

# Adult Height, Insulin, and 17 $\beta$ -Estradiol in Young Women

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## Abstract

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

**Methods:** Among 204 healthy women, ages 25 to 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 $\beta$ -Estradiol concentrations were measured in daily saliva samples throughout an entire menstrual cycle through RIA. Age and multivariate linear regression models were used to study the association between adult height and 17 $\beta$ -estradiol levels throughout an entire menstrual cycle and whether serum levels of fasting insulin may modify such an association.

**Results:** The women had a mean age of 30.7 years, adult height of 166.9 cm, and serum insulin of 85.7 pmol/L. For each increase of one SD in insulin levels in the upper tertile of adult height, the adjusted level of 17 $\beta$ -estradiol increased by 3.1 pmol/L (95% confidence interval, 1.1-5.2), equivalent to a 17.3% higher mean average concentration of 17 $\beta$ -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 $\beta$ -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 $\beta$ -estradiol vary in response to adult height and insulin levels, of possible importance for breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2009; 18(5):1477-83)

## 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 an increase 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 (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). Therefore, insulin resistance seems to influence the metabolic and hormonal processes that promote breast cancer (1). Thus, several biological mechanisms support an association among adult height, insulin levels, and premenopausal sex hormone levels. Furthermore, as adult height may partly reflect lifetime insulin sensitivity, we hypothesize that serum insulin modifies the association between adult height and 17 $\beta$ -estradiol, the primary endogenous estrogen throughout the premenopausal years.

We have previously hypothesized (8) and more recently observed that levels of 17 $\beta$ -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 17 $\beta$ -estradiol levels throughout an entire menstrual cycle are associated with adult height and whether variations 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 17 $\beta$ -estradiol, which represents the free biologically active hormone, rather than levels of both free and protein-bound circulating steroids, as found in serum.

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## Materials and Methods

**Participants and Study Design.** The participants in the study were healthy, regularly menstruating Norwegian women ages 25 to 35 y (12). They were invited to participate in the Norwegian Energy Balance and Breast Cancer Aspect I study by announcements in newspapers and locally in Northern Norway during 2000 to 2002. The study participants had to meet the following criteria: self-reported regular menstruation (normal cycle length of 22-38 d within the previous 3 mo), not taking hormonal contraceptives, no pregnancy or lactation over the previous 6 mo, and no history of endocrinologic, gynecologic, or chronic disorders (e.g., diabetes, hypothyroidism/hyperthyroidism). A total of 204 women were included in the study and came to the Department of Clinical Research, University Hospital North Norway, Tromsø, 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 precoded food diary with a photographic booklet on portion size was developed and used to collect dietary data, including alcohol on seven separate occasions during the menstrual cycle. The average daily intake of energy and nutrients was computed with the use of 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 the 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, University Hospital North Norway.

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 (kg/m<sup>2</sup>) was used to estimate relative weight. A whole body scan was obtained during midcycle (days 7-12) by dual energy X-ray absorptiometry (with the use of DPX-L 2288; Lunar Radiation Corporation) operated by the trained nurse, and the percentage of fat tissue was estimated with the use of Lunar software.

**Serum Samples.** Fasting serum blood samples were drawn from an antecubital vein thrice during the menstrual cycle (visits 1, 2, and 3). The blood was centrifuged and the serum separated. Serum concen-

trations of insulin were measured at the Hormone Laboratory, Aker University Hospital, Oslo, in serum that was stored at -70°C for up to 3 y until analysis. All samples were assayed during a time period of 2 mo. Serum insulin was measured by RIA with the use of kits from Linco Research Inc. (13). The coefficients of variation 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, University Hospital North Norway, Tromsø.

**Estradiol Indices and Assay Procedure.** Concentrations of 17 $\beta$ -estradiol were measured in daily saliva samples. From the 1st 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, United States (16), which also analyzed the saliva samples. Concentrations of 17 $\beta$ -estradiol were estimated from these saliva samples through an <sup>125</sup>I-based RIA kit (DSL-39100; Diagnostic Systems Laboratory), following modifications to the manufacturer's protocol (12). All samples were run in duplicate. All of each woman's samples were run in the same batch, with women randomly assigned to batches. Coefficients of variation were calculated from high-value to low-value pools (appropriate to the range of 17 $\beta$ -estradiol) that were run with each batch (12).

The sensitivity of the 17 $\beta$ -estradiol assay (the lowest concentration of 17 $\beta$ -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%, and the interassay variability ranged from 23% for the lower values (15 pmol/L) to 13% for the 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 affect 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 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 (days -10 to +9). Satisfactory identification of the midcycle estradiol drop could not be made for 14 women, and their cycles were not aligned. These 14 women had a mean height of 166.4 cm, 17 $\beta$ -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 metabolic equivalent task hours per week compared with 52.4 metabolic equivalent task hours per 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 17 $\beta$ -estradiol throughout a menstrual cycle, including very low as well as high levels of 17 $\beta$ -estradiol, all cycles, both anovulatory and ovulatory, are included in linear regression models.

**Statistical Analysis.** The study population was categorized into tertiles of adult height: (a) <164 cm, (b)

**Table 1. Characteristics of the study population in tertiles of adult height and means (SD): the Norwegian Energy Balance and Breast Cancer Aspect I study (N = 204)**

Study characteristics	Height (cm)			P* trend
	<164, n = 65	≥164 and <170, n = 69	≥170, n = 70	
Age (y)	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 <sup>2</sup> )	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 (y)	12.9 (1.3)	13.0 (1.3)	13.4 (1.5)	0.02
Age at 1 birth (y) <sup>†</sup>	24.5 (4.4)	24.1 (4.1)	24.9 (3.2)	0.7
No. of children	0.9 (1.0)	0.9 (1.2)	1.0 (1.2)	0.5
Cycle length (d)	28.0 (2.8)	28.3 (3.0)	28.4 (3.7)	0.4
Saliva hormone concentrations (pmol/L)				
Overall 17β-estradiol	16.8 (8.1)	19.7 (8.5)	17.2 (9.6)	0.8
Serum hormone concentration <sup>‡</sup>				
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/wk)	53.7 (35.8)	52.8 (37.9)	50.8 (34.7)	0.6
Alcohol units per wk 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

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

Abbreviation: BMI, body mass index.

\*One-way ANOVA or  $\chi^2$  test.

<sup>†</sup>For those who have children, n = 98; in each group: 32-30-36.

<sup>‡</sup>Blood sampling first visit (days 1-5).

≥164 cm and <170 cm, and (c) ≥170 cm, to study in more detail the characteristics of the study group in relation to adult height. One-way ANOVAs and  $\chi^2$  tests were used to test for differences in means and frequencies of selected characteristics across tertiles of adult height (Table 1).

Age-adjusted and multivariate-adjusted linear regression analyses were used to study the associations among mean salivary 17β-estradiol concentrations, height, and different measures of body composition and fasting insulin. We did tests for assumption of linearity in our regression models, and 17β-estradiol was found to be 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

**Table 2. Estimated variation in mean salivary 17β-estradiol concentrations (pmol/L) with 95% confidence interval by 1 SD change in explanatory variable by tertiles of adult height (N = 204)**

Variable	Mean SD	Variation in 17β-estradiol levels (pmol/L)					
		Adult height <164 (n = 65)		Adult height ≥164 and <170 (n = 69)		Adult height ≥170 (n = 70)	
		Age adjusted	Adjusted*	Age adjusted	Adjusted*	Age adjusted	Adjusted*
BMI (kg/m <sup>2</sup> )	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)

NOTE: Variations were measured by linear regression analyses. Data are regression coefficient (95% confidence interval). Number may vary as a result of missing serum values. SD = standard deviation.

\*Adjusted for age, leisure-time physical activity, number of cigarettes, and age at menarche.

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 with the use of mixed models for repeated measures.

To study whether variation in fasting insulin levels modified the association between height and salivary 17 $\beta$ -estradiol concentrations, fasting insulin was divided into both tertiles: (a) <59 pmol/L, (b)  $\geq$ 59 and <90 pmol/L, and (c)  $\geq$ 90 pmol/L; and quartiles: (a) <53 pmol/L, (b)  $\geq$ 53 pmol/L and <73 pmol/L, (c)  $\geq$ 73 and <101 pmol/L, and (d)  $\geq$ 101 pmol/L. Insulin levels were also dichotomized at the upper tertile ( $\geq$ 90 pmol/L) and 75th percentile ( $\geq$ 101 pmol/L) to see if there was a linear association or if there was an upper threshold effect.

Measurements of 17 $\beta$ -estradiol at the start and end of the cycles had higher coefficients of variation and higher rates of missing data as a result of variations in cycle length; therefore, we included 17 $\beta$ -estradiol measurements from aligned cycle days -10 to +9 in the linear mixed models. Results were considered statistically significant when two-sided  $P < 0.05$ . The 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.

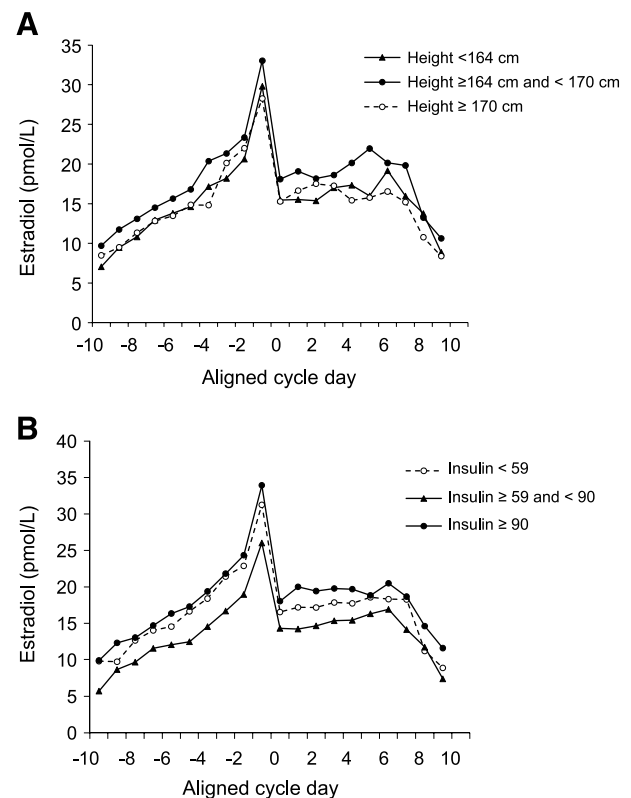
## Results

**Characteristics of the Study Population.** The 204 participating women had a mean age of 30.7 years, height of 166.9 cm, mean salivary 17 $\beta$ -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 ( $\geq$ 170 cm) had a 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 was higher for higher categories of heights ( $P_{\text{trend}} = 0.02$ ; Table 1).

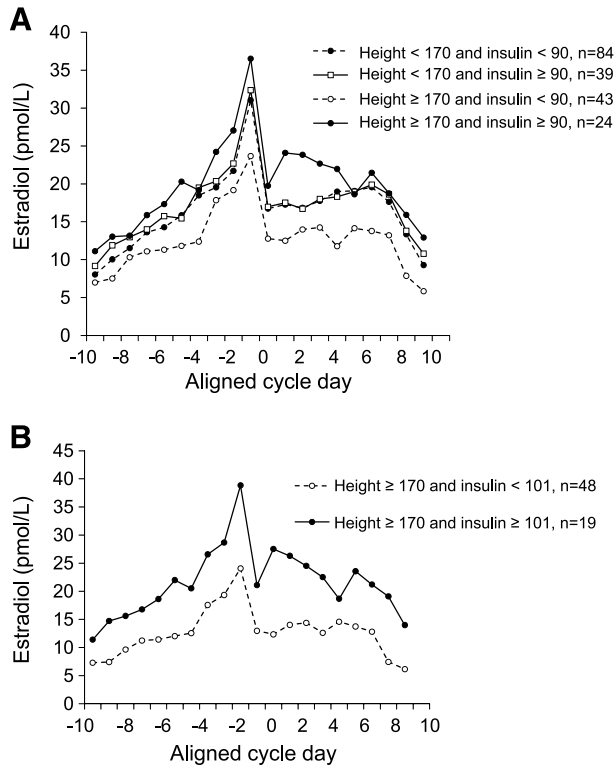
**Average 17 $\beta$ -Estradiol Concentrations by Changes in Selected Risk Factors.** We then studied the variation in overall average salivary 17 $\beta$ -estradiol concentration throughout the entire menstrual cycle by one SD variation in serum insulin (SD, 59.2 pmol/L), body mass index (SD, 3.8 kg/m<sup>2</sup>), 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 with the use of linear regression analyses (age adjusted and multivariate adjusted; Table 2). After adjustments for potential confounding factors, within the highest tertile of adult height ( $\geq$ 170 cm), all these explanatory variables were positively associated with the overall average 17 $\beta$ -estradiol concentration (Table 2). For each higher SD in insulin levels in the upper tertile of adult height, the

overall adjusted level of 17 $\beta$ -estradiol was 3.1 pmol/L (95% confidence interval, 1.1-5.2) higher, equivalent to a 17.3% higher mean average concentration of 17 $\beta$ -estradiol in the upper tertile of adult height. For each higher SD in body mass index in the upper tertile of adult height, the overall adjusted level of 17 $\beta$ -estradiol was 3.0 pmol/L (95% confidence interval, 0.8-5.3) higher, equivalent to a 16.8% higher mean average concentration of 17 $\beta$ -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).

**17 $\beta$ -Estradiol Concentrations by Cycle Day with Variation in Height and Insulin Levels.** We used a linear mixed model for repeated measures to study salivary 17 $\beta$ -estradiol concentrations throughout an entire menstrual cycle in relation to adult height and insulin levels (Figs. 1 and 2). We studied the average 17 $\beta$ -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 17 $\beta$ -estradiol levels and variation in adult height (tertiles) or levels of insulin (tertiles) (Fig. 1A and 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 ( $\geq$ 170 cm) with high serum insulin ( $\geq$ 90 pmol/L) had higher levels



**Figure 1. A.** Age-adjusted salivary 17 $\beta$ -estradiol concentrations by cycle day in women categorized by tertiles of adult height. N=190. **B.** Age-adjusted salivary 17 $\beta$ -estradiol concentrations by cycle day in women categorized by tertiles of insulin levels. N=190.



**Figure 2. A.** Age-adjusted salivary  $17\beta$ -estradiol concentrations by cycle day in women categorized by adult height  $\geq 170$  cm (highest tertile) combined with high and low levels of insulin ( $\geq 90$  versus  $< 90$  pmol/L) compared with women with an adult height  $< 170$  cm and levels of serum insulin. **B.** Age-adjusted salivary  $17\beta$ -estradiol concentrations by cycle day in women categorized by adult height  $\geq 170$  cm in combination with high ( $\geq 101$  pmol/L) and lower ( $< 101$  pmol/L) serum insulin levels.

of  $17\beta$ -estradiol during an entire menstrual cycle compared with those with the same height and insulin levels  $< 90$  pmol/L ( $P = 0.0003$ ; Fig. 2A). The difference in  $17\beta$ -estradiol levels became even more pronounced among tall women ( $\geq 170$  cm) with even higher levels of serum insulin. Women with adult height  $\geq 170$  cm and serum insulin levels  $\geq 101$  pmol/L (upper quartile) had, on average, 41% higher  $17\beta$ -estradiol levels throughout the entire menstrual cycle compared with women with the same height and serum insulin  $< 101$  pmol/L ( $P < 0.0001$ ; Fig. 2B).

## Discussion

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

Little is known about the association among adult height, insulin, and  $17\beta$ -estradiol and, to our knowledge, no other studies have looked into this interrelationship. However, one possible explanation in support of our present findings of  $17\beta$ -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 the 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  $17\beta$ -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 sex hormone-binding globulin, 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  $17\beta$ -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  $17\beta$ -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 variations 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  $17\beta$ -estradiol levels throughout the menstrual cycle. Thus, not finding any clear association between adult height and  $17\beta$ -estradiol levels or between serum insulin and  $17\beta$ -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  $17\beta$ -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 a 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,  $17\beta$ -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. Epidemiologic 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 the effects mediated by inflammatory cell-related cytokines, such as increased angiogenesis. The opportunity for a multi-disciplinary 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 physiologic 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 the protective breast differentiation that occurs at the time of the first live birth (29). Li and et al. (29) observed that, although age at menarche correlated with 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 17 $\beta$ -estradiol (14). Moreover, in the present study, after adjustments for age at menarche the association of height, insulin, and 17 $\beta$ -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 17 $\beta$ -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, the salivary levels of 17 $\beta$ -estradiol are quite stable within the 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 with the use of uniform procedures. To limit any potential influence of season, the 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 for not more than 3 years and then analyzed at the Aker University Hospital with the use of well-documented methods. Adjustment was made for potential confounders.

**Conclusion.** Our main findings suggest that taller women are put at risk for substantially higher 17 $\beta$ -estradiol levels when their insulin levels increase. This may influence levels of 17 $\beta$ -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.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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