Usual Physical Activity and Endogenous Sex Hormones in Postmenopausal Women: The European Prospective Investigation into Cancer–Norfolk Population Study

Mei-Fen Chan,[1] Mitch Dowsett,[4] Elizabeth Folkerd,[4] Sheila Bingham,[2] Nicholas Wareham,[3] Robert Luben,[1] Ailsa Welch,[1] and Kay-Tee Khaw[1]

[1]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge School of Clinical Medicine; [2]Dunn Human Nutrition Unit and [3]Epidemiology Unit, Medical Research Council, Cambridge, United Kingdom; and [4]Academic Department of Biochemistry, Royal Marsden Hospital, London, United Kingdom

Abstract

Background: **Short-term trials indicate that intensive physical activity may influence endogenous sex hormone concentrations. However, the relationship between usual daily physical activity and endogenous hormones in postmenopausal women in the general population is still uncertain.**
Objective and Methods: **To determine the relationship between usual physical activity and endogenous sex hormones in postmenopausal women. A cross-sectional population-based study of 2,082 postmenopausal women ages 55 to 81 years, residing in the general community of Norfolk, United Kingdom, and not currently using hormone replacement therapy were chosen to participate. Physical activity in the past 1 year was assessed using a validated questionnaire, and endogenous sex hormone and sex hormone–binding globulin (SHBG) concentrations were determined.**
Results: **Usual physical activity levels were inversely associated with circulating concentrations of testosterone and estradiol, testosterone/SHBG ratio, and positively associated with SHBG. These associations were only slightly attenuated after adjusting for potential covariates including body mass index, smoking status, alcohol intake, and reproductive variables. Testosterone concentrations and testosterone/SHBG ratios were 19% [95% confidence interval (95% CI), 9-27%, $P < 0.001$] and 24.0% (95% CI, 13-34% $P < 0.001$) lower, respectively, whereas estradiol concentrations were 6% (95% CI, 0-12%; $P < 0.05$) lower in the highest compared with lowest activity levels, respectively. A decreasing trend for the estradiol/SHBG ratio and 17α-hydroxyprogesterone concentrations was also observed. Androstenedione levels did not differ significantly according to physical activity.**
Conclusions: **Higher usual physical activity levels among postmenopausal women seem to be related to lower endogenous testosterone and estradiol concentrations. This may be one mechanism that could partly explain the reported inverse relationship between physical activity and breast cancer risk in some studies.** (Cancer Epidemiol Biomarkers Prev 2007;16(5):900–5)

Introduction

Sex hormones have well documented biological effects. Exogenous postmenopausal estrogen and progestin supplementation has been extensively explored in observational studies and recently reported in a randomized clinical trial, the Women's Health Initiative, to increase cardiovascular disease and breast cancer risk (1). Higher endogenous estrogen and androgen concentrations have also been associated with an increased risk of mammary gland cancer in animal studies (2), and breast cancer in both premenopausal and postmenopausal women (3, 4).

Physical activity is associated with lower risk of several diseases including cardiovascular disease and breast cancer (5, 6). Observational studies suggest a protective relationship between increasing physical activity and breast cancer risk (7-9) among premenopausal and postmenopausal women. The magnitude of the risk reduction ranged from 10% to 70% for the most active women, and was, on average, between 30% and 40% for women who exercised for 3 to 4 h per week at moderate to vigorous levels compared with sedentary women (7), with a dose-response relationship (8).

Plausible mechanisms by which physical activity may modulate disease risk are through effects on hormone-related pathways, energy balance, and obesity, among others (10). A recent randomized clinical trial of overweight postmenopausal women reported that women who were in a 12-month moderate intensity physical activity intervention in which there was a 2% or more loss of body fat, had a significantly greater reduction of circulating estrogens and androgens compared with a control group doing stretching exercises (11, 12). It was not clear how far the reduction in hormone concentrations was explained by the reduction in body fat or by other possible mechanisms. However, less is known about the effects of physical activity within habitual activity ranges on endogenous sex hormone levels in postmenopausal women. Previous cross-sectional studies in postmenopausal women from the general population have reported inconsistent associations between usual physical activity and hormone levels (13-17).

We investigated whether differences in endogenous sex hormone concentrations could be observed within the reference range of usual physical activity in a large free-living general population of postmenopausal women.

Received 9/7/06; revised 2/7/07; accepted 2/19/07.

Grant support: EPIC-Norfolk is supported by programme grants from the Cancer Research UK and Medical Research Council, with additional support from the Stroke Association, Research into Ageing, British Heart Foundation, and the Academy of Medical Science.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Statement of significance: Usual physical activity in the range found in free-living postmenopausal women in the general community was associated with lower endogenous testosterone and estradiol concentrations independently of known covariates. Because these endogenous hormone hormones have been associated with disease risk, most notably, breast cancer risk, this might provide a plausible mechanism through which physical activity influences disease risk.

Requests for reprints: Kay-Tee Khaw, Clinical Gerontology Unit Box 251, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge CB2 2QQ, United Kingdom. Phone: 44-1223-217292; Fax: 44-1223-336928. E-mail: kk101@medschl.cam.ac.uk

doi:10.1158/1055-9965.EPI-06-0745

Materials and Methods

Study Population. The European Prospective Investigation into Cancer (EPIC)–Norfolk is a population-based prospective cohort of 25,663 men and women ages 40 years and above who were recruited between 1993 and 1997 from the general population in Norfolk, United Kingdom, detailed elsewhere (18), and part of an international 10-country collaboration investigating diet and cancer. Participants completed a detailed baseline questionnaire on their health status, medical history, and lifestyle and reproductive factors. They also attended a health examination conducted by trained nurses who obtained anthropometric measurements based on a standard protocol. Between 1997 and 2000, ~16,000 of the original cohort attended a second health examination with repeat questionnaire self-completion and health measurements. From participants in this second visit, 2,114 women were selected at random from the subset of women older than 55 years who were postmenopausal for 1 year or more and had not been on hormone replacement therapy (HRT) for the 3 months prior to the study. Nonfasting blood samples were taken and plasma and sera stored at −80°C until analysis. These women formed the basis of our study. Ethical approval of the EPIC-Norfolk Study was obtained from the Norwich Local Research Ethics Committee, and all participants gave written informed consent.

Physical Activity. The EPIC physical activity questionnaire (EPAQ2) used in the study cohort has been validated and tested for repeatability (19). The self-completed questionnaire obtains information on the participants' physical activity in three major domains: the home, at work, and during recreation over the past 12 months (http://www.srl.cam.ac.uk/epic/questionnaires/epaq2/). The participants' energy expenditure was assessed from the frequency, intensity, and duration per episode of self-reported physical activity in these domains. The questions, which comprised ordered categories of continuous variables on activities, were context-specific. An example with regard to walking is, rather than asking participants to estimate how far they had walked, the questionnaire asked participants to recall the time they had spent walking in different sections of their life at and around home, to work, at work, and walking for leisure. With regard to each activity, participants ticked one of the following categories: ''no times in the last 12 months'', ''less than once a month'', ''once per month'', ''two to three times a month'', ''once a week'', ''two to three times per week'', ''four to five times a week'', or ''six or more times a week.'' Replies obtained from all questions were aggregated based on the dimension of physical activity of interest. Energy expenditure at home, work, and during recreation were calculated by multiplying participation (h/wk) by the metabolic cost of each activity expressed in metabolic equivalents (MET) obtained from published articles, one MET being the ratio of the energy cost of a given activity to resting metabolic rate. Collectively, participants were then divided into four quartiles of physical activity index groups with level 1 defined as the least active group, progressively increasing to level 4, the highest.

The questionnaire had been previously validated using total energy expenditure as assessed by heart rate monitoring of participants with individual calibration, a method which is highly correlated with the gold standard method of whole body calorimetry and doubly labeled water. The validation process comprised 4-day heart rate monitoring of participants on four separate episodes over a 1-year period. Positive age- and sex-adjusted associations were found between the sum of self-reported recreational and occupational physical activity reported and objectively measured daytime energy expenditure ($r = 0.28$, $P < 0.001$). Self-reported time spent on vigorous activity correlated with cardiorespiratory fitness ($r = 0.16$, $P < 0.05$). Repeatability was high ($r = 0.73$).

Other Lifestyle and Reproductive Factors and Anthropometric Measures. Smoking status was derived from replies to the questions ''Do you smoke cigarettes?'' and ''If you have stopped smoking, how old were you when you gave up?''. Based on these responses, smokers were then categorized as ''current-smokers'', ''former-smokers'', and ''never-smokers''. Alcohol consumption was based on the question ''At present, about how many alcoholic drinks do you have each week?''. Participants quantified the number of pints of beer, cider or lager, number of glasses of wine, sherry, whisky, and other liquor consumed each week, which were then converted to units of alcohol per week. Half a pint of beer, cider, lager, and one glass of wine, spirits, and sherry were equivalent to one unit of alcohol (8 g by weight).

To determine age at menarche, participants were asked: ''How old were you when you had your first menstrual period?''. Age at first birth was ascertained based on their response to ''Have you had any children?'', and if ''yes'', the date of birth of each child was recorded. Parity was calculated based on the number of children recorded. Menopausal status was determined by the questions ''Are you still menstruating?'', ''If NO, how old were you when you stopped having periods?'' and ''How many periods have you had in the past 12 months?''. ''Menopause'' status was defined as not having had any periods in the last 12 months. Past oral contraceptive use was based on participants' responses to the questions: ''Have you ever taken 'the pill'?'' and ''If you are no longer taking 'the pill', at what age did you stop?''. Similar questions were asked for HRT. From the answers, the categories: ''ever'' and ''never'' users of oral contraceptives and HRT were determined.

The participants' height and weight in light clothing without shoes were measured with a free-standing stadiometer and calibrated digital scale (Salter) to the nearest 0.1 cm and 100 g, respectively. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

Sex Hormone Analysis. This is detailed elsewhere (20). Nonfasting plasma samples were used for the determination of estradiol, testosterone, sex hormone–binding globulin (SHBG), 17α-hydroxyprogesterone (17OHP), androstenedione, and estrone sequentially. More than 94% of these participants had sufficient plasma or serum to complete estradiol, testosterone, 17OHP, and SHBG. Seventy-one percent of the participants had sufficient plasma or serum for androstenedione measurements, and 59% of the participants had sufficient plasma or serum for estrone measurements.

Estradiol was measured using RIA after ether extraction, and estrone by RIA after extraction with ether and liquid column chromatography on a Lipidex 5000 (Perkin-Elmer) with elution using chloroform/hexane/methanol (50:50:1). The within- and between-batch coefficients of variation of estradiol were 8.6% and 13%, respectively, at a concentration of 18 pmol/L, and the sensitivity limit was 3.0 pmol/L. Within- and between-batch coefficients of variations for estrone were 14% and 22% at 55 pmol/L, and sensitivity limit was at 15 pmol/L.

Testosterone was measured using solid-phase RIA kit (Diagnostic Products). Within- and between-batch coefficients of variation were 6.1% and 10%, respectively, at a concentration of 3.1 nmol/L, and the sensitivity limit was 0.14 nmol/L. Androstenedione was estimated using solid-phase RIA. Within- and between-batch coefficients of variation were 5.6% and 11%, respectively, at a concentration of 3.5 nmol/L, and a sensitivity limit of 0.1 nmol/L. 17OHP was measured by RIA. Within- and between-batch coefficients of variation were 4.2% and 6.2%, respectively, at a concentration of 1.5 nmol/L and the sensitivity limit was 0.06 nmol/L. SHBG was measured by a liquid-phase immunoradiometric kit (Orion Diagnostica). Within- and between-batch coefficients

of variation were 2.1% and 7.4%, respectively, at a concentration of 11 nmol/L, and a sensitivity limit of 0.5 nmol/L.

Data Analyses. As the distributions of sex hormones and SHBG concentrations were skewed, they were log-transformed to approximate the normal distribution. Extreme outliers >4 SD from the mean were removed for estradiol, estrone, 17OHP, and testosterone measurements. ANOVA was used to compare means across hormone quartiles for continuous variables including age and BMI, and the χ^2 test for frequencies for lifestyle and reproductive factors.

Mean hormone and SHBG levels were adjusted for age, and age and BMI in multivariate linear models, with additional adjustment for known and potential lifestyle and reproductive covariates (21, 22). These included age at menarche (continuous), age at menopause (continuous) and parity (continuous), smoking status (categorical with current-, former-, and never-smokers), past use of oral contraceptive (categorical with ever, never), and past HRT (categorical with ever, never). Log mean of hormones and SHBG and their 95% confidence intervals (95% CI) were transformed back to their original scale, producing geometric means and their 95% CIs. For all analyses, a two-sided P test was used, taking the level of statistical significance as <0.05. Linear regression modeling was conducted and the hormone concentration of the highest to lowest activity levels with their 95% CI were assessed. The naturally log-β coefficients of hormones were reconverted back to the original coefficient by taking its exponential function, e^{β}. This was used to estimate the multivariate adjusted percentage difference between the highest and lowest activity groups.

Results

The descriptive characteristics of 2,082 postmenopausal women with hormone measures and physical activity data are in Table 1. Women who were least active were more likely to be older (69.0 years at level 1, and 61.7 years at level 4 of physical activity) with slightly higher BMI compared with women who were most active (27.1 versus 26.7 kg/m^2). They were also less likely to have used oral contraceptive or HRT in the past. No significant differences were found between differing physical activity levels with respect to smoking status, age at menarche and menopause, or parity. Least active women had the lowest alcohol consumption intake. They were also more likely to be older at the age at first birth.

Increasing physical activity levels were significantly associated with decreasing age-adjusted concentrations of circulating estradiol and testosterone, and decreasing ratios of estradiol/SHBG and testosterone/SHBG (Table 2). Differences in estradiol concentrations were partly explained by BMI differences. SHBG concentrations followed a U-shaped curve (P quadratic trend = 0.03; data not shown) with the highest concentrations at the highest activity level. The difference in estradiol/SHBG ratio between different physical activity levels was also partially explained by BMI (P = 0.03) and no longer statistically significant after adjusting for other covariates (P = 0.25) although the decreasing trend was still apparent but borderline (P linear trend = 0.07; data not shown). Testosterone, testosterone/SHBG and SHBG concentrations were only slightly attenuated but remained significant after adjusting for BMI and further for covariates. Concentrations of 17OHP were not significantly different across increasing activity levels, although there was a significant decreasing linear trend (P = 0.03; data not shown).

Testosterone concentrations and testosterone/SHBG ratios in the highest physical activity level group were 81% (95% CI, 73-91%) and 74% (95% CI, 66-87%) that of the lowest activity level group, respectively; estradiol concentration was 0.94 (95% CI, 88-100%).

Discussion

In this cohort of postmenopausal women who were not currently using exogenous hormones, we found an inverse relationship between usual physical activity levels and circulating concentrations of estradiol, testosterone, and the testosterone/SHBG ratio, and a U-shaped association with SHBG concentrations. These associations were only slightly attenuated after adjusting for additional potential covariates including lifestyle and reproductive factors. The estradiol/SHBG ratio also decreased with higher physical activity levels. A significant decreasing trend for 17OHP concentrations was also observed.

The significant differences observed were greatest and most consistent for total testosterone and testosterone/SHBG with ~19% and 24% lower geometric mean levels in the highest compared with the lowest physical activity group. Estradiol and 17OHP were ~6%, and 9% lower in the highest compared with the lowest physical activity groups, respectively. Although the cross-sectional design of our study does not allow us to infer a causal relationship between physical activity and hormone levels, our observations are nevertheless consistent with the results from the randomized clinical trial by McTiernan et al. of the effects of a moderate-intensity physical activity intervention comprising outdoor walking, treadmill, and stationary bicycling on endogenous hormones compared with the control group of stretching exercises in postmenopausal women (11, 12). In their study of overweight postmenopausal women not on HRT, overweight women in the exercise intervention group who lost >2% body fat had significant reductions of testosterone and free testosterone levels of 10.1% and 12.2%, respectively, between baseline and at 12 months of the intervention compared with declines of 1.6% and 8.0% among controls (11). Smaller decreases (0.5-2%) in body fat among exercisers also produced significant declines of testosterone and free testosterone levels between 4.7% and 10.4% compared with 2.8% and 4.3% among controls. Given similar levels of body fat loss, women randomized to the 12-month exercise program had greater declines in testosterone and bioavailable testosterone. Although not significant, effects in a similar direction were reported for androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulfate with greater declines in exercisers than controls. Exercisers also had significant declines of 3.8%, 7.7%, and 8.2% in estrone, estradiol, and free estradiol levels, respectively, at 3 months of the intervention compared with controls; the effect being limited to women who had lost body fat (12).

These results were also consistent with some previous reports (13, 14, 16, 17). Cauley et al. found a negative relationship between estrone and estradiol levels and physical activity (16). Nelson et al. reported that compared with sedentary postmenopausal women, endurance-trained postmenopausal women had lower estrogen, specifically estrone, levels (17). Madigan et al. reported that in 125 postmenopausal women, androstenedione, estrone, and estrone sulfate were inversely associated with nonrecreational, but not with recreational, physical activity (13). Verkasalo et al. reported no differences for estradiol, SHBG, progesterone, luteinizing hormone, or follicle-stimulating hormone according to physical activity at work in postmenopausal women, although they reported that women who had >5 h a week of vigorous exercise had slightly elevated estradiol levels (14). Testosterone and androstenedione levels were not reported in this study. The Tromso study did not report any significant associations between physical activity and hormones in postmenopausal women (15). Inconsistencies in findings from different studies might reflect differences in the assessment of physical activity, and possibly, variable measurement errors in different components of physical activity.

In our study, postmenopausal women who were at physical activity level 2 (range, 18.5 to <43.5 MET h/wk) were expending an average of ~2.6 to 6.2 MET h/d and those at level 3 were expending ~6.2 to 12.4 MET h/d. In McTiernan et al.'s randomized controlled trial, participants undertook moderate-intensity sports/recreational exercise of 45 min a day for 5 days in a week over a 12-month period (11). A current recommendation by the Centers for Disease Control and Prevention and American College of Sports Medicine for adults from the United States is that they should accumulate 30 min or more of moderate-intensity physical activity defined as 3 to 6 METs, 5 or more days of the week. Although direct comparison between McTiernan et al.'s and our study is not possible because of differences in population characteristics, study design, and objectives, it is nonetheless notable that in our study of postmenopausal women in free-living conditions, the association between estrogens and physical activity seemed to be partially accounted for by BMI, although the association between testosterone and physical activity remained largely independent of BMI. The magnitude of difference in testosterone and testosterone/SHBG in the highest compared with the lowest activity group in our study was greater than that observed for the exercisers group who had lost >2% body fat at 12 months versus control group in McTiernan et al.'s study. The testosterone difference in our study of 11% (95% CI, 0-16%) comparing physical activity level 2 (equivalent to moderately intense activity) to level 1 approximates that of McTiernan's study.

One possible mechanism for the relationship between physical activity and the lower estradiol concentrations observed in postmenopausal women in our study could be a decrease in the aromatization of androgens, possibly as a result of the reduction of body fat substrate with weight loss (11, 12, 23). The higher physical activity level in our study was also associated with an increase in SHBG concentrations, supporting earlier reports (14, 24), reflecting a reduction of bioavailable active fractions.

Physiologic studies indicate that the mode and intensity of physical activity affects the circulating endogenous hormone profile (25, 26). Among premenopausal women, the mechanism by which physical activity affects steroidal sex hormones has been well studied through the examination of the effects of exercise on adrenocortical responses and the menstrual cycle, often with regard to high-intensity physical activity among women athletes. Physical activity has acute as well as longer-term effects on steroid sex hormones: proandrogens (dehydroepiandrosterone and dehydroepiandrosterone sulfate), androgens (testosterone and androstenedione), and estrogens (estradiol) through its action on the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis (9, 27-35).

The relevance of studies on physical activity and hormones in premenopausal women to postmenopausal women is unclear, where the ovaries are no longer the primary source of sex hormone production. In addition, the acute effects of high-intensity physical activity in untrained women may alter hormone levels and patterns differently from trained women, and may also differ from usual physical activity in free-living women. As in premenopausal women, physical activity also exerts effects through the hypothalamic-pituitary-adrenal axis in postmenopausal women (28). Acute bouts of physical activity among postmenopausal White and Black women ages between 51 and 61 years tested at submaximal exercise at 70% VO_2max for 30 min of aerobic exercise on a cycle ergometer resulted in increased cortisol and dehydroepiandrosterone above resting levels (36), although dehydroepiandrosterone sulfate did not change significantly with exercise. Nevertheless, there is much less evidence on the effects of usual physical activity within the reference range of physical activity on

Table 1. Descriptive characteristics by usual physical activity levels in 2,082 postmenopausal women ages 55-81 y not on HRT in EPIC-Norfolk Study (1997-2000)

	No.	Quartiles of usual physical activity				*P* for association
		1 (<18.5 MET h/wk)	2 (18.5 to <43.9 MET h/wk)	3 (43.9 to <86.8 MET h/wk)	4 (>86.8 MET h/wk)	
		Mean (SD)				
Age (y)	2,082	69.0 (6.4)	66.9 (6.2)	64.1 (6.3)	61.7 (6.2)	<0.001
BMI (kg/m^2)	2,082	27.1 (4.5)	26.6 (4.0)	26.2 (3.9)	26.7 (4.8)	0.003
Alcohol consumption (units)	2,082	3.96 (5.30)	4.38 (5.00)	5.06 (6.25)	4.08 (5.10)	0.011
Age at menopause (y)	2,077	49.8 (6.4)	49.9 (5.8)	50.0 (4.0)	49.6 (5.4)	0.84
		No. (%)				
Smoking status	2,061					
Current		61 (7.1)	46 (6.7)	26 (7.1)	21 (13.2)	0.16
Former		279 (32.7)	218 (31.9)	124 (34.1)	45 (28.3)	
Never		514 (60.2)	420 (61.4)	214 (58.8)	93 (58.5)	
Age at menarche (y)	1,985					
<13		303 (37.0)	234 (35.5)	140 (39.8)	66 (42.3)	0.32
≥13		515 (63.0)	425 (64.5)	212 (60.2)	90 (57.7)	
Age at first birth (y)	1,751					
<20		38 (5.3)	25 (4.2)	19 (6.1)	10 (7.5)	0.02
20 to <30		568 (79.3)	474 (80.2)	267 (85.9)	109 (82.0)	
≥30		110 (15.4)	92 (15.6)	25 (8.0)	14 (10.5)	
Parity	2,089					
0		149 (17.2)	96 (14.0)	53 (14.5)	28 (17.3)	0.34
1-2		463 (53.4)	356 (51.8)	194 (53.2)	88 (54.3)	
≥3		255 (29.4)	235 (34.2)	118 (32.3)	46 (28.4)	
Past oral contraceptive use	1,955					
Ever		187 (23.0)	196 (30.3)	146 (42.3)	63 (41.7)	<0.001
Never		625 (77.0)	451 (69.7)	199 (57.7)	88 (58.3)	
Past HRT use	2,080					
Ever		116 (13.4)	121 (17.6)	82 (22.5)	50 (30.9)	<0.001
Never		750 (86.6)	566 (82.4)	283 (77.5)	112 (69.1)	

Table 2. Physical activity levels and concentrations of circulating estradiol and testosterone

	No.	Increasing physical activity levels				*P* for association	% Difference, level 4/level 1 (95% CI)
		1 (0 to <18.5 MET h/wk)	2 (18.5 to <43.9 MET h/wk)	3 (43.9 to <86.8 MET h/wk)	4 (>86.8 MET h/wk)		
Estradiol (pmol/L)							
Age adjusted	2,060	17.2 (16.5-17.9)	15.3 (14.6-15.9)	15.1 (14.2-16.0)	15.4 (14.1-16.9)	<0.001	
Age and BMI adjusted	2,060	16.9 (16.3-17.5)	15.4 (14.8-16.0)	15.5 (14.7-16.4)	15.5 (14.2-16.8)	0.03	
Age and covariate adjusted*	1,803	16.8 (16.0-17.7)	15.4 (14.5-16.3)	15.9 (14.9-17.0)	15.9 (14.4-17.4)	0.02	−6% (−12 to 0)
Testosterone (nmol/L × 10^{-1})							
Age adjusted	1,940	7.53 (7.22-7.84)	6.75 (6.45-7.06)	6.38 (5.99-6.78)	6.26 (5.69-6.89)	<0.001	
Age and BMI adjusted	1,940	7.55 (7.17-7.77)	6.77 (6.47-7.08)	6.46 (6.07-6.87)	6.26 (5.70-6.88)	<0.001	
Age and covariate adjusted*	1,712	7.94 (7.49-8.42)	7.13 (6.70-7.60)	7.00 (6.49-7.55)	6.46 (5.81-7.18)	<0.001	−19% (−27 to −9)
SHBG (nmol/L)							
Age adjusted	2,066	41.9 (41.3-43.8)	40.9 (39.3-42.0)	42.1 (40.1-44.2)	47.0 (43.6-50.7)	0.01	
Age and BMI adjusted	2,066	42.5 (41.3-43.8)	40.7 (39.3-42.0)	41.2 (39.3-43.1)	47.2 (44.0-50.6)	0.001	
Age and covariate adjusted*	1,822	43.5 (41.6-45.4)	41.4 (39.6-43.4)	42.3 (39.9-44.7)	46.7 (43.2-50.5)	0.02	+7% (−1 to +16)
Androstenedione (nmol/L)							
Age adjusted	1,490	3.23 (3.04-3.37)	3.12 (2.96-3.25)	3.07 (2.87-3.27)	3.20 (2.90-3.54)	0.44	
Age and BMI adjusted	1,490	3.23 (3.10-3.36)	3.10 (2.97-3.25)	3.08 (2.89-3.29)	3.20 (2.90-3.54)	0.54	
Age and covariate adjusted*	1,320	3.24 (3.05-3.44)	3.09 (2.86-3.30)	3.18 (2.94-3.44)	3.08 (2.76-3.45)	0.54	−5% (−15 to +9)
17OHP (nmol/L)							
Age adjusted	1,980	1.09 (1.05-1.13)	1.05 (1.02-1.09)	1.04 (0.99-1.09)	0.98 (0.91-1.06)	0.07	
Age and BMI adjusted	1,980	1.09 (1.05-1.12)	1.06 (1.02-1.09)	1.04 (0.99-1.09)	0.98 (0.91-1.06)	0.09	
Age and covariate adjusted*	1,748	1.11 (1.06-1.17)	1.08 (1.03-1.14)	1.07 (1.00-1.13)	1.01 (0.92-1.10)	0.16	−9% (−17 to −1)
Estrone (pmol/L)							
Age adjusted	1,156	78.7 (75.3-82.4)	72.3 (68.8-75.9)	74.9 (70.2-79.9)	73.9 (66.3-82.3)	0.09	
Age and BMI adjusted	1,156	77.9 (74.6-81.2)	72.6 (69.2-76.1)	76.4 (71.8-81.4)	73.5 (66.2-81.5)	0.17	
Age and covariate adjusted*	1,023	76.6 (71.9-81.5)	71.1 (66.5-76.0)	75.3 (69.7-81.4)	73.3 (65.3-82.3)	0.18	−4% (−15 to +8)
Estradiol/SHBG (/10)							
Age adjusted	2,030	41.1 (38.9-43.4)	37.2 (35.1-39.5)	35.8 (32.9-38.9)	33.2 (29.2-37.6)	0.003	
Age and BMI adjusted	2,030	39.8 (38.0-47.2)	37.7 (35.8-41.7)	37.6 (35.0-40.4)	33.3 (29.8-37.1)	0.03	
Age and covariate adjusted*	1,793	38.8 (36.3-41.5)	37.1 (34.5-39.9)	37.5 (34.4-40.9)	34.3 (30.4-38.8)	0.25	−12% (−22 to 0)
Testosterone/SHBG (×100)							
Age adjusted	1,931	1.82 (1.73-1.96)	1.65 (1.56-1.74)	1.52 (1.41-1.65)	1.35 (1.19-1.52)	<0.001	
Age and BMI adjusted	1,931	1.77 (1.69-1.84)	1.66 (1.58-1.75)	1.58 (1.47-1.71)	1.34 (1.20-1.50)	<0.001	
Age and covariate adjusted*	1,704	1.84 (1.71-1.97)	1.71 (1.59-1.84)	1.67 (1.52-1.82)	1.39 (1.23-1.59)	0.001	−26% (−34 to −13)

Note: Geometric mean endogenous hormone and SHBG levels and their 95% CI, age adjusted, age and BMI adjusted, and age, BMI, and covariates adjusted in 2,082 postmenopausal women ages 55 to 81 y not on HRT in the EPIC-Norfolk (1997-2000) according to physical activity levels, and e^{β} of highest versus lowest physical activity level calculated from the exponential of the β coefficient on the logged scale.
*Covariates were age, BMI, smoking status, alcohol consumption, parity, age at menarche, age at menopause, past oral contraceptive use, and past HRT use.

hormone concentrations in free-living postmenopausal women in the general population.

Study Issues. We measured endogenous hormones among free-living postmenopausal women participating in a population-based study. Selection bias is unlikely to explain our results between physical activity and hormone concentrations, as it is not likely that associations would be in the opposite direction in women not participating in the study.

Measurement error requires consideration. Although EPAQ2 has been extensively validated against 24-h heart rate monitoring with individual calibration, a method which gives more precise quantification of physical activity energy expenditure (37), and the validity of EPAQ2 is comparable to other physical activity instruments used in other large epidemiologic studies (38), although all physical activity questionnaires have known limitations (39).

Our study measured hormone levels at a single point in time, which may not be representative of a woman's hormone profile over time. However, sex hormone levels are comparatively more stable among postmenopausal compared with premenopausal women (4, 40). Random measurement errors in assessing hormone concentrations and physical activity in our study are only likely to attenuate any associations, thereby underestimating any true associations.

Implications. Physical activity is associated with lower breast cancer and cardiovascular disease risk. High endogenous estradiol and testosterone levels are associated with increased breast cancer risk (3). Increasing levels of usual physical activity across the reference range were associated with differences in endogenous hormone concentrations in postmenopausal women in our study. The magnitude of the association was substantial for testosterone and testosterone/SHBG, ~19% and 24% lower, respectively, in postmenopausal women in the highest compared with the lowest physical activity level, and could provide one plausible mechanism by which physical activity might be protective against breast cancer and other hormone-related diseases.

References

1. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321–33.
2. Xie B, Tsao SW, Wong YC. Induction of high incidence of mammary tumour in female Noble rats with a combination of 17β-oestradiol and testosterone. Carcinogenesis 1999;20:1069–78.
3. Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002;94:606–16.
4. Bernstein L, Ross RK. Endogenous hormones and breast cancer. Epidemiol Rev 1993;15:48–65.
5. Friedenreich CM, Thune I, Brinton LA, Albanes D. Epidemiologic issues related to the association between physical activity and breast cancer. Cancer 1998;83:600–10.
6. Oguma Y, Shinoda-Tagawa T. Physical activity decreases cardiovascular disease risk in women: review and meta-analysis. Am J Prev Med 2004;26:407–18.
7. McTiernan A. Behavioral risk factors in breast cancer: can risk be modified? Oncologist 2003;8:326–34.
8. Thune I, Furberg AS. Physical activity and cancer risk: dose-response and cancer, all sites and site-specific. Med Sci Sports Exerc 2001;33:S530–50.
9. Gammon MD, John EM, Britton JA. Recreational and occupational physical activities and risk of breast cancer. J Natl Cancer Inst 1998;90:100–17.
10. Fernandes G, Venkatraman JT. Possible mechanisms through which dietary

lipids, calorie restriction, and exercise modulate breast cancer. In: Jacobs MM, editor. Exercise, calories, fat, and cancer. 1992: 185–201.
11. McTiernan A, Tworoger SS, Rajan KR, et al. Effect of exercise on serum androgens in postmenopausal women: a 12-month randomized clinical trial. Cancer Epidemiol Biomarkers Prev 2004;13:1099–105.
12. McTiernan A, Tworoger SS, Ulrich CM, et al. Effect of exercise on serum estrogens in postmenopausal women: a 12-month randomized clinical trial. Cancer Res 2004;64:2923–8.
13. Madigan MP, Troisi R, Potischman N, Dorgan JF, Brinton LA, Hoover RN. Serum hormone levels in relation to reproductive and lifestyle factors in postmenopausal women (United States). Cancer Causes Control 1998;9: 199–207.
14. Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ. Circulating levels of sex hormones and their relation to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). Cancer Causes Control 2001;12:47–59.
15. Bjornerem A, Straume B, Midtby M, et al. Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: the Tromso Study. J Clin Endocrinol Metab 2004;89:6039–47.
16. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. Am J Epidemiol 1989;129: 1120–31.
17. Nelson ME, Meredith CN, Dawson-Hughes B, Evans WJ. Hormone and bone mineral status in endurance-trained and sedentary postmenopausal women. J Clin Endocrinol Metab 1988;66:927–33.
18. Day N, Oakes S, Luben R, et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer 1999;80:95–103.
19. Wareham NJ, Jakes RW, Rennie KL, Mitchell J, Hennings S, Day NE. Validity and repeatability of the EPIC-Norfolk physical activity questionnaire. Int J Epidemiol 2002;31:168–74.
20. Dunning AM, Dowsett M, Healey CS, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. J Natl Cancer Inst 2004;96:936–45.
21. Tazuke S, Khaw KT, Barrett-Connor E. Exogenous estrogen and endogenous sex hormones. Medicine 1992;71:44–51.
22. Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. J Mammary Gland Biol Neoplasia 2002;7:3–15.
23. Irwin ML, Yasui Y, Ulrich CM, et al. Effect of exercise on total and intra-abdominal body fat in postmenopausal women: a randomized controlled trial. JAMA 2003;289:323–30.
24. Newcomb PA, Klein R, Klein BE, et al. Association of dietary and life-style factors with sex hormones in postmenopausal women. Epidemiology 1995;6: 318–21.
25. Tremblay MS, Copeland JL, Van Helder W. Effect of training status and exercise mode on endogenous steroid hormones in men. J Appl Physiol 2004;96:531–9.
26. Lindholm C, Hirschberg AL, Carlstrom K, von Schoultz B. Altered adrenal steroid metabolism underlying hypercortisolism in female endurance athletes. Fertil Steril 1995;63:1190–4.
27. Loucks AB, Mortola JF, Girton L, Yen SS. Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. J Clin Endocrinol Metab 1989;68:402–11.
28. Yanovski JA, Yanovski SZ, Boyle AJ, et al. Hypothalamic-pituitary-adrenal axis activity during exercise in African American and Caucasian women. J Clin Endocrinol Metab 2000;85:2660–3.
29. Russell JB, Mitchell DE, Musey PI, Collins DC. The role of β-endorphins and catechol estrogens on the hypothalamic-pituitary axis in female athletes. Fertil Steril 1984;42:690–5.
30. Bonen A, Keizer HA. Pituitary, ovarian, and adrenal hormone responses to marathon running. Int J Sports Med 1987;8:161–7.
31. Baker ER, Mathur RS, Kirk RF, Landgrebe SC, Moody LO, Williamson HO. Plasma gonadotropins, prolactin, and steroid hormone concentrations in female runners immediately after a long-distance run. Fertil Steril 1982;38: 38–41.
32. Copeland JL, Consitt LA, Tremblay MS. Hormonal responses to endurance and resistance exercise in females aged 19–69 years. J Gerontol A Biol Sci Med Sci 2002;57:158–65.
33. Warren MP. Health issues for women athletes: exercise-induced amenorrhea. J Clin Endocrinol Metab 1999;84:1892–6.
34. Boyden TW, Pamenter RW, Stanforth P, Rotkis T, Wilmore JH. Sex steroids and endurance running in women. Fertil Steril 1983;39:629–32.
35. Keizer H, Janssen GM, Menheere P, Kranenburg G. Changes in basal plasma testosterone, cortisol, and dehydroepiandrosterone sulfate in previously untrained males and females preparing for a marathon. Int J Sports Med 1989;10:S139–45.
36. Giannopoulou I, CArhart R, Sauro LM, Kanaley JA. Adrenocortical responses to submaximal exercise in postmenopausal black and white women. Metabolism 2003;52:1643–7.
37. Sjorstrom M, Ekelund U, Yngve A. Assessment of physical activity, In Public health nutrition: the nutrition society textbook. Gibney M, Margetts B, editors. Blackwell Publishing. 2004. p. 83–105.
38. Albanes D, Conway JM, Taylor PR, Moe PW, Judd J. Validation and comparison of eight physical activity questionnaires. Epidemiology 1990;1:65–71.
39. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 2000;32:S498–504.
40. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. Cancer Epidemiol Biomarkers Prev 1995;4:649–54.