A BRIEF ORIGINAL CONTRIBUTION

Leptin Concentrations in Women in the San Antonio Heart Study: Effect of Menopausal Status and Postmenopausal Hormone Replacement Therapy

Steven M. Haffner, Leena Mykkänen, and Michael P. Stem

Leptin, the product of the human OB gene, is increased in obese individuals, suggesting resistance to its effect. However, there is variability in leptin levels at each level of body mass index, suggesting that genetic and environmental factors other than overall adiposity may regulate leptin concentrations. Leptin concentrations are higher in women relative to men, a difference that is only partially explained by the increased fat depots in women. The authors hypothesized that higher estrogen levels in women might be responsible for the sexual dimorphism in leptin concentrations. To test this hypothesis, they measured leptin concentrations in premenopausal women not on oral contraceptives (PRE) \( n = 53 \), postmenopausal women on hormone replacement therapy (POSTY) \( n = 28 \), and postmenopausal women not on hormone replacement therapy (POSTN) \( n = 28 \) in the San Antonio Heart Study, a population-based study of diabetes and cardiovascular risk factors. Analyses were restricted to nondiabetic Mexican Americans. Subjects were well matched on obesity as assessed by body mass index \( (kg/m^2) \): PRE = 31.0, POSTY = 29.8, and POSTN = 31.6. Leptin concentrations (ng/ml) were not significantly different among the three groups (PRE = 27.6, POSTY = 28.3, and POSTN = 27.8). The authors conclude that differences in estrogen levels are not likely to explain the sexual dimorphism in leptin concentrations.

MATERIALS AND METHODS

The San Antonio Heart Study is a population-based study of diabetes and cardiovascular disease in Mexican Americans and non-Hispanic whites. From 1979 to 1982 (phase I) and from 1984 to 1988 (phase II), we randomly selected households in several San Antonio...
neighborhoods (13, 14). All men and nonpregnant women aged 25–64 years who resided in the randomly selected households were eligible to participate. Only Mexican Americans were sampled in the barrio, but approximately equal numbers of each ethnic group were studied in the other types of neighborhoods. Mexican Americans were defined as individuals whose ancestry and cultural traditions derived from Mexican national origin (15). Detailed descriptions of the two study phases (I and II) have been published previously (13, 14). This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. All subjects gave informed consent.

In October 1987, we began an 8-year follow-up of the phase I cohort to determine the incidence of non-insulin-dependent diabetes mellitus (type 2 diabetes) and cardiovascular disease (16). Beginning in October 1991, we started a similar 7-year follow-up of the phase II cohort (17). The results reported in this paper are based on cross-sectional analyses of a subset of subjects who participated in the 7-year follow-up of the phase II cohort. To simplify the analyses, subjects with diabetes by World Health Organization criteria (18) were excluded from this subset, although we have previously shown (19) that non-insulin-dependent diabetes is not associated with changes in leptin concentrations. In addition, to simplify the analyses, we restricted the analyses to Mexican Americans, although we have shown no ethnic difference in leptin levels between Mexican Americans and non-Hispanic whites (20).

Anthropometric measurements (height and weight, subscapular and triceps skinfolds, waist and hip circumferences) were made with the participant wearing an examining gown after having removed his/her shoes and upper garments (21). The triceps skinfold was measured directly posterior over the right triceps muscle, and the subscapular skinfold was measured just below the inferior angle of the right scapula. A Lange skinfold caliper (Cambridge, Maryland) was used, and the average of three readings, each made to the nearest 0.5 mm, was taken as the measurement for each skinfold. Waist circumference was measured at the level of the umbilicus. Hip circumference was measured at the level of the greater trochanters. Body mass index and the sum of the subscapular and triceps skinfolds were used as measures of overall adiposity and subcutaneous adiposity, respectively. Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared. The waist-to-hip ratio was used as a measure of body fat distribution.

At the follow-up of the phase II cohort, blood specimens were obtained after a 12- to 14-hour fast for determination of plasma glucose and serum insulin and proinsulin concentrations. Glucose was measured by a glucose oxidase method. A 75-g glucose equivalent load (Koladex or Orangedex, Custom Laboratories, Baltimore, Maryland) was then administered, and blood specimens were obtained 2 hours later for plasma glucose and serum insulin concentrations.

We considered women to be postmenopausal if they had had a hysterectomy and bilateral oophorectomy and/or had had no menstrual periods in the year preceding their examination. We assessed whether postmenopausal women were taking postmenopausal estrogen by questionnaire. Women were considered to be postmenopausal if they had had fewer than two menstrual periods in the last year. Postmenopausal women taking hormones were initially matched to postmenopausal women not taking hormones by age (±2 years) and level of obesity (body mass index ± 2 kg/m²). Seven hundred twenty-two women had contingency samples available. Women were considered to be premenopausal if they had had more than 10 periods in the year preceding their examination. We restricted analyses to premenopausal women who did not take oral contraceptives. Finally, premenopausal women were matched to the two groups of postmenopausal women on obesity (body mass index ± 2 kg/m²). The premenopausal women, as expected, were younger than the postmenopausal women. The age range of the premenopausal women was 26–50 years; the age range of the postmenopausal women was 46–70 years.

Serum samples later used for leptin determinations were stored at −70°C for an average duration of 2.9 years. These samples were not previously thawed until the leptin assay was performed. Leptin was measured by a commercial radioimmunoassay (Linco Research, Inc., St. Louis, Missouri) (7). The intraassay coefficient of variation was 3.4–8.3 percent, and the interassay coefficient of variation was 3.6–6.2 percent.

Estradiol was measured by a solid phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California). The lower limit of detectability was 8 pg/ml, and the intraassay and interassay coefficients of variation were 5.3 and 6.4 percent, respectively. Estrone was measured by a double antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, Texas). The lower limit of sensitivity was 1.2 pg/ml, and the intraassay and interassay coefficients of variation were 6.5 and 9.1 percent, respectively. The samples for sex hormones were not previously thawed until these assays were done; the average duration of storage was 3.8 years.

Data analyses were performed using SAS statistical packages. The skewness and kurtosis of variables (lep-
tin, body mass index, and so forth) were checked and found to be approximately normally distributed; therefore, parametric statistical tests were performed.

RESULTS

In table 1, the clinical and metabolic characteristics of subjects are shown by menopausal status. Subjects were matched on obesity as measured by body mass index. They also were similar with respect to overall adiposity as assessed by the sum of skinfolds and with respect to body fat distribution as determined by the waist-to-hip ratio or the ratio of subscapular-to-triceps skinfolds. Leptin concentrations (ng/ml) were not significantly different in premenopausal women (27.6 ng/ml), postmenopausal women on hormone replacement therapy (28.3 ng/ml), and postmenopausal women not on hormones (27.8 ng/ml). Estradiol and estrone levels were significantly different by menopausal status \((p < 0.001)\). Estradiol and estrone levels were significantly higher in postmenopausal women on hormones than in premenopausal women \((p < 0.001)\). Premenopausal women also had significantly higher estradiol and estrone levels than postmenopausal women not on hormones. The duration of postmenopausal hormone use was not significantly related to leptin levels \((p > 0.05)\). The dose of Premarin therapy (used in 19 of 28 women) was also not significantly related to leptin concentrations \((p > 0.05)\). Estradiol and estrone levels were not significantly related to leptin concentrations in the overall group or in any of the individual groups shown in table 1.

In table 2, the clinical data are shown after adjustment for age and body mass index. Leptin concentrations were similar in each group. In table 3, we present a two-way analysis of variance with obesity (above and below the median for body mass index) and menopausal status as grouping variables. Obese subjects had significantly higher leptin concentrations than nonobese subjects \((p < 0.001)\), but neither menopausal status nor hormone replacement therapy was related to leptin concentrations.

DISCUSSION

We have shown in this report that leptin concentrations are not related to menopausal status or postmenopausal hormone use. These findings argue against the hypothesis that increased estrogen concentrations in women are an explanation for their higher leptin concentrations. Further supporting the absence of a relation between hormone status and leptin levels are the absence of a dose response for premarin on leptin levels or a relation between the duration of estrogen use and leptin levels. These analyses, however, are limited by the small number of subjects. Hassink et al. (21) showed a rise in leptin levels \((independently of adiposity)\) in girls with increased Tanner stage, suggesting a possible role for estrogen in modulation of leptin concentrations.

Several reports have suggested higher leptin concentrations in women than in men (5-10, 21, 22). Two reports, which used bioimpedance to assess percentage of adiposity, reported no sex difference between men and women after adjustment for percentage of adiposity.
TABLE 2. Clinical and metabolic characteristics of subjects (mean ± SE*) by menopausal status and hormone replacement (adjusted for age and BMI*), San Antonio Heart Study follow-up, 1991–1995

<table>
<thead>
<tr>
<th></th>
<th>Pre menopausal (n = 53)</th>
<th>Postmenopausal</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>95.1 ± 1.3</td>
<td>93.2 ± 1.7</td>
<td>98.2 ± 1.7</td>
</tr>
<tr>
<td>WHR*</td>
<td>0.87 ± 0.01</td>
<td>0.86 ± 0.01</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td>STR*</td>
<td>1.25 ± 0.08</td>
<td>1.20 ± 0.10</td>
<td>1.27 ± 0.10</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>102.9 ± 7.15</td>
<td>92.3 ± 9.30</td>
<td>113.3 ± 9.46</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>27.4 ± 1.6</td>
<td>29.9 ± 2.0</td>
<td>26.5 ± 2.1</td>
</tr>
<tr>
<td>Estrone (pg/ml)</td>
<td>53.3 ± 7.2</td>
<td>168.3 ± 14.0</td>
<td>25.0 ± 8.1</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>74.3 ± 6.4</td>
<td>118.2 ± 12.6</td>
<td>25.4 ± 7.2</td>
</tr>
<tr>
<td>Skinfolds (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular</td>
<td>28.6 ± 1.5</td>
<td>27.5 ± 2.0</td>
<td>21.8 ± 2.0</td>
</tr>
<tr>
<td>Triceps</td>
<td>24.7 ± 1.6</td>
<td>24.3 ± 2.0</td>
<td>20.2 ± 2.1</td>
</tr>
<tr>
<td>Sum of skinfolds</td>
<td>53.3 ± 2.8</td>
<td>51.8 ± 3.6</td>
<td>51.0 ± 3.7</td>
</tr>
</tbody>
</table>

* SE, standard error; BMI, body mass index; WHR, waist-to-hip ratio.
† Based on overall test of differences (F statistic from analysis of variance).

TABLE 3. Leptin levels (ng/ml) by hormonal status and level of obesity (adjusted for age), San Antonio Heart Study follow-up, 1991–1995

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal: no hormone</th>
<th>Postmenopausal</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean ± SE†</td>
<td>No.</td>
</tr>
<tr>
<td>Low BMI (&lt;30.5 kg/m²)</td>
<td>26</td>
<td>17.3 ± 2.6</td>
<td>13</td>
</tr>
<tr>
<td>High BMI (≥30.5 kg/m²)</td>
<td>27</td>
<td>34.8 ± 2.4</td>
<td>15</td>
</tr>
</tbody>
</table>

* Computed by two-way analysis of variance with menopausal status and obesity as grouping variable. p value for interaction was 0.872.
†SE, standard error; BMI, body mass index.

In contrast, Saad et al. (9) have reported higher leptin levels in women than in men after adjustment for adiposity as assessed by the DEXA technique. However, Rosenbaum et al. (22) did not show a difference in leptin levels in adult men and women after adjustment for adiposity. Similarly, two other studies reported higher leptin levels in women than in men after adjustment for adiposity assessed by subcutaneous skinfolds (8, 10). In one of these studies (8), based on a population-based study (San Antonio Heart Study), the sex difference in leptin levels was 18.0 ng/ml. After adjustment for body mass index, the sex difference in leptin levels was essentially unchanged (17.6 ng/ml). After adjustment for subscapular/triceps skinfolds (which are increased in women relative to men), the sex difference in leptin was reduced to 9.0 ng/ml but was still highly significant (p < 0.001).

The current report has a number of limitations. Since the report is restricted to Mexican Americans, questions can be raised about its generalizability. In previous studies, no ethnic difference in leptin levels was observed between Mexican Americans and non-Hispanic whites (20) or between blacks and Caucasians (7). Furthermore, the relation between body mass index and leptin concentrations was similar in Mexican Americans and non-Hispanic Whites (20). Although the validity of self-report may be limited, we have measured estradiol and estrone levels and shown results consistent with their self-reported status.

The absence of a difference in leptin concentrations between pre- and postmenopausal women as well as the absence of an effect of postmenopausal hormone replacement therapy argues against an estrogenic effect regulating leptin levels. Moreover, the subjects were well matched for obesity using two different measures of adiposity; there were no differences in overall obesity or body fat distribution between the two groups. However, our study was cross-sectional. Definitive data would consist of observational studies going through natural menopause as well as direct studies of postmenopausal hormone replacement.

It is also possible that differences in fat depots between men and women may help to explain gender differences in leptin levels. Men have lower levels of overall adiposity but greater visceral adiposity than women (23). Lönnqvist et al. (2) have shown that subcutaneous fat produces more leptin mRNA than visceral fat (2), which could explain why women have higher leptin levels inasmuch as they have more subcutaneous fat than visceral fat. Our group, in fact, has
previously shown (8) that adjustment for subcutaneous fat reduced the sex difference in leptin concentrations much more than adjustment for waist circumference (which has been suggested as a good proxy for visceral fat) (24).

Another possible explanation for the sexual dimorphism in leptin levels could be differences in the hypothalamus between men and women. There are present sites for leptin in the hypothalamus, and leptin suppresses hypothalamic neuropeptide Y synthesis (25). The release of neuropeptide Y by the hypothalamus stimulates food intake (26). Urban et al. (27) have suggested that there are sex differences in the regional distribution of neuropeptide Y mRNA-containing cells in the hypothalamus of mice. However, it is not known whether this structural sex difference in the hypothalamus is associated with functional differences in leptin signaling and leptin levels.

In conclusion, we have shown that leptin concentrations neither are significantly different in premenopausal and postmenopausal women nor are affected by postmenopausal hormone use. Our data suggest that sex hormones do not explain the sexual dimorphism in leptin levels, although direct studies of hormone replacement in both men and women would provide more definitive data.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Heart, Lung, and Blood Institute (R01 HL24799).

REFERENCES


