Total Energy Expenditure and the Level of Physical Activity Correlate With Plasma Leptin Concentrations in Five-Year-Old Children

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Abstract

Leptin, the product of the *ob* gene, is a hormone secreted by adipocytes that is known to decrease food intake and increase energy expenditure in *ob/ob* mice. In humans, variants in the *OB* gene have not been detected and very little is known about the action of leptin on food intake and energy expenditure, although circulating leptin concentrations are positively correlated to body fat stores. The purpose of this study was to assess the relationship between fasting plasma leptin concentrations and energy expenditure in 123 5-yr-old Pima Indian children (67 males/76 females). Body composition was assessed by isotopic water dilution (^{18}O) whereas total energy expenditure (TEE) and resting metabolic rate (RMR) were measured using doubly labeled water and indirect calorimetry, respectively. The physical activity level was calculated as the ratio of TEE:RMR. Plasma leptin concentrations were positively correlated to percent body fat ($r = 0.84$, $P < 0.0001$), but were similar in boys and girls after adjusting for percent body fat. Most importantly, we found that, independent of the percentage of body fat, plasma leptin concentrations correlated with TEE (in absolute values, $r = 0.37$, $P < 0.0001$, or adjusted for body size $r = 0.42$; $P < 0.0001$) and with physical activity level ($r = 0.26$, $P < 0.01$), but not RMR. These results suggest that, as in animal models, leptin plays a role in energy expenditure in humans. (*J. Clin. Invest.* 1997. 99:592–595.) Key words: obesity • preschool-aged children • doubly labeled water • Pima Indian • energy metabolism

Introduction

Leptin, the product of the mouse *ob* gene, is a hormone secreted by adipocytes (1) that decreases food intake (2–5) and increases body temperature (2), metabolic rate (2, 3), and physical activity (2), resulting in a rapid decline in body weight in the *ob/ob* mouse (2–5). Leptin is believed to regulate food intake through a negative feedback signal between adipose tissue stores and the satiety centers in the hypothalamus, possibly mediated by neuropeptide Y (5, 6). Regulation of energy metabolism by leptin may stem from stimulation of sympathetic nervous system activity, independent of food intake (7).

In humans, variants in the *OB* gene have not been detected (8); instead, circulating leptin concentrations are positively correlated to body fat stores and body mass index (9, 10), suggesting defects in leptin signaling pathways in obese individuals (11). However, low plasma leptin concentrations have been reported in Pima Indians who subsequently gained weight as compared with Pima Indians who maintained their weight (12), suggesting that leptin may play a role in the development of obesity. Whether this is due to excess food intake, low levels of energy expenditure, or both is not known.

In the present study, we have investigated the relationship between fasting plasma leptin concentrations and energy metabolism in 123 5-yr-old children, a group that is prone to obesity. As expected, plasma leptin concentrations were positively correlated to percent body fat. However, independent of body fat, leptin concentrations correlated with total energy expenditure (TEE,[1] absolute or adjusted for body size) and physical activity level (PAL), suggesting that leptin plays a role in energy expenditure in humans.

Methods

Subjects. We studied 123 5-yr-old Pima Indian children (67 males/76 females, aged 5.5±0.3 yr) during the summer months of 1992 to 1995. A previous report on physical activity in 5-yr-old children contained some of the data presented here as well as most of the experimental details (13). The experimental protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Indian Community, and informed consent/assent was obtained.

Briefly, studies were conducted in healthy children after a 10-h overnight fast on two occasions, 1 wk apart, between 0800 and 1400 hours. Anthropometric, body composition, and resting metabolic rate (RMR) measurements were performed during both clinic admissions; results represent the means of the two measurements. Height was measured without shoes. Weight was measured while the child was wearing light summer clothing. Relative weight was determined using a reference population described by Jelliffe (14). Skinfolds (Lange Skinfold Calipers; Beta Technology Inc., Cambridge, MD) were measured on the nondominant side at five sites: triceps, biceps, suprailiac, subscapular, and calf. Body water, calculated from ^{18}O dilution spaces, was used to assess body composition with the assumption that water is 75% of the fat-free mass in girls and 74% in boys (15).

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1. *Abbreviations used in this paper:* PAL, physical activity level; RMR, resting metabolic rate; TEE, total energy expenditure.

Total energy expenditure. TEE was measured by the doubly labeled water method as previously described (16). The dose contained 0.132 g 100% ^2H_2O/kg total body wt (Isotec, Inc., Miamisburg, OH) and 2.508 g 10% $H_2{}^{18}O$/kg total body wt (Isotec, Inc.). Complete urine collections were made at ~ 1.5, 2.5, 3.5, and 4.5 h after dosing on day 1, and twice during a 3-h period 7 d later. The disappearance rates of the stable isotopes were determined as previously described (17), and energy expenditure was calculated from CO_2 production using a respiratory quotient of 0.866.

Resting metabolic rate. After ingesting the doubly labeled water, the children rested comfortably on a bed for 10 min, after which the RMR was measured for 20 min using a DeltaTrac Metabolic Monitor (SensorMedics Corp., Yorba Linda, CA) as previously described (18). The RMR measurement was repeated under fasting conditions at the return visit.

Physical activity. The PAL was calculated as the ratio of TEE to RMR.

Glucose, insulin, and leptin concentrations. Immediately after the first RMR measurement, a blood sample was drawn while the child was still in the fasted state. Samples were kept on ice and centrifuged at 4°C within several minutes of collection. Plasma glucose concentrations were measured using the glucose oxidase method (Beckman Instruments Inc., Fullerton, CA). All other samples were frozen and stored at −15°C until assayed. Plasma insulin concentrations were measured by an automated radioimmunoassay technique (ICN Biomedicals, Costa Mesa, CA). Plasma leptin concentrations were measured by solid-phase sandwich enzyme immunoassay using an affinity-purified polyvalent antibody immobilized in microtiter wells. The concentration of leptin in samples was calculated from a standard curve generated in each assay with recombinant human leptin. This assay is sensitive to leptin concentrations of 20 pg/ml.

Statistical methods. Plasma leptin concentrations were not normally distributed, hence the data were log transformed before parametric analyses. Calculation of Pearson correlation coefficients as well as multiple linear regression analyses were performed using the programs of the SAS Institute (Cary, NC). Energy expenditure results and plasma leptin concentrations were adjusted for their major determinants by multiple linear regression models and are expressed as residual values, i.e., the difference between the measured value and the predicted value (on the basis of the entire group of 123 children).

Results

The physical and metabolic characteristics of the subjects and the correlations between leptin concentrations and these parameters are presented in Table I. We found a significant positive correlation between each of the parameters and the log-transformed values of fasting plasma leptin concentration. As consistently reported in adults, plasma leptin concentration was best correlated to percent body fat ($r = 0.84$, $P < 0.0001$) in these 5-yr-old children. We also found positive but weaker associations between plasma leptin concentrations and plasma concentrations of glucose ($r = 0.40$, $P < 0.0001$) and insulin ($r = 0.48$, $P < 0.0001$). However, after adjusting plasma leptin concentrations for percent body fat, there was no additional effect of either glucose, insulin, or sex.

Most importantly, we found significant positive correlations between TEE, RMR, and PAL and plasma leptin concentrations adjusted for percent fat (Table II). TEE adjusted for its major determinants (i.e., weight and sex) was still strongly correlated with leptin concentrations independent of percent body fat ($r = 0.42$, $P < 0.0001$) (Fig. 1). Similarly, PAL, an index of physical activity, was also correlated with fasting plasma leptin concentrations adjusted for percent fat ($r = 0.26$, $P < 0.01$) (Fig. 1). In contrast, RMR adjusted for its major determinants (i.e., fat-free mass, fat mass, and sex) did not correlate with leptin concentrations adjusted for percent fat (Table II).

Table I. Physical and Metabolic Characteristics of the 123 5-yr-old Pima Indian Children and Correlations between Fasting Plasma Leptin Concentrations (log_{10}) and these Characteristics

	Mean±SD	Range	r	$P <$
Weight (kg)	23.3±5.8	14.6–42.9	0.78	0.0001
Height (cm)	114.5±5.0	102–126	0.52	0.0001
Relative weight* (%)	115±21	89–189	0.78	0.0001
Body fat‡ (%)	30±8	17–50	0.84	0.0001
Sum of skin folds§ (mm)	68±33	27–166	0.80	0.0001
Plasma glucose (mmol/liter)	4.5±0.4	3.4–5.4	0.40	0.0001
Plasma insulin (pmol/liter)	61±65	7–409	0.48	0.0001
Plasma leptin (ng/ml)‖	4.0	(0.8, 41.3)	—	—

*Relative weight based on a reference population described by Jelliffe (14). ‡Percent body fat estimated from total body water determined by isotopic dilution (^{18}O). §Sum of skin folds measured at five sites: biceps, triceps, subscapular, suprailiac, and calf. ‖Plasma leptin expressed as the geometric mean with the 95% confidence interval in parentheses.

Discussion

The results of this study in young children confirm the association between plasma leptin concentrations and body composition previously reported in adults (9, 10) and prepubertal children (19). The important new finding is that energy expenditure in children, and more specifically physical activity, is positively correlated with fasting plasma leptin concentrations. Studies in animal models have reported that administration of exogenous leptin increases energy expenditure and physical activity (2, 3). Our findings in children support the concept that leptin plays a role in energy expenditure in humans.

In *ob/ob* mice, exogenous leptin is known to increase energy expenditure and, more specifically, energy expenditure associated with physical activity (2). Leptin administered to mice stimulates sympathetic nervous system outflow, which, in turn,

Table II. Energy Metabolism Characteristics of the 123 5-yr-old Pima Indian Children and Correlations between these Characteristics and Fasting Plasma Leptin Concentrations (log_{10}) Adjusted for Percent Body Fat

	Mean±SD	Range	r	$P =$
Total energy expenditure (kJ/d)	5982±961	4243–9326	0.37	0.0001
Adjusted TEE* (kJ/d)	5982±560	4764–7489	0.42	0.0001
Resting metabolic rate (kJ/d)	4479±650	3389–6862	0.21	0.02
Adjusted RMR‡ (kJ/d)	4479±314	3559–5329	0.12	0.18
Physical activity level (TEE:RMR)	1.34±0.14	1.03–1.75	0.26	0.003

*TEE adjusted for body weight and sex. ‡RMR adjusted for fat-free mass, fat mass, and sex.

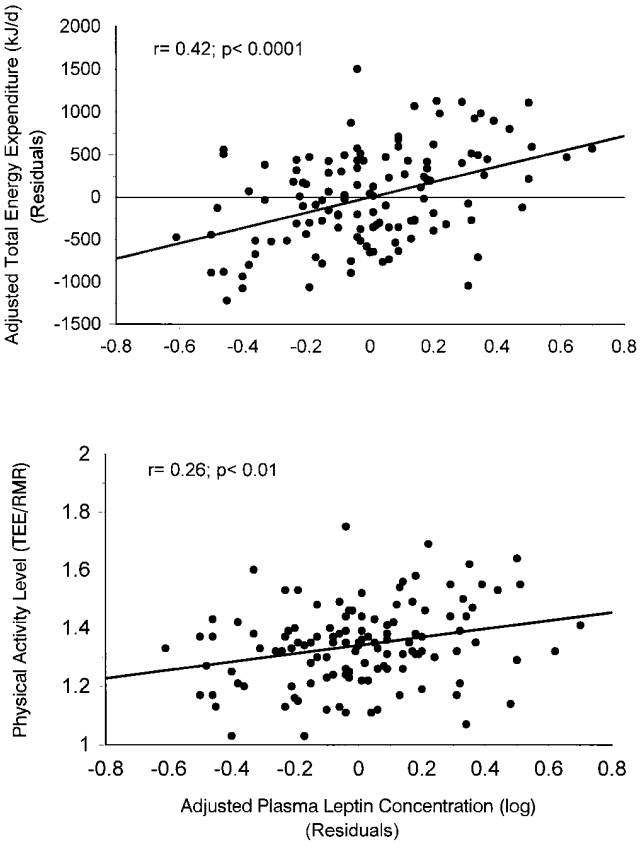

Figure 1. (*Top*) Total energy expenditure, expressed as residuals, plotted against plasma leptin concentrations, also expressed as residuals. Residuals were calculated as the difference between the measured value and the predicted value after adjustment of these variables for their major determinants by multiple linear regression models (on the basis of the entire group of 123 children). The zero point on this graph represents the mean total energy expenditure in this group (5,982±961 kJ/d). (*Bottom*) Physical activity level (TEE: RMR) plotted against the residuals of plasma leptin concentration (log_{10}) adjusted for percent body fat.

seems to increase thermogenesis in brown adipose tissue (7). It has been reported previously that there is a genetic component to sympathetic nervous system activity (20) that may impact upon levels of spontaneous physical activity as measured in a metabolic chamber (21). Spontaneous physical activity, which is also a familial trait (22), can account for as much as 420–3,350 kJ of total daily energy expenditure (23), and also predicts body weight gain in Pima Indians (21). Our results linking leptin concentrations and total energy expenditure among Pima Indian children suggest that, as with animal models, leptin may mediate energy expenditure in humans and, more specifically, physical activity, perhaps by activation of the sympathetic nervous system. Leptin may therefore be important in regulating energy balance, not only by controlling food intake, but also by increasing total daily energy expenditure.

The association between leptin concentrations and energy expenditure in children does not prove a causal relationship whereby leptin produces an increase in energy expenditure. However, it is unlikely that energy expenditure actually elevates leptin concentrations since it is known that elevated levels of physical activity increase sympathetic nervous system activity (21), which has been shown to inhibit leptin secretion in vitro (24) and in vivo (25). In conclusion, these results confirm that, as in animal studies, leptin seems to increase energy expenditure and physical activity in young children.

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