Estrogen and exercise may be related to body fat distribution and leptin in young women

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Objective: To evaluate the effects of estrogen deficiency and exercise on body composition and leptin in young women.

Design: Cross-sectional clinical study.

Setting: Volunteers in an academic research environment.

Patient(s): Three age- and body mass index–matched groups: normal-weight women with exercise-associated amenorrhea, regularly menstruating exercising control women, and regularly menstruating normally active control women.

Intervention(s): Collection of blood samples and measurement of body fat and regional fat distribution by dual-energy x-ray absorptiometry.

Main Outcome Measure(s): Central fat accumulation (i.e., ratio of trunk to extremity fat) and serum concentrations of E2 and leptin.

Result(s): In both regularly menstruating control groups, but not in the amenorrheic women, there was a negative correlation between the serum E2 concentrations and the trunk-to-extremity fat ratio (r = −0.4), independent of age, exercise, body fat, and serum T concentrations. In all women, E2 concentrations were positively and exercise inversely correlated to leptin concentrations, independent of body fat.

Conclusion(s): Estradiol level is inversely associated with central fat accumulation only in women with regular menstrual cycles. In all young premenopausal subjects, estrogen secretion influences leptin concentrations independently of body fat. (Fertil Steril 2006;86:694–9. ©2006 by American Society for Reproductive Medicine.)

Key Words: Exercise, body composition, amenorrhea, leptin

Central obesity augments insulin resistance (1, 2) and is an important and independent risk factor for cardiovascular disease and mortality in epidemiologic studies (1, 3, 4). Compared with men, women have less central accumulation of body fat (1, 5, 6). However, most studies have demonstrated an increase in central abdominal fat during the menopausal transition (7–10). The accumulation of central fat after menopause can be prevented or reversed by hormonal replacement therapy (1, 6, 11–14). Therefore, estrogen secretion may be an important mediator of body composition and have important metabolic and health implications. Despite this, a relationship between estrogen concentrations and central accumulation of body fat has never been demonstrated in premenopausal women.

Central obesity is furthermore decreased by physical activity in studies focusing on overweight or obese subjects (15–20), although there are little data in young normal-weight individuals. In young normal-weight women, extensive physical activity associated with a large energy deficiency can lead to amenorrhea and hypoestrogenemia. Leptin, an adipocyte-secreted hormone involved in energy homeostasis, is thought to represent a possible link between energy deficiency and menstrual disturbances and is highly related to body fat (21, 22). The effects of E2 levels and chronic exercise on body fat distribution and leptin levels in normal-weight premenopausal women are unknown. It is also not known if these effects are independent of body fat.

Therefore we evaluated the influence of estrogen and exercise both on body fat distribution and on leptin serum concentrations in young premenopausal women. We also examined the relationship between total body fat, body fat distribution, and leptin both in normally active and in exercising normal-weight women with regular menstrual cycles as well as in normal-weight women with exercise-associated amenorrhea, a syndrome thought to be due to chronic energy deficiency.

MATERIALS AND METHODS

Subjects

Fifty subjects (ages 18–36 years) were studied to determine the relationship between E2 level, body composition,
and metabolic parameters in normal-weight subjects with exercise-associated amenorrhea and in normal-weight exercising and normally active controls. Twelve women who had exercise-associated amenorrhea for at least 6 months (ExAmen group) were studied. Control subjects included 19 exercising women with regular menstrual cycles (ExControl) who were matched for age and body mass index (BMI). These control subjects had 10–12 menses in the previous 12 months. The third group consisted of 19 women with regular menstrual cycles who performed <5 hours of physical activity per week (NormControl).

Women were recruited from local gyms and dance studios in the New York Metropolitan area via posters and advertisements. Inclusion into the study was based on the following criteria: [1] no current evidence of disordered eating or history of an eating disorder; [2] stable weight for ≤3 months; [3] not taking hormonal contraceptives or any other medications; and [4] no evidence of polycystic ovary syndrome (PCOS), hyperprolactinemia, or thyroid disorders at the initial screening. Women in both exercise groups exercised ≥7 h/wk (any type of exercise, including dancing, aerobics, biking, etc.).

**Study Design**

After a screening examination and medical history, all subjects were evaluated at the Columbia University Medical Center. Morning blood samples were collected for analysis of E2 and leptin. Subjects with regular menstrual cycles were studied in the early follicular phase (days 3 to 7) of the cycle. All samples were run in the same assay, and measurements for leptin were analyzed in duplicate measurements. Body composition was determined by using both whole-body dual-energy x-ray absorptiometry (DXA) (DPX-L; GE Systems, Madison, WI) and bioimpedance analysis. This protocol was approved by the Institutional Review Board of Columbia University Medical Center and St. Luke’s Hospital Center (New York, NY), and written informed consent was obtained from all participants.

**Measures**

Body fat distribution was determined by DXA using the method of Hadigan et al. (23) as described by Grinspoon et al. (24) and Misra et al. (2, 25), calculating percentage trunk fat as the ratio of trunk fat to total fat × 100 and percentage extremity fat as the ratio of total extremity fat (left and right arm fat and left and right leg fat) to total fat × 100. Trunk-to-extremity fat ratio, a measure of central obesity, was determined by dividing percentage trunk fat by percentage extremity fat.

Dual-energy x-ray absorptiometry is currently considered to be the most reliable technique available in clinical studies for measuring body fat, with a measurement precision of ±1.2% and a cross-subject accuracy in subjects of comparable fatness of better than ±1%. The reports from the DPX-L scanner were analyzed using version 3.6 software (GE Systems) and were used to determine total body fat percentage.

**Assay Methods**

Estradiol, T, FSH, and LH were measured by a commercial solid-phase chemiluminescent immunoassay (Immulite; Diagnostic Products, Los Angeles, CA). Assay sensitivity for E2 is 20 pg/mL, for T 10 ng/dL, for FSH 0.2 mIU/mL, and for LH 0.7 mIU/mL. The intraassay coefficient of variation (CV) for E2 is 9.3% and the interassay CV 10.5%. The intraassay CV for FSH is 6.4% and the interassay CV 7.5%. The intraassay CV for LH is 6.2% and the interassay CV 7.4%. The intraassay CV for T is 7.4% and the interassay CV 9.8%. Leptin levels were measured using a commercial ELISA kit (Diagnostic System Labs, Webster, TX). Assay sensitivity is 0.1 ng/mL. The intraassay CV is 3.6% and the interassay CV 4.9%.

**Statistical Analysis**

Data are shown either as mean ± SEM for normally distributed values or as median (interquartile ranges) for not normally distributed values. Variables with a skewed distribution were log transformed for all analyses. Patients’ characteristics or laboratory values were compared between groups using the t test. For multigroup comparisons of normally distributed data, we used parametric one-way analysis of variance. Post hoc testing was performed using the Fisher multiple comparison test.

Pearson correlation tests, partial correlation analyses, and multiple linear regression analyses were used to assess the relationship between the regional body composition and the hormonal and metabolic parameters. A value of P<.05 was considered statistically significant.

All statistical analyses were done with Statistica for Windows, version 6 (StatSoft, Tulsa, OK) or Intercooled Stata, version 8 (StataCorp LP, College Station, TX).

**RESULTS**

**Demographic and Anthropometric Characteristics**

Demographic and anthropometric characteristics of the subjects in the three groups are shown in Table 1. There was no significant difference in age or BMI among all three groups. Lean body mass, the percentage body fat, and the serum leptin concentrations differed among the three groups: On post hoc analysis, the normally active control group (NormControl) had less lean body mass and a higher percentage of body fat as well as higher leptin serum concentrations compared with both the ExControl group (P<.001, P=.01, and P=.001, respectively) and the ExAmen group (P=.02, P=.003, and P=.02, respectively).

In these normal-weight women, there was no difference in gonadotropin and sex steroid serum concentrations measured either in the early follicular phase (regularly menstruating
women, i.e., ExControl and NormControl groups) or randomly (ExAmen group) (Table 2).

**Body Composition and Sex Steroids**

In both regularly menstruating control (ExControl and NormControl) groups, serum E₂ concentrations correlated positively to the percentage of extremity fat (r = 0.41, P = .02) and negatively to both the percentage of trunk fat (r = −0.39, P = .02) and the trunk-to-extremity fat ratio (r = −0.40, P = .02) (Fig. 1). Removing the two women with serum E₂ concentrations >100 pg/mL yielded similar results (data not shown). Age, extent of exercise, total body fat, and serum T concentrations were not associated with central fat accumulation (data not shown). Thus, additional adjustment for age, extent of exercise, total body fat, and serum T concentrations did not alter the association between E₂ and the trunk-to-extremity ratio (β-coefficient for E₂ = −0.18, 95% confidence interval [CI] = −0.34 to −0.01; pcorr: r = −0.4, P = .035).

Estradiol concentrations were highly correlated to the E₂-T ratio (r = 0.88, P < .0001). Therefore, almost identical results were obtained comparing the E₂-T ratio with the regional body distribution (data not shown).

Similar results were also obtained for the subjects in the ExControl group only. In this group, serum E₂ level correlated as well to the percentage of extremity fat (r = 0.55, P = .02) and negatively to both the percentage of trunk fat (r = −0.40, P = .02) and the trunk-to-extremity fat ratio (r = −0.54, P = .02). In the subjects with exercise-associated amenorrhea, E₂ level did not correlate to any components of the regional body composition (data not shown).

**TABLE 1**

Demographic and anthropometric characteristics of women with exercise-induced amenorrhea (ExAmen), regularly exercising controls with regular menstrual cycles (ExControl), and normally active controls with regular menstrual cycles (NormControl).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ExAmen (n = 12)</th>
<th>ExControl (n = 19)</th>
<th>NormControl (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21.4 ± 1.1</td>
<td>25.1 ± 1.3</td>
<td>24.1 ± 1.0</td>
<td>.12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.8 ± 0.5</td>
<td>21.7 ± 0.5</td>
<td>21.6 ± 0.3</td>
<td>.31</td>
</tr>
<tr>
<td>Total lean mass (kg)</td>
<td>41.6 ± 1.0</td>
<td>42.9 ± 1.0</td>
<td>38.1 ± 0.9</td>
<td>.002</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.8 ± 2.6</td>
<td>23.8 ± 1.5</td>
<td>29.4 ± 1.1</td>
<td>.007</td>
</tr>
<tr>
<td>Total body fat (kg)</td>
<td>11.8 ± 1.4</td>
<td>13.9 ± 1.2</td>
<td>15.0 ± 0.7</td>
<td>.16</td>
</tr>
<tr>
<td>Trunk fat (kg)</td>
<td>5.30 ± 0.62</td>
<td>6.45 ± 0.65</td>
<td>7.03 ± 0.38</td>
<td>.14</td>
</tr>
<tr>
<td>Extremity fat (kg)</td>
<td>5.88 ± 0.75</td>
<td>6.80 ± 0.59</td>
<td>7.36 ± 0.39</td>
<td>.23</td>
</tr>
<tr>
<td>Trunk-to-extremity fat ratio</td>
<td>0.93 ± 0.05</td>
<td>0.96 ± 0.06</td>
<td>0.98 ± 0.05</td>
<td>.84</td>
</tr>
<tr>
<td>Exercise (h/wk)</td>
<td>18.5 ± 3.4</td>
<td>17.2 ± 2.2</td>
<td>1.6 ± 0.5</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Note: Data shown as mean ± SEM.*

**TABLE 2**

Laboratory parameters of women with exercise-associated amenorrhea (ExAmen), regularly exercising controls with regular menstrual cycles (ExControl), normally active controls with regular menstrual cycles (NormControl).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ExAmen (n = 12)</th>
<th>ExControl (n = 19)</th>
<th>NormControl (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₂ (pg/mL)</td>
<td>35.5 (21–51)</td>
<td>30 (22–50)</td>
<td>44 (31–53.5)</td>
<td>.59</td>
</tr>
<tr>
<td>T (ng/dL)</td>
<td>93 (62.5–100.5)</td>
<td>66 (55–80)</td>
<td>64 (58–73)</td>
<td>.07</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>3.0 (1.7–10.6)</td>
<td>2.3 (1.8–3.9)</td>
<td>3.1 (1.4–4.3)</td>
<td>.54</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>3.2 (2.5–4.8)</td>
<td>3.9 (2.5–6.2)</td>
<td>3.7 (1.8–4.4)</td>
<td>.35</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>5.4 (3.6–12)</td>
<td>5.2 (2.6–9.1)</td>
<td>11.2 (6.6–15.7)</td>
<td>.004</td>
</tr>
</tbody>
</table>

*Note: Data are shown as median (interquartile ranges). To convert E₂ concentration from pg/mL to pmol/L, multiply by 3.67; to convert T concentration from ng/dL to nmol/L, multiply by 0.035.*
Exercise

Exercise (h/wk) correlated negatively to total body fat and borderline negatively to percentage body fat in all three groups ($r = -0.37, P = .01$ and $r = -0.28, P = .06$, respectively).

Leptin

The serum leptin concentrations are shown in Table 2. In these normal-weight subjects, leptin levels were similar in the ExAmen and the ExControl group but much lower than the NormControl group.

In the total study population, there was a positive correlation between leptin level and BMI ($r = 0.55, P < .0001$), total body fat ($r = 0.74, P < .0001$), trunk fat ($r = 0.76, P < .0001$), extremity fat ($r = 0.69, P < .0001$), and percentage body fat ($r = 0.82, P < .0001$) but not the trunk-to-extremity fat ratio ($r = 0.15, P = .33$). Estradiol level correlated positively to the serum leptin concentrations independently of total body fat (after adjusting: $\beta$-coefficient 0.37, 95% CI 0.05–0.69; $p_{corr}: r = 0.34, P = .02$). Similarly, the inverse correlation between exercise (h/wk) and serum leptin concentrations was also independent of total body fat (after adjusting: $\beta$-coefficient $-0.02$, 95% CI $-0.04$–$-0.004$; $p_{corr}: r = -0.36, P = .02$). However, adjusting for the extent of exercise abolished the relationship between serum $E_2$ and serum leptin concentrations (after adjusting: $\beta$-coefficient 0.26, 95% CI $-0.07$–$0.59$, $P = .1$).

DISCUSSION

We showed for the first time in premenopausal women that $E_2$ concentrations were inversely correlated to central accumulation of body fat and positively to extremity fat. The effect was independent of age, extent of exercise, total body fat, and serum T concentrations. However, we observed this relationship only in regularly menstruating women. Our findings underscore the protective effect of physiologic concentrations of estrogen on central fat accumulation.

In accordance with our data, one report in obese premenopausal women found a negative relationship between visceral fat and free $E_2$–free T ratio (26). Another study, including both premenopausal and postmenopausal women, showed a negative correlation between estrogen and central accumulation of body fat (27). In addition, central fat accumulation is linked to other factors, such as insulin resistance, also defined by low levels of sex hormone–binding globulin (SHBG) (28–30). Interestingly, SHBG concentrations are also themselves modulated by estrogen (31, 32).

Sex steroids such as estrogen and T have a strong influence on regional fat distribution (6) and at least partly explain the gender difference in central accumulation of body fat (1, 5). Cross-sectional data demonstrated an increase in central obesity in postmenopausal women when compared with premenopausal women (7, 10), even after adjustment for total body fat (33), age (8), or both (9). Longitudinal studies have also confirmed an increase in central obesity during the menopausal transition (14). Similarly, an increase in visceral adipose tissue area was noted in the majority of women whose hypoestrogenemia was due to the use of a GnRh agonist (34).

In our study, we could not find a correlation between serum estrogen concentrations and regional fat distribution in subjects with exercise-associated amenorrhea. It may seem paradoxical also that these women even had a tendency for less central accumulation of body fat. Their regional fat distribution is probably influenced by additional factors such
as energy restriction, abnormally low fat in all areas, and the influence of other hormones. Indeed, a recent study showed that adolescent girls with anorexia nervosa had less central accumulation of body fat compared with healthy adolescents. This effect was explained by increasing growth hormone concentrations (25).

A potential limitation of our study is that we did not include patients with PCOS, another group of premenopausal women characterized by oligomenorrhea or amenorrhea. These subjects have a more central fat distribution compared with controls, which is linked to their increased insulin resistance (35). Healthy controls have lower serum E₂ concentrations in the follicular phase. However, we have previously demonstrated that controls have higher overall mean serum E₂ concentrations over a 1-month period compared with patients with PCOS and anovulation (35).

Hormonal replacement therapy has been found either to prevent or to reverse accumulation of central fat in menopausal women, and estrogen seems to be the most active component (1, 6, 11–14). Female sex hormones, predominantly estrogen, regulate regional differences in fat cell metabolism and generally decrease the production of lipoprotein lipase in adipose tissues (1, 6, 34, 36). Premenopausal women have higher lipoprotein lipase activity in the femoral than in the abdominal fat depot (1). Lack of estrogen is associated with alterations in omental and subcutaneous adipocyte metabolism, reflecting a predominant visceral fat storage (34).

Additional factors that may influence fat distribution in menopausal women are age (37) as well as changes in physical activity (15) and reduced energy expenditure both at rest and during physical activity (38). In overweight or obese subjects, exercise reduced central obesity, though this was predominantly combined with energy restriction (17, 19, 20). All other studies that showed a relationship between exercise and central obesity are cross-sectional: Thereby, sedentary subjects are generally older and/or have higher BMI values than subjects who exercise (15, 16, 18).

In our population of normal-weight young premenopausal women, we could not find a relationship between the extent of exercise and central fat accumulation. However, exercise was inversely associated with total body fat, percentage of body fat, and serum leptin concentrations in our total study population. Similarly, serum leptin concentrations as well as the percentage of body fat were higher in the normally active BMI- and age-matched group compared with both regularly exercising groups. However, exercising women with regular menstrual cycles had similar total body fat and similar leptin concentrations compared with women with exercise-associated amenorrhea.

This stands in apparent contrast to other studies (21, 39), where both fat content and leptin concentrations were lower in amenorrheic subjects. That control group performed almost no exercise and had higher total body fat and leptin concentrations compared with our exercising regularly men- struating controls. Therefore, based on our data and another recent report (40), we think that in normal-weight subjects leptin deficiency is not a major contributor to exercise-induced amenorrhea. Leptin is a key protein that plays a role in body weight homeostasis (6, 22). We showed that E₂ concentrations were associated with leptin concentrations, independently of body fat. Indeed, the gender difference in leptin levels is thought to relate both to the difference in body fat in men and women as well as to direct effects of estrogens in the regulation of leptin (1, 6).

In summary, in normal-weight women, E₂ concentrations were positively and exercise inversely correlated to serum leptin concentrations, even after adjustment for total body fat. Therefore, both estrogen and exercise can probably influence leptin and thus energy balance through additional mechanisms that are independent of their effect on body fat. We found that exercise was related to total body fat but not to regional fat distribution. In regularly menstruating women we could demonstrate a positive correlation between the serum E₂ concentrations and peripheral fat and an inverse association between serum E₂ concentrations and central accumulation of body fat, independent of total body fat. This underscores a protective effect of physiologic concentrations of estrogen on body composition in normal premenopausal women and may in turn attenuate risk factors associated with central fat accumulation, including cardiovascular disease.

REFERENCES