Physical activity energy expenditure may mediate the relationship between plasma leptin levels and worsening insulin resistance independently of adiposity

P. W. Franks,1,2 R. J. F. Loos,1 S. Brage,1 S. O’Rahilly,3 N. J. Wareham,1 and U. Ekelund1

1Medical Research Council Epidemiology Unit, Cambridge, United Kingdom; 2Genetic Epidemiology and Clinical Research Group, Department of Public Health and Clinical Medicine, Division of Medicine, Umeå University Hospital, Umeå, Sweden; and 3Departments of Clinical Biochemistry and Medicine, Addenbrooke’s Hospital, Cambridge, United Kingdom

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Leptin regulates a constellation of neuroendocrine processes that control energy homeostasis. The infusion of leptin in rodents lacking endogenous leptin promotes physical activity energy expenditure (PAEE) and improves insulin signaling, whereas hyperleptinemia is associated with physical inactivity and insulin resistance (IR). We tested whether baseline leptin levels predict changes in PAEE and IR over time, independent of obesity. We also assessed whether the relationship between leptin and change in IR is mediated by PAEE. The population consisted of 288 nondiabetic UK Caucasian adults (mean age: 49.4 yr; SD: 0.7 yr), in whom leptin, insulin, glucose, PAEE (via heart rate monitoring with individual calibration by indirect calorimetry), and anthropometric characteristics had been measured at baseline and 5 yr later. In linear regression models, baseline leptin levels inversely predicted follow-up PAEE (P = 0.033). On average, individuals with low leptin levels (below sex-specific median) increased their daily activity 35% more during the 5-yr follow-up period than those with above-median leptin levels. Baseline leptin level also predicted worsening IR (fasting, 30-min, and 2-h dian) increased their daily activity 35% more during the 5-yr follow-up period than those with above-median leptin levels. Baseline leptin level also predicted worsening IR (fasting, 30-min, and 2-h)

energy expenditure; hyperleptinemia; prospective cohort study

THE ACCUMULATION AND UTILIZATION of adipose tissue are controlled by a complex network of neural signals that drive the propensity for the intake and expenditure of energy. Many of the hormones that underpin this feedback process act centrally to control hunger and satiation, as well as fatigue and energy expenditure. Thus these hormones, which include leptin, control both sides of the energy balance equation. Rodents lacking endogenous leptin are characteristically hyperphagic, physically inactive, obese, and insulin resistant (40, 45), features that can be reversed with recumbent leptin therapy (14, 17, 30). Similar effects have been observed in humans. The few leptin-deficient patients described so far all exhibited early-onset morbid obesity, hyperphagia, and hyperinsulinemia (8, 26, 39).

In these individuals, administration of recombinant leptin had major and sustained beneficial effects on the multiple phenotypic abnormalities associated with leptin deficiency (9). Within the general population, however, leptin secretory defects are rare, and resistance to leptin may be common, particularly in obese individuals (7).

In a study of healthy young men, Boden et al. (2) demonstrated that long-duration insulin infusion during euglycemia dramatically increases leptin synthesis; at around 60 h of hyperinsulinemia, leptin levels approached 180% of basal levels. Elsewhere, Kennedy et al. (19) reported induction of leptin syntheses during insulin infusion in women but not in men. Although Segal et al. (36) observed hyperleptinemia in insulin-resistant individuals independently of the level of obesity, pharmacological induction of insulin secretion did not influence leptin synthesis. Elsewhere, Ruige et al. (34) reported cross-sectional associations between hyperleptinemia and insulin resistance independently of body mass index in a population-based cohort of Dutch men and women. These studies indicate that leptin and insulin are involved in a complex regulatory loop and highlight the pivotal role leptin plays in glucose homeostasis. These studies also suggest that leptin acts as an insulin sensitizer when leptin levels are at low and normal levels and may contribute to insulin resistance when leptin is chronically elevated.

A similar relationship exists between leptin and physical inactivity. Leptin-deficient mice are hypoactive, yet administration of exogenous leptin stimulates physical activity in these animals (30). By contrast, in population-based cross-sectional epidemiological studies, leptin levels, independent of obesity, are negatively related to physical activity (11, 16). However, other cross-sectional studies have not confirmed this (27, 35), and one reported a positive correlation between leptin levels and physical activity in Pima Indian children (35).

Given that physical activity is a strong determinant of insulin sensitivity, both with and in the absence of changes in adiposity (18), and leptin appears important in controlling insulin sensitivity and promoting physical activity, we hypothesized that physical activity may mediate the relationship between leptin and insulin resistance. Thus we investigated the prospect...
tive relationship between leptin and insulin resistance and tested whether physical activity modified this relationship.

MATERIALS AND METHODS

Study population. The volunteers in the present study were all participants in the Medical Research Council Ely Study (10, 44), a prospective population-based cohort study of the etiology and pathogenesis of Type 2 diabetes and related metabolic disorders. Data in the present report are derived from the baseline (1994–96) and follow-up visits (2001–03). The restudy rate was 80% of the baseline cohort. A data set, including clinical and anthropometric data at baseline and follow-up, was available in 389 nondiabetic participants, of whom 288 also had an objective measurement of physical activity at baseline and follow-up. All participants provided written, informed consent. Ethical permission was granted by the Cambridge Local Research Ethics Committee.

Anthropometric, body composition, and metabolic measurements. The procedure for data collection was the same at baseline and follow-up visits. Participants attended the laboratory at 8:30 AM following a 10-h overnight fast. Standard anthropometric data were obtained by trained observers with participants in indoor clothing. Height and weight were measured using a rigid stadiometer and calibrated scales, respectively. Quantities of fat mass and fat-free mass (FFM) were assessed using a standard impedance technique (Bodystat, Isle of Man, UK). Participants then received an explanation of the procedure for the collection of blood. A sample of fasting blood was taken, following which participants drank 75 g of anhydrous glucose (BMS Laboratories, Beverley, UK) dissolved in 250 ml of water over the course of 2–5 min. Further blood samples were taken at 30 and 120 min. Type 2 diabetes was defined as a 2-h plasma glucose concentration $\geq 11.1$ mmol/L. Blood was centrifuged on site for separation of serum and plasma, and plasma samples were aliquoted, packed in ice, and transferred to the laboratory where they were stored at $-70^\circ$C within 4 h. Plasma samples were assayed for insulin and glucose, as described in detail previously (44). Fasting plasma leptin was measured using a DELFIA in-house two-site immunometric assay using commercially available antibodies (R&D Systems, Europe). The analytical sensitivity was 0.1 ng/ml; intra-assay coefficient of variation was 4.4% or better across the range 0.7–125 ng/ml. Between-batch coefficients of variation are 7.1% at 2.7 ng/ml, 3.9% at 14.9 ng/ml, and 5.7% at 54.9 ng/ml ($n = 30$). The comparison of a commercially available leptin assay kit (Linco Human Leptin RIA Kit, Linco Research, St. Louis, MO) with the in-house DELFIA leptin assay used here showed a high level of agreement ($r = 0.95, P < 0.0001$) and showed no evidence of heteroscedasticity or other systematic error (I. Halsall, personal communication). All assays were performed in the same laboratory, as described above. To control for potential time-dependent and assay-specific variability, we also statistically standardized data within the assay. Data on socioeconomic status, smoking, and alcohol consumption were collected using methods described previously (44).

Assessment of energy expenditure. The level of free-living physical activity energy expenditure (PAEE) was assessed objectively in each participant using the Flex Heart Rate technique. This method has been shown to be reliable and valid for assessing PAEE when compared with the gold-standard methods of doubly labeled water and indirect calorimetry (37). The oxygen consumption-heart rate relationship was assessed at rest with the participant lying and then seated, using an oxygen analyzer calibrated daily with 100% nitrogen and fresh air as standard gases (PK Morgan, Kent, UK). Participants bicycled on a cycle ergometer (baseline) and walked/run on a treadmill (follow-up) at progressively increasing workloads to provide the slope and the intercept of the line relating energy expenditure to heart rate. At each workload, participants exercised at a steady state for 5 min before the intensity increased, during which three separate readings of heart rate, minute volume, and expired air oxygen concentration were recorded.

Energy expenditure (kJ/min) was calculated at each time point as oxygen consumption (ml/min) $\times 20.35$. The slope and intercept of the least squares regression line of the exercise points were calculated. Flex heart rate, which is the point used to discriminate between rest (below flex) and exercise (above flex), was calculated as the mean of the highest resting pulse rate and the lowest during exercise. Exercising energy expenditure was predicted from the slope and intercept of the regression line calculated during the exercise test. Participants wore heart rate monitors (Polar Electro, Kempele, Finland) continuously during the waking hours over the following 4 days. During this time, they were encouraged to maintain their usual activity behaviors. PAEE is expressed as total daily energy expenditure minus resting energy expenditure (kJ/min).

Statistical analysis. All analyses were conducted using the statistical software SAS version 9.1 (SAS Institute, Carey, NC). The unadjusted means and standard deviations (SD) for variables were calculated at baseline and follow-up exams. All leptin, insulin, and glucose data were logarithmically transformed (ln) owing to their skewed distributions. For these variables, we present geometric means and 95% confidence intervals. Owing to the widely reported sex differences that exist in relation to leptin, the interaction between sex and leptin was tested for all outcomes.

Generalized linear models adjusted for age, sex, baseline, and follow-up fat mass and FFM, follow-up time, and the baseline outcome variable (indexes of insulin resistance or PAEE) were used to test the associations between the predictor variables (PAEE or leptin) and the outcome variables at follow-up.

RESULTS

Table 1 shows the characteristics of participants at baseline and follow-up. On average, fat mass and insulin levels increased, and FFM declined during the 5-yr follow-up period ($P < 0.05$). Table 2 shows the characteristics of participants stratified by below and above the sex-specific median level of leptin. All variables, except for age, sex, height, and PAEE, were significantly higher in individuals with high leptin levels compared with those with lower leptin levels. Table 3 shows the correlations between baseline leptin, insulin variables, and PAEE. The top right corner of the table shows unadjusted correlations, and the bottom left corner of the table shows correlations adjusted for age, sex, fat mass, and FFM. The strength of the correlations were generally reduced after adjustment.

Leptin and insulin resistance. In generalized linear models ($N = 360$), a unit increase in leptin at baseline was positively
related with follow-up measures of log fasting insulin ($\beta = 0.11, SD = 0.04; P = 0.008$), log insulin at 30 min ($\beta = 0.13, SD = 0.04; P = 0.001$), log 2-h ($\beta = 0.14, SD = 0.06; P = 0.0096$), and homeostasis model assessment-insulin resistance (HOMA-IR) ($\beta = 0.12, SD = 0.04; P = 0.0062$) (see Figs. 1 and 2). Models were adjusted for age, body composition at baseline, and follow-up, follow-up time, sex, and the baseline measure of the dependent variable. None of the associations for insulin resistance outcomes was modified by sex (sex * leptin interaction: $P > 0.51$ for all models). Additional adjustments for alcohol intake and smoking did not materially affect these results.

**Leptin and energy expenditure.** In generalized linear models, including all 288 individuals in whom data on baseline and follow-up leptin and PAEE were available, a unit increase in log leptin at baseline was related with 0.1 (SD: 0.04) unit decrease in PAEE at follow-up ($P = 0.025$). Models were adjusted for age, baseline PAEE, body composition at baseline, and follow-up, follow-up time, and sex. Individuals with below-median leptin levels at baseline had a 35% greater increase in physical activity from baseline to follow-up than those with above-median leptin levels at baseline. Neither association was significantly modified by sex ($P \geq 0.1$). Additional adjustments for alcohol intake and smoking did not materially affect these results.

**DISCUSSION**

The data from the present study indicate that baseline leptin levels relate prospectively with the tendency to move around and expend energy via physical activity. Leptin relates in a concordant manner with the development of insulin resistance.

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**Table 2. Participant characteristics at baseline stratified below (low leptin) and above (high leptin) the sex-specific median for leptin levels**

<table>
<thead>
<tr>
<th></th>
<th>Low Leptin</th>
<th>High Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>52.6 (5.5)</td>
<td>52.6 (4.4)</td>
</tr>
<tr>
<td>Sex, %female</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.3 (9.4)</td>
<td>168.6 (8.9)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.3 (2.1)</td>
<td>28.2 (3.9)</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>18.9 (4.5)</td>
<td>26.7 (8.3)</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>50.3 (11.7)</td>
<td>53.5 (11.3)*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>5.5 (3.4)</td>
<td>18.0 (13.7)</td>
</tr>
<tr>
<td>Fasting insulin, mmol/l</td>
<td>31.7 (15.9)</td>
<td>58.7 (43.5)†</td>
</tr>
<tr>
<td>30-min Insulin, mmol/l</td>
<td>251.2 (115.1)</td>
<td>393.3 (227.5)†</td>
</tr>
<tr>
<td>2-h Insulin, mmol/l</td>
<td>197.0 (126.8)</td>
<td>322.7 (317.7)†</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.9 (0.5)</td>
<td>1.9 (1.5)†</td>
</tr>
<tr>
<td>PAEE, kJ/min</td>
<td>6.1 (3.2)</td>
<td>6.0 (3.0)</td>
</tr>
</tbody>
</table>

Values are means (SD); $N = 288$. Independent samples $t$-test for difference between strata: $P < *0.05$, †0.0001.

**Table 3. Spearman correlations between leptin, PAEE, and measures of insulin resistance**

<table>
<thead>
<tr>
<th></th>
<th>Leptin</th>
<th>Fasting Insulin</th>
<th>30-min Insulin</th>
<th>2-h Insulin</th>
<th>HOMA-IR</th>
<th>PAEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>0.47</td>
<td>0.40</td>
<td>0.37</td>
<td>0.40</td>
<td>-0.25</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.25</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>30-min Insulin</td>
<td>0.26</td>
<td>0.58</td>
<td>0.45</td>
<td>0.55</td>
<td>-0.01</td>
<td></td>
</tr>
<tr>
<td>2-h Insulin</td>
<td>0.21</td>
<td>0.67</td>
<td>0.43</td>
<td>0.69</td>
<td>-0.12</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.48</td>
<td>0.98</td>
<td>0.60</td>
<td>0.73</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>PAEE</td>
<td>-0.05</td>
<td>-0.05</td>
<td>-0.05</td>
<td>-0.11</td>
<td>-0.05</td>
<td></td>
</tr>
</tbody>
</table>

Data in top right corner of table are unadjusted. Data in bottom left corner of table are adjusted for age, sex, fat mass, and fat-free mass.
subsequent DNA sequence binding of the STAT kinase complexes. The long-form leptin receptor (OBRb) activates the 5′-AMP-activated protein kinase gene in muscle (13). 5′-AMP-activated protein kinase promotes mitochondrial biogenesis via peroxisome proliferator-activated receptor-α coactivator-1 (28) and skeletal muscle fatty acid oxidation by inhibiting the activity of acetyl coenzyme A carboxylase. Thus, as reported elsewhere for thiazolidinediones (32), leptin may stimulate oxidative phosphorylation, mitochondrial biogenesis, and insulin signaling, the net effects of which would be improvements in aerobic exercise capacity and metabolic homeostasis. By contrast, hyperleptinemia may reflect resistance to leptin at a cellular level.

We observed an inverse association between baseline leptin levels and change in PAEE from baseline to follow-up. This finding is consistent with previously reported cross-sectional results in an overlapping population (11) and in people with anorexia nervosa (16). However, three studies, all in children, reported either no association or a positive association between leptin levels and physical activity (27, 33, 35). Thus it is possible that hormonal changes during puberty influence the effects of leptin on behavior.

Although leptin may affect aerobic capacity as described above, we speculate that the association between leptin and physical activity is most likely due to its hypothalamic effects. The leptin receptor is widely expressed in the brain, mainly in the choroids plexus and hypothalamic regions, such as the arcuate nucleus, the ventromedial, the dorsomedial, and the ventral premammillary nuclei, but is also widely distributed in various telenchephalic structures (6, 21, 40). Prominent among these is the arcuate nucleus, a region that has previously been proposed as an important site for mediating leptin's effects on physical activity (3), although other sites are also plausibly involved in the regulation of physical activity (20, 41).

A recent animal study assessed the physiological significance of leptin signaling on locomotor activity in Lepr<sup>Δ<br>neon</sup> mice. These mice lack the leptin receptor, are hypoactive, and show a similar phenotype to db/db mice (3). Selectively restoring leptin signaling in the arcuate nucleus markedly increased locomotor activity. The authors concluded that leptin-sensitive arcuate neurons are sufficient for mediating the majority of leptin’s action on motor control (3). A possible pathway through which leptin influences the behavior of physical activity is via activation of the melanocortin-4 receptor in the arcuate nucleus, a notion that is supported by animal and human studies (1, 23, 25, 29). Thus high baseline leptin levels may reflect central leptin resistance in the arcuate nucleus, which in turn leads to reduced physical activity.

**Leptin and insulin resistance.** The leptin hormone helps regulate energy metabolism, growth, and fertility. Chronic hyperleptinemia, which reflects increased adipose mass and leptin resistance, may represent a mechanism through which obesity and insulin resistance pathophysiologically relate. In obese Zucker rats, characterized in part by nonfunctioning leptin receptors, hypertriglyceridemia and β-cell failure occur. However, receptor induction via adenoviral transfection combined with leptin infusion in isolated pancreatic β-cells of these animals substantially reduces intracellular triglyceride content (42, 43). In human tissue, fatty acid oxidation in rectus abdominus muscle obtained from obese individuals is blunted following leptin infusion by comparison with fatty acid oxida-
tion rates in muscle from lean individuals (4). Although these experiments used supraphysiological doses of leptin, they suggest that muscle from obese humans is resistant to leptin. As proposed more than 40 yr ago by Randle and colleagues (31), inhibited fatty acid oxidation and consequent intra-myocellular lipid accumulation cause insulin resistance via diminution of cellular insulin signaling. Thus, because leptin plays important roles in fatty acid oxidation (5), the relationship between hyperleptinemia and insulin resistance may be mediated by impairments in fatty acid oxidation (38). Leptin also promotes low-grade inflammation via synthesis of TNF-α and IL-6 (22). Thus lipotoxicity and cellular inflammation brought on by leptin resistance are important mediators of the relationship between obesity and insulin resistance.

Our laboratory has previously reported on the cross-sectional relationship between leptin and physical activity (11), and the prospective relationship between leptin and the metabolic syndrome, the latter of which included fasting insulin (10). In this report, we present new data on the prospective relationship between leptin and physical activity, and on the prospective relationships between leptin and a variety of other measures of insulin action. We also report analyses combining physical activity and leptin as determinants of changes in insulin action. Thus the novel aspects of the present study include the comprehensive analyses of the prospective associations between leptin, physical activity, and insulin resistance. These analyses are informative of leptin’s temporal relationships with physical activity and insulin action and are likely less prone to residual confounding by adiposity or reverse causality than those previously reported (10, 11).

Limitations. We observed a modest average increase in PAEE during follow-up, which is partially due to an increase in body weight, and thus an increase in the metabolic cost of locomotion. However, this increase may also reflect the transition of participants in this study from sedentary occupations to more active retirement lifestyles or variations in study procedures between baseline and follow-up exams. Nonetheless, variations in PAEE that are associated with the participants’ occupations or study protocols will not bias the observed relationships, unless these factors are also related with leptin level, which is unlikely to be true.

Although in this study we describe data that indicate an inhibitory effect of high leptin levels on physical activity, some have hypothesized that exercise may improve leptin sensitivity via changes in suppressor of cytokine signaling-3 signaling (see Ref. 4 for a review of this topic). Thus leptin levels and the propensity for habitual physical activity may form part of a regulatory loop, which influences insulin action. In observational studies, however, it is impossible to fully disentangle temporal trends or the independent effects of highly correlated factors, which, for the behavior of physical activity, includes nutrient intake. However, it is also possible that physical activity promotes delivery and cellular uptake of dietary fats (12), providing a possible mechanism for interaction between physical activity and leptin on insulin levels.

A further limitation of this paper is that, because leptin is primarily secreted in white adipose tissue in humans, and we adjusted for fat mass in our analyses, we are likely to have adjusted out some of the variance that leptin explains in changes in PAEE and insulin. However, an unadjusted model would not account for confounding by fat mass of the leptin-PAEE or leptin-insulin relationships. Thus we decided to adopt a conservative approach and risk understating the strength and magnitude of the relationships.

Finally, to achieve a balance between precision and feasibility, we used fasting and postglucose challenge plasma insulin levels as a proxy measure of insulin action and a bioimpedance assessment of body fat percent, neither of which are gold-standard measures of the respective phenotypes. Although fasting and postchallenge insulin concentrations correlate fairly well with direct measure of insulin sensitivity and strongly predict the development of Type 2 diabetes (15), they cannot be used to distinguish between peripheral and hepatic insulin action.

In summary, the results of this study indicate that high plasma leptin levels may predict a relative decline in physical activity and worsening insulin resistance during 5-yr follow-up. Furthermore, physical activity may mediate the relationship between hyperleptinemia and the development of insulin resistance. These observations are consistent with data in rodents and suggest that, in humans, the leptin signaling pathway may contain molecular targets that control the propensity for energy expenditure and the capacity for insulin signaling. This information extends our understanding of the etiological underpinnings of physical inactivity and insulin resistance and may be informative for future studies that seek to manipulate these phenotypes through pharmacological intervention.

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GRANTS

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