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Bone, Muscle, and Fat: Sex-related Differences in Prepubertal Children[1]

PURPOSE: To determine whether there are sex-related differences in vertebral cross-sectional dimensions, in paraspinous muscle area, and in the amount of fat in the subcutaneous and visceral compartments of prepubertal boys and girls.

MATERIALS AND METHODS: Subcutaneous fat, visceral fat, paraspinous musculature, and vertebral cross-sectional dimensions were studied in 31 pairs of prepubertal healthy white girls and boys 5–10 years of age, rigorously matched for age, height, and weight. Data were analyzed with the Student *t* test and multiple regression analysis.

RESULTS: Sex had a differential effect on fat accumulation and musculoskeletal development. Compared with boys, girls had, on average, 28% greater total fat and 30% higher subcutaneous fat ($P < .001$ for both), but 10% less paraspinous musculature ($P = .002$) and 15% smaller vertebral cross-sectional dimensions ($P < .001$). In contrast, the sexes were monomorphic for visceral fat ($P = .24$). Stepwise regression analysis indicated that only 22% of the difference in vertebral cross-sectional area could be explained by sex-related differences in paraspinous musculature.

CONCLUSION: Together, these data indicate that sex is an important determinant of the morphology in humans well before the beginning of puberty.

Variations in the accumulation and distribution of fat and muscle between men and women are well documented and are important contributors to the different sexual characteristics of humans. The time of life when these differences first appear is currently unknown, although studies have shown that they are not apparent at birth (1–3) but are consistently present in adolescents (3–16). Unfortunately, in spite of numerous reports, the question of whether sex-related differences in body composition are evident prior to puberty is, as yet, unanswered. Researchers in studies (3,5,7–10,17,18) with use of skin calipers have consistently documented greater values for subcutaneous fat in girls than in boys, even prior to puberty.

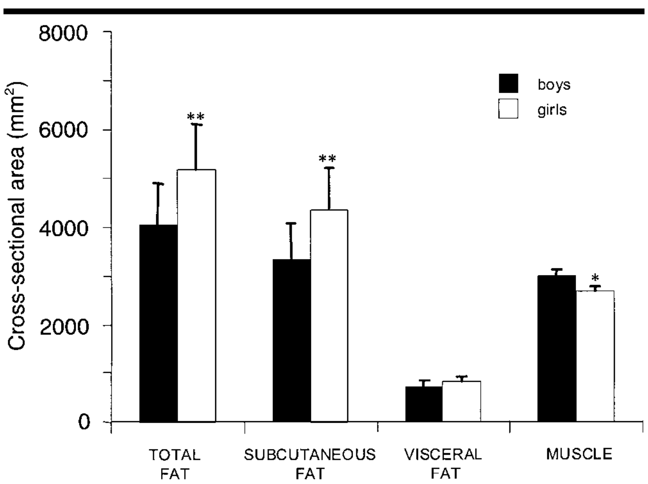
In contrast, two studies in which muscle and fat volume in the extremities of prepubertal children were measured by using conventional radiography yielded contradictory findings. In one study (19), investigators reported greater subcutaneous fat but similar muscle mass, whereas in the other study (11), researchers determined similar fat but less muscle in girls when they were compared with boys. Subsequent studies with dual x-ray absorptiometry to assess total body fat and muscle accumulation in prepubertal children also yielded conflicting results. Some investigators observed no sex-related differences in body composition (12,13), others reported that girls had more fat and similar muscle mass (4,6), and still others suggested that, compared with boys, girls had more fat but less muscle (20). Discrepancies in these results are most likely a reflection of the lack of rigorous control for the degree of sexual development and anthropometric variables and/or the limitations associated with small sample size. In addition, projection techniques, such as dual x-ray absorptiometry, do not allow for the three-dimensional assessment of body composition or the independent analysis of subcutaneous fat and visceral (intraabdominal) fat.

In adults, visceral fat is an important risk factor for cardiovascular disease and type 2 diabetes mellitus (21–24), and the relationship between this trait and adverse health, Radiology

TABLE 1
Chronologic Age, Bone Age, Anthropometric Measurements, and Dietary Intake in 31 Boys and 31 Girls Matched for Age, Height, and Weight

Clinical Data	Boys	Girls
Age (y)	8.05 ± 1.27	8.14 ± 1.21
Bone age (y)	8.10 ± 0.92	8.12 ± 1.10
Weight (kg)	28.62 ± 8.88	28.26 ± 8.03
Height (cm)	126.43 ± 8.80	126.20 ± 8.96
Body mass index (kg/m^2)	17.61 ± 3.12	17.59 ± 3.29
Body surface area (m^2)	1.01 ± 0.20	1.02 ± 0.21
Caloric intake (kcal)*	2,017 ± 286	1,985 ± 273
Dietary intake		
Protein (g)	90 ± 15	85 ± 13
Carbohydrates (g)	259 ± 44	247 ± 35
Fat (g)	69 ± 14	73 ± 16
Fiber (g)	4.6 ± 2.2	4.4 ± 2.4
Vitamin D (μg)	6.6 ± 4.3	6.7 ± 4.5
Calcium (mg)	1,061 ± 501	1,034 ± 486
Phosphorus (mg)	1,389 ± 417	1,278 ± 404
Sugar (g)	89 ± 26	87 ± 29
Magnesium (mg)	253 ± 71	236 ± 65
Sodium (mg)	3,068 ± 502	2,985 ± 461

Note.—All values are the mean ± SD.
* To convert to kilojoule, 1 kcal = 4.2 kJ.



Mean and SD values for CT measurements of abdominal fat and paraspinous musculature in 31 matched pairs of prepubertal children. Although there was a significant sexual dimorphism in subcutaneous fat, total fat, and muscle, no sex-related differences in visceral fat were present. * indicates $P = .002$, and ** indicates $P < .001$.

including dyslipidemia and glucose intolerance, also was noted in children (25,26). Regardless of race, visceral fat consistently was higher in men than in women (27–29), but the time of life when such differences first appear remains obscure.

In a previous investigation (30), quantitative computed tomography (CT) was used to examine sex-related differences in bone growth in prepubertal children, and the investigators determined that boys have a larger cross-sectional area of the vertebrae than have girls, even after accounting for differences in body size. In that study (30), trends toward greater measures for paraspinous musculature in boys and for abdominal fat in girls were also observed. The purpose of this study was to determine whether there are sex-related differences in paraspinous muscle volume and in the amount of fat in the subcutaneous and visceral compartments in prepubertal boys and girls.

MATERIALS AND METHODS

Study Subjects

The study subjects were healthy white prepubertal children who were recruited from schools of Los Angeles County. The investigational protocol for this study was approved by the institutional review board for clinical investigations at the Childrens Hospital Los Angeles, and informed consent was provided by all parents.

Possible candidates and/or their parents were asked about their age, ethnic background, and medical history in a telephone conversation. Candidates were excluded if they were younger than 5 years because they may not have been able to stay still during CT scanning and if they were older than 10 years because puberty may already have begun. Candidates were also excluded if either of their parents or either set of grandparents were not of the same race, if they had received a diagnosis of chronic illness, if they had been ill for more than 2 weeks during the previous 6 months, if they had taken any medications or vitamin preparations on a regular basis, or if they had been hospitalized at any time since birth.

A physical examination was performed to determine the stage of sexual development, and the grading system delineated by Tanner (31) was used for classification. Children in whom puberty (Tanner stage II or higher) began were excluded from the study. Measurements of height and weight were determined, and children in whom either height or weight differed by more than 2 SDs from the mean age-adjusted normal values were excluded from further evaluation (32). Body surface area and body mass index were calculated as previously described by other researchers (33).

Bone age was assessed on the basis of radiographs obtained on the same day as, but before, CT scans were obtained to determine the measurements. The radiographs were evaluated according to the method of Greulich and Pyle (34), and bone age was determined by one author (V.G.). Subjects in whom chronologic age and bone age differed by more than 1 year were excluded from the study. In the remaining subjects, measurements of fat, muscle, and bone were determined at CT, and these subjects were subsequently given instructions by a dietician for completing a written record of their food intake for the 3 days immediately following the CT examination (35).

Subjects were matched according to chronologic age, height, and weight to control for these important determinants of fat and muscle mass. Girls were enrolled and examined in this study before their male counterparts. Thereafter, boys were recruited, examined, and matched with girls who had been examined. For this analysis, the ages of each pair of subjects differed by less than 6 months, and neither height nor weight differed by more than 5%. With this approach, we examined 31 matched pairs of children who were 5–10 years old.

CT Measurements of Bone, Muscle, and Fat

All CT studies included the abdomen and were performed with the same scanner (model CT-T 9800; GE Medical Systems, Milwaukee, Wis), with the same reference phantom for simultaneous calibration, and with specially designed software for fat, muscle, and bone measurements. Identification of the sites to be scanned was performed with lateral scout views. After identification, cross-sectional images were obtained from the midportion of the first through the third lumbar vertebra with 80 kVp, 70 mA, and 2 seconds. The time required for the procedures was approximately 10 minutes, and the radiation exposure of this limited CT examination was 100–150 mrem (1.0–1.5 mSv). The examination was localized to the midportions of the first three lumbar vertebrae; the effective radiation dose was approximately 4 mrem (0.04 mSv) (36,37).

For the purposes of this study, *subcutaneous fat volume* was defined as the average amount of adipose tissues located between the skin and the rectus muscles of the abdomen, the external oblique muscles, the broadest muscle of the back, and the erector muscles of the spine at the midportions of the first, second, and third lumbar vertebrae. *Visceral fat area* was defined as the intraabdominal adipose tissue surrounded by the rectus muscles of the abdomen, the external oblique muscles, the lumbar quadrate muscle, the psoas muscles, and the lumbar spine at the midportions of the first three lumbar vertebrae and consists mainly of perirenal, pararenal, retroperitoneal, and mesenteric fat. *Total abdominal fat area* was defined as all subcutaneous and visceral adipose tissues at the midportions of the first three lumbar vertebrae.

Further, *paraspinous musculature volume* was defined as the sums of the volumes of the erector muscles of the spine, the psoas major muscle, and the quadratus lumbosum muscle at the midportions of the first three lumbar vertebrae. *Vertebral cross-sectional area* was defined as the mean cross-sectional dimensions of the first three lumbar vertebral bodies. *Vertebral height* was defined as the mean height of the first three lumbar vertebral bodies. *Vertebral cancellous bone density* was defined as the amount of bone and marrow in milligrams per cubic centimeter per pixel, the CT unit of measurement at the midportion of the first three lumbar vertebrae. The coefficients of variation for repeated measurements for each of these traits were determined by using measurements on CT scans obtained in three children to calculate the values, and they ranged from 1.5% to 3.5%.

TABLE 2
Values for Fat, Muscle, and Bone Calculated from CT Scans in 31 Boys and 31 Girls Matched for Age, Height, and Weight

Value	Boys*	Girls*	*P* Value
Total fat (mm^2)	4,044 ± 4,732	5,165 ± 5,268	<.001
Subcutaneous fat (mm^2)	3,325 ± 4,134	4,361 ± 4,582	<.001
Visceral fat (mm^2)	720 ± 646	803 ± 765	<.241
Paraspinous musculature (mm^2)	2,977 ± 741	2,676 ± 461	<.002
Vertebral cross-sectional area (mm^2)	663 ± 114	565 ± 82	<.001
Vertebral cancellous bone density (mg/cm^3)	156 ± 26	152 ± 21	<.555
Vertebral height (mm)	17 ± 1.5	17 ± 1.8	<.118

* Data are the mean ± SD.

Sample Size, Power Calculations, and Statistical Analysis

Power calculations for sample size determinations to assess sex-related differences in muscle and fat volume were based on previous data showing that the average values for paraspinous musculature were 23.1 cm^2 ± 6.2 and 20.7 cm^2 ± 3.7 for boys and girls, respectively, in a cohort of 28 prepubertal children (30). A sample size of 31 boys and 31 girls was calculated to be sufficient to detect a significant sex-related difference in muscle area with an 80% power at a *P* value of .05 level of significance. Because findings in previous studies suggested greater sex-related differences in fat than in muscle, a sample size of 31 matched pairs was also considered sufficient power to depict any dimorphism in fat between boys and girls.

The values for bone, muscle, and fat measured by using CT represent the mean of values calculated from scans obtained at L1, L2, and L3 vertebral levels, and those for carbohydrates, fat, protein, vitamins, and minerals represent the mean of the three daily determinations. The data were analyzed with the Student *t* test for paired samples, Pearson correlation, and multiple regression analysis. A significance level of *P* less than .05 was used for all comparisons.

RESULTS

By design, there were no differences in the chronologic age, height, or weight of the 31 matched pairs of prepubertal children. In addition, body surface area, body mass index, bone age, and nutritional intake did not differ between groups (Table 1). When all children were considered together, weak positive correlations were demonstrated between caloric intake and values for fat and muscle ($r = 0.11–0.31$). There were, however, no significant correlations between caloric or calcium intake and any bone trait.

Sex influenced CT measurements of total and subcutaneous fat, paraspinous musculature, and vertebral cross-sectional area. In contrast, differences in visceral fat were not significant ($P = .24$). On average, girls had 28% and 30% greater total ($P < .001$) and subcutaneous ($P < .001$) fat, respectively, and 10% and 15% less paraspinous musculature ($P < .002$) and vertebral cross-sectional area ($P < .001$), respectively, than boys (Figure). Sex, however, had no effect on values for vertebral cancellous bone density or vertebral height calculated from CT scans (Table 2).

Table 3 shows the correlations between all phenotypes in boys and girls. Age and anthropometric measurements correlated strongly with CT values for all traits except vertebral cancellous bone density; the strongest correlations were observed between weight and the values of fat and muscle and between standing height and vertebral height and vertebral cross-sectional area. Strong correlations were observed between the cross-sectional area of the vertebrae and the area of the adjacent paraspinous musculature.

To examine the relationship between the sexual dimorphism in bone and that in muscle size, a stepwise forward multiple regression analysis was performed.

TABLE 3
Correlations Between Age, Bone Age, and Anthropometric Measurements and Values for Fat, Muscle, and Bone Calculated from CT Scans in 31 Boys and 31 Girls Matched for Age, Height, and Weight

Data and Subjects	Age	Bone Age	Weight	Height	Total Fat	Subcutaneous fat	Visceral Fat	Paraspinous Musculature	Vertebral Cross-sectional Area	Vertebral Height
Boys										
Age	1.00	0.98	0.60	0.74	0.33	0.32	0.39	0.64	0.56	0.73
Bone age		1.00	0.67	0.79	0.42	0.40	0.48	0.69	0.61	0.74
Weight			1.00	0.86	0.87	0.86	0.86	0.89	0.75	0.70
Height				1.00	0.59	0.57	0.64	0.87	0.85	0.88
Total fat					1.00	1.00	0.94	0.65	0.50	0.38
Subcutaneous fat						1.00	0.91	0.63	0.49	0.36
Visceral fat							1.00	0.69	0.59	0.50
Paraspinous musculature								1.00	0.86	0.75
Vertebral cross-sectional area									1.00	0.68
Vertebral height										1.00
Girls										
Age	1.00	0.98	0.73	0.81	0.54	0.55	0.39	0.57	0.69	0.75
Bone age		1.00	0.68	0.80	0.46	0.48	0.31	0.53	0.68	0.73
Weight			1.00	0.85	0.90	0.91	0.74	0.76	0.79	0.68
Height				1.00	0.62	0.63	0.46	0.80	0.80	0.88
Total fat					1.00	1.00	0.91	0.60	0.62	0.49
Subcutaneous fat						1.00	0.88	0.60	0.63	0.50
Visceral fat							1.00	0.55	0.54	0.40
Paraspinous musculature								1.00	0.81	0.77
Vertebral cross-sectional area									1.00	0.77
Vertebral height										1.00

TABLE 4
Anthropometric Measurements and Values for Fat, Muscle, and Bone Calculated from CT Scans in Matched Pairs of Children in Early and Late Prepubertal Periods

	Early Prepubertal Period			Late Prepubertal Period		
	Boys (n = 16)*	Girls (n = 16)*	P Value	Boys (n = 15)*	Girls (n = 15)*	P Value
Age	6.9 ± 0.81	7.1 ± 0.78	.12	9.0 ± 0.46	9.0 ± 0.70	.79
Weight	24.6 ± 5.89	24.8 ± 6.05	.49	32.2 ± 9.07	32.1 ± 8.97	.58
Height	121.48 ± 6.69	120.62 ± 4.98	.36	130.94 ± 7.04	131.06 ± 7.26	.88
Total fat	3,019 ± 3,297	3,917 ± 4,259	.02	5,068 ± 5,981	6,596 ± 6,249	.004
Subcutaneous fat	2,462 ± 2,953	3,189 ± 3,799	.02	4,194 ± 5,206	5,713 ± 5,313	.003
Visceral fat	557 ± 627	728 ± 579	.16	873 ± 810	883 ± 975	.91
Paraspinous musculature	2,594 ± 526	2,536 ± 261	.63	3,359 ± 678	2,749 ± 502	<.001
Vertebral cross-sectional area	614 ± 83	533 ± 67	<.001	721 ± 115	589 ± 70	<.001
Vertebral cancellous bone density	153 ± 26	151 ± 24	.45	156 ± 26	154 ± 21	.55
Vertebral height	16 ± 1.8	16 ± 1.5	.47	17 ± 1.0	17 ± 1.4	.27

Note.—Children in early prepubertal period were 5.6–7.9 years old, and children in late prepubertal period were 8.0–9.9 years old.
* Data are the mean ± SD.

Sex-related differences in paraspinous musculature, total fat, subcutaneous fat, and vertebral height were the independent variables, whereas sex-related difference in vertebral cross-sectional area was the dependent variable. The results showed that sex-related differences in musculature were moderately related to sex-related differences in vertebral cross-sectional area (r = 0.46). Only 22% of the difference in the vertebral cross-sectional area between boys and girls could be explained by sex-related differences in muscle. Once muscle was included in the model, no other independent variable significantly predicted the sex-related differences in the cross-sectional dimensions of the vertebrae.

Strong correlations were consistently observed in values calculated from CT scans for all traits at each vertebral level and for all three vertebral bodies considered together (r = 0.9–0.99). This was true whether the mean values for boys and girls were considered together or assessed independently.

In a post hoc analysis of the data, the 31 pairs of subjects were divided into two groups by age, early prepubertal period (5.6–7.9-year-old children) and late prepubertal period (8.0–9.9-year-old children). Sex-related differences in vertebral cross-sectional area and subcutaneous fat remained significant in both groups (Table 4). In contrast, although the sexual dimorphism in paraspinous musculature was striking in the 15 pairs of 8.0–9.9-year-old children ($P < .001$), there were no significant sex-related differences in the 16 pairs of younger subjects.

DISCUSSION

The objective of this study was to determine the possible effects of sex on body composition in prepubertal children. We determined that sex has a significant differential effect on fat and muscle accumulation in healthy children, even prior to the onset of sexual development. Girls had 28% and 32% greater total and subcutaneous fat in the abdomen, respectively, but 10% less paraspinous musculature than boys. Findings in previous studies (3–13,17–20,38) disagree with regard to the relative importance of sex on fat accumulation and musculoskeletal development before the pubertal transition.

Potential confounders, such as differences in body composition measurement techniques, age, and body weight, as well as mixed pubertal stages and ethnic groups, may contribute to discrepancies among results. We attempted to overcome these potential confounders by studying only prepubertal white children and by comparing boys and girls matched for age, height, and weight. Thus, our results cannot be attributed to differences in age, race, body size, or level of sexual development. Moreover, our findings cannot be ascribed to variations in diet, as there were no significant differences observed in the nutritional intake of the subjects.

Although all participants were younger than 10 years of age and were assessed as prepubertal by a pediatric endocrinologist, the influence that peripubertal hormonal secretions may have had on our findings cannot be accounted for. Theoretically, and particularly with reference to sex-related differences in muscle volume, the results could be caused, at least in part, by the pulsatile hormonal surges that are known to occur for several months to 1 or 2 years prior to the appearance of secondary sex characteristics (39). In the current study, we found striking differences in paraspinous musculature between matched pairs of boys and girls who were 8 and 9 years old, but there were no discernible sex-related differences for this trait in the younger pairs of subjects. In contrast, sex-related differences for subcutaneous fat were present in pairs of both younger and older prepubertal subjects, and, thereby, this finding caused an underrating of the influence that peripubertal gonadal steroids had on these traits.

The results of this study also showed that there were no significant differences in values for visceral fat between boys and girls. Thus, in contrast to values for subcutaneous fat, the sexes were monomorphic in regard to values for intraabdominal adipose tissue before puberty. In adults, ample data indicate that, regardless of race, men have greater visceral fat than women, even after differences in total body fat are considered (27–29). To our knowledge, however, in only two studies has assessment of visceral fat been performed in white children. In one in which a small cohort of prepubertal children (eight girls, 23 boys) were examined, no sex-related differences were observed (20), whereas in the other in which 11-year-old children were examined, findings suggested that girls have greater visceral fat than boys (38).

Recently, visceral fat has been identified as the specific fat depot related to important negative health outcomes, such as cardiovascular disease and type 2 diabetes (21–23,40). The relationship between this trait and adverse health, including dyslipidemia and glucose intolerance, also has been described in children (25,26). Studies are needed to determine the exact time of life when male subjects begin to accumulate greater visceral fat than female subjects, because this determination may help define the time during which interventions for these negative health outcomes may be most effective.

Because of the large number of CT scans that would be required to assess the total volume of fat and paraspinous musculature in the abdomen, in this study, measurements were obtained solely from scans at the midportions of the first, second, and third lumbar vertebrae. However, findings in previous studies (41,42) demonstrated strong correlations (r = 0.89–0.99) between findings on a single CT scan and the mean value for adiposity determined with a complete series of abdominal scans. The data from the current study also indicate that, in both boys and girls, measurements of fat and musculature at one abdominal level correlated strongly with values at other levels (r = 0.93–0.97). Thus, CT scanning findings at only a few levels not only offer a high prediction for the value of abdominal fat but also are likely to accurately predict the value of paraspinous musculature.

Our results corroborate findings in previous studies (43,44) in which analogous vertebral cancellous bone density between prepubertal girls and boys was reported, but they indicate demonstrably smaller vertebral cross-sectional area in girls. These findings, and those of others (43,45), emphasize that sex-related differences in bone mass in the axial skeleton result from differences in the size of the bones and not in the density of vertebral cancellous bone. Observations with CT indicate that, throughout life, female subjects have smaller vertebral cross-sectional area when compared with male subjects, even after accounting for differences in body size (43). On average, the cross-sectional area of the vertebral bodies is 15% smaller in prepubertal girls than in prepubertal boys matched for age, height, and weight (30,44). This disparity increases further with growth and is greatest at skeletal maturity, when the cross-sectional dimensions of the vertebrae are about 25% smaller in women than in men, even after considering differences in body size (43).

In the current study, we observed strong correlations between values for paraspinous musculature and the cross-sectional dimensions of the bones in the axial skeleton in both girls and boys. The positive relationship between muscle size and bone size is consistent with analytic models, and this finding suggests that postural muscle activity related to maintenance of upright position and locomotion are dominant forces that control cross-sectional bone growth in the axial skeleton (46).

However, our results do not support previous contentions that the greater cross-sectional dimensions of the vertebrae in male subjects are merely a reflection of the higher stresses that result from larger psoas muscles and erector muscles of the spine (47). In the cohort of children examined, sex-related differences in paraspinous musculature accounted for only 22% of the sex-related variations in the cross-sectional dimensions of the vertebral bodies. Thus, although muscle mass is a major predictor of bone mass in children, factors other than mechanical stresses from adjacent paraspinous musculature and those related to sex have a significant role in the regulation of the cross-sectional growth of the axial skeleton.

The reasons for the striking sexual dimorphism in muscle, fat, and bone volume in prepubertal children are unknown. Considerable evidence indicates, however, that during the first months of life the gonads secrete sex steroids (48–50), and there is an increase in leptin (51) and free insulin-like growth factor I bioavailability (52). The precise function of this hormonal surge is yet to be determined. However, as many of the physical changes during the pubertal transition (such as the accelerated growth and the dimorphic increases in fat, muscle, and bone mass) are mediated, at least in part, through sex steroids, leptin, and insulin-like growth factor I, it is reasonable to suppose that there is a similar relationship between these regulators and the in-

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creases in fat and musculoskeletal development during infancy.

Supporting this idea are data (53) showing that blockage of the neonatal secretion of gonadotropins and testosterone in newborn male monkeys results in diminished muscle mass and smaller bones. Additional studies are needed to determine the relative roles of the neonatal surges of leptin, sex steroids, and bioavailable insulin-like growth factor I in the cause of the sexual dimorphism in fat, muscle, and bone accumulation in prepubertal children.

In summary, findings of this study indicate that, compared with boys who have the same body mass, healthy prepubertal girls have *(a)* greater total and subcutaneous fat but comparable visceral fat, *(b)* less paraspinous musculature, and *(c)* smaller vertebral cross-sectional dimensions but similar vertebral height and vertebral cancellous bone density. In addition, we determined that differences in the size of the bones in the axial skeleton between boys and girls are, for the most part, independent of differences in paraspinous musculature. Considered together, these data provide unambiguous evidence that sex is an important determinant of the anthropomorphic characteristics of humans well before the beginning of puberty.

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