Body fat, energy balance and estradiol levels: a study based on hormonal profiles from complete menstrual cycles

A. Ziomkiewicz1,2,6, P.T. Ellison3, S.F. Lipson3, I. Thune4,5 and G. Jasienska2

1Institute of Anthropology, Polish Academy of Science, Kuznicza 35, 50-951 Wroclaw, Poland; 2Department of Epidemiology and Population Studies, Jagiellonian University, Collegium Medicum, 31-531 Krakow, Poland; 3Department of Anthropology, Harvard University, Cambridge, MA 02138, USA; 4Institute of Community Medicine, University of Tromso, N-9037 Tromso, Norway; 5The Cancer Center, Ulleval University Hospital, N-0407 Oslo, Norway

6Correspondence address. Institute of Anthropology, Polish Academy of Science, Kuznicza 35, 50-951 Wroclaw, Poland. E-mail: annaz@antro.pan.wroc.pl

BACKGROUND: Female fecundity is regulated by nutritional status. Although widely cited, this hypothesis is not strongly supported by empirical data from non-obese, healthy women of reproductive age. METHODS: Healthy, reproductive aged women (n = 141) from Southern Poland collected daily morning saliva samples for one complete menstrual cycle. Levels of 17-β-estradiol were analyzed by radioimmunoassay. Anthropometric measurements, including body fat percentage, were taken randomly with respect to phase of the menstrual cycle. Energy balance was specified based on changes in body fat percentage from the beginning to the end of the observation period. RESULTS: Women with very low and high body fat had significantly lower levels of E2 compared with women with low and average body fat. In women of very low to average body fat, a 10% increase in body fat was associated with a 5–7 pmol/l increase in estradiol levels. The association between fat percentage and E2 was even stronger in women with positive energy balance, who also showed significant differences between body fat groups in estradiol profiles across the whole menstrual cycle. No such relationship was found in women with negative energy balance. CONCLUSIONS: In healthy women, we found a non-linear association between body fat and estradiol levels. Both very low and high body fat was associated with decreased estradiol levels. The relationship between estradiol and body fat was strongly influenced by women’s energy balance.

Keywords: 17-β-estradiol; body fat percentage; energy balance; menstrual cycle

Introduction

A small reduction in the levels of estradiol during the follicular phase, even when not related to significant changes in the length of the menstrual cycle, is associated with decreased probability of conception (Yoshimura and Wallach, 1987). Higher levels of follicular estradiol have been observed in cycles resulting in conception compared with cycles without conception in healthy subjects (Lipson and Ellison, 1996; Baird et al., 1997, 1999; Lu et al., 1999; Li et al., 2001; Venners et al., 2006). In women undergoing in vitro fertilization, higher levels of estradiol after the time of embryo transfer (Chen et al., 2003) or hCG administration (Blazar et al., 2004) were also strongly positively related to pregnancy success.

Inter-individual variation in levels of ovarian steroid hormones (Jasienska and Jasienski, 2008) can be attributed to several factors, including those related to energy availability and metabolism. Although the influence of physical activity on ovarian hormone levels is relatively well recognized (Elias and Wilson, 1993; Jasienska and Ellison, 1998, 2004; Chen and Brzyski, 1999; Burrows and Bird, 2000; Warren and Perlroth, 2001; Jasienska, 2003; De Souza and Williams, 2004; Jasienska et al., 2006), the role of nutritional status is still controversial.

A regulatory role of nutritional status on reproductive ability was first hypothesized by Frisch (1984), and the clearest evidence for a relationship between nutritional status and ovarian hormone levels comes from studies on women with anorexia nervosa (Warren, 1983; van Binsbergen et al., 1990; Kirchengast and Huber, 2004; Miller et al., 2004). In these women, low body mass or low body mass index (BMI) is related to low levels of estradiol and inhibition of menstrual cycles. However, the results of studies on healthy, relatively well-nourished women are contradictory. Studies report no relationship (Panter-Brick et al., 1993; Dorgan et al., 1995; Ivandic et al., 1998; Ukkola et al., 2001), a positive relationship (Brunning et al., 1992; Zanker and Swaine, 1998; Barnett et al., 2001, 2002; Furberg et al., 2005) or a negative relationship (Howard et al., 1987; Potischman et al., 1996; Westhoff et al., 1996; Thomas et al., 1997a,b) between nutritional status and ovarian reproductive hormone levels.
therefore, it is not clear whether variation in the ovarian hormone levels can be attributed to the influence of nutritional status.

Several authors have suggested that nutritional status does not regulate reproductive functioning independently from the effects of physical activity or energy balance (Wade et al., 1996; Ellison, 2001, 2003; Jasienska, 2001; Jasienska and Ellison, 2004; Schneider, 2004; Wade and Jones, 2004). Accordingly, a relationship between nutritional status and estradiol may be a by-product of the association between energy balance and reproductive hormone levels. In none of the cited studies, investigating the relationship between nutritional status and ovarian function was the effect of physical activity and/or energy balance accounted for, thus it is impossible to determine if the observed changes in the levels of reproductive hormones were independently modified by nutritional status.

To clarify the role of nutritional status as the factor affecting the levels of reproductive hormones in a menstrual cycle, we conducted a study investigating an association between estradiol levels measured over the course of a complete menstrual cycle and nutritional status indicated by body fat percentage in 141 healthy, well-nourished, but not obese women from Southern Poland. To account for the confounding effect of energy balance, separate analyses were conducted for a sub-sample of 131 women for whom energy balance was estimated on the basis of changes in body fat during the course of the study.

We hypothesize that nutritional status has an independent, regulatory effect on the levels of estradiol during the menstrual cycle and that the energy balance could influence this effect.

**Materials and Methods**

**Study participants**

The participants in this study were 141 women from urban and rural areas of Southern Poland. Women were recruited for the study by local media advertisement in the urban area, and through their parish in the rural area between June 2001 and June 2003. Selection of women for the study was based on the following criteria: age between 24 and 37 years, normal weight for height (reference data for weight for height recommended by WHO Expert Committee on Physical Report, 1995), self-assessed regular menstrual cycles not <25 and not >35 days, no fertility problems, no gynecological and endocrinological disorders, not taking hormonal oral contraceptives, or other hormonal medications for the period of 6 months prior to the recruitment and not being pregnant or lactating during the 6 months prior to the recruitment. The research protocol was approved by the Bioethical Committee of Jagiellonian University.

**General questionnaire and physical activity assessment**

A general questionnaire requesting information about place of birth, age, birthweight and birth length, age of menarche, education, marital status, reproductive history, use of hormonal medication and tobacco consumption was distributed to the study participants.

Physical activity was assessed on the basis of a pre-set daily log completed by women every day during their menstrual cycle. It requested data about hours of sleep, wake-up time and time spent during the day on physical activities in five categories. A detailed description of the methods was published elsewhere (Jasienska et al., 2006).

**Anthropometric measurements and energy balance adjustment**

Anthropometric measurements were taken from each woman twice, randomly with respect to phase of the menstrual cycle. The maximum time between the first measurement and the beginning of the menstrual cycle and between the second measurement and the end of menstrual cycle was <65 days.

Measurements of height, weight, body fat percentage, breast, under-breast, waist and hip circumferences were taken by a trained anthropologist. Body fat percentage was measured by bioimpedance using a TANITA scale (model TBF 551 with measurement accuracy of 0.1%). Body mass was measured using the same TANITA scale, with a measurement accuracy of 0.1 kg. BMI was calculated as the ratio of height (m) to body mass (kg) squared. Waist and hip circumference measurements were used to calculate waist-to-hip ratio (WHR). Breast and under-breast circumference measurements were used to calculate breast-to-under-breast ratio (BUR).

Energy balance was determined based on changes in the percentage of body fat between the first and the second measurement. A woman was classified as having positive energy balance when the difference in body fat percentage between the first and second measurement was equal to or greater than −1%. When this difference was less than −1%, a woman was classified as having negative energy balance.

**Salivary estradiol assay procedure and estradiol indices**

Packages containing plastic vials and laboratory-tested chewing gum were distributed to women before the beginning of their menstrual cycle. During one complete menstrual cycle, every day in the morning after waking-up, women collected saliva samples in plastic tubes pretreated with sodium azide following published protocols (Lipson and Ellison, 1989). Saliva samples from 20 days (−5 to −24 reverse cycle days) were analyzed for E2 concentrations using an I-125-based RIA kit (#39100, Diagnostic Systems Laboratories, Webster, Texas, USA) with published modifications to the manufacturer’s protocol (Jasienska et al., 2004). Average intra-assay variability was 9% and inter-assay variability varied from 23% for lower (15 pmol/l) to 13% for higher (50 pmol/l) values. The assay sensitivity was 4 pmol/l. Before statistical analysis, cycles were aligned on the basis of identification of the mid-cycle drop day (Day 0), which provides a reasonable estimate of the day of ovulation (Lipson and Ellison, 1996). Values of E2 concentration from 18 consecutive days were used in the analysis, and the following estradiol indices were calculated: ‘mean E2’ (mean of days from −9 to 8), ‘mid-cycle E2’ (mean of days from −2 to 2), ‘Day −1 E2’ (Day −1 E2 value, day before the mid-cycle drop day), ‘Day 0 E2’ (Day 0 E2 value, mid-cycle drop day), ‘mean follicular E2’ (mean of days from −9 to −1) and ‘mean luteal E2’ (mean of days from 0 to 8). Out of 141 women, complete data about 17-β-estradiol indices were available for 130, and data about these women were used in hormonal analysis.

**Statistical analysis**

Women were divided into four groups based on the quartiles of the distribution of body fat percentage. The established designation of body fat quartiles was maintained in all analyses to allow comparisons between groups (all women, positive energy balance women, negative energy balance women); thus sample sizes varied in different analyses. Differences among very low, low, average and high body fat groups in basic characteristics (i.e. age, size at birth, age at menarche, menstrual cycle length, body anthropometrics, body composition, physical...
activity and number of cigarettes smoked per day) were tested by one-way factorial analysis of variance (ANOVA) followed by Duncan’s tests. Separate comparisons of basic characteristics were also made for body fat quartiles of women with negative and positive energy balance. The same procedure was applied to test the significance of the differences in E2 indices among body fat quartiles.

Additionally, simple regression models were used to test the effect of body fat on E2 levels, with each E2 index as a dependent variable and body fat as the independent predictor. Separate analyses were conducted for women with positive and negative energy balance.

Repeated measures ANOVA was used to test the differences in E2 profiles among the body fat groups in positive energy balance women. Separate models for values of E2 levels during −9 to 8 days (whole cycle), −9 to −1 days (follicular phase) and 0 to 8 (luteal phase) as dependent variables and body fat group as the independent variable were tested. Contrast analyses were used to test the statistical significance of differences among the four body fat groups.

Results

Body fat and estradiol

General characteristics

General descriptive statistics for all women categorized with respect to their body fat are presented in Table I. Women characterized by very low, low, average and high body fat did not differ significantly with respect to age, birthweight and birth height, age of first menstruation, number of cigarettes smoked per day and physical activity. They differed significantly with respect to usual length of menstrual cycle ($F_{3,134} = 2.73$, $P < 0.05$) and anthropometric traits. Women with average body fat had significantly shorter self-reported length of menstrual cycle compared with women with very low body fat (28.1 versus 30.2 days, respectively). As expected, women in the high body fat group had significantly higher body mass, BMI and higher WHR than women in the other groups (all between group comparisons were statistically significant at $P < 0.05$), but they did not differ significantly with respect to their BUR.

Table I. General characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>All women</th>
<th>Very low body fat ≤22%, n = 36</th>
<th>Low body fat ≤26.5%, n = 35</th>
<th>Average body fat ≤30.8%, n = 35</th>
<th>High body fat &gt;30.8%, n = 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.8 (3.34)</td>
<td>29.6 (3.07)</td>
<td>29.7 (3.75)</td>
<td>29.4 (3.25)</td>
<td>30.6 (3.25)</td>
</tr>
<tr>
<td>Age of menarche (years)</td>
<td>13.4 (1.31)</td>
<td>13.6 (1.37)</td>
<td>13.2 (1.26)</td>
<td>13.3 (1.35)</td>
<td>13.6 (1.28)</td>
</tr>
<tr>
<td>Usual cycle length (days)*</td>
<td>29.2 (3.13)</td>
<td>30.2 (3.82)</td>
<td>29.6 (3.52)</td>
<td>28.1 (2.16)</td>
<td>29.1 (2.46)</td>
</tr>
<tr>
<td>Birthweight (kg)</td>
<td>3.27 (0.611)</td>
<td>3.34 (0.626)</td>
<td>3.22 (0.502)</td>
<td>3.24 (0.500)</td>
<td>3.28 (0.819)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>53.2 (4.38)</td>
<td>53.1 (3.74)</td>
<td>53.7 (2.90)</td>
<td>52.6 (4.37)</td>
<td>53.4 (6.37)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.1 (6.51)</td>
<td>161.7 (6.12)</td>
<td>162.1 (6.30)</td>
<td>164.2 (7.11)</td>
<td>164.3 (6.26)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.1 (8.37)</td>
<td>51.4 (3.67)</td>
<td>56.3 (3.10)</td>
<td>62.5 (4.40)</td>
<td>70.3 (6.15)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6 (2.84)</td>
<td>19.7 (1.25)</td>
<td>21.4 (1.47)</td>
<td>23.2 (1.65)</td>
<td>26.0 (1.92)</td>
</tr>
<tr>
<td>Body fat %</td>
<td>26.4 (6.52)</td>
<td>17.9 (3.19)</td>
<td>24.4 (1.19)</td>
<td>28.8 (1.22)</td>
<td>34.6 (2.31)</td>
</tr>
<tr>
<td>BUR</td>
<td>1.16 (0.037)</td>
<td>1.15 (0.037)</td>
<td>1.15 (0.036)</td>
<td>1.16 (0.036)</td>
<td>1.17 (0.037)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.72 (0.043)</td>
<td>0.70 (0.029)</td>
<td>0.71 (0.037)</td>
<td>0.72 (0.041)</td>
<td>0.75 (0.051)</td>
</tr>
<tr>
<td>Physical activity</td>
<td>5.8 (2.95)</td>
<td>4.7 (1.47)</td>
<td>6.1 (3.09)</td>
<td>5.9 (2.95)</td>
<td>6.4 (3.68)</td>
</tr>
<tr>
<td>(MET*hour/day)</td>
<td>1.8 (4.44)</td>
<td>1.1 (3.65)</td>
<td>1.9 (4.30)</td>
<td>2.1 (5.20)</td>
<td>2.0 (4.59)</td>
</tr>
</tbody>
</table>

Mean and standard deviation (in parentheses) for four body fat groups compared by one-way ANOVA tests.

*ac, $P < 0.05$.

**All between group comparison significant at $P < 0.05$.

Estradiol levels in body fat groups

Mean levels of 17ß-estradiol in the four body fat groups are presented in Table II. Compared with women characterized by average body fat, women with very low and high body fat percentage had significantly lower levels of estradiol during the follicular phase (21.1 versus 17.0 and 15.9 pmol/l, $F_{3,124} = 3.22$, $P = 0.025$), mid-cycle (25.4 pmol/l versus 19.7 and 18.2 pmol/l, $F_{3,126} = 4.03$, $P = 0.009$) and on Day −1 (38.8 pmol/l versus 29.1 and 26.6 pmol/l, $F_{3,123} = 4.60$, $P = 0.004$) and Day 0 (20.4 pmol/l versus 15.2 and 13.4 pmol/l, $F_{3,122} = 3.35$, $P = 0.021$). Similar differences were also observed in comparisons between women with low body fat and women with very low and high body fat.

Additional evidence for the relationship between nutritional status and estradiol levels came from simple regression analysis of all non-overweight women (body fat <31%). In these women, we observed a linear, positive association between body fat and mean E2 ($R^2 = 0.085$, $P = 0.003$), follicular E2 ($R^2 = 0.075$, $P = 0.007$), mid-cycle E2 ($R^2 = 0.094$, $P = 0.002$) and luteal E2 ($R^2 = 0.076$, $P = 0.006$). Statistically significant relationships were also observed for Day −1 E2 ($R^2 = 0.097$, $P = 0.002$) and Day 0 E2 ($R^2 = 0.085$, $P = 0.004$). A 10% increase in percentage of body fat in the range of 9.1% to 30.8% was associated with a 7 pmol/l increase in mid-cycle E2 and a 5 pmol/l increase in follicular phase E2, luteal phase E2 and mean E2 during the menstrual cycle.

Body fat, energy balance and estradiol levels

General characteristics

To test the effect of energy balance on the relationship between body fat and estradiol, separate analyses were conducted for 131 women characterized by positive or negative energy balance. Women with negative energy balance had significantly longer usual cycle length (30.0 versus 28.8 days, respectively, $F_{1,124} = 4.67$, $P < 0.05$) and lower body weight at birth (3.38 versus 3.07 kg, $F_{1,105} = 6.48$, $P < 0.05$) than...
women with positive energy balance. However, they did not differ significantly in any of the anthropometric or lifestyle parameters.

In contrast, significant differences in anthropometries were observed within both negative and positive energy balance groups among groups of women characterized by very low, low, average and high body fat content. As expected, higher weight (\(F_{3,70} = 66.66, P < 0.01\)), body fat (\(F_{3,70} = 232.85, P < 0.001\)), BMI (\(F_{3,70} = 56.52, P < 0.001\)) and WHR (\(F_{3,70} = 9.71, P < 0.001\)) were observed in women with higher body fat compared with women with lower body fat (all between group comparisons significant at \(P < 0.05\)) in the positive energy balance group. In the negative energy balance group, the same trend was observed for weight (\(F_{3,52} = 36.27, P < 0.001\)), BMI (\(F_{3,52} = 9.40, P < 0.001\)) and body fat (\(F_{3,52} = 129.45, P < 0.001\)). Additionally, in the positive energy balance group, women with very low and high body fat had significantly higher birthweight compared with women with low and average body fat (3.58 kg for very low body fat group and 3.57 kg for high body fat group versus 3.18 kg for low body fat group and 3.21 kg for average body fat group, \(F_{3,59} = 3.35, P < 0.05\), between group comparison significant at \(P < 0.05\)).

**Estradiol levels in body fat and energy balance groups**

Average values of E2 indices for the body fat quartiles of women with positive and negative energy balance are presented in Table III. ANOVA analyses of estradiol levels in these groups revealed significant differences in all estradiol indices between women differing with respect to body fat percentage in the positive energy balance group, but not in the negative, energy balance group. Significantly lower levels of mean E2 (\(F_{3,66} = 3.51, P < 0.05\)), mid-cycle E2 (\(F_{3,66} = 4.04, P < 0.05\)), Day –1 E2 (\(F_{3,64} = 3.55, P < 0.05\)) and follicular phase E2 (\(F_{3,64} = 3.23, P < 0.05\)) were observed in women with very low and high body fat compared with women with low and average body fat (between group comparison significant at \(P < 0.05\)). Additionally, levels of Day

### Table II. Estradiol indices in four body fat groups.

<table>
<thead>
<tr>
<th>E2 (pmol/l)</th>
<th>All women n = 130</th>
<th>Very low body fat n = 34</th>
<th>Low body fat n = 34</th>
<th>Average body fat n = 31</th>
<th>High body fat n = 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean E2</td>
<td>18.5 (8.99)</td>
<td>17.0 (8.45)</td>
<td>20.3 (9.70)</td>
<td>20.7 (9.32)</td>
<td>16.1 (7.87)</td>
</tr>
<tr>
<td>Mid-cycle E2*</td>
<td>22.1 (11.43)</td>
<td>19.7 (10.54)*</td>
<td>25.4 (11.77)</td>
<td>25.2 (12.51)*</td>
<td>18.2 (9.20)*</td>
</tr>
<tr>
<td>Day –1 E2*</td>
<td>33.3 (18.27)</td>
<td>29.1 (15.38)*</td>
<td>39.2 (17.75)</td>
<td>38.8 (22.26)*</td>
<td>26.6 (14.22)*</td>
</tr>
<tr>
<td>Day 0 E2*</td>
<td>17.1 (11.36)</td>
<td>15.2 (10.33)*</td>
<td>19.4 (11.74)*</td>
<td>20.4 (13.93)*</td>
<td>13.4 (8.70)*</td>
</tr>
<tr>
<td>Mean follicular E2*</td>
<td>18.7 (8.97)</td>
<td>17.0 (8.08)*</td>
<td>20.9 (10.27)*</td>
<td>21.1 (9.71)*</td>
<td>15.9 (6.56)*</td>
</tr>
<tr>
<td>Mean luteal E2</td>
<td>18.3 (9.84)</td>
<td>17.1 (9.60)</td>
<td>19.6 (10.01)</td>
<td>20.2 (9.39)</td>
<td>16.3 (10.26)</td>
</tr>
</tbody>
</table>

Mean and standard deviation (in parentheses) for four body fat groups compared by one-way ANOVA tests.

*ab, \(P < 0.05\); ac, \(P < 0.05\); bd, \(P < 0.05\); cd, \(P < 0.05\).

### Table III. Estradiol indices in body fat quartiles of women with positive and negative energy balance.

<table>
<thead>
<tr>
<th>E2 (pmol/l)</th>
<th>Very low fat n = 32</th>
<th>Low fat n = 33</th>
<th>Average fat n = 30</th>
<th>High fat n = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean E2</td>
<td>16.8 (5.88)</td>
<td>20.7 (9.61)</td>
<td>19.7 (8.96)</td>
<td>15.7 (7.86)</td>
</tr>
<tr>
<td>Positive energy balance*</td>
<td>14.0 (7.52)*</td>
<td>20.2 (10.86)b</td>
<td>20.7 (9.80)c</td>
<td>13.4 (4.43)d</td>
</tr>
<tr>
<td>Negative energy balance</td>
<td>20.8 (9.06)</td>
<td>21.3 (7.81)</td>
<td>18.0 (7.52)</td>
<td>18.2 (9.96)</td>
</tr>
<tr>
<td>Mid-cycle E2</td>
<td>19.5 (10.83)</td>
<td>26.1 (11.45)</td>
<td>24.2 (12.12)</td>
<td>17.7 (8.74)</td>
</tr>
<tr>
<td>Positive energy balance*</td>
<td>16.0 (10.32)*</td>
<td>24.4 (12.48)b</td>
<td>24.7 (13.30)c</td>
<td>14.4 (6.37)d</td>
</tr>
<tr>
<td>Negative energy balance</td>
<td>24.3 (10.34)</td>
<td>28.7 (9.64)</td>
<td>23.4 (10.45)</td>
<td>21.2 (9.76)</td>
</tr>
<tr>
<td>Day –1 E2</td>
<td>29.4 (15.78)</td>
<td>39.8 (17.67)</td>
<td>37.1 (21.41)</td>
<td>25.8 (19.23)</td>
</tr>
<tr>
<td>Positive energy balance*</td>
<td>24.9 (16.00)*</td>
<td>39.1 (19.02)b</td>
<td>36.0 (21.87)c</td>
<td>22.1 (14.59)d</td>
</tr>
<tr>
<td>Negative energy balance</td>
<td>34.4 (13.49)</td>
<td>40.8 (16.21)</td>
<td>38.8 (21.65)</td>
<td>29.7 (9.93)</td>
</tr>
<tr>
<td>Day 0 E2</td>
<td>14.6 (10.24)</td>
<td>20.0 (11.52)</td>
<td>19.6 (12.90)</td>
<td>13.1 (8.61)</td>
</tr>
<tr>
<td>Positive energy balance*</td>
<td>11.4 (8.10)*</td>
<td>15.8 (11.25)c</td>
<td>20.8 (14.72)d</td>
<td>9.9 (4.48)h</td>
</tr>
<tr>
<td>Negative energy balance</td>
<td>18.7 (12.03)</td>
<td>26.5 (8.89)</td>
<td>17.7 (9.81)</td>
<td>16.7 (10.80)</td>
</tr>
<tr>
<td>Mean follicular E2</td>
<td>17.2 (8.33)</td>
<td>21.1 (10.30)</td>
<td>20.3 (9.33)</td>
<td>15.6 (6.53)</td>
</tr>
<tr>
<td>Positive energy balance*</td>
<td>14.6 (7.47)*</td>
<td>21.1 (11.63)b</td>
<td>20.9 (9.93)c</td>
<td>13.8 (4.96)d</td>
</tr>
<tr>
<td>Mean luteal E2</td>
<td>16.4 (9.16)</td>
<td>20.2 (9.79)</td>
<td>19.0 (9.17)</td>
<td>15.8 (10.34)</td>
</tr>
<tr>
<td>Positive energy balance*</td>
<td>13.4 (7.91)*</td>
<td>19.2 (11.13)c</td>
<td>20.4 (10.36)</td>
<td>13.0 (5.32)h</td>
</tr>
<tr>
<td>Negative energy balance</td>
<td>20.6 (9.78)</td>
<td>21.6 (7.59)</td>
<td>16.7 (6.64)</td>
<td>18.9 (13.44)</td>
</tr>
</tbody>
</table>

Mean and standard deviation (in parentheses) for four body fat groups compared by one-way ANOVA tests.

*ab, \(P < 0.05\); ac, \(P < 0.05\); bd, \(P < 0.05\); cd, \(P < 0.05\).

**eg, \(P < 0.05\); gh, \(P < 0.05\).
Body fat, energy balance and estradiol levels in women

**Table IV.** The association between estradiol indices and body fat percentage in women with positive and negative energy balance and body fat below 31%.

<table>
<thead>
<tr>
<th>Positive energy balance</th>
<th>Negative energy balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Mean E2</td>
<td>55</td>
</tr>
<tr>
<td>Follicular E2</td>
<td>53</td>
</tr>
<tr>
<td>Mid-cycle E2</td>
<td>55</td>
</tr>
<tr>
<td>Day -1 E2</td>
<td>53</td>
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<tr>
<td>Day 0 E2</td>
<td>53</td>
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<tr>
<td>Luteal E2</td>
<td>55</td>
</tr>
</tbody>
</table>

Results of simple regression analysis.

Figure 1: The association between E2 and body fat. E2 profiles in very low, low, average and high body fat groups of women with positive energy balance.

In the range of 9.1% to 30.8% body fat, a 10% increase in body fat was associated, on average, with a 7 pmol/l increase in mean E2, luteal E2 and follicular E2. Also, a 10% increase in body fat was associated, on average, with a 9 pmol/l increase in E2 on Day 0, a 10 pmol/l increase in mid-cycle E2 and an almost 15 pmol/l increase in E2 on Day −1.

**Discussion**

We found that nutritional status indicated by body fat percentage is an important factor associated with the levels of estradiol during the menstrual cycle. In particular, we found a non-linear U-shaped association between body fat percentage and levels of estradiol during the menstrual cycle. Women with very low (below 22%) and high body fat (above 31%) had 25–35% lower levels of estradiol than women with low or average body fat. Furthermore, when the analysis was restricted to women characterized by body fat percentage <31%, we found a positive linear relationship between body fat and estradiol levels, such that a 10% increase in body fat was associated with a 5–7 pmol/l increase in estradiol levels.

We also demonstrated that energy balance confounded the association between estradiol levels and body fat in such a way that in women with positive energy balance, this association was even stronger than it was in the whole sample of women, whereas in women with negative energy balance, no relationship between body fat and estradiol levels was found.

Our findings correspond with the results of other studies concerned with levels of E2 in overweight and obese women. A decreased E2 surge was noted in studies by Grenman et al. (1986), Kopelman et al. (1986), Wabitsch et al. (2006) and recently by Tworoger et al. (2006). Several authors have demonstrated that, in women, increased adiposity and obesity are related to high androgenic activity (Evans et al., 1983; Hauner et al., 1988; Wabitsch et al., 1995; Norman and Clark, 1998). This may explain the inversion in the pattern of association between body fat percentage and estradiol levels observed in our study.

In accordance with our results, van der Steeg et al. (2007) demonstrated declining probability of spontaneous pregnancy for low and high BMI subfertile, ovulatory women. In women of BMI <21, a kg/m2 decrease in BMI was associated with 3% lower pregnancy rate, whereas in women of BMI >29, a kg/m2 increase in BMI was associated with 4% lower pregnancy rate. Gesink Law et al. (2006) also
demonstrated significantly reduced fecundity in overweight and obese women of reproductive age, whereas Wang et al. (2000) showed that in underweight (BMI < 20) and overweight (BMI > 25) women, the probability of pregnancy during assisted reproduction treatment was ~20% lower compared with women with BMI in the normal range.

Levels of estradiol during the menstrual cycle, and especially during its follicular phase, are related to follicular diameter, oocyte quality and endometrial morphology and thickness (Ohno and Fujimoto, 1998; Cahill et al., 2000). Lower levels of E2 during the menstrual cycle and during ovulation frequently correlate with lower pregnancy rates, both in healthy, naturally conceiving women (Lipson and Ellison, 1996; Lu et al., 1999; Li et al., 2001; Venners et al., 2006) and in women undergoing in vitro fertilization procedures (Chen et al., 2003; Blazar et al., 2004).

Our results suggest that lower fecundity, conception and pregnancy rates in underweight, overweight and obese women can be mediated by an unfavorable estradiol environment. Furthermore, the negative linear association between pregnancy rate and BMI in low BMI women found in van der Steeg et al. study (2007) can be easily explained by the linear association between body fat and estradiol levels found in non-overweight participants of our study.

Results of our study clarify and extend previous evidence on the association between nutritional status and levels of reproductive steroids. Several studies conducted on different groups of premenopausal women (diabetic, obese, dieting, very lean and normal weight) demonstrated contradictory results (Howard et al., 1987; Brunning et al., 1992; Potischman et al., 1996; Westhoff et al., 1996; Thomas et al., 1997a,b; Zanker and Swaine, 1998; Barnett et al., 2001, 2002). This inconsistency partly results from methodological limitations, especially calculations of mean E2 levels based on no more than a few samples from each menstrual cycle. Owing to substantial intra-cycle variation in E2 levels, such sampling is vastly insufficient and can lead to errors in estimating mean E2 levels for individual women (Jasienska and Jasienksi, 2008). In most of these studies, estradiol levels were analyzed from a single blood sample, whereas Williams et al. (2002), relying on repeated measurements of reproductive steroids in 34 women, showed that at least eight samples taken from a single subject are necessary to detect ~80% of biological variation in estradiol levels during a particular menstrual cycle. In our study, the number of samples taken from a single subject considerably exceeded this requirement.

Another limitation of other studies is the use of BMI as the indicator of nutritional status. BMI does not represent sufficient information about nutritional status and accumulated body fat (Piers et al., 2000; Kyle et al., 2003; Ketel et al., 2007). Frequently, individuals classified as overweight on the basis of BMI are of normal adiposity, especially if they have high muscle mass (Hortobagyi et al., 1994; Nevill et al., 2005; Wit and Bush, 2005). Conversely, women classified as normal using BMI criteria frequently have increased adiposity (Frankenfield et al., 2001). In our study, this limitation was circumvented by direct measurement of body fat percentage. The concurrent lack of a relationship between estradiol and BMI and positive relationship between estradiol and percent body fat observed in our study provides further evidence that BMI may be a poor indicator of nutritional status.

In addition to making careful measurements of estradiol levels and nutritional status, we were able to estimate the energy balance of women based on changes in body fat.
Body fat, energy balance and estradiol levels in women
during the observational period. Although we are aware of the
fact that changes in the percentage of body fat are only the
proxy measures of energy balance, this estimation allowed us
to investigate the interactions between nutritional status,
energy balance and the estradiol levels across the menstrual
cycle, which to our knowledge have not previously been
reported. Energy balance has been shown to influence
ovarian steroid profiles in several studies (Ellison et al.,
1989; Lager and Ellison, 1990; Panter-Brick et al., 1993).
Our results indicate that negative energy balance caused by
increased physical activity and/or inadequate caloric intake
has a confounding effect on the association between nutritional
status and reproductive hormone levels. This fact can explain
the lack of a relationship between nutritional status and
levels of reproductive hormones demonstrated in populations
or groups of women characterized by high physical activity
(Lager and Ellison, 1990; Jasienska and Ellison, 1998, 2004),
women losing weight due to voluntarily caloric restriction
(Lager and Ellison, 1990), and women from hunter gatherer
and horticulturalist groups in Africa and Nepal (Ellison et al.,
1989; Panter-Brick et al., 1993) who experienced periods of
restricted caloric intake and high physical activity. In contrast,
an association between body fat and reproductive hormones
has frequently been demonstrated in women from western
populations who generally have higher energy intake and
lower physical activity; none of these studies, however, con-
trolled for energy balance (Brunning et al., 1992; Zanker and
Swaine, 1998; Barnett et al., 2001, 2002; Furberg et al., 2005).

The first limitation of our study is the imprecise estimation
of energy balance. Although evaluation of metabolic rate or
resting energy expenditure would have been a more accurate
method, Abbott et al. (1988) showed that fat balance highly
 correlates with energy balance both in men and in women.
Thus, we believe that a change in body fat percentage provides
a good proxy of energy balance in our study participants.

Another limitation of our study is that the interval between
the first anthropometric measurement and the beginning of the
menstrual cycle and between the end of the menstrual cycle and
the second anthropometric measurement varied significantly
between study participants. The minimal interval between
these two was <1 day and maximal interval was 65 days.
Although differences in these intervals could possibly confound
our findings, additional analyses conducted with exclusion
of participants with longer intervals between measurements did
not change the results of the analyses (data not shown).

Although our results point to the important association
between nutritional status and energy balance and estradiol
levels, our study did not investigate physiological mechanisms
that were behind the observed relationship. Several different
physiological mechanism have been proposed in other
studies, including those postulating the regulatory role of
leptin and insulin both indirectly influencing hypothalamic
secretion and directly influencing ovarian production of estra-
diol (Smith et al., 2002; Porte et al., 2005).

Our results support the hypothesis of a regulatory role of
nutritional status on potential fertility in reproductive age
women, but only in those with positive energy balance. In
women of reproductive age, energetic resources are partitioned
between maintenance of normal physiological processes and
reproduction. The increased energy requirements of reproduc-
tion can be partially supported by energetic reserves stored in
women’s bodies in the form of fat. This fat depot is built-up
under favorable environmental conditions when energy
intake is high, energy expenditure due to physical activity is
low and total energy balance is positive. The consequences
of inadequate nutritional status during pregnancy are highly
adverse both to the child and to the mother. Low body mass
of a woman prior to and during pregnancy is associated with
a high risk of preterm labor, intrauterine growth retardation,
low birthweight of the infant and maternal depletion syndrome
(Jelliffe and Maddocks, 1964; Winkvist et al., 1992; Ehrenberg
et al., 2003; Kramer, 2003). Short-term reproductive suppres-
sion in women with low energy reserves may function as an
evolutionary adaptation protecting against these risks and
improving the chances of successful reproduction in the future

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