Relation between hormones and body composition, including bone, in prepubertal children[1–3]

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ABSTRACT

Background: Sex differences in body composition exist before puberty, but the reason for this phenomenon is unknown. The physical changes that occur during puberty are mediated, in part, through sex steroids, insulin-like growth factor I (IGF-I), and leptin. However, data are lacking that address the extent to which concentrations of these hormones influence body composition, bone mass, and density in prepubertal children.

Objective: We investigated the effects of IGF-I, dehydroepiandrosterone sulfate, and sex steroids on body composition and fat distribution and the effects of these hormones and leptin on total body bone mineral content (TBMC) and volumetric bone mineral density (vBMD) at the femoral neck and lumbar spine (LS) in 255 healthy children (137 girls), aged 7–8 y.

Design: Body composition, fat distribution, TBMC, and vBMD were derived by using dual-energy X-ray absorptiometry. Association between variables was examined by using regression analysis.

Results: No sex differences were found in age, height, or weight. However, girls had significantly more total body fat, trunk fat, and higher LS vBMD but significantly less fat-free soft tissue, TBMC, and femoral neck vBMD than did boys. Girls also had significantly ($P < 0.001$) higher IGF-I, estradiol, testosterone, and leptin concentrations than did boys. Estradiol concentrations predicted percentage body fat, which supported an effect of estrogen on fat storage. Leptin had an independent effect on LS vBMD, which suggests a positive effect for leptin on trabecular bone.

Conclusions: The hormones examined explained 3–17% of the variations in body-composition measures, fat distribution, and bone density, which suggests that other factors are important predictors of prepubertal sexual dimorphism. *Am J Clin Nutr* 2004;80:966–72.

KEY WORDS Body composition, fat distribution, bone density, estradiol, testosterone, leptin, prepubertal children

INTRODUCTION

Significant sex differences in body composition are evident well before the onset of puberty. Prepubertal girls generally have higher total body fat and percentage body fat but lower fat-free mass (1, 2) and total-body bone mineral content (TBMC; 3, 4) than do boys matched for age, weight, and height. Fat distribution also differs between sexes: prepubertal girls generally have greater trunk fat than do boys (5, 6), although the results are conflicting (7, 8). The reason for the prepubertal sexual dimorphism in body composition is unknown.

The physical changes that occur during puberty, including accelerated growth and the changes in fat, muscle, and bone mass, are mediated, at least in part, by steroids, insulin-like growth factor I (IGF-I), and leptin. Estrogen and testosterone concentrations gradually increase from midchildhood, before the external signs of puberty are obvious, with differences between sexes being observed (9–11). During puberty, circulating estrogen is thought to favor fat storage, particularly in peripheral adipose tissue, and, at low concentrations, to increase calcium absorption and decrease bone turnover (12, 13). Circulating testosterone favors the increase in lean tissue mass and trunk adipose tissue (12). However, there is a lack of data on the extent to which the low concentrations of sex steroids might influence body composition in prepubertal children.

Leptin concentrations are directly associated with fat mass as well as increased bone mineral (14). The association between fat and bone mass and density was attributed to a combination of mechanical load exerted on the skeleton by the fat mass and the effect of hormones produced by fat cells, including estradiol and leptin (15). In children and adolescents, evidence is limited of an independent association between leptin and bone mass and density. The only pediatric study that involved both healthy girls and boys included 18 prepubertal children and did not support an independent role for leptin on bone mineral density (BMD; 16). Other studies were unrepresentative of children or involved only a small number of girls (17–19). Thus, the role of leptin on bone in prepubertal children is currently unresolved.

We hypothesized that similar relations between sex steroids and body composition to those seen in puberty exist during childhood and that leptin concentrations might have an independent effect on bone. To test the hypotheses we conducted a cross-sectional study of 255 healthy 7- and 8-y-old children to investigate *1*) the role of IGF-I, dehydroepiandrosterone sulfate (DHEAS), estradiol, and testosterone concentrations on body

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composition and fat distribution and *2*) the role of these hormones and leptin concentrations on TBMC, lumbar spine (LS) volumetric BMD (vBMD), and femoral neck (FN) vBMD.

SUBJECTS AND METHODS

Two hundred fifty-five healthy 7- and 8-y-old children (137 girls) were recruited for the study. The children were participants in the longitudinal Nepean Study that was designed to investigate the effect of birth size, body size, and genes on blood pressure and bone mass. All were born at term at Nepean Hospital (Penrith, NSW, Australia) between August 1989 and April 1990 and were part of a cohort whose birth details and selection criteria were previously published (20). The children were predominately (>96%) of European descent. Written consent was obtained from their parents, and both The Children's Hospital at Westmead Ethics Committee and the Ethics Committee of the Wentworth Area Health Service approved the study.

Anthropometric measurements

Height was measured with use of a Harpenden stadiometer (Holtain Ltd, Crymych, United Kingdom) to the nearest 0.1 cm with use of a standard technique, and weight was measured with minimal clothing to the nearest 0.1 kg with Detecto electronic scales (Detecto Scale Co, Webb City, MO). Body mass index was calculated as weight (in kg) divided by height (in m^2). Height, weight, and body mass index were calculated as *z* scores from age- and sex-specific reference values (21). Waist circumference was measured with a flexible steel tape at the level of the narrowest point between the lower costal border and the iliac crest. If no obvious narrowing was observed, the measurement was taken at the midpoint between the 2 landmarks (22). Puberty was not formally assessed, but the children were viewed in undergarments during skinfold assessment (8 sites), and none was overtly pubertal.

Body composition, bone mass, and density

TBMC, fat-free (FF) soft tissue, and total body fat were measured with use of dual-energy X-ray absorptiometry (DXA; LUNAR DPX; Lunar Corp, Madison, WI) equipped with adult, proprietary software, version 3.6. Adult software was used because it was considered to be the most appropriate software for the body weight of all the subjects. The fast scan mode and standard subject positioning were used for total body measurements, and they were analyzed with use of the extended analysis option. TBMC:FF soft tissue was calculated to assess the muscle bone unit (23). The standard manufacturer's skeletal landmarks were used to define trunk and leg fat. Care was taken to ensure that soft tissue was delineated into the appropriate regions. Manual analysis, with use of the "Regions Of Interest" feature, was performed on total body scans to gain specific information about the abdominal region that was defined by anatomic bony landmarks. The upper border was defined as the distal margin of the lower ribs, and the lower border was just superior to the suprailiac crest. The lateral margins were placed outside the body so that all abdominal but no arm tissue was included (20, 24). Percentage body fat was calculated as DXA-measured fat mass divided by the sum of soft tissue and TBMC. Abdominal fat was expressed as a percentage of total body fat (abdominal fat %). The ratio of trunk fat (in g) to leg fat (in g) was calculated.

Separate scans of the LS (L1–L4) and FN were made with use of the slow scan mode. The vBMD (in g/cm^3) was calculated as TBMC/estimated volume. Detailed methodology was described previously (25). Long-term quality control was performed on the DPX with use of an in-house total body phantom (aluminum and rice) and the LUNAR spine phantom. The mean precision for the machine over the period of the study was 3.7% for TBMC, 1.3% for soft tissue, and 1.3% for LS vBMD.

Biochemistry

Morning blood samples were obtained after an overnight fast by standard venipuncture technique. Plasma was frozen at −20 °C until assayed. All hormone determinations were performed by the endocrine laboratory of the Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Australia.

Radioimmunoassays were used to measure IGF-I, DHEAS, estradiol, testosterone, and leptin. The plasma samples were batched and measured in duplicate. To minimize variability between assays, each specific assay was performed by one operator with use of the same batch of reagents. Both DHEAS and testosterone were determined with use of in-house competitive binding radioimmunoassay. Commercial radioimmunoassay kits were used to determine concentrations of IGF-I (Bioclone Australia Pty Ltd, Sydney), estradiol (DiaSorin, Saluggia, Italy), and leptin (Linco Research Inc, St Charles, MO). The estradiol assay was modified to enhance sensitivity to 8.1 pmol/L. The modifications to the standard assay protocol were the following: *1*) the 2 lowest kit standards were diluted with zero standard to give standards of values 9.2, 18.4, 36.7, 73.4, and 146.8 pmol/L, and *2*) preincubation of samples or standards with the antiserum was done at 37 °C for 2 h. Tracer was then added with a further incubation at 37 °C for 2 h. The interassay and intraassay CVs (respectively) were IGF-I, 7.1% and 5.4%; DHEAS, 9.4% and 6.3%; estradiol, 8.4% and 5.1%, testosterone, 7.2% and 4.3%; and leptin, 6.6% and 5.5%. Hormone concentrations consistent with prepuberty in the laboratory of the Institute of Endocrinology and Diabetes are DHEAS, <1.5 μmol/L; estradiol, <50 pmol/L; and testosterone, <1.0 nmol/L.

Two girls and 9 boys had DHEAS concentrations below the minimum detection concentration (<0.1 μmol/L). Four girls and 5 boys had testosterone concentrations below the minimum detection concentration (<0.4 nmol/L). An additional girl did not have enough blood collected for testosterone analysis.

Statistical analysis

Data were analyzed and assessed for normality with use of SPSS software (version 11.5; SPSS Institute Inc, Chicago). Differences between girls and boys were assessed by Student's *t* test if data were normally distributed and otherwise by Mann-Whitney *U* test. Associations between variables were assessed with use of curve estimation. On the basis of *F* values and significance, linear associations were found to be the best fit. Data were analyzed by both correlation analysis (Spearman ρ) and multiple linear regression. IGF-I, DHEAS, estradiol, testosterone, and leptin were initially tested in the models. Sex (girls = 0, boys = 1) was added as the final variable. Variables entered were selected on the basis of *F* values and significance. Age was not considered as an independent variable because it is linearly related to hormone concentrations. Indicators for collinearity were

TABLE 1
Anthropometric and body-composition characteristics of the children in the study[1]

	Girls (n = 137)	Boys (n = 118)	P
Age (y)	7.8 ± 0.6	7.9 ± 0.6	0.25[2]
Height (cm)	127.2 ± 6.0	128.6 ± 7.1	0.07[2]
Height z score	0.34 ± 0.9	0.45 ± 1.2	0.38[2]
Weight (kg)	27.4 ± 5.3	27.8 ± 5.8	0.51[3]
Weight z score	0.58 ± 1.1	0.56 ± 1.4	0.90[2]
BMI (kg/m^2)	16.9 ± 2.3	16.7 ± 2.5	0.33[3]
BMI z score	0.16 ± 1.0	0.04 ± 1.1	0.34[2]
Total body fat (kg)	6.58 ± 3.55	5.12 ± 3.81	<0.001[3]
Percentage body fat (%)	23.6 ± 8.4	17.4 ± 8.6	<0.001[3]
Fat-free mass (kg)	19.02 ± 2.56	20.90 ± 3.02	<0.001[3]
Trunk fat (kg)	2.63 ± 1.66	1.91 ± 1.17	<0.001[3]
Trunk fat:leg fat	0.90 ± 0.22	0.82 ± 0.23	0.007[2]
Abdominal fat (%)	5.30 ± 1.15	4.97 ± 1.09	0.017[3]
Waist circumference (cm)	56.5 ± 5.3	58.0 ± 5.9	0.044[2]

[1] All values are $\bar{x}$ ± SD.
[2] Student's t test.
[3] Mann-Whitney U test.

examined, and residuals of final models were normally distributed. Only variables with a significance value $P < 0.05$ were included in the final models. Predictive regression models are presented, explaining the variance in body composition and bone measures.

RESULTS

No sex difference was found in age, height, or weight. Girls had significantly higher total body fat, percentage body fat, trunk fat, trunk fat:leg fat, and abdominal fat percentage but significantly lower FF soft tissue and waist circumference than did boys. On average, girls had 1.5 kg more body fat and 1.9 kg less FF soft tissue than did boys (**Table 1**).

TBMC, total bone area, and FN vBMD were significantly lower and LS vBMD was significantly higher in girls than in boys **(Figure 1**). IGF-I, estradiol, testosterone, and leptin concentrations were significantly higher in girls than in boys **(Figure 2**). The sex difference in leptin concentrations remained after adjustment for total body fat [geometric $\bar{x}$ (95% CI): girls, 3.39 (3.16, 3.64); boys, 2.25 (2.08, 2.42); $P < 0.001$] or percentage body fat [girls, 3.07 (2.86, 3.29); boys, 2.41 (2.23, 2.60); $P < 0.001$]. No significant sex difference was seen in DHEAS.

Correlation analysis

Correlation coefficients between measures of body composition, fat distribution, bone measures, and independent variables are shown in **Table 2**. FF soft tissue, percentage body fat, and TBMC were significantly correlated with height, IGF-I, DHEAS, testosterone, and leptin with ρ values ranging from 0.187 to 0.803. Estradiol concentrations were correlated with percentage body fat but not with FF soft tissue. Similar results were seen when the sexes were examined separately (results not shown).

Abdominal fat percentage, but not trunk fat:leg fat, was correlated with IGF-I, estradiol, and testosterone concentrations. When the sexes were examined separately, the correlation between abdominal fat percentage and estradiol concentrations was significant only in the boys (boys: $\rho = 0.33$, $P < 0.001$; girls: $\rho = -0.06$, $P = 0.49$), and the correlations between abdominal fat percentage and IGF-I and testosterone concentrations were not significant in either girls or boys.

LS vBMD was positively correlated with IGF-I, estradiol, testosterone, and leptin concentrations. The relation between LS vBMD and leptin concentrations was stronger in girls ($\rho = 0.27$, $P = 0.001$) than in boys ($\rho = 0.17$, $P = 0.066$). Estradiol and testosterone concentrations were also correlated with LS vBMD but to a lesser extent, and the correlations were not significant when the sexes were examined separately. FN vBMD was significantly correlated with FF soft tissue (positive association) and IGF-I (negative association) but not with estradiol, testosterone, or leptin concentrations.

Multiple regression models

Multiple regression models were developed to examine the effect of *1*) IGF-I, DHEAS, estradiol, and testosterone concentrations on FF soft tissue, percentage body fat, and 2 measures of fat distribution (trunk fat:leg fat and abdominal fat percentage)

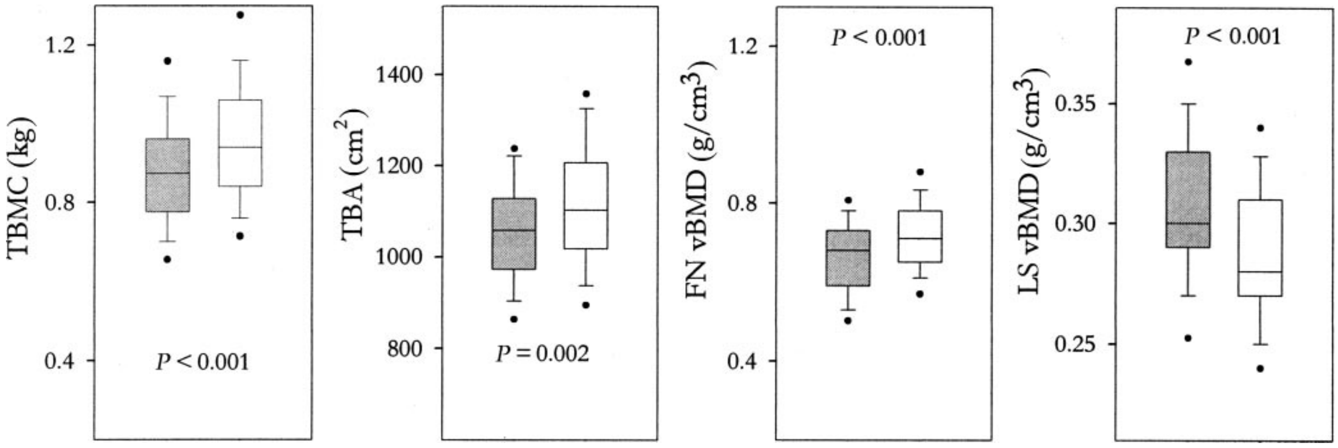

FIGURE 1. The bone characteristics of the study subjects (girls: n = 137, ▩; boys: n = 118, □). Box plots represent median and 10th, 25th, 75th, and 90th centiles. The 5th and 95th centiles are shown as dots. P values were determined by Student's t test. TBMC, total bone mineral content; TBA, total bone area; FN vBMD, femoral neck volumetric bone mineral density; LS vBMD, lumbar spine volumetric bone mineral density.

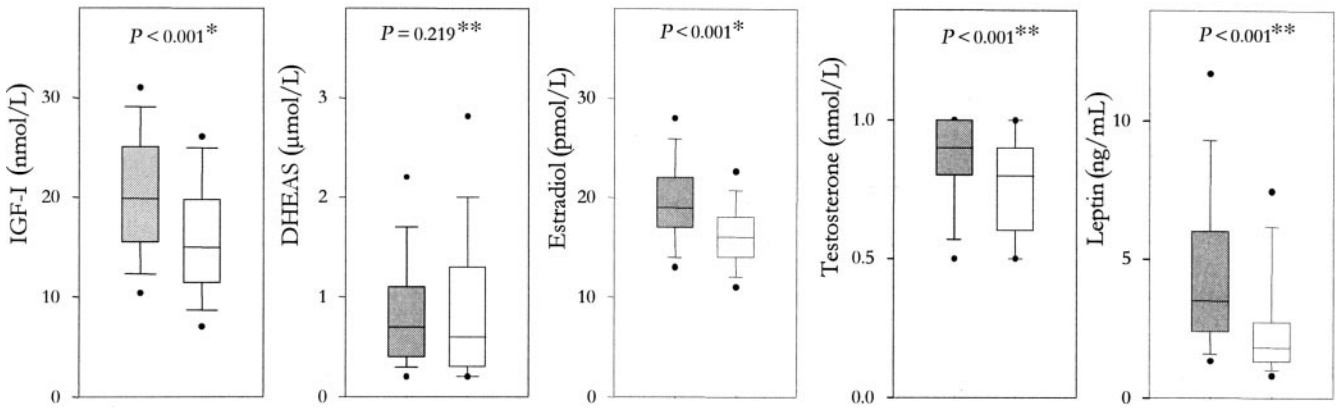

FIGURE 2. The hormone profile of the study subjects (girls: $n = 137$, ▩; boys: $n = 118$, □). Box plots represent median and 10th, 25th, 75th, and 90th centiles. The 5th and 95th centiles are shown as dots. Two girls and 9 boys had dehydroepiandrosterone sulfate (DHEAS) concentrations below the detectable minimum (<0.1 µmol/L). Four girls and 5 boys had testosterone concentrations below minimum detection concentration (< 0.4nmol/L). An additional girl did not have enough blood collected for testosterone analysis. IGF-I, insulin-like growth factor I. *Student's *t* test, **Mann-Whitney *U* test.

and *2*) IGF-I, DHEAS, estradiol, testosterone, and leptin concentrations on TBMC, total bone area, LS vBMD, and FN vBMD. Predictive models are shown in **Table 3**.

Fat-free soft tissue and percentage body fat

IGF-I and DHEAS predicted 10% of the variation in FF soft tissue, and IGF-I and estradiol predicted 17% of the variation in percentage body fat. When sex was included as an independent variable, the variation explained by the model increased to 27% for FF soft tissue and 20% for percentage body fat, which indicated an effect for other sex-related factors on FF soft tissue and percentage body fat.

Trunk fat:leg fat and abdominal fat percentage

Sex alone predicted 2% of the variation in the trunk fat:leg fat. The only significant predictor of abdominal fat percentage was testosterone, which again explained a small amount (4%) of the variation. Total body fat was not tested in these equations because it is closely associated with the outcome variables.

Total body bone mineral content and total bone area

The significant sex differences in TBMC remained after adjustment for height ($P = 0.001$), but no sex difference was seen in TBMC:FF soft tissue ($\bar{x}$ (± SD): girls, 0.046 ± 0.005; boys, 0.046 ± 0.004; $P = 0.265$). The best predictive model, explaining 73% of the variation in TBMC, included FF soft tissue and total body fat. The relation between FF soft tissue, total body fat, and TBMC was the same for both girls and boys (ie, the slope of the regression line was the same). FF soft tissue had ≈4 times the effect of total body fat. Thus, for every 1-kg increase in FF soft tissue, there was a 42-g increase in TBMC, and for every 1-kg increase in total body fat, there was a 10-g increase in TBMC. Body composition was the important determinate of TBMC in these 7- and 8-y-old children. Similar to TBMC, FF soft tissue and total body fat predicted 84% of the variation in total bone area (in cm^2) (Table 3).

Femoral neck volumetric bone mineral density and lumbar spine volumetric bone mineral density

Both total body fat and leptin concentrations were independently associated with LS vBMD. Because these 2 variables are highly correlated ($\rho = 0.80$, $P < 0.001$), they could not be considered together in the same model. On the basis of F values and significance, leptin concentrations were stronger predictors of LS vBMD than was total body fat. Together, leptin concentrations and sex predicted 13% of the variation in LS vBMD. The

TABLE 2
Correlation coefficients (ρ) between body composition, bone measures and independent variables[1]

	Height	Fat-free soft tissue	Total body fat	IGF-1	DHEAS	Estradiol	Testosterone	Leptin
	cm	*kg*	*kg*	*nmol/L*	*µmol/L*	*pmol/L*	*nmol/L*	*ng/mL*
Fat-free soft tissue (kg)	0.800[2]		0.387[2]	0.223[2]	0.243[2]	−0.072	0.187[3]	0.166[3]
Total body fat (kg)	0.459[2]	0.387[2]		0.403[2]	0.249[2]	0.259[2]	0.262[2]	0.803[2]
Percentage body fat (%)	0.291[2]	0.175[3]	0.971[2]	0.377[2]	0.210[3]	0.294[2]	0.240[2]	0.822[2]
Trunk fat:leg fat	0.035	0.067	0.467[2]	0.161[4]	0.07	0.112	0.056	0.399[2]
Abdominal fat (%)	0.031	0.047	0.517[2]	0.178[3]	0.159[4]	0.166[3]	0.139[4]	0.445[2]
Total-body BMC (kg)	0.733[2]	0.778[2]	0.494[2]	0.192[3]	0.254[3]	−0.036	0.188[3]	0.278[2]
Lumbar spine vBMD (g/cm^3)	0.032	−0.010	0.270[2]	0.133[4]	0.046	0.141[4]	0.143[4]	0.348[2]
Femoral neck vBMD (g/cm^3)	0.165[3]	0.299[2]	−0.010	−0.155[4]	−0.004	−0.044	0.012	−0.094

[1] IGF-I, insulin-like growth factor I; DHEAS, dehydroepiandrosterone sulfate; BMC, bone mineral content; vBMD, volumetric bone mineral density.
[2] $P < 0.001$.
[3] $P < 0.01$.
[4] $P < 0.05$.

TABLE 3
Predictive models explaining the variance in body-composition and bone measures[1]

Variables	Coefficient	P	R^2 change
Fat-free soft tissue[2]			
IGF-I (nmol/L)	0.15 ± 0.03	<0.001	0.05
DHEAS (μmol/L)	0.76 ± 0.22	0.001	0.05
Sex	2.59 ± 0.34	<0.001	0.17
	Final adjusted R^2		0.27
Percentage body fat[3]			
IGF-I (nmol/L)	0.38 ± 0.08	<0.001	0.14
Estradiol (pmol/L)	0.25 ± 0.12	0.045	0.03
Sex	−3.56 ± 1.14	0.002	0.03
	Final adjusted R^2		0.20
Trunk fat:leg fat[4]			
Sex	−0.08 ± 0.03	0.007	0.02
	Final adjusted R^2		0.02
Abdominal fat[4]			
Testosterone (nmol/L)	0.72 ± 0.25	0.004	0.04
Sex	−0.19 ± 0.15	0.185	0
	Final adjusted R^2		0.04
Total-body BMC[5]			
Fat-free soft tissue (kg)	41.65 ± 1.95	<0.001	0.69
Total body fat (kg)	10.34 ± 1.53	<0.001	0.04
Sex	12.43 ± 12.05	0.303	0
	Final adjusted R^2		0.73
Total bone area[5]			
Fat-free soft tissue (kg)	38.29 ± 1.43	<0.001	0.79
Total body fat (kg)	8.44 ± 1.08	<0.001	0.05
Sex	−5.54 ± 7.96	0.487	0
	Final adjusted RM^2		0.84
Lumbar spine vBMD[6]			
Leptin (ng/mL)	0.003 ± 0.001	<0.001	0.09
Sex	−0.01 ± 0.004	0.001	0.04
	Final adjusted R^2		0.13
Femoral neck vBMD[6]			
Fat-free soft tissue (kg)	0.006 ± 0.002	0.006	0.04
IGF-I (nmol/L)	−0.002 ± 0.001	0.020	0.04
Sex	0.031 ± 0.014	0.025	0.02
	Final adjusted R^2		0.10

[1] IGF-I, insulin-like growth factor; DHEAS, dehydroepiandrosterone; vBMD, volumetric bone mineral density; BMC, bone mineral content. All interactions were tested but were not significant in the models.

[2–6] Variables tested in model: [2] IGF-I, DHEAS, testosterone, and sex (girls = 0, boys = 1); [3] IGF-I, DHEAS, estradiol, and sex; [4] IGF-I, DHEAS, testosterone, estradiol, and sex; [5] height, fat-free soft tissue, total body fat, IGF-I, DHEAS, estradiol, testosterone, leptin, and sex; [6] fat-free soft tissue, total body fat, IGF-I, DHEAS, estradiol, testosterone, leptin, and sex.

best predictive model for FN vBMD, explaining 10% of the variation, included FF soft tissue, IGF-I, and sex.

DISCUSSION

To our knowledge, this is the largest study of healthy prepubertal children to investigate the role of IGF-I, DHEAS, sex steroids, and leptin concentrations on body composition, fat distribution, and bone density. Even though no sex differences were seen in age, height, or weight, girls had significantly more total body fat and abdominal fat, higher LS vBMD, and significantly less FF soft tissue, TBMC, and FN vBMD than did boys. Girls also had significantly higher IGF-I, estradiol, testosterone, and leptin concentrations than did boys. Estradiol concentrations were predictive of percentage body fat, which supported an effect for estrogen on fat storage. The independent effect of leptin concentration on LS vBMD suggests a positive effect for leptin on trabecular bone.

Predictors of body composition and fat distribution

In agreement with previous reports in prepubertal children (1, 2, 6, 7), girls in this study had significantly more total body fat and less FF soft tissue than did boys. Girls also had significantly higher estradiol and testosterone concentrations than did boys. We hypothesized that differences in sex steroids would explain the sexual dimorphism in body composition. Estradiol concentrations predicted, in part, percentage body fat, whereas testosterone did not explain the sex differences in FF soft tissue. During puberty, circulating estrogen is thought to favor fat storage, particularly in peripheral adipose tissue, and circulating testosterone favors the increase in lean tissue mass and trunk adipose tissue (12).

In the current study, girls also had lower total bone area and waist circumference but higher trunk fat, trunk fat:leg fat, and abdominal fat percentage than did boys. We speculate that this difference indicates a smaller frame for the girls. Increased abdominal fat, measured by computed tomography scanning, in prepubertal girls compared with boys was previously reported (6). We observed a positive association between testosterone concentrations and abdominal fat percentage. Increased abdominal fat is associated with insulin resistance and an androgenic profile, a clinical condition observed more frequently in prepubertal girls than in prepubertal boys (26). The long-term metabolic implications of increased abdominal fat in the prepubertal period might be limited because striking changes in fat distribution occur during puberty.

IGF-I was positively associated with both FF soft tissue and percentage body fat, as previously described by our group (27). IGF-I is known to have strong anabolic effects, promoting protein synthesis and increases in FF soft tissue and fat oxidation. The observation that girls had higher IGF-I concentrations, higher total body fat, and less FF soft tissue than did boys might be counterintuitive. The equation predicting FF soft tissue indicated that boys had higher ($\bar{x}$: 2.6 kg) FF soft tissue than did girls after adjustment for IGF-I and DHEAS concentrations. In contrast, boys had a lower ($\bar{x}$: 3.6%) percentage fat after adjustment for IGF-I and estradiol concentrations. Nevertheless, the best models explaining FF soft tissue and percentage body fat explained only 27% and 20%, respectively. The hormones examined—IGF-I, DHEAS, estradiol, and testosterone—are only 4 of many factors, including hormonal interaction, genetics, nutrition, and physical activity, that could have a significant influence on determining body composition.

The time of life when sex differences in body composition first occur is currently unknown. Differences were reported in children as young as 3 y old and could present during infancy (1, 28, 29). It was suggested that the surge of sex steroid secretion observed during the first few months of life could have a potential role in increasing fat and muscle development during infancy (13). Alternatively, body-composition differences could be mediated by nonhormonal sex-specific factors rather than by sex steroids. Support for this explanation comes from a cross-sectional study that found fat distribution in children aged 5–12 y did not change with age after adjustment for body size, despite the presumed increase in sex steroids (7).

Predictors of bone mass and density

This is the first study to report an independent effect of leptin concentrations on LS vBMD in healthy prepubertal children, supporting previous smaller studies in pubertal girls and obese children (17, 18). The fat mass–independent effect of leptin was observed on the metabolically more active trabecular bone at the LS than at the FN, a predominantly cortical site. A greater influence of leptin on trabecular bone than on cortical bone was also reported in an animal model (30).

However, the role of leptin in human bone remodeling is not well defined (31), and not all studies in children support these findings. Roemmich et al (16) reported no association between leptin concentrations and LS BMD, but that study involved 59 children ranging in age from 12 to 18 y and included only a small number of prepubertal children. The strength of our study is the large number of children within a narrow age range. Consistent with previous studies in children, we did not find an independent effect of leptin on TBMC or FN vBMD (16–18).

Cortical bone, which accounts for 85% of the skeleton and 75% of the bone at the FN, might be more responsive to body size and body-composition measures than is trabecular bone (32), a concept supported by the results presented (Table 3). IGF-I is also associated with increased body size as well as being an important determinant of cortical bone mass (33). A positive association between IGF-I and TBMC was noted in this study. However, we report a negative association between IGF-I and FN vBMD. Children with lower FN vBMD had higher IGF-I concentrations and increased FN volume, which supports the hypothesis that IGF-I is a determinant of cortical bone mass but not cortical bone density (33, 34).

Estradiol concentrations did not predict bone mass or density. During puberty, girls accrue more bone mass than do boys for a given muscle mass (23, 35), which was attributed to the effect of estrogen (36). In those previous studies, no sex difference in bone mass was apparent before puberty. We speculate that the combined increase in estradiol and leptin concentrations might contribute to the higher bone mass and density accrued during puberty.

Previous reports of sex differences in TBMC in prepubertal children were inconsistent (3, 4, 37). We noted a significantly lower TBMC and total bone area in girls than in boys. The sex difference remained significant after adjustment for height, but the difference was not significant after adjustment for the main predictors, FF soft tissue and total body fat, nor was there a significant sex difference in the TBMC:FF soft tissue. Body composition, not height, was the important determinate of TBMC in these 7- and 8-y-old children. In contrast to bone mass, sex differences were reported in vBMD measured by DXA in prepubertal children; there was higher LS vBMD and lower FN vBMD in girls than in boys (3, 38). Our results confirm these findings from DXA studies. Differences in imaging techniques and statistical modeling, as well as small prepubertal sample sizes, might be the cause of inconsistencies between studies.

A potential limitation of the study was the low concentrations of DHEAS, estradiol, and testosterone that were observed. Although the estradiol assay was modified to increase sensitivity, it would not be as sensitive as other reported methods (11), and subtle changes in hormone concentrations might be missed. Another potential limitation to the study is that DXA, a two-dimensional technique, was used to measure body composition and areal BMD, and vBMD was calculated. Nevertheless, DXA was validated as a measure of body composition (39, 40). However, we can think of no reason why there would be a systematic sex bias in hormone assays, body composition, or bone measurements.

A considerable body of evidence now exists to support sexual dimorphism in body composition, fat distribution, and bone density in prepubertal children. Significant sex differences in estradiol, testosterone, and leptin concentrations are also evident before the external signs of puberty appear. Estradiol concentrations were predictive of percentage body fat, which supports an effect of estrogen on fat storage. The independent effect of leptin concentration on LS vBMD suggests a positive effect for leptin on trabecular bone. However, the hormones examined explained only 3–17% of the variation in body-composition measures, fat distribution, and bone density. Whether the observed sexual dimorphism in body composition in prepubertal children is also mediated by an earlier surge in sex steroid concentrations or is due to nonhormonal factors is yet to be determined.

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SPG participated in all aspects of this study and was primarily responsible for drafting the manuscript; WH participated in data interpretation and preparation of the manuscript; BB developed the estradiol assay and was responsible for the management and biochemical analysis (ie, IGF-I and estradiol) of blood samples; LAB participated in the study design and supervised the study implementation, data interpretation, and preparation of the manuscript; JP provided statistical support and participated in the preparation of the manuscript; JL was responsible for the management and biochemical analysis (ie, testosterone, DHEAS, and leptin) of blood samples; CTC participated in the study design and supervised the study implementation, data interpretation, and preparation of the manuscript. None of the authors had any conflict of interest.

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