Changes in Serum Leptin Concentration During Behavioral Therapy in Obese Children

TAKAYA NAKANE, KOHTARO ASAYAMA, HIDEMASA HAYASHIBE, NORIHIKO UCHIDA, KOJI KODERA AND KAZUSHIGE DOBASHI

Department of Pediatrics, Yamanashi Medical University, Tamahocho, Yamanashi 409-3898, Japan

Abstract. To determine the pathophysiological implications of serum leptin level in obesity, we monitored the changes in serum leptin level during outpatient treatment with life style modification in children. Fifty-five obese Japanese children (34 boys and 21 girls; mean age, 9.64 years) were studied. The control children consisted of 42 nonobese subjects (27 boys and 15 girls). The serum leptin concentration was 4.35±0.46 ng/ml (mean±SEM) in the control girls and 2.93±0.21 ng/ml in the control boys. The serum leptin concentrations in the obese boys and girls were higher than those in their lean counterparts. The concentration in the obese boys (16.28±1.41 ng/ml) was similar to that in the obese girls (20.33±2.0 ng/ml). The logarithmic value of serum leptin concentration at the first blood sampling in obese children was correlated with percent overweight and percent body fat. In 36 obese children (24 boys and 12 girls) whose serum leptin concentrations were monitored serially during treatment of obesity, the percent overweight was significantly decreased after the initial sampling. In each individual, the changes in leptin concentration were roughly parallel to those in percent overweight. The ratio of the leptin concentration at the second blood sampling divided by the one at the first sampling in each individual was closely correlated with the respective delta percent overweight. These results suggest that the preceding course of obesity determines the serum leptin level of obese children on longitudinal basis, and that the leptin level reflects the degree of obesity on cross-sectional basis.

Key words: Leptin, Obesity, Child, Adolescent, Life style modification

LEPTIN, a 16 kDa protein of ob gene product, is predominantly expressed in adipocytes and secreted into circulation [1, 2]. It is possible that leptin is a lipostatic hormone secreted by the adipocytes themselves. However, serum leptin concentration is increased in most obese adults and children [3-8], indicating that such simple obese subjects are unresponsive to the lipostatic action of leptin. It is generally accepted that serum leptin level reflects the extent of adiposity [9-11]. In addition to the adiposity, acute calorie restriction [12] and exercise [13, 14] also modify serum leptin level.

There have been previous reports in which serum leptin concentration is monitored during treatment with calorie restriction in adult obese subjects [15-17]. In adult studies, mixed effect of adiposity, unphysiological calorie restriction and forced exercise preclude from determining the physiological implication of serum leptin level during weight reduction in simple obesity. To our knowledge, changes in serum leptin level during treatment of obese children have yet to be reported. In the present study, we monitored serum leptin levels during outpatient treatment with only life style modification (i.e., without calorie restriction and forced exercise) in children.
Materials and Methods

Subjects

Fifty-five obese Japanese children, 34 boys and 21 girls, who visited the Clinic for Obese Children in Yamanashi Medical University were enrolled in the present study. A child was considered obese when their body weight exceeded 120% of the standard body weight, which is defined as the mean body weight corresponding to the height for that age obtained from national statistics for Japanese school children. The ages of the subjects ranged from 3 to 15 years. They had no endocrine, metabolic or kidney disease other than obesity. Before entering a course of treatment, they were instructed to visit the Clinic in the morning, after overnight fast. Blood was then drawn and, at the same time, they were subjected to anthropometric measurements including height, body weight, waist girth, hip girth, and triceps and subscapular skinfold thicknesses. The control children consisted of 42 nonobese subjects, 27 boys and 15 girls, ranging in age from 5 to 16 years. They had no endocrine, metabolic, kidney or acute inflammatory diseases. Blood was drawn after an overnight fast.

Anthropometric measurements

Anthropometric measurements were performed, as described previously [18, 19], by the medical staff of the Department of Pediatrics, Yamanashi Medical University. Percent body fat was obtained using Brozek’s equation [20], after body density was calculated according to Nagamine’s formula [21] for Japanese children.

Treatment program

The program of treatment was based on the lifestyle modification using a simple checklist. Obese children and their family were instructed to regularly eat three meals and one afternoon snack daily, avoiding intake of extra dishes, juices, oily (greasy) food additives, sugar and candies, and drinking >200 ml of milk. Diet caloric intake was not prescribed but we advised them to observe the recommended daily allowance of food energy for each age and sex set by the Ministry of Education, Science and Culture of Japan. Children were instructed to play TV computer games alone at home for no longer than 1 hour a day. Each child (or family) kept a checklist to evaluate (yes or no) if they could observe the seven items (three meals and a snack, no night eating, TV game and doing their chores, and so on) on daily basis. They visited our outpatient clinic once every 3 months for anthropometric measurement and reported their checklist scores (max. 7 points × 7 days per week) and weekly changes in body weight. Pediatricians instructed the family on these points repeatedly at each visit.

Serum leptin concentration

Leptin concentration was measured using a commercial RIA kit (Linco Research, St. Charles, MO). Blood was drawn in the morning after an overnight fast.

Statistical methods

Data are presented as the means and standard error of means (means±SEM). Since the data for leptin was considerably skewed, it was transformed logarithmically before performing statistical analysis. The difference between the two means was estimated by unpaired Student’s t-test. The values were considered to be statistically significant at p < 0.05. The statistical analyses were performed using SPSS version 7.5J (SPSS Inc., Chicago, IL).

The Human Study Committee of Yamanashi Medical University approved this study. Informed consent was obtained either from subjects or their parents as appropriate.

Results

Age and anthropometric data

The control boys (11.30 ± 0.51 years) were significantly older than the control girls (9.47 ± 0.51 years, p < 0.01). The percent overweight of the control boys (1.25 ± 1.69%) was similar to that of the control girls (−1.74 ± 2.53%). The anthropometric data for obese children were summarized in Table 1. The age, height, weight, waist and thigh...
girths, and triceps and subscapular skinfold thicknesses were similar in both genders. The hip girth was longer in the girls than in the boys. Accordingly, the percent overweight was similar in both genders and the waist-hip ratio (WHR) was greater in the boys than in the girls. The percent body fat was greater in the girls than in the boys.

**Serum leptin concentration**

The serum leptin concentration was significantly higher in the control girls (4.35 ±0.46 ng/ml) than in the control boys (2.93 ±0.21 ng/ml) (P < 0.001). On the other hand, the concentration in the obese boys (16.28±1.41 ng/ml) was similar to that in the obese girls (20.33±2.0 ng/ml). The serum leptin concentrations in the obese boys and girls were higher than those in their lean counterparts (P<0.001). The logarithmic value of serum leptin concentration at the first blood sampling in obese children was correlated with percent overweight and percent body fat, but not with WHR or delta percent overweight per 90 days after the first blood sampling (Table 2). The leptin concentration did not change according to the sexual maturation (i.e., Tanner stage) of the subjects (data not shown).

**Changes in leptin concentration during treatment**

Serial data for serum leptin concentration were obtained in 36 obese children (24 boys and 12 girls) during treatment of obesity. Their initial percent overweight was 52.8±2.7% (Table 3). The subsequent sampling dates were categorized by 100-day intervals. The percent overweight was significantly decreased at each category after the initial sampling except for the >500 days category whereas the percent body fat in the girls was not significantly decreased during the treatment (data not shown).

### Table 1. Anthropometric data for obese children

<table>
<thead>
<tr>
<th></th>
<th>combined (n=55)</th>
<th>boys (n=34)</th>
<th>girls (n=21)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>9.64±0.31</td>
<td>9.59±0.45</td>
<td>9.71±0.38</td>
<td>ns</td>
</tr>
<tr>
<td>height</td>
<td>139.2±1.7</td>
<td>138.5±2.3</td>
<td>140.4±2.3</td>
<td>ns</td>
</tr>
<tr>
<td>weight</td>
<td>49.8±1.7</td>
<td>49.5±2.1</td>
<td>50.2±2.4</td>
<td>ns</td>
</tr>
<tr>
<td>waist</td>
<td>81.1±1.07</td>
<td>81.2±1.3</td>
<td>81.0±1.8</td>
<td>ns</td>
</tr>
<tr>
<td>hip</td>
<td>85.0±1.0</td>
<td>83.7±1.1</td>
<td>87.2±1.7</td>
<td>0.037</td>
</tr>
<tr>
<td>thigh</td>
<td>41.2±0.4</td>
<td>40.9±0.6</td>
<td>41.8±0.6</td>
<td>ns</td>
</tr>
<tr>
<td>% overweight</td>
<td>51.2±2.4</td>
<td>52.3±3.5</td>
<td>49.5±3.2</td>
<td>ns</td>
</tr>
<tr>
<td>WHR</td>
<td>0.955±0.007</td>
<td>0.970±0.005</td>
<td>0.929±0.010</td>
<td>0.001</td>
</tr>
<tr>
<td>%FAT</td>
<td>34.8±0.74</td>
<td>33.8±0.93</td>
<td>36.6±1.2</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Data are expressed as means±SEM. Statistical analysis was performed by unpaired t-test. WHR = waist to hip circumference ratio, %FAT = percent body fat.

### Table 2. Correlation between the logarithmic value of baseline leptin level and anthropometric variables

<table>
<thead>
<tr>
<th>variables</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% overweight</td>
<td>0.580</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%FAT</td>
<td>0.661</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.185</td>
<td>ns</td>
</tr>
<tr>
<td>delta overweight*</td>
<td>0.077</td>
<td>ns</td>
</tr>
</tbody>
</table>

Number of observations is 55 for each variable.
* Change in % overweight during 90 days after the baseline blood sampling.

### Table 3. Percent overweight and percent body fat in outpatient obese children

<table>
<thead>
<tr>
<th>n</th>
<th>%overweight</th>
<th>%FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>36</td>
<td>52.8±2.7</td>
</tr>
<tr>
<td>b</td>
<td>18</td>
<td>44.9±3.7</td>
</tr>
<tr>
<td>c</td>
<td>15</td>
<td>45.9±5.6</td>
</tr>
<tr>
<td>d</td>
<td>20</td>
<td>48.8±4.2</td>
</tr>
<tr>
<td>e</td>
<td>16</td>
<td>40.0±3.4</td>
</tr>
<tr>
<td>f</td>
<td>8</td>
<td>46.8±5.6</td>
</tr>
<tr>
<td>g</td>
<td>11</td>
<td>49.4±6.0</td>
</tr>
</tbody>
</table>

Data are expressed as means±SEM. Statistical analysis was performed by Wilcoxon signed-ranks test. Statistically different values from the baseline data are indicated by *(p<0.05) and **(p<0.01).
cent body fat was unaltered. Fig. 1 and 2 summarize the changes in serum leptin concentration for 24 obese boys and 12 obese girls during treatment. The changes in leptin concentration were roughly parallel to those in percent overweight.

**Serum leptin level as a marker of changes in percent overweight**

To further elucidate the pathophysiological implication of serum leptin level in obese children, we explored the relationship between percent overweight and serum leptin level. Fig. 3 summarizes the correlation between percent overweight and the logarithmic value of serum leptin concentration in whole samples. The correlation was very close on the whole. However, individual change in leptin concentration in response to change in percent overweight varied from person to person. The leptin concentration at the second blood sampling was divided by the one at the first sampling in each individual whose paired samples were available. Fig. 4 summarizes the relationship between this ratio and the respective delta percent overweight. The correlation was highly significant.
Discussion

In the present study, serum leptin concentration was higher in obese children than in their lean counterparts, as was reported previously in adults [3-5] and in children [6-8]. Serum leptin concentration was closely correlated with both percent overweight and percent body fat, as was also reported previously [9-11]. On the other hand, leptin levels did not appear to be correlated with body fat distribution, or to show sex-related difference in obese children. Relative changes in individual leptin concentration during treatment were closely correlated with the respective delta percent overweight.

As estrogen increases [22], androgen decreases [23] leptin secretion from adipose tissue in man. Women exhibit a higher leptin level than in men at given degree of obesity in several series of adults [9, 24-26] and children [27]. After the onset of puberty, leptin tends to increase in girls and, conversely, to decrease in boys [8]. Only a couple of reports showed a sex-related difference in leptin concentration in children [7, 27]. Serum leptin concentration also changes depending on age of children [7]. Effect of adiposity on leptin concentration appears to totally offset the changes due to age and sex in the present obese subjects.

The baseline leptin concentrations in the present series of obese children did not appear to predict subsequent change in percent overweight. Several previous adult studies also failed to establish the predictive value of leptin concentration with regard to the outcome of treatment [28].

Both exercise [13, 14] and fasting [17] suppress leptin secretion independently of the change in adiposity. Such effect combined with that of weight reduction may bring about over-suppression of leptin secretion, thereby facilitating rebound of obesity status. Obese subjects who experience marked decreases in leptin during weight loss can generate stronger signals of hunger to their brain than during weight maintenance, and consequently restore their adipose mass and leptin concentrations to baseline levels. Drastic weight loss results in a significant decline in serum leptin level corrected for fat mass [15-17]. Rosenbaum et al. [16] reported that plasma leptin concentrations were significantly lower during weight loss than weight maintenance at the same body composition. It remains unclear whether the difficulty of weight loss maintenance is due to failure to adhere to the diet and exercise regimen [29], or to metabolic change following weight loss [30].

Linear growth allows preadolescent children to grow out of their obesity without weight reduction. In the present treatment program, diet was not restricted and fixed exercise regimens were not prescribed. Thus, the obesity was ameliorated essentially by life style modification with a modest change in body weight. Decrease in percent overweight was not large enough to induce a significant decline in percent body fat. A close correlation between relative change in leptin concentration and delta percent overweight, as observed in the present study, indicated that leptin level declined in proportion to the change in the degree of obesity at individual level. Lack of over-correction of serum leptin level implies that children treated as such are free from the excessive urge of overeating and metabolic suppression following inappropriately low leptin secretion for given adiposity. This balanced decline of leptin secretion is favorable for avoiding regain of percent overweight. In fact, we previously reported that the majority of the boys and nearly half of the girls participating in the present mode of therapy for longer than 200 days grew out of their obesity [31].

In conclusion, the preceding course of obesity determines the serum leptin level of obese children on longitudinal basis, while the leptin level reflects the degree of obesity on cross-sectional basis.

Acknowledgments

This work was supported in part by Grants-in-Aid #08670866 and #10670714 from the Ministry of Education, Science and Culture of Japan.
References


