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

A. Ziomkiewicz[1,2,6], P.T. Ellison[3], S.F. Lipson[3], I. Thune[4,5] and G. Jasienska[2]

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

[6]Correspondence 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 whole the 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 subsample 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, underbreast, 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 underbreast circumference measurements were used to calculate breast-to-underbreast 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.

Estradiol levels in body fat groups
Mean levels of 17-β-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

Table I. General characteristics of study participants.

	All women $n = 141$	Very low body fat $\leq 22\%$, $n = 36$	Low body fat $\leq 26.5\%$, $n = 35$	Average body fat $\leq 30.8\%$, $n = 35$	High body fat $> 30.8\%$ $n = 35$
Age (years)	29.8 (3.34)	29.6 (3.07)	29.7 (3.75)	29.4 (3.25)	30.6 (3.25)
Age of menarche (years)	13.4 (1.31)	13.6 (1.37)	13.2 (1.26)	13.3 (1.35)	13.6 (1.28)
Usual cycle length (days)*	29.2 (3.13)	30.2 (3.82)[a]	29.6 (3.52)[b]	28.1 (2.16)[c]	29.1 (2.46)[d]
Birthweight (kg)	3.27 (0.611)	3.34 (0.626)	3.22 (0.502)	3.24 (0.500)	3.28 (0.819)
Birth length (cm)	53.2 (4.38)	53.1 (3.74)	53.7 (2.90)	52.6 (4.37)	53.4 (6.37)
Height (cm)	163.1 (6.51)	161.7 (6.12)	162.1 (6.30)	164.2 (7.11)	164.3 (6.26)
Weight (kg)**	60.1 (8.37)	51.4 (3.67)	56.3 (3.10)	62.5 (4.40)	70.3 (6.15)
BMI (kg/m^2)**	22.6 (2.84)	19.7 (1.25)	21.4 (1.47)	23.2 (1.65)	26.0 (1.92)
Body fat%**	26.4 (6.52)	17.9 (3.19)	24.4 (1.19)	28.8 (1.22)	34.6 (2.31)
BUR	1.16 (0.037)	1.15 (0.037)	1.15 (0.036)	1.16 (0.036)	1.17 (0.037)
WHR**	0.72 (0.043)	0.70 (0.029)	0.71 (0.037)	0.72 (0.041)	0.75 (0.051)
Physical activity (MET*hour/day)	5.8 (2.95)	4.7 (1.47)	6.1 (3.09)	5.9 (2.95)	6.4 (3.68)
Number of cigarettes	1.8 (4.44)	1.1 (3.65)	1.9 (4.30)	2.1 (5.20)	2.0 (4.59)

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 $P < 0.05$.

Table II. Estradiol indices in four body fat groups.

E2 (pmol/l)	All women $n = 130$	Very low body fat $n = 34$	Low body fat $n = 34$	Average body fat $n = 31$	High body fat $n = 31$
Mean E2	18.5 (8.99)	17.0 (8.45)	20.3 (9.70)	20.7 (9.32)	16.1 (7.87)
Mid-cycle E2*	22.1 (11.43)	19.7 (10.54)[a]	25.4 (11.77)[b]	25.2 (12.51)[c]	18.2 (9.20)[d]
Day −1 E2*	33.3 (18.27)	29.1 (15.38)[a]	39.2 (17.75)[b]	38.8 (22.26)[c]	26.6 (14.22)[d]
Day 0 E2*	17.1 (11.36)	15.2 (10.33)[a]	19.4 (11.74)[b]	20.4 (13.16)[c]	13.4 (8.70)[d]
Mean follicular E2*	18.7 (8.97)	17.0 (8.08)[a]	20.9 (10.27)[b]	21.1 (9.71)[c]	15.9 (6.56)[d]
Mean luteal E2	18.3 (9.84)	17.1 (9.60)	19.6 (10.01)	20.2 (9.39)	16.3 (10.26)

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, $P < 0.05$.

women with positive energy balance. However, they did not differ significantly in any of the anthropometric or life style parameters.

In contrast, significant differences in anthropometrics 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 III. Estradiol indices in body fat quartiles of women with positive and negative energy balance.

E2 (pmol/l)	Very low fat $n = 32$	Low fat $n = 33$	Average fat $n = 30$	High fat $n = 30$
Mean E2				
All women	16.8 (8.58)	20.7 (9.61)	19.7 (8.96)	15.7 (7.86)
Positive energy balance*	14.0 (7.52)[a]	20.2 (10.86)[b]	20.7 (9.80)[c]	13.4 (4.43)[d]
Negative energy balance	20.8 (9.06)	21.3 (7.81)	18.0 (7.52)	18.2 (9.96)
Mid-cycle E2				
All women	19.5 (10.83)	26.1 (11.45)	24.2 (12.12)	17.7 (8.74)
Positive energy balance*	16.0 (10.32)[a]	24.4 (12.48)[b]	24.7 (13.30)[c]	14.4 (6.37)[d]
Negative energy balance	24.3 (10.34)	28.7 (9.64)	23.4 (10.45)	21.2 (9.76)
Day −1 E2				
All women	29.4 (15.78)	39.8 (17.67)	37.1 (21.41)	25.8 (19.23)
Positive energy balance*	24.9 (16.00)[a]	39.1 (19.02)[b]	36.0 (21.87)[c]	22.1 (14.59)[d]
Negative energy balance	34.4 (13.49)	40.8 (16.21)	38.8 (21.65)	29.7 (9.93)
Day 0 E2				
All women	14.6 (10.24)	20.0 (11.52)	19.6 (12.90)	13.1 (8.61)
Positive energy balance**	11.4 (8.10)[e]	15.8 (11.25)[f]	20.8 (14.72)[g]	9.9 (4.48)[h]
Negative energy balance	18.7 (12.03)	26.5 (8.89)	17.7 (9.81)	16.7 (10.80)
Mean follicular E2				
All women	17.2 (8.33)	21.1 (10.30)	20.3 (9.33)	15.6 (6.53)
Positive energy balance*	14.6 (7.47)[a]	21.1 (11.63)[b]	20.9 (9.93)[c]	13.8 (4.96)[d]
Negative energy balance	21.1 (8.68)	21.1 (8.44)	19.4 (8.78)	17.4 (7.62)
Mean luteal E2				
All women	16.4 (9.16)	20.2 (9.79)	19.0 (9.17)	15.8 (10.34)
Positive energy balance**	13.4 (7.91)[e]	19.2 (11.13)[f]	20.4 (10.36)	13.0 (5.32)[h]
Negative energy balance	20.6 (9.78)	21.6 (7.59)	16.7 (6.64)	18.9 (13.44)

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, $P < 0.05$.
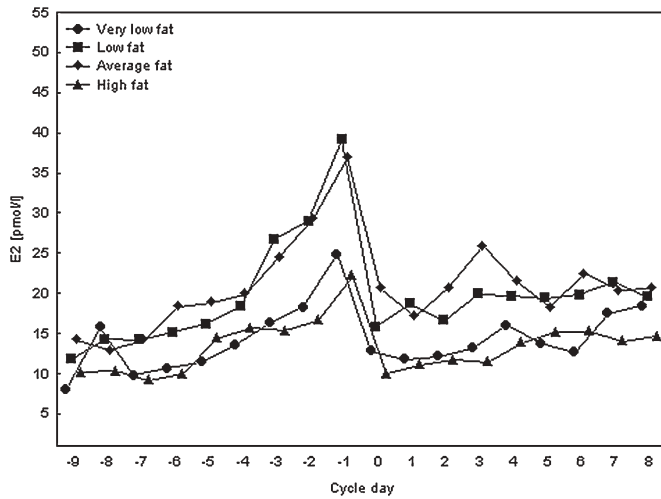**eg, $P < 0.05$; gh, $P < 0.05$.

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.

0 E2 ($F_{3,64} = 3.54$, $P < 0.05$) and luteal phase E2 ($F_{3,66} = 3.03$, $P < 0.05$) were significantly lower in women with very low and high body fat compared to women with average body fat (between group comparison significant at $P < 0.05$).

Repeated measures ANOVA revealed significant differences in estradiol profiles during the menstrual cycle between body fat groups ($F_{3,66} = 3.81$, $P < 0.05$) in women with positive energy balance. Women with very low and high body fat had significantly lower levels of E2 over the whole menstrual cycle compared with women with average and low fat (Fig. 1). Similar differences between body fat groups were also observed with respect to levels of E2 during the follicular phase ($F_{3,66} = 3.69$, $P < 0.05$) and levels of E2 during the luteal phase ($F_{3,66} = 3.17$, $P < 0.05$).

The relationship between body fat and E2 levels was further confirmed by regression analyses in non-overweight women with positive energy balance, but not in those with negative energy balance (Table IV). In women with positive energy balance and body fat <31%, we observed a linear association between body fat and mean E2 ($R^2 = 0.13$, $P < 0.01$), mid-cycle E2 ($R^2 = 0.15$, $P < 0.01$), follicular E2 ($R^2 = 0.11$, $P < 0.05$) and luteal E2 ($R^2 = 0.14$, $P < 0.01$) (Fig. 2). The same trend was also observed for estradiol on Day -1 ($R^2 = 0.14$, $P < 0.01$) and on Day 0 ($R^2 = 0.14$, $P < 0.01$).

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. (1980), Leenen et al. (1994) 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

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

	Positive energy balance			Negative energy balance		
	n	R^2	P	n	R^2	P
Mean E2	55	0.13	0.006	36	0.001	NS
Follicular E2	53	0.11	0.017	36	0.003	NS
Mid-cycle E2	55	0.15	0.003	36	0.010	NS
Day -1 E2	53	0.14	0.006	36	0.039	NS
Day 0 E2	53	0.14	0.005	35	0.010	NS
Luteal E2	55	0.14	0.005	36	0.002	NS

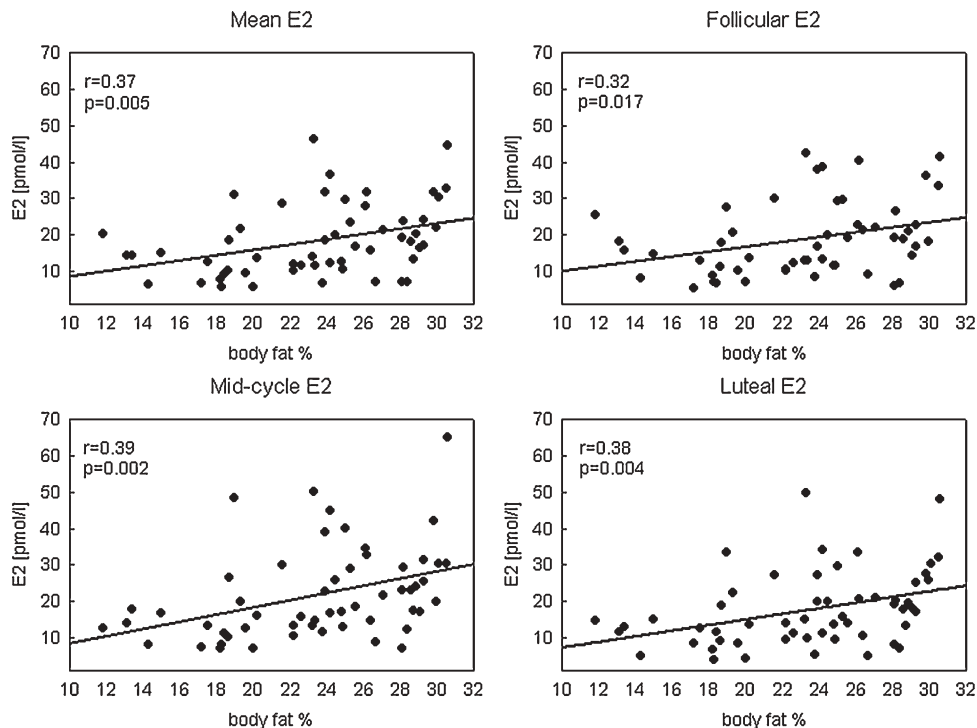Results of simple regression analysis.

Figure 2: The association between body fat percentage and E2 levels in women with positive energy balance and body fat below 31%.

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 Jasienski, 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

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, controlled 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 estradiol (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 reproduction 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 suppression 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 (Ellison, 1990, 2001; Jasienska, 2003).

References

Abbott WG, Howard BV, Christin L, Freymond D, Lillioja S, Boyce VL, Anderson TE, Bogardus C, Ravussin E. Short-term energy balance: relationship with protein, carbohydrate, and fat balances. *Am J Physiol* 1988;**255**:E332–E337.

Baird DD, Wilcox AJ, Weinberg CR, Kamel F, McConnaughey DR, Musey PI, Collins DC. Preimplantation hormonal differences between the conception and non-conception menstrual cycles of 32 normal women. *Hum Reprod* 1997;**12**:2607–2613.

Baird DD, Weinberg CR, Zhou H, Kamel F, McConnaughey DR, Kesner JS, Wilcox AJ. Preimplantation urinary hormone profiles and the probability of conception in healthy women. *Fertil Steril* 1999;**71**:40–49.

Barnett JB, Woods MN, Rosner B, McCormack C, Longcope C, Houser RF, Gorbach SL. Sex hormone levels in premenopausal African-American women with upper and lower body fat phenotypes. *Nutr Cancer* 2001;**41**:47–56.

Barnett JB, Woods MN, Rosner B, McCormack C, Floyd L, Longcope C, Gorbach SL. Waist-to-hip ratio, body mass index and sex hormone levels associated with breast cancer risk in premenopausal Caucasian women. *J Med Sci* 2002;**2**:170–176.

Blazar AS, Hogan JW, Frankfurter D, Hackett R, Keefe DL. Serum estradiol positively predicts outcomes in patients undergoing in vitro fertilization. *Fertil Steril* 2004;**81**:1707–1709.

Brunning PF, Bonfrer JMG, Hart AAM, van Noord PAH, van der Hoeven H, Collette HJA, Battermann JJ, de Jong-Bakker M, Nooijen WJ, de Waard F. Body measurements, estrogen availability and the risk of human breast cancer: a case-control study. *Int J Cancer* 1992;**51**:14–19.

Burrows M, Bird S. The physiology of the highly trained female endurance runner. *Sports Med* 2000;**30**:281–300.

Cahill DJ, Wardle PG, Harlow CR, Hunt LP, Hull MG. Expected contribution to serum oestradiol from individual ovarian follicles in unstimulated cycles. *Hum Reprod* 2000;**15**:1909–1912.

Chen EC, Brzyski RG. Exercise and reproductive dysfunction. *Fertil Steril* 1999;**71**:1–6.

Chen CH, Zhang X, Barnes R, Confino E, Milad M, Puscheck E, Kazer RR. Relationship between peak serum estradiol levels and treatment outcome in vitro fertilization cycles after embryo transfer on day 3 or day 5. *Fertil Steril* 2003;**80**:75–79.

De Souza MJ, Williams NI. Physiological aspects and clinical sequel of energy deficiency and hypoestrogenism in exercising women. *Hum Reprod Update* 2004;**10**:433–448.

Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, Albanes D, Campbell WS, Franz C, Kahle L *et al.* The relation of body size to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control* 1995;**6**:3–8.

Ehrenberg HM, Dierker L, Milluzzi C, Mercer BM. Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. *Am J Obstet Gynecol* 2003;**189**:1726–1730.

Elias AN, Wilson AF. Exercise and gonadal function. *Hum Reprod* 1993;**8**:1747–1761.

Ellison PT. Human ovarian function and reproductive ecology: new hypotheses. *Am Anthropol* 1990;**92**:933–952.

Ellison PT. *On Fertile Ground.* Cambridge: Harvard University Press, 2001.

Ellison PT. Energetics and Reproductive Effort. *Am J Hum Biol* 2003;**15**:342–351.

Ellison PT, Peacock NR, Lager C. Ecology and ovarian function among Lese women of the Ituri Forest, Zaire. *Am J Phys Anthropol* 1989;**78**:519–526.

Evans DJ, Hoffmann RG, Kalkhoff RK, Kissebah AH. Relationship of androgenic activity to body fat topography, fat cell morphology, and metabolic aberrations in premenopausal women. *J Clin Endocrinol Metab* 1983;**57**:304–310.

Frankenfield DC, Rowe WA, Cooney RN, Smith JS, Becker D. Limits of body mass index to detect obesity and predict body composition. *Nutrition* 2001;**17**:26–30.

Frisch RE. Body fat, puberty and fertility. *Biol Rev* 1984;**59**:161–188.

Furberg A, Jasienska G, Bjurstam N, Torjesen PA, Emaus A, Lipson SF, Ellison PT, Thune I. Metabolic and hormonal profiles: HDL cholesterol as a plausible biomarker of breast cancer risk. The Norwegian EBBA Study. *Cancer Epidemiol Biomarkers* 2005;**14**:33–40.

Gesink Law DC, Maclehose RF, Longnecker MP. Obesity and time to pregnancy. *Hum Reprod* 2006;**22**:414–420.

Grenman S, Rönnemaa T, Irjala K, Kaihola HL, Grönroos M. Sex steroid, gonadotropin, cortisol, and prolactin levels in healthy massively obese women: correlation with abdominal fat cell size and effect of weight reduction. *J Clin Endocrinol Metab* 1986;**63**:1257–1261.

Hauner H, Ditschuneit HH, Pal SB, Moncayo R, Pfeiffer EF. Fat distribution, endocrine and metabolic profile in obese women with and without hirsutism. *Metabolism* 1988;**37**:281–286.

Hortobagyi T, Israel RG, O'Brien KF. Sensitivity and specificity of the Quetelet index to assess obesity in men and women. *Eur J Clin Nutr* 1994;**48**:369–375.

Howard BV, Pan XR, Harper I, Kuusi T, Taskinen MR. Lack of sex differences in high density lipoproteins in Pima Indians. Studies of obesity, lipase activities, and steroid hormones. *Arteriosclerosis* 1987;**7**:292–300.

Ivandic A, Prpic-Krizevac I, Sucic M, Juric M. Hyperinsulinemia and sex hormones in healthy premenopausal women: relative contribution of obesity, obesity type, and duration of obesity. *Metabolism* 1998;**47**:13–19.

Jasienska G. Why energy expenditure causes reproductive suppression in women. an evolutionary and bioenergetic perspective. In: Ellison PT (ed). *Reproductive Ecology and Human Evolution.* New York, USA: Aldine de Gruyter, 2001,59–84.

Jasienska G. Energy metabolism and the evolution of reproductive suppression in the human female. *Acta Biotheor* 2003;**51**:1–18.

Jasienska G, Ellison PT. Physical work causes suppression of ovarian function in women. *P Roy Soc Lond B Bio* 1998;**265**:1847–1851.

Jasienska G, Ellison PT. Energetic factors and seasonal changes in ovarian function in women from rural Poland. *Am J Hum Biol* 2004;**16**:563–580.

Jasienska G, Jasienski M. Inter-population, inter-individual, inter-cycle, and intra-cycle natural variation in progesterone levels: a quantitative assessment and implications for population studies. *Am J Hum Biol* 2008;**20**:35–42.

Jasienska G, Ziomkiewicz A, Ellison PT, Lipson SF, Thune I. Large breasts and narrow waists indicate high reproductive potential in women. *Proc R Soc London B.* 2004;**271**:1213–1217.

Jasienska G, Ziomkiewicz A, Thune I, Lipson SF, Ellison PT. Habitual physical activity and estradiol levels in women of reproductive age. *Eur J Cancer Prev* 2006;**15**:439–445.

Jelliffe DB, Maddocks I. Notes on ecologic malnutrition in the New Guinea Highlands. *Clin Pediatr* 1964;**35**:432–438.

Ketel IJ, Volman MN, Seidel JC, Stehouwer CD, Twisk JW, Lambalk CB. Superiority of skinfold measurements and waist over waist-to-hip ratio for determination of body fat distribution in a population-based cohort of Caucasian Dutch adults. *Eur J Endocrinol* 2007;**156**:655–661.

Kirchengast S, Huber J. Body composition characteristics and fat distribution patterns in young infertile women. *Fertil Steril* 2004;**81**:539–544.

Kopelman PG, Pilkington TR, White N, Jeffcoate SL. Abnormal sex steroid secretion and binding in massively obese women. *Clin Endocrinol* 1980;**12**:363–369.

Kramer MS. The epidemiology of adverse pregnancy outcomes: an overview. *J Nutr* 2003;**133**:1952S–1956S.

Kyle U, Schutz Y, Dupertuis YM, Pichard C. Body composition interpretation: contributions of the fat-free mass index and the body fat mass index. *Nutrition* 2003;**19**:597–604.

Lager C, Ellison PT. Effect of moderate weight loss on ovarian function assessed by salivary progesterone measurements. *Am J Hum Biol* 1990;**2**:303–312.

Leenen R, van der Kooy K, Seidell JC, Deurenberg P, Koppeschaar HPF. Visceral fat accumulation in relation to sex hormones in obese men and women undergoing weight loss therapy. *J Clin Endocrinol Metab* 1994;**78**:1515–1520.

Li H, Nakajima ST, Chen J, Todd HE, Overstreet JW, Lasley BL. Differences in hormonal characteristics of conceptive versus nonconceptive menstrual cycles. *Fertil Steril* 2001;**75**:549–553.

Lipson SF, Ellison PT. Development of protocols for the application of salivary steroid analysis to field conditions. *Am J Hum Biol* 1989;**1**:249–255.

Lipson SF, Ellison PT. Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Hum Reprod* 1996;**11**:2090–2096.

Lu Y, Bentley GR, Gann PH, Hodges KR, Chatterton RT. Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol. *Fertil Steril* 1999;**71**:863–868.

Miller KK, Grinspoon S, Gleysteen S, Grieco KA, Ciampa J, Breu J, Herzog DB, Klibanski A. Preservation of neuroendocrine control of reproductive function despite severe undernutrition. *J Clin Endocrinol Metab* 2004;**89**:4434–4438.

Nevill AM, Stewart AD, Olds T, Holder R. Relationship between adiposity and body size reveals limitations of BMI. *Am J Phys Anthropol* 2005;**129**:151–156.

Norman RJ, Clark AM. Obesity and reproductive disorders: a review. *Reprod Fert Develop* 1998;**10**:55–63.

Ohno Y, Fujimoto Y. Endometrial oestrogen and progesterone receptors and their relationship to sonographic appearance of the endometrium. *Hum Reprod* 1998;**4**:560–564.

Panter-Brick C, Lotstein DS, Ellison PT. Seasonality of reproductive function and weight loss in rural Nepali women. *Hum Reprod* 1993;**8**:684–690.

Piers LS, Soares MJ, Frandsen SL, O'Dea K. Indirect estimates of body composition are useful for groups but unreliable in individuals. *Int J Obes Relat Metab Disord* 2000;**24**:1145–1152.

Porte D Jr, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system. a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes* 2005;**54**:1264–1276.

Potischman N, Swanson CA, Siiteri P, Hoover RN. Reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. *J Natl Cancer Inst* 1996;**88**:756–758.

Schneider JE. Energy balance and reproduction. *Physiol Behav* 2004;**81**:289–317.

Smith GD, Jackson LM, Foster DL. Leptin regulation of reproductive function and fertility. *Theriogenology* 2002;**57**:73–86.

Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, Wang DY. Re: Reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. *J Natl Cancer Inst* 1997a;**89**:396–397.

Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, Wang DY. A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on island of Guernsey. *Br J Cancer* 1997b;**76**:401–405.

Tworoger SS, Eliassenn AH, Missmer SA, Baer H, Rich-Edwards J, Michels KB, Barbieri RL, Dowsett M, Hankinson SE. Birth weight and body size through life in relation to sex hormones and prolactin concentrations in premenopausal women. *Cancer Epidemiol Biomarkers* 2006;**15**:2494–2501.

Ukkola O, Gagnon J, Rankinen T, Thompson PA, Hong Y, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Age, body mass index, race and other

determinants of steroid hormone variability: the HERITAGE Family Study. *Eur J Endocrinol* 2001;**145**:1–9.

van Binsbergen CJM, Coelingh Bennink HJT, Odink J, Haspels AA, Koppeschaar HPF. A comparative and longitudinal study on endocrine changes related to ovarian function in patients with anorexia nervosa. *J Clin Endocrinol Metab* 1990;**71**:705–711.

van der Steeg JW, Steures P, Eijkemans MJC, Habbema JDF, Hompes PGA, Burggraaff JM, Oosterhuis GJE, Bossuyt PMM, van der Veen F, Mol BWJ. Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. *Hum Reprod* 2007;**23**:324–328.

Venners SA, Liu X, Perry MJ, Korrick SA, Li ZP, Yang F, Yang JH, Lasley BL, Xu XP, Wang XB. Urinary estrogen and progesterone metabolite concentrations in menstrual cycles of fertile women with non-conception, early pregnancy loss or clinical pregnancy. *Hum Reprod* 2006;**21**: 2272–2280.

Wabitsch M, Hauner H, Heinze E, Böckmann Benz R, Mayer H, Teller W. Body fat distribution and steroid hormone concentrations in obese adolescent girls before and after weight reduction. *J Clin Endocrinol Metab* 1995;**80**:3469–3475.

Wade GN, Jones JE. Neuroendocrinology of nutritional infertility. *Am J Physiol* 2004;**287**:R1277–R1296.

Wade GN, Schneider JE, Li H-Y. Invited review: control of fertility by metabolic cues. *Am J Physiol* 1996;**270**:E1–E19.

Wang JX, Davies M, Norman RJ. Body mass and probability of pregnancy during assisted reproduction treatment: retrospective study. *BMJ* 2000;**321**:1320–1321.

Warren MP. Effects of undernutrition on reproductive function in the human. *Endocr Rev* 1983;**4**:363–377.

Warren MP, Perlroth NE. The effects of intense exercise on the female reproductive system. *J Endocrinol* 2001;**170**:3–11.

Westhoff C, Gentile G, Lee J, Zacur H, Helbig D. Predictors of ovarian steroid secretion in reproductive-age women. *Am J Epidemiol* 1996;**144**:381–388.

WHO Expert Committee on Physical Status. Physical status: the use and interpretation of anthropometry WHO technical report series. 1995;**854**:450–452.

Williams AE, Maskarinec G, Franke AA, Stanczyk FZ. The temporal reliability of serum estrogens, progesterone, gonadotropins, SHBG and urinary estrogen and progesterone metabolites in premenopausal women. *BMC Women's Health* 2002;**2**:13.

Winkvist A, Rasmussen KM, Habicht J-P. A new definition of the maternal depletion syndrome. *Am J Public Health* 1992;**82**:691–694.

Wit K, Bush EA. College athletes with an elevated body mass index often have a higher upper arm muscle area, but not elevated triceps and subscapular skinfolds. *J Am Diet Assoc* 2005;**105**:599–602.

Yoshimura Y, Wallach EE. Studies of the mechanism(s) of mammalian ovulation. *Fertil Steril* 1987;**47**:22–34.

Zanker CL, Swaine IL. The relationship between serum oestradiol concentration and energy balance in young women distance runners. *Int J Sports Med* 1998;**19**:104–108.