

Adult height, insulin, and 17 β -estradiol in young women

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Background: Adult height and insulin are thought to modify development of breast cancer. However, little is known about the association between height and 17 β -estradiol, a key factor in breast carcinogenesis, and whether insulin modifies such an association.

Methods: Among 204 healthy women, aged 25–35 years, who participated in the Energy Balance and Breast Cancer Aspect-I study, adult height (in centimeters) and fasting serum concentrations of insulin (pmol/L) were measured. 17 β -estradiol concentrations were measured in daily saliva samples throughout an entire menstrual cycle using radioimmunoassay (RIA). Age and multivariate linear regression models were used to study the association between adult height and 17 β -estradiol levels throughout an entire menstrual cycle, and whether serum levels of fasting insulin may modify such an association.

Results: The women had mean age of 30.7 years, adult height 166.9 cm and serum insulin 85.7 pmol/L. For each increase of one SD (standard deviation) in insulin levels in the upper tertile of adult height, the adjusted level of 17 β -estradiol increased 3.1 pmol/L (95% confidence interval or CI, 1.1, 5.2), equivalent to a 17.3% higher mean average concentration of 17 β -estradiol. Women with an adult height \geq 170 cm (upper tertile) and insulin levels $>$ 101 pmol/L (upper quartile) experienced, on average, 41% higher 17 β -estradiol levels throughout the entire menstrual cycle compared with women with the same adult height and insulin levels $<$ 101 pmol/L.

Conclusion: Our findings support that premenopausal levels of 17 β -estradiol vary in response to adult height and insulin levels, of possible importance for breast cancer risk.

Introduction

Genetic, environmental, nutritional, and hormone-related factors affecting growth from preconception to completion of linear growth are important in determining adult height. Height is linked to growth hormones, insulin-like growth factors, and sex hormone-binding proteins. These hormones also influence sexual maturation including age of puberty and fat storage, and may influence levels of estrogens, which in turn induce cellular proliferation and are associated with an increased risk of breast cancer (1). Moreover, adult height has consistently been positively associated with breast cancer risk independent of body mass (2, 3). However, little is known about the association between adult height and estrogen levels.

Interestingly, an increased final height and a rise in insulin resistance both parallel the increase in breast cancer incidence worldwide. Moreover, insulin has been observed to promote birth size, changes in growth during childhood (4), and in particular sexual maturation, ovarian steroidogenesis and production of sex hormone-binding globulin (SHBG) (5). Furthermore, insulin is a strong growth factor enhancing tumor cell proliferation (6), and studies suggest that hyperinsulinemic women have increased breast cancer risk (6, 7). So, insulin resistance seems to influence the metabolic and hormonal processes that promote breast cancer (1). Thus, several biological mechanisms support an association of adult height, insulin levels, and premenopausal sex hormone levels. Furthermore, as adult height may partly reflect life-time insulin sensitivity, we hypothesize that serum insulin modifies the association between adult height and 17β -estradiol, the primary endogenous estrogen throughout the premenopausal years.

We have previously hypothesized (8), and more recently observed that levels of 17β -estradiol are sensitive to energetic conditions during development and adult life (9-11). The aim of the present study was therefore to elucidate whether the daily free and biologically active 17β -estradiol levels throughout an entire menstrual cycle are associated with adult

height and whether variation in serum levels of fasting insulin (tertiles and quartiles) may modify such an association. A unique aspect of this study is the daily assessments of salivary 17β -estradiol, which represents the free biologically active hormone, rather than levels of both free and protein-bound circulating steroids, as found in serum.

Materials and methods

Participants and study design

Participants in the study were healthy, regularly menstruating Norwegian women aged 25–35 years (12). They were invited to participate in the Norwegian Energy Balance and Breast Cancer Aspect-I study (EBBA-I study), by announcements in newspapers and locally in Northern Norway during 2000–2002. 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, gynecological, or chronic disorders (e.g. diabetes, hypo-/hyperthyroidism). A total of 204 women were included in the study and came to the Department of Clinical Research, University Hospital North Norway, Tromsø (UNN), at a scheduled time (13, 14).

Questionnaires – dietary assessments

We used a general questionnaire (self-administered and by interview) to collect information on ethnicity, education, menstruation and reproductive history, previous hormone use, family history of cancer, and lifestyle habits (lifetime total physical activity, smoking, alcohol). Recall and memory-probing aids and interviews by trained personnel were used, including a lifetime calendar. Age at menarche was assessed by questionnaire and interview by the same trained nurse. A pre-coded food diary with a photographic booklet on portion size was

developed and used in order to collect dietary data, including alcohol on seven separate occasions during the menstrual cycle. The average daily intake of energy and nutrients was computed by using a food database and software system developed at the Institute of Nutrition Research, University of Oslo, Norway (15).

Height and other anthropometric measures

Study participants made three subsequent visits to the study center over the course of one menstrual cycle: visit 1 (days 1–4), visit 2 (midcycle) and visit 3 (days 22–25). They came on the first possible day after onset of menstrual bleeding for clinical examination, anthropometric measurements, and fasting blood samples. All clinical procedures were conducted by trained nurses at the Department of Clinical Research, UNN.

Anthropometric measures were taken twice (visits 1 and 3) with women wearing light clothing and no footwear. We measured height to the nearest 0.5 cm with the women in standing position. Waist circumference (in centimeters) was measured to the nearest 0.5 cm in a horizontal line 2.5 cm above the umbilicus. Weight was measured to the nearest 0.1 kg on an electronic scale that was standardized on a regular basis. Body mass index (BMI, kg/m^2) was used to estimate relative weight. A whole body scan was obtained during mid-cycle (days 7–12) by DEXA (dual energy X-ray absorptiometry, using DPX-L 2288, Lunar Radiation Corporation, Madison, WI, USA), operated by the trained nurse, and the percentage of fat tissue was estimated using Lunar software.

Serum samples

Fasting serum blood samples were drawn from an antecubital vein three times during the menstrual cycle (visits 1, 2, and 3). The blood was centrifuged and the serum separated. 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 3 years until analysis. All samples were assayed during a time period of 2 months. Serum insulin were measured by radioimmunoassay (RIA) using kits from Linco Research Inc. (St Charles, MO, USA) (13). The coefficients of variation (CVs) derived from the laboratories were 8% to 12% for insulin. Serum concentrations of estradiol were measured in fresh sera at the Department of Clinical Chemistry, UNN, Tromsø.

Estradiol indices and assay procedure

Concentrations of 17β -estradiol were measured in daily saliva samples. From the first day of bleeding, and each day during the menstrual cycle, the women collected morning saliva samples at home according to collection protocols previously established at the Reproductive Ecology Laboratory, Harvard University, USA (16), which also analyzed the saliva samples. Concentrations of 17β -estradiol were estimated from these saliva samples using an ^{125}I -based RIA kit (#39100, Diagnostic Systems Laboratory, Webster, TX, USA), following modifications to the manufacturer's protocol (12). All samples were run in duplicate. All of a woman's samples were run in the same batch, with women randomly assigned to batches. Coefficients of variation (CVs) were calculated from high- to low-value pools (appropriate to the range of 17β -estradiol) that were run with each batch (12).

The sensitivity of the 17β -estradiol assay (the lowest concentration of 17β -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 the lower values (15 pmol/l) to 13% for higher values (50 pmol/l). Salivary assays have higher variability than serum assays because they measure levels that are one to two orders of magnitude lower in concentration. This may impact on the results so that the lower values (in the tail of the cycle) will have greater variability.

In connection with regression modeling, all cycles were aligned to the day of ovulation following published methods (12), based on the identification of the estradiol drop at the mid-cycle (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 mid-cycle estradiol drop could not be made for 14 women, and their cycles were not aligned. These 14 women had mean height of 166.4 cm, 17β -estradiol concentration of 16.5 pmol/L, and approximately the same level of leisure time physical activity as the rest of the study group (53.1 MET h/week compared to 52.4 MET h/week). Anovulatory cycles are associated with low estradiol exposure, as we can also see among these 14 women, and because 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, all cycles, both anovulatory and ovulatory cycles are included in linear regression models.

Statistical analysis

The study population was categorized into tertiles of adult height: 1) <164 cm, 2) ≥ 164 cm and <170 cm and 3) ≥ 170 cm, in order to study in more detail the characteristics of the study group in relation to adult height. One- way analyses of variance and χ^2 tests were used to test for differences in means and frequencies of selected characteristics across tertiles of adult height (Table 1).

Age- and multivariate-adjusted linear regression analyses were used to study the associations between mean salivary 17β -estradiol concentrations, height and different measures of body composition and fasting insulin. We performed tests for assumption of linearity in our regression models, and 17β -estradiol was found normally distributed. 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, age at menarche, smoking, physical activity, energy intake, alcohol, previous use of hormonal contraceptives, age at first birth, and number of children were tested in the model. 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 (see Table 2). Possible interactions were studied.

We used linear mixed models for repeated measures to study salivary 17β -estradiol concentrations throughout the entire menstrual cycle in relation to height, and to see whether fasting serum insulin influenced such an association. Different covariance structures were explored, and we used the model with the best fit to our data (Toeplitz's). Dunnett's method was used for multiplicative comparisons. As the multivariate analyses gave only minor changes of our age-adjusted estimates, in relation to both linear regression models and linear mixed models for repeated measures, only age-adjusted results are presented in figures using mixed models for repeated measures.

In order to study whether the variation in fasting insulin levels modified the association between height and salivary 17β -estradiol concentrations, the fasting insulin were divided into both tertiles; 1) < 59 pmol/L, 2) ≥ 59 and < 90 pmol/L and 3) ≥ 90 pmol/L, and quartiles; 1) < 53 pmol/L, 2) ≥ 53 pmol/L and < 73 pmol/L, 3) ≥ 73 and < 101 pmol/L and 4) ≥ 101 pmol/L. Insulin levels were also dichotomized at the upper tertile (≥ 90 pmol/L) and 75th percentile (≥ 101 pmol/L), to see if there was a linear association or if there was an upper threshold effect.

Measurements of 17β -estradiol at the start and end of the cycles had higher CV's and higher rates of missing data as a result of variation in cycle length; therefore we included 17β -estradiol measurements from aligned cycle day -10 to $+9$ in the linear mixed models. Results

were considered statistically significant when two-sided $p < 0.05$. SAS statistical package version 9.1 was used.

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 a mean age of 30.7 years, height of 166.9 cm, mean salivary 17β -estradiol concentration of 17.9 pmol/L, and mean fasting serum insulin of 85.7 pmol/L. There were only minor variations in selected characteristics across tertiles of adult height. The tallest women tended to have higher fasting serum insulin levels (Table 1). Salivary and serum estradiol levels did not differ by tertiles of adult height. Women within the highest tertile of height (≥ 170 cm) had larger waist circumference compared with those with a shorter adult height ($p_{\text{trend}} = 0.008$), whereas percentage total fat did not vary by tertiles of adult height. Age at menarche were higher for higher categories of heights ($p_{\text{trend}} = 0.02$) (Table 1).

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

We then studied the variation in overall average salivary 17β -estradiol concentration throughout the entire menstrual cycle by 1 standard deviation (SD) variation in serum insulin (SD = 59.2 pmol/L), BMI (SD = 3.8 kg/m²), waist circumference (SD = 9.8 cm), and percentage total fat (SD = 7.6%), both in the total study population and within tertiles of adult

height using linear regression analyses (age adjusted and multivariate adjusted) (Table 2). After adjustments for potential confounding factors, within the highest tertile of adult height (≥ 170 cm), all these explanatory variables were positively associated with overall average 17β -estradiol concentration (Table 2). For each higher SD in insulin levels in the upper tertile of adult height, the overall adjusted level of 17β -estradiol were 3.1 pmol/L (95% CI, 1.1, 5.2) higher, equivalent to a 17.3% higher mean average concentration of 17β -estradiol in the upper tertile of adult height. For each higher SD in BMI in the upper tertile of adult height, the overall adjusted level of 17β -estradiol were 3.0 pmol/L (95% CI, 0.8, 5.3) higher, equivalent to a 16.8% higher mean average concentration of 17β -estradiol in the upper tertile of adult height. These clear associations were not observed in the middle or lowest tertiles of adult height (Table 2).

17β -estradiol concentrations by cycle day with variation in height and insulin levels

We used a linear mixed model for repeated measures to study salivary 17β -estradiol concentrations throughout an entire menstrual cycle in relation to adult height and insulin levels (Figures 1 and 2). We studied the average 17β -estradiol level by cycle day over the entire menstrual cycle across tertiles and quartiles of final height and fasting serum insulin. We observed no clear pattern between daily 17β -estradiol levels and variation in adult height (tertiles) or levels of insulin (tertiles) (Figure 1a, b) (quartiles not shown in figures). When we looked into adult height in combination with serum insulin levels, women in the highest tertile of adult height (≥ 170 cm) with high serum insulin (≥ 90 pmol/L) had higher levels of 17β -estradiol during an entire menstrual cycle compared with those with the same height and insulin levels < 90 pmol/L ($p = 0.0003$) (Figure 2a). The difference in 17β -estradiol levels became even more pronounced among tall women (≥ 170 cm) with even higher levels of serum insulin. Women with adult height ≥ 170 cm and serum insulin levels ≥ 101 pmol/L

(upper quartile) had, on average, 41% higher 17β -estradiol levels throughout the entire menstrual cycle compared with women with the same height and serum insulin <101 pmol/L ($p < 0.0001$) (Figure 2b).

Discussion

In our study of healthy young women with regular menstrual cycles, we found that tall women (adult height ≥ 170 cm), who also had high serum insulin levels (≥ 101 pmol/L), had 41% higher levels of free biologically active 17β -estradiol during a menstrual cycle compared with those of the same height but lower serum insulin. These results support that adult height in combination with insulin levels may be important biomarkers for breast cancer risk.

Little is known about the association of adult height, insulin, and 17β -estradiol, and to our knowledge no other studies have looked into this interrelationship. However, one possible explanation in support of our present findings of 17β -estradiol levels being linked to final height is that height is an indicator of early life nutrition during periods of growth (fetal period, childhood, and puberty), which may also be a marker of later responsiveness to normal physiology reflected by production and variation in sex steroid levels (17-19). Final height may therefore reflect both early and later responsiveness, and be an indicator of childhood energy intake; it has been suggested that these early exposures possibly affect both height and mammary mass (20). Second, our present findings that 17β -estradiol levels are linked to serum insulin (dose-response and no threshold effect) may reflect that insulin stimulates the synthesis of sex steroids, and inhibits the synthesis of SHBG, a binding protein that regulates the bioavailability of circulating sex steroids to tissues (21). Interestingly, elevated insulin levels during the period before menstrual resumption (postpartum) may synergize with gonadotropins to stimulate higher levels of ovarian steroid production. This, in turn, leads to a resumption of menstruation and a resolution of the transient phase of insulin

resistance (5), indicating a close coupling between energy metabolism and normal ovarian function, including levels of 17β -estradiol in menstruating women.

In addition, the observations that insulin regulates energy metabolism and stimulates anabolic processes throughout life, as a function of available energy and elementary substrates (e.g. amino acids), support possible biological mechanisms relating to adult height, levels of 17β -estradiol, and insulin levels. It has been suggested that better nutrition accelerates final height and growth hormone release (22), and the adolescent growth spurt involves stimulation by insulin and sex steroids (23). Overall, these findings support our observation that adult height and serum insulin concentration in combination may be related to variation in normal ovarian function. Patterns of inherited and adaptive metabolic responses and growth may be present from early life and persist into adult life, putting subgroups of women at risk for high 17β -estradiol levels throughout the menstrual cycle. Thus, not finding any clear association between adult height and 17β -estradiol levels or between serum insulin and 17β -estradiol levels may not be contradictory. Our results suggest that it is the combination of these factors – growth expressed by final height, ovarian responsiveness, and levels of insulin – that influence levels of 17β -estradiol.

The observation that both height and insulin are positively associated with breast cancer risk (1, 2, 22, 24) , and the fact that the incidence of breast cancer was lower than expected among women who experienced puberty during World War II in Norway (18), support that energy restriction, as part of lifestyle observed during World War II, may play a part in influencing both final height and breast cancer risk. Moreover, major hormonal factors that promote linear growth in childhood may be directly linked to breast carcinogenesis (1). Furthermore, central obesity and higher circulating levels of insulin, 17β -estradiol, and testosterone are risk markers for breast cancer (22). Recent theories propose that a western lifestyle may increase cancer risk through alterations in the metabolism of insulin (25-27).

Weight gain, through a typical western diet, limited levels of physical activity and, more recently reported, stress-related changes in neuroendocrine function may lead to insulin resistance and hyperinsulinemia. Epidemiological evidence is accumulating and suggests that the risk of breast cancer is related to circulating levels of insulin, among others (24, 28). An important characteristic of the insulin-resistant state is the presence of a systematic, low-grade inflammatory state. Increasing evidence has pointed recently to the potential role of this inflammatory state in the malignant process, including an increased stimulus to tumor cell proliferation, as well as effects mediated by inflammatory cell-related cytokines, such as increased angiogenesis. The opportunity for a multidisciplinary approach involving nutrition, exercise, and stress reduction in an integrative setting may be crucial to limiting the insulin-resistant state and improving cancer outcomes (6).

Final height is also probably influenced by inherited patterns in endogenous hormones and growth factors that influence age at puberty when breast tissue is rapidly developing, as well as promoting effects later in life. In particular, we suggest that insulin sensitivity may be a determinant of both adult height and breast cancer risk, and that serum insulin may thus be an interesting biomarker.

Another interesting observation is that age when maximum height is attained, rather than final height, relates to breast cancer risk (29, 30). The physiological basis for this observation may be that, if women reach their maximum height later, their breasts mature later and, consequently, there is less time between their pubertal breast development and protective breast differentiation that occurs at the time of the first live birth (29). Li and colleagues (29) observed that, although age at menarche correlated with the age at maximum height, the effect of age at maximum height persisted after adjustment for age at menarche. Previously, we have observed that age at menarche in combination with adult obesity is strongly associated with levels of 17β -estradiol (14). Moreover, in the present study, after

adjustments for age at menarche, the association of height, insulin, and 17β -estradiol is still strong among tall women with high levels of insulin. However, height may also reflect the number of ductal stem cells that develop in the breast in utero, which implicates prenatal exposures in breast cancer etiology (31).

The daily saliva sampling allowed for estimation of daily 17β -estradiol concentrations throughout an entire menstrual cycle which strengthened our study. We used well-developed and validated methods and assays to characterize the women's exposure to free, biologically active ovarian steroids and the comparisons of levels by aligned cycle days (16). This study has the benefit of having collected samples every day over an entire menstrual cycle, as opposed to a random or selected day within a cycle. Furthermore, salivary levels of 17β -estradiol are quite stable within participants over time (32).

The use of one clinical research department at a university hospital, with one specially trained nurse, enhanced the quality of our data. It also allowed us to sample all clinical variables within the same narrow time frame of the cycle for each participant, using uniform procedures. To limit any potential influence of season, women did not participate during months with no daylight (December and January). Height was measured according to standardized methods. Insulin was estimated in fasting serum samples after being stored not more than 3 years and then analyzed at the Aker University Hospital using well-documented methods. Adjustment was made for potential confounders.

Conclusion

Our main findings suggest that taller women are put at risk for substantially higher 17β -estradiol levels when their insulin levels rise. This may influence levels of 17β -estradiol during each menstrual cycle, and support the hypothesis that height, together with elevated

levels of insulin without any threshold effect, may influence major biomarkers for breast cancer risk.

Acknowledgments

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Table 1 Characteristics of the study population in tertiles of adult height (cm), means (SD)*:The Norwegian EBBA- I Study (N=204^a)

Study Characteristics	Height (cm)			p ^b (trend)
	<164 n=65	≥164 and <170 n=69	≥170 n=70	
Age (years)	31.1 (1.3)	30.3 (3.3)	30.7 (3.0)	0.4
Years of schooling	16.2 (2.8)	15.8 (3.2)	16.2 (3.2)	1.0
Ethnic minority, Sami (%)	0.1 (0.3)	0.1 (0.3)	0.1 (0.2)	0.3
Anthropometric measurements				
BMI (kg/m ²)	23.9 (3.2)	25.2 (4.0)	24.1 (4.0)	0.9
Waist circumference (cm)	76.5 (7.9)	80.9 (10.2)	81.1 (10.5)	0.008
Total fat (%)	33.2 (7.7)	35.2 (7.7)	34.0 (7.4)	0.5
Menstrual and reproductive characteristics				
Menarche (years)	12.9 (1.3)	13.0 (1.3)	13.4 (1.5)	0.02
Age at 1. birth (years) ^d	24.5 (4.4)	24.1 (4.1)	24.9 (3.2)	0.7
Number of children	0.9 (1.0)	0.9 (1.2)	1.0 (1.2)	0.5
Cycle length (days)	28.0 (2.8)	28.3 (3.0)	28.4 (3.7)	0.4
Saliva hormone concentrations (pmol/L)				
Overall 17 β-estradiol	16.8 (8.1)	19.7 (8.5)	17.2 (9.6)	0.8
Serum hormone concentration ^c				
Serum estradiol (mmol/L)	0.14 (0.05)	0.15 (0.1)	0.15 (0.1)	0.2
Serum glucose (mmol/L)	5.0 (0.6)	5.0 (0.5)	5.1 (0.6)	0.3
Serum insulin (pmol/L)	79.6 (65.1)	85.4 (43.0)	91.8 (67.0)	0.2
Energy intake (kJ/day)	7,786 (1,682)	8,028 (1,789)	8,442 (2,150)	0.04
Previous use of hormonal contraceptives (%)	78.1	92.8	80.0	0.8
Leisure time (MET h/week)	53.7 (35.8)	52.8 (37.9)	50.8 (34.7)	0.6
Alcohol units per week among users ,n= 190	2.3 (3.0)	3.2 (3.6)	3.2 (3.5)	0.1
Current smokers (%)	23.1	18.8	24.3	0.9

SD, standard deviation.

^aNumbers of participants may vary as a result of missing information for certain variables.^bOne-way analysis of variance or χ^2 test.^cBlood sampling first visit (days 1–5).^dFor those who have children, $n = 98$, in each group: 32-30-36.

Table 2 Estimated **variation**^{*} in mean salivary 17 β -estradiol concentrations (pmol/L) with 95% CI by 1 SD change in explanatory variable by tertiles of adult height (n=204)^a

Variation in 17 β -estradiol levels (pmol/L)								
Variable	Mean	(SD)	Adult height < 164 (n = 65)		Adult height \geq 164 and < 170 (n = 69)		Adult height \geq 170 (n = 70)	
			Age adjusted	Adjusted ^b	Age adjusted	Adjusted ^b	Age adjusted	Adjusted ^b
BMI (kg/m ²)	24.4	(3.8)	2.4 (0.1, 4.7)	2.1 (-0.2, 4.5)	0.7 (-1.4, 2.7)	0.9 (-1.2, 3.0)	2.9 (0.9, 5.0)	3.0 (0.8, 5.3)
Waist (cm)	79.5	(9.8)	2.0 (-0.5, 4.4)	1.7 (-0.9, 4.3)	0.1 (-2.0, 2.1)	0.1 (-2.0, 2.3)	2.4 (0.3, 4.5)	2.4 (0.1, 4.7)
Total fat (%)	34.1	(7.6)	1.8 (-0.2, 3.7)	1.7 (-0.3, 3.8)	-0.3 (-2.4, 1.8)	-0.2 (-2.5, 2.0)	2.4 (0.1, 4.7)	2.2 (-0.4, 4.8)
Insulin (pmol/L)	85.7	(59.2)	-0.4 (-2.2, 1.5)	-0.3(-2.2, 1.6)	0.4 (-2.5, 3.2)	1.2 (-2.3, 4.7)	3.1 (1.2, 5.0)	3.1 (1.09, 5.2)

*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 1a)

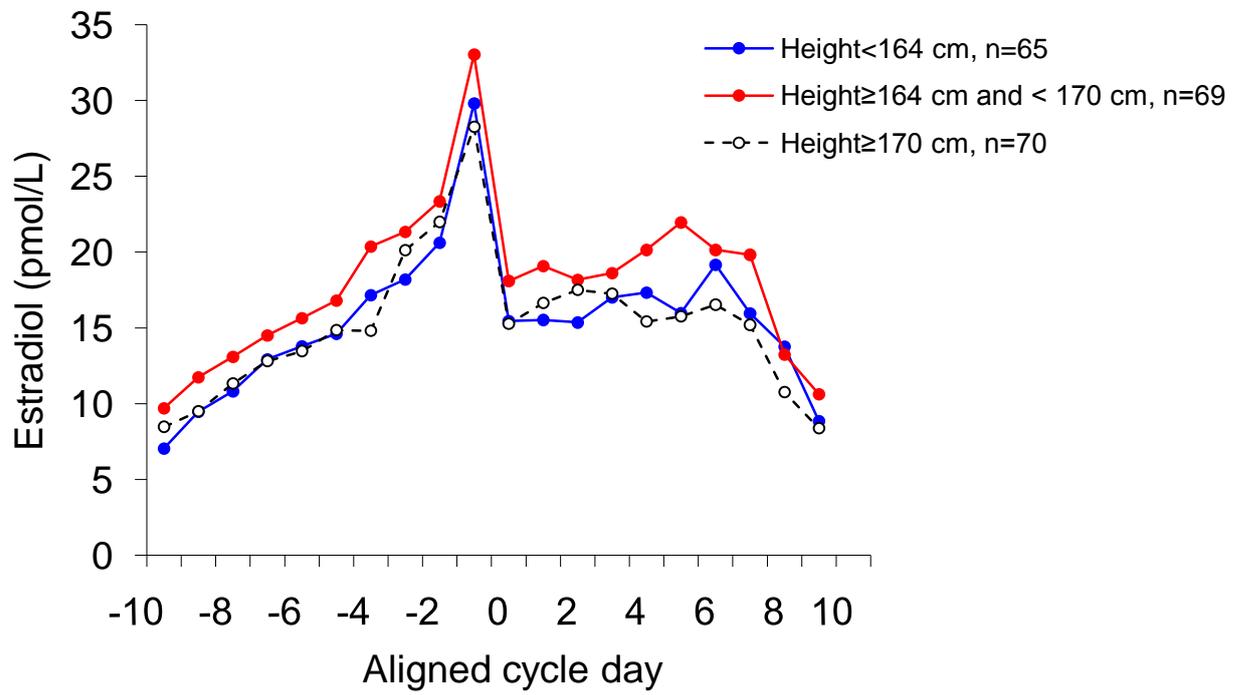
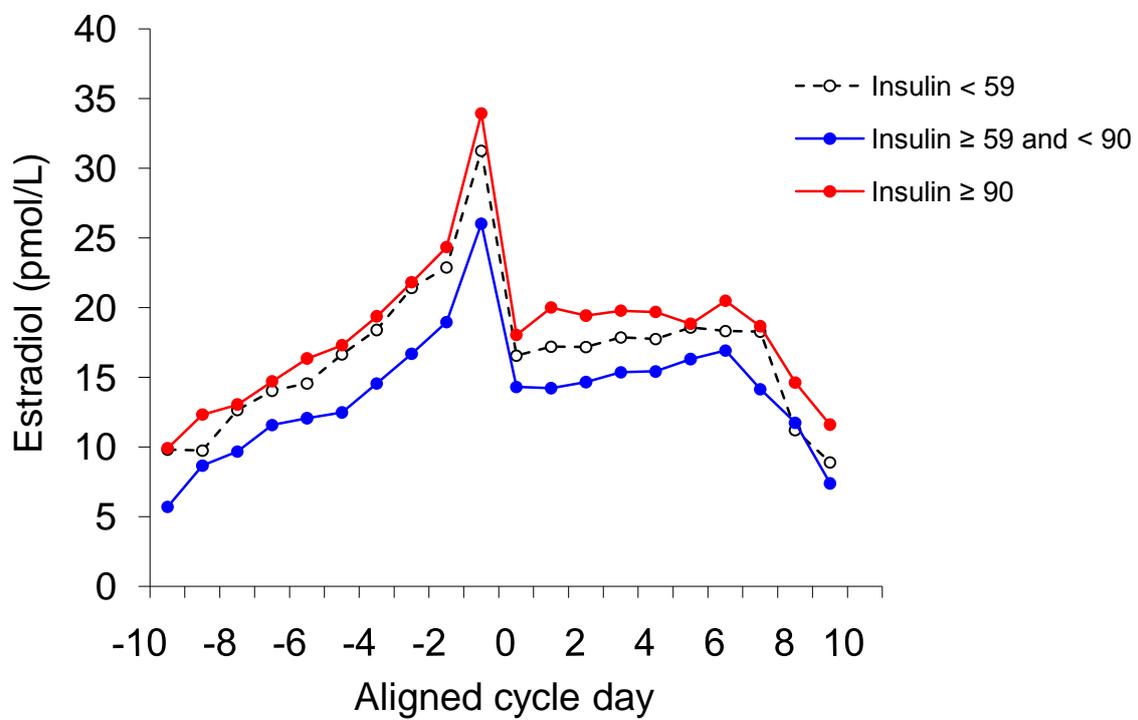


Figure 1b)



Legends figures

Figure 1

a) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by tertiles of adult height.

b) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by tertiles of insulin levels.

Figure 2a)

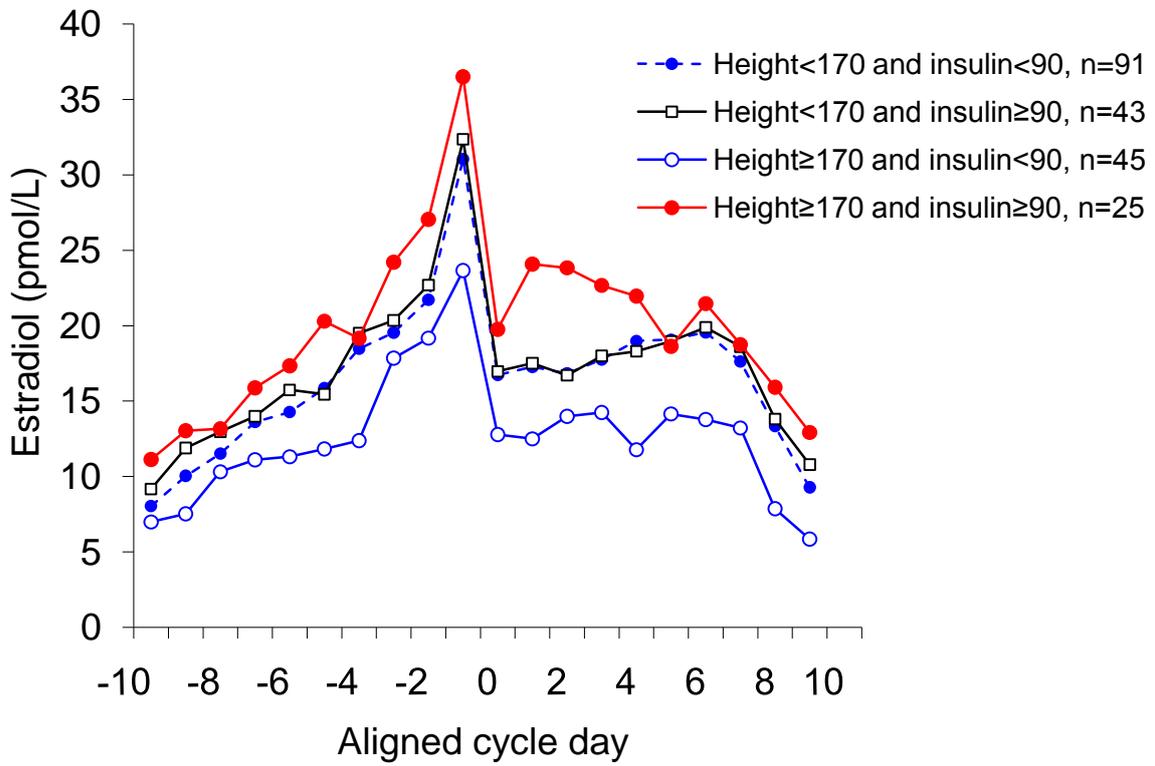
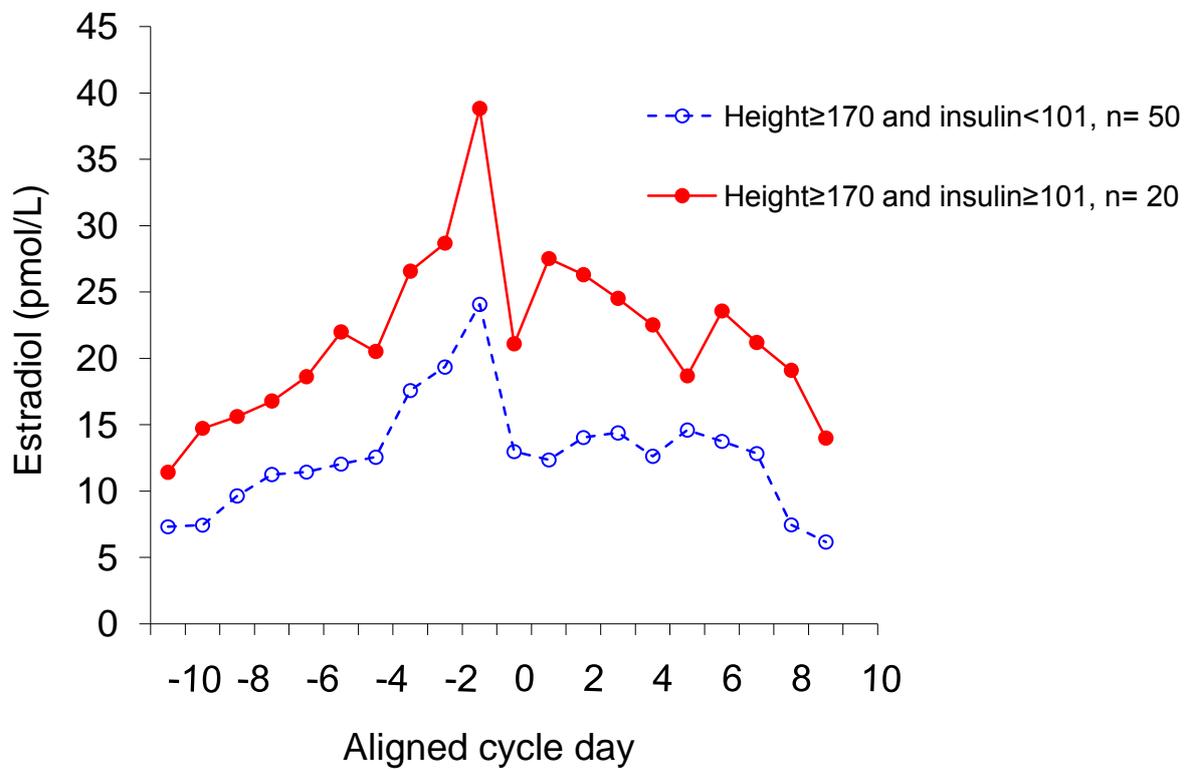


Figure 2b)



Legends figures

Figure 2

a) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by adult height ≥ 170 cm (highest tertile) combined with high and low levels of insulin (≥ 90 vs < 90 pmol/L) compared to women with an adult height < 170 cm and levels of serum insulin.

b) Age-adjusted salivary 17β -estradiol concentrations by cycle day in women categorized by adult height ≥ 170 cm in combination with high (≥ 101 pmol/L) and lower (< 101 pmol/L) serum insulin levels.

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