Influence of Leptin on Changes in Body Fat during Growth in African American and White Children

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Abstract

JOHNSON, MARIA S., TERRY T-K. HUANG, REINALDO FIGUEROA-COLON, JAMES H. DWYER, AND MICHAEL I. GORAN. Influence of leptin on changes in body fat during growth in African American and white children. *Obes Res.* 2001;9:593-598.

Objective: The aim of this study was to determine whether initial levels or temporal changes in fasting leptin were associated with longitudinal changes in body-fat mass in children.

Research Methods and Procedures: The study group consisted of 85 children (42 white and 43 African American) with a mean initial age of 8.1 ± 0.1 years. The children had between three and six annual visits for repeated measurements of body composition by DXA and fasting leptin level. Fat mass and fasting leptin level were not normally distributed and were log-transformed. Data were analyzed using SAS Proc mixed growth models, with log fat as the dependent variable.

Results: Initial leptin level was a significant predictor of the change in fat mass over time ($p < 0.0001$), with high initial leptin levels resulting in increased fat gain, independent of initial fat levels. This relationship remained significant when the data were analyzed separately by race (whites, $p < 0.0001$; African Americans, $p = 0.008$). The relationship between the initial level of leptin and the change in fat mass was not modified by race, sex, or Tanner Stage. The rate of change in leptin during the study was significantly related to the rate of change in fat mass in African Americans ($p = 0.008$) but not in whites ($p = 0.490$).

Discussion: In conclusion, high fasting leptin level at the start of the study was significantly associated with increasing fat mass in this cohort, indicating that the children may be developing resistance to the effects of leptin.

Key words: leptin, children, body fat, longitudinal, ethnicity

Introduction

The role of leptin in human obesity has been widely studied since its discovery in 1994. At first it was thought that human obesity may occur as a result of a lack of leptin (as in the *ob/ob* mouse). However, studies found that the majority of humans did not lack leptin; in contrast, they had circulating leptin concentrations that were highly correlated with adipose tissue mass (1–5). It is possible that overweight people had low leptin levels initially, which may have resulted in their gaining weight. However, the increase in fat mass then led to an increase in leptin levels, signaling the increased fat stores. In intervention studies, leptin has been shown to decrease with weight loss (6) and increase with weight gain associated with overfeeding (7).

Longitudinal studies were performed to examine whether baseline leptin concentrations predicted changes in weight or fat mass over a follow-up period. Studies of diverse populations, such as adult Mauritians (8), postobese women (9), and white and African American young adults (10), have failed to find any associations between initial leptin concentrations and subsequent weight gain over differing periods (4 to 8 years). However, a positive relationship was found in a cohort of middle-aged Swedish women (11) with high leptin concentration measured at 38 to 46 years of age, predicting increased weight gain over the ensuing 24 years (11).

Currently, there is no clear picture of the effect of leptin on weight gain in humans. Differences in race, age, sex, and follow-up time between studies mean that generalizations and comparisons are difficult. In addition, the outcome

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variable (weight gain) differs widely among studies, from changes in body weight and body mass index to the percentage of fat and total fat mass. In this study we aimed to determine whether initial fasting leptin level and the rate of change in leptin level over time were related to the rate of increase in total body fat in growing white and African American children. The rate of increase in total body fat was previously estimated to be 15.6% per year in this cohort (12). By including both the initial level of leptin (at first visit) and the subsequent rate of change in leptin during growth, we aimed to determine whether leptin level changed as the level of fat mass changed, and independent of this change, whether initial levels of leptin were predictive of the rate of change in fat mass.

Research Methods and Procedures

Subjects

This study consisted of 85 children, 42 white (19 boys and 23 girls) and 43 African American (21 boys and 22 girls) children who had at least three annual study visits with repeat measurements of both leptin and body fat. The mean age at the initial measurement was 8.1 ± 0.1 years. Children were recruited from Birmingham, Alabama, and had been free of any major illnesses since birth (13). Cross-sectional data on leptin and body fat and leptin and energy expenditure were reported previously (1,14). Serum leptin was significantly related to body composition and fat distribution, but not to energy expenditure in children, independent of fat mass. Studies were performed during the school year (fall and spring). The nature, purpose, and possible risks of the study were explained carefully to the parents before consent was obtained. This study was approved by the Institutional Review Board at the University of Alabama at Birmingham. All measurements were performed at the General Clinical Research Center (GCRC) and the Department of Nutrition Sciences at the University of Alabama at Birmingham between 1994 and 1999.

Protocol

Children were admitted to the GCRC in the late afternoon for an overnight visit. Anthropometric measurements, including assessment of sexual maturation were obtained. After 8:00 PM only water and energy-free, noncaffeinated beverages were permitted until after the morning testing. On the following morning, blood was collected for analysis of leptin concentration. Two weeks later the children arrived at the Energy Metabolism Research Unit at 7:00 AM in the fasted state. Body composition was determined by DXA.

Assessment of Sexual Maturation

Sexual maturation was assessed according to pubic hair and breast development in girls and genitalia in boys using Tanner's criteria on a scale of 1 to 5, with 1 being prepubertal and 5 being adult. The same qualified pediatrician (R.F.-C.) assessed Tanner Stage in all the children.

Assessment of Body Composition

Body composition was measured by DXA using a DPX-L densitometer (Lunar, Madison, WI) that we had validated previously in the pediatric body-weight range (15,16). DXA scans were performed and analyzed using pediatric software (version 1.5e; Lunar) (15,16).

Serum Leptin Concentrations

Leptin concentrations were determined with a radioimmunoassay 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 intra-assay coefficient of variation was 4.2% at 64% bound (2.61 ng/mL) and 3.5% at 28% bound (14.9 ng/mL).

Statistics

Total fat mass, lean tissue mass, and fasting leptin levels were not normally distributed and were log-transformed before analysis. Random coefficients mixed models were used to determine the overall growth rate of body fat and the influence of leptin on this growth rate. Mixed models were used to account for intraperson correlations among repeated measures (mean of 4.3 measures per child). Between- and within-subject degrees of freedom were used, and the covariance structure of the repeated dependent measures was unstructured.

The following specifications were made for the model that was used to examine the effect of leptin on the rate of change in fat mass:

- Dependent variable: log fat mass
- Class variable: visit number (0 to 5); visit number was also used in the repeated statement to account for missing data.
- Fixed variables: initial age; initial fat mass; race (0 for white and 1 for African American); sex (0 for boys and 1 for girls); Tanner Stage (1 to 5); initial leptin; and rate of change of leptin (Δ leptin).
- Random effects: time (years) since initial visit; intercept (defined at baseline).
- Interactions: the interaction between time and other variables in the model was used to interpret whether a particular variable had a significant effect on the change in fat over time. For example, the time × race interaction represented the effect of race on the rate of change of fat over time. Two-way interactions between time and the fixed variables were included in the model.

The initial leptin level and the rate of change of leptin were calculated by regressing log leptin against time and saving the slopes and intercepts. Three-way interactions were also included among time; the leptin variables; and

Table 1. Baseline descriptive statistics of the cohort*

	Total sample	White boys	White girls	African American boys	African American girls	Significant effects
Age (years)	8.11 ± 0.14	8.38 ± 0.38	8.11 ± 0.31	7.70 ± 0.33	8.05 ± 0.18	None
Tanner Stage (1 to 5)	1.09 ± 0.03	1.05 ± 0.05	1.04 ± 0.04	1.00 ± 0.00	1.23 ± 0.11	None
Total fat mass (kg)	9.49 ± 0.53	9.92 ± 1.54	8.84 ± 1.12	10.75 ± 1.83	10.98 ± 1.53	None
Lean tissue mass (kg)	20.77 ± 0.44	21.72 ± 1.29	19.46 ± 0.92	23.00 ± 1.12	20.66 ± 1.05	Gender
Leptin (ng/mL)	7.42 ± 1.10	7.09 ± 1.22	7.10 ± 1.17	6.87 ± 1.20	8.46 ± 1.18	None

* Data presented as means ± SE.

race, sex, and Tanner Stage to test whether the relationship between the changes in leptin or initial leptin and the changes in fat mass differ between boys and girls, between whites and African Americans, or by degree of sexual maturity. If the rate of change in leptin is significantly associated with the change in fat mass over time, then this association indicates that both fat mass and leptin levels are changing together over time, whereas if the initial leptin level is significantly associated with the rate of change in fat mass, then this association indicates a degree of causality; initial leptin levels predict subsequent changes in fat mass. The models were all run with the three-way interactions; however, only the significant interactions are reported in the tables. Data were analyzed using SAS statistical software, version 7.0 (SAS, Cary, NC), with a significance level of $p < 0.05$.

Results

Descriptive Statistics

Means and SEs are presented for the entire sample at baseline (visit 1) and then separately for the four race and gender groups (Table 1). There were no significant differences in any of the variables between African American and white children ($p > 0.2$). However, boys did have significantly greater lean tissue mass than the girls ($p = 0.034$). Fat mass, age, Tanner Stage, or leptin concentration did not differ significantly by sex ($p > 0.1$).

Effect of Leptin on the Acquisition of Fat

Results from the model are presented in Table 2. Initial leptin was significantly and positively related to the rate of change in fat mass ($\beta = 0.085$, $p < 0.0001$), even after adjusting for initial fat mass. A doubling in the initial leptin levels resulted in a 6.1% (95% confidence interval [CI]: 3.6 to 8.5) difference in the rate of change in fat mass (kilograms/year). This relationship between initial leptin and the rate of change in fat mass was not significantly influenced by race ($p = 0.220$), sex ($p = 0.476$), or Tanner Stage ($p = 0.519$). Although the rate of change in leptin was not significantly related to the rate of change in fat mass ($p = 0.273$), there were significant interactions between this relationship and Tanner Stage ($\beta = 0.057$, $p = 0.035$) and between this relationship and race ($\beta = 0.214$, $p = 0.012$), but not with sex ($p = 0.349$). An increase in the rate of change in leptin resulted in a greater rate of change in fat mass at higher Tanner Stages. None of the above relationships were affected when lean mass was included as a covariate. Because of the significant race interaction, the data were split by race and reanalyzed (Tables 3 and 4).

Table 2. Results of the SAS Proc mixed growth model to examine the relationship between leptin and the rate of acquisition of fat

	β	p
Time (years)	0.003 ± 0.030	0.896
Initial age (years)	**0.014 ± 0.005**	**0.006**
Initial fat (kg)	**0.850 ± 0.051**	**<0.0001**
Race (0 white, 1 AA)	0.011 ± 0.014	0.433
Sex (0 boy, 1 girl)	0.007 ± 0.014	0.613
Tanner Stage (1 to 5)	0.004 ± 0.013	0.749
Initial leptin	**0.154 ± 0.037**	**<0.0001**
Δ leptin	0.168 ± 0.092	0.074
Time × initial age	−0.005 ± 0.003	0.087
Time × initial leptin	**0.085 ± 0.017**	**<0.0001**
Time × Δ leptin	0.104 ± 0.094	0.273
Time × Δ leptin × race	**0.214 ± 0.084**	**0.012**
Time × Δ leptin × Tanner	**0.057 ± 0.027**	**0.035**

Significant model factors are shown in bold.
Only the significant three-way interactions are presented.
Δ leptin, change in leptin; AA, African American.

Table 3. Results of the SAS Proc mixed growth model to assess the influence of leptin on the rate of acquisition of fat in whites

	β	p
Time (years)	0.021 ± 0.036	0.567
Initial age (years)	0.008 ± 0.007	0.288
Initial fat (kg)	**1.013 ± 0.086**	**<0.0001**
Sex (0 boy, 1 girl)	0.024 ± 0.019	0.225
Tanner Stage (1 to 5)	0.020 ± 0.023	0.371
Initial leptin	0.043 ± 0.054	0.427
Δ leptin	0.037 ± 0.114	0.748
Time × initial age	−0.008 ± 0.004	0.052
Time × initial leptin	**0.097 ± 0.023**	**<0.0001**
Time × Δ leptin	0.079 ± 0.114	0.490

Significant model factors are shown in bold.
None of the three-way interactions were significant and, therefore, are not presented.
Δ leptin, change in leptin.

Table 4. Results of the SAS Proc mixed growth model to assess the influence of leptin on the rate of acquisition of fat in African Americans

	β	p
Time (years)	0.003 ± 0.031	0.931
Initial age (years)	**0.020 ± 0.007**	**0.008**
Initial fat (kg)	**0.733 ± 0.063**	**<0.0001**
Sex (0 boy, 1 girl)	−0.004 ± 0.021	0.846
Tanner Stage (1 to 5)	−0.001 ± 0.017	0.958
Initial leptin	**0.242 ± 0.053**	**<0.0001**
Δ leptin	**0.355 ± 0.165**	**0.038**
Time × initial age	−0.004 ± 0.004	0.221
Time × initial leptin	**0.061 ± 0.023**	**0.008**
Time × Δ leptin	**0.402 ± 0.149**	**0.008**

Significant model factors are shown in bold.
None of the three-way interactions were significant and are therefore not presented.
Δ leptin, change in leptin.

In white children (Table 3), initial leptin was significantly and positively related to the rate of change in fat mass over time ($\beta = 0.097$, $p < 0.0001$). A doubling in initial leptin levels resulted in a 7.0% (95% CI: 3.7 to 10.3) difference in the rate of change in fat mass (kilograms/year). This relationship was not significantly influenced by Tanner Stage ($p = 0.067$) or sex ($p = 0.791$). In addition, the relationship between the rate of change in leptin and the rate of change in fat mass was not significant ($p = 0.490$) and was not significantly influenced by sex ($p = 0.095$) or Tanner Stage ($p = 0.219$). When the analysis was repeated including lean mass as a covariate, the relationships remained the same.

In African American children (Table 4), both initial leptin level ($\beta = 0.061$, $p = 0.008$) and the change in leptin ($\beta = 0.402$, $p = 0.008$) were significantly and positively related to the rate of change in fat mass over time, independent of the effect of initial fat mass. Therefore, a doubling in initial leptin level and in the rate of change in leptin resulted in a 4.3% (95% CI: 1.1 to 7.6) and 32.1% (95% CI: 7.9 to 61.8) difference in the rate of change in fat mass, respectively. These relationships were not influenced by sex or Tanner Stage ($p > 0.100$). When the data were adjusted for lean mass, none of the relationships changed.

Discussion

The major finding of this study was that high baseline leptin concentration was predictive of an increased rate of subsequent fat gain in boys and girls and in African Americans and whites, independent of the initial level of fat mass. In contrast, the temporal changes in leptin and fat mass during this period of growth were significantly related in African Americans but not whites.

Although the main effect of time was not significant in any of the models, this does not mean that the children were not increasing in body fat over time. Rather, the significant two- and three-way interactions between time and other variables indicated that the rate of increase in body fat was significantly modified by the other variables, including initial leptin.

Results from previous human longitudinal studies on the relationship between leptin and fat gain have not been consistent. No relationship was found between baseline leptin and subsequent weight or fat gain in adult Mauritians (8), postobese postmenopausal women (9), or African American and white young adults (10). In contrast, low baseline leptin was found to predict increased weight gain over 1 year in prepubertal Australian children (2) and also increased the percentage of fat in girls going through puberty (17). The results from these two studies seem to indicate that children are responding to low leptin levels in a normal way and are acting to increase fat stores. However, in the current study, high baseline levels of leptin, rather than low, were predictive of increased fat gain. A doubling of the initial leptin level would result in an increase in the change of fat mass from 15.6% per year to 21.1% per year. This translates to an extra gain of 1.74 kg of fat mass over 3 years (based on the average fat mass of 9.49 kg at baseline). These results are consistent with a study of Swedish women that found that high leptin levels in 38- to

46-year-olds predicted increased weight gain (11). High leptin levels should signal that the body has ample fat stores present and further inhibit the increase in fat stores. However, the children in this study with high leptin levels actually gained more fat mass. High initial leptin levels could just be a marker for high initial fat mass and the positive relationship between initial leptin levels and the increase in fat mass could be explained as the fatter children at the start of the study gaining more fat mass. However, when initial fat mass was included in the model, the relationship between the initial level of leptin and the increase in fat mass remained significant (positive). This may be an indication of the development of leptin resistance, because although high leptin levels are present, fat stores are still increasing. Resistance to leptin has been documented in some animals including obese *Psammomys obesus* (18), and Sprague–Dawley rats have been shown to become more resistant to leptin as they age (19). There is little evidence in human studies of leptin resistance; however, this remains the most likely explanation for these results.

In girls, leptin has been shown to increase throughout puberty (17,20–22), whereas in boys, leptin increases before puberty and then declines after puberty (17,20,21). In this study, Tanner Stage, as a marker for sexual development, did not modify the relationship between baseline leptin and the rate of change in fat mass, which was the same in both boys and girls. However, as mentioned previously, the majority of visits of these children were at Tanner Stage 3 or lower (94%), and additional follow-up would be needed to assess any changes relating to the later stages of puberty.

Change in Leptin

The rate of change in leptin during growth was found to be positively related to the rate of change in fat mass in African American children but not in white children. There have been few studies examining whether change in leptin is related to change in fat mass. Most studies have only examined whether baseline levels of leptin predict subsequent weight gain. However, leptin change was found to be highly correlated with weight change in one study of African American and white young adults (10). Adipose tissue is the primary source of circulating leptin, and as this tissue increases, more leptin is produced. Therefore, changes in fat mass should be associated with changes in leptin in all groups. It has been shown that leptin mRNA is expressed less in visceral fat compared with subcutaneous fat (23,24), and because whites have more visceral fat than do African Americans (25,26), there may be a lower correlation of leptin to fat mass in whites because of greater amounts of visceral fat. However, the reason behind the uncoupling of changes in fat mass and leptin in white children is not clear.

In African American children, the relationship between the rate of change in leptin and the rate of change in fat mass was the same for boys and girls and for children at different stages of maturation. However, Tanner Stage was a significant modifier of the relationship between the rate of change in leptin and the rate of change in fat mass in the combined analysis, with the rate of change in leptin having a greater effect on the rate of change of fat mass in those children at high Tanner Stage compared with those at a low Tanner Stage. This relationship was not seen when the data were analyzed by race, although a trend was observed in whites that failed to reach significance ($p = 0.067$). Once the data were analyzed separately, there was probably insufficient power to detect these effects.

To determine whether the addition of the significant interaction terms in the model of the whole cohort explained more of the variance in total fat mass, we compared the -2 log likelihood values. When the two two-way interactions, time $\times$ initial leptin level and time $\times$ rate of change in leptin level were added, the model explained significantly more of the variance in total fat mass ($\chi^2_{(2)} \approx 34$, $p < 0.001$). However, although the addition of the significant three-way interactions (time $\times$ change in leptin level $\times$ race and time $\times$ change in leptin $\times$ Tanner Stage) did not improve the fit of the model, they provide important insight into how the change in leptin level and change in fat may differ between races and at different stages of sexual maturation.

In conclusion, high initial levels of leptin were found to be predictive of increased fat gain in all children, regardless of race, sex, or degree of sexual maturation. This is counterintuitive because high leptin levels should inhibit additional increases in fat mass. The results that we observed may be an indication that the children are becoming resistant to the effect of leptin during early pubertal growth.

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References

1. **Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI.** Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. *J Clin Endocrinol Metab.* 1997;82:2148–52.
2. **Byrnes SE, Baur LA, Bermingham M, Brock K, Steinbeck K.** Leptin and total cholesterol are predictors of weight gain in pre-pubertal children. *Int J Obes Relat Metab Disord.* 1999; 23:146–50.
3. **Ho SC, Tai ES, Eng PHK, Ramli A, Tan CE, Fok ACK.** A study in the relationships between leptin, insulin, and body fat in Asian subjects. *Int J Obes Relat Metab Disord.* 1999;23: 246–52.

4. **Jensen MD, Hensrud D, O'Brien PC, Nielsen S.** Collection and interpretation of plasma leptin concentration data in humans. *Obes Res.* 1999;7:241–5.
5. **Caprio S, Tamborlane WV, Silver D, et al.** Hyperleptinemia: an early sign of juvenile obesity: relations to body fat depots and insulin concentrations. *Am J Physiol.* 1996;271:E626–E30.
6. **Eriksson J, Valle T, Lindstrom J, et al.** Leptin concentrations and their relation to body fat distribution and weight loss—a prospective study in individuals with impaired glucose tolerance. *Horm Metab Res.* 1999;31:616–9.
7. **Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF.** Response of leptin to short-term and prolonged overfeeding in humans. *J Clin Endocrinol Metab.* 1996;81:4162–5.
8. **Hodge AM, De Courten MP, Dowse GK, et al.** Do leptin levels predict weight gain? A 5-year follow-up study in Mauritius. *Obes Res.* 1998;6:319–25.
9. **Nagy TR, Davies SL, Hunter GR, Darnell B, Weinsier RL.** Serum leptin concentrations and weight gain in postobese, postmenopausal women. *Obes Res.* 1998;6:257–61.
10. **Folsom AR, Jensen MD, Jacobs DRJ, Hilner JE, Tsai AW, Schreiner PJ.** Serum leptin and weight gain over 8 years in African American and Caucasian young adults. *Obes Res.* 1999;7:1–8.
11. **Lissner L, Karlsson C, Lindroos AK, et al.** Birth weight, adulthood BMI, and subsequent weight gain in relation to leptin levels in Swedish women. *Obes Res.* 1999;7:150–4.
12. **Johnson MS, Figueroa-Colon R, Huang TT-K, Dwyer JH, Goran MI.** Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. *J Clin Endocrinol Metab.* 2001;86: 3182–7.
13. **Sun M, Gower BA, Nagy TR, Trowbridge CA, Dezenberg C, Goran MI.** Total, resting, and activity-related energy expenditures are similar in Caucasian and African American children. *Am J Physiol.* 1998;274:E232–E7.
14. **Nagy TR, Gower BA, Shewchuk RM, Goran MI.** Serum leptin and energy expenditure in children. *J Clin Endocrinol Metab.* 1997;82:4149–53.
15. **Goran MI, Driscoll P, Johnson R, Nagy TR, Hunter G.** Cross-calibration of body composition techniques against dual-energy X-ray absorptiometry in young children. *Am J Clin Nutr.* 1996;63:299–305.
16. **Pintauro S, Nagy TR, Duthie C, Goran MI.** Cross-calibration of fat and lean measurements by dual-energy X-ray absorptiometry to pig carcass analysis in the pediatric body weight range. *Am J Clin Nutr.* 1996;63:293–9.
17. **Ahmed ML, Ong KKL, Morrell DJ, et al.** Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. *J Clin Endocrinol Metab.* 1999;84:899–905.
18. **Walder K, Lewandowski P, Morton G, et al.** Leptin resistance in a polygenic, hyperleptinemic animal model of obesity and NIDDM: *Psammomys obesus*. *Int J Obes Relat Metab Disord.* 1999;23:83–9.
19. **Qian H, Azain MJ, Hartzell DL, Baile CA.** Increased leptin resistance as rats grow to maturity. *Proc Soc Exp Biol Med.* 1998;219:160–5.
20. **Clayton PE, Trueman JA.** Leptin and puberty. *Arch Dis Child.* 2000;83:1–3.
21. **Roemmich JN, Rogol AD.** Role of leptin during childhood growth and development. *Endocrinol Metab Clin North Am.* 1999;28:749–64.
22. **Demerath EW, Towne B, Wisemandle W, Blangero J, Chumlea WC, Siervogel RM.** Serum leptin concentration, body composition, and gonadal hormones during puberty. *Int J Obes Relat Metab Disord.* 1999;23:678–85.
23. **Masuzaki H, Ogawa Y, Isse N, et al.** Human obese gene expression: adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes.* 1995;44:855–8.
24. **Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S.** Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes.* 1997;46:342–7.
25. **Goran MI, Nagy TR, Treuth MS, et al.** Visceral fat in white and African-American prepubertal children. *Am J Clin Nutr.* 1997;65:1703–8.
26. **Conway JM, Yanovski SZ, Avila NA, Hubbard VS.** Visceral adipose tissue differences in black and white women. *Am J Clin Nutr.* 1995;61:765–71.