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Effects of Weight Loss on Leptin, Sex Hormones, and Measures of Adiposity in Obese Children

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Adipose tissue influences steroid conversion by paracrine and autocrine mechanisms. Leptin is secreted by adipocytes and influenced by sex hormones and adiposity. Short-term weight loss in the treatment of childhood obesity reduces leptin and adipose tissue. We therefore asked, Do alterations in sex hormones occur owing to weight loss? and can these alterations be explained by changes in fat mass or sc fat and are alterations in sex hormones directly related to the fall in leptin? Twenty obese boys and 40 obese girls were studied before and after 3 wk of low-calorie diet and physical activity. The weight loss program significantly lowered fat mass, abdominal fat distribution, sc fat (all $p < 0.0001$), leptin, insulin, and estradiol (all $p < 0.0001$) but not testosterone. Changes in leptin were related to changes in body mass and to changes in fat mass in boys. In girls, changes in leptin were related to changes in sc fatness and also to changes in insulin. In boys, the reduction in sc fat was positively correlated to changes in testosterone ($r = 0.54$; $p < 0.01$) and inversely related to the fall in estradiol ($r = –0.41$; $p < 0.05$). In girls, changes in testosterone ($r = 0.33$; $p < 0.05$) and in estradiol ($r = 0.40$; $p < 0.01$) were related to changes in insulin. Stepwise regression showed that initial leptin was the best determinant for the fall in leptin (adjusted $R^2 = 0.87$; $p < 0.0001$). The results show that alterations in sex hormones are related to changes in certain fat depots in boys whereas in girls changes in insulin might participate in changes in sex hormones. A greater fall in leptin owing to short-term weight loss is not associated with greater alterations in sex hormones and initial leptin is the best determinant to explain the variability in changes in leptin. The possibility of sex differences in changes in sex hormones secondary to the reduction in fatness warrants further study.

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Introduction

Leptin, the product of the *ob* gene, is secreted by adipocytes *(1)* and several other tissues *(2)*. Leptin circulates in the blood in proportion to stored adipose tissue and is elevated in obese adults *(3)* and obese children *(4,5)*. Leptin is under multihormonal control *(6)*, and leptin in children is influenced by the degree of fatness and body fat distribution *(7–11)*. In addition to insulin, which increases the expression rate of leptin *(12,13)*, sex hormones are involved in the regulation of leptin. Estradiol stimulates leptin secretion *(14)*, whereas testosterone has been shown to be a negative modulator of leptin in vitro and in vivo *(15)*.

The marked gender difference in leptin with girls showing higher levels of circulating leptin is not only related to the sc fat depot of children *(16)* but also to the consequence of the potent role of gonadal steroids on leptin *(16,17)*. Adipose tissue in general, and especially sc fat, is an important endocrine organ that influences steroid conversion by paracrine and autocrine mechanisms *(18)*. It is likely, therefore, that some of the effects of sex hormones on leptin are mediated, in part, by the degree of adiposity and, in particular, by the amount of stored sc adipose tissue.

Food restriction decreases plasma leptin *(19)*, and weight loss decreases also sc and visceral adipose tissue in obese girls *(20)*. Three weeks of dieting and physical activities has been shown to reduce leptin *(21)* but the fall in leptin was independent of the concomitant reduction in adiposity in obese girls, suggesting that factors other than adipose tissue might be involved in the short-term regulation of leptin *(22)*. Given the strong relationship between leptin and sc fat, and between leptin and sex hormones, we sought to determine whether changes in sex hormones occur owing to caloric restriction and physical activities in the treatment of childhood obesity and whether these alterations are related to the fall in leptin. An additional aim was to study whether the reduction in fat mass and sc fat participates in alterations of sex hormones.

Table 1
Baseline Values of Anthropometric and Hormonal Parameters of Obese Boys and Girls

Parameter	Boys (n = 22)	Girls (n = 40)	p
Age (years)	11.9 ± 1.7	12 ± 1.8	0.77
Body Mass Index (BMI)	26.2 ± 5.2	26.9 ± 5.25	0.64
Fat Mass (FM; kg)	30.4 ± 13	31.8 ± 12.4	0.66
Percentage FM (%FM)	45.5 ± 6.1	46 ± 7.2	0.79
Waist circumference (cm)	89.5 ± 13.1	87.4 ± 12.2	0.53
Hip–circumference (cm)	89.8 ± 11.4	95.7 ± 11.4	0.054
WHR	1 ± 0.05	0.91 ± 0.07	<0.0001
Leptin (ng/mL)	13.5 (2.6–64)	17.6 (7.9–62)	
Log Leptin	1.1 ± 0.3	1.27 ± 0.25	0.036
Testosterone (ng/mL)	0.33 (0.09–4.6)	0.32 (0.09–0.69)	
Log Testosterone	–0.4 ± 0.52	–0.53 ± 0.23	0.67[a]
Estradiol (pg/mL)	8 (1–38)	19 (2–181)	
Log Estradiol	0.9 ± 0.46	1.24 ± 0.43	0.0069
Serum insulin (μIU/mL)	8.1 (5.1–34.7)	10.2 (4.5–32.6)	
Log Insulin	0.91 ± 0.2	1.03 ± 0.19	0.24
SAT (mm)	235.75 (125.5–308.8)	216.7 (101.7–276)	0.16

[a] By means of Kruskal–Wallis test.

Table 2
Age-Adjusted Correlations Between Leptin, Testosterone, Estradiol and in Parameters of Interest in Obese Boys and Obese Girls at Baseline[a,b]

	Boys (n = 22)				Girls (n = 40)			
Parameters	Leptin	Testosterone	Estradiol	Insulin	Leptin	Testosterone	Estradiol	Insulin
Body Mass	0.75‡	–0.20	0.41*	0.63¶	0.76‡	0.33*	0.15	0.57‡
BMI	0.77‡	–0.32	0.42*	0.72‡	0.85‡	0.19	0.15	0.58‡
FM	0.84‡	–0.42*	0.43*	0.64†	0.85‡	0.24	0.13	0.56‡
%FM	0.83‡	–0.60¶	0.37*	0.40*	0.86‡	0.12	–0.01	0.565
SAT	0.48*	–0.245	0.50¶	–0.02	0.435¶	–0.18	–0.22	0.17
Waist–circumference	0.82‡	–0.375*	0.42*	0.60¶	0.74‡	0.24	0.02	0.55‡
Hip–circumference	0.66†	–0.11	0.405*	0.61¶	0.79‡	0.22	0.10	0.55‡
WHR	0.645¶	–0.575¶	0.22	0.18	0.35*	0.15	–0.07	0.285
Leptin	—	–0.76‡	0.32	0.63¶	—	0.05	0.09	0.55‡
Insulin	—	–0.39*	0.09	—	—	0.33*	0.40¶	—
Testosterone	—	—	0.07	—	—	—	0.26	—

[a] Values are coefficients of the Pearson's product moment correlation.
[b] Superscripts denote level of significance: *$p < 0.05$, ¶$p < 0.01$, †$p < 0.001$, ‡$p < 0.0001$.

Results

Chronologic age and estimates of adiposity (body mass index [BMI], fat mass [FM], %FM, and sc adipose tissue [SAT]) were almost identical in boys and girls (Table 1). Albeit slightly out of significance ($p = 0.054$), hip circumference was greater in girls. Waist circumference did not differ between sexes but waist-to-hip ratio (WHR) was significantly greater in obese girls ($p < 0.0001$). Likewise, leptin ($p = 0.036$) and estradiol ($p = 0.0069$) were significantly higher in girls, but testosterone and insulin did not differ between boys and girls.

Age-Adjusted Correlations Between Baseline Parameters in Obese Boys and Girls (Table 2)

In obese boys, leptin was significantly related to measures of adiposity and abdominal fat distribution. The same was found for insulin with the exception that insulin failed to show a significant relationship to SAT and WHR. Leptin was also significantly related to insulin and was inversely correlated to testosterone ($p < 0.0001$). Testosterone was significantly and inversely correlated to FM, %FM, waist circumference, WHR, and insulin. Estradiol was significantly associated with all measures of adiposity but not with WHR, leptin, and insulin.

Table 3
Mean Changes (and SDs) in Estimated Parameters
as Shown by the 2 × 2 Study Design in Obese Children and According to Their Sex[a]

Parameters	Mean changes (all children)	F-value, df; p	Mean changes (obese boys, $n = 22$)	Mean changes (obese girls, $n = 40$)
Body Mass (kg)	−3.76 ± 1.22‡	1.18, 1; 0.28	−4 ± 1.4	−3.6 ± 1.1
FM (kg)	−3.6 ± 1.6‡	2.75, 1; 0.10	−4 ± 1.4	−3.3 ± 1.65
%FM (%)	−3.1 ± 2.3‡	7.1, 1; 0.0 1	−4.1 ± 2.7¶	−2.5 ± 1.9
FFM (kg)	−0.18 ± 1.38	0.86, 1; 0.36	−0.04 ± 1.7	−0.3 ± 1.2
Waist–circumference (cm)	−7.9 ± 5.4‡	7.35, 1; 0.009	−5.5 ± 3.2	−9.2 ± 6†
Hip–circumference (cm)	−5.8 ± 2.87‡	0.49, 1; 0.49	−5.5 ± 3.2	−6 ± 2.7
(Log) Leptin (ng/mL)	−14.6 ± 9.2‡	0.007, 1; 0.935	−11.6 ± 8.7	−15.9 ± 9.2
(Log) Insulin (μIU/mL)	−4.1 ± 5.6‡	0.198, 1; 0.66	−3.9 ± 5.85	−4.25 ± 5.5
(Log) Testosterone (ng/mL)	0.1 ± 0.53	1.27, 1; 0.26	0.3 ± 0.9	0.0 ± 0.1
(Log) Estradiol (pg/mL)	−18.25 ± 30.9‡	1.33, 1; 0.25	−9.2 ± 9.5	−22.65 ± 36.4
SAT (mm)	−23.2 ± 36.7‡	6.37, 1; 0.014	−38.4 ± 41.55*	−14.8 ± 31.2

[a] Superscripts denote level of significance for changes in parameters (‡$p < 0.0001$) and also denote level of significance for the sex-difference in changes of parameters (*$p < 0.05$, ¶$p < 0.01$, †$p < 0.001$).

Table 4
Relationship Between Changes in Leptin (Δ Leptin),
Testosterone (Δ Testosterone), and Estradiol (Δ Estradiol) with Changes in Parameters of Interest[a]

	Boys ($n = 22$)			Girls ($n = 40$)		
Parameters	Δ Leptin	Δ Testosterone	Δ Estradiol	Δ Leptin	Δ Testosterone	Δ Estradiol
Δ Body Mass	0.81‡	−0.26	0.0	0.45¶	−0.08	0.08
Δ FM	0.46*	0.0	−0.41*	0.455¶	−0.09	0.01
Δ Waist	0.02	0.16	0.04	−0.12	−0.06	−0.02
Δ Hip	0.02	0.31	−0.22	0.23	−0.19	−0.18
Δ SAT	−0.19	0.54¶	0.09	−0.43¶	−0.17	−0.24
Δ Leptin	—	−0.365	−0.04	—	−0.26	−0.03
Δ lnsulin	0.27	0.08	−0.09	0.285*	0.33*	0.40¶
Δ Testosterone	—	—	−0.11	—	—	0.36*

[a] Values are coefficients of the Spearman's rank sum test. Superscripts denote level of significance: *$p < 0.05$, ¶$p < 0.01$, ‡$p < 0.0001$.

In obese girls, all measures of adiposity, abdominal fat distribution, and insulin were significantly related to leptin. Insulin was not related to SAT but was significantly related to all other measures of adiposity and abdominal fat distribution. Testosterone was only correlated to body mass and insulin (both $p = 0.02$). No relationship between measures of adiposity and estradiol was found, and estradiol was correlated to insulin ($p = 0.006$).

Influence of Weight Loss Program on Estimated Parameters (Table 3)

All measures of adiposity were significantly lowered (all $p < 0.0001$), and the reduction in %FM was greater in boys (sex × time interaction; $p = 0.01$). The reduction in waist circumference was significantly greater in girls than in boys (sex × time interaction; $p = 0.009$). No sex differences were found for the reduction in hip circumference. There was also a significant sex × time interaction for changes in SAT ($p = 0.014$), with boys showing a greater loss in SAT. Fat-free mass (FFM) of children did not change as a whole.

Leptin was reduced ($p < 0.0001$) and the magnitude of this fall was quite similar in boys and girls. Insulin was reduced ($p < 0.0001$) with no significant differences between boys and girls. No significant alterations were obtained for testosterone, but estradiol was significantly lowered ($p < 0.0001$).

Relationship Between Changes in Estimated Parameters (Table 4)

In boys and girls, changes in leptin were significantly related to changes in body mass and FM. In girls, also changes in SAT and insulin were related to changes in leptin. However, there was an inverse relationship between changes in

Table 5
Multiple Regression Analysis with Changes in Leptin (Δ Leptin) as Dependent Variable in Obese Children[a]

	Multiple Regression Model			Stepwise Regression Model		
	Independent variables	β 95%CI	*p*	Independent variables	β 95%CI	*p*
First model	Age	−0.016 ± 1.09	0.98	Sex	4.09 ± 3.98	0.045
	Sex	4.26 ± 4.2	0.047	Δ Body Mass	4.9 ± 1.55	<0.000
	Δ Insulin	0.19 ± 0.36	0.29	Δ SAT	−0.065 ± 0.049	0.0105
	Δ Body Mass	4.05 ± 2.23	0.0006	Intercept: −3.23		
	Δ Fat Mass	0.56 ± 1.48	0.45	adj. $R^2 = 0.48$, $p < 0.0001$		
	Δ SAT	−0.063 ± 0.05	0.014			
Second model	Age	−0.14 ± 0.54	0.61	Initial Leptin	31.04 ± 3.18	<0.000
	Sex	−0.23 ± 2.19	0.83	Intercept: −19.54		
	Δ Insulin	0.14 ± 0.18	0.13	adj. $R^2 = 0.87$, $p < 0.0001$		
	Δ Body Mass	0.001 ± 1.27	0.99			
	Δ Fat Mass	0.20 ± 0.73	0.58			
	Δ SAT	0.015 ± 0.027	0.28			
	Initial Leptin	−30.7 ± 4.89	<0.0001			

[a] The *p*-level of significance, the regression coefficient (β), and the 95% confidence interval (95% CI) for each independent variable is shown. The adjusted R^2 is given for each model of the stepwise regression.

SAT and changes in leptin ($r = -0.43$; $p = 0.003$), indicating that those girls who showed the smallest reduction in SAT or even showed an increase in SAT had the greatest reduction in leptin.

Although testosterone did not change significantly, we used changes in testosterone to determine whether subtle alterations were related to the fall in leptin. In boys and girls, there was a trend for an inverse association between changes in testosterone and the fall in leptin, but this was slightly out of significance in boys ($r = -0.365$; $p = 0.068$) and girls ($r = -0.26$; $p = 0.055$). Notwithstanding, initial leptin was the best correlate of the fall in leptin in boys ($r = 0.92$; $p < 0.0001$) and girls ($r = 0.96$; $p < 0.0001$).

In boys, changes in testosterone were related to changes in SAT and changes in estradiol were related to changes in FM. In girls, however, changes in sex hormones were related to changes in insulin. Only in girls was the change in testosterone related to the change in estradiol.

Stepwise, Multiple Regression Analyses with Changes in Leptin as Dependent Variables (Table 5)

Stepwise regression was performed on the basis of the correlations shown in Table 4 with sex and age as additional cofactors. The first model included changes in body mass, FM, SAT, and insulin. Changes in leptin were best explained by changes in body mass ($p < 0.0001$), changes in SAT ($p = 0.01$), and sex of children ($p = 0.044$). These parameters contributed to 48% of the variance in changes in leptin. When changes in testosterone or in estradiol entered the equation, both variables had a p level of > 0.3 in the regression model, and the significance of the other variables in the model did not change significantly. However, there was an exception for the variable sex that was not significant after control for sex hormones (data not shown). In the second model, we controlled for initial leptin, and in this case, only initial leptin was retained as the main determinant for the fall in leptin (adjusted $R^2 = 0.87$; $p < 0.0001$).

We did not assess the magnitude and significance of independent determinants for changes in sex hormones because of the low number of significant relationships between changes in sex hormones and in other parameters (Table 4).

Discussion

The present study aimed to investigate the influence of weight loss on sex hormones, leptin, and measures of adiposity in obese children. As can be seen in Table 1, leptin was significantly higher in girls, as were levels of estrogens. However, measures of adiposity, such as body mass, BMI, FM, %FM, and overall subcutaneous fat (SAT), were not different between boys and girls. Waist circumference was almost identical in boys and girls, but WHR ratio was significantly greater in boys owing to the greater hip circumference in girls.

As can be expected from the obese state of the children, their levels of leptin were higher than in a sample of schoolchildren with a range in BMI from 14.6 to 29.4 in boys and from 15.8 to 47 in girls, respectively *(23)*. However, levels of testosterone and estradiol were lower in obese boys and girls of the present investigation probably because the mean age of the children was lower in our study. Leptin levels are approximately the same as shown previously for obese children *(15)*. However, mean levels of testosterone were higher for boys in that study (2.15 ng/mL) whereas mean estradiol was lower (4 pg/mL) *(15)*. By contrast, mean estradiol was

lower in the girls in our study than in girls of the other study (45.1 pg/mL). Whether these differences in hormones are owing to subtle mean differences in BMI, FM, and age of children is unclear. Because our results of fatness values are in line with findings in randomly selected obese Austrian children *(24,25)*, it is unlikely that the clustered sample of obese children participating in the weight loss program could have introduced a sampling bias responsible for some of these differences.

As expected, strong relationships exist between leptin and measures of adiposity at baseline (Table 2). Those correlations were adjusted for age because total adiposity increases with age and also testosterone and estradiol (in girls). However, it was somewhat unexpected to find that SAT was not as strong related to leptin than other measures of adiposity and waist circumference. Although sc fat was shown to be one of the best correlates of leptin *(16)*, the present findings suggest that visceral fat as indicated by waist circumference also contributes to leptin in boys and girls. This assumption is based on the fact that the thickness of SAT layers from the abdominal region were not positively related to leptin in the present study (data not shown) and also not in previous findings *(11,22)*.

We found testosterone to be inversely related to leptin in boys after control for age. This is in agreement with several studies showing testosterone to be a negative modulator of leptin *(15,26)*. Because testosterone was also inversely associated with %FM, WHR, and insulin, a worsening in body composition (and in the metabolic profile) might blunt the effects of testosterone on leptin in these obese boys.

In girls, measured sex hormones did not show a significant relationship to leptin but were related to insulin. Sex hormones were not related to measures of adiposity in girls, whereas estrogen was significantly correlated to adiposity and, especially, to SAT in boys. Together, this might indicate that the regulation of sex hormones is more susceptible to metabolic perturbations in obese girls, whereas the aromatizing properties of an increased adiposity might affect the hormonal milieu predominantly in obese boys.

To determine whether changes in leptin were related to alterations in sex hormones, we studied the effects of short-term weight loss on measures of adiposity, leptin, and sex hormones. Body mass and measures of adiposity were substantially reduced after 3 wk (Table 3). As shown by the 2×2 factorial design, the improvement in relative fatness favored obese boys, whereas the reduction in waist circumference was greater in girls. Because the loss in FM did not significantly differ between boys and girls, and the loss in SAT was greater in boys, it can be assumed that girls had a greater loss in visceral fat. However, because of lack of a criterion method to estimate the visceral fat compartment, this assumption needs to be clarified in ongoing studies.

The impressive fall in leptin was almost identical in obese boys and girls as were changes in insulin and estradiol (Table 3). However, there were great interindividual differences in changes of estradiol especially in girls, which ranged between a decrease of –178 pg/mL and an increase of +16 pg/mL. There was some weakness in the present study because we did not ask girls about their menstruation cycle and did not assess stages of maturity levels. Nevertheless, we cannot rule out the possibility that these results were not only the net result of the loss in body weight but were also affected by the menstrual cycle *(27)* and stages of maturity levels of the girls. Levels of testosterone did not change and neither did changes in estradiol nor that of testosterone show a significant relationship with the fall in leptin (Table 4). However, the relationship between changes in leptin and in testosterone were slightly out of significance in boys ($p = 0.068$) and girls ($p = 0.055$). Nevertheless, these relationships were of an inverse nature and, therefore, do not support the assumption that a greater fall in leptin is associated with greater alterations in testosterone.

It is known that estrogen biosynthesis is catalyzed by aromatase cytochrome P-450 and that the expression of aromatase in adipose tissue is under the control of glucocorticoids *(28)* and class I cytokines such as oncostatin M, interleukin 6, and interleukin 11, as well as tumor necrosis factor-α *(29)*. Leptin was shown to be able to stimulate aromatase activity in adipose stromal cells at high concentrations only in some abdominal and breast fat cultures in obese females *(30)*. In addition, marked short-term reduction in serum estradiol concentration after inhibition of estrogen biosynthesis by selective blockade of the aromatase enzyme had no effect on serum leptin levels in young men *(31)*. Taken together these studies do not support any independent relationship between leptin and estradiol.

However, we studied these hormones before and after diet-induced weight loss, and one might argue that it is not the fall in leptin *per se* that participates in changes in sex hormones but, rather, the loss in adipose tissue. Nevertheless, we investigated the effects of both increased energy expenditure and caloric restriction on hormones and adiposity. Therefore, it is impossible to differentiate between these two effects and to decide which effect was the main one responsible for alterations in sex hormones. However, in girls the changes in sex hormones were significantly related to changes in insulin. This implies that it is rather the improvement in the metabolic state, not the loss in adipose tissue, that contributes to the weight loss–associated fall in estradiol and testosterone. Only in boys were changes in SAT related to alterations in testosterone, and changes in estradiol were inversely associated with the reduction in FM (Table 4). Therefore, the assumption that particular changes in SAT might participate in changes in sex hormones can hardly be verified from the present findings. Unfortunately, our study included twofold more girls than boys. Thus, we cannot rule out the possibility that some of our findings were also affected by this difference in sample size. Consequently, a permissive role of adipose tissue and perhaps adipose tissue distribution in this context cannot be excluded.

As shown by the stepwise regression analysis (Table 5), changes in body mass, changes in SAT, and sex of the children were the main determinants for the fall in leptin. Changes in SAT had a negative slope in the regression model, raising some doubts as to whether a loss in SAT has a biologic significance in mediating the fall in leptin during short-term weight loss. Nevertheless, after control for initial leptin, none of those variables were significantly associated with the fall in leptin. This finding is in agreement with other studies showing initial leptin to be the strongest predictor of the variance in the change in leptin *(19,32,33)*.

In conclusion, we show that a greater fall in leptin owing to short-term weight loss is not associated with greater changes in sex hormones. The loss in sc fat in boys contributed to alterations in testosterone, but the loss in FM was in inverse relationship to changes in estradiol. In girls, changes in sex hormones were related to the fall in insulin, suggesting that changes in factors such as lipoprotein lipase activity, sex hormone–binding globulin, or changes in the growth hormone/insulin-like growth factor regulatory system perhaps secondary to the loss in fatness are responsible for the short-term changes in sex hormones. The possibility of sex differences in adipose tissue-mediated changes in sex hormones in childhood obesity warrants further study.

Materials and Methods

Subjects

Forty obese girls (mean ± SD; age: 12 ± 1.8 yr; BMI: 26.9 ± 5.25) and 22 obese boys (age: 11.9 ± 1.7 yr; BMI: 26.2 ± 5.2) were studied. Obesity was defined as BMI > 90th percentile for age and sex. Main characteristics of the children are given in Table 1. Children were judged as healthy by medical examination and written informed consent was given by the parents. The study was approved by the local ethical committee.

Weight Reduction Program

Children participated in a weight reduction program during summer holidays for 3 wk *(22)*. Physical training consisted of different activities such as biking, jogging, and playing different ball games. Physical activities were performed three times a day and lasted approx 1.5 h. All training sessions were supervised by an experienced instructor. Children were assigned to a mixed diet of 900–1200 kcal/d depending on age and degree of overweight. Energy intake consisted of approx 50% carbohydrate, 20% protein, and 30% fat *(34)*.

Laboratory Methods

Blood samples were taken after an overnight fast and determined for leptin (nanograms/milliliter) and insulin (microliters/millilter) by means of radioimmunoassay (RIA) (Linco Research, St. Louis, MO). Intra- and interassay coefficients of variation (CVs) for leptin were 4.6 and 7.4% and that for insulin were 6.2 and 8.6%, respectively. Testosterone (nanograms/milliliter) was determined by means of RIA (DPC Chiron, Medfield, MA) and had an intraassay CV of 15% at a concentration of 0.57 ng/mL and 6% at 3.25 ng/mL. Interassay CV at similar concentrations was 21.5 and 8.8%, respectively. Estradiol (picograms/milliliter) was determined by means of RIA (Sorin, Italy). The intraassay CV was 14.7% at a concentration of 15.7 pg/mL and 6.4% at 206 pg/mL. Interassay CV at similar concentrations was 7.7 and 6%, respectively.

Measurement of Body Composition

Measurements for FFM *(35)* were performed by means of bioelectrical impedance (BIA Akern-RJL 101/S) with an applied current of 0.8 mA at 50 kHz. FM was calculated as the difference between body mass and FFM. %FM was expressed as the relative amount of FM for a given body weight.

Measurement of WHR

Waist and hip circumferences were measured to the nearest 0.5 cm in triplicate and the median value was taken. The WHR was calculated as waist (cm) × hip $(cm)^{-1}$.

Measurement of SAT Layers

All measurements were performed in the fasting state at the beginning, and after 3 wk. Measurements were performed by means of the optical device Lipometer as described previously *(36-38)*. The Lipometer uses light-emitting diodes, which illuminate the sc fatty layer (SAT layer), forming certain geometrical patterns varying in succession. A photodiode measures the corresponding light intensities backscattered in the SAT. These light signals are amplified, digitized, and stored on a computer. Calibration and evaluation were done by means of computed tomography. Measurements for the thickness of SAT layers (in millimeters) were taken at 15 body sites, from 1-neck to 15-calf on the right side of the body in standing position. Measurements were performed in triplicate and the median value was taken. The CVs of SAT layers ranged between 1.9% for SAT layer 5-front chest and 12.2% for SAT layer 13-rear thigh *(38)*. Subcutaneous fatness (SAT, in millimeters) was calculated through linear addition of the median values of the thickness for all 15 measured SAT layers (Table 1).

Statistical Analyses

Leptin, insulin, testosterone, and estradiol were skewed and therefore $\log_{10}$ transformed. Analysis of variance (ANOVA) was used to compare baseline parameters between boys and girls when appropriate. In case of a significant difference, post-hoc analysis correction was employed. The Kruskal-Wallis test was used if variances were not normally distributed. A 2 (sex) × 2 (time) design with repeated measurements on time was used to compare changes in parameters between groups. ANOVA with repeated measurements was used to study changes within groups. Correlations between variables of interest were calculated using

Pearson's product moment correlation and Spearman's rank sum test when appropriate. Partial correlation was performed to adjust for the influence of chronologic age on outcome measures. For partial correlations, $\log_{10}$-transformed values of skewed variables were used. Univariate and partial correlations were performed for boys and girls separately. The independence and significance of variables were tested by stepwise, multiple regression analysis based on results of the bivariate correlations. A maximum of four independent variables was allowed to enter the equation. Because the study included twice as many girls as boys, and maturity levels were not assessed in obese children, we additionally controlled for sex and chronologic age in the regression models. The significance level of p values was set at 5%. Data are given as mean and SD. Skewed data are shown as median and range.

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