

# Serum Leptin and Energy Expenditure in Children\*

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## ABSTRACT

Leptin has been hypothesized to play an important role in energy balance by affecting both energy intake and energy expenditure. The purpose of our study was to determine the relationship between fasting serum leptin concentrations and measures of energy expenditure in prepubertal children. We measured total energy expenditure (TEE; by the doubly labeled water technique), resting energy expenditure (REE; after an overnight fast), activity energy expenditure (AEE; TEE - REE), body composition (by dual energy x-ray absorptiometry), and fasting serum leptin concentration (by RIA) in 76 children. Simple correlations showed that all measures of energy expenditure (TEE, REE, and AEE) were positively related to the serum leptin concentration ( $r = 0.50, P < 0.001$ ;  $r = 0.45, P < 0.001$ ; and  $r = 0.30, P < 0.01$ , respectively). However, after adjusting for body composition

(fat-free mass and fat mass), gender, and ethnicity, serum leptin concentrations were not related to any measure of energy expenditure (TEE,  $P = 0.61$ ; REE,  $P = 0.97$ ; AEE,  $P = 0.65$ ). These latter findings were further confirmed using structural equation models with leptin and energy expenditure as dependent variables, and fat-free mass and fat mass as independent variables. Results from these models showed no direct effect of leptin and no indirect effect of fat mass (through leptin) on any measure of energy expenditure, when a path between fat mass and energy expenditure was present in the model. Thus, our data do not support the hypothesis that the serum leptin concentration (independent of fat mass) is related to measures of energy expenditure in children. (*J Clin Endocrinol Metab* 82: 4149-4153, 1997)

LEPTIN administration has been shown to decrease body mass in some animal models of obesity. In the *ob/ob* mouse, the decrease in body mass after leptin administration is due to a decrease in food intake and an increase in energy expenditure (1). Daily ip injections of leptin to *ob/ob* mice result in increases in oxygen consumption, body temperature, and locomotor activity. These data suggest that in leptin-deficient animal models, leptin administration tends to normalize energy balance (food intake and energy expenditure) relative to that in nonobese controls.

In humans, the role of leptin in energy balance is unknown. However, two recent studies have examined the relationship between leptin and energy expenditure in humans. Nicklas *et al.* (2) found a positive correlation between resting energy expenditure (REE; adjusted for lean body mass) and serum leptin concentration in African-American, but not Caucasian, postmenopausal women. Salbe *et al.* (3) found serum leptin to be positively correlated with total energy expenditure (TEE; adjusted for body size) and physical activity level, but not with REE, in Pima children. These studies suggest that in humans, serum leptin may play a role in the energy balance equation.

The present study examined the relationship between the serum leptin concentration and energy expenditure [REE,

TEE, and activity energy expenditure (AEE)] in Caucasian and African-American prepubertal children. After correcting for confounding variables, our data failed to show a significant relationship between leptin and measures of energy expenditure in children.

## Materials and Methods

### Subjects

We studied 47 African-American (25 girls and 22 boys) and 29 Caucasian (9 girls and 20 boys) children. Children were recruited by newspaper and radio advertisements and by word of mouth. Subjects were screened by a medical history evaluation and were ineligible if they were taking medications known to affect body composition or physical activity (e.g. prednisone, Ritalin, or GH), if they were diagnosed with syndromes known to affect body composition and/or fat distribution (e.g. Cushing's syndrome, Down's syndrome, type 1 diabetes, or hypothyroidism), or if any major illness had occurred since birth. Pubertal status (Tanner stage) was determined by physician evaluation of both breast and pubic hair in females (4) and genitalia in males (5). Only children rated as Tanner stage 1 are included in this study. This research was approved by the institutional review board at the University of Alabama at Birmingham. The parents of all participants provided informed consent before testing commenced. We have previously reported the relationship between serum leptin concentrations, body composition, and fat distribution in this population (6).

### Protocol

Children were admitted to the General Clinical Research Center (GCRC) in the late afternoon for an overnight visit. Upon arrival, anthropometric measurements were obtained, two baseline urine samples were collected, and the children were given an oral dose of doubly labeled water (DLW). Dinner was served at approximately 1700 h, and an evening snack was allowed as long as it was consumed before 2000 h. After 2000 h, only water and noncaloric, noncaffeinated beverages were allowed until after the morning testing. The following morning, REE was determined via indirect calorimetry followed by blood collection for

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hormone analyses and the collection of two urine sample for DLW. Two weeks later, the children arrived at the Energy Metabolism Research Unit at 0700 h in the fasted state, two urine samples were collected for DLW, and body composition was determined by dual energy x-ray absorptiometry (DXA).

### Body composition

Body composition [fat mass and fat-free mass (FFM)] was measured by DXA using a Lunar DPX-L densitometer (Lunar Radiation Corp., Madison, WI). We have previously validated the use of DXA against carcass analysis in a pig model that encompassed the pediatric weight range (7). Subjects were scanned in light clothing while lying on their backs with arms at their sides. DXA scans were performed and analyzed with pediatric software version 1.5e.

### Energy expenditure measurements

REE was measured in the early morning after an overnight fast at the GCRC using a Deltatrac Metabolic Monitor (Sensormedics Corp., Yorba Linda, CA). During testing, all subjects were instructed to lie still and remain awake. An adult-size canopy hood was used to collect expired air. After a 10-min equilibration period, oxygen consumption and carbon dioxide production data were collected for 20 min. Energy expenditure was calculated by the equation of de Weir (8).

TEE was measured under free living conditions using the DLW technique for a 14-day period. Four timed urine samples were collected after oral administration of DLW (0.15 g H<sub>2</sub><sup>18</sup>O and 0.12 g <sup>2</sup>H<sub>2</sub>O): two on the morning following treatment and two in the morning 14 days later. Samples were analyzed in triplicate for H<sub>2</sub><sup>18</sup>O and <sup>2</sup>H<sub>2</sub>O by isotope ratio mass spectrometry using a Fisons Optima isotope ratio mass spectrometer (University of Alabama at Birmingham). Samples were prepared and analyzed as previously reported (9). Carbon dioxide production rates were calculated using Eq R2 from the report by Speakman *et al.* (10) and were converted to energy expenditure using Eq 12 of de Weir (8) and a food quotient of 0.89 in Caucasians and 0.87 in African-Americans (the food quotients used are the mean values for each group based on two 24-h dietary recalls for each child).

AEE was calculated from the following equation: AEE = 0.9 × TEE - REE. A correction for the thermic effect of feeding (0.9 × TEE) was necessary as this component of energy expenditure was not measured. The correction assumes that the thermic effect of food is equal to 10% of the TEE. Because of the tremendous error propagation in calculating AEE, "true scores" (11) were used to improve the precision of AEE, as we have previously described in detail (9). It should be noted that use of these true scores does not change the findings compared to using unadjusted AEE values.

### Serum leptin concentrations

Leptin concentrations were determined with a RIA kit (Linco Research, St. Charles, MO) using serum from the fasted children. All serum samples (100 μL) were analyzed in duplicate in a single assay. The intraassay coefficient of variation was 4.2% at 64% bound (2.61 ng/mL) and 3.5% at 28% bound (14.9 ng/mL).

### Statistical methods

Data for variables not normally distributed, were log<sub>10</sub> transformed to increase the normality of their distribution. Differences in subject characteristics were determined by two-way ANOVA, with gender and ethnicity as class variables (the interaction of gender and ethnicity was included in the ANOVA). Simple correlations (Pearson product moment) were assessed for serum leptin concentration and the physical and metabolic characteristics of the children. To determine the independent effect of leptin on measures of energy expenditure, multiple linear regression analyses were performed (general linear models procedure), with leptin, FFM, fat mass, gender, and ethnicity as independent variables and TEE, REE, and AEE as dependent variables. The above statistics were performed using SAS for Windows (version 6.11, SAS Institute, Cary, NC). Effects were considered significant at *P* < 0.05.

A LISREL approach to structural equation modeling (12) was used to examine the pattern of causal relationships that were hypothesized to exist among the variables measured in this study. This approach differs from traditional data analytic strategies in that it focuses on the entire system or on the pattern of all relationships that are assumed to exist

**TABLE 1.** Descriptive statistics by gender and ethnicity

	African-American boys (n = 22)	African-American girls (n = 25)	Caucasian boys (n = 20)	Caucasian girls (n = 9)	Effects <sup>a</sup>
Age (yr)	7.4 ± 1.6 (5.1–10.0)	7.6 ± 1.7 (4.6–10.0)	8.3 ± 1.6 (5.6–10.9)	7.9 ± 1.2 (5.2–9.2)	NS
Wt (kg)	31.6 ± 11.5 (19.8–68.4)	32.8 ± 13.0 (14.1–62.6)	29.7 ± 7.6 (17.0–45.1)	28.8 ± 6.9 (18.5–41.5)	NS
Ht (m)	1.29 ± 0.10 (1.13–1.53)	1.29 ± 0.12 (1.05–1.55)	1.30 ± 0.10 (1.10–1.48)	1.25 ± 0.07 (1.10–1.33)	NS
BMI (kg/m <sup>2</sup> )	18.4 ± 3.9 (11.8–29.2)	19.1 ± 4.7 (12.8–29.0)	17.4 ± 2.7 (13.9–25.5)	18.2 ± 3.0 (15.1–24.1)	NS
Fat (%)	24 ± 10 (9–49)	33 ± 11 (15–53)	24 ± 11 (11–56)	28 ± 7 (22–38)	Gender <sup>b</sup>
FFM (kg) <sup>c</sup>	21.8 ± 6.1 (14.6–35.3)	20.5 ± 6.5 (10.6–31.6)	20.4 ± 4.5 (12.5–25.9)	18.7 ± 3.9 (13.4–24.2)	Gender <sup>b</sup>
Fat (kg) <sup>c</sup>	6.6 ± 6.1 (2.0–30.8)	9.4 ± 9.4 (2.7–29.2)	6.1 ± 4.5 (2.6–19.8)	8.3 ± 3.9 (4.2–15.3)	Gender <sup>b</sup>
TEE (Cal/day) <sup>c</sup>	1698 ± 479 (991–2729)	1648 ± 475 (922–2582)	1660 ± 349 (1178–2511)	1614 ± 401 (1236–2296)	NS
REE (Cal/day) <sup>c</sup>	1253 ± 246 (929–2113)	1170 ± 211 (896–1611)	1227 ± 211 (918–1766)	1146 ± 158 (918–1330)	NS
AEE (Cal/day) <sup>c</sup>	432 ± 215 (126–721)	485 ± 160 (275–927)	429 ± 173 (200–716)	479 ± 164 (352–785)	NS
Leptin (ng/mL) <sup>c</sup>	4.6 ± 4.3 (1.8–20.4)	8.8 ± 10.7 (2.6–39.7)	4.3 ± 4.4 (1.5–23.2)	8.4 ± 4.9 (4.8–16.6)	Gender <sup>d</sup>

Values are the means ± SE, with the data range in parentheses.

<sup>a</sup> Effects from a two-way ANOVA with gender and ethnicity as class variables (the interaction of gender and ethnicity was not included in the models, as it was not significant for any variable).

<sup>b</sup> *P* < 0.05.

<sup>c</sup> Data for these variables were transformed (log<sub>10</sub>) before statistical analysis. Data presented are the back-transformed means (geometric mean).

<sup>d</sup> *P* < 0.01.

**TABLE 2.** Simple correlations (Pearson product moment) of select variables with serum leptin (log) in 76 children

Variable	Correlation coefficient
Wt (kg) <sup>a</sup>	0.72 <sup>b</sup>
FFM (kg) <sup>a</sup>	0.42 <sup>b</sup>
Fat mass (kg) <sup>a</sup>	0.88 <sup>b</sup>
% Body fat	0.86 <sup>b</sup>
BMI (kg/m <sup>2</sup> )	0.76 <sup>b</sup>
TEE (Cal/day) <sup>a</sup>	0.50 <sup>b</sup>
REE (Cal/day) <sup>a</sup>	0.45 <sup>b</sup>
AEE (Cal/day) <sup>a</sup>	0.30 <sup>c</sup>

<sup>a</sup> Data for these variables were transformed ( $\log_{10}$ ) before statistical analysis.

<sup>b</sup>  $P < 0.001$ .

<sup>c</sup>  $P < 0.01$ .

among variables of interest. Moreover, the approach is based on a confirmation paradigm that requires an *a priori* specification of a model that explicitly describes the hypothesized predictive framework. Under these circumstances, it is assumed that the specified model represents the actual underlying causal mechanisms that are responsible for generating the pattern of relationships observed in the data. The statistical significance of coefficients that are associated with individual paths between variables in the model can be evaluated and interpreted in the same manner as ordinary regression coefficients. Preliminary testing indicated that separating the children based on gender and ethnicity did not dramatically alter the overall picture revealed by the models. Therefore, the structural equation models contained all children, regardless of gender and ethnicity.

## Results

Plasma leptin concentration, body composition, and energy expenditure components were assessed in 76 children. The physical and metabolic characteristics of these children are outlined in Table 1. The group of children displayed a wide range in weight, FFM, fat mass, percent body fat, and serum leptin concentration. Results from two-way ANOVA models showed that there were no significant differences between the two ethnic groups or differences due to the interaction of ethnicity and gender in any of the data listed in Table 1. However, there were significant effects of gender on FFM, fat mass, percent body fat, and serum leptin concentrations; girls had greater fat mass, percent body fat, and serum leptin concentrations, but lesser FFM than boys.

Serum leptin concentrations were positively correlated with body weight, FFM, fat mass, percent body fat, TEE, REE, and AEE (Table 2). However, serum leptin concentrations were not independently associated with TEE, REE, or AEE when included in a multiple linear regression model with FFM, fat mass, gender, and ethnicity (Table 3). If fat mass was removed from the multiple regression models, then serum leptin was significantly and positively related to TEE ( $P < 0.01$ ) and REE ( $P < 0.01$ ), but not to AEE ( $P = 0.43$ ).

Results from the structural equation models are presented in Figs. 1 and 2. When the path between fat mass and energy expenditure was in the models, there was no significant effect of leptin on TEE, REE, or AEE (Fig. 1). Likewise, there were no significant indirect effects of FFM or fat mass (through leptin) on any measure of energy expenditure. Because the paths between fat mass and TEE and AEE were not significant, we removed them from the model. When the path was removed, leptin was significantly and positively associated with TEE, but not with AEE (Fig. 2). Additionally, there were

**TABLE 3.** Multiple linear regression analyses with energy expenditure components as dependent variables

Independent variable	$\beta$	SE ( $\beta$ )	$P$ value
Dependent variable = TEE (Cal/day) <sup>a</sup>			
Intercept	2.471	0.104	<0.001
Gender <sup>b</sup>	0.016	0.017	0.36
Ethnicity <sup>c</sup>	-0.013	0.015	0.39
FFM (kg) <sup>a</sup>	0.470	0.097	<0.001
Fat mass (kg) <sup>a</sup>	0.172	0.066	<0.05
Leptin (ng/mL) <sup>a</sup>	-0.025	0.049	0.61
Model $r^2 = 0.66$ ; $P < 0.0001$			
Dependent variable = REE (Cal/day) <sup>a</sup>			
Intercept	2.533	0.072	<0.001
Gender <sup>b</sup>	0.035	0.012	<0.01
Ethnicity <sup>c</sup>	-0.007	0.010	0.52
FFM (kg) <sup>a</sup>	0.335	0.067	<0.001
Fat mass (kg) <sup>a</sup>	0.108	0.046	<0.05
Leptin (ng/mL) <sup>a</sup>	-0.001	0.034	0.97
Model $r^2 = 0.68$ ; $P < 0.0001$			
Dependent variable = AEE (Cal/day) <sup>a</sup>			
Intercept	2.237	0.238	<0.0001
Gender <sup>b</sup>	-0.048	0.039	0.22
Ethnicity <sup>c</sup>	-0.010	0.034	0.76
FFM (kg) <sup>a</sup>	0.267	0.222	0.23
Fat (kg) <sup>a</sup>	0.164	0.152	0.28
Leptin (ng/mL) <sup>a</sup>	-0.052	0.114	0.65
Model $r^2 = 0.16$ ; $P < 0.05$			

$\beta$ , Regression coefficient; SE( $\beta$ ), of regression coefficient.

<sup>a</sup> Data were transformed ( $\log_{10}$ ) before analysis.

<sup>b</sup> Where girls = 0 and boys = 1.

<sup>c</sup> Where Caucasians = 0 and African-Americans = 1.

significant indirect effects of FFM and fat mass on TEE, but not on AEE. FFM had a significant negative effect and fat mass had a significant positive indirect effect on TEE.

## Discussion

Previous studies in the *ob/ob* mouse (1, 13), Pima Indian children (3), and postmenopausal African-Americans (2, 14) suggest that serum leptin concentrations are positively associated with energy expenditure and physical activity. Our data also show positive relationships between serum leptin and energy expenditure, but these relationships are not independent of fat mass.

In the present study, our primary goal was to test the hypothesis that leptin has an effect on energy expenditure (TEE, REE, and AEE) that is independent of body composition and other potentially confounding factors. Our first step was to use multiple regression models to determine whether serum leptin concentrations were significantly and positively related to measures of energy expenditure when the potential influences of FFM, fat mass, gender, and ethnicity were controlled statistically. These additional independent variables (FFM, fat mass, gender, and ethnicity) were chosen because they are known to influence both serum leptin concentrations (6) and measures of energy expenditure (15, 16). The results showed no independent effect of leptin on any measure of energy expenditure (TEE, REE, and/or AEE). Thus, our data do not support an independent effect of leptin on measures of energy expenditure in African-American and Caucasian children.

We next evaluated our data using structural equation models. Our model included two correlated exogenous vari-

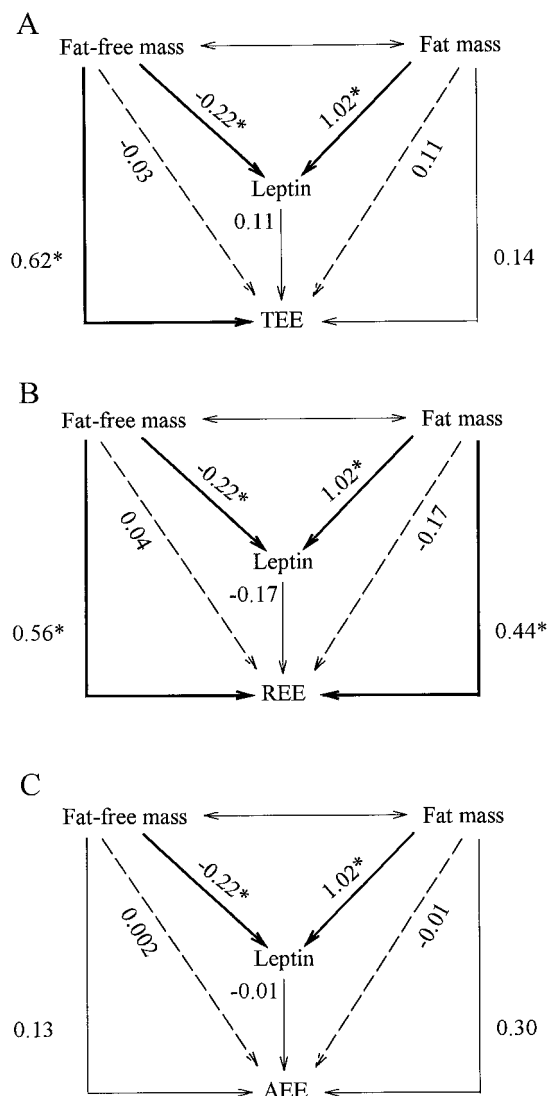


FIG. 1. Structural equation model describing the effects of FFM and fat mass (exogenously correlated variables) on leptin and energy expenditure and the effect of leptin on energy expenditure. Indirect effects (via leptin) of FFM and fat mass on measures of energy expenditure are shown with *dashed arrows*. \*,  $P < 0.05$ .

ables (FFM and fat mass), both of which are predictive of the same dependent variables (leptin and energy expenditure). The model specified direct effects of FFM and fat mass on both leptin and measures of energy expenditure and a direct effect of leptin on energy expenditure. Additionally, use of this model allowed us to determine indirect effects of FFM and fat mass on energy expenditure (through leptin). Our results showed that there were no significant relationships between leptin and TEE, REE, or AEE. In two of the models, the coefficient between leptin and energy expenditure was negative, albeit not significant (REE,  $-0.17$ ; AEE,  $-0.01$ ). Lastly, there were no significant indirect effects of FFM and fat mass on energy expenditure (TEE, REE, or AEE) through the leptin pathway.

The majority of studies dealing with the relationship of leptin to energy expenditure in humans have used REE as the outcome variable. However, the findings from these studies

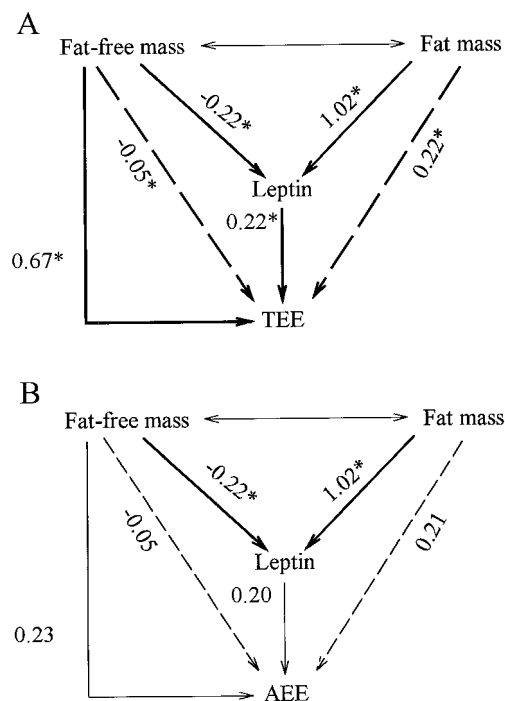


FIG. 2. Structural equation model describing the effects of FFM and fat mass (exogenously correlated variables) on leptin and energy expenditure and the effect of leptin on energy expenditure. In this model, the path between fat mass and energy expenditure has been removed. Indirect effects (via leptin) of FFM and fat mass on measures of energy expenditure are shown with *dashed arrows*. \*,  $P < 0.05$ .

are mixed, with some reporting no relationship (Refs. 2, 3, 14, and 17 and present study), one reporting a negative relationship (18), and two reporting positive relationships (2, 14) after controlling for some measure of body composition. The reason why these studies differ is unclear, but could be a result of many things, including differences in subject population and statistical methods used to control for confounding variables.

The best method of normalizing energy expenditure and leptin for body composition is not straightforward, and the choice of covariates may affect the outcome. For instance, a positive relationship between REE and leptin, after controlling REE for differences in FFM in postmenopausal African-American women, has been documented (2, 14). One possible explanation for the positive relationship between REE and leptin is that although REE was normalized for FFM, REE was not normalized for fat mass, which also is positively correlated to REE (15). Failure to normalize REE for both FFM and fat mass may have led to a confounding effect by not removing the effect of fat mass on REE. Thus, both the dependent variable (REE adjusted for FFM) and the independent variable (log leptin) are positively correlated with fat mass, leading to the positive finding. In fact, when REE was adjusted for both FFM and fat mass, there was no longer a significant relationship between leptin and REE (Nicklas, B., personal communication).

It has been suggested that correcting measures of energy expenditure for fat mass may negate the relationship between leptin and energy expenditure because of the ex-

tremely good correlation between leptin and fat mass (14). Furthermore, it has been argued that fat mass may be an inappropriate covariate, as fat mass is relatively inactive and accounts for only 4% of whole body oxygen consumption (14). These points are well taken, and when our data were analyzed without fat mass as an independent variable in the multiple regression models, leptin was significantly and positively related to TEE and REE, but not to AEE. Similarly, in our structural equation model, when we removed the non-significant pathway between fat mass and energy expenditure, the pathway from leptin to TEE was significant. The indirect effects of fat mass and FFM on TEE were also significant ( $P < 0.05$ ), but with opposite signs: fat mass had a positive whereas FFM had a negative indirect effect on TEE. Thus, although it is clear that leptin does not have an effect on energy expenditure that is independent of fat mass, we cannot rule out the possibility that leptin may play a role in human energy expenditure.

The negative indirect effect of FFM on TEE through leptin deserves comment. Using multiple regression techniques (6) and structural equation models (present study), we have shown that FFM is negatively related to serum leptin concentrations when both FFM and fat mass are entered into the model. Thus, although FFM is the major determinant of energy expenditure through direct pathways, it has a potentially negative effect on energy expenditure through its negative relationship with leptin. Therefore, the observation that future studies examining the relationship of leptin and energy expenditure should be conducted in individuals matched for fat mass (14) may need to be revised to individuals matched for body composition (FFM and fat mass).

In conclusion, our data do not support the hypothesis that serum leptin concentrations play an independent role in regulating energy expenditure (TEE, AEE, and REE) in African-American and Caucasian prepupal children after controlling for fat mass. However, because of the close correlation of leptin to fat mass, we cannot rule out the possibility that leptin has an effect on energy expenditure in children. Future studies using individuals matched for body composition (fat mass and FFM) and/or studies that manipulate leptin concentrations will be needed to determine the role of leptin in energy expenditure in humans.

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