CLINICAL STUDY

The influence of menopause and body mass index on serum leptin concentrations

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Abstract

Objective: The aim of this study was to evaluate the influence of menopausal status, serum estradiol and body mass index (BMI) on serum leptin concentration in a large sample of pre- and postmenopausal women.

Design: 434 healthy women (mean age ± S.D., 52.2 ± 10.3 years) were recruited at the University of Marburg on the occasion of a routine gynecological visit. Two hundred and eighteen (50.2%) women were premenopausal (mean age, 36.5 ± 10.4 years) and not on oral contraceptives or hormone replacement therapy (HRT) and 216 (49.8%) women were postmenopausal (mean age 61.8 ± 8.9 years) not on HRT. To evaluate the influence of menopausal status, estradiol level and BMI on serum leptin concentrations, women were allocated to one of the four groups: (a) premenopausal women BMI <25 kg/m² (n=137), (b) premenopausal women BMI >25 kg/m² (n=81), (c) postmenopausal women BMI <25 kg/m² (n=94) and (d) postmenopausal women BMI >25 kg/m² (n=122).

Results: Irrespective of the menopausal status, women with a BMI >25 kg/m² had significantly higher leptin concentrations in all age groups compared with women with a BMI <25 kg/m² (P<0.001). The multiple linear regression analyses showed that BMI was the only statistically significant independent predictor for leptin. In comparison to postmenopausal women, premenopausal women showed a significantly lower mean age, weight, BMI and FSH concentration (P<0.001), a higher mean height and serum estradiol (P<0.01 and P<0.001 respectively) but significantly lower serum leptin concentration (P<0.01). The multiple linear regression model showed no significant influence of menopausal status or serum estradiol on serum leptin concentration, even after controlling for BMI.

Conclusions: Serum leptin concentrations are significantly higher in pre- and postmenopausal obese women, compared with normal weight controls. Serum leptin concentrations are not influenced by menopausal status or serum estradiol level.

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Introduction

It is a common clinical observation that a large number of women gain body weight after menopause. Several clinical studies have demonstrated this effect (1–3). Central obesity increases after menopause, indicating that the loss of estrogen may not only increase body fat and weight, but also change body fat distribution (4). It has been reported that long-term hormone replacement therapy (HRT) prevents the increase in body weight and skin-fold thickness observed after the menopause (5). However, the exact mechanism of the menopausal effect on body weight and fat distribution after the loss of estrogen has not been fully determined in vivo. It has been suggested that there is a link between estradiol and leptin metabolism, production and/or action in postmenopausal women.

Leptin is the product of the ob gene and is expressed in adipose tissue (6–9). It appears to be a component of a regulatory loop linking fat mass to food intake and energy expenditure (10–12). Serum leptin concentrations are closely related to body fat content (13), have a circadian rhythm (14), are regulated by fasting plasma insulin concentrations (15–17) and differ between males and females (18, 19). Leptin concentrations have been reported to be 2–4 times higher in females than in males (18–21). Most data suggest that there are gender differences in leptin concentrations that are unexplained by differences in obesity. Additionally, females seem to be more sensitive to exercise-induced changes in leptin concentrations than males, even in the absence of any change in fat mass (20, 22). The mechanism for the gender dichotomy in the leptin–fat mass relationship,
and for the greater sensitivity of leptin to change in energy balance in women is not apparent. Since there is a residual variability in leptin levels, environmental factors, genetics, gender and other factors besides obesity may also influence serum leptin concentrations. It has been suggested that there is a link between sex steroids and leptin production/action in humans (23). Only a few studies have evaluated the relationship between serum estradiol and leptin concentrations. These studies investigated different proportions of males and females, some only postmenopausal, some only premenopausal women and included mainly small sample sizes leading to conflicting results (19–20, 23). We therefore studied a large sample of normal weight and obese, pre- and postmenopausal women not on oral contraceptives or HRT to determine the influence of menopausal status, serum estradiol and body mass index (BMI) on serum leptin concentrations. We hypothesized that if estradiol plays a regulatory role in leptin production, leptin levels would be expected to vary as a function of the presence or absence of estrogen.

Methods

Subjects

Four hundred and thirty-four women (age range 30–86 years; mean ± s.d., 52.2 ± 10.3 years) participated in the study. They were recruited from women attending the department of obstetrics and gynecology of the University of Marburg for routine gynecological check-up. Before entry to the study, all women had answered a detailed questionnaire on important risk factors and had written informed consent. Exclusion criteria contained known cardiovascular disease, diabetes and medication such as lipid-lowering drugs, corticosteroids, thyroid hormone oral contraceptives or HRT which might alter metabolism. Anthropometric measurements (weight and height) were made with the participant wearing an examining gown after having removed their shoes. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Women with a BMI >25 kg/m² were classified as obese and women with a BMI of <25 kg/m² were classified as not obese. We considered women to be postmenopausal if they had a hysterectomy and bilateral oophorectomy and/or had no menstrual periods in the year preceding their examination and/or had a serum follicle-stimulating hormone (FSH) concentration of >30 IU/l and a serum estradiol level of <10 pg/ml. Women taking HRT were excluded from the study. All blood samples were collected in the morning to gain comparable results. Women were considered premenopausal if they had regular periods in the year preceding their examination and/or had a serum FSH of <30 IU/l and a serum estradiol level of >10 pg/ml. We restricted analyses to non-pregnant premenopausal women who did not take oral contraceptives.

Ethical approval

The study received the approval of the ethical committee of the University of Marburg and was carried out according to the requirements of the declaration of Helsinki.

Study design

In the initial analysis, we compared non-pregnant premenopausal women (n = 218) with postmenopausal women who had not used HRT (n = 216). In order to reduce any bias introduced by confounding variables such as age, weight, height and BMI, we separately analyzed the groups according to their BMI. Women with a BMI >25 kg/m² were classified as obese while women with a BMI of <25 kg/m² were classified as not obese. Eligible subjects were assigned to the following groups: (a) premenopausal women with a BMI >25 kg/m²; (b) premenopausal women with a BMI <25 kg/m²; (c) postmenopausal women with a BMI >25 kg/m²; and (d) postmenopausal women with a BMI <25 kg/m². Additionally, we evaluated the influence of age and BMI on serum leptin concentration irrespective of the menopausal status as well as the effect of serum estradiol concentration and BMI on serum leptin.

Assays

Serum leptin was measured by a commercial RIA (Linco Research, Inc., St Louis, MO, USA). The intraassay coefficient of variation was 3.4–8.3%, and the interassay coefficient of variation was 3.6–6.2%.

Serum estradiol was measured by a solid phase RIA (Diagnostic Products Corporation, Los Angeles, CA, USA). The lower limit of detectability was 8 pg/ml, and the intraassay and interassay coefficients of variation were 5.3% and 6.4% respectively.

Serum FSH was measured by a highly sensitive IRMA assay (Biochem Immunosystems Co., Freiburg, Germany). Intraassay and interassay coefficients of variation were 6.1% and 6.5% respectively. The detection limit was 0.5 IU/l.

Statistical analysis

Data analyses were performed using SPSS 9.0 for Windows. Two-tailed tests were used throughout and the level of statistical significance set at 0.05. The skewness and kurtosis of the distribution of all variables were controlled and found to be normally distributed. Differences in patients characteristics and serum leptin, FSH and estradiol concentrations between pre- and postmenopausal women were calculated using t-test. In order to evaluate differences between the defined groups we used ANOVA with the Bonferroni-adjusted probabilities for post hoc analysis. Because of the large number of statistical comparisons, the likelihood of Type I errors is increased for each analysis. The Bonferroni method
Table 1 Baseline characteristics of premenopausal women (n=218) compared with postmenopausal women (n=216).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Premenopausal Women</th>
<th>Postmenopausal Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>S.D.</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.6***</td>
<td>36.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.7***</td>
<td>70.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.8**</td>
<td>161.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9***</td>
<td>16.3</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>9.7***</td>
<td>50.5</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>50.3***</td>
<td>8.4</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>16.4**</td>
<td>19.5</td>
</tr>
</tbody>
</table>

**P < 0.01, ***P < 0.001.**

The role of serum leptin in humans is still controversial. Serum leptin concentration is increased in obese subjects and is closely related to fat mass (FM) and BMI (13, 19). It is regulated by serum insulin concentration (25) and declines with weight loss (13). Several reports have shown a higher leptin concentration in women than in men (18). The gender difference has been explained partly by the variable degree and

Postmenopausal women with a BMI <25 kg/m² showed a significantly lower weight, BMI and serum leptin concentration (P<0.001) as well as a higher mean serum FSH concentration (P<0.001) in comparison to postmenopausal women with a BMI >25 kg/m². There were no significant differences in mean age, mean height and mean serum estradiol concentration between the groups (Table 2).

We performed ANOVA to compare pre- and postmenopausal women with a BMI below and above 25 kg/ m². The model was highly significant (F = 27.37 with P<0.001). The post hoc analysis (significance level P = 0.05) revealed significant mean differences for serum leptin concentrations between the defined groups as follows: between group a vs b and d, group b vs a and c, group c vs b and d and between group d vs a and c.

**Our results indicate that the significant differences in serum leptin concentration in the premenopausal and postmenopausal women were only influenced by BMI.** There was no influence of menopausal status on serum leptin concentration (Fig. 2).

We additionally performed a multiple regression analysis with leptin as the continuous variable to evaluate the independent influence of BMI on serum leptin concentration. The following variables were entered into stepwise multiple linear regression models: age, weight, height, BMI, menopausal status (pre- vs postmenopausal), estradiol and FSH. Hereby, only BMI remained as a statistically significant independent predictor for leptin (R² = 0.17; Model: F = 125.07 with P<0.0001). The multiple linear regression model showed no significant effect for menopausal status or serum estradiol on serum leptin concentration.

Finally, we plotted the serum leptin concentration of premenopausal women (serum estradiol concentration >10 pg/ml) and postmenopausal women (serum estradiol concentration <10 pg/ml) in relation to the BMI (Fig. 3).

Discussion

Our results on a large sample of healthy pre- and postmenopausal women demonstrate that obese women have significantly higher leptin concentrations than women with normal weight. Our study indicates that neither menopause nor serum estradiol alters serum leptin concentration. This effect persisted even after controlling for BMI in a multiple linear regression analyses.

The role of serum leptin in humans is still controversial. Serum leptin concentration is increased in obese subjects and is closely related to fat mass (FM) and BMI (13, 19). It is regulated by serum insulin concentration (25) and declines with weight loss (13). Several reports have shown a higher leptin concentration in women than in men (18). The gender difference has been explained partly by the variable degree and
distribution of the amount of body fat depots. Women tend to have a higher overall obesity which is more pronounced in subcutaneous fat than in visceral fat, in contrast to men who have a lower overall but greater visceral adiposity. Lönnqvist et al. reported that subcutaneous fat produced more leptin mRNA than visceral fat (8). This may also help to explain a part of the sexual dimorphism in leptin levels. Ostlund et al. and Haffner et al. reported higher leptin concentrations in women than in men even after adjustment for obesity assessed by subcutaneous skinfolds (21, 23). In contrast, two reports by Considine et al. and Maffei et al. showed no difference between women and men after adjustment for percentage of body fat assessed by bioimpedance (13, 26).

It has also been hypothesized that gender differences of serum leptin concentrations between men and women can partly be explained by differences in serum estradiol levels. A study by Barash et al. (27) suggested that serum leptin concentrations seem to modulate gonadal activity. Ovarian and testicular weight increased in this study after treatment with leptin and histological examination demonstrated a larger amount of follicular development in ovarian tissue and elevated cellular activity in seminiferous tubules in the testis. More recently, Ahima et al. hypothesized that leptin acts as a signal triggering puberty (28). A study by Shimizu et al. also suggested that estradiol raises leptin levels in humans. They found that serum leptin was about 33% higher during luteal phase of menstrual cycle while estradiol concentrations were two-fold higher in comparison to follicular phase (29). A report by Hardie et al. (30) with a similar design suggested that leptin levels are more closely associated with serum progesterone levels. These authors also reported that during gestation, serum leptin concentration correlates closely with estradiol and not with progesterone.

Today, the influence of serum estradiol concentration on serum leptin is still poorly understood.

| Table 2 Characteristics of pre- and postmenopausal women according to BMI and menopausal status. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Premenopausal                                   | Premenopausal                                   | Postmenopausal                                  | Postmenopausal                                  |
| BMI < 25 (n = 137)                              | BMI > 25 (n = 81)                               | BMI < 25 (n = 94)                               | BMI > 25 (n = 122)                              |
| Mean    | S.D.    | Mean    | S.D.    | Mean    | S.D.    | Mean    | S.D.    |
| Age (years) 33.9*** 9.9  | 41.0 9.9  | 61.3 9.8  | 62.2 8.2  |
| Weight (kg) 58.6*** 6.3  | 77.5 10.4  | 60.9§§§ 5.9  | 78.6 10.3  |
| Height (cm) 166.4* 6.0  | 164.7 6.7  | 164.6 5.3  | 163.1 6.4  |
| BMI (kg/m²) 21.2*** 1.9  | 28.6 3.4  | 22.4§§§ 1.7  | 29.5 3.3  |
| FSH (IU/l) 9.4 15.1  | 10.1 10.5  | 56.0§§§ 23.5  | 46.3 20.4  |
| Estradiol (pg/ml) 60.8 72.7  | 56.7 61.6  | 9.1 14.1  | 8.0 7.4  |
| Leptin (ng/ml) 12.6*** 13.7  | 22.9 14.2  | 11.8§§§ 10.7  | 25.5 14.2  |

*P ≤ 0.05; ***P ≤ 0.001 Differences between premenopausal women under or over BMI 25.
§§§ P ≤ 0.001 Differences between postmenopausal women under or over BMI 25.

Figure 2 Serum leptin concentration in pre- and postmenopausal women in relation to BMI. ***Differences between premenopausal under or over BMI 25 (P < 0.001). §§§Differences between postmenopausal under or over BMI 25 (P < 0.001).

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Hickey et al. (20) recently analyzed data on postmenopausal women participating in a Cardiovascular Risk Factor Identification Program. Women were matched for age, aerobic capacity and obesity. Despite a four-fold variation in serum estradiol, serum leptin concentration was not different in HRT users and non-users. A previous study by Haffner et al. (23) analyzing a small but well-matched number of Mexican American women selected from the San Antonio Heart Study also showed no relation of serum leptin concentration to menopausal status or postmenopausal hormone use. These and other findings including our own results argue against the hypothesis that differences of serum leptin concentrations between men and women can be explained by differences in serum estradiol levels (31, 32). In contrast, a more recent report by Paolisso et al. (24) on a much younger population showed a high correlation of serum leptin with estradiol or testosterone concentrations in men and with estradiol concentration in women after adjustment for age, amount of body fat and waist to hip ratio (WHR). The authors hypothesized that the relationship between sex hormones and serum leptin concentration could also be mediated through the effect of sex hormones on body fat content and concentration, since in vitro studies demonstrated a direct relationship between serum estradiol levels and BMI (24, 33).

In conclusion, our study on a large sample of healthy pre- and postmenopausal women demonstrates that neither menopause nor serum estradiol alters plasma leptin concentration even after controlling for BMI in a multiple linear regression analyses. Hereby, only BMI remained as a statistically significant independent predictor for leptin. However, the role of leptin in humans is still poorly understood. The existing reports show inconclusive results on the interaction of serum leptin concentration and estradiol levels. If, how and why serum estradiol levels might influence serum leptin concentration needs to be investigated in further longitudinal and interventional studies.

References


