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Energy Expenditure and Plasma F2-Isoprostanes across the Menstrual Cycle

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Abstract

Introduction—Habitual energy expenditure appears to favorably alter oxidant/antioxidant balance. Sparse evidence suggests that hormones that fluctuate during the menstrual cycle, particularly estrogens, may influence concentrations of oxidative biomarkers and their relation to energy expenditure.

Methods—We investigated the relation between energy expenditure and plasma free F2-isoprostane concentrations in 259 healthy, regularly menstruating 18 to 44 year old participants of the BioCycle Study. Habitual energy expenditure was measured using a baseline International Physical Activity Questionnaire and categorized as low, moderate, or high. Women were followed for one or two subsequent menstrual cycles. Past-week and past-day physical activity were measured during follow-up using questionnaires and diaries, respectively. F2-isoprostane concentrations were measured in blood samples collected at both menses (approximate cycle day 2; low serum estradiol concentration) and the late follicular phase (approximate cycle day 12; peak estradiol concentration). Generalized estimating equations were used to model the energy expenditure/isoprostane association, adjusting for confounders.

Results—Habitual energy expenditure was positively associated with F2-isoprostane concentration (adjusted difference in median F2-isoprostane, high versus low energy expenditure: 17.4%; 95% confidence interval [CI] 3.3, 31.4%). This association was not modified by cycle phase (interaction p=0.61) or differences in peak estradiol concentration across women (interaction p=0.20). Past-week and past-day physical activity measures were not associated with F2-isoprostane concentration (category trend p-values 0.50 and 0.18, respectively).

Conclusion—These results suggest that higher habitual energy expenditure may be associated with higher concentration of F2-isoprostanes in healthy reproductive-aged women. Estradiol concentration changes during the menstrual cycle do not appear to influence this relationship.

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Keywords

physical activity; menses; exercise; oxidative stress; premenopause; women

Introduction

Regular physical activity benefits health across the lifespan (32). One mechanism relevant to many of the associated health benefits may be reduced oxidative stress, *i.e*. a favorable change in the oxidant/antioxidant balance. As reviewed by Aldred, evidence suggests that increased oxygen metabolism after a bout of activity acutely increases oxidative stress (2). However, through an adaptive response to habitual physical activity, a reduction in oxidative stress occurs, particularly with regard to oxidative damage of lipoproteins (2). As reviewed previously, *in vitro* and animal studies both support and refute this hypothesis (2), and epidemiologic evidence is sparse (17,22,35). Short-term training studies and studies of acute activity effects conducted in a variety of populations such as older adults (20), young active men (10), and children (15) have shown decreased concentrations of oxidative stress markers after exercise, though other studies have shown no effect (3,19,35).

The results from these studies may not be generalizable to reproductive-aged women due to the potential influences of estrogen or other reproductive hormones. Concentrations of oxidative markers have been shown to vary throughout the menstrual cycle (28). Some studies suggest that estrogen has antioxidant properties (7,26), and others show that it is associated with pro-oxidant activity (28,30). A small number of studies have examined the relation between physical activity and measures of oxidative stress in reproductive-aged women (8,14,16,29,30). The influence of estrogen on oxidative balance, which has generally not been accounted for, may be one explanation for their contradictory results. Findings from one small study suggested that a physical activity bout caused a decline in a marker of lipid peroxidation only during the follicular phase of the menstrual cycle, when estrogen concentration typically peaks. No association was observed after an activity bout during the luteal phase, when estrogen concentration is often lower (14).

Given this evidence that both physical activity and estrogen may influence concentrations of oxidative biomarkers, we hypothesized that physical activity is inversely associated with oxidative stress in premenopausal young women and that menstruation-related estradiol fluctuations may influence this relationship. The BioCycle Study offered an opportunity to prospectively examine the association between habitual and recent physical activity and oxidative stress in young, healthy women. We used plasma F2-isoprostanes, a marker of radical-mediated lipid peroxidation that is currently favored due to its excellent stability and lack of generation by enzymatic processes (23). The unique study design also allowed us to evaluate the potential influence of estradiol on this relationship. We examined the influences of differences in estradiol both across women and within the menstrual cycle.

Methods

Study population

We used data from the BioCycle Study, which has been previously described in detail (33). The objective of this study was to longitudinally assess the associations among endogenous hormones, oxidative stress biomarkers, and antioxidants during the menstrual cycle. Between June 2005 and July 2006, 259 regularly menstruating female volunteers aged 18-44 years were recruited from western New York. Exclusion criteria included self-reported menstrual cycle length <21 or >35 days in the past 6 months, plans or active attempts to conceive, use of oral or implanted contraceptives or hormone supplements in the past 3

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months, and histories of gynecologic or other chronic diseases. Of 318 women who were eligible for participation, 276 (87%) enrolled; 259 enrollees (94%) participated in the study through one or two menstrual cycles. All participants provided written informed consent. Study procedures were approved by the University at Buffalo Institutional Review Board.

Participants completed a baseline visit that included anthropometric measurements and completion of questionnaires regarding lifestyle, health, food consumption, and physical activity, among other characteristics. At this baseline visit, participants also received a fertility monitor and instructions on its use (Clearblue® Easy Fertility Monitor, Inverness Medical, Inc., Waltham, MA). The monitor indicated low, high, and peak fertility using levels of estrone-3-glucuronide and luteinizing hormone measured daily in urine. Participants used the monitor to assist in scheduling study visits to correspond with specific phases of the menstrual cycle: 8 visits per cycle for 2 cycles. Participants were asked to visit the clinic on approximate menstrual cycle days 2 (menses), 7 (mid-follicular), 12 (late follicular), 13 (luteinizing hormone & follicle stimulating hormone surge), 14 (ovulation), 18 (early luteal), 22 (mid-luteal) and 27 (late luteal) of a 28-day cycle, adjusted for cycle length (34). Detailed information on the methods by which fertility monitors were used to identify menstrual cycle phases has been published previously (12,34). During these cycle visits, study personnel collected fasting blood and urine samples and administered questionnaires regarding several characteristics, including past-week physical activity. At each visit, blood and urine samples were collected, processed, fractionated and stored at −80°C in a standardized fashion (34). Cycle visits were scheduled between 7:00 and 8:30 am to reduce diurnal variation in biomarkers. Participants were also asked to complete structured daily diaries during the study. Diaries were used to collect information on daily vigorous physical activity, among other characteristics.

This analysis includes data from 250 participants who were enrolled in the study during two menstrual cycles and 9 participants who were enrolled during one cycle. Most women (n=233) completed the study in two consecutive cycles. Nearly all women completed 7 or 8 visits per cycle: 94% in the first and 97% in the second cycle. Missed cycle visits and completion of the study in non-consecutive cycles were primarily due to travel or illness.

Energy expenditure assessment

We assessed habitual, past-week, and past-day physical activity from the baseline questionnaire, cycle visit questionnaires, and daily diaries, respectively. We assessed habitual energy expenditure using a self-administered long-form International Physical Activity Questionnaire (IPAQ) completed, on average, 10 days before the onset of the first study cycle visit (range, 92 days before to 3 days after) (13). The long-form IPAQ assesses duration and frequency of participation in moderate and vigorous physical activities within the domains of work, transportation, household, and leisure. Participants were asked to describe the characteristics of activities performed during the last 7 days. Responses to the IPAQ were scored as energy expenditure (MET-hr/wk), which was then classified as high, moderate, or low according to the standard protocol. High energy expenditure was defined as either ≥3 days per week of vigorous activity totaling ≥25 MET-hr/wk or 7 days/week of walking, moderate, or vigorous activities totaling ≥50 MET-hr/wk. Moderate energy expenditure was defined as either ≥3 days/week of vigorous activity for at least 20 minutes/day, ≥5 days/week of moderate activity for at least 30 minutes/day, or ≥5 days/week of walking, moderate- or vigorous-intensity activities totaling ≥10 MET-hr/wk. Low energy expenditure was defined as that which did not meet criteria for the moderate or high categories (13).

We assessed past-week energy expenditure at each study visit using the IPAQ short form (12). The short-form IPAQ assesses participation in walking and moderate- and vigorous-

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intensity activities during the last 7 days. Reponses were scored and classified as high, moderate, or low energy expenditure using the standard scoring protocol (13). We assessed past-day vigorous activity using information from daily diaries. Participants were asked to record the number of minutes spent in vigorous activities. For ease of interpretation, we report this measure in minutes. However, it can be converted to MET-hr/wk by multiplying by (8/60)*7 (13). All participants provided information on habitual physical activity. Information on past-week physical activity was missing for 34 (3.3%) of the 1,036 timepoints used in the analysis. Past-day information was missing for 106 timepoints (10.2%). Timepoints with missing past-day information were associated with younger age (mean, 22.3 years) and lower likelihood of being married (5.5%) compared to timepoints without missing information (mean age 27.9 years, 27.8% married). Otherwise, participant characteristics were similar between the two groups.

Lipid peroxidation assessment

Lipid peroxidation was quantified by measuring plasma free F2-isoprostanes (8-iso-$PGF_{2\alpha}$) using a gas chromatography–mass spectrometry-based method at the Molecular Epidemiology and Biomarker Research Laboratory at the University of Minnesota (Minneapolis, MN) (interassay coefficient of variation [CV] 8.3%) (23). Isoprostanes were measured in blood samples collected at each of the 16 cycle visits (8 visits per cycle over 2 cycles). Because we aimed to examine the influence of estradiol concentration on the energy expenditure/isoprostane association, for this analysis we used data collected during menses (approximate cycle day 2, with low estradiol concentration) and the late follicular phase (approximate cycle day 12, the cycle day with peak serum estradiol concentration). All participants reported at least 2 days of menstrual bleeding. Estradiol concentration was measured in serum using radioimmunoassay (interassay CV <5%). Isoprostane concentrations were missing for 129 timepoints (12.4%) and estradiol concentrations were missing for 109 timepoints (10.5%). The proportions of missing isoprostane and estradiol concentrations were directly associated with younger age and lower likelihood of being married.

Statistical analyses

We examined distributions of participant characteristics (percentile or mean and standard deviation [SD]) according to habitual energy expenditure. We tested differences of continuous and categorical characteristics across energy expenditure categories using t-tests and Pearson chi-square tests, respectively. We examined distributions of isoprostane concentrations (median, intraquartile range [IQR]) within groups defined by habitual energy expenditure and menstrual cycle phase. We tested differences in median isoprostane concentrations across energy expenditure categories using a rank-sum test. We fit linear regression models using generalized estimating equations to estimate the influence of energy expenditure on isoprostane concentrations in both menses and the late follicular phase. Data from both study cycles were used for the 250 women who completed two cycles. The models used a cluster term and an unstructured correlation matrix to account for within-person and within-cycle correlations in isoprostane concentrations. Because isoprostane concentrations were log-transformed to account for their skewed distribution, model coefficients are interpreted as the percent difference (95% confidence interval [CI]) in median isoprostane concentration between two groups categorized by energy expenditure (9). We used low energy expenditure as the referent category.

We examined several characteristics as potential confounders of the relation between energy expenditure and isoprostane concentrations. To evaluate whether each characteristic was a confounder, we included the variable as a covariate or set of covariates in a liner regression model relating log-isoprostane concentration to categorized habitual energy expenditure.

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Only covariates that changed one or both of the energy expenditure coefficients by ≥10% were retained in the final model. Continuous covariates were modeled continuously, as quartiles, and using biologically meaningful cutpoints when appropriate (*e.g*. body mass index [BMI]). For each covariate, the coding scheme that produced the greatest change from the unadjusted odds ratio was used in the final model. If multiple coding schemes for one covariate resulted in similar changes, the most parsimonious one was used. Only participants with complete confounder information were included in the final models.

The following characteristics evaluated as confounders were self-reported in the baseline questionnaire: age, race, Hispanic ethnicity, education, family income, marital status, height and weight (used to calculate BMI), history of eating disorders, weight fluctuation of ≥10 lbs in the past year, history of smoking, alcohol consumption and illicit drug use in the past year, usual hours of sleep per day (averaged over weekends and weekdays), age at menarche, and numbers of past pregnancies and live deliveries. Percent body fat was measured at or soon after the final study visit using dual energy X-ray absorptiometry (Hologic, Inc., Waltham MA). Information on dietary characteristics, including daily energy, carbohydrates, fat, protein, fiber, vitamins A, C, and E, and fruit and vegetable servings, was collected at baseline using a food frequency questionnaire developed by the Nutrition Assessment Shared Resource of the Fred Hutchinson Cancer Research Center (http://www.fhcrc.org/science/shared_resources/nutrition/ffq). We also examined season of participation, measured at baseline, as a potential confounder. Covariates included in the final adjusted models included race, age, parity, average hours of sleep, percent body fat, menstrual cycle length, and dietary vitamin A intake.

We evaluated whether estradiol modified the relation between energy expenditure and isoprostane concentration in two ways. To measure the influence of within-person estradiol fluctuations, we fit a model including a term for the product of timing within the menstrual cycle (menses or late follicular phase) and categorized energy expenditure. To measure the influence of between-person estradiol differences on the relation between energy expenditure and late-follicular isoprostane concentration, we fit a model including a term for the product of peak estradiol (dichotomized at the median) and categorized energy expenditure. We tested the statistical significance of these interaction terms using Wald tests.

Results

Participants' first and second study cycles had median (IQR) lengths of 28 (26-31) days. The menses visits occurred on median cycle day 2 (IQR 2-3) and late follicular phase visits occurred on median day 14 (IQR 13-16). Median (IQR) serum estradiol concentrations were 33 (25-45) pg/ml during menses and 223 (150-307) pg/ml during the late follicular phase. Median (IQR) plasma F2- isoprostane concentrations were 45 (36-57) pg/ml and 47 (37-61) pg/ml during menses and the late follicular phase, respectively.

Participants were generally young (mean, 27.5 years), of normal weight (mean BMI, 24.1 kg/m^2), and physically active (90% reported moderate or high habitual energy expenditure). Habitual energy expenditure was positively associated with older age, white race, high family income, married status, past or current smoking, alcohol consumption, having had at least one live birth, summer season, energy consumption, and fruit and vegetable consumption (Table 1).

Median (IQR) habitual energy expenditure was 57 (25-112) MET-hr/wk. Median (IQR) past-week energy expenditure reported at menses of the first cycle (day 2 visit) was 26 (8-56) MET-hr/wk. Median (IQR) past-day time spent in vigorous activity reported at the

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same visit was 0 (0-28) minutes; 67% of women reported no vigorous activity participation on the previous day. Distributions of past-week and past-day physical activity measures did not differ substantially at later study visits (data not shown). Habitual energy expenditure was moderately correlated with past-week energy expenditure: Spearman correlation coefficients ranged from 0.40 to 0.52 across the four measurement timepoints (all $p<0.001$). Habitual energy expenditure was weakly correlated with past-day vigorous physical activity (correlations 0.11-0.20; p 0.02-0.09).

Higher habitual energy expenditure was associated with higher median isoprostane concentrations in both menses and the late follicular phase (Table 2). After adjustment for confounders, habitual energy expenditure was positively associated with isoprostane concentrations in both phases. For example, adjusted high versus low energy expenditure percent differences in median isoprostane were 14.8% (95% CI −1.5, 31.2%) in menses and 19.8% (95% CI 6.1, 33.5%) in the late follicular phase. Associations did not differ significantly between menses and the late follicular phase (cycle phase interaction p-value 0.61). Similarly, between-person differences in peak estradiol concentration did not influence the positive relation between habitual energy expenditure and late-follicular phase isoprostane (peak estradiol interaction p-value 0.20).

Given the lack of evidence that within-person or between-person differences in estradiol concentration influenced the association of interest, we examined associations over both phases and all participants combined (Table 3). Compared with women reporting low habitual energy expenditure, median isoprostane concentrations were higher for the moderate-expenditure group (8.5%, 95% CI −5.5, 22.4%) and significantly higher for the high-expenditure group (17.4%, 95% CI 3.3, 31.4%), after adjustment for confounders (trend p-value 0.003). A one MET-hr/wk increase in habitual energy expenditure was associated with a 4.1% (95% CI 1.7, 6.6%) increase in median isoprostane after adjustment for confounders. In contrast, more recent physical activity was neither strongly nor significantly associated with isoprostane concentrations. Median isoprostane was 3.3% lower (95% CI, −8.0, 3.6%) in the high versus low past-week energy expenditure groups and 2.9% lower (95% CI −8.1, 2.3%) among women reporting >30 minutes of physical activity on the previous day versus those reporting none. Trend p-values across past-week and past-day activity categories were 0.50 and 0.18, respectively. Median isoprostane concentrations were not strongly associated with a one METhr/wk increase in past-week energy expenditure (−0.01%, 95% CI −0.1, 0.05%) or a 10-minute increase in past-day vigorous activity (−0.1%, 95% CI −1.0, 0.6%). Associations with past-week and past-day energy expenditure were not modified by within-person or within-cycle differences in estradiol concentration (data not shown). Adjusting for past-week or past-day energy expenditure, same-day or prior-visit estradiol concentration, or time between the baseline questionnaire and first cycle visit did not meaningfully change estimated associations between isoprostane concentrations and habitual energy expenditure (data not shown). We also found no influence of study cycle (first or second) on our results (data not shown).

Discussion

In this population of healthy, regularly menstruating women, habitual energy expenditure was positively associated with plasma F2-isoprostane concentrations measured in one or two subsequent menstrual cycles. This relationship did not differ between menses, when estradiol concentration is lowest, and the late follicular phase, when it is highest. Similarly, the relationship did not differ across women with high versus low peak estradiol concentrations. Energy expenditure in the week before isoprostane measurement, and time spent in vigorous activity on the day before, were not associated with isoprostane concentrations.

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These results contribute to a limited and inconsistent body of literature. Three studies suggest that habitual physical activity is associated with lower concentrations of oxidative biomarkers among young or premenopausal women (8,29,30). In a cross-sectional analysis of 1,647 middle-aged premenopausal and postmenopausal women by Sowers *et al*., self-reported past-year physical activity was inversely associated with urinary F2α-isoprostane concentrations (measured in the follicular phase among premenopausal women). As this was not an association of primary interest in this analysis, its magnitude and standard error were not reported (30). As reported by Schmitz *et al*., after fifteen 18-25 year old regularly menstruating women participated in a 15-week moderate exercise program, urinary follicular phase F2-isoprostane declined approximately 34% (p-value 0.02) (29). In a study conducted by Devries *et al*. of 23 lean and obese women aged 40 years on average, a 12-week progressive endurance training program resulted in urinary 8-isoprostane declines of approximately 25% (p-value 0.02) (8). In contrast to our observational study, both Devries and Schmitz examined changes in urinary isoprostane concentrations following a fairly short (<4 month) prescribed exercise regimen (8,29). The impact on isoprostanes of recently adopted changes in activity, such as those prescribed in these two studies, may differ from that of habitual activity, which we aimed to assess here. Furthermore, both studies measured urinary rather than plasma isoprostane concentrations. Finally, Schmitz *et al*. restricted their study to sedentary women, while our study population was generally active. Devries *et al*. examined a population that was older than ours, on average.

Unlike previous studies, the unique design of the BioCycle study allowed us to assess associations of isoprostane concentrations with both habitual and more recent physical activity. Two studies of the acute effects of physical activity found conflicting results using biomarkers of lipid peroxidation other than F2-isoprostanes. Kanaley *et al*. found no changes in concentrations of plasma malondialdehyde, a lipid peroxidation byproduct, from immediately before to after a moderate 90-minute exercise bout in six amehorrheic and six eumenorrheic athletes (16). In a study by Joo *et al*., eighteen young women with regular cycles were monitored immediately before and after three 30-minute bouts of moderate activity performed during menses, at the follicular phase, and during the luteal phase. The follicular phase was defined as the 4^{th} day to the 2^{nd} day before the day of ovulation as predicted by ovulation tests in two previous menstrual cycles. The luteal phase was defined as the 4^{th} to 9^{th} day after the predicted day of ovulation. Blood malondialdehyde declined after the activity bout only in the follicular phase (14). We were unable to measure isoprostanes immediately following an exercise bout in this study. However, our results do not provide evidence that activities performed in the past day or past week strongly influence isoprostane concentrations in young women.

Joo's study suggested that cyclical hormonal fluctuations associated with menses influence the relation between physical activity and oxidative stress (14). We were able to test the influence of differences in estradiol concentrations during menses versus the late-follicular and found none. It has been hypothesized that estradiol and estrogen metabolites have antioxidant properties (6,30). Evidence of this association is conflicting. *In vitro* studies show that estrogen suppresses lipid peroxidation in rats (4,31). Some, though not all, studies show that estrogen replacement lowers circulating oxidized low density lipoprotein concentrations in postmenopausal women (11,27). However, estrogen metabolite concentrations were positively, though weakly, correlated with F2α-isoprostane in a cross-sectional analysis of 778 non-smoking premenopausal middle-aged women (adjusted correlation 0.08, 95% CI 0.00-0.15) (30). Similarly, a positive correlation between estradiol and isoprostane concentrations was observed in the BioCycle population after adjustment for participant characteristics and other reproductive hormones (beta coefficient for 1-unit difference in log-estradiol 0.011 pg/ml, 95% CI 0.001, 0.022 pg/ml) (28). While estradiol concentrations were positively associated with isoprostane concentrations in this population,

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we did not find evidence that they confounded the isoprostane-energy expenditure relationship.

The positive relation we observed between habitual energy expenditure and F2-isoprostane concentrations was also independent of more recent physical activity, broad measures of dietary composition, and other participant characteristics. This relation was unexpected given the hypothesized favorable effect of habitual physical activity on oxidative stress. Plasma F2-isoprostanes are widely used as a stable marker of radical-mediated lipid peroxidation. The biomarker was shown to be reliable in a study of 103 non-smoking premenopausal women (2,18). While the study was designed to reduce diurnal and menstrual-related variation in biomarkers, it is unlikely that this variation was entirely controlled. However, we would expect such uncontrolled within-person variation to be unrelated to habitual energy expenditure, and thus bias our estimates toward, rather than away from, the null. Although we were able to examine many potential confounders of our relation of interest, it is possible that we were unable to account for one or more participant characteristics that may explain the relationship we observed. Furthermore, habitual energy expenditure may have also influenced antioxidant capacity, which would also influence total oxidative stress. We plan to examine this possibility in future work.

Due to stringent inclusion and exclusion criteria, BioCycle participants were generally young and healthy. While 90% of BioCycle participants reported moderate or high habitual physical activity, only 47% of American women in 2007 were least moderately physically active (5). The fairly small proportion of inactive participants used as our referent group hampered the precision of our estimates, though high-category and trend p-values for habitual energy expenditure were statistically significant. The relations observed here may not extend to women who are very active or sedentary. Women who were excluded from one or more analyses due to missing past-day physical activity, isoprostane, or estradiol measurements were more likely to be young and unmarried than those with complete data. While this non-random missingness may have biased our estimates of association, proportions of women with missing data were fairly small (10-12%). Furthermore, isoprostane concentrations were not strongly associated with marital status, though they were higher among younger and older women (data not shown).

We acknowledge limitations in our physical activity assessment methods. Questionnaire and diary-based physical activity assessment may be limited by under- or over-reporting of activities. However, it is often the most feasible choice for epidemiologic studies. Although the IPAQ references activities performed during the last 7 days, we, like many others, used it at the baseline visit to assess habitual physical activity (21,32). Reliabilities of short and long forms using a ‘usual week’ versus ‘the last 7 days’ frame of reference were comparable in adult populations sampled from 12 countries (7). Similarities in estimates of association between the first and second study cycles suggest that the IPAQ did capture habitual rather than recent activity patterns. Nevertheless, use of the 7-day window may have introduced error in our measure of habitual activity. Additionally, lack of associations with past-day and past-week activities may be due to nondifferential misclassification of exposure. The short-form IPAQ was designed primarily for international monitoring of physical activity rather than ranking of persons within populations (7). Median past-week energy expenditure estimates obtained using the short-form IPAQ were lower than habitual estimates obtained using the long form. This suggests that the brevity of the short form may have led women to underreport activities during their study visits. However, reliability and criterion validity of the short-form IPAQ are at least as good as other established questionnaires (7) and have been demonstrated in young adult women (24,25). The single item in daily diaries used to assess past-day vigorous activity did not capture moderate or walking activities that were captured in the IPAQ forms. Vigorous activity comprised only 29% of habitual energy

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expenditure in this population. The lack of information on moderate and walking activities may have contributed to misclassification of total past-day energy expenditure, which may be more relevant to F2-isoprostane concentrations than vigorous activity alone.

These results suggest that higher habitual energy expenditure is associated with higher concentrations of F2-isoprostanes in healthy reproductive-aged women. Estradiol concentration changes during the menstrual cycle do not appear to influence this relationship. Future work designed to examine other aspects of oxidative balance will provide additional evidence to help clarify the complex relation between physical activity and oxidative stress in reproductive-aged women.

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Table 1

Frequency distributions (mean ± SD or N [%]) of participants' characteristics according to self-reported habitual energy expenditure.

	Habitual energy expenditure (MET-hours/week)[1]					
Characteristic	**Low n=25**		**Moderate n=92**		**High n=142**	
Age (years)		26.0±6.8		27.1±7.5		27.6±8.9
<20	3	12.0	8	8.7	28	19.7
20-29	17	68.0	56	60.9	63	44.4
30-39	3	12.0	20	21.7	28	19.7
≥40	2	8.0	8	8.7	23	16.2
Race[3]						
White	10	40.0	51	55.4	93	65.5
Black	8	32.0	16	17.4	27	19.0
Asian / Pacific Islander	5	20.0	22	23.9	13	9.2
Other	2	8.0	3	3.3	9	6.3
Education (highest grade completed)						
High school	1	4.0	17	18.5	15	10.6
Post-secondary	24	96.0	75	81.5	127	89.4
Family income						
<$19,999	5	20.0	20	21.7	30	21.3
$20,000-39,999	8	32.0	27	29.4	26	18.3
$40,000-74,999	6	24.0	25	27.2	41	28.9
$75,000-99,999	5	20.0	14	15.2	26	18.3
>$100,000	1	4.0	5	5.4	12	12.7
Missing	0	0.0	1	1.1	1	0.7
Marital status						
Married/living as married	6	24.0	23	25.0	37	26.1
Separated/divorced	0	0.0	6	6.5	10	7.0
Single	19	76.0	63	68.5	95	66.9
Body mass index (kg/m^2)		23.5±4.1		24.1±4.0		24.2±3.8
<18.5	2	8.0	1	1.1	6	4.2
18.5-24.9	16	64.0	61	66.3	83	58.5

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Characteristic	Habitual energy expenditure (MET-hours/week)[1]					
	Low n=25		Moderate n=92		High n=142	
25.0-29.9	5	20.0	19	20.7	40	28.2
≥30.0	2	8.0	11	12.0	13	9.2
Body fat (% of total composition)	30.0±7.0		30.0±5.7		29.2±6.0	
Ever smoked regularly						
Never	21	84.0	77	83.7	113	79.6
Past	3	12.0	11	12.0	23	16.2
Present	1	4.0	4	4.4	6	4.2
Alcohol consumption in past year						
<12 drinks	12	48.0	32	34.8	41	18.9
≥12 drinks	13	52.0	59	64.1	100	70.4
Missing	0	0.0	1	1.1	1	0.7
Hours of sleep per day[3]	7.5±0.2		7.6±0.1		7.3±0.1	
Nulliparous	21	84.0	73	79.4	99	69.7
Season of baseline visit						
Winter (Dec.-Feb.)	8	32.0	30	32.6	38	26.8
Spring (Mar.-May)	10	40.0	20	21.7	29	20.4
Summer (June-Aug.)	2	8.0	23	25.0	43	30.3
Autumn (Sept.-Nov.)	5	20.0	19	20.7	32	22.5
Length of first study cycle (days)	29.5±3.6		29.1±4.8		28.7±4.7	
Energy (kcal)[2]	1335±1022		1622±753		1677±741	
Total dietary vitamin A (IU)	6522±6416		9432±8381		9010±6625	
A verage daily fruit & veg (servings)[2]	2.1±1.8		3.6±3.6		4.0±4.0	

[1] Habitual activity was classified according to IPAQ scoring guidelines (16); see methods section for details.

[2] Low- versus high-category energy expenditure p-value <0.05, using chi-square for categorical variables and unpaired t-test for continuous variables.

[3] Moderate- versus high-category energy expenditure p-value <0.05, using chi-square for categorical variables and unpaired t-test for continuous variables.

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Table 2

Distributions (median [intraquartile range]) of F2-isoprostanes (pg/ml) according to habitual energy expenditure and menstrual cycle phase (259 participants, 460 cycles).

	Habitual energy expenditure (MET-hours/week)[1]		
Time within cycle[2]	**Low (n=42)**	**Moderate (n=166)**	**High(n=252)**
Menses[3]	41.6 [33.2-55.2]	43.5 [33.9-55.4]	46.9 [37.9-60.1]
Late follicular phase[3]	42.6 [35.2-46.6]	46.3 [35.4-59.7]	48.4 [38.7-63.0]

[1] Habitual activity was classified according to IPAQ scoring guidelines (16); see methods section for details.

[2] The menses measurement occurred on approximate cycle day 2, a day with low estradiol concentration. The late follicular phase occurred on approximate cycle day 13, a day with peak serum estradiol concentration. See methods section for details.

[3] Moderate- versus high-category energy expenditure rank-sum p-value <0.05.

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Table 3

Unadjusted and adjusted percent differences (95% confidence intervals [CI]) in median F2-isoprostane concentration across categories of habitual, past-week, and past-day energy expenditure.

Exposure measure	No. obs.[2]	Unadjusted % difference (95% CI)	Adjusted[1] % difference (95% CI)
Habitual energy expenditure categorized according to IPAQ cutpoints (16)[3]			
Low	74	0.0 (referent)	0.0 (referent)
Moderate	326	1.5 (−11.9, 14.9)	8.5 (−5.5, 22.4)
High	474	10.9 (−2.0, 23.7)	17.4 (3.3, 31.4)
Trend p-value:		0.02	0.003
Past-week energy expenditure categorized according to IPAQ cutpoints (16)[4]			
Low	280	0.0 (referent)	0.0 (referent)
Moderate	363	0.5 (−3.9, 4.9)	0.3 (−4.1, 4.7)
High	217	−2.3 (−8.1, 3.4)	−3.3 (−8.0, 3.6)
Trend p-value:		0.48	0.50
Past-day minutes spent in vigorous physical activity[5]			
None	576	0.0 (referent)	0.0 (referent)
1-30	121	−2.3 (−7.1, 2.4)	−2.8 (−7.3, 1.8)
31-180	125	−1.9 (−6.8, 3.1)	−2.9 (−8.1, 2.3)
Trend p-value:		0.36	0.18

[1] Adjusted for race (white, black, other), age (<20, 20-29, 30-39, and ≥40 years), parity (0, 1, or ≥2 live births), average hours of sleep (continuous), and percent body fat, menstrual cycle length, and dietary vitamin A intake (quartiles).

[2] Number of observations within each category in the adjusted model. Women could contribute up to 4 observations each (2 observations from 2 cycles).

[3] The unadjusted model was fit on 907 observations from 248 women. The adjusted model was fit on 874 observations from 236 women.

[4] The unadjusted model was fit on 893 observations from 248 women. The adjusted model was fit on 860 observations from 236 women.

[5] The unadjusted model was fit on 851 observations from 247 women. The adjusted model was fit on 822 observations from 236 women.