Daily Energy Expenditure Is Related to Plasma Leptin Concentrations in Older African-American Women but Not Men

Barbara J. Nicklas, Michael J. Toth, and Eric T. Poehlman

Recent research suggests that leptin may control body weight by regulating energy expenditure and energy intake in mice. To explore the possible role of leptin in the regulation of energy expenditure in humans, we used doubly-labeled water methodology to determine whether fasting plasma leptin concentrations were related to total daily energy expenditure (TEE) and its components, resting energy expenditure (REE) and physical activity energy expenditure (PAEE), in free-living older African-American men (n = 21) and women (n = 25). Plasma leptin concentrations were higher in women than men, even after the adjustment for differences in fat mass (28 ± 3 ng/ml for women vs. 17 ± 3 ng/ml for men; $P < 0.01$). The logarithm of plasma leptin concentrations correlated with fat mass in both women ($r = 0.80$) and men ($r = 0.78$) ($P < 0.0001$). After statistical adjustment for sex differences in fat-free mass and fat mass, women had lower TEE (22%) and REE (15%) ($P < 0.01$) and a trend ($P = 0.08$) toward lower PAEE, compared with men. After controlling for the effects of fat-free mass on energy expenditure, plasma leptin concentrations were related to REE ($r = 0.68$, $P < 0.001$) and tended to be related to TEE ($r = 0.37$, $P = 0.07$) in African-American women but not men ($r = 0.18$ and -0.03, respectively). Plasma leptin concentrations were not related to PAEE in either men or women. These results suggest that leptin may contribute to the regulation of TEE in older African-American women through its effects on resting energy metabolism, but the role of leptin in the regulation of energy expenditure is less apparent in older African-American men. *Diabetes* 46:1389–1392, 1997

Leptin, the protein product of the *obesity* (*ob*) gene (1), regulates body fat stores through the hypothalamic control of energy balance (2,3). In mice, the administration of recombinant leptin induces weight loss by decreasing energy intake and increasing energy expenditure (3,4). In humans, adipose tissue expression of the leptin gene (5), adipose tissue leptin production (6), and plasma leptin concentrations are elevated in the obese state (7,8) and decrease after weight loss (7,8). However, the role of leptin in the regulation of body weight in humans is not known.

Older African-Americans, particularly women, have one of the highest prevalences of obesity in the U.S. (9). Although this may be partially due to environmental and social factors, low rates of energy expenditure may also contribute to the epidemic of obesity in African-Americans (10). Thus, African-Americans represent a particularly interesting population in which to examine the role of leptin in the regulation of human energy expenditure. Therefore, using doubly-labeled water, we examined the relationship of plasma leptin concentrations to total daily energy expenditure (TEE) and its components, resting energy expenditure (REE) and physical activity energy expenditure (PAEE), in free-living older African-American men and women.

RESEARCH DESIGN AND METHODS

Subjects. Older African-American men (n = 21, 66 ± 2 years of age) and women (n = 25, 65 ± 1 years of age) were recruited from the Baltimore metropolitan area. All subjects were sedentary (<20 min exercise 2 days/week), weight stable (<2.0 kg weight change in the past year), and nonsmoking. They were apparently healthy and exhibited *1*) no symptoms of coronary heart disease or diabetes, *2*) a normal resting electrocardiogram, *3*) a normal electrocardiogram response to an exercise stress test, and *4*) an absence of medication that could affect cardiovascular or metabolic function. The women were postmenopausal (no menstruation for at least 2 years) and not on estrogen replacement therapy. All subjects provided informed written consent to participate in the study according to the guidelines of the University of Maryland Institutional Review Board for Human Research.

Experimental protocol. All measurements were performed over a 10-day period. On the 1st day, after a baseline urine sample was obtained, each subject received an oral dose of doubly-labeled water. REE and body composition were measured the following morning after an overnight fast. After these tests, a venous blood sample was drawn and two urine samples were collected to mark the beginning of the doubly-labeled water measurement period. Subjects then resumed their daily activities (i.e., free-living conditions) and after 10 days returned to provide two urine samples.

Leptin concentrations. Venous blood samples for the measurement of leptin concentration were drawn into chilled tubes containing 1 mg of EDTA per cubic centimeter of blood. Plasma was separated by centrifugation at 4°C and samples were stored at –70°C until analysis. Leptin was measured in duplicate using radioimmunoassay (Linco, St. Louis, MO). Samples from men and women were run in the same assay. The intra-assay coefficient of variation for the measurement of leptin was 5.2%.

TEE. Free-living TEE was determined over a 10-day period using the doubly-labeled water technique, as previously described (11). Briefly, each subject consumed a mixed oral dose of ^2H_2O and $H_2{}^{18}O$ (0.078 and 0.092 g/kg body mass, respectively) after providing a baseline urine sample (between 1200 and 1600 h). Two urine samples were obtained the morning after dosing to mark the beginning of the measurement period and 10 days later to mark the end (all between 0800

From the Department of Medicine (M.J.T., E.T.P.), Division of Gerontology, University of Maryland School of Medicine, and the Geriatric Research Education and Clinical Center (B.J.N.), Baltimore Veterans Affairs Medical Center, Baltimore, Maryland.

Address correspondence and reprint request to Dr. Barbara J. Nicklas, Division of Gerontology (18), Baltimore VA Medical Center, 10 N. Greene St., Baltimore, MD 21201. E-mail: bnicklas@umabnet.ab.umd.edu.

Received for publication 4 December 1996 and accepted in revised form 18 April 1997.

ANCOVA, analysis of covariance; PAEE, physical activity energy expenditure; REE, resting energy expenditure; TEE, total (daily) energy expenditure.

and 1200 h). The urine samples were stored in sealed vacutainers at –20°C until analysis in triplicate by isotope ratio mass spectrometry at the Biomedical Mass Spectrometry Facility at the University of Maryland. Samples were analyzed for isotopic enrichment of ^2H_2O and $H_2{}^{18}O$, using the off-line zinc reduction procedure of Kendall and Copelan (12), and CO_2 equilibration technique (13), respectively. The ^2H_2O and $H_2{}^{18}O$ enrichments of the samples were expressed in delta per mil. Turnover rates and zero time enrichment of ^2H_2O and $H_2{}^{18}O$ were determined from the slope and intercept, respectively, of the semilogarithmic plot of urinary enrichment versus time (in days). Isotope dilution spaces were calculated using the equation of Coward (14). A fixed dilution space ratio of 1.0427 was used (15), and the rate of carbon dioxide production was calculated using Equation 2 of Speakman et al. (15). Carbon dioxide production rates were used to calculate TEE from Equation 12 of Weir (16), assuming a respiratory quotient of 0.85 (17).

REE. REE was measured for 45 min in the morning after a 12-h overnight fast by indirect calorimetry, using the ventilated hood method (Deltatrac, Sensormedics, Yorba Linda, CA), as previously described (11).

PAEE. PAEE was calculated based on the three-component model of daily energy expenditure: [(0.9 × TEE) – REE], as previously described (11).

Body composition. Percentage body fat, fat-free mass, and fat mass were measured using dual energy X-ray absorptiometry (DXA, Model DPX-L, Lunar Radiation, Madison, WI).

Statistics. Data were analyzed using SPSS version 6.1 for Windows. Since leptin concentrations were not normally distributed, a Mann-Whitney *U* test was used to compare leptin levels between men and women, and the natural logarithm of leptin was used for regression analysis and analysis of covariance (ANCOVA). Univariate correlations between variables were determined by linear regression analyses with calculation of Pearson's product correlation coefficients. Energy expenditure variables were adjusted for fat-free mass to control for variation in energy expenditure attributable to differences in the amount of metabolically active tissue. We used regression techniques (ANCOVA and multiple regression) to normalize energy expenditure data in light of the results of recent studies showing that the traditional approach of dividing energy expenditure by fat-free mass is invalid and leads to spurious correlations (18–20). ANCOVA adjusts energy expenditure data by removing the linear effect of fat-free mass. We have previously outlined the mathematical basis for this adjustment (20). All data are presented as means ± SE, and statistical significance was denoted by $P < 0.05$.

RESULTS

The body composition, leptin, and energy expenditure data of the subjects are shown in Table 1. Body weight did not differ between women and men, but BMI was higher in women ($P < 0.05$). As expected, percentage body fat and fat mass were greater ($P < 0.0001$) in the women, and fat-free mass was greater ($P < 0.0001$) in the men. Plasma leptin concentrations were higher in the women than in the men, even when adjusted for differences in fat mass ($P < 0.01$). Absolute rates of TEE, REE, and PAEE were lower in the women than the men. After adjustment for sex differences in fat-free mass and fat mass, TEE and REE remained lower ($P < 0.01$) in the women, and there was a trend for lower PAEE in the women ($P = 0.08$).

The logarithm of plasma leptin concentrations correlated strongly with fat mass in both women ($r = 0.80$) and men ($r = 0.78$) (both $P < 0.0001$). Leptin concentrations correlated with REE ($r = 0.62$, $P < 0.01$) but not with TEE ($r = 0.30$) or PAEE ($r = 0.06$) in women (Fig. 1). In men, plasma leptin concentrations were not significantly related to any component of daily energy expenditure (TEE, $r = 0.11$; REE, $r = 0.36$; PAEE, $r = -0.03$).

Because fat-free mass was related to REE in both men and women ($r = 0.56$, $P < 0.01$ and $r = 0.42$, $P < 0.05$, respectively) and to TEE in women ($r = 0.52$, $P < 0.01$), the relationship of leptin concentrations to TEE and REE was also analyzed after removing the effects of fat-free mass, using multiple regression analyses. The relationship between the logarithm of plasma leptin concentrations and REE persisted (partial $r = 0.68$, $P < 0.001$; Fig. 2), and there was a tendency for a relationship between the logarithm of plasma leptin concentrations and TEE (partial $r = 0.37$, $P = 0.07$; Fig. 2) in women. The logarithm of plasma leptin did not correlate with REE (partial $r = 0.18$) or TEE (partial $r = -0.03$) in men after controlling for fat-free mass.

TABLE 1
Body composition, leptin, and energy expenditure data of older African-American women and men

	Women	Men
n	25	21
Weight (kg)	83.5 ± 3.5	86.5 ± 3.6
BMI (kg/m^2)	32.0 ± 1.5	27.9 ± 1.0‡
Body fat (%)	46.7 ± 1.8	26.6 ± 1.3*
Fat mass (kg)	39.0 ± 3.0	22.8 ± 1.7*
Fat-free mass (kg)	42.2 ± 1.6	57.8 ± 2.0*
Leptin (ng/ml)	36.2 ± 4.8	6.7 ± 0.9*
Adjusted leptin (ng/ml)	27.7 ± 2.5	16.7 ± 2.7†
TEE (kcal/day)	1,999 ± 77	2,679 ± 129*
Adjusted TEE (kcal/day)	2,044 ± 119	2,625 ± 135†
REE (kcal/day)	1,365 ± 40	1,594 ± 44*
Adjusted REE (kcal/day)	1,363 ± 41	1,596 ± 47†
PAEE (kcal/day)	435 ± 62	817 ± 103†
Adjusted PAEE (kcal/day)	477 ± 106	767 ± 120§

Data are *n* or means ± SE. Leptin was adjusted for the variation in fat mass, using ANCOVA. TEE, REE, and PAEE were adjusted for the variation in fat-free mass and fat mass, using ANCOVA. *$P < 0.0001$; †$P < 0.01$; ‡$P < 0.05$; §$P = 0.08$.

DISCUSSION

Leptin is secreted from adipocytes as a hormonal signal of adipose tissue stores. While leptin influences both energy intake and energy expenditure in mice (3,4), its physiological role in humans is enigmatic. We examined the relationship between leptin and daily energy expenditure in free-living humans. In addition, the greater prevalence of obesity among older African-Americans makes this population an interesting model in which to examine the role of leptin in the regulation of human energy expenditure.

Our results show that plasma leptin concentrations were related to REE and showed a trend for a relationship to TEE in older African-American women but not in older African-American men. Moreover, leptin concentrations were not related to PAEE in either men or women. Although these correlational data do not indicate a causal effect of leptin on energy expenditure, the results suggest that leptin may participate in the regulation of body weight through its relationship with REE in older African-American women. Studies of leptin administration are necessary to confirm our preliminary finding of a possible role of leptin in the regulation of energy expenditure in humans.

The application of doubly-labeled water permitted the examination of free-living energy expenditure and its relationship to leptin concentrations in humans. In women, plasma leptin concentrations were positively related to REE, and there was a trend for an increase in TEE with increasing plasma leptin concentrations. These relationships persisted after statistically controlling for the effects of fat-free mass on energy expenditure. We adjusted energy expenditure for differences in fat-free mass because of the large influence of fat-free mass on REE and TEE (21). While it is possible that dif-

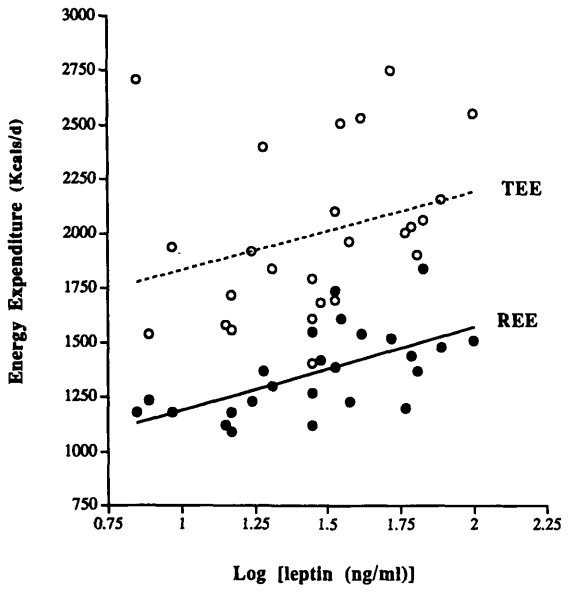

FIG. 1. Relationship of plasma leptin concentrations to absolute REE (r = 0.68, P < 0.01) and absolute TEE (r = 0.30, P = 0.15) in African-American women.

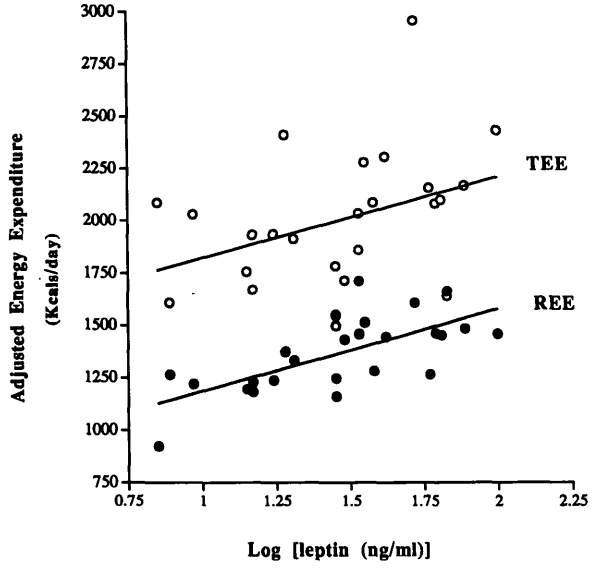

FIG. 2. Relationship of plasma leptin concentrations to REE (partial r = 0.68, P < 0.001) and TEE (partial r = 0.37, P = 0.07) after the adjustment for fat-free mass in African-American women.

ferences in fat mass may contribute to the relationship between plasma leptin and energy expenditure in women, this study cannot adequately address this issue. We did not statistically control energy expenditure variables for differences in fat mass because of the minor contribution of adipose tissue (4%) to whole body oxygen consumption (22). Moreover, because plasma leptin concentrations are highly correlated with fat mass, controlling energy expenditure for fat mass would attenuate the measured interaction between leptin and energy expenditure. Thus, the question of whether the relationship of plasma leptin to energy expenditure is independent of fat mass should be answered experimentally by examining the relationship of plasma leptin levels and energy expenditure in individuals matched for fat mass.

Although obesity is associated with low levels of physical activity (23), no relationship was found between plasma leptin concentrations and PAEE in free-living men and women. This finding is in contrast to the effects of leptin in *ob/ob* mice who are less physically active when leptin-deficient and more active after daily injections of leptin (4). Taken together, these findings suggest that in African-American women, leptin may contribute to variation in TEE through its effects on resting metabolism rather than physical activity. Moreover, because low rates of REE are associated with subsequent weight gain (24), low leptin levels may predispose some African-American women to gain body weight via a lower REE. It is also possible that a lower REE is a marker of low leptin levels that may be associated with weight gain.

Sex differences in plasma leptin levels and their relationship to energy expenditure are consistent with other documented differences in leptin physiology between men and women. Plasma leptin concentrations are higher in women than in men of similar fat mass (25), and *ob* gene expression in abdominal adipose tissue is greater in obese women than in obese men (5). Our data extend the results of other studies to report sex differences in the relationship between plasma leptin and energy expenditure. Collectively, these results suggest that sex differences in plasma leptin levels are not race-specific. Our data also show that African-American women have lower TEE and REE and a trend for lower PAEE than African-American men after controlling for differences in body composition. This finding supports previous work that showed a lower REE in Caucasian women for their metabolic size (26,27). The reason for the sex difference in the relationship of plasma leptin concentrations to energy expenditure is unclear, but it may be associated with these sex differences in REE and PAEE or may be related to the higher adiposity in women. Whether lower rates of energy expenditure in women are related to alterations in leptin signaling has yet to be determined.

In conclusion, higher plasma leptin concentrations are associated with elevated rates of energy expenditure at rest and tend to be associated with elevated rates of TEE in African-American women. However, leptin is not related to PAEE in either men or women. These results suggest that leptin may partially contribute to the regulation of daily energy expenditure in older African-American women through its effects on resting energy metabolism.

ACKNOWLEDGMENTS

This study was supported by National Institute on Aging Research and Training in Gerontology and Exercise Physiology Grant T32 AG002109 (to B.J.N. and M.J.T.), National Institutes of Health Grant R01 (AG07857) (to E.T.P.), and National Institutes of Health Grant K04 (AG00564) (to E.T.P.).

We thank the men and women who volunteered for this study and the staff of the Geriatrics Service at the Baltimore Veterans Affairs Medical Center. We especially appreciate the laboratory assistance from Tekum Fonong and the assistance with data collection from Kendall Donaldson and Sarah Schneider.

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