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# Physical activity, heart rate, metabolic profile, and estradiol in premenopausal women

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### Justification for more than 6 authors

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Anne-Sofie Furberg	Concept and design Data Acquisition Significant manuscript reviser
Sissi Espetvedt	Data analyses and interpretation Significant manuscript reviser
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## Abstract

**Purpose:** To study whether physical inactive women with a tendency to develop metabolic syndrome, have high levels of  $17\beta$ -estradiol ( $E_2$ ) of importance for breast cancer risk.

**Methods:** 204 healthy women of reproductive age were assessed for self-reported leisure-time physical activity (LPA), resting heart rate (HR), blood pressure (BP), anthropometry, and glucose, lipids and insulin (Norwegian EBBA study).  $E_2$  were measured in daily saliva samples throughout an entire menstrual cycle. A clustered metabolic risk score (zMS; total cholesterol:HDL-C-ratio, insulin resistance, total fat tissue, BP and triglycerides) was defined. Linear regression and linear mixed models were used and confounding factors were tested.

**Results:** Physically active women had lower fat percentages ( $p_{trend}=0.003$ ) and HRs ( $p_{trend}=0.003$ ) than sedentary women. We estimated an increase in  $E_2$  of 1.27 pmol/L (95% CI, 0.06-2.47) for each 11.7 beats/min (1SD) increase in HR, and this correspond to 7% change in mean concentration of  $E_2$  for the total group. Associations with  $E_2$  was also found for fat tissue, total cholesterol:HDL-C-ratio, insulin resistance and triglycerides. A dose–response relationship was observed among the three levels of LPA and HR, and zMS ( $p_{trend}=0.03$  for LPA,  $p_{trend}=0.004$  for HR). Women in the highest tertile of the clustered metabolic risk score had average salivary  $E_2$  profiles that were markedly higher, throughout the cycle, than those of the other groups, with a cycle peak-day difference in  $E_2$  of 22–29%.

**Conclusion:** LPA and HR were associated with metabolic risk score and this score was associated with daily level of  $17\beta$ -estradiol, pointing to important biologic mechanisms operating between a sedentary lifestyle and increased breast cancer risk.

Key words: leisure-time physical activity, pulse,  $17\beta$ -estradiol, clustered metabolic score

## **Introduction**

**“Paragraph Number 1”** A sedentary lifestyle and high levels of estradiol are factors associated with increased breast cancer risk (34). The biological mechanisms underlying the relation between physical activity and breast cancer risk are complex and have not been fully identified (18). In contrast, estradiol is known to be associated with increased mitogenic and proliferative effect in breast cells (16) and has been identified as an important factor in the development and prognosis of breast cancer (18). However, reports on the association between estrogens and breast cancer risk among premenopausal women are sparse, partly because of the complexity of measuring hormonal levels throughout a menstrual cycle. Interestingly, in a recent publication from the Nurses’ Health Study II (7), the risk of breast cancer was more than doubled among premenopausal women in the highest quartile of the follicular free estradiol level compared with women in the lowest quartile.

**“Paragraph Number 2”** Even though the biological mechanisms that act in the association between physical activity and breast cancer risk are currently not fully outlined, there are several plausible mechanisms, including, among others, a suppressive effect of physical activity on ovarian steroid hormone production (21,23,25) and an effect on energy balance (12,18). The evidence for an inverse relationship between physical activity and breast cancer is stronger for postmenopausal than for premenopausal women (34). However, a number of case–control (2,11) and cohort studies (19,37) among various populations and on different continents, have observed a beneficial effect of physical activity on premenopausal breast cancer risk and on biological mechanisms important for breast cancer risk (22). The magnitude of the observed risk reduction has been, on average, between 20% and 40% for the most physically active women compared with the least active, and there is evidence for a dose–response relationship (18,28,34).

**“Paragraph Number 3”** Moreover, physical activity is one of the few risk factors for breast cancer that can be modified; it is also a promising preventive measure for many other chronic diseases, because physical activity improves the metabolic risk profile (18,38). In addition, breast cancer has been linked to metabolic components including lipids, insulin, blood pressure, and tissue fat (35,36). In a cohort study, we demonstrated that low high-density lipoprotein-cholesterol (HDL-C) is a potential marker of increased breast cancer risk among overweight women (14). In the Norwegian Energy Balance and Breast Cancer Aspect (EBBA) study (14), we confirmed the hypothesis that low HDL-C may be associated with increased levels of 17 $\beta$ -estradiol (E<sub>2</sub>) throughout the entire menstrual cycle. Furthermore, in Poland, not that much westernized population yet and in a parallel study to our EBBA study, Jasienska et al. (23) observed habitual physical activity to be associated with lower levels of E<sub>2</sub> throughout Polish women’s menstrual cycles.

**“Paragraph Number 4”** Since physical activity assessment is complex, a need exists to include objective measures of activity and energy expenditure when elucidating plausible biological mechanisms related to physical activity, E<sub>2</sub>, and later breast cancer risk. In the current study we have used resting heart rate (HR) and a set of metabolic factors related to the variation in physical activity as objective measurements. The aim of the present study was therefore (1) to study how self-reported leisure-time physical activity (LPA) and heart rate are related to levels of E<sub>2</sub> and with the metabolic profile among premenopausal women, and (2) to investigate how a set of metabolic risk factors (fat tissue, BP [(systolic BP + diastolic BP)/2], insulin resistance, triglycerides and total cholesterol:HDL-C ratio) are associated with E<sub>2</sub> throughout an entire menstrual cycle.

## **Materials and methods**

## **Participants and study design**

**“Paragraph Number 5”** Women who participated in the cross-sectional EBBA study during 2000–2002 were recruited through local media campaigns. A total of 204 6 women, 25–35 years of age, were included and met the following eligibility criteria: self-reported regular menstruation (normal cycle length: 22–38 days within the previous 3 months), no use of steroid contraceptives and no pregnancy or lactation over the previous 6 months, no history of gynecological disorders, and no chronic medical conditions (e.g. diabetes, hypo-/hyperthyroidism).

## **Ethical considerations**

**“Paragraph Number 6”** All participating women signed an informed consent form and the study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

## **General questionnaires**

**“Paragraph Number 7”** We used both self- and interviewer-administered questionnaires to collect general information, including education, reproductive history, previous use of hormonal contraceptives and lifestyle habits (smoking, alcohol). Recall aids including a lifetime calendar were used. We collected dietary data on 7 different week days during the menstrual cycle (days 3–6 and 21–23) using a pre-coded food diary developed for the present study population, based on a validated pre-coded food diary (29). The average daily intake of energy and nutrients was computed using a food database and software system developed at the Department of Nutrition, University of Oslo, Norway (29).

## **Leisure time physical activity, Heart rate and metabolic risk factors**

**“Paragraph Number 8”** In the general questionnaire we asked about leisure time physical activity (LPA) over the last year. Leisure time was recorded and graded from 1 to 4 as follows:

1 = low/sedentary activities, including reading, watching television, or other sedentary activities

2 = moderate activities, including walking, bicycling, or physical activities for at least 4 hours a week

3 = hard activities, including exercises to keep fit for at least 4 hours a week

4 = very hard activities, defined as regular hard training or exercise for competition several times per week.

**“Paragraph Number 9”** The study participants made three subsequent visits in a fasting state during the collection period: first visit (days 1–5 of the menstrual cycle), second visit (days 7–12), and third visit (days 22–25). The participants met on the first day possible, after onset of menstrual bleeding, for the first visit with clinical examinations, including height, weight, waist and hip circumference, resting HR, BP, and fasting blood sampling. All clinical procedures and measurements were conducted by trained nurses at the Department of Clinical Research, University Hospital North Norway (UNN), Tromsø, Norway, at a scheduled time (14).

**“Paragraph Number 10”** Heart rate (HR) and blood pressure (BP) were measured three times (PROPAQ 104), sitting in a resting position, with the mean of the final two measurements used in the analysis.

**“Paragraph Number 11”** Anthropometric measurements were taken with participants wearing light clothing and no footwear: height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg on an electronic scale. Waist circumference was measured in a horizontal line 2.5 cm above the umbilicus; hip circumference was measured at the largest circumference, both measured to the nearest 0.5 cm.



**“Paragraph Number 12”** Around the time of the second visit, participants underwent a whole-body scan using dual energy X-ray absorptiometry (DEXA – DPX-L 2288, Lunar Radiation Corporation, Madison, WI, USA), which was operated by our nurse; the percentage total fat tissue was estimated using standard Lunar software.

**“Paragraph Number 13”** Blood samples were drawn after overnight fasting (14). Serum concentrations of glucose, triglycerides, total cholesterol, HDL-C, and E<sub>2</sub> were measured in fresh sera at the Department of Clinical Chemistry, UNN. Serum glucose was measured enzymatically by the hexokinase method, serum triglycerides were assayed by enzymatic hydrolysis with lipase, serum cholesterol was determined enzymatically using cholesterol esterase and cholesterol oxidase, with HDL-C quantified by a direct assay using polyethylene glycol-modified enzymes and dextran sulfate. Finally serum concentrations of insulin were measured at the Hormone Laboratory, Aker University Hospital, Oslo by RIA using kits from Linco Research, Inc. (St Charles, MO, USA) (14).

### **Saliva hormone samples and analysis**

**“Paragraph Number 14”** Women collected daily saliva samples at home for one entire menstrual cycle preferentially in the morning. They started on the first day of bleeding, according to previously established collection protocols developed at the Reproductive Ecology Laboratory at Harvard University, USA (31).

**“Paragraph Number 15”** E<sub>2</sub> concentrations were measured in daily saliva samples for 20 days (reverse cycle days –5 to –24) of the cycle using an <sup>125</sup>I-based radioimmunoassay (RIA) kit (#39100, Diagnostic Systems Laboratory, Webster, TX, USA), along with published modifications to the manufacturer’s protocol (20).

**“Paragraph Number 16”** The sensitivity, and inter- and intra-assay variability of E<sub>2</sub> have been described previously (13,14). Of the 4080 potential samples, improper collection or loss during the assay procedure resulted in a loss of 6.9% of daily E<sub>2</sub> samples for the analyses.

**“Paragraph Number 17”** Before statistical analysis, all cycles were aligned to the day of ovulation, based on the identification of the E<sub>2</sub> drop at the mid-cycle (day 0), which provides a reasonable estimate of the day of ovulation, according to published methods (31). The E<sub>2</sub> values for 20 consecutive days during each woman’s cycle, aligned on day 0, were used in data analyses. Satisfactory identification of the mid-cycle E<sub>2</sub> drop could not be made for 14 women and their cycles were not aligned. Overall average salivary E<sub>2</sub> (mean E<sub>2</sub>) was calculated for all 204 women, whereas additional indices were calculated for the 190 women with aligned cycles. The following E<sub>2</sub> indices were calculated: ‘mean E<sub>2</sub>’ (mean of day –10 to + 9), ‘mean follicular E<sub>2</sub>’ (mean of day –7 to –1), and ‘mean luteal E<sub>2</sub>’ (mean of day 0 to + 11).

### **Statistical analysis**

**“Paragraph Number 18”** In order to study E<sub>2</sub> concentrations in relation to physical activity, heart rate and a clustered metabolic risk score, linear regression and linear mixed models were used (SAS version 9.1).

**“Paragraph Number 19”** As only 10 participants reported that they performed group 4 (very hard) activities, in the analyses we combined activity groups 3 and 4. Hence, the study population was divided into three groups of LPA (low, moderate and high activity).

**“Paragraph Number 20”** As indicator of insulin resistance, we used the HOMA-score, calculated as the product of fasting glucose concentration (in mmol/L) and fasting insulin concentration (in µU/mL) divided by the constant 22.5 (26).

**“Paragraph Number 21”** To create clustered metabolic risk scores we computed the Z score (standard score) for selected individual risk factor variables associated with metabolic profile, broadly based on the World Health Organization definition of the metabolic syndrome. The Z score is calculated by subtracting the sample mean from the individual score (raw score), and dividing the difference by the sample standard deviation. It is negative when the raw score is below the sample mean and positive when the raw score is above the sample mean, and lower Z scores indicate a more favorable metabolic profile. The HOMA-score, fasting triglycerides and insulin were logarithmically transformed when used in the Z score, because of their skewed distribution. The Z scores of the individual risk factors were then summed to construct clustered risk scores. The five risk factors included in the main clustered risk score (zMS or z metabolic syndrome) were the ratio total cholesterol:HDL-C, logarithm of the HOMA-score, percentage of total fat as estimated from the DEXA scan, BP, and logarithm of the triglyceride levels. In parallel, we calculated a second clustered risk score containing the same variables without the obesity component (zMS-O).

**“Paragraph Number 22”** Age-adjusted linear regression analyses were used to study the associations of average salivary E<sub>2</sub> concentration (overall, in the follicular and luteal phases of the menstrual cycle) and different variables related to the metabolic profile. We used a linear mixed model for repeated measures to study salivary E<sub>2</sub> concentrations throughout the entire menstrual cycle in relation to HR, level of LPA, and the clustered risk scores. Different covariance structures were explored and the results using a heterogeneous Toeplitz method are presented. Dunnett’s method was used for multiple comparisons, with examination of residuals. Age was included as a co-variate in the final models. In addition, adjustment for birth weight, body mass index (BMI), energy intake, smoking, alcohol, previous use of hormonal

contraceptives, education, and age at menarche gave similar estimates and are not presented. Possible interactions were studied.

**“Paragraph Number 23”** E<sub>2</sub> measurements at the start and end of the cycles have higher coefficients of variation and higher rates of missing as a result of variation in cycle length; we therefore included E<sub>2</sub> measurements from day of cycle –10 to +9. Sample size estimation has shown that a sample size of 20-25 is sufficient to detect significant differences in indices of ovarian function between groups.

## Results

**“Paragraph Number 24”** The participating 204 healthy women of reproductive age were, on average, 30.7 years of age; 15.7% reported low LPA, 59.3% moderate LPA and 25.0% high LPA. Descriptive analyses were stratified by reported LPA (Table 1). Women with a high level of reported LPA had lower total fat percentage ( $p_{trend}=0.003$ ), lower HR ( $p_{trend}=0.003$ ), and tended to have a lower total cholesterol:HDL-C ratio ( $p_{trend}=0.09$ ) and higher HDL-C ( $p_{trend}=0.12$ ) than women with a more sedentary level of LPA (Table 1). There were no statistically significant differences in overall average salivary hormones or HOMA-score between the three groups.

**“Paragraph Number 25”** We studied estimated changes in overall average, follicular, and luteal salivary E<sub>2</sub> concentrations by changes in the selected metabolic risk factor variables (Table 2). We estimated that an increase (of 1 SD) in total fat tissue, HOMA-score, total cholesterol:HDL-C ratio, triglycerides and HR was associated with an age-adjusted increase in E<sub>2</sub> concentrations (Table 2). The mean daily saliva concentration of E<sub>2</sub> throughout the menstrual cycle was 17.9 pmol/L (SD = 8.8 pmol/L) (data not presented). An age-adjusted increase in the

overall level of E<sub>2</sub> of 1.27 pmol/L (95% confidence interval, CI, 0.06–2.47) was observed for each 11.7 beats/min (1SD) increase in heart rate, and this correspond to 7% (1.3 pmol/L of 17.9 pmol/L) change in mean overall concentration of E<sub>2</sub> for the total group. For each 0.8 (1 SD) increase in the total cholesterol:HDL-C ratio, the mean age-adjusted increase in overall E<sub>2</sub> is 2.46 pmol/L (95% CI, 1.26–3.66), which equals a 13.7% change in mean overall concentration of E<sub>2</sub> for the total group.

**“Paragraph Number 26”** Figure 1A shows the association between the clustered metabolic score (zMS) and LPA. An inverse dose–response relationship was observed ( $p_{trend}=0.03$ ). Likewise, figure 1B shows that zMS is directly related to HR in a dose-dependent manner ( $p_{trend}=0.004$ ).

**“Paragraph Number 27”** We examined the average E<sub>2</sub> concentrations by cycle day throughout the whole menstrual cycle by tertile of HR (Figure 2a,  $p$  between levels= 0.15) and at the three levels of LPA (Figure 2b,  $p$  between levels= 0.48), without finding a clear pattern, even when stratifying by birth weight (data not shown). No interaction was found between HR and day of E<sub>2</sub> measurement ( $p=0.89$ ) or level of activity and day of E<sub>2</sub> measurement ( $p=0.18$ ).

**“Paragraph Number 28”** In contrast, when we examined the E<sub>2</sub> concentration by cycle day throughout the whole menstrual cycle, in tertiles of the clustered risk score, zMS, a clear pattern was observed; women in the highest tertile of the zMS had age-adjusted average salivary E<sub>2</sub> profiles that were markedly higher, throughout the cycle, than those of the other groups (Figure 3). Using a linear mixed model for repeated measures, we examined how, in women in the group of the highest tertile of the zMS, the average E<sub>2</sub> concentration by cycle day differed from that in the other groups of women described, and we observed significant age-adjusted differences for all comparisons (Figure 3; age-adjusted  $p$  values: tertile III–I,  $p=0.001$ ; tertile III–II,  $p< 0.001$ ).

The same pattern was observed when the obesity component was removed (i.e. zMS-O, not shown).

**“Paragraph Number 29”** When studying the peak day (the day before the drop day of the menstrual cycle), we observed that the mean peak-day levels of E<sub>2</sub> in tertiles of zMS were as follows (Figure 3): lowest tertile 28.7 pmol/L, medium tertile 26.3 pmol/L, and highest tertile 36.6 pmol/L ( $p_{trend}=0.001$ ), which corresponds to a 21.6% and 28.7% difference between the highest tertile of the clustered metabolic risk score and the lowest and medium tertiles, respectively.

## **Discussion**

**“Paragraph Number 30”** Among 204 healthy premenopausal women we observed that those women with high leisure time physical activity (LPA) and women with lower heart rate (HR) had a more favorable clustered metabolic risk score, zMS, with a dose response relationship, than inactive women. In addition, we observed that this clustered risk score of metabolic factors was strongly associated with the daily level of free biologically active E<sub>2</sub> throughout an entire menstrual cycle, with a cycle peak-day difference of 22–29% between the tertiles of zMS.

**“Paragraph Number 31”** The observation that healthy women of reproductive age in the current study showed a clear association between LPA and zMS, and that zMS were associated to levels of estradiol, is of great interest and support previously hypothesized biological mechanisms operating between physical activity and breast cancer (14, 21, 24). Furthermore, western lifestyle, characterized by physical inactivity, is also associated with obesity and a metabolic imbalance, including unfavorable lipids, high blood pressure, and insulin resistance – factors that are also associated with increased breast cancer risk (15,18,30,37). Additionally,

several studies have observed a stronger protective effect of physical activity among lean women in comparisons with normal weighted women (21, 42). Furthermore, several recent studies provide support for the role of physical activity in the prevention of the metabolic syndrome (1,5). Randomized controlled trials have shown that exercise training has a mild or moderately favorable effect on many metabolic risk factors (27), whereas others have shown less association after controlling for obesity (17). Moreover, the metabolic syndrome is a cluster of risk factors that predisposes individuals to type 2 diabetes, and individuals with metabolic syndrome have an increased risk of all-cause mortality (27).

Recently, the association between metabolic syndrome and its components (especially insulin), have been observed to predispose individuals to breast cancer. Hence, if physical activity can improve the metabolic risk profiles (3,38), it might indirectly reduce breast cancer risk. Importantly, we have recently pointed to sex hormones and insulin as important pathways towards the development of breast cancer (13). In the current study, we observed an association between the clustered metabolic risk score and the level of LPA, but the association was not there for all the single factors alone. There could be not only an additive effect, but also a multiplicative effect when several metabolic risk factors are put together in a cluster score based on biological effects. Another explanation may be that a clustered risk score may compensate for fluctuations in the single risk factors, or there could be less error variation in the clustered risk score compared with each single risk factor. However, this observation underlines the fact that physical activity may influence several factors often hypothesized (14,21) which, independently and/or together, influence the level of  $E_2$  and then lead to an increase in breast cancer risk.

**“Paragraph Number 33”** In our study, the clustered risk score, was positively associated with daily levels of  $E_2$  during the entire menstrual cycle: a high clustered metabolic risk score gave a

high level of E<sub>2</sub>. Interestingly, this observation was similar when the obesity component was excluded from the metabolic risk score, indicating that the protective effect of physical activity is not entirely mediated by changes in adiposity also supported by others (7). In a recent study, Ekelund et al. observed that an increase in physical activity is associated with reduced metabolic risk, independent of changes in fatness and fitness (6). However, in another study, Campbell et al. found that a 12-week aerobic exercise training intervention improved aerobic fitness and body composition, but did not alter the urinary estrogen metabolites in premenopausal women (4). These results regarding metabolites cannot be compared with our measurements of the free biologically active E<sub>2</sub> throughout the entire menstrual cycle.

**“Paragraph Number 34”** Despite the HR not showing any clear pattern with level of E<sub>2</sub> throughout the menstrual cycle, when analyzing it as a single factor, it clearly associated with a clustering of metabolic factors in the same way as leisure time activity. Previous studies have shown that heart rate has a clear association both with fitness and leisure time activity. These findings underline the role of heart rate as an objective measurement in clinical studies both in relation to metabolic profile and leisuretime activity (38).

**“Paragraph Number 35”** Current evidence supports an inverse relationship between physical activity and breast cancer risk (18), and one of the biologic mechanisms proposed to explain the protective effect is reduced exposure to E<sub>2</sub> (2), based on the suppressive effect of physical activity on ovarian steroid hormones (18,23,25). Endurance training as well as general physical activity have been reported to give low levels of E<sub>2</sub> (23,33); in a recent parallel study, a strong association was observed between habitual physical activity and level of E<sub>2</sub> among premenopausal women in Poland (23). In addition, when stratifying by birth weight, Polish women who were relatively fat babies did not exhibit ovarian suppression in response to



moderate levels of physical activity at adulthood, in contrast to women who were skinnier babies (22). This suggests that ovarian responsiveness may depend on conditions during fetal life. In comparison, in the present study among Norwegian women, we observed that women with the most unfavourable metabolic profile (highest tertile zMS) had higher level of E<sub>2</sub> throughout the menstrual cycle corresponding to a higher heart rate and lower leisure activity, compared to other women. This result suggests that ovarian responsiveness also may interact with normal physiology throughout adulthood of importance for premenopausal E<sub>2</sub> levels throughout an entire menstrual cycle. In the present study, we did not observe a clear association between level of E<sub>2</sub> and level of LPA, or between level of E<sub>2</sub> and HR as a single factor, even when stratifying by birth weight. These findings suggest that Norwegian and Polish women may have different ovarian responsiveness to physical activity or that other factors may have an influence.

**“Paragraph Number 36”** The evidence for an inverse relationship between physical activity and breast cancer is stronger for postmenopausal than for premenopausal women (34). The women in our study were premenopausal, and there is no clear reason why physical inactivity is often less strongly related to increased risk of breast cancer among premenopausal women. The main source of E<sub>2</sub> in premenopausal women is the ovaries, although it is also produced in adipose tissue. For postmenopausal women the main site for E<sub>2</sub> production is adipose tissue. Thus, physical activity may influence premenopausal and postmenopausal breast cancer risk differently through different physiological and hormonal mechanisms, as seen with obesity (9). To elucidate in detail the influence of physical activity, it might be necessary to do subgroup analyses of birth characteristics, obesity, and parity which more often is performed and underlined as well as different aspects of the activity: type, intensity, frequencies, duration and age at exposure (10).

**“Paragraph Number 37”** Our study has several strengths, including daily saliva sampling for estimation of daily E<sub>2</sub> concentrations through an entire menstrual cycle. We used well-developed and validated methods and assays to characterize the women’s exposure to free, biologically active, ovarian steroids and performed comparisons of levels by aligned cycle days (31). This recommended approach, of examining all women at the same time during the menstrual cycle, is rarely achieved because of its logistic complexity (24). However, it is a major strength of this study, given the large intracycle fluctuations in levels of ovarian hormones and the wide inter-individual variation in cycle length in menstruating women. Furthermore, salivary levels of E<sub>2</sub> was quite stable within participants over time (8). In addition, one trained nurse traced all the participants in the study and met them in the same clinical research department at a university hospital. This standardization enhanced the quality of our data and allowed us to sample all clinical variables within the same narrow frame of the cycle for each participant, using uniform procedures. To limit any potential influence of season, women did not participate during the winter months when there is no daylight (December and January). When adjusted for potential confounders such as age at menarche, parity, birth weight, energy intake, and use of alcohol, smoking, and previous oral contraceptives, we observed only small modifications of the interrelationships of clustered metabolic risk score and level of E<sub>2</sub>, which did not change the result.

**“Paragraph Number 38”** Assessment of physical activity is difficult and self-reported physical activity is less precise than objective measurements of physical activity. In addition, we used recorded LPA data over the last year, so the effect of physical activity in different time periods of life on E<sub>2</sub> has not been examined in the current study. However, the self-reported LPA questionnaire that we used has previously been validated (32,39), found to be associated with HR

and to be a valid measure of physical fitness; 5869 women, 20 to 49 years of age, who both sustained high levels of physical activity and changed from sedentary to higher levels of physical activity, relative to sedentary women, improved their metabolic risk profiles (lipids, BMI) and HR (38), and the level of LPA and physical fitness, and HR and physical fitness were positively related (32). We used information about LPA, and not total activity, which includes habitual and occupational activity.

## **Conclusion**

**“Paragraph Number 39”** In conclusion, our data suggest that higher physical activity and lower heart rate are associated with a healthier metabolic risk profile, which furthermore is positively associated with levels of E<sub>2</sub> throughout the entire menstrual cycle among healthy premenopausal women. These associations point to possible biological pathways operating in the inverse relationship between physical activity and breast cancer, but the interactions of these possible pathways are complex. Physical activity may influence the carcinogenesis of breast cancer by having a suppressive effect on ovarian steroid hormones as well as through reduced exposure to insulin and by reducing obesity.

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## Figure legends

**Figure 1** Mean (95% CI) clustered risk score (zMS) in (a) low, moderate and high activity and in (b) tertiles of resting heart rate. The clustered risk score is a sum of the Z score to total-cholesterol:HDL-C ratio, logarithm of triglycerides, blood pressure [(systolic BP + diastolic BP)/2], logarithm of HOMA-score and total percentage tissue fat ( $n = 204$ ).

**Figure 2** Age-adjusted salivary  $17\beta$ -estradiol concentrations by cycle day in women in (a) tertiles of the heart rate and (b) different levels of self-reported leisure-time physical activity. Linear mixed models for repeated measurements.

**Figure 3.** Age-adjusted salivary  $E_2$  concentrations by cycle day in women in groups of clustered risk score, zMS [total-cholesterol:HDL-C ratio, logarithm of HOMA-score, total fat tissue % (DEXA), blood pressure [(systolic BP + diastolic BP)/2] and logarithm of triglycerides]. Linear mixed models for repeated measurements.  $N=192$ .  $P<0.0001$  for differences in  $E_2$  concentration by cycle day of women in the highest tertile of zMS and cycles of women in the other tertiles (medium and lowest).



**Table 1:** Characteristic of the study population by level of self-reported leisure time activity among premenopausal women: means (SD)\* or proportions (from the Norwegian EBBA study [ $n = 204^{\dagger}$ ])

	Low activity ( $n = 32$ )		Moderate activity ( $n = 121$ )		High activity ( $n = 51$ )		$p$ for trend‡
	Mean	(SD)	Mean	(SD)	Mean	(SD)	
Age (years)	30.98	(2.88)	30.81	(2.96)	30.32	(3.45)	0.31
Age at menarche	13.16	(1.44)	13.03	(1.23)	13.29	(1.62)	0.55
Energy intake (kJ/day)	7767.34	(1670.18)	8153.36	(1928.52)	8153.92	(1979.93)	0.43
Education (total years)	16.08	(2.99)	15.61	(2.69)	17.11	(3.59)	0.05
% with children	40.63		52.07		43.14		0.97
Ethnic minority, Sami (%)	3.13		9.92		5.88		0.83
Current smokers (%)	28.13		23.97		13.73		0.10
Alcohol (units/week)	2.33	(3.14)	2.69	(3.26)	3.72	(3.74)	0.05
<i>Body composition</i>							
BMI (kg/m <sup>2</sup> )	24.90	(4.21)	24.38	(3.46)	24.14	(4.20)	0.39
Waist:hip ratio	0.78	(0.06)	0.77	(0.06)	0.77	(0.07)	0.40
Waist circumference (cm)	80.84	(10.65)	79.69	(8.87)	78.33	(11.32)	0.24
Total tissue fat (%)	36.38	(8.88)	34.61	(6.79)	31.63	(8.12)	0.003
<i>Clinical measurements</i>							
Heart rate (beats/min)	71.27	(11.48)	70.26	(11.12)	64.39	(11.43)	0.003
Systolic blood pressure (mmHg)	114.47	(10.67)	112.72	(10.36)	113.75	(13.34)	0.90
Diastolic blood pressure (mmHg)	71.36	(8.95)	70.78	(7.39)	70.84	(8.91)	0.81
<i>Saliva hormone concentrations (pmol/L)</i>							
Overall average Estradiol	17.92	(8.93)	17.20	(8.86)	19.55	(8.49)	0.29
Overall average Progesterone	121.24	(61.06)	129.31	(72.37)	137.75	(62.68)	0.28
Estradiol follicular index	18.70	(9.63)	16.91	(8.7)	19.72	(8.86)	0.45
Estradiol luteal index	18.97	(9.54)	18.20	(9.92)	19.70	(9.08)	0.66
<i>Serum concentrations§</i>							
Total cholesterol:HDL-cholesterol ratio	3.17	(0.71)	3.02	(0.81)	2.87	(0.83)	0.09
HDL-cholesterol (mmol/L)	1.45	(0.29)	1.55	(0.32)	1.57	(0.39)	0.12
Total cholesterol (mmol/L)	4.45	(0.67)	4.49	(0.77)	4.34	(0.88)	0.42
Fasting triglycerides (mmol/L)	0.90	(0.55)	0.86	(0.99)	0.87	(1.37)	0.93
Fasting glucose (mmol/L)	5.06	(0.54)	5.06	(0.62)	4.92	(0.42)	0.20
Insulin ( $\mu$ U/mL)	13.25	(10.20)	11.70	(6.35)	12.00	7.58)	0.55

\*SD, standard deviation.

†Numbers may vary as a result of missing information.

‡Linear regression or  $\chi^2$  test.

§Blood sampling first visit (days 1–5).

**Table 2** Estimated changes\* in salivary overall average 17 $\beta$ -estradiol (E<sub>2</sub>) with 95% confidence interval by 1 standard deviation (SD) increase in the subcomponents of the clustered metabolic risk score (*n* = 204)<sup>†</sup>

Variable <sup>‡</sup>	Mean (SD)		Overall average E <sub>2</sub> change		Average E <sub>2</sub> change (follicular index) <sup>§</sup>		Average E <sub>2</sub> change (luteal index) <sup>§</sup>	
Total fat tissue, %	34.2	(7.6)	1.43	(0.23, 2.63) <sup>a</sup>	1.40	(0.14, 2.66) <sup>a</sup>	1.02	(-0.34, 2.38)
<i>Insulin resistance</i>								
Glucose, mmol/L	5.02	(0.56)	0.84	(-0.39, 2.06)	0.93	(-0.33, 2.2)	0.44	(-0.90, 1.80)
Insulin, $\mu$ U/mL	12.02	(7.35)	1.00	(-0.21, 2.21)	0.82	(-0.46, 2.10)	1.34	(-0.02, 2.72)
HOMA score <sup>**</sup>	2.67	(1.71)	1.25	(0.03, 2.46) <sup>a</sup>	1.07	(-0.21, 2.35)	1.55	(0.19, 2.93) <sup>a</sup>
<i>Blood pressure</i>								
Systolic BP, mmHg	113.3	(11.2)	0.12	(-1.10, 1.34)	0.07	(-1.23, 1.37)	0.02	(-1.37, 1.41)
Blood pressure <sup>‡‡</sup>	92.07	(9.01)	-0.11	(-1.33, 1.10)	-0.15	(-1.44, 1.13)	-0.32	(-1.70, 1.06)
<i>Lipids</i>								
Total C:HDL-C ratio	3.0	(0.8)	2.46	(1.26-3.66) <sup>c</sup>	2.29	(1.01, 3.57) <sup>c</sup>	2.35	(0.97, 3.72) <sup>b</sup>
Triglycerides	0.77	(0.39)	1.62	(0.40, 2.85) <sup>b</sup>	1.49	(0.23, 2.75) <sup>a</sup>	1.60 <sup>a</sup>	(0.25, 2.96)
Heart rate, beats/min	69.0	(11.5)	1.27	(0.06, 2.47) <sup>a</sup>	1.29	(0.03, 2.55) <sup>a</sup>	1.31	(-0.04, 2.66)

\*Age adjusted regression coefficient and 95% confidence interval (CI).

<sup>†</sup>Number may vary due to missing information

<sup>‡</sup>Measurements at days 1–5 after onset of the menstrual cycle.

<sup>§</sup>Includes women with aligned cycles only, *n* = 190.

<sup>\*\*</sup>As indicators of insulin resistance we used homeostasis model assessment (HOMA), calculated as the product of fasting glucose concentration (mmol/L) and fasting insulin concentration ( $\mu$ U/mL) divided by the constant 22.5.

<sup>‡‡</sup>Blood pressure= [(systolic BP + diastolic BP)/2]

<sup>a</sup>*p* < 0.05

<sup>b</sup>*p* < 0.01

<sup>c</sup>*p* < 0.001

