Serum leptin level: possible association with haematopoiesis in adolescents, independent of body mass index and serum insulin

Hiroshi HIROSE††, Ikuo SAITO††, Toshihide KAWAI*, Keiko NAKAMURA*, Hiroshi MARUYAMA* and Takao SARUTA*

*Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan, and †Health Center, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

1. The obese gene product leptin, secreted exclusively from adipocytes, was discovered to serve as a satiety factor and to play an important role in regulating body weight. In adults, the serum leptin level reportedly increases with the degree of obesity. Leptin receptors are expressed in various tissues, and recent in vitro studies suggest a role for leptin in haematopoiesis.

2. The present study was designed to clarify the relationship between serum leptin and body mass index, peripheral blood cell counts, serum cholesterol, high-density lipoprotein-cholesterol, insulin and cortisol levels in 299 Japanese male adolescents aged 15–16 years.

3. With simple linear correlation, log [serum leptin] showed a strong correlation with body mass index ($r = 0.56$), log [insulin] ($r = 0.36$) and leucocyte count ($r = 0.22$) ($P < 0.001$ for all). There were also correlations with systolic blood pressure, erythrocyte count, haematocrit and high-density lipoprotein-cholesterol ($P < 0.01$ for all). Even after adjustment for body mass index and log [insulin], log [leptin] correlated with leucocyte ($P = 0.004$) and erythrocyte ($P = 0.057$) counts. Stepwise multiple regression analyses revealed log [leptin] to correlate significantly with body mass index, log [insulin] and the leucocyte count ($P < 0.005$ for all, $r^2 = 0.399$).

4. To our knowledge, this is the first clinical study to show the possible association of serum leptin level with blood cell counts, independent of body mass index and serum insulin. We conclude that these data further support a role for leptin in haematopoiesis.

INTRODUCTION

Obesity is characterized by excessive accumulation of fat tissue in the body (body fat), which results from an imbalance between caloric intake and energy expenditure. Obesity has become an important health problem in industrialized societies, because it is a risk factor for diabetes mellitus, hypertension, hyperlipidaemia and atherosclerotic diseases. Recently, Zhang et al. [1] identified the mouse obese (ob) gene using a positional cloning technique. Further studies revealed that the ob gene product 'leptin' [2] is expressed exclusively in adipocytes and serves as a satiety factor which reduces food intake and body weight through its receptor in the hypothalamus [2–6]. The ob/ob mice, who have a nonsense mutation in the ob gene-coding region, cannot produce functional leptin [1], and db/db mice [7,8] and fa/fa rats [9,10] have mutations in the leptin receptor with defective signalling in the hypothalamus [11].

In adults, the serum leptin level is reported to increase with the degree of obesity [body mass index (BMI) and/or percentage body fat] [12–14], suggesting leptin resistance in the signalling pathway(s) in obese subjects. To our knowledge, however, there is little information concerning leptin levels in adolescents. In addition to regulating body weight, leptin receptors are expressed in various tissues [6,7], and there is accumulating evidence suggesting that leptin also influences reproductive and haematopoietic systems in which its receptors are expressed [15–20]. The present study was thus designed to clarify the relationship between serum leptin and BMI, peripheral blood cell counts, lipid profiles, serum insulin and cortisol levels in Japanese male adolescents.

METHODS

Subjects

The present study included 299 male Keio high school students, aged 15–16 years. Informed consent was obtained before the study, and the protocol was approved by the review committee of the Keio University Health Center. In the morning at fast, height, weight, blood pressure, peripheral blood cell counts, serum cholesterol, high-density lipoprotein (HDL)-cholesterol, insulin, leptin and cortisol levels were measured.

Measurements

Peripheral blood cell counts, serum cholesterol and HDL-cholesterol were assayed by routine automated
laboratory methods. Serum insulin and leptin concentrations were measured with commercially available kits (Eiken Co., Tokyo, Japan, and Linco Research Inc., St. Charles, Missouri, U.S.A., respectively) based on RIA, as described previously [21-23]. Serum cortisol was measured by competitive enzyme immunoassay (EIA) using an automatic EIA system analyzer, AIA-1200 (Tosoh Corporation, Tokyo, Japan). The intra- and inter-assay coefficients of variation were, respectively, 5.3 and 5.8% for insulin, 3.4–8.3% and 3.0–6.2% for leptin, and 1.9–5.3% and 6.3–8.5% for cortisol.

**Statistical analyses**

All statistical analyses were performed using the StatView® program for Macintosh (version 4.5-J, Abacus Concepts Inc., Berkeley, California, U.S.A.). Relationships between variables were analysed by simple correlation and by multiple and stepwise linear regressions. The significance of simple correlation coefficients was assessed using Fisher's Z transformation. The significance of independent relationships in the multiple and stepwise regressions was assessed applying a t-test to the regression coefficients. Stepwise regressions were performed in the forward direction with F for entry set to 4. Data are expressed as means ± S.D. except when otherwise specified.

**RESULTS**

Clinical and metabolic characteristics are shown in Table 1. Because both serum insulin and leptin concentrations were normally distributed after log-transformation, we used log [insulin] and log [leptin] for the analyses.

With simple linear correlation, the logarithm of serum leptin (2.7 ± 1.6 ng/ml, n = 299) showed a strong correlation with BMI (r = 0.56), log [insulin] (r = 0.36) and the leucocyte count (r = 0.22) (P < 0.001 for all). There were also correlations with systolic blood pressure (r = 0.17), erythrocyte count (r = 0.19), haematocrit (r = 0.15) and HDL-cholesterol (r = −0.18) (P < 0.01 for all, Table 1). Even after adjustment for BMI and log [insulin], log [leptin] correlated with leucocyte (P = 0.004) and erythrocyte (P = 0.057) counts. As shown in Table 2, stepwise multiple regression analysis revealed log [leptin] to correlate significantly with BMI (F = 106.7), log [insulin] (F = 29.5) and the leucocyte count (F = 8.1) (r² = 0.399, Figure 1).

**DISCUSSION**

Recent studies in the field of obesity have revealed that adipocytes function not only as energy storage depots, but also secrete many hormone-like substances, including free fatty acids, tumour necrosis factor-α, plasminogen activator inhibitor-1 and leptin. In adults, serum leptin levels are reportedly higher in women than in men, when the groups are defined by BMI [13]. The present study confirmed that the serum leptin level in adolescents also correlates significantly with BMI and serum insulin. Unfortunately, we were not able to measure percentage body fat or other anthropometric parameters in this study.

To our knowledge, this is the first clinical study to show the possible association of serum leptin level with

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**Table 1** Clinical and metabolic characteristics (means ± S.D.), simple and multiple linear regressions with log [serum leptin] in 299 Japanese male adolescents

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± S.D.</th>
<th>r</th>
<th>P</th>
<th>Adjusted for BMI</th>
<th>Adjusted for BMI and log [insulin]</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>20.6 ± 2.2</td>
<td>0.56</td>
<td>&lt;0.001</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Log [insulin] (pmol/l)</td>
<td>1.16 ± 0.22</td>
<td>0.36</td>
<td>&lt;0.001</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.0 ± 0.1</td>
<td>–0.08</td>
<td>NS</td>
<td>–0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122 ± 12</td>
<td>0.17</td>
<td>&lt;0.01</td>
<td>–0.01</td>
<td>–</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67 ± 9</td>
<td>0.10</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Leucocyte count (10³ cells/l)</td>
<td>6.03 ± 1.31</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>Erythrocyte count (10¹² cells/l)</td>
<td>5.07 ± 0.33</td>
<td>0.19</td>
<td>0.002</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>45.3 ± 2.7</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>165 ± 25</td>
<td>0.10</td>
<td>NS</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>58 ± 14</td>
<td>−0.18</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td>−0.02</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>14.5 ± 3.8</td>
<td>0.06</td>
<td>NS</td>
<td>0.06</td>
<td>0.04</td>
</tr>
</tbody>
</table>
blood cell counts. Although we recognized the same trend in female adolescents (most of them seem non-fasted, results not shown), i.e. an association of serum leptin with blood cell counts, we found no such association in middle-aged adults (H. Hirose, I. Saito, et al., unpublished work). Recent in vitro studies have suggested that leptin can induce proliferation and differentiation of haematopoietic stem cells [17-20], although the precise mechanisms are not clear. At a minimum, leptin’s stimulation of erythroid differentiation in combination with erythropoietin is clear [19,20].

We also speculated as to the possibility of increased blood cell counts and blood pressure being glucocorticoid-mediated, because glucocorticoid has been reported to up-regulate leptin both in vitro [24-26] and in vivo [27]. There were, however, no positive correlations between serum leptin and cortisol levels in this study. As for leptin-induced insulin resistance, there have been several studies showing discrepancies [28-31].

To summarize, we have shown a weak but significant correlation of serum leptin level with blood cell counts in Japanese male adolescents, independent of BMI and serum insulin. We conclude that these clinical data further support a role for leptin in haematopoiesis.

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REFERENCES


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