

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

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Abstract

Background Estrogens induce cellular proliferation and are associated with an increased risk of breast cancer. Birth weight and adult body weight have independently been associated with both estrogen levels and breast cancer risk. Thus, we hypothesize that low birth weight, in combination with adult overweight, may influence premenopausal

17β -estradiol over an entire menstrual cycle of possible importance for breast cancer.

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 were determined.

Results The participating women had mean birth weight of 3,389 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 $<3,530$ 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 ($\geq 3,530$ g) and adult excess body weight ($p = 0.03$). The association was even more pronounced in women with birth weights $<3,220$ 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

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cycles, which is of possible importance for breast cancer risk.

Keywords Birth weight · Adult body composition · Energy balance · 17β -estradiol

Introduction

Several studies have observed a strong relationship between birth weight and later risk of chronic diseases [1, 2], including breast cancer, indicating that fetal conditions may influence later susceptibility to adult disease ('Forsdahl–Barker hypothesis') [3–5]. One explanation for the association observed between birth weight and breast cancer risk may be that variation in birth weight may influence premenopausal estrogen levels [6, 7]. Furthermore, estrogens stimulate ductal growth and cell proliferation of breast epithelial cells, which is of importance for breast cancer risk [8, 9].

However, the association between birth weight and breast cancer risk has been complicated by contradictory results [10, 11], because some have observed a protective effect of low birth weight [12, 13], whereas others have not [10]. Birth weight, a marker of fetal growth and intra-uterine environment [14, 15], may interact with later growth pattern and availability of energy, and a joint effect of these factors may influence premenopausal estrogen levels and later breast cancer risk [4, 16]. Women with low birth weight compared with women with higher birth weight may have different set points for their physiological ovarian responsiveness to changes in energy balance throughout their premenopausal years [4, 17]. This variation in ovarian responsiveness may influence their estrogen levels during each menstrual cycle. Such a variation in ovarian responsiveness is also supported by the observation that women with low birth weights experience their growth spurt and menarche earlier than other girls, especially among those with excess weight during childhood [18–22].

There is also a growing recognition that breast cancer may be promoted by other factors related to an unfavorable metabolic profile as hyperinsulinemia and insulin resistance. These factors may influence ovarian responsiveness and favor a metabolic environment promoting tumor growth [23], but their relation with estrogens has not been much studied.

In spite of the somewhat contradictory patterns observed related to birth weight and breast cancer risk, little is known about the associations between variation in birth weight, adult body composition, and premenopausal levels of estrogens over an entire menstrual cycle. However, recent discussions argue for studies regarding that environmental factors acting during development should be

accorded greater weight in studies of disease causation [24]. In order to elaborate such a relationship, we chose to study the variation in the primary endogenous estrogen throughout premenopausal years, 17β -estradiol. A unique aspect of this study is the daily assessments of salivary 17β -estradiol, which represent the free biologically active hormone, rather than levels of both free and protein-bound circulating steroid as in serum. Additionally, it allows for fine discrimination of functional differences in steroid signaling throughout an entire menstrual cycle [25].

Thus, the aim of the present study was to test whether the 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 between birth weight and adult body composition 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 the 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 three months); not taking hormonal contraceptives; no pregnancy or lactation over the previous six 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 [26].

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 seven days of dietary data (days 3–6 and days 21–23 of the menstrual cycle, where day 1 represented the onset of menstrual bleeding) [27]. Average daily intake of energy and nutrients was computed by using

a food database and software system developed at the Institute for Nutrition Research, University of Oslo, Norway [28].

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. BMI (kg/m^2) was used to estimate the 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.

Insulin and other serum variables

Fasting serum blood samples were drawn from an antecubital vein in the morning at all three visits. Serum concentrations of insulin were measured at the Hormone Laboratory, Aker University Hospital, Oslo, in serum that was stored at -70°C for up to three years until analysis. All samples were assayed during a time period of two months. Serum insulin was measured by RIA using kits from Linco Research Inc (St Charles, MO, USA). The coefficients of variation (CVs) derived from the laboratories were 8–12% for insulin [26]. Serum concentrations of glucose and estradiol were measured in fresh sera at the Department of Clinical Chemistry, UNN, Tromsø.

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 [29]. 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 -labeled RIA kit (#39100, Diagnostic Systems Laboratory, Webster, TX, USA), along with published modifications of the manufacturer's protocol [26]. All samples were run in duplicate. All of a participant's samples were run in the same batch, with women randomly assigned to batches. CVs were calculated based on high and low value pools (appropriate to the range of each steroid) included in each assay [26].

Salivary assays have higher variability than serum assays because their measuring levels are one to two orders of magnitude lower in concentration. In general, this may impact the results so that the lower values (in the tail of the cycle) will have greater variability. In our study measurements of 17β -estradiol at the beginning and end of the cycles had higher CVs as the inter-assay variability ranged from 23% for low values (15 pmol/l) to 13% for high values (50 pmol/l). Furthermore, also higher rates of missing data, as a result of variation in cycle length, were observed. Therefore, we included 17β -estradiol measurements from aligned cycle day -10 to $+9$ in the linear mixed models.

The sensitivity of the estradiol assay (the lowest concentration of estradiol distinguishable from 0 at the 95% level) was 4 pmol/l. The average intra-assay variability (estimated from the 50% binding point of the standard curve) was 9%.

Before statistical analysis, all cycles were aligned to the day of ovulation following published methods [26], 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.

One of the important issues in the present study was to elaborate the importance of variations in 17β -estradiol throughout a menstrual cycle including very low as well as high levels of 17β -estradiol. Anovulatory cycles are associated with low-estradiol exposure, and thus all cycles, both anovulatory and ovulatory cycles, are included. Thus, no sub analyses are performed.

We assessed estrogen concentrations both in serum and in saliva. However, in the present study we use 17β -estradiol concentrations in saliva instead of serum concentrations, as steroid levels in saliva represent the free biologically active fraction rather than the levels of both free and protein-bound circulating 17β -estradiol as in serum. Furthermore, as saliva can readily be collected from individuals on many occasions, it is possible to compare

17 β -estradiol levels across entire menstrual cycles among different women, rather than relying on one or a few timed blood samples [25].

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: <3,220 g, \geq 3,220 to 3,530 g, and \geq 3,530 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.

In the present study, 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 and serum insulin [26, 30]. Based on a combination of biological plausibility, known breast cancer risk factors and reaching statistical significance in multivariate models, potential confounding factors were tested and adjusted for when appropriate. 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. The following variables contributed and were included in the final model; age, smoking, physical activity, and age at menarche. However, multivariate adjustments gave minor changes in comparison with age adjustments (Table 2), [26]. We evaluated possible interactions between measures of attained adult body composition and serum insulin 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 17 β -estradiol, waist circumferences were dichotomized at the 75th percentile (\geq 84 cm). We used linear mixed models for repeated measures to study variations in salivary 17 β -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 17 β -estradiol at the beginning and the end of the cycles had higher CVs and higher rates of missing data as a result of variation in cycle length; therefore we included 17 β -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

Characteristics of the study population

The 204 participating women had mean age 30.7 years, mean birth weight 3,389 g, and self-reported mean age at menarche 13.1 years. Mean BMI was 24.4 kg/m², mean waist circumference 79.5 cm, and mean total fat percentage 34.1%. When dividing the women into tertiles of birth weight (<3,220 g, \geq 3,220 to 3,530 g, and \geq 3,530 g), women in the highest tertile of birth weight had the lowest overall average salivary and thus free 17 β -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 17 β -estradiol by cycle day over the entire menstrual cycle across birth weight tertiles. Women who reported the highest birth weight (\geq 3,530 g) tended to have the lowest levels of daily free 17 β -estradiol over the entire menstrual cycle. No clear pattern was observed between 17 β -estradiol by cycle day over the entire menstrual cycle by tertiles of adult-attained waist circumference (Figures not shown).

Average 17 β -estradiol concentrations by changes in selected risk factors

We then studied the changes in overall average salivary 17 β -estradiol concentration of one entire menstrual cycle by 1 standard deviation (SD) change in explanatory variables for each tertile of birth weight. Among women in the

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

| Birth weight | | | | |
|--|--------------------------|---|--------------------------------|------------------------------------|
| Study characteristics | <3,220 g ($n = 68$) | $\geq 3,220$, <3,530 g ($n = 68$) | $\geq 3,530$ g ($n = 68$) | p -value ^b (trend) |
| 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 17β -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 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) | 7,968 (1,452) | 7,976 (2,093) | 8,335 (2,088) | 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 ($n = 190$) | 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 Number 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

two lowest tertiles of birth weight (<3,530 g), we observed a pattern of positive association between measures of adult-attained body composition and serum 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 $3.8 \text{ kg}/\text{m}^2$ (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 ($\geq 3,530$ g), the level of 17β -estradiol did not vary with changes in adult body composition and serum hormones (Table 2). A threshold level was observed as those women within the lowest and middle birth weight

tertiles reached higher levels of 17β -estradiol when they gain weight in adulthood in comparison with women within the highest birth weight tertiles who seem to have less change in 17β -estradiol levels when they become overweight in adulthood.

We found the same pattern 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).

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

| Variable | Mean (SD) | Change in 17 β -estradiol levels (pmol/l) | | | | | |
|--------------------------|-------------|---|-----------------------|---|-----------------------|---------------------------------|-----------------------|
| | | <3,220 g (<i>n</i> = 68) | | \geq 3,220 g, <3,530 g (<i>n</i> = 68) | | \geq 3,530 g (<i>n</i> = 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) |
| 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 coefficients, and 95% confidence intervals (CI)

^a Numbers may vary as a result of missing serum values^b Adjusted for age, leisure time physical activity, number of cigarettes, and age at menarche
95% CI: 95% confidence interval; SD: standard deviation

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 (<3,220 g), the relationship between measures of excess adult waist circumference and overall 17 β -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).

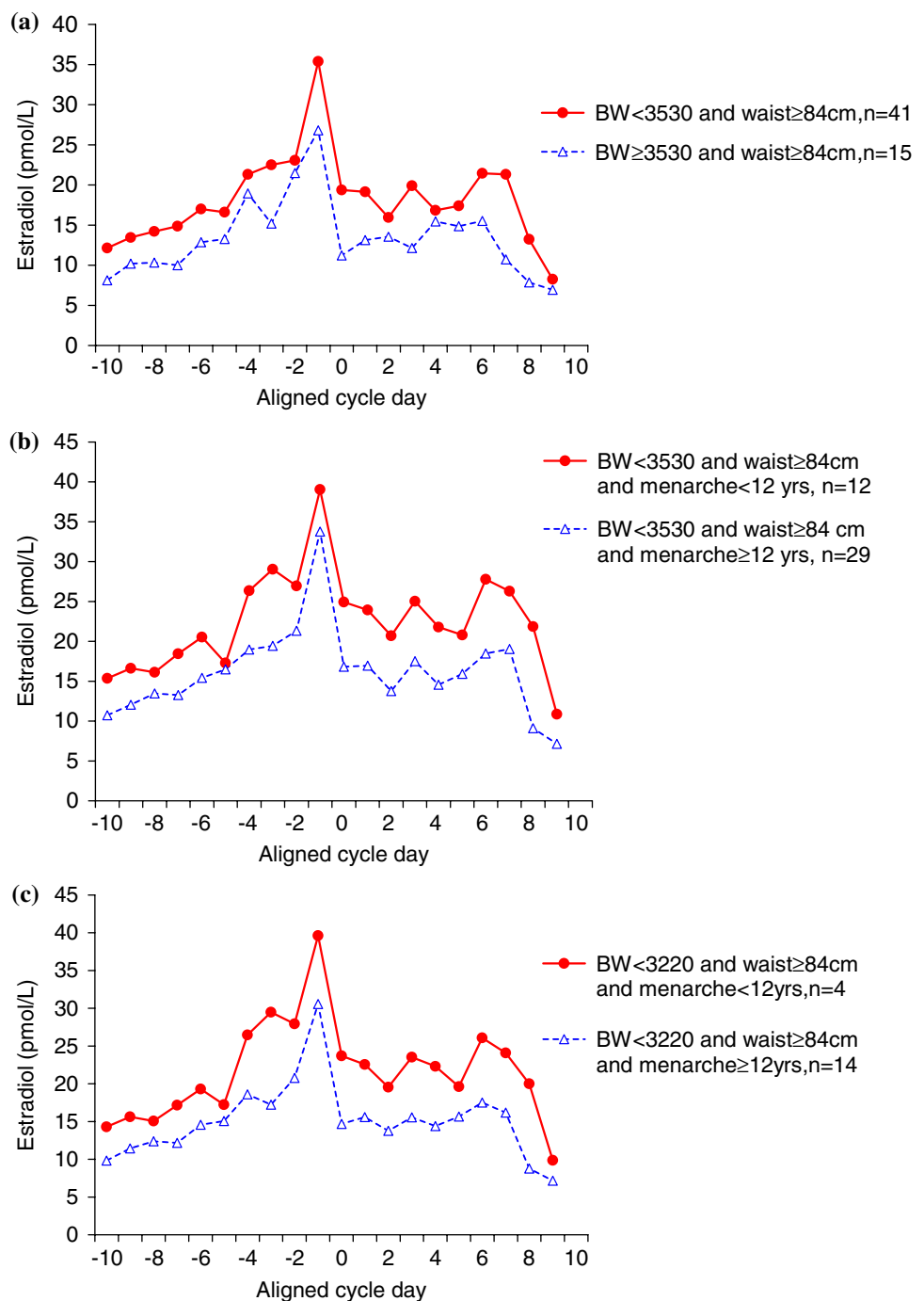
17 β -estradiol concentrations by cycle day with variation in anthropometric factors

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 (Fig. 1a–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 <3,530 g who later developed excess body weight (waist \geq 84 cm) showed higher 17 β -estradiol concentrations over the menstrual cycle compared with women of higher birth weights (\geq 3,530 g) and adult excess body weight (*p* = 0.03; Fig. 1a). 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 (<3,530 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; Fig. 1b). Women with lower birth weights (<3,530 g), early age at menarche (<12 years), and adult overweight also tended to have higher insulin levels than those who were older at menarche (\geq 12 years), although the difference was not significant (data not presented). Among those in the lowest birth weight tertile (<3,220 g) who later had a larger waist circumference (\geq 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; Fig. 1c).

Insulin in relation to birth weight and adult overweight

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 (<3,220 g)

Fig. 1 (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 ≥ 84 cm). (b) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by birth weight (BW) $<3,530$ g, adult obesity (waist ≥ 84 cm), and age at menarche. (c) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by birth weight (BW) $<3,220$ g (lowest tertile), adult obesity (waist ≥ 84 cm), and age at menarche



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).

Discussion

In our study of young healthy women with regular menstrual cycles, we found that low birth weight ($<3,530$ g)

combined with large-attained adult waist (≥ 84 cm) resulted in a 33% increase in free 17β -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, $<3,220$ g, combined with early age at menarche (<12 years). These results support that birth weight, a marker of conditions during fetal period, in combination with energy availability and metabolism during growth and development influence the

cumulative estrogen levels throughout premenopausal years.

Few studies have elaborated the associations between birth weight in combination with adult body composition and premenopausal 17β -estradiol levels, but some studies have reported alteration in sex hormones among females with low birth weight [6, 31]. In a study by Jasienska et al. among Polish women, they observed lower estradiol levels in the lowest birth weight quartile (1,300–3,000 g) versus among those with a higher birth weight [6]. Results from the Nurses Health' Study [31] suggest that low birth weight may be associated with lower serum estradiol levels through adolescence, but not into adulthood. In our present study, we did not find any clear positive association between birth weight and premenopausal estradiol levels throughout an entire menstrual cycle. However, 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 [17]. Furthermore, Barker has proposed that fetal tissues respond to the intrauterine environment by permanently altering their structure and function [2, 32], and recently, perinatal conditions has been argued to influence later plasticity for disease [24]. Our present findings that women within the lowest birth weight tertile had higher levels of 17β -estradiol when they become obese in adulthood, in opposite to those with a higher birth weight, support that later ovarian responsiveness may depend on intrauterine fetal conditions. This may indicate that women of low birth weight may have a different set point for their physiological responsiveness than women with higher birth weights. Therefore, our findings support a threshold level that put those within the lowest and middle birth weight tertiles at risk for increasing levels of 17β -estradiol when they gain weight.

The present findings are shared by other studies, which have observed that birth weight may influence later susceptibility of chronic diseases including breast cancer [4, 12, 33–35]. Furthermore, our results also support why the association between birth weight and breast cancer has been complicated by contradictory results [10, 11], as most studies have not stratified by adult body composition. Studies that have stratified by adult body composition when looking into the association between birth weight and breast cancer risk have observed that fetal experience may influence later risk of adult obesity with potential consequences for risk of breast cancer [36], but not always [4].

Also other periods than the fetal period in combination with energy balance throughout life may influence premenopausal 17β -estradiol levels. Some studies have observed that childhood obesity may accelerate age at menarche, especially in those of low birth weight [4, 18, 37, 38], and early age at menarche is a known risk factor

for breast cancer development. In our study, we observed that women of birth weight $<3,530$ g tended to have an earlier age at menarche than those in the highest birth weight tertile, $\geq 3,530$ g. A Swedish study suggested that girls born small or short for gestational age experienced their growth spurt and menarche earlier than other girls [39]. These results indicate that there may be compensation for those with fetal growth restriction through higher growth rate after birth, and the transit through puberty is faster. It is also possible that in women with fetal growth restriction who catch-up rapidly after birth, hyperinsulinemia, and increased IGF-1 levels are the trigger for an earlier adrenal androgen secretion [40, 41]. In addition, early age at menarche independent of birth weight but in combination with excess adult weight has been observed to give higher estrogen levels throughout each menstrual cycle and, consequently, a higher cumulative amount of estrogens throughout their lives [30, 42].

There is also a growing recognition that breast cancer may be promoted by hyperinsulinemia, insulin resistance, and high serum leptin levels, which may favor a metabolic environment promoting higher estrogen levels and tumor growth [43, 44]. Insulin resistance and elevated serum steroid levels often co-exist due to insulin up-regulation of ovarian steroid secretion [45]. 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 [41, 46, 47] 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, showing that women with the lowest birth weight had significantly higher serum insulin levels compared with women with higher birth weights. The results became even clearer when we looked into birth weight in relation to being overweight as an adult. Women of low birth weight ($<3,220$ 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. These findings are also supported by others as central obesity, a clinical marker of insulin resistance and metabolic syndrome is associated with hyperinsulinemia which in turn is considered to reduce sex hormone binding globulin (SHBG) levels, resulting in increasing levels of free estrogens [23].

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 procedure. 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 the 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 [29]. This study had the benefit of collecting samples every day over an entire menstrual cycle, rather than only on selected days within a cycle [48]. In addition, salivary levels of estradiol were shown to be quite stable within participants over time [49].

Age at menarche was recalled retrospectively, and misclassification is therefore similar. Several studies, however, have found that age at menarche are recalled with high reliability [50]. 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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