

Habitual physical activity and estradiol levels in women of reproductive age

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Variation in the risk of breast cancer observed among women and among populations may be explained by variation in lifetime exposure to estrogens. The suppressive effect of exercise on estradiol levels in women is well documented, but it is unknown whether habitual (i.e. typical daily) physical activity has a similar effect. Epidemiological data suggest that physical activity is one of the few modifiable factors capable of reducing the risk of breast cancer in women. We investigated whether variation in the amount of habitual activity corresponds to variation in estradiol levels in women of reproductive age. One hundred and thirty-nine regularly menstruating women 24–37 years of age collected daily saliva samples for one complete menstrual cycle and kept a daily log of physical activity. Saliva samples were analyzed for concentration of estradiol. We observed a negative relationship between habitual physical activity and salivary levels of estradiol. Mean estradiol was 21.1 pmol/l in the low, 17.9 pmol/l in the moderate and 16.6 pmol/l in the high activity group (all pairwise differences were statistically significant at $P < 0.009$). A strong association exists between physical activity and levels of estradiol among women of reproductive age. A modern lifestyle, characterized by

reduced physical activity, may therefore contribute to a rise in the levels of estradiol produced during menstrual cycles and thus to higher cumulative lifetime exposure to estradiol, resulting in a higher risk of breast cancer. *European Journal of Cancer Prevention* 15:439–445 © 2006 Lippincott Williams & Wilkins.

European Journal of Cancer Prevention 2006, 15:439–445

Keywords: breast cancer, energy expenditure, estrogen, premenopausal women

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Sponsorship: Supported by the Polish State Committee for Scientific Research and Norwegian Cancer Society.

Received 4 May 2005 Accepted 23 August 2005

Introduction

Physical activity is one of the few modifiable risk factors for breast cancer in women. Recent case–control studies (Coogan *et al.*, 1997; Carpenter *et al.*, 1999; Friedenreich *et al.*, 2001; John *et al.*, 2003; Yang *et al.*, 2003) and prospective studies (Fraser and Shavlik, 1997; Thune *et al.*, 1997; Rockhill *et al.*, 1999) have shown that physical activity in adulthood is associated with reduced risk of breast cancer, even when levels of exercise and occupational physical activity are moderate (Thune *et al.*, 1997). More recently, risk-reducing effects of physical activity were shown in physically active BRCA1/BRCA2 mutation carriers who had delayed onset of breast cancer (King *et al.*, 2003).

Several hypotheses for why higher levels of physical activity are associated with a reduced risk of breast cancer have been proposed, including a mechanism based on a suppressive effect of physical activity on ovarian steroid hormones (Key and Pike, 1988, IARC, 2002; Kaaks and Lukanova, 2002). We recently observed that interpopulation variation in ovarian steroid levels was associated with variation in risk of breast cancer, and suggested that

differences in physical activity may be an important cause of such variation (Jasienska and Thune, 2001).

Ovarian steroid hormones, especially 17- β -estradiol (E2), have been identified as important factors in the development and prognosis of breast cancer (Henderson *et al.*, 1988; Key and Pike, 1988; Jasienska *et al.*, 2000; Clemons and Goss, 2001). Exercise and participation in sports (Rosetta, 1993), even if only of moderate intensity (Ellison and Lager, 1986; De Souza *et al.*, 1998), are associated with reduced levels of ovarian E2 and progesterone. Epidemiological studies, however, show that any physical activity, not only recreational exercise but also occupational and total physical activity, reduces the risk of breast cancer.

Although the existence of the suppressive effect of exercise on levels of ovarian hormones both in professional and recreational athletes is well documented (Ellison and Lager, 1986; Rosetta, 1993; De Souza *et al.*, 1998), such an effect in relationship to occupational activity or habitual activity has been shown in only a few

studies and only for progesterone (Panter-Brick and Ellison, 1994; Jasienska and Ellison, 1998; Jasienska and Ellison, 2004). Recently, we observed that a seasonal increase in intensity of occupational physical activity in Polish rural women corresponded to a reduction in ovarian progesterone levels by almost 25% (Jasienska and Ellison, 2004).

To our knowledge, a relationship between habitual physical activity (which is typical daily physical activity comprising of occupational work, housework, child care, walking, and exercise) and E2 levels in women of reproductive age has never been documented. The purpose of the present study was to assess whether variation in normal daily levels of physical activity can sufficiently explain observed variation in the levels of E2 produced during menstrual cycles to suggest that levels of habitual physical activity are an important factor in explaining variation in breast cancer risk.

Materials and methods

Participants

Participants of the study were urban and rural women from Poland. Urban women were recruited for the study by newspaper and television advertisements and rural women were recruited through their parish between June 2001 and June 2003. Women were selected for participation if they met the following criteria: age between 24 and 36 years, regular menstrual cycles and no fertility problems, no gynecological and chronic disorders (i.e. diabetes, hypo/hyperthyroidism), not taking any hormonal medication or using hormonal contraception during the 6 months before recruitment, and not having been pregnant or lactating during the 6 months before recruitment. Out of 154 women who collected saliva samples for an entire menstrual cycle, 15 participants were not included in the present analysis: activity data for four women were not complete enough to permit reliable calculations and reliable identification of the day of the mid-cycle E2 drop could not be made for 11 participants. Therefore, we used data from 139 women. The research protocol was approved by the Jagiellonian University Bioethical Committee.

Anthropometric measurements and general questionnaire

Participants' body weight, height, subscapular and triceps skinfolds, and percent body fat (by bioimpedance) were measured by a trained anthropologist. A general questionnaire (self-administered and by interview) was used to collect information on education, reproductive history, and past use of hormonal medication, tobacco, and alcohol.

Physical activity

Physical activity was assessed based on a pre-set daily log of physical activity that was completed by the

participants every day during the menstrual cycle. Women recorded number of hours of sleep, time of waking and number of minutes spent each day in five categories of physical activity: 'walking to and from work', 'other walking', 'physical work at home', 'physical occupational work', and 'exercise and sports participation'. Women were asked to assign the intensity level (from 1 to 4) for each of the listed activities according to provided definitions (based on changes in pulse and sweating). Period of inactivity was assessed by adding the time of sleep and time performing activities of intensity 1 to intensity 4 and subtracting the sum from 24 h (Richardson *et al.*, 2001). The category of inactivity included all activities that participants did not list as physical activity, for example, most sedentary activities at home and at work, watching television, and reading. For each class of activities the following metabolic equivalents (METs) were assigned: sleep = 1 MET; inactivity = 1.1 METs; activities of intensity 1 = 1.5 METs, activities of intensity 2 = 4 METs; activities of intensity 3 = 6 METs; and activities of intensity 4 = 10 METs (Ainsworth *et al.*, 2000; Richardson *et al.*, 2001). Mean 24 h physical activity (in MET-h/day) was calculated for each participant. Women were divided into three groups based on the tertiles of the distribution of mean 24-h physical activity: low activity, moderate activity, and high activity.

Estradiol indices and assay procedure

Women collected daily morning saliva samples for one entire menstrual cycle. Saliva samples from 20 days (reverse cycle days -5 to -24) of each cycle were analyzed for the concentration of E2 using an I-125-based radioimmunoassay kit (#39100, Diagnostic Systems Laboratories, Webster, Texas, USA) with published (Jasienska *et al.*, 2004) modifications to the manufacturer's protocol. The sensitivity of the E2 assay is 4 pmol/l. Average intraassay variability was 9%, and interassay variability ranged from 23% for lower (15 pmol/l) to 13% for higher (50 pmol/l) values. Of the 2780 potential samples, improper collection or loss during the assay procedure resulted in 5.3% of daily samples of E2 missing in the analyses. Before statistical analyses, cycles were aligned on the basis of identification of the day of the midcycle E2 drop (day 0), which provides a reasonable estimate of the day of ovulation, according to the published methods (Lipson and Ellison, 1996). E2 values from 18 consecutive days of each cycle aligned on day 0 were used in analyses. The following E2 indices were calculated: 'mean E2' (mean of days -9 to +8), 'mean mid-cycle E2' (mean of days -2 to +2), 'mean follicular E2' (mean of days -9 to -1), and 'mean luteal E2' (mean of days 0 to +8).

Statistical analysis

Differences among the low activity, moderate activity, and high activity groups in age, levels of education, tobacco smoking, alcohol consumption, anthropometric and body

composition variables, and in mean time per day spent in different categories of physical activity, and mean 24-h activity were tested in factorial, fixed model, one-way analysis of variance (ANOVA), followed by Tukey–Kramer post-hoc tests.

The effects of potential confounding factors such as age, height, and body composition variables (body weight, body mass index, percentage of body fat, and sum of skinfold thickness) on E2 indices were tested in multiple regression analyses. We also performed multiple regression analyses testing the effects of mean physical activity, body fat percentage, number of smoked cigarettes, and units of consumed alcohol. Separate analyses were performed with mean E2, mean mid-cycle E2, mean follicular E2, and mean luteal E2 as dependent variables.

The effects of physical activity on indices of E2 were tested by factorial, three-way ANOVA analysis. Group division into low activity, moderate activity, and high activity was used as one factor and, days of menstrual cycle as the second factor (with 18 levels). The third factor was represented by the tertiles of body fat percentage: low fat, moderate fat, and high fat. Body fat percentage was used as a factor in this analysis because multiple regression analyses showed that this variable had a significant effect on some E2 indices (see Results); in addition, we recently documented that women with high body mass index had higher E2 levels than slimmer women (Furberg *et al.*, 2005). ANOVA was followed by contrast analyses; an α level of 0.0167 (with the Bonferroni correction) was used to indicate statistical

significance. To control for potential confounders, we repeated ANOVA analyses adding smoking to the model (women were assigned to two groups based on current smoking status).

Results

General characteristics and body composition

General characteristics of all study participants and characteristics of the three activity level groups are presented in Table 1. Women from groups with low activity, moderate activity, and high activity did not differ in age, menarcheal age, height, body weight, body mass index, percentage of body fat, and thickness of subscapular and triceps skinfolds. They also did not differ in mean number of cigarettes smoked. These groups, however, show significant variation in the education, the age of first full-term pregnancy, proportion of women who had full-term pregnancy, and mean number of children. The group with high activity had more children than the other groups ($P < 0.0001$). In a covariance analysis stratified by parity with mean daily physical activity as the covariate, the four groups of women with different parity (nulliparous women, $n = 74$; women with one child, $n = 28$; women with two children, $n = 33$; women with three or more children, $n = 14$) did not differ in mean E2 levels ($F_{3,135} = 1.28$, $P = 0.28$; all pairwise differences were statistically insignificant).

Physical activity

The mean time (in minutes) per day spent in different categories of physical activity is presented in Table 2. Groups of low activity, moderate activity, and high activity

Table 1 Characteristics of all study participants together and divided into three groups with low, moderate, and high physical activity

	All women $n = 139$, mean (range)	Low activity group $n = 47$, mean (range)	Moderate activity group $n = 48$, mean (range)	High activity group $n = 44$, mean (range)	P for trend
Age (years)	29.9 (24–37)	29.7 (24–35)	29.8 (24–36)	30.0 (25–37)	0.89
Education, total (years)	15.5 (8–24)	16.6 (9–20)	15.9 (8–24)	13.5 (8–19)	0.0001
Full-time employment (years)	4.3 (0–15)	4.0 (0–14)	4.7 (0–14)	4.0 (0–15)	0.68
Alcohol consumption – teetotaler (%) ^a	4.4	2.2	2.2	9.1	0.22
Alcohol consumption (units/week)	1.8 (0–10.8)	1.8 (0–8.3)	2.0 (0–10.8)	1.2 (0–6.7)	0.12
Ever smokers (%) ^a	32.2	28.3	36.4	29.5	0.68
Current smokers (%) ^a	19.2	13.0	17.8	20.4	0.40
Number of cigarettes	9.0 (0–20)	6.7 (0–17)	9.4 (0–20)	10.0 (0–20)	0.47
Age at menarche (years)	13.4 (10–18)	13.2 (10–16)	13.6 (10.5–18)	13.4 (11–16)	0.29
Ever had a full-term pregnancy (%) ^a	50.0	42.5	37.5	84.1	0.0001
Age at first full-term pregnancy (years)	23.2 (18–33)	23.7 (20–28)	24.9 (20–33)	22.2 (18–29)	0.01
Number of children	0.97 (0–6)	0.6 (0–3)	0.6 (0–3)	1.8 (0–6)	0.0001
Height (cm)	162.9 (147.3–182.7)	163.2 (152–182.7)	163.0 (147.3–182)	162.2 (151.2–177.8)	0.26
Body weight (kg)	61.3 (42–99.5)	59.5 (42–93.7)	61.8 (44.5–82.1)	62.5 (47.4–99.5)	0.32
Body mass index (kg/m ²)	23.11 (17.2–38)	22.3 (17.9–38)	23.2 (17.9–30.8)	23.8 (17.2–37.3)	0.17
Body fat (%)	27.2 (9.1–45.5)	26.2 (9.1–44.4)	27.4 (12.3–41.8)	27.8 (11.8–45.5)	0.52
Subscapular skinfold (mm)	13.8 (5.5–37.3)	13.2 (6.9–27.8)	13.7 (6.7–26.5)	14.1 (5.5–37.3)	0.74
Triceps skinfold (mm)	18.3 (4.6–33.6)	18.6 (5.9–32.1)	18.6 (9.1–33.6)	17.4 (4.6–33.4)	0.57
Self-reported usual length of menstrual cycles (days)	29.2 (24–42)	29.2 (24–42)	29.6 (24–42)	28.9 (24–40)	0.53
Length of menstrual cycle during sample collection (days)	28.4 (22–38)	28.5 (23–38)	28.9 (22–37)	28.0 (22–37)	0.42
Mean estradiol (pmol/l)	18.7 (6–62)	21.1 (6–62)	17.9 (6–40)	16.6 (6–36)	0.0001
Mean mid-cycle estradiol (pmol/l)	22.1 (6–65)	25.2 (6–65)	21.5 (6–44)	19.5 (7–48)	0.0001
Mean follicular estradiol (pmol/l)	19.2 (10.66)	21.6 (17.44)	18.0 (12.57)	17.3 (12.68)	0.0001
Mean luteal estradiol (pmol/l)	18.1 (4–51)	20.6 (4–51)	17.8 (5–43)	15.8 (4–34)	0.0001

^aDifferences among groups were tested by G-squared tests. Differences among groups in all other variables were tested by analysis of variance.

Table 2 Time (in min/day) spent in different categories of physical activity

	All women, mean (range)	Low activity group, mean (range)	Moderate activity group, mean (range)	High activity group, mean (range)	<i>P</i> for trend
Walking to and from work ^a	10.7 (0–44)	11.7 (0–44)	11.5 (0–36)	8.7 (0–36)	0.37
Other walking ^a	48.0 (0–204)	41.3 (0–144)	52.8 (0–159)	49.8 (0–204)	0.89
Occupational work ^a	103.1 (0–611)	39.7 (0–389)	88.9 (0–326)	184.4 (0–611)	0.0001
Housework ^a	111.4 (0.3–776)	53.4 (0.3–227)	97.29 (2–362)	187.3 (4–776)	0.0001
Sport/exercise ^a	10.9 (0–86)	6.9 (0–42)	15.5 (0–86)	9.9 (0–80)	0.04
Mean 24-h activity ^b	41.5 (27–89)	31.9 (27–35)	38.7 (35–43)	54.9 (43–89)	0.0001

^aIn minutes per day.^bIn metabolic equivalent-hour per day.**Table 3 Differences in physical activity among groups with different body fat percentage**

	Low fat group <i>n</i> =47, mean (range)	Moderate fat group <i>n</i> =49, mean (range)	High fat group <i>n</i> =43, mean (range)	<i>P</i> for trend
Walking to and from work ^a	11.8 (0–35)	11.4 (0–44)	9.4 (0–36)	0.47
Other walking ^a	52.4 (0–150)	33.2 (0–128)	46.3 (0–160)	0.17
Occupational work ^a	74.6 (0–426)	124.1 (0–352)	127.8 (0–611)	0.18
Housework ^a	77 (2–305)	132.6 (0.4–467)	125.9 (0.3–776)	0.02
Sport/exercise ^a	16.5 (0–86)	10.5 (0–60)	6.5 (0–51)	0.03
Mean 24-hour activity ^b	38.4 (29–70)	43.3 (27–73)	42.9 (27–89)	0.06

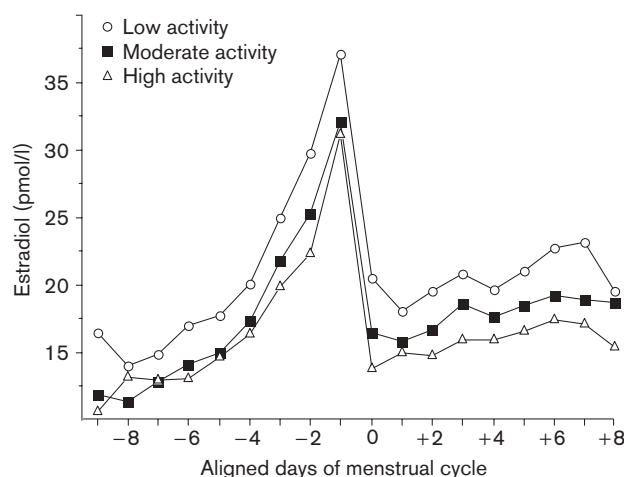
^aIn minutes per day.^bIn metabolic equivalent-hour per day.

did not differ in mean time of walking to and from work and in mean time of other walking. The three groups differed marginally in mean time performing sport and exercise-related activities. The moderate activity group spent more time in sport/exercise than the low activity group ($P = 0.01$), but all other differences in sport/exercise among groups were not significant. Groups differed in time spent in housework and in occupational work. All pairwise differences among groups are statistically significant for these two categories of physical activity.

We detected some variation in indices of physical activity between the low fat group and the two other fat groups (Table 3). Mean levels of 24-h physical activity in the low fat group were lower than in the two other 'fat' groups (difference between low fat and moderate fat groups was statistically significant, $P = 0.046$ and the difference between the low fat and high fat groups was approaching significance, $P = 0.06$). Women from the low fat group spent, on average, significantly fewer minutes a day in housework than women from the two other groups. Women from the low fat group reported more minutes a day in sport/exercise activity than other women (Table 3). All differences in indices of physical activity between the moderate fat and the high fat groups were statistically insignificant.

Relationships between age, body composition, smoking, alcohol consumption, and estradiol indices

All multiple regression models testing potential effects of age, height, and body composition variables on E2 indices showed statistically insignificant results (for mean E2, $R^2 = 0.07$, $P = 0.22$; for mid-cycle E2, $R^2 = 0.05$, $P = 0.31$; for follicular E2, $R^2 = 0.029$, $P = 0.7$; for luteal E2, $R^2 = 0.07$, $P = 0.13$). None of the tested variables had

Fig. 1

Profiles of mean E2 for groups with low, moderate, and high levels of physical activity. Confidence intervals were omitted for clarity.

a significant effect on E2 indices, except for body fat percentage, which had a significant effect in two models: on mean E2 ($P = 0.042$) and on luteal E2 ($P = 0.014$).

Relationships between physical activity and estradiol indices

A significant variation was observed among groups of low activity, moderate activity, and high activity in mean E2 ($F_{2,2125} = 27.58$, $P = 0.0001$) (Fig. 1). Contrast analyses showed that all among-group differences were statistically significant (low activity vs. moderate activity and low activity vs. high activity, $P = 0.0001$; moderate activity vs. high activity, $P = 0.008$). Differences between

low activity vs. moderate activity groups and between low activity vs. high activity groups were statistically significant for follicular E2 ($P = 0.0009$ and $P = 0.0003$, respectively), for luteal E2 ($P = 0.004$ and $P = 0.0003$, respectively), and for mid-cycle E2 ($P = 0.01$ and $P = 0.0003$, respectively).

An ANOVA with smoking added as an additional factor showed that groups with variation in physical activity varied in E2 levels ($P = 0.002$). In this model, smokers and nonsmokers also varied in E2 (smokers had higher E2 than nonsmokers, 21.9 and 17.9 pmol/l, respectively, $P = 0.006$). Contrast analyses, however, revealed that only in the low activity group did smokers and nonsmokers vary significantly in E2 levels ($P = 0.002$); in the moderate activity and in the high activity groups, differences between smokers and nonsmokers were not statistically significant ($P = 0.11$ and $P = 0.95$, respectively).

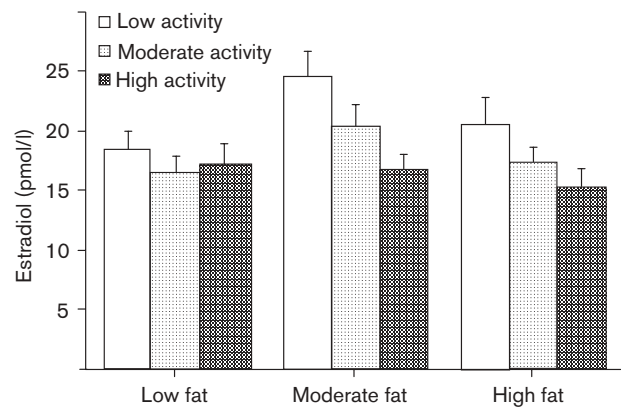
Multiple regression analysis testing the effects of mean physical activity, body fat percentage, number of smoked cigarettes, and units of alcohol consumed on mean E2 showed that only physical activity had a significant negative effect on mean E2 ($P = 0.04$). The impacts of alcohol consumed and body fat were insignificant ($P = 0.42$ and $P = 0.37$, respectively). The number of cigarettes smoked had a borderline positive significance ($P = 0.06$).

Groups with low fat, moderate fat, and high fat differed in mean E2 levels: the moderate fat group had mean E2 of 20.2 pmol/l, whereas the low fat group had mean E2 of 17.5 pmol/l, and the high fat group had mean E2 of 17.8 pmol/l ($F_{2,2125} = 16.25$, $P = 0.0001$). Contrast analyses showed that within the low fat group, there were no significant differences in E2 levels in relationship to differences in physical activity (Fig. 2). In the moderate fat and high fat groups, however, almost all differences among physical activity levels were statistically significant. The moderate fat, low activity group had higher E2 than both the moderate fat, moderate activity group ($P = 0.0005$) and the moderate fat, high activity group ($P = 0.0001$). The moderate fat, moderate activity group had higher E2 than the moderate fat, high activity group ($P = 0.0005$). Within the high fat group, the high fat, low activity group had higher E2 than both the high fat, moderate activity group ($P = 0.002$) and the high fat, high activity group ($P = 0.0001$).

Discussion

We document a significant negative relationship between the levels of habitual physical activity and the concentration of salivary E2, after adjustments for potential confounding factors. Mean levels of E2 varied significantly in women from the highest, middle, and lowest

Fig. 2



Mean (with 95% confidence intervals) E2 levels in groups of women with low, moderate, and high percentage of body fat. Each group was further divided by level (low, moderate, or high) of physical activity.

tertiles of physical activity. The low activity group had mean E2 levels 21% higher than the high activity group and almost 18% higher than the moderate activity group. When levels of E2 during the luteal phase of the cycle were compared, the group with low activity had more than 30% higher E2 than the high activity group.

Although a high incidence of menstrual disturbances in female endurance athletes has been well documented (Rosetta, 1993), even exercise of moderate intensity has been shown to reduce levels of ovarian steroid hormones (Ellison and Lager, 1986; De Souza *et al.*, 1998). A recent study showed that moderate levels of leisure-time physical activity enhance estrogen metabolism, especially among women with higher body weight (Matthews *et al.*, 2001). The suppressive effect of sports participation and exercise may explain the findings of epidemiological studies showing that women with higher levels of recreational activity have a lower risk of breast cancer. Results of many case-control (Coogan *et al.*, 1997; Carpenter *et al.*, 1999; Friedenreich *et al.*, 2001; John *et al.*, 2003; Yang *et al.*, 2003) and prospective studies (Fraser and Shavlik, 1997; Thune *et al.*, 1997; Rockhill *et al.*, 1999), however, show that not only sports and exercise, but also occupational activity and total physical activity are related to reduced risk of breast cancer. The mechanism for such an effect has been unclear, because there were no data showing that these types of activity have a suppressive effect on levels of E2 in premenopausal women. To date, a suppressive effect of occupational physical activity was shown only in relation to ovarian progesterone levels (Panter-Brick and Ellison, 1994; Jasienska and Ellison, 1998; Jasienska and Ellison, 2004).

Factors that could have a potential effect on E2 levels do not seem to confound the observed relationship between

physical activity and levels of E2. Even though there was a higher percentage of women who smoked in the high activity group than in the other two activity groups, smoking was unlikely to be responsible for the reduction in the levels of E2, because in our sample, smokers had higher levels of E2 than women who did not smoke and, furthermore, the difference in E2 between smokers and nonsmokers was significant only in the low activity group.

In our study, mean body fat showed a significant interaction with physical activity. Although in the moderate fat and high fat groups, the impact of physical activity on E2 levels is evident (Fig. 2), in the low fat group, variation in physical activity was not related to variation in E2 levels. Possible explanations of this interaction include (a) insufficient levels of activity in the low fat group for an activity effect to be expressed; (b) a suppressive effect of physical activity on extra-gonadal estrogen production by adipose tissue that is not expressed in the low fat group; and (c) a suppressive effect of activity on gonadal estrogen production that is mediated in part by a correlate of fatness such as insulin production.

The results of this study clearly suggest that suppressed levels of E2 associated with habitual physical activity may be one of the mechanisms explaining why higher habitual activity corresponds to a reduced risk of breast cancer in women. Other mechanisms, such as enhanced immune function and changes in metabolic hormones and growth factors, have been suggested to mediate a relationship between physical activity and breast cancer (Hoffman-Goetz *et al.*, 1998, IARC, 2004). Changes in insulin, glucose, insulin-like growth factors and their binding proteins, and alterations in immune response, however, were shown to occur in association with sports and exercise (McCarty, 1997), whereas the occurrence of such changes with other types of physical activity still needs to be demonstrated. In our study, participation in sports and exercise activity was generally low (Table 2). Our study groups differed, on average, only marginally in time spent in exercise and sports activities, and furthermore, these differences cannot explain the observed differences in E2 levels, because the moderate activity group reported spending more time in sports and exercise than the other two groups. Differences among study groups in physical activity resulted mostly from variation in housework and especially in occupational work. Mean activity reported by our participants was comparable, although slightly higher than previously published values. Richardson *et al.* (2001) reported the mean total activity of studied women to be 2231 MET-min a day, whereas in our study it was 2492 MET-min a day.

Finding that higher habitual activity is associated with reduced levels of E2 in women of reproductive age may

explain variation in breast cancer incidence among both individual women and among populations.

Acknowledgements

We are grateful to the women who participated in this study, to students of the Faculty of Public Health, Jagiellonian University who worked as research assistants, to Dr Mary O'Rourke from Harvard University, and to Rev. Jozef Puchala of the Jurkow parish in Poland.

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