostic International, San Antonio, Texas, USA) [27]. Serum IGF-1 concentration was analysed using an ELISA kit (Mediagnost, Reutlingen, Germany). Plasma glucose concentration was measured using the hexokinase method (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin concentration was measured by the electrochemiluminescence immunoassay with the sandwich principle (Roche Diagnostics GmbH, Mannheim, Germany). We calculated the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) using the formula fasting serum insulin x fasting plasma glucose/22. Serum high-molecular-weight adiponectin concentration was analysed using an ELISA kit after a specific proteolytic digestion of other multimeric adiponectin forms (Millipore, Billerica, MA, USA). Plasma leptin concentration was measured by a competitive radioimmunoassay (Multigamma 1261-001,

PerkinElmer Wallac Oy, Turku, Finland) and plasma soluble leptin receptor concentration using an ELISA kit (Multicalc evaluation programme PerkinElmer Wallac Oy, Turku, Finland). We calculated the free leptin index by dividing leptin with soluble leptin receptor and multiplying by 100 [28]. Commercially available ELISA kits were employed for the measurement of plasma irisin (Phoenix Pharmaceuticals, Burlingame, California, USA), IL-6, and TNF-α concentrations (Sanquin Reagents, Amsterdam, The Netherlands). Plasma high-sensitivity C-reactive protein (hsCRP) was measured using an enhanced immunoturbidimetric assay with the CRP (Latex) High Sensitive Assay reagent (Roche Diagnostics GmbH, Mannheim, Germany) and the limit of quantitation of 0.3 mg/l.

2.4 Assessments of general health, puberty, and adrenarche

The parents filled out a questionnaire that included items on the children's chronic diseases and allergies diagnosed by a physician as well as detailed information on the children's use of medications. A research physician assessed pubertal status during a medical examination. Central puberty was defined as breast development at Tanner stage ≥2 for girls and testicular volume ≥4 mL assessed using an orchidometer for boys. Premature adrenarche was defined as serum DHEAS ≥ 1 μmol/l (≥ 37 μg/dl) [29] and at least one clinical sign of androgen action. Birth weight was obtained from Kuopio University Hospital record, and birth weight -SDS was calculated according to Finnish growth reference data [30].

2.5 Statistical methods

We performed statistical analyses using the IBM SPSS Statistics® software, Version 21 (IBM Corp., Armonk, NY, USA). The normality of distributions of the variables was verified visually and by the Kolmogorov-Smirnov test. The t-test for independent samples and the Mann–Whitney's U-test were used to examine differences in the basic characteristics between sexes. Linear regression analysis was used to investigate the determinants of BMD, and the normality of residuals for regression models was assessed using histograms. Model 1 included each determinant of BMD separately, adjusted for age and sex. Model 2 was additionally adjusted for body height. Model 3 included all variables in Model 2 and LM, and Model 4 included all variables in Model 2 and FM. Corresponding linear regression analyses were also performed for girls and boys separately. FM had a strong positive correlation with leptin in girls ($r=0.789$, $p<0.001$), boys ($r=0.850$, $p<0.001$), and girls and boys combined ($r=0.810$, $p<0.001$). We therefore tested whether FM modified the association between leptin and BMD by analyzing this association in the sex-specific thirds of FM using linear regression

analysis adjusted for age, sex, and body height. In all analyses, associations with a p-value of <0.05 were considered statistically significant.

3. Results

3.1 Characteristics of children

The boys were heavier and taller and had higher waist circumference and LM and lower BF% and FM than the girls, but there was no difference in BMI-SDS between the genders (Table 1). The girls had higher IGF-1, insulin, leptin, and free leptin index and lower leptin receptor and IL-6 than the boys. Of the children, 38 (8.1%) had asthma, 128 (27.1%) any allergic symptom (rhinitis, conjunctivitis, atopy, food or medicine allergy), 21 (4.4%) an attention deficit hyperactivity disorder (ADHD/ADD) or another mild neurocognitive disorder or developmental delay, 8 (1.7%) a mild congenital dysmorphism, and 10 (2.1%) any other chronic disease. There was no difference in BMD between children with these diseases and those without them.

3.2. Determinants of bone mineral density in all children

Body height (β=0.572, p<0.001) and weight (β=0.709, p<0.001) were positively associated with BMD adjusted for age and sex. LM was also a strong positive correlate for BMD adjusted for age and sex (Table 2, Model 1). This association remained similar after additional adjustment for body height (Model 2) but weakened slightly after further adjustment for FM (Model 4). Moreover, FM had a strong positive association with BMD adjusted for age and sex (Table 2, Model 1). This association weakened after additional adjustment for body height (Model 2) but remained similar when further adjusted for LM (Model 3). Birth weight was positively associated with BMD adjusted for age and sex (Table 2, Model 1), but this association disappeared after additional adjustments (Models 2-4).

Serum 25(OH)D was positively associated with BMD adjusted for age and sex (Table 2, Model 1). This association remained almost similar after additional adjustment for body height and FM (Models 2 and 4) but was no longer statistically significant when adjusted for LM (Model 3). DHEAS was positively associated with BMD adjusted for age and sex (Table 2, Model 1). This association weakened when additionally adjusted for body height (Model 2) but was no longer statistically significant after adjustment for LM or FM (Models 3-4). IGF-1 was a positive correlate for BMD adjusted for age and sex (Table 2, Model 1) but not after further adjustments (Models 2-4). Insulin and HOMA-IR were positively associated with BMD adjusted for age and sex (Table 2, Model 1).

These associations weakened after additional adjustment for body height (Model 2). The association of insulin weakened and that of HOMA-IR was no longer statistically significant after further adjustment for LM (Model 3). The associations of insulin and HOMA-IR with BMD disappeared when adjusted for FM (Model 4).

Adiponectin was a negative correlate for BMD adjusted for age and sex (Table 2, Model 1) but not after further adjustments (Models 2-4). Leptin was positively associated with BMD adjusted for age and sex (Table 2, Model 1). This association weakened after additional adjustment for body height and LM (Models 2-3) and was no longer statistically significant after adjustment for FM (Model 4). There was a positive association between leptin and BMD in the highest sex-specific third of FM (β=0.274, $p<0.001$) but a non-significant inverse association in the middle third (β=-0.144, p=0.058) and the lowest third (β=-0.112, p=0.118) adjusted for age and body height. Lower leptin receptor and higher free leptin index were associated with higher BMD adjusted for age and sex (Table 2, Model 1). These associations weakened after additional adjustment for body height and when further adjusted for LM (Models 2-3) and were no longer statistically significant after adjustment for FM (Model 4). Irisin was positively associated with BMD adjusted for age and sex (Table 2, Model 1). This association weakened slightly when additionally adjusted for body height (Model 2) and remained similar after further adjustment for LM or FM (Models 3-4).

IL-6 and TNF-α were not associated with BMD (Table 2, Models 1-4). Higher hs-CRP was associated with higher BMD adjusted for age and sex (Table 2, Model 1), after additional adjustment for body height (Model 2), and also when further adjusted for LM (Model 3). However, this association disappeared after adjustment for FM (Model 4).

3.2.2 Determinants of bone mineral density in girls

In girls, body height (β=0.615, $p<0.001$) and weight (β=0.727, $p<0.001$) were positively associated with BMD adjusted for age. LM had a strong positive association with BMD adjusted for age, body height, and FM (Table 3, Models 1, 2, and 4). FM was also a strong positive correlate for BMD adjusted for age, body height, and LM (Table 3, Models 1-3). Birth weight SDS, 25(OH)D, DHEAS, IGF-1, and irisin were positively associated with BMD when adjusted for age (Table 3, Model 1) but not after further adjustments (Models 2-4). Insulin and HOMA-IR were positive correlates for BMD adjusted for age, body height, and LM (Table 3, Models 1-3) but not when adjusted for FM (Model 4). Leptin and free leptin index were positively and leptin receptor was negatively associated with BMD adjusted for age, body height, and LM (Table 3, Models 1-3) but not adjusted for FM (Model

4). There was a positive association between leptin and BMD in the highest third of FM (β=0.346, p<0.001) but a non-significant inverse association in the middle third (β=-0.169, p=0.126) and the lowest third (β=-0.122, p=0.261) adjusted for age and body height.

3.2.3 Determinants of bone mineral density in boys

In boys, body height (β=0.520, p<0.001) and weight (β=0.686, p<0.001) were positively associated with BMD adjusted for age. LM had a strong positive association with BMD adjusted for age, body height, and FM (Table 4, Models 1, 2, and 4). FM was also a strong positive correlate for BMD adjusted for age, body height, and LM (Table 4, Models 1-3). Serum 25(OH)D was positively associated with BMD adjusted for age, body height, and FM (Table 4, Models 2 and 4) but not adjusted for LM (Model 4). Birth weight SDS, DHEAS, insulin, HOMA-IR and hs-CRP were positively associated with BMD adjusted for age (Table 4, Model 1) but not after further adjustments (Models 2-4). IGF-1 was negatively associated with BMD only when adjusted for age, body height, and FM (Table 4, Model 4). Leptin and free leptin index were positively and leptin receptor was negatively associated with BMD adjusted for age, body height, and LM (Table 4, Models 1-3), but the associations of free leptin index and leptin receptor were no longer statistically significant and that of leptin became negative when adjusted for LM (Model 4). There was a non-significant positive association between leptin and BMD in the highest third of FM (β=0.199, p=0.061), a non-significant inverse association in the middle third (β=-0.135, p=0.203) and no association in the lowest third (β=-0.024, p=0.821).

4. Discussion

Our study is one of the few studies on the associations of LM, FM, and various biomarkers secreted by adipose tissue, skeletal muscle, or bone with BMD in a population sample of prepubertal children. LM but also FM were strong and independent positive determinants of BMD in all children, girls, and boys. Plasma irisin was also an independent positive correlate for BMD in all children but not in girls and boys separately. The associations of other biomarkers were explained by body height, LM, or FM. In boys, the positive association between leptin and BMD became negative and the negative association between IGF-1 and BMD strengthened after controlling for FM.

In line with previous studies among children and adolescents [4,5,7], LM was a strong positive correlate for BMD in the current study. The positive association between LM and BMD may be

explained by increased mechanical load to bone caused by increased LM and the loading effect of weight-bearing exercise on bone mass and metabolism [11].

A recently identified myokine irisin is produced by skeletal muscle after exercise and may increase energy expenditure [31]. Irisin has been found to increase bone mass in mice [32], but evidence on the association between serum irisin and BMD in humans is limited. Irisin has been positively associated with bone mass and strength in young athletes and negatively related to vertebral fragility fractures in postmenopausal women [31,33]. To the best of our knowledge, the association between irisin and BMD has not been studied earlier in children. We found that higher serum irisin levels were associated with higher BMD even after controlling for LM or FM. The weak positive association between irisin and BMD was slightly stronger in girls than in boys, but statistical power was limited in these sex-specific analyses.

Of other biomarkers previously related to skeletal muscle and bone metabolism, insulin had a weak positive association with BMD even after controlling for LM. However, the association between insulin and BMD was explained by FM. IGF-1 was positively associated with BMD in all children and in girls but not after controlling for body size and composition. Moreover, there was a weak negative association between IGF-1 and BMD in boys when controlled for FM. Previous studies in children and adolescents have reported an independent positive association between IGF-1 and bone growth [20] and a muscle-dependent positive association between IGF-1 and BMD [20,34]. However, insulin resistance has suppressed the muscle-dependent relationship between IGF-1 and BMC and cortical bone measurements in children 9-13 years of age [34,35]. One reason for the inconsistency between our results and the findings of earlier studies could be that our participants were prepubertal and slightly younger than those of the previous studies. It is also possible that the weak negative association between IGF-1 and BMD in boys after controlling for FM in our study is partly explained by the positive relationships among adiposity, insulin resistance, and IGF-1.

FM has been positively associated with BMD in some previous studies among mainly prepubertal children [6,36]. Obesity has also been associated with increased bone mass independent of LM in a study among children and adolescents [37]. Moreover, adiposity was associated with increased bone mass in another study in adolescents, but this association was explained by LM [7]. One explanation for the positive association between FM and BMD among children and adolescents could be the increased mechanical load to the bone due to adiposity [3]. Another reason could be that adipose tissue stimulates bone growth [36]. However, one study reported a decreased volumetric BMD in

obese prepubertal children despite increased bone size [38]. Another study showed an inverse association between BF% and BMD in adolescents [5]. In a Finnish study among prepubertal and pubertal children, those with decreased body fat content and those with increased fat content had decreased BMD independent of LM [39]. In the current study, FM was positively associated with BMD independent of LM, even though LM was a stronger correlate for BMD than FM. This observation is consistent with the results of a previous study among children [6]. Studies that have shown an association between excess fat mass and decreased BMD have been conducted in older and more overweight children and adolescents [5,39] than the participants of our study. Only 14% of the girls and 10% of the boys in our population sample of prepubertal children 6-8 years of age were overweight or obese [40]. Therefore, we cannot draw a conclusion on the association between obesity and BMD based on our findings. It is possible that the detrimental effect of excess fat mass appears in later childhood or in adolescence during or after puberty along with changes in body composition [1]. In our study, the association between LM and BMD was stronger in boys than in girls. One reason for this finding could be that boys have more skeletal muscle and girls have more adipose tissue already in prepubertal stage [1], that is consistent with our observation.

Leptin is an adipocyte-secreted hormone that decreases appetite and increases energy expenditure [14] but may also influence bone modeling through central and peripheral mechanisms [14,15]. Leptin has been suggested to inhibit bone formation indirectly through the sympathetic nervous system [14,15]. In contrast, leptin directly enhances bone formation and inhibits bone resorption peripherally, even though the mechanisms are rather complex and not yet well defined [14,15]. These local effects of leptin on bone have been suggested to be dominant, and higher circulating leptin levels may therefore be related to a stronger skeleton [15]. Leptin may also regulate the hypothalamic-pituitary-peripheral endocrine axes, including thyroid, gonadal, cortisol, and growth hormone axes, which are possible additional indirect ways by which leptin affects bone [41]. Soluble leptin receptor is the major protein binding leptin in blood, and leptin receptor levels seem to vary independent of serum leptin levels during childhood [28]. Functional differences between free and bound leptin are not clear, but some studies have suggested that free leptin index better reflects the physiological actions of leptin [28]. A meta-analysis concluded that circulating leptin levels were positively associated with BMD [42], but most of the 46 studies included in the analysis were performed in adults. The association between leptin and total body BMD was also positive in five studies among girls [42]. Interestingly, the relationship between leptin and BMD adjusted for body mass was negative in the only small study among boys [43]. Furthermore, body fat content was not taken into account in the meta-analysis [42]. In a previous study, free leptin index was associated with bone

turnover markers [13], which may be one mechanism for the inverse association between leptin and BMD. We found that leptin receptor level was negatively and leptin and free leptin index were positively associated with BMD independent of LM, but these associations were explained by FM. Moreover, the association between leptin and BMD became negative in boys after controlling for FM. Leptin was positively associated with BMD in the highest sex-specific third of FM but had a weak negative association in the middle and lowest thirds. These findings suggest that FM strongly modifies the association between leptin and BMD.

Adiponectin is an adipokine that has been inversely related to FM in children [44], and this inverse association has been found to strengthen in puberty [45]. Adiponectin regulates energy homeostasis, glucose and lipid metabolism, and inflammatory pathways [15]. Increased adiponectin has been associated with reduced bone mass in children [44]. This may be explained by the decreased circulating levels of insulin and IGF-1 due to increased adiponectin levels [15]. In the current study among prepubertal children, we found a weak negative association between adiponectin and BMD, but it was largely explained by LM and FM. It is possible that the negative association between adiponectin and BMD might be stronger after puberty.

Excess adiposity is associated with insulin resistance and hyperinsulinemia in youth [46]. Insulin has been suggested to be anabolic for bone formation, and higher serum insulin levels have been associated with higher BMD in adults [15]. However, the associations of insulin resistance with BMC and BMD remain controversial in children and adolescents [47–49]. In a study among prepubertal overweight children, BMC was lower in children with prediabetes than in children without it [47]. In overweight adolescents, increased HOMA-IR was associated with decreased BMD [48]. In another study among adolescents, insulin was positively associated with BMD, but the association was inverse after controlling for FM [49]. In line with these results, we found that higher fasting insulin and HOMA-IR were associated with higher BMD, but the associations became weak negative in boys and disappeared in girls after controlling for FM. These findings suggest that the association between insulin resistance and BMD is largely dependent on adiposity that should be taken into account when interpreting the results.

IL-6 has a double-edged role in bone metabolism as it may stimulate both osteocyte differentiation and osteoclastic bone resorption [19]. IL-6 but also TNF-α are inflammation-related cytokines secreted by adipose tissue, and they may enhance bone resorption [14]. We found no association between IL-6 or TNF-α and BMD in children. One explanation for this may be that the prevalence of

overweight was low in our general population of children, and thus the inflammatory-related effects of these cytokines may have been modest. Higher hs-CRP has been associated with lower BMD in adolescent girls [50] and in overweight children with prediabetes but not in overweight children without it [47]. Inconsistent with these findings, we observed a weak positive association between hs-CRP and BMD in children. The reason for this inconsistency probably is the low proportion of overweight and obese children in our population sample [40]. Moreover, the observed positive association between hs-CRP and BMD was explained by FM. This is an expected result as adiposity is known to be related to systemic low-grade inflammation [51].

The definition of vitamin D deficiency based on serum 25(OH)D concentration varies between 25 and 50 nmol/l and the lower limit for optimal serum 25(OH)D concentration has been suggested to be as high as 75 nmol/l [3,16,52–57]. No consensus exists on the optimal serum level of 25(OH)D. As vitamin D is essential for bone metabolism [16], the positive association of 25(OH)D with BMD in the current study was expected, and this is in line with the results of previous studies [4]. However, the association between 25(OH)D and BMD was weak especially in girls, but this is probably explained by the low proportion of children having 25(OH)D concentrations below 50 nmol/l [25], which has been considered as a limit of deficiency based on bone outcomes [53]. The association between 25(OH)D and BMD was stronger in boys, and it was partly explained by LM. One explanation for this finding may be that physically active children, particularly boys, have increased LM and spend more time outdoors and are therefore exposed to sunlight that increases serum 25(OH)D concentrations.

DHEAS is an androgen precursor produced mainly by the adrenal cortex and whose circulating levels are increased during adrenarche [27]. Both obesity and premature adrenarche are associated with advanced bone age [58,59]. However, there are little and inconsistent data on the association between DHEAS and BMD in children [58,60]. In the current study among prepubertal children, higher DHEAS was associated with higher BMD. However, the positive association weakened after controlling for body height, LM, and FM, suggesting that DHEAS does not have an independent effect on BMD in prepubertal children.

Some diseases, conditions and medications, such as juvenile arthritis, renal insufficiency, inflammatory conditions, disabilities, immobility, oral corticosteroid use, or certain antiepileptic drugs, may decrease BMD [61]. We therefore excluded children who had such diseases, conditions, or medications to avoid associated confounding. The use of inhaled corticosteroids has been

associated with decreased BMD in some studies [62]. However, a recent review and meta-analysis concluded that the use of inhaled corticosteroids was not associated with decreased lumbar BMD or increased risk of fractures [63]. In our study, about 8% of the children had asthma, a few of them used regular inhaled corticosteroids, and they had similar BMD to children without asthma. We therefore included children with asthma in the current study population.

Body weight and BMI have been directly associated with BMD in children and adolescents [3,6], but neither of them is a specific measure of LM or FM. We therefore investigated the associations of LM and FM measured by DXA with BMD among children. DXA is also the most widely used method to evaluate BMD and it has been reported to be well reproducible also in children [64–66]. The assessment and interpretation of BMD measurements are not simple in growing children because of both methodological aspects and differences in maturation and growth. In children, The International Society of Clinical Densitometry (ISCD) recommends measuring BMD and BMC from total body excluding the head and the posterior-anterior spine [66]. Areal BMD measurements may underestimate the BMD of short children and overestimate the BMD of tall children. Therefore, ISCD recommends adjusting BMD of total body excluding head and spinal BMD using height z-score. We used DXA of the whole body, excluding the head, which is one of the methods recommended to be used for measuring BMD among children by the ISCD [66]. Moreover, we adjusted the data first for age and sex and then additionally for body height, all components of height z-score. However, we did not measure volumetric BMD but areal BMD and did not use computed tomography to measure the more detailed quality of the bone.

The results of different studies depend not only on the methods used but also on the age and maturation of the participants and the prevalence of overweight in the study population, because each of them affects BMD. We investigated a general population of prepubertal children 6-8 years of age with a low prevalence of overweight, whereas many other studies have mainly included overweight or obese children and adolescents with advanced puberty [5,7,37,39,47]. It is therefore difficult to compare the findings of our study with those of many other studies.

5. Conclusions

Our study showed that LM is the strongest positive determinant of BMD, but also FM is positively and independently associated with BMD in a population sample of mainly normal-weight prepubertal Finnish children. Of biomarkers related to body composition, irisin had a positive association with BMD independent of LM and FM. To the best of our knowledge, this is the first study to examine the

association between irisin and BMD in children, and this finding needs to be confirmed in other populations. As expected, 25(OH)D was a positive correlate for BMD, but the association was weak probably due to the relatively low prevalence of vitamin D deficiency in our study population and was partly explained by body composition. In boys, the positive association of leptin with BMD became negative after controlling for FM. This finding suggests that FM strongly modifies the association between leptin and BMD and that adiposity should be taken into account when interpreting the associations of leptin with bone structure and metabolism.

6. Acknowledgements

The authors are grateful to all the children and their parents for participating in the PANIC study. The authors are also indebted to the members of the PANIC research team for their skillful contribution in performing the study. The authors are grateful to Ayhan Korkmaz for performing irisin measurements, Leila Antikainen for performing DHEAS and IGF-1 measurements, Tuomas Onnukka for performing leptin measurements, and Kaija Kettunen for performing leptin receptor and adiponectin measurements. We also thank Tarja Kokkola for the help with methodological issues on laboratory measurements.

7. Funding sources

This work was financially supported by grants from Ministry of Social Affairs and Health of Finland, Ministry of Education and Culture of Finland, Finnish Innovation Fund Sitra, Social Insurance Institution of Finland, Finnish Cultural Foundation, Juho Vainio Foundation, Foundation for Paediatric Research, Doctoral Programs in Public Health, Paavo Nurmi Foundation, Paulo Foundation, Diabetes Research Foundation, Yrjö Jahnsson Foundation, Finnish Foundation for Cardiovascular Research, Research Committee of the Kuopio University Hospital Catchment Area (State Research Funding), Kuopio University Hospital (previous state research funding (EVO), funding number 5031343), and the city of Kuopio.

8. Conflict of interest

The authors declare there are no conflicts of interest.

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Table 1. Characteristics of children.

	All (n=472)	Girls (n=227)	Boys (n=245)	P-value
Age, y	7.6 (0.4)	7.6 (0.4)	7.6 (0.4)	0.169
Birth weight SDS	-0.05 (1.00)	-0.01 (0.99)	-0.09 (1.01)	0.372
Weight, kg	26.7 (4.9)	26.2 (4.8)	27.3 (4.9)	**0.017**
Height, cm	128.6 (5.6)	127.7 (5.6)	129.5 (5.4)	**0.001**
BMI-SDS	-0.20 (1.07)	-0.23 (1.02)	-0.17 (1.11)	0.511
Waist circumference, cm	56.5 (5.7)	55.5 (5.4)	57.5 (5.7)	**<0.001**
Lean body mass, kg	17.7 (2.2)	16.8 (2.0)	18.6 (2.1)	**<0.001**
Body fat mass, kg	5.2 (3.3)	5.6 (3.2)	4.7 (3.3)	**0.002**
Body fat percentage	20.6 (8.5)	23.2 (7.8)	18.2 (8.5)	**<0.001**
BMD, total body excluding the head, g/cm^2	0.72 (0.05)	0.72 (0.05)	0.72 (0.05)	0.094
25(OH)D, nmol/l	67.8 (22.7)	65.6 (18.8)	69.8 (25.7)	0.056
DHEAS, µmol/l	0.57 (0.33-0.85)	0.57 (0.33-0.84)	0.58 (0.32-0.85)	0.998
IGF-1, nmol/l	23.1 (7.6)	24.4 (7.3)	22.0 (7.5)	**0.001**
Insulin, mU/l	4.50 (2.44)	4.74 (2.25)	4.29 (2.58)	**0.049**
HOMA-IR	0.98 (0.56)	1.01 (0.51)	0.94 (0.59)	0.196
Adiponectin, µg/ml	8.91 (4.09)	8.84 (3.77)	8.97 (4.36)	0.740
Leptin, ng/ml	3.70 (2.70-5.85)	4.30 (3.20-6.80)	3.20 (2.40-4.70)	**<0.001**
Leptin receptor, ng/ml	40.8 (10.5)	38.6 (9.7)	42.9 (10.9)	**<0.001**
Free leptin index	9.0 (5.9-16.2)	10.9 (7.5-20.6)	6.9 (5.1-12.6)	**<0.001**
Irisin, ng/ml	151.5 (53.0)	151.3 (43.3)	151.8 (60.5)	0.918
IL-6, pg/ml	0.90 (0.63-1.47)	0.83 (0.60-1.27)	1.00 (0.63-1.57)	**0.016**
TNF-α, pg/ml	14.3 (5.2-34.2)	12.7 (5.0-30.4)	15.7 (5.4-37.4)	0.177
hs-CRP, mg/l	0.29 (0.29-0.54)	0.29 (0.29-0.59)	0.29 (0.29-0.49)	0.098

The values are presented as mean (SD) for normally distributed variables and median (IQR) for skewed variables. Differences between girls and boys were tested with independent samples t test for normally distributed variables and Mann–Whitney's U test for skewed variables. P-value for differences between girls and boys.

Abbreviations: SDS, standard deviation score; BMI-SDS, body mass index standard deviation score; BMD, bone mineral density; 25(OH)D, 25-hydroxyvitamin D; DHEAS, dehydroepiandrosterone sulphate; IGF-1, insulin-like growth factor 1; HOMA-IR: homeostatic model assessment for insulin resistance; adiponectin, high-molecular weight adiponectin; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; hs-CRP, high-sensitivity C-reactive protein (values over 10 excluded)

Number of children (n) varies from 417 to 472 in different variables; n=472, 227 girls and 245 boys: age, weight, height, BMI-SDS, waist, lean body mass, body fat mass, BMD; n=463, 222 girls and 241 boys: birth weight SDS; n=417, 198 girls and 219 boys: 25(OH)D; n= 440, 211 girls and 229 boys DHEAS, IGF-1; n= 456, 216 girls and 240 boys :insulin; n= 452, 215 girls and 237 boys: HOMA-IR; n= 452, 214 girls and 238 boys: adiponectin, leptin, leptin receptor; n= 433, 205 girls and 228 boys: irisin; n= 448, 210 girls and 238 boys: IL-6; n= 450, 211 girls and 239 boys: TNF-α; n= 456, 217 girls and 239 boys: hs-CRP (values over 10 excluded).

Table 2. Determinants of bone mineral density (total body excluding the head) in all children.

	Model 1		Model 2		Model 3		Model 4	
	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value
Lean body mass, kg	0.729	<**0.001**	0.708	<**0.001**			0.562	<**0.001**
Body fat mass, kg	0.594	<**0.001**	0.358	<**0.001**	0.365	<**0.001**		
Birth weight SDS	0.169	<**0.001**	-0.011	0.786	-0.059	0.103	0.009	0.807
25(OH)D, nmol/l	0.097	**0.044**	0.086	**0.036**	0.067	0.075	0.087	**0.017**
DHEAS, µmol/l	0.178	<**0.001**	0.100	**0.012**	0.071	0.052	0.065	0.068
IGF-1, nmol/l	0.188	<**0.001**	0.037	0.375	0.007	0.844	-0.041	0.275
Insulin, mU/l	0.218	<**0.001**	0.102	**0.010**	0.071	**0.048**	-0.043	0.260
HOMA-IR	0.212	<**0.001**	0.087	**0.028**	0.062	0.087	-0.054	0.153
Adiponectin, µg/ml	-0.091	**0.049**	-0.067	0.082	-0.042	0.232	-0.052	0.126
Leptin, ng/ml	0.397	<**0.001**	0.275	<**0.001**	0.245	<**0.001**	-0.114	0.058
Leptin receptor, ng/ml	-0.432	<**0.001**	-0.260	<**0.001**	-0.192	<**0.001**	0.061	0.168
Free leptin index	0.400	<**0.001**	0.284	<**0.001**	0.245	<**0.001**	0.012	0.825
Irisin, ng/ml	0.105	**0.026**	0.079	**0.048**	0.072	**0.047**	0.075	**0.034**
IL-6, pg/l	0.042	0.370	0.031	0.422	0.007	0.836	-0.001	0.982
TNF-α pg/ml	0.009	0.844	0.022	0.568	0.014	0.679	0.032	0.341
hs-CRP, mg/l	0.111	**0.014**	0.088	**0.023**	0.098	**0.005**	0.003	0.941

The values are standardized regression coefficients (β) and p-values from linear regression models.

Model 1: Each variable was entered in linear regression analysis adjusted for age and sex.
Model 2: Each variable was entered in linear regression analysis adjusted for age, sex, and height.
Model 3: Each variable was entered in linear regression analysis adjusted for age, sex, height, and lean mass.
Model 4: Each variable was entered in linear regression analysis adjusted for age, sex, height, and fat mass.

Abbreviations: SDS, standard deviation score; BMI-SDS, body mass index standard deviation score; BMD, bone mineral density; 25(OH)D, 25-hydroxyvitamin D; DHEAS, dehydroepiandrosterone sulphate; IGF-1, insulin-like growth factor 1; HOMA-IR: homeostatic model assessment for insulin resistance; adiponectin, high-molecular weight adiponectin; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; hs-CRP, high-sensitivity C-reactive protein (values over 10 excluded)

Number of children (n) varies from 417 to 472 in different variables; n=472: BMD, lean body mass, body fat mass; n=463: birth weight SDS; n=417: 25(OH)D; n= 440: DHEAS, IGF-1; n= 456: insulin; n= 452: HOMA-IR; n= 452: adiponectin, leptin, leptin receptor; n= 433: irisin; n= 448: IL-6; n= 450: TNF-α; n= 456: hs-CRP (values over 10 excluded).

Table 3. Determinants of bone mineral density (total body excluding the head) in girls.

	Model 1		Model 2		Model 3		Model 4	
	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value
Lean body mass, kg	0.663	**<0.001**	0.571	**<0.001**			0.459	**<0.001**
Body fat mass, kg	0.612	**<0.001**	0.439	**<0.001**	0.382	**<0.001**		
Birth weight SDS	0.187	**0.004**	-0.009	0.870	-0.047	0.357	-0.016	0.749
25(OH)D, nmol/l	0.097	**0.044**	0.042	0.464	0.048	0.350	0.035	0.473
DHEAS, µmol/l	0.256	**<0.001**	0.095	0.095	0.078	0.132	0.030	0.551
IGF-1, nmol/l	0.278	**<0.001**	0.083	0.157	0.060	0.258	0.040	0.422
Insulin, mU/l	0.231	**<0.001**	0.139	**0.010**	0.120	**0.015**	0.019	0.693
HOMA-IR	0.228	**<0.001**	0.136	**0.012**	0.120	**0.014**	0.015	0.758
Adiponectin, µg/ml	-0.061	0.352	-0.060	0.263	-0.042	0.394	-0.018	0.706
Leptin, ng/ml	0.425	**<0.001**	0.305	**<0.001**	0.279	**<0.001**	-0.064	0.417
Leptin receptor, ng/ml	-0.432	**<0.001**	-0.271	**<0.001**	-0.226	**<0.001**	-0.074	0.198
Free leptin index	0.434	**<0.001**	0.321	**<0.001**	0.286	**<0.001**	0.034	0.629
Irisin, ng/ml	0.175	**0.008**	0.108	0.052	0.094	0.063	0.073	0.126
IL-6, pg/l	0.120	0.070	0.061	0.269	0.038	0.452	0.021	0.656
TNF-α pg/ml	0.054	0.416	0.065	0.232	0.024	0.630	0.052	0.270
hs-CRP, mg/l	0.070	0.283	0.070	0.198	0.092	0.065	-0.030	0.563

The values are standardized regression coefficients (β) and p-values from linear regression models.

Model 1: Each variable was entered in linear regression analysis adjusted for age.
Model 2: Each variable was entered in linear regression analysis adjusted for age and height.
Model 3: Each variable was entered in linear regression analysis adjusted for age, height, and lean mass.
Model 4: Each variable was entered in linear regression analysis adjusted for age, height, and fat mass.

Abbreviations: SDS, standard deviation score; BMI-SDS, body mass index standard deviation score; BMD, bone mineral density; 25(OH)D, 25-hydroxyvitamin D; DHEAS, dehydroepiandrosterone sulphate; IGF-1, insulin-like growth factor 1; HOMA-IR: homeostatic model assessment for insulin resistance; adiponectin, high-molecular weight adiponectin; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; hs-CRP, high-sensitivity C-reactive protein (*values over 10 excluded)

Number of girls (n) varies from 205 to 227 in different variables; n=227: BMD, lean body mass, body fat mass; n=222: birth weight SDS; n=198: 25(OH)D; n=211: DHEAS, IGF-1; n=216: insulin; n=215: HOMA-IR; n=214: adiponectin, leptin, leptin receptor; n=205: irisin; n= 210: IL-6; n=211: TNF-α; n=217: hs-CRP (values over 10 excluded).

Table 4. Determinants of bone mineral density (total body excluding the head) in boys.

	Model 1		Model 2		Model 3		Model 4	
	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value
Lean body mass, kg	0.664	**<0.001**	0.746	**<0.001**			0.591	**<0.001**
Body fat mass, kg	0.568	**<0.001**	0.435	**<0.001**	0.337	**<0.001**		
Birth weight SDS	0.145	**0.023**	-0.029	0.619	-0.074	0.153	0.031	0.555
25(OH)D, nmol/l	0.123	0.070	0.117	**0.045**	0.079	0.145	0.123	**0.023**
DHEAS, µmol/l	0.139	**0.036**	0.108	0.062	0.070	0.182	0.088	0.095
IGF-1, nmol/l	0.094	0.152	-0.007	0.905	-0.045	0.393	-0.108	**0.049**
Insulin, mU/l	0.211	**0.001**	0.079	0.172	0.039	0.456	-0.089	0.132
HOMA-IR	0.209	**0.001**	0.057	0.975	0.028	0.598	-0.108	0.069
Adiponectin, µg/ml	-0.127	0.050	-0.080	0.152	-0.055	0.278	-0.080	0.115
Leptin, ng/ml	0.346	**<0.001**	0.229	**<0.001**	0.187	**<0.001**	-0.266	**0.006**
Leptin receptor, ng/ml	-0.418	**<0.001**	-0.249	**<0.001**	-0.161	**0.004**	-0.056	0.401
Free leptin index	0.353	**<0.001**	0.236	**<0.001**	0.180	**<0.001**	-0.165	0.063
Irisin, ng/ml	0.056	0.400	0.058	0.312	0.053	0.310	0.073	0.168
IL-6, pg/l	-0.019	0.770	0.006	0.914	-0.023	0.650	0.019	0.992
TNF-α pg/ml	-0.020	0.762	-0.010	0.854	0.015	0.766	0.015	0.768
hs-CRP*, mg/l	0.138	**0.032**	0.101	0.068	0.083	0.096	0.024	0.650

The values are standardized regression coefficients (β) and p-values from linear regression models.

Model 1: Each variable was entered in linear regression analysis adjusted for age.
Model 2: Each variable was entered in linear regression analysis adjusted for age and height.
Model 3: Each variable was entered in linear regression analysis adjusted for age, height, and lean mass.
Model 4: Each variable was entered in linear regression analysis adjusted for age, height, and body fat mass.

Abbreviations: SDS, standard deviation score; BMI-SDS, body mass index standard deviation score; BMD, bone mineral density; 25(OH)D, 25-hydroxyvitamin D; DHEAS, dehydroepiandrosterone sulphate; IGF-1, insulin-like growth factor 1; HOMA-IR: homeostatic model assessment for insulin resistance; adiponectin, high-molecular weight adiponectin; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; hs-CRP, high-sensitivity C-reactive protein (values over 10 excluded)

Number of boys (n) varies from 219 to 245 in different variables; n= 245: BMD, lean mass, body fat mass; n=241: birth weight SDS; n=219: 25(OH)D; n=229: DHEAS, IGF-1; n=240: insulin; n=237: HOMA-IR; n=238: adiponectin, leptin, leptin receptor; n=228: irisin; n=238: IL-6; n=239: TNF-α; n=239: hs-CRP (values over 10 excluded).