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Influence of birth weight and adult body composition on 17 β -estradiol levels in young women

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Background: Birth weight and adult body weight have independently been associated with breast cancer risk. Thus, we hypothesize that low birth weight, in combination with adult overweight, may influence premenopausal hormonal levels over an entire menstrual cycle.

Methods: Among 204 healthy women, aged 25–35 years, who participated in the Norwegian EBBA-I Study, birth weight and age at menarche, were assessed. Levels of 17β -estradiol were measured in daily saliva samples over one menstrual cycle using radioimmunoassay (RIA). Measurements of body composition; waist circumference (cm), body mass index (BMI, kg/m^2) and total fat percentage (DEXA, %) were assessed. Fasting blood samples were drawn, and serum concentrations of lipids and hormones determined.

Results: The participating women had mean birth weight of 3389 g and age at menarche 13.1 years. Women within the highest tertile of birth weight had the lowest 17β -estradiol throughout the menstrual cycle ($p=0.03$), and they tended to have a later age at menarche ($p=0.06$). When we looked into birth weight in combination with adult attained weight, we found that women with lower birth weights, combined with excess weight during adulthood, had higher levels of free 17β -estradiol over an entire menstrual cycle compared with women with high birth weights and adult overweight. Women with birth weights < 3530 g, who later developed excess body weight (waist ≥ 84 cm), showed 33% higher 17β -estradiol concentrations over a menstrual cycle compared with women with higher birth weights (≥ 3530 g) and adult excess body weight ($p = 0.03$). The association was even more pronounced in women with birth weights < 3220 g, early age at menarche (< 12 years) and adult overweight.

Conclusion: Our findings support variation of premenopausal levels of 17β -estradiol in response to birth weight and energy status in adult life, suggesting that women with low birth weight in combination with adult overweight are put at risk for higher estradiol levels throughout menstrual cycles, of possible importance for breast cancer risk.

Introduction

Several studies have observed a strong relationship between birth weight and later risk of chronic diseases, indicating that fetal conditions may influence later susceptibility to adult disease ('Forsdahl- Barker hypothesis') (1), (2), (3). Thus, women with low birth weight may have different set points for their physiological ovarian responsiveness to changes in energy balance later in life compared with women with higher birth weight.

Furthermore, the westernization of society including high levels of available energy combined with low overall energy expenditure, could provide new challenges for normal physiology and response, not only during the fetal period and early childhood, but also throughout life. It has been observed that birth size, a marker of fetal growth and intrauterine environment (4), together with later growth pattern and availability of energy, may influence later risk of chronic diseases, as for example diabetes or breast cancer (5), (6), (7), (8). However, the pattern observed is complex. Obesity during adolescence and early adult life has been observed to reduce premenopausal breast cancer risk, whereas later adult obesity, weight gain and unfavourable metabolic profiles have been observed to increase postmenopausal breast cancer risk (9).

In addition, indicators of affluent and excessive energy early in life – increased body weight during adolescence and a shift towards lower age at menarche (10), (11), (12), (13) – may interact with later energy availability and body composition, thereby affecting levels of endogenous hormones. Along with the downward shift in age at menarche, increases in both body weight and waist circumference have been observed (14), (9). Age at menarche tends to occur earlier, especially in those with decreasing birth weight who gain more weight during pre-pubertal years (5), (13), (15). Interestingly, we recently observed that women who experienced early age at menarche (≤ 12 years) and had a large waist to hip ratio, had 24% higher levels of estradiol over the menstrual cycle (16).

To our knowledge, little is known about the associations of variation in birth weight, adult attained body composition and premenopausal levels of 17β -estradiol over an entire menstrual cycle. In addition, the large variation in breast cancer incidence and levels of sex hormones across populations has been discussed and reflects both genetic variation (17), (18) and variation in availability of energy factors (19).

As steroid levels in saliva represent free steroid levels, rather than levels of both free and protein-bound circulating steroid, they allow for fine discrimination of functional differences in steroid signalling. Furthermore, as saliva can be readily collected from

individuals on many occasions, it is possible to compare steroid levels across entire menstrual cycles among different women, rather than relying on one or a few timed blood samples (20).

Thus, the aim of the present study was to test whether levels of free 17β -estradiol over an entire menstrual cycle were associated with birth weight in combination with attained adult body composition. Such an interactive effect could be important for susceptibility to breast cancer.

Materials and Methods

Participants and study design

Women aged 25–35 years, living in Tromsø and surroundings during 2000-2002, were invited to participate in the Norwegian EBBA-I study by announcements both in newspapers and locally. Study participants had to meet the following criteria: self-reported regular menstruation (normal cycle length of 22–38 days within the previous 3 months), not taking hormonal contraceptives, no pregnancy or lactation over the previous 6 months, and no history of endocrinological (e.g. diabetes, hypo-/hyperthyroidism), gynecological or chronic disorders. A total of 204 women who met the inclusion criteria were subsequently enrolled into the study and came to the Department of Clinical Research, University Hospital of North Norway (UNN), at a scheduled time (21).

Questionnaires

We used a general questionnaire (self-administered and by interview) to collect information on ethnicity, education, menstruation and reproductive history, previous use of hormonal contraceptives, family history of cancer, lifetime total physical activity, smoking [‘current smoker’ (yes/no), ‘how many cigarettes per day’] and alcohol [‘do you drink alcohol’ (yes/no), ‘units of alcohol’]. Trained personnel conducted interviews using recall and memory-probing aids, including a lifetime calendar. A pre-coded food diary with a photographic booklet on portion size was developed and used in order to collect 7 days of dietary data (days 3–6 and days 21–23 of the menstrual cycle, where day 1 represented the onset of menstrual bleeding) (22). Average daily intake of energy and nutrients were computed by using a food database and software system developed at the Institute for Nutrition Research, University of Oslo, Norway (23).

Birth size, age at menarche and body composition measurements

We collected data on birth size from personal health records. Age at menarche was assessed by both questionnaire and interview using the same trained nurse during the whole study period.

Study participants made three subsequent visits to the study laboratory over the course of one menstrual period: visit 1 (days 1–4), visit 2 (midcycle) and visit 3 (days 22–25). They came in on the first possible day after onset of menstrual bleeding for clinical examinations, anthropometric measurements and provision of a fasting blood sample. All clinical procedures were conducted by trained nurses at the Department of Clinical Research, UNN, Tromsø. Anthropometric measures were taken with participants wearing lightweight clothing and no footwear: height and waist circumference were measured to the nearest half centimeter and weight to the nearest 0.1 kg on an electronic scale. Body Mass Index (kg/m^2) was used to estimate relative weight. Waist circumference (cm) was measured in a horizontal line 2.5 cm above the umbilicus.

The participants underwent a whole body scan using Dual Energy X-ray Absorptiometry or DEXA (DPX-L 2288, Lunar Radiation Corporation, Madison, WI, USA) during midcycle (days 7-12). The same trained nurse carried out the scans, and the percentage of fat tissue was estimated using Lunar software.

Serum samples

Fasting serum blood samples were drawn from an antecubital vein in the morning at all three visits. Serum concentrations of glucose and estradiol were measured in fresh sera at the Department of Clinical Chemistry, UNN, Tromsø. Serum concentrations of insulin and leptin were measured at the Hormone Laboratory, Aker University Hospital, Oslo, in serum that was stored at -70°C for up to 3 years until analysis. Serum insulin and leptin were measured by RIA using kits from Linco Research Inc (St Charles, MO, USA) (21).

Estradiol indices and assay procedure

From the first day of bleeding and each day during the menstrual cycle, participants collected saliva samples at home, in the morning, according to collection protocols previously established at the Reproductive Ecology Laboratory at Harvard University, USA (24). Levels of 17β -estradiol were measured in daily saliva samples from 20 days (reverse cycle day -5 to -24) of the cycle using an ^{125}I -labelled RIA kit (#39100, Diagnostic Systems Laboratory, Webster, TX, USA), along with published modifications of the manufacturer's protocol (21).

All samples were run in duplicate. All of a participant's samples were run in the same batch, with women randomly assigned to batches. Coefficients of variation were calculated based on high and low value pools (appropriate to the range of each steroid) included in each assay (21).

The sensitivity of the estradiol assay (the lowest concentration of estradiol distinguishable from 0 at the 95% level) was 4 pmol/L. Average intra-assay variability (estimated from the 50% binding point of the standard curve) was 9%, and the interassay variability ranged from 23% for low values (15 pmol/L) to 13% for high values (50 pmol/L). Before statistical analysis, all cycles were aligned to the day of ovulation following published methods (21), based on the identification of the estradiol drop at the midcycle (day 0), which provides a reasonable estimate of the day of ovulation. The estradiol values for 20 consecutive days from each cycle, aligned on day 0, were used in data analyses (day -10 to +9). Satisfactory identification of the midcycle estradiol drop could not be made for 14 women and so their cycles were not aligned. However, in order to study the total variation in estradiol concentration throughout an entire menstrual cycle and as anovulatory cycles are associated with low estradiol exposure, all cycles; both anovulatory and ovulatory cycles, were included in this study.

Statistical analysis

The associations between birth weight, adult attained body composition and 17 β -estradiol levels throughout a menstrual cycle were studied using linear regression analysis and linear mixed models for repeated measures (SAS statistical package version 9.1).

To study in detail the association between birth weight and 17 β -estradiol levels, the study population was divided into birth weight tertiles: < 3220 g, \geq 3220 to 3530 g and \geq 3530 g. These groups of women with different birth weights were then compared with regard to selected characteristics. We used one-way analysis of variance for continuous variables and χ^2 tests for categorical variables to test for differences in means and frequencies of selected characteristics across tertiles of birth weight.

Linear regression models were used to study the associations of average salivary 17 β -estradiol concentration, birth weight, and measures of adult attained body composition, serum insulin and leptin. Potential confounding factors were tested and adjusted for when appropriate. Based on biological plausibility, covariates such as age, smoking, physical activity, age at menarche, energy intake, alcohol, previous use of hormonal contraceptives,

age at first birth and number of children were tested in the models. Age, smoking, physical activity and age at menarche were included in the final models due to proper model-data fit.

We evaluated possible interactions between measures of attained adult body composition and serum insulin and leptin and birth weight (tertiles) by including multiplicative interaction tests in the models.

To study whether variation in adult excess body weight and fat distribution modified the association between birth weight and salivary concentrations of 17β -estradiol, waist circumference were dichotomized at the 75th percentile (≥ 84 cm). We used linear mixed models for repeated measures to study variations in salivary 17β -estradiol concentrations over the entire menstrual cycle across different subgroups of women defined by birth weight (tertiles) and adult body composition (75th percentile); age was included in the models. As the multivariate analyses only gave minor changes of our age-adjusted estimates, both in relation to linear regression models and in relation to linear mixed models for repeated measures, only age adjusted results are presented in figures using mixed models for repeated measures. Different co-variance structures were explored, and the results are presented using heterogeneous Toeplitz. Dunnett's method was used for multiple comparisons.

Measurements of 17β -estradiol at the start and the finish of the cycles had higher coefficients of variation and higher rates of missing data as a result of variation in cycle length; therefore we included 17β -estradiol measurements from cycle day -10 to $+9$ in the linear mixed models. Results were considered statistically significant when the two-sided p value was < 0.05 .

Ethical considerations

All the participating women signed an informed consent form. The study protocol was reviewed and approved by the Regional Committee for Medical Research Ethics North-Norway and the Norwegian Data Inspectorate.

Results

The 204 participating women had mean age 30.7 years, mean birth weight 3389 g and self-reported mean age at menarche 13.1 years. Mean BMI was 24.4 kg/m^2 , mean waist circumference 79.5 cm and mean total fat percentage 34.1%. When dividing the women into tertiles of birth weight (< 3220 g, ≥ 3220 to 3530 g and ≥ 3530 g), women in the highest tertile of birth weight had the lowest overall average salivary and thus free 17β -estradiol

concentrations ($p = 0.03$), and tended to have a later age at menarche ($p = 0.06$) (Table 1). In addition, women with the lowest birth weight had significantly higher serum insulin levels ($p = 0.02$) compared with women with higher birth weights.

We also studied the average 17β -estradiol by cycle day over the entire menstrual cycle across birth weight tertiles. Women who reported the highest birth weight (≥ 3530 g) tended to have the lowest levels of daily free 17β -estradiol over the entire menstrual cycle (Figure 1a).

No clear pattern was observed between 17β -estradiol by cycle day over the entire menstrual cycle by tertiles of adult attained waist circumference (Figure 1b).

We then studied the changes in overall average salivary 17β -estradiol concentration by 1 standard deviation (SD) change in explanatory variables for each tertile of birth weight. Among women in the two lowest tertiles of birth weight (< 3530 g), we observed a pattern of positive association between measures of adult attained body composition and serum leptin and insulin levels and mean overall 17β -estradiol concentration in both age-adjusted models and models adjusted for potential confounders (smoking, physical activity and age at menarche). For each 3.8 kg/m^2 (1 SD) increase in BMI, the overall adjusted level of 17β -estradiol in the lowest birth weight tertile increased by 2.74 pmol/L (95% CI, 0.63, 4.85), and in the middle birth weight tertile by 2.84 pmol/L (95% CI, 0.56, 5.11), equivalent to a 15.3% change in mean average concentration of 17β -estradiol in the lowest birth weight tertile and a 15.9% change in the middle birth weight tertile, respectively. In contrast, among women in the highest tertile of birth weight (≥ 3530 g), the level of 17β -estradiol did not vary with changes in adult body composition and serum hormones (Table 2).

When we examined changes in waist circumference by tertiles of birth weight, we observed that for each 9.8 cm (1 SD) increase in waist circumference, the overall adjusted level of 17β -estradiol in the lowest birth weight tertile tended to increase. Among women in the middle birth weight tertile each 1 SD increase in waist circumference was associated with an increase of 3.05 pmol/L (95% CI, 0.53, 5.57) in overall average 17β -estradiol levels, which equals a 17.0% change in overall average 17β -estradiol concentration. The results were even more pronounced when we performed the analyses by birth weight in quartiles (data not shown).

To study in detail whether age at menarche influenced our result, we performed analysis stratified by age at menarche. Among women with low birth weight (< 3220 g), the relationship between measures of excess adult waist circumference and overall 17β -estradiol

concentrations was strongest in the subgroup of those having an age at menarche < 12 years. For each 9.8 cm (1 SD) increase in waist, overall average 17 β -estradiol concentration increased by 3.9 pmol/L, which equals a 21.8% change in mean overall 17 β -estradiol levels among women in the lowest birth weight tertile ($p = 0.03$; not presented in tables).

In our study we observed a significant relationship between birth weight and serum insulin levels. When we examined birth weight in relation to adult overweight, the results became even clearer. Women of low birth weight (< 3220 g) who later became overweight (as defined by a waist circumference ≥ 84 cm) had twice as high insulin levels (145.4 pmol/L) than women of low birth weight and adult waist circumference < 84 cm (77.7 pmol/l; $p = 0.0018$; data not shown in tables).

To study the differences in daily salivary 17 β -estradiol concentrations over an entire menstrual cycle between subgroups of women characterized by birth weight and adult attained waist, we used a linear mixed model for repeated measures (Figures 2a-c). We observed that birth weight combined with adult waist circumference showed a clear association with levels of premenopausal 17 β -estradiol throughout an entire menstrual cycle. Women with birth weights < 3530 g who later developed excess body weight (waist ≥ 84 cm) showed higher 17 β -estradiol concentrations over the menstrual cycle compared with women of higher birth weights (≥ 3530 g) and adult excess body weight ($p = 0.03$; Figure 2a). These results equal a change in mean overall concentration of 17 β -estradiol for the total group of 33 %. Comparable results were also observed among women with an early age at menarche compared to later age at menarche; among women of lower birth weight (< 3530 g) and adult excess waist, those with an early age at menarche (< 12 years) had higher levels of 17 β -estradiol over their menstrual cycle compared with those who had a later age at menarche ($p = 0.07$; Figure 2b). Women with lower birth weights (< 3530 g), early age at menarche (< 12 years) and adult overweight also tended to have higher insulin levels than those who were older at menarche (≥ 12 years), although the difference was not significant (data not presented). Among those in the lowest birth weight tertile (< 3220 g) who later had a larger waist circumference (≥ 84 cm, highest quartile), there was a trend for those with early age at menarche (< 12 years) to have even higher levels of 17 β -estradiol over a whole menstrual cycle compared with those with later age at menarche ($p = 0.09$; Figure 2c).

Discussion

In our study of young healthy women with regular menstrual cycles we found that low birth weight (< 3530 g) combined with large attained adult waist (≥ 84 cm) resulted in a 33% increase in free 17 β -estradiol levels over an entire menstrual cycle compared with women of higher birth weight and the same adult waist circumference. This association was even more pronounced among women of even lower birth weights, < 3220 g, combined with early age at menarche (< 12 years).

Little is known about the interrelationship of birth weight, adult attained body composition and 17 β -estradiol levels over an entire menstrual cycle. Therefore, one could ask whether these results can be explained by plausible biological mechanisms, observations from other studies or by bias and/or confounding. Interestingly, several studies have observed that perinatal factors, including birth weight, may influence later susceptibility of chronic diseases including breast cancer (25), (26), (5), (27), (28). Moreover, the association related to breast cancer has been complicated by contradictory results (29), (30) because some have observed a protective effect of low birth weight (25), whereas others have not (31). We have previously hypothesized that birth size may influence later responsiveness of ovarian function and have shown that, among adult women ovarian response to physical activity depends on their size at birth (32). Women of low birth weights may have a different set point for their physiological responsiveness than women with higher birth weights. Barker has proposed that fetal tissues respond to the intrauterine environment by permanently altering their structure and function (2). Such a mechanism may explain associations between low birth weight and increased risk of chronic diseases (33), including breast cancer risk.

Other studies have shown a clear interrelationship of low birth weight, later overweight and susceptibility of chronic diseases (34). One biological explanation of this could be that individuals programmed for poor early nutrient intake would be put at risk if their food intake was subsequently increased to a level inappropriate for their programming(35).

Lissner et al. (36) furthermore found that birth weight was inversely related to serum leptin levels in adulthood, and that leptin can stimulate mitogenic and angiogenic processes in peripheral organs. Data obtained in cell and animal models and analyses of human breast cancer biopsies suggest involvement in breast cancer etiology (37). In our study we observed that serum leptin levels tended to be lower among women in the highest tertile of birth weight, although this result was not significant (Table 1).

There is a growing recognition that breast cancer may be promoted by hyperinsulinemia and insulin resistance, which may favour a metabolic environment

promoting tumor growth (38). Additionally, insulin resistance and elevated serum steroid levels often coexist because of insulin up-regulation of ovarian steroid secretion (39). Low birth weight, thinness at 2 years of age and an increase in BMI after the age of 2 have been observed to be associated with development of insulin resistance in later life (40), (41), (42) underlining the importance of birth size as a risk factor for insulin resistance and other chronic diseases. Our study supports these findings by showing a significant relationship ($p = 0.02$) between tertiles of birth weight and serum insulin levels. The results became even clearer when we looked into birth weight in relation to being overweight as an adult. Women of low birth weight (< 3220 g) who later became overweight (with a waist circumference ≥ 84 cm) had significantly higher insulin levels, compared with those of low birth weight and adult waist circumference < 84 cm. It is suggested that metabolic markers of high breast cancer risk include higher serum concentrations of insulin and IGF1 and also excessive abdominal fat accumulation (43).

Mechanisms during gestation, such as increasing growth factor exposure and availability of energy influencing birth weight, may also influence later responsiveness to other factors. However, effect modification throughout childhood and adult life may interact and, among others, levels of 17β -estradiol over a menstrual cycle may vary.

Several biological mechanisms and observation from different studies support a relationship between birth weight, childhood and adult weight gain and 17β -estradiol. Some studies have observed that childhood obesity may accelerate age at menarche, especially in those of low birth weight (5),(13),(44),(45), and early age at menarche is a known risk factor for breast cancer development. In our study we observed that women of birth weight < 3530 g tended to have an earlier age at menarche than those in the highest birth weight tertile, ≥ 3530 g. A Swedish study suggested that girls who at birth were relatively small for gestational age had higher childhood growth velocities than other girls (46). These results indicate that there may be compensation for those with fetal growth restriction through higher growth rate after birth. It is also possible that such compensatory growth could be mediated by increased levels of adrenal steroids and hyperinsulinemia in girls who were relatively small at birth (47), (40). Our observation that women with low birth weight and adult obesity tend to have higher levels of both insulin and 17β -estradiol over a menstrual cycle supports these findings. On the other hand, higher birth weight together with low pre-pubertal body weight have been observed to result in later age at menarche and lower estradiol concentrations (48).

Ovarian steroids are thought to increase the rate of breast cancer growth mainly by stimulating the mitotic activity of breast epithelial cells both directly and through interaction with growth hormones (49). One implication of the accumulating evidence on the importance of ovulatory menstrual cycles is that any factor that modifies menstrual cycle patterns and reduces the frequency of ovulation may reduce a woman's lifetime risk of developing breast cancer (50). Consequently, reducing lifetime estrogen levels might be the most important step in lowering breast cancer in women.

The use of just one clinical research department at a university hospital with one specially trained nurse enhanced the quality of our data. It allowed us to sample all clinical variables within the same narrow frame of the cycle for each participant, using uniform procedures. To limit any potential influence of variation in daylight, women did not participate during those months with markedly less daylight (December and January).

The present population consists of only 204 women which limit the possibility to perform subgroup analyses. Moreover, it also underlines the necessity to interpret our results with cautiousness and the need for further studies among other ethnic groups. However, the population is very homogenous which may strengthen the interpretation in smaller groups. Taking daily saliva samples allowed estimation of daily estradiol concentrations over one menstrual cycle, enabling precise and reliable assessment of interindividual variations in hormonal levels. We used well-developed and validated methods and assays to characterize the women's exposure to free, biologically active, ovarian steroids and the comparison of levels by aligned cycle days (24). This study had the benefit of collecting samples every day over an entire menstrual cycle, rather than only on selected days within a cycle (51). In addition, salivary levels of estradiol were shown to be quite stable within participants over time (52).

Age at menarche was recalled retrospectively, and misclassification is therefore likely. Several studies, however, have found that age at menarche are recalled with high reliability (53). In multivariate and stratified analyses we adjusted for potential confounders as age, smoking, physical activity and age at menarche. They were included in the final model without any large changes in estimates.

Conclusion

Our main findings support that lower birth weight, combined with large waist circumference during adulthood, is associated with increased levels of 17β -estradiol over one menstrual cycle, especially among women with early age at menarche (< 12 years). These women also had higher levels of insulin. This supports the hypothesis that women of lower birth weight may have a different set point for their physiological responsiveness than women of higher birth weight; this may, in turn, influence levels of reproductive hormones such as 17β -estradiol. Our findings support the hypothesis that conditions during fetal, childhood and adult life may in combination influence later breast cancer risk.

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Table 1 Characteristics of the study population in tertiles of birth weight, means (SD)*: The Norwegian EBBA-Study ($N = 204^a$)

Study characteristics	Birth weight			<i>p</i> value ^b (trend)
	< 3220 g (<i>n</i> = 68)	≥ 3220–3530 g (<i>n</i> = 68)	≥ 3530 g (<i>n</i> = 68)	
Age (years)	30.8 (3.2)	30.8 (2.9)	30.6 (3.2)	0.71
Years of schooling	15.7 (3.2)	16.2 (2.8)	16.3 (3.1)	0.22
Ethnic minority, Sami (%)	10.3	7.4	5.9	0.62
Anthropometric measurements				
BMI (kg/m ²)	23.9 (3.6)	25.5 (4.1)	23.9 (3.4)	0.96
Waist circumference (cm)	78.2 (10.3)	81.4 (9.9)	79.0 (9.1)	0.60
Total fat (%)	33.8 (8.3)	36.1 (7.1)	32.5 (7.1)	0.35
Saliva hormone concentrations (pmol/L)				
Overall 17 β -estradiol	18.8 (8.3)	19.5 (10.2)	15.5 (7.3)	0.03
Serum hormone concentrations ^c				
Serum estradiol (nmol/L)	0.14 (0.1)	0.15 (0.1)	0.15 (0.1)	0.50
Serum leptin (pmol/L)	826.8 (561.7)	968.8 (571.7)	772.8 (544.9)	0.58
Serum glucose (mmol/L)	5.0 (0.6)	5.1 (0.5)	5.0 (0.6)	0.59
Serum insulin (pmol/L)	95.6 (80.8)	90.1 (53.0)	71.5 (30.9)	0.02
Menstrual and reproductive characteristics				
Menarche (years)	12.96 (1.3)	12.98 (1.3)	13.40 (1.5)	0.06
Age at first birth (years) ^d	23.0 (4.2)	25.7 (3.8)	24.6 (3.2)	0.10
Percentage women with children	45.6	50.0	48.5	0.73
Cycle length (days)	28.3 (3.0)	28.7 (3.3)	27.8 (3.1)	0.36
Previous use of hormonal contraceptives (%)				
	80.9	88.1	82.4	0.82
Energy intake (kJ/day)	7968 (1452)	7976 (2093)	8335 (2088)	0.26
Leisure time (MET h/week)	51.8 (36.8)	51.8 (33.6)	53.7 (38.2)	0.76
Alcohol units per week among users (<i>n</i> =190)	2.8 (3.4)	3.3 (3.6)	3.2 (3.2)	0.47
Current smokers (%)	20.6	14.7	30.9	0.15

*SD, standard deviation.

^a Numbers of participants may vary as a result of missing information for certain variables.

^b One-way analysis of variance or χ^2 test.

^c Blood sampling first visit (days 1–5).

^d For those who have children, *n* = 98, in each group: 31–34–33.

Table 2 Estimated changes* in mean salivary 17 β -estradiol concentrations (pmol/L) with 95% CI by 1 standard deviation (SD) increase in explanatory variable by tertiles of birth weight ($n = 204$)^a

Variable	Mean	(SD)	Change in 17 β -estradiol levels (pmol/L)					
			Birth weight					
			< 3220 g ($n = 68$)		≥ 3220 g , < 3530 g ($n = 68$)		≥ 3530 g ($n = 68$)	
		Age adjusted	Adjusted ^b	Age adjusted	Adjusted ^b	Age adjusted	Adjusted ^b	
BMI (kg/m ²)	24.4	(3.8)	2.33 (0.23, 4.42)	2.74 (0.63, 4.85)	2.97 (0.82, 5.12)	2.84 (0.56, 5.11)	0.11 (-1.88, 2.09)	0.17 (-1.72, 2.07)
Waist (cm)	79.5	(9.8)	1.17 (-0.79, 3.13)	1.67 (-0.31, 3.66)	3.20 (0.85, 5.55)	3.05 (0.53, 5.57)	-0.16 (-2.08, 1.76)	-0.28 (-2.12, 1.55)
Total fat (%)	34.1	(7.6)	1.02 (-0.85, 2.90)	1.69 (-0.26, 3.64)	2.41 (-0.22, 5.03)	2.25 (-0.50, 5.01)	0.24 (-1.68, 2.15)	-0.09 (-2.01, 1.83)
Leptin (pmol/L)	856.1	(562.9)	1.93 (-0.08, 3.94)	2.93 (0.87, 4.98)	1.43 (-1.00, 3.85)	1.24 (-1.27, 3.75)	-0.26 (-2.1, 1.57)	-0.12 (-1.88, 1.64)
Insulin (pmol/L)	85.7	(59.2)	0.07 (-1.44, 1.57)	0.15 (-1.42, 1.73)	3.60 (0.96, 6.24)	3.75 (1.00, 6.50)	-0.56 (-3.95, 2.83)	-0.22 (-3.54, 3.09)

*Linear regression analyses. Regression coefficient and 95% confidence interval (CI).

^aNumber may vary as a result of missing serum values.

^bAdjusted for age, leisure time physical activity, number of cigarettes and age at menarche.

95% CI = 95% confidence interval; SD, standard deviation.

Figure 1

a) Average 17β -estradiol by cycle day over the entire menstrual cycle across tertiles of birth weight (BW), ($< 3220\text{g}$, $\geq 3220\text{g}$ and $< 3530\text{g}$, $\geq 3530\text{g}$).

b) Average 17β -estradiol by cycle day over the entire menstrual cycle by tertiles of adult attained waist circumference (Waist), ($< 74\text{ cm}$, ≥ 74 and $< 82\text{ cm}$, $\geq 82\text{ cm}$).

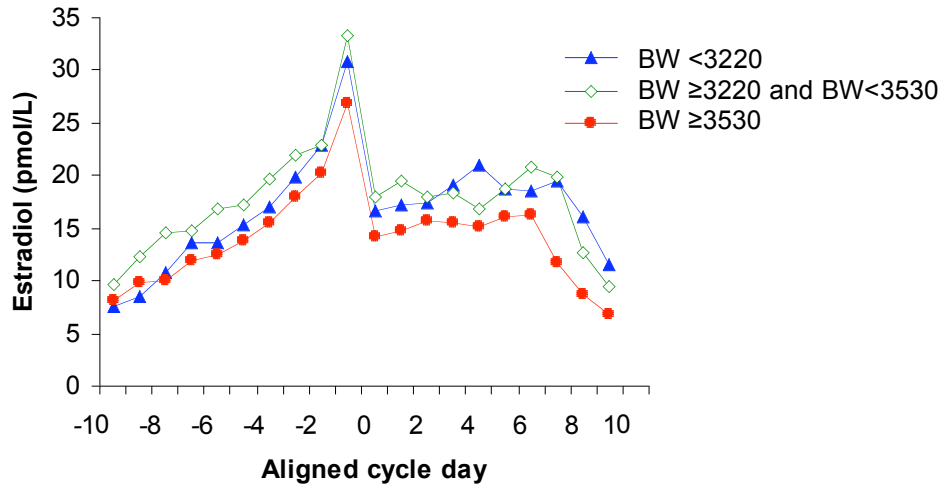
Figure 2

a) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by anthropometric measurements, highest compared with lower birth weight (BW) tertiles combined with being overweight in adulthood (waist $\geq 84\text{cm}$).

b) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by birth weight (BW) $< 3530\text{ g}$, adult obesity (waist $\geq 84\text{cm}$) and age at menarche.

c) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by birth weight (BW) $< 3220\text{ g}$ (lowest tertile), adult obesity (waist $\geq 84\text{cm}$) and age at menarche.

Figure 1a



b

