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Sitting, physical activity, and serum oestrogen metabolism in postmenopausal women: the Women's Health Initiative Observational Study

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Background: Prolonged sitting and lower levels of physical activity have been associated with increased levels of parent oestrogens (oestrone and oestradiol), the key hormones in female cancers, in postmenopausal women. However, it is unknown whether sitting and physical activity are associated with circulating oestrogen metabolite levels.

Methods: Among 1804 postmenopausal women enrolled in the Women's Health Initiative Observational Study, 15 serum oestrogens/oestrogen metabolites were quantified using liquid chromatography–tandem mass spectrometry. Physical activity and sitting were self-reported via questionnaire. Using baseline, cross-sectional data, geometric means (GM) of oestrogens/oestrogen metabolites (pmol l^{-1}) were estimated using inverse probability weighted linear regression, adjusting for potential confounders and stratified on menopausal hormone therapy (MHT) use.

Results: Longer time spent sitting ($\geqslant$10 *vs* $\leqslant$5 h per day) was associated with higher levels of unconjugated oestrone, independent of moderate- to vigorous-intensity physical activity and body mass index, among both never/former (GM = 70.6 *vs* 57.7) and current MHT users (GM = 242 *vs* 179) (*P*-trend $\leqslant$0.03). Among never/former MHT users, sitting ($\geqslant$10 *vs* $\leqslant$5 h per day) was positively associated with 2-methoxyestradiol (GM = 16.4 *vs* 14.4) and 4-methoxyestradiol (GM = 2.36 *vs* 1.98) (*P*-trend $\leqslant$0.04), independent of parent oestrogens. Inverse associations between moderate- to vigorous-intensity physical activity ($\geqslant$15 *vs* 0 metabolic equivalent task-hours per week) and parent oestrogens were found as expected. After adjustment for parent oestrogens, physical activity was not associated with oestrogen metabolites.

Conclusions: Our data suggest that prolonged sitting and lower moderate- to vigorous-intensity physical activity are associated with higher levels of postmenopausal oestrogens/oestrogen metabolites, the oestrogen metabolism patterns that have previously been associated with higher endometrial and breast cancer risk.

In postmenopausal women, physical activity is associated with reduced levels of oestrogens (McTiernan *et al*, 2006; Chan *et al*, 2007; Monninkhof *et al*, 2009; van Gils *et al*, 2009; Friedenreich *et al*, 2010; Choudhury *et al*, 2011), one of the key hormones implicated in the aetiology of some female cancers (e.g., endometrial, breast, and non-serous ovarian) (Key *et al*, 2002; Fuhrman *et al*, 2012; Falk *et al*,

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2013; Brown and Hankinson, 2015; Trabert *et al*, 2015; Brinton *et al*, 2016). In a randomised controlled trial, postmenopausal women who exercised for 150–225 min per week during a 12-month period had an 18% reduction (*vs* 4% in the control group) in circulating oestradiol levels from baseline (Friedenreich *et al*, 2010). The inverse relationships between physical activity and postmenopausal endogenous oestrogens have been consistently reported in other randomised trials (McTiernan *et al*, 2004; Monninkhof *et al*, 2009) and most observational studies (Nelson *et al*, 1988; Cauley *et al*, 1989; Madigan *et al*, 1998; McTiernan *et al*, 2006; Chan *et al*, 2007; van Gils *et al*, 2009; Choudhury *et al*, 2011). However, beyond parent oestrogens (oestradiol and oestrone), little is known about the association between physical activity and oestrogen metabolism. It is also unclear whether the relationship can be explained by body weight. Independent of physical activity, sedentary time has been shown to be positively associated with urinary levels of parent oestrogens (Dallal *et al*, 2016). To the best of our knowledge, no study to date has examined the association of sedentary time with serum levels of endogenous oestrogens in postmenopausal women. Although the exact relationships between urinary and serum oestrogens are unclear, serum levels are thought to more closely represent circulating *vs* excreted levels of oestrogens, which may be more relevant to cancer risk. Further, while current menopausal hormone therapy (MHT) users have higher circulating oestrogen levels (Nachtigall *et al*, 2000; Edlefsen *et al*, 2010), it is unknown whether sedentary time and physical activity are associated with further differences in circulating levels in this group.

Parent oestrogens are metabolised into an array of oestrogen metabolites when hydroxylated at the 2-, 4-, or 16-carbon position. Parent oestrogens and some oestrogen metabolites stimulate cell proliferation by binding oestrogen receptors (Santen *et al*, 2015). Evidence has shown that certain oestrogen metabolites, namely 2- and 4-pathway catechols, can also damage DNA directly by forming quinone DNA adduct and indirectly via redox cycling (Cavalieri *et al*, 2000, 2006). When catechols are methylated, they do not undergo further redox cycling thus mutagenic potentials may be reduced (Yager, 2000). The degree of oestrogenic and mutagenic potentials of individual oestrogen metabolites likely depends on their affinity to oestrogen receptors (Cavalieri *et al*, 2000) and, for catechols, methylation status (Cavalieri *et al*, 2000; Yager, 2000). Hence, comprehensive evaluations of oestrogens/oestrogen metabolites may provide greater insight into the roles of sedentary time and physical activity in endogenous oestrogen metabolism. Using baseline, cross-sectional data from the Women's Health Initiative (WHI) Observational Study (OS), we examined whether sedentary time, specifically sitting, and physical activity were associated with serum levels of 15 oestrogens/oestrogen metabolites in postmenopausal women stratified by MHT usage.

MATERIALS AND METHODS

Study population. This study includes baseline, cross-sectional data from participants in a case–control study of ovarian and endometrial cancers nested within the WHI-OS (Trabert *et al*, 2015; Brinton *et al*, 2016). The WHI-OS is a prospective cohort study that recruited 93 676 postmenopausal women aged 50–79 years at 40 clinical centres in the United States between 1993 and 1998 (Langer *et al*, 2003; The Women's Health Initiative Study Group, 1998). At baseline clinic visit, anthropometrics (e.g., height and weight) were measured and blood samples were collected. In addition, baseline self-administered questionnaires collected information on participants' demographics, medical history, and health behaviours (e.g., physical activity and sitting).

Details of the nested case–control study are described elsewhere (Trabert *et al*, 2015; Brinton *et al*, 2016). Cases were women with ovarian or endometrial cancer diagnosed between study enrolment and 2012. Participants had no history of cancer (except non-melanoma skin cancer), bilateral oophorectomy, or hysterectomy (for endometrial controls only), and had $\geqslant$1.1 ml serum available. Controls were selected from women who remained cancer-free at the date of case diagnosis and were frequency matched to cases based on age at baseline (5-year categories), year of blood draw (1993–1996 and 1997–1998), race/ethnicity (white, black, Hispanic, and other/unknown), hysterectomy before the index date (for ovarian controls only), and MHT use (never, $\leqslant$1 year since last MHT use, $>$1 year since last MHT use, and current).

A total of 1804 women (492 cases and 444 controls among never/former MHT users, and 452 cases and 416 controls among current MHT users), representing 55256 women when weighted back to the entire cohort, were included in the study. Because all serum samples were collected at baseline prior to cancer diagnosis (range: 45 days–14.9 years; mean: 6.7 years), we included both cases and controls and accounted for case–control selection by weighting in this cross-sectional analysis.

Approval for conducting the study was obtained from human subjects review at the Fred Hutchinson Cancer Research Center (WHI Clinical Coordinating Center), as well as at all participating clinical centres. Written informed consent was obtained from study participants.

Exposure assessment. At baseline, levels of sitting and physical activity were self-reported via questionnaire. Sitting during a usual day (e.g., sitting at work, sitting at the table eating, driving or riding in a car or bus, and sitting up watching TV or talking) was reported in one of eight categories ($<$4, 4–5, 6–7, 8–9, 10–11, 12–13, 14–15, and $\geqslant$16 h per day). Because of sparse data, categories were collapsed into three levels: $\leqslant$5; 6–9; and $\geqslant$10 h per day. Details of physical activity assessment have been described previously (Arem *et al*, 2013). Briefly, participants were asked to report their usual number of days per week (1, 2, 3, 4, and $\geqslant$5) and minutes per session ($<$20, 20–39, 40–59, and $\geqslant$60) they spent on moderate exercise (e.g., biking outdoors and folk dancing), strenuous exercise (e.g., aerobics and swimming laps), and walking outside the home (average-paced (2–3 mph), fairly fast (3–4 mph), and very fast ($>$4 mph)). Total metabolic equivalent (MET)-hours per week of moderate- to vigorous-intensity physical activities including walking were estimated by multiplying the summed number of hours per week of each activity type with its corresponding average MET values (Ainsworth *et al*, 2000). According to the 2008 Physical Activity Guidelines for Americans recommended minimum ($\geqslant$150 min per week of moderate-intensity exercise, equivalent to 7.5–14.9 MET-h per week) (President's Council on Fitness Sports & Nutrition, 2016), activity levels were categorised into four levels: 0 (no exercise); 0.1–7.4 (less than the guideline); 7.5–14.9 (meeting the guideline); and $\geqslant$15 MET-h per week (exceeding the guideline).

Laboratory assays. Details of the laboratory assays have been described previously (Trabert *et al*, 2015). The assay quantifies the combined concentrations of conjugated and unconjugated forms of each of the 15 oestrogens/oestrogen metabolites (oestrone, 17β-oestradiol (oestradiol), 2-hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone, 2-methoxyestradiol, 2-hydroxyestrone-3-methyl ether, 4-hydroxyestrone, 4-methoxyestrone, 4-methoxyestradiol, 16α-hydroxyestrone, oestriol, 16-ketoestradiol, 16-epiestriol, and 17-epiestriol) and unconjugated concentrations of five analytes (oestrone, oestradiol, oestriol, 2-methoxyestrone, and 2-methoxyestradiol) in serum using a stable isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay (Leidos Biomedical Research, Inc., Frederick, MD, USA) (Xu *et al*, 2007). Coefficients of variation were $<$6% and intraclass correlation coefficients (ICCs) were $>$0.93 for all analytes (Trabert *et al*, 2015). Spearman's correlations amongst the

oestrogens/oestrogen metabolites ranged from 0.34 to 0.99 (Brinton *et al*, 2016).

Statistical analyses. Because oestrogens/oestrogen metabolite levels vary by MHT usage (Nachtigall *et al*, 2000; Edlefsen *et al*, 2010), all analyses were stratified on MHT use ($n=936$ never/former users and 868 current users). Inverse probability sampling weights were used to adjust the data to represent the population in the entire cohort using methods for secondary phenotype analysis of case–control data described by Li and Gail (2012). Sampling weights were calculated by taking the inverse of sampling fractions: one for all cases and varying weights for controls depending on their strata defined by matching factors.

Distributions of serum oestrogens/oestrogen metabolite data were right-skewed; details of the data have been described elsewhere (Brinton *et al*, 2016). After log-transformation of data to improve normality, geometric means (GM; pmol l^{-1}) of individual serum oestrogen/oestrogen metabolite concentrations by exposure categories were estimated using inverse probability weighted linear regression. All analyses were adjusted for age (5-year categories) and *a priori* selected potential confounders: blood draw year (1993–1996 and 1997–1998); race (white and non-white); smoking status (never, former, and current); and time since menopause (<10, 10–19, $\geqslant 20$ years, and missing). Additional adjustment for soy and alcohol intakes and MHT formulation (among current MHT users) did not change the results, thus were not included in the final models. To account for correlated data, we evaluated models with additional adjustment for body mass index (BMI; Spearman's $r=0.12$ for sitting and -0.27 for physical activity) and mutual adjustment for sitting and physical activity (Spearman's $r=-0.11$). For oestrogen metabolite analyses, we assessed whether the associations were independent of associations with parent oestrogens. We performed a test for trend by including the exposure in the model as a continuous variable. We also performed cubic spline models to assess for nonlinearity of the associations; however, the model fit was not statistically significantly better than linear models ($P>0.05$) thus are not presented. We tested for any difference in oestrogen/oestrogen metabolite levels across exposure categories using global F-test. In secondary analysis, we further investigated the relationships with patterns of oestrogen metabolism by comparing the mean proportion of parent oestrogens across exposure categories, with adjustment for the summed concentration of oestrogens/oestrogen metabolites.

Finally, to assess the influence of subclinical disease, we performed sensitivity analyses after excluding endometrial and ovarian cancer cases ($n=492$ never/former and 452 current MHT users) and excluding women with low BMI ($<18.5\,\text{kg m}^{-2}$; $n=12$ never/former and 13 current MHT users). We also excluded women with a history of diabetes at baseline ($n=56$ never/former and 28 current MHT users) because diabetes and diabetic medications (e.g., metformin) may influence oestrogen levels via biologic crosstalk between insulin signalling and sex hormones. We also repeated analyses after excluding outliers ($\leqslant 10$ values for never/former and $\leqslant 10$ values for current MHT users) identified using the extreme Studentized deviate many-outlier procedure (Rosner, 1983) and further stratifying by never *vs* former MHT users ($n=629$ never and 307 former users). All statistical tests were two-sided with 5% type I error. *Q*-values reflecting the false discovery rates (FDR) were calculated to account for multiple comparisons. Analyses were conducted with SAS version 9 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Study population characteristics. The mean age was 64.4 years for never/former MHT users and 60.1 years for current MHT users. Among both never/former and current MHT users, women who reported sitting $\geqslant 10$ h per day (*vs* $\leqslant 5$ h per day) and women reporting 0 MET-h per week (*vs* $\geqslant 15$ MET-h per week) moderate- to vigorous-intensity physical activity were more likely to have BMI $\geqslant 30\,\text{kg m}^{-2}$ (Table 1). As expected, serum concentrations of all evaluated oestrogens/oestrogen metabolites were higher in current MHT users compared to never/former users (Table 2–4; Supplementary Tables 1–2).

Parent oestrogens. Adjusting for age and potential confounders, sitting at least for 10 h per day compared with sitting $\leqslant 5$ h per day was positively associated with serum levels of unconjugated oestrone (GM = 70.0 *vs* 54.6 pmol l^{-1}) and unconjugated oestradiol (GM = 16.4 *vs* 12.5 pmol l^{-1}) with significant linear trends (P-trend $\leqslant 0.04$) among never/former MHT users (Table 2). The positive association with unconjugated oestrone remained after adjustment for BMI and physical activity (GM = 70.6 *vs* 57.7 pmol l^{-1}, P-trend = 0.02). Among current MHT users, a similar positive association with unconjugated oestrone was observed after adjustment for BMI and physical activity (GM = 242 *vs* 179 pmol l^{-1}, P-trend = 0.03). Among these current MHT users, percentages of parent oestrogens out of the total measured oestrogens/oestrogen metabolites concentrations were also significantly greater in women with longer sitting hours ($\geqslant 10$ *vs* $\leqslant 5$ h per day; 58% *vs* 53%, $P=0.01$); we observed no difference in percentages among never/former MHT users (48% *vs* 49%, $P=0.43$; data not tabled).

Among never/former MHT users, women who reported at least 15 MET-h per week moderate- to vigorous-intensity physical activity in comparison to women who reported no physical activity (0 MET-h per week) were associated with lower levels of oestrone (GM = 289 *vs* 403 pmol l^{-1}) and oestradiol (GM = 49.7 *vs* 72.5 pmol l^{-1}) adjusting for age and potential confounders (all $P<0.001$; Table 2). The associations appeared to be driven by the differences between women who reported some *vs* no physical activity ($\geqslant 0.1$ *vs* 0 MET-h per week). The associations remained after adjustment for BMI and sitting (GM = 332 *vs* 370 pmol l^{-1} for oestrone; 56.2 *vs* 67.4 pmol l^{-1} for oestradiol; all $P\leqslant 0.02$). No association between physical activity and parent oestrogens was observed among current MHT users.

Oestrogen metabolites. Among never/former MHT users, hours spent sitting ($\geqslant 10$ *vs* $\leqslant 5$ h per day) was positively associated with 2-methoxyestradiol (GM = 15.8 *vs* 12.6 pmol l^{-1}, P-trend = 0.03) and 4-methoxyestradiol (GM = 2.27 *vs* 1.75 pmol l^{-1}, P-trend = 0.02) adjusting for age, potential confounders, and BMI (Table 3); the associations persisted after adjustment for parent oestrogens (GM = 16.4 *vs* 14.4 pmol l^{-1}, P-trend = 0.04; and 2.36 *vs* 1.98 pmol l^{-1}, P-trend = 0.03, respectively; Table 3). Among current MHT users, none of the oestrogen metabolites were associated with time spent sitting (Supplementary Table 1).

Among never/former MHT users, most serum oestrogen metabolites were inversely associated with moderate- to vigorous-intensity physical activity (e.g., $\geqslant 15$ *vs* 0 MET-h per week: GM = 66.6 *vs* 80.8 pmol l^{-1} for 2-hydroxyestrone; 16.7 *vs* 19.9 pmol l^{-1} for 2-hydroxyestradiol; 8.13 *vs* 9.77 pmol l^{-1} for 4-hydroxyestrone; and 33.3 *vs* 40.1 pmol l^{-1} for 16α-hydroxyestrone; all $P\leqslant 0.02$) adjusting for age, potential confounders, and BMI (Table 4). However, after additional adjustment for parent oestrogens, these patterns did not persist. Among current MHT users, none of the oestrogen metabolites were associated with moderate- to vigorous-intensity physical activity (Supplementary Table 2).

Most parent oestrogen associations with physical activity, but not with sitting, were <5% FDR (Tables 2–4). Results were similar after excluding endometrial or ovarian cancer cases at the time of last follow-up (Supplementary Table 3), after excluding women who reported a history of diabetes at baseline (data not shown), after excluding women with low BMI ($<18.5\,\text{kg m}^{-2}$; data not

Table 1. Characteristics of study population by sitting and moderate- to vigorous-intensity physical activity in postmenopausal women not using menopausal hormone therapy, stratified by menopausal hormone therapy use: the Women's Health Initiative Observational Study

Postmenopausal women not using menopausal hormone therapy ($N = 936$, weighted $N = 30\,405$)							
	Sitting (h per day)			Moderate- to vigorous-intensity physical activity (MET-h per week)			
	⩽5	6–9	⩾10	0	0.1–7.4	7.5–14.9	⩾15
Characteristic	*N* (weighted %[a])			*N* (weighted %[a])			
Age at baseline blood draw (years)							
<55	17 (4.6)	36 (7.2)	27 (16.9)	15 (4.3)	12 (8.7)	20 (8.6)	33 (10.5)
55–59	57 (13.3)	63 (17.9)	55 (23.4)	48 (22.9)	42 (18.4)	25 (11.5)	60 (17.8)
60–64	71 (18.6)	94 (22.5)	56 (21.2)	52 (21.9)	51 (21.6)	52 (20.7)	66 (19.5)
65–69	71 (23.6)	92 (27.3)	34 (15.9)	37 (21.8)	52 (25.5)	51 (23.0)	57 (23.1)
70–74	73 (28.0)	66 (15.4)	29 (11.9)	32 (21.4)	42 (18.5)	41 (18.4)	53 (19.4)
75–79	35 (12.0)	38 (9.7)	22 (10.8)	17 (7.7)	16 (7.3)	31 (17.8)	31 (9.8)
Race							
White	283 (89.1)	342 (88.0)	200 (91.9)	164 (84.2)	181 (84.7)	200 (91.1)	280 (94.0)
Year at blood draw							
1993–1996	188 (53.6)	244 (67.3)	139 (64.6)	117 (57.0)	131 (62.4)	133 (58.4)	190 (66.1)
1997–1998	136 (46.4)	145 (32.7)	84 (35.4)	84 (43.0)	84 (37.6)	87 (41.6)	110 (33.9)
Smoking status							
Never	174 (56.0)	190 (48.5)	110 (43.5)	92 (46.5)	111 (52.2)	117 (50.9)	154 (50.6)
Former	126 (35.7)	172 (42.4)	97 (48.0)	92 (42.9)	83 (38.3)	87 (38.8)	133 (43.7)
Current	24 (8.3)	27 (9.1)	16 (8.5)	17 (10.6)	21 (9.5)	16 (10.3)	13 (5.7)
Time since menopause							
<10 years	96 (27.7)	117 (27.3)	83 (39.0)	63 (29.4)	62 (29.3)	61 (26.8)	110 (33.4)
10–20 years	119 (33.5)	151 (41.2)	84 (34.0)	83 (41.9)	82 (34.7)	92 (38.2)	97 (33.8)
⩾20 years	100 (35.4)	104 (27.4)	42 (20.7)	44 (23.5)	66 (33.5)	57 (30.5)	79 (27.9)
Missing	9 (3.5)	17 (4.1)	14 (6.3)	11 (5.2)	5 (2.4)	10 (4.5)	14 (4.9)
BMI at baseline ($kg\,m^{-2}$)							
<25	130 (45.9)	141 (39.5)	70 (47.8)	38 (25.8)	72 (41.9)	78 (39.9)	136 (54.5)
25–30	116 (37.3)	114 (29.3)	60 (22.3)	59 (27.9)	67 (30.3)	89 (35.2)	90 (27.2)
⩾30	78 (16.8)	134 (31.2)	93 (29.9)	104 (46.3)	76 (27.8)	53 (24.9)	74 (18.4)
Postmenopausal women currently using menopausal hormone therapy ($N = 868$, weighted $N = 24\,851$)							
	Sitting (h per day)			Moderate- to vigorous-intensity physical activity (MET-h per week)			
	⩽5	6–9	⩾10	0	0.1–7.4	7.5–14.9	⩾15
Characteristic	*N* (weighted %[a])			*N* (weighted %[a])			
Age at baseline blood draw (years)							
<55	26 (11.0)	33 (15.5)	40 (26.5)	16 (14.0)	17 (29.5)	11 (6.6)	45 (22.2)
55–59	53 (24.9)	75 (23.1)	65 (30.9)	36 (27.6)	43 (24.8)	46 (33.2)	68 (21.7)
60–64	77 (26.4)	88 (23.1)	49 (21.5)	37 (27.1)	58 (24.5)	44 (23.2)	75 (21.7)
65–69	72 (21.7)	71 (21.0)	33 (9.6)	19 (14.2)	38 (15.0)	50 (21.0)	69 (21.2)
70–74	53 (11.7)	56 (11.8)	25 (9.8)	29 (13.1)	32 (12.3)	29 (12.1)	44 (8.9)
75–79	21 (4.2)	25 (5.5)	6 (1.8)	8 (4.1)	14 (4.0)	14 (3.9)	16 (4.2)
Race							
White	282 (90.5)	325 (93.4)	208 (96.8)	134 (93.0)	199 (94.3)	182 (91.9)	300 (93.6)
Year at blood draw							
1993–1996	174 (57.5)	219 (63.9)	145 (65.9)	90 (56.1)	126 (61.3)	117 (54.8)	205 (70.5)
1997–1998	128 (42.5)	129 (36.1)	73 (34.1)	55 (43.9)	86 (38.7)	77 (45.2)	112 (29.5)
Smoking status							
Never	153 (45.0)	176 (45.9)	92 (47.8)	72 (47.2)	109 (50.4)	99 (55.3)	141 (37.4)
Former	137 (48.3)	159 (50.7)	116 (45.2)	62 (40.7)	95 (44.3)	89 (40.8)	166 (59.9)
Current	12 (6.6)	13 (3.4)	10 (6.9)	11 (12.1)	8 (5.3)	6 (3.9)	10 (2.8)
Time since menopause (years)							
<10	98 (40.5)	132 (38.1)	115 (58.5)	54 (38.8)	84 (48.0)	62 (36.3)	145 (48.9)
10–20	127 (35.9)	133 (39.8)	66 (25.9)	48 (32.1)	78 (31.4)	84 (44.9)	116 (33.4)
⩾20	77 (23.6)	83 (22.1)	37 (15.6)	43 (29.1)	50 (20.5)	48 (18.8)	56 (17.8)
BMI at baseline ($kg\,m^{-2}$)							
<25	166 (49.3)	177 (50.8)	98 (33.5)	45 (35.3)	63 (29.4)	93 (41.2)	206 (62.7)
25–30	91 (32.3)	96 (27.7)	68 (36.4)	47 (28.1)	92 (44.7)	69 (35.4)	74 (25.4)
⩾30	45 (18.1)	75 (21.5)	52 (30.2)	53 (36.6)	57 (25.9)	32 (23.4)	37 (11.8)

Abbreviations: BMI = body mass index; MET = metabolic equivalent of task.
[a]Percentages reflect weighted counts and refer to the study cohort.

Table 2. Geometric means (pmol l^{-1}) and 95% CI of serum parent oestrogen (oestrone and oestradiol) concentrations by sitting and moderate- to vigorous-intensity physical activity in postmenopausal women, stratified by menopausal hormone therapy use: the Women's Health Initiative Observational Study

	Sitting (h per day)				Moderate- to vigorous-intensity physical activity (MET-h per week)				
Postmenopausal women not using menopausal hormone therapy (*N* = 936, weighted *N* = 30 405)									
	⩽5	6–9	⩾10	*P*-trend[a]	0	0.1–7.4	7.5–14.9	⩾15	*p*-diff[b]
N	324	389	223		201	215	220	300	
Weighted *N*[c]	11 434	12 162	6808		6050	6723	7443	10 188	
	Model 1				**Model 1**				
Oestrone	288 (247, 336)	322 (271, 382)	352 (282, 441)	0.08	403 (329, 493)	248 (205, 300)	337 (275, 412)	289 (238, 351)	**<0.001**[d]
Conjugated	227 (191, 269)	251 (207, 303)	273 (214, 348)	0.13	315 (251, 394)	190 (153, 235)	265 (213, 331)	227 (183, 281)	**<0.001**[d]
Unconjugated	54.6 (48.8, 61.0)	61.0 (53.6, 69.5)	70.0 (59.1, 83.0)	**0.01**	74.4 (63.6, 87.1)	50.9 (44.2, 58.5)	62.5 (53.8, 72.5)	55.9 (48.4, 64.6)	**<0.001**[d]
Oestradiol	51.4 (42.8, 61.7)	56.9 (47.0, 68.8)	63.8 (50.0, 81.5)	0.08	72.5 (58.4, 89.9)	46.7 (38.7, 56.4)	58.7 (46.8, 73.7)	49.7 (40.5, 61.0)	**<0.001**[d]
Conjugated	34.4 (28.3, 41.9)	36.8 (29.9, 45.2)	42.0 (32.3, 54.6)	0.13	48.2 (38.5, 60.4)	31.2 (25.1, 38.8)	37.1 (29.2, 47.3)	33.2 (26.7, 41.3)	**0.002**[d]
Unconjugated	12.5 (10.2, 15.4)	15.6 (12.7, 19.2)	16.4 (12.8, 21.0)	**0.04**	19.4 (15.2, 24.8)	11.5 (9.32, 14.1)	16.0 (12.6, 20.3)	12.4 (9.85, 15.6)	**<0.001**[d]
	Model 1 + BMI[e]				**Model 1 + BMI[e]**				
Oestrone	313 (269, 365)	326 (276, 385)	365 (294, 454)	0.16	367 (301, 448)	264 (220, 318)	362 (298, 441)	328 (272, 397)	**0.005**[d]
Conjugated	248 (208, 295)	254 (211, 307)	284 (223, 361)	0.24	284 (227, 356)	204 (166, 250)	288 (232, 358)	261 (212, 322)	**0.008**[d]
Unconjugated	57.8 (52.0, 64.2)	61.6 (54.6, 69.5)	71.8 (61.2, 84.1)	**0.02**	69.9 (60.5, 80.8)	53.2 (46.4, 60.9)	65.7 (57.0, 75.6)	60.9 (53.0, 70.0)	**0.01**[d]
Oestradiol	55.4 (46.4, 66.1)	57.5 (48.3, 68.6)	66.0 (51.8, 84.0)	0.15	66.8 (54.3, 82.3)	49.4 (41.1, 59.4)	62.6 (50.2, 78.1)	55.5 (45.4, 67.8)	**0.02**[d]
Conjugated	36.8 (30.3, 44.6)	37.2 (30.6, 45.1)	43.3 (33.3, 56.2)	0.20	45.0 (36.1, 56.0)	32.8 (26.4, 40.6)	39.3 (31.0, 49.8)	36.6 (29.4, 45.4)	0.05
Unconjugated	14.1 (11.7, 17.0)	15.9 (13.3, 19.1)	17.3 (13.7, 21.7)	0.10	17.0 (13.6, 21.2)	12.6 (10.4, 15.2)	17.8 (14.2, 22.3)	14.9 (12.1, 18.3)	**0.01**[d]
	Model 1 + BMI[e] + physical activity[f]				**Model 1 + BMI[e] + sitting[g]**				
Oestrone	313 (268, 366)	324 (274, 384)	358 (288, 444)	0.16	370 (303, 451)	269 (223, 323)	365 (300, 444)	332 (274, 402)	**0.007**[d]
Conjugated	248 (208, 296)	253 (209, 305)	278 (219, 353)	0.24	286 (228, 358)	207 (168, 254)	290 (233, 360)	264 (213, 327)	**0.01**[d]
Unconjugated	57.7 (51.8, 64.2)	61.4 (54.4, 69.3)	70.6 (60.3, 82.6)	**0.02**	70.6 (61.2, 81.4)	54.4 (47.4, 62.4)	66.4 (57.7, 76.3)	61.8 (53.7, 71.0)	**0.01**[d]
Oestradiol	55.1 (46.2, 65.7)	57.4 (48.2, 68.4)	64.6 (50.9, 82.0)	0.16	67.4 (54.5, 83.2)	50.3 (41.7, 60.8)	63.2 (50.6, 78.9)	56.2 (45.7, 69.0)	**0.02**[d]
Conjugated	36.6 (30.2, 44.4)	37.1 (30.5, 45.0)	42.4 (32.7, 55.0)	0.21	45.3 (36.3, 56.6)	33.4 (26.8, 41.6)	39.6 (31.2, 50.3)	37.1 (29.7, 46.4)	0.06
Unconjugated	14.0 (11.6, 17.0)	15.9 (13.2, 19.0)	16.9 (13.5, 21.2)	0.10	17.1 (13.7, 21.4)	12.8 (10.6, 15.5)	17.9 (14.3, 22.5)	14.9 (12.1, 18.4)	**0.02**[d]
Postmenopausal women currently using menopausal hormone therapy (*N* = 868, weighted *N* = 24 851)									
	Sitting (h per day)				Moderate- to vigorous-intensity physical activity (MET-h per week)				
	⩽5	6–9	⩾10	*P*-trend[a]	0	0.1–7.4	7.5–14.9	⩾15	*p*-diff[b]
N	302	348	218		145	212	194	317	
Weighted *N*[c]	8172	9937	6742		4642	6869	4626	8715	
	Model 1				**Model 1**				
Oestrone	2625 (2007, 3432)	3608 (2697, 4825)	3363 (2468, 4582)	0.09	2832 (2070, 3875)	3051 (2164, 4302)	3460 (2406, 4974)	3190 (2366, 4301)	0.74
Conjugated	2373 (1803, 3122)	3375 (2505, 4549)	3022 (2193, 4164)	0.13	2571 (1858, 3556)	2769 (1940, 3952)	3253 (2240, 4726)	2912 (2140, 3962)	0.65
Unconjugated	183 (148, 226)	252 (203, 311)	232 (184, 294)	0.08	196 (154, 249)	223 (174, 285)	235 (176, 313)	218 (173, 274)	0.62
Oestradiol	370 (287, 476)	484 (359, 653)	455 (339, 612)	0.08	397 (293, 537)	456 (329, 632)	460 (330, 641)	408 (305, 544)	0.71
Conjugated	302 (228, 398)	415 (299, 575)	370 (266, 514)	0.15	324 (231, 455)	370 (257, 534)	392 (270, 568)	345 (251, 474)	0.77
Unconjugated	38.8 (30.9, 48.6)	48.6 (38.7, 61.0)	48.8 (37.0, 64.4)	0.09	42.3 (33.1, 54.1)	48.6 (37.1, 63.7)	48.2 (37.0, 63.0)	40.0 (31.1, 51.4)	0.35
	Model 1 + BMI[e]				**Model 1 + BMI[e]**				
Oestrone	2588 (1982, 3380)	3572 (2673, 4775)	3396 (2488, 4636)	0.07	2859 (2094, 3906)	3058 (2171, 4308)	3428 (2387, 4923)	3118 (2297, 4232)	0.81
Conjugated	2336 (1778, 3069)	3337 (2476, 4497)	3052 (2210, 4213)	0.09	2597 (1881, 3587)	2771 (1943, 3950)	3220 (2219, 4675)	2842 (2071, 3899)	0.73
Unconjugated	179 (146, 221)	248 (201, 306)	235 (186, 297)	0.05	199 (156, 252)	223 (174, 285)	231 (174, 308)	211 (166, 266)	0.71
Oestradiol	362 (281, 467)	477 (354, 644)	462 (342, 622)	0.05	405 (300, 547)	458 (332, 633)	451 (325, 627)	388 (290, 521)	0.59
Conjugated	294 (223, 388)	407 (293, 565)	376 (269, 524)	0.08	332 (237, 466)	371 (258, 533)	383 (265, 554)	326 (235, 451)	0.70
Unconjugated	39.5 (31.4, 49.7)	49.2 (39.0, 62.1)	48.3 (36.5, 63.9)	0.18	41.5 (32.5, 53.0)	48.6 (36.9, 63.9)	49.1 (37.3, 64.7)	41.8 (32.2, 54.2)	0.42
	Model 1 + BMI[e] + physical activity[f]				**Model 1 + BMI[e] + sitting[g]**				
Oestrone	2583 (1969, 3388)	3632 (2699, 4887)	3500 (2531, 4839)	**0.04**	2819 (2091, 3802)	3133 (2233, 4396)	3585 (2524, 5092)	3319 (2457, 4483)	0.62
Conjugated	2334 (1766, 3085)	3407 (2507, 4631)	3151 (2250, 4413)	0.06	2555 (1874, 3482)	2822 (1989, 4004)	3365 (2345, 4829)	3023 (2214, 4126)	0.54
Unconjugated	179 (145, 222)	251 (203, 311)	242 (190, 308)	**0.03**	196 (156, 246)	228 (179, 290)	242 (184, 317)	224 (178, 281)	0.50
Oestradiol	365 (284, 470)	480 (356, 648)	470 (348, 634)	**0.04**	400 (299, 536)	468 (341, 642)	468 (339, 647)	409 (307, 544)	0.61
Conjugated	296 (224, 391)	411 (295, 574)	384 (273, 539)	0.07	328 (236, 455)	377 (264, 538)	397 (275, 573)	344 (249, 474)	0.71
Unconjugated	39.8 (31.7, 49.8)	49.7 (39.4, 62.7)	49.5 (37.2, 65.7)	0.17	41.1 (32.4, 52.0)	49.5 (37.4, 65.3)	50.7 (38.9, 66.2)	43.7 (34.0, 56.3)	0.35

Abbreviations: BMI = body mass index; CI = confidence interval; MET = metabolic equivalent task. Model 1 adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, and 75–79 years), blood draw year (1993–1996 and 1997–1998), race (white and non-white), smoking status (never, former, and current), and time since menopause (<10, 10–19, ⩾20 years, and missing). Note: *P*-values <0.05 are denoted in bold font. Conjugated concentrations were calculated as follows: for example, conjugated oestrone = oestrone–unconjugated oestrone.

[a] *P*-trend was estimated using the Wald test for continuous sitting (h per day).

[b] *P*-diff was estimated using global F-test for categories of moderate- to vigorous-intensity physical activity.

[c] Reflects weighted counts and refer to the study cohort.

[d] False discovery rate *q*-value <0.05.

[e] Adjusted for current BMI (continuous, kg m^{-2}).

[f] Adjusted for moderate- to vigorous-intensity physical activity as a categorical variable (0, 0.1–7.4, 7.5–14.9, and ⩾15 MET-h per week).

[g] Adjusted for sitting as a categorical variable (⩽5, 6–9, and ⩾10 h per day).

Table 3. Geometric means (pmol l^{-1}) and 95% CI of serum oestrogen metabolite concentrations by sitting in postmenopausal women not using menopausal hormone therapy: the Women's Health Initiative Observational Study (*N* = 936, weighted *N* = 30405)

	Sitting (h per day)							
	Model 1 + BMI[a]				Model 1 + BMI[a] + parent oestrogens[b]			
	Geometric means (95% CI)			*P*-trend[c]	Geometric means (95% CI)			*P*-trend[c]
	⩽5	6–9	⩾10		⩽5	6–9	⩾10	
N	324	389	223		324	389	223	
Weighted *N*[d]	11 434	12 162	6808		11 434	12 162	6808	
2-Hydroxyestrone	64.9 (56.5, 74.5)	68.7 (59.9, 78.7)	78.0 (63.8, 95.2)	0.05	76.0 (69.4, 83.1)	78.1 (71.8, 85.0)	82.0 (73.1, 91.9)	0.13
2-Hydroxyestradiol	16.3 (14.2, 18.7)	17.1 (14.9, 19.6)	19.5 (15.9, 23.8)	0.07	18.9 (17.1, 20.8)	19.3 (17.7, 21.1)	20.4 (18.0, 23.2)	0.22
2-Methoxyestrone	41.3 (36.9, 46.3)	42.1 (37.5, 47.3)	47.4 (40.4, 55.7)	0.22	47.0 (43.2, 51.1)	46.8 (43.2, 50.7)	49.4 (44.9, 54.4)	0.80
Conjugated	30.6 (26.9, 34.8)	30.4 (26.8, 34.5)	34.8 (29.3, 41.4)	0.26	34.6 (31.2, 38.3)	33.6 (30.5, 37.1)	36.2 (32.3, 40.7)	0.80
Unconjugated	9.74 (8.56, 11.1)	10.6 (9.29, 12.2)	11.7 (9.87, 13.9)	0.14	11.2 (10.1, 12.5)	11.9 (10.7, 13.2)	12.3 (10.9, 13.8)	0.55
2-Methoxyestradiol	12.6 (10.8, 14.6)	13.4 (11.6, 15.5)	15.8 (12.7, 19.5)	**0.03**	14.4 (12.9, 16.1)	15.0 (13.5, 16.7)	16.4 (14.2, 19.0)	**0.04**
Conjugated	10.0 (8.44, 11.9)	10.8 (9.20, 12.8)	12.5 (9.83, 15.9)	**0.04**	11.5 (10.1, 13.2)	12.1 (10.7, 13.8)	13.1 (11.0, 15.5)	0.09
Unconjugated	1.99 (1.71, 2.32)	2.14 (1.88, 2.44)	2.43 (2.03, 2.91)	0.12	2.24 (1.94, 2.58)	2.35 (2.10, 2.63)	2.52 (2.20, 2.89)	0.36
2-Hydroxyestrone-3-methyl ether	7.43 (6.53, 8.46)	7.64 (6.74, 8.66)	8.50 (7.18, 10.1)	0.16	8.40 (7.63, 9.24)	8.45 (7.76, 9.20)	8.83 (7.97, 9.79)	0.59
4-Hydroxyestrone	7.92 (6.86, 9.15)	8.34 (7.26, 9.58)	9.54 (7.76, 11.7)	0.06	9.22 (8.34, 10.2)	9.45 (8.59, 10.4)	10.0 (8.76, 11.4)	0.17
4-Methoxyestrone	4.23 (3.78, 4.73)	4.19 (3.74, 4.69)	5.00 (4.17, 6.00)	0.11	4.73 (4.31, 5.19)	4.59 (4.20, 5.01)	5.18 (4.53, 5.93)	0.32
4-Methoxyestradiol	1.75 (1.51, 2.02)	1.84 (1.60, 2.12)	2.27 (1.82, 2.83)	**0.02**	1.98 (1.75, 2.23)	2.04 (1.82, 2.29)	2.36 (2.00, 2.78)	**0.03**
16α-Hydroxyestrone	32.6 (28.1, 37.7)	34.3 (29.6, 39.6)	38.6 (31.2, 47.8)	0.08	38.3 (34.7, 42.3)	39.2 (35.7, 42.9)	40.7 (35.8, 46.1)	0.24
Oestriol	136 (118, 157)	148 (128, 171)	166 (135, 203)	0.06	161 (147, 177)	170 (156, 186)	175 (157, 195)	0.14
Conjugated	107 (90.9, 126)	117 (99.6, 138)	131 (105, 164)	0.08	128 (115, 143)	136 (122, 151)	139 (123, 157)	0.21
Unconjugated	26.1 (23.0, 29.6)	27.0 (23.8, 30.7)	31.7 (26.6, 37.9)	0.06	29.7 (26.6, 33.1)	30.0 (27.1, 33.2)	33.0 (28.9, 37.8)	0.23
16-Ketoestradiol	34.3 (29.4, 39.9)	36.7 (31.7, 42.6)	41.3 (33.2, 51.2)	0.08	40.5 (36.6, 44.8)	42.1 (38.3, 46.2)	43.5 (38.3, 49.3)	0.21
16-Epiestriol	15.1 (13.1, 17.3)	16.2 (14.2, 18.6)	17.2 (14.2, 20.8)	0.13	17.4 (15.8, 19.1)	18.2 (16.7, 19.9)	18.0 (16.1, 20.1)	0.44
17-Epiestriol	12.6 (11.0, 14.4)	13.7 (12.0, 15.7)	14.3 (11.9, 17.1)	0.14	14.5 (13.2, 15.9)	15.4 (14.1, 16.8)	14.9 (13.2, 16.9)	0.54

Abbreviations: BMI = body mass index; CI = confidence interval. Model 1 adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, and 75–79 years), blood draw year (1993–1996 and 1997–1998), race (white and non-white), smoking status (never, former, and current), and time since menopause (<10, 10–19, ⩾20 years, and missing). Note: All false discovery rate *q*-values >0.05. *P*-values <0.05 are denoted in bold font. Conjugated concentrations were calculated as follows: for example, conjugated 2-methoxyestrone = 2-methoxyestrone–unconjugated 2-methoxyestrone.

[a]Adjusted for current BMI (continuous, kg m^{-2}).

[b]Adjusted for parent oestrogens as a continuous variable (log-transformed).

[c]*P*-trend was estimated using the Wald test for continuous sitting (h per day).

[d]Reflects weighted counts and refer to the study cohort.

shown), and further stratifying by never *vs* former MHT users (data not shown). Results also did not change after excluding outliers (data not shown).

DISCUSSION

To the best of our knowledge, this study is the first to examine the relationships of sitting and physical activity with detailed serum oestrogen metabolite measures in postmenopausal women stratified by MHT usage. In this cross-sectional analysis, longer hours spent sitting was associated with higher levels of unconjugated oestrone among both never/former and current MHT users, adjusted for moderate- to vigorous-intensity physical activity and current BMI. Moderate- to vigorous-intensity physical activity was inversely associated with serum levels of parent oestrogens, independent of current BMI and sitting, among never/former MHT users. Whereas physical activity was not associated with individual oestrogen metabolites after adjustment for the parent oestrogens, time spent sitting was positively associated with methylated catechols in never/former MHT users.

Although growing evidence has shown an increased endometrial (Friberg *et al*, 2006; Moore *et al*, 2010; Schmid and Leitzmann, 2014) and possibly breast (George *et al*, 2010; Dallal *et al*, 2012; Cohen *et al*, 2013; Zhou *et al*, 2015) cancer risk associated with sitting, there are little data on sitting and oestrogens, a potential mediating pathway. A single study has evaluated urinary oestrogens in postmenopausal women not using MHT and found positive associations between accelerometer-measured sedentary time and excreted levels of oestrone and oestradiol (Dallal *et al*, 2016). However, no study to date has evaluated the relationships between sitting and serum oestrogens, which may be more relevant for cancer risk. The current study provides a novel finding that supports a positive association between prolonged sitting and circulating unconjugated oestrogens, the most bioavailable oestrogens, in postmenopausal women. Prolonged sitting may increase circulating oestrogens through a reduction in physical activity energy expenditure and an increase in adiposity, as adipocytes produce oestrogens via aromatase activity (Siiteri, 1987). Independent mechanisms, such as a deceleration in metabolism and excretion of oestrogens (Arciero *et al*, 1998; Bergouignan *et al*, 2011) and an alteration in lipid profiles (Healy *et al*, 2008, 2011; Duvivier *et al*, 2013; Crichton and Alkerwi, 2015), are also likely to exist because the associations with sitting persisted after adjustment for physical activity and BMI. It is also possible that the physical activity-adjusted associations were driven by other types of activity not captured in our data (e.g., light-intensity activity and occupational activity). Our data also suggest that prolonged sitting may further contribute to the differences in absolute serum

Table 4. Geometric means (pmol/l) and 95% confidence intervals (CI) of serum oestrogen metabolite concentrations by moderate- to vigorous-intensity physical activity in postmenopausal women not using menopausal hormone therapy: the Women's Health Initiative Observational Study (*N* = 936, weighted *N* = 30405)

	Moderate- to vigorous-intensity physical activity (MET-h per week)									
	Model 1 + BMI[a]					Model 1 + BMI[a] + parent oestrogens[b]				
	Geometric means (95% CI)					Geometric means (95% CI)				
	0	0.1–7.4	7.5–14.9	⩾15	*p*-diff[c]	0	0.1–7.4	7.5–14.9	⩾15	*p*-diff[c]
N	201	215	220	300		201	215	220	300	
Weighted *N*[d]	6050	6723	7443	10 188		6050	6723	7443	10 188	
2-Hydroxyestrone	80.8 (68.1, 95.7)	58.2 (49.4, 68.6)	71.9 (59.9, 86.3)	66.6 (57.4, 77.2)	**0.007**[e]	84.1 (75.4, 93.7)	76.5 (69.0, 84.8)	76.1 (68.9, 84.2)	75.7 (69.1, 83.0)	0.25
2-Hydroxyestradiol	19.9 (16.7, 23.7)	14.9 (12.6, 17.5)	17.8 (14.9, 21.4)	16.7 (14.4, 19.4)	**0.02**[e]	20.7 (18.3, 23.3)	19.2 (17.2, 21.5)	18.8 (16.9, 20.9)	18.8 (17.1, 20.7)	0.42
2-Methoxyestrone	48.1 (41.5, 55.8)	37.5 (33.0, 42.7)	45.2 (38.9, 52.5)	41.0 (35.8, 47.0)	**0.01**[e]	49.7 (44.6, 55.4)	46.9 (42.6, 51.6)	47.4 (43.2, 52.0)	45.6 (41.6, 49.9)	0.60
Conjugated	34.6 (29.3, 40.8)	28.3 (24.5, 32.6)	33.1 (28.2, 38.8)	29.7 (25.5, 34.6)	0.10	35.7 (31.4, 40.6)	35.0 (31.2, 39.3)	34.6 (31.0, 38.6)	32.9 (29.3, 36.8)	0.65
Unconjugated	12.1 (10.3, 14.2)	8.62 (7.43, 10.0)	11.2 (9.51, 13.2)	10.3 (8.84, 12.1)	**0.001**[e]	12.5 (10.9, 14.3)	11.0 (9.73, 12.4)	11.8 (10.5, 13.2)	11.6 (10.3, 12.9)	0.35
2-Methoxyestradiol	15.5 (12.9, 18.7)	11.8 (10.0, 13.9)	14.0 (11.6, 16.9)	13.2 (11.1, 15.7)	**0.03**[e]	16.1 (14.0, 18.5)	14.9 (13.2, 17.0)	14.7 (13.1, 16.5)	14.7 (13.0, 16.7)	0.62
Conjugated	12.3 (9.88, 15.2)	9.47 (7.87, 11.4)	11.3 (9.22, 13.9)	10.5 (8.67, 12.8)	0.07	12.7 (10.7, 15.1)	12.1 (10.4, 14.0)	11.9 (10.4, 13.6)	11.8 (10.2, 13.7)	0.84
Unconjugated	2.48 (2.10, 2.94)	1.80 (1.54, 2.09)	2.23 (1.89, 2.62)	2.09 (1.81, 2.42)	**0.005**[e]	2.56 (2.20, 2.98)	2.19 (1.90, 2.53)	2.32 (2.05, 2.63)	2.30 (2.04, 2.58)	0.28
2-Hydroxyestrone-3-methyl ether	8.43 (7.20, 9.85)	7.04 (6.13, 8.08)	7.81 (6.67, 9.14)	7.72 (6.71, 8.90)	0.17	8.70 (7.75, 9.75)	8.72 (7.94, 9.58)	8.17 (7.38, 9.05)	8.54 (7.76, 9.41)	0.62
4-Hydroxyestrone	9.77 (8.21, 11.6)	7.20 (6.08, 8.52)	8.71 (7.23, 10.5)	8.13 (6.99, 9.45)	**0.02**[e]	10.2 (8.98, 11.5)	9.38 (8.34, 10.5)	9.21 (8.25, 10.3)	9.21 (8.31, 10.2)	0.39
4-Methoxyestrone	4.71 (4.05, 5.49)	3.91 (3.42, 4.46)	4.55 (3.88, 5.32)	4.36 (3.83, 4.96)	0.10	4.85 (4.28, 5.50)	4.75 (4.25, 5.31)	4.74 (4.26, 5.27)	4.78 (4.30, 5.31)	0.99
4-Methoxyestradiol	2.13 (1.78, 2.55)	1.68 (1.41, 1.98)	1.90 (1.58, 2.29)	1.90 (1.62, 2.22)	0.08	2.20 (1.90, 2.55)	2.08 (1.81, 2.40)	1.99 (1.74, 2.28)	2.10 (1.84, 2.40)	0.60
16α-Hydroxyestrone	40.1 (33.5, 47.9)	29.0 (24.4, 34.6)	36.0 (29.7, 43.7)	33.3 (28.5, 39.0)	**0.01**[e]	41.7 (37.2, 46.9)	38.6 (34.5, 43.1)	38.3 (34.3, 42.7)	38.1 (34.4, 42.1)	0.40
Oestriol	170 (143, 201)	122 (102, 145)	154 (127, 186)	144 (123, 170)	**0.01**[e]	177 (159, 197)	164 (148, 183)	164 (148, 181)	166 (150, 184)	0.57
Conjugated	134 (110, 162)	95.6 (78.8, 116)	121 (98.4, 149)	116 (96.7, 138)	**0.03**[e]	140 (124, 159)	131 (116, 148)	130 (115, 146)	134 (120, 151)	0.69
Unconjugated	31.0 (26.7, 36.0)	22.8 (19.6, 26.4)	29.8 (25.3, 35.2)	27.5 (23.8, 31.7)	**0.004**[e]	32.0 (28.0, 36.6)	28.4 (25.2, 32.1)	31.2 (27.9, 35.0)	30.5 (27.2, 34.3)	0.39
16-Ketoestradiol	42.1 (35.0, 50.5)	30.9 (25.8, 37.0)	38.2 (31.4, 46.6)	36.2 (30.8, 42.6)	**0.03**[e]	43.9 (39.1, 49.2)	41.2 (36.7, 46.3)	40.6 (36.3, 45.5)	41.5 (37.4, 46.1)	0.65
16-Epiestriol	18.2 (15.3, 21.7)	13.9 (11.8, 16.2)	16.3 (13.7, 19.3)	15.7 (13.5, 18.2)	**0.03**[e]	18.9 (16.9, 21.1)	17.8 (16.0, 19.7)	17.2 (15.6, 19.0)	17.7 (16.0, 19.4)	0.43
17-Epiestriol	15.1 (12.7, 17.9)	11.5 (9.83, 13.4)	13.8 (11.8, 16.2)	13.2 (11.4, 15.3)	**0.03**[e]	15.7 (14.0, 17.6)	14.7 (13.1, 16.4)	14.5 (13.2, 16.0)	14.8 (13.5, 16.3)	0.58

Abbreviations: BMI = body mass index; CI = confidence interval; MET = metabolic equivalent task. Model 1 adjusted for age at blood draw (<55, 55–59, 60–64, 65–69, 70–74, and 75–79 years), blood draw year (1993–1996 and 1997–1998), race (white and non-white), smoking status (never, former, and current), and time since menopause (<10, 10–19, ⩾20 years, and missing). Note: *P*-values <0.05 are denoted in bold font. Conjugated concentrations were calculated as follows: for example, conjugated 2-methoxyestrone = 2-methoxyestrone-unconjugated 2-methoxyestrone.

[a]Adjusted for current BMI (continuous, $kg\,m^{-2}$).

[b]Adjusted for parent oestrogens as a continuous variable (log-transformed).

[c]*P*-diff was estimated using global F-test for categories of moderate- to vigorous-intensity physical activity.

[d]Reflects weighted counts and refer to the study cohort.

[e]False discovery rate *q*-value <0.05.

concentrations of unconjugated oestrogens among current MHT users who already have high circulating oestrogens. Among current MHT users, women with longer sitting hours also appeared to be less likely to metabolise parent oestrogens into downstream oestrogen metabolites as proportions of parent oestrogens out of the total measured oestrogens/oestrogen metabolites concentrations were higher in these women, possibly due to the saturation of cytochrome P450 enzymes that regulate hydroxylation of parent oestrogens to downstream metabolites.

Consistent with previous studies (McTiernan *et al*, 2006; Chan *et al*, 2007; Monninkhof *et al*, 2009; van Gils *et al*, 2009; Friedenreich *et al*, 2010; Choudhury *et al*, 2011), we observed inverse associations between physical activity and endogenous parent oestrogens among never/former MHT users. To date, three intervention trials (McTiernan *et al*, 2004; Monninkhof *et al*, 2009; Friedenreich *et al*, 2010) have evaluated the effect of exercise among inactive postmenopausal women not using MHT and have shown a reduction in circulating oestrogen levels as early as within 3 months (McTiernan *et al*, 2004). While there are limited data on current MHT users, a single observational study has reported similar inverse associations between physical activity and parent oestrogens among current MHT users (Choudhury *et al*, 2011); however, the associations were not observed in the current study. The exact biologic mechanisms explaining the inverse association between physical activity and parent oestrogens are not clear. Our data suggest that the associations are likely independent of BMI, as the associations remained after adjustment for current BMI. Our findings agree with all (Madigan *et al*, 1998; McTiernan *et al*, 2006; Friedenreich *et al*, 2010; Choudhury *et al*, 2011) but one (van Gils *et al*, 2009) previous observational studies that assessed associations adjusted for BMI. In a randomised trial (McTiernan *et al*, 2004), a reduction in oestrogen levels was restricted to women in the exercise arm who lost ⩾2% body fat during the study period (measured by dual-energy X-ray absorptiometry), which suggests that the effects of physical activity were primarily mediated by the change in body fat. Further studies are needed to clarify the role of body fat and whether physical activity may lower oestrogen levels through other independent pathways.

Our findings show that physical activity is related to increased levels of circulating parent oestrogens but not oestrogen metabolites. Previous studies evaluated oestrogen metabolites in urine only (Atkinson *et al*, 2004; Dallal *et al*, 2016). A randomised trial examined the effect of exercise on select oestrogen metabolites, 2-hydroxyestrone and 16α-hydroxyestrone, in postmenopausal women and found no difference in the urinary excretion of these oestrogen metabolites or their ratio (Atkinson *et al*, 2004). A cross-sectional analysis reported inverse associations of physical activity with urinary levels of methylated catechols and certain 16-pathway oestrogen metabolites (Dallal *et al*, 2016). However, these studies did not investigate relationships with oestrogen metabolites independent of parent oestrogens, which serve as precursors for oestrogen metabolites. In the current study, we found similar

associations in unadjusted models; however, physical activity was not associated with serum oestrogen metabolites after adjustment for parent oestrogens. In contrast, the positive associations between sitting and methylated catechols persisted after adjustment for parent oestrogens.

We acknowledge several limitations of this study. We used a single measurement of circulating oestrogens/oestrogen metabolites at baseline. However, moderate to high 1-year ICCs reported in a previous study for most oestrogens/oestrogen metabolites (range 0.35–0.72), except for 2-methoxyestrone (0.10) and 2-hydroxyestrone-3-methyl-ether (0.14) (Falk *et al*, 2013), suggest that our data may adequately represent levels over at least 1 year. Although we cannot exclude the possibility of false positives, most associations with physical activity remained at 5% FDR. Finally, physical activity and sitting were based on self-reported measures; however, measurement error in this context is unlikely to be related to serum oestrogen levels. A prior study has shown a moderate test–retest reliability (ICC = 0.77) for moderate- to vigorous-intensity physical activity data reported using the WHI questionnaire (Meyer *et al*, 2009).

Despite these limitations, this study has important strengths. Use of high-performance LC–MS/MS assay allowed comprehensive evaluations of individual oestrogens/oestrogen metabolites with high reliability, sensitivity, and specificity. By stratifying the analysis by MHT usage, we were able to take into account variations in oestrogens/oestrogen metabolite levels by exogenous hormone use. Further, use of a large sample size and careful adjustment for potential confounders assessed at blood collection increased the validity of the results.

In this comprehensive analysis of serum oestrogens/oestrogen metabolites, we observed positive associations between sitting and parent oestrogens and inverse associations between physical activity and parent oestrogens, independent of current BMI. Independent of parent oestrogens, sitting was positively associated with methylated catechols and physical activity was not associated with any of the evaluated oestrogen metabolites in postmenopausal women. These findings suggest that both sitting and physical activity are likely to influence circulating levels of parent oestrogens but only sitting was associated with oestrogen metabolites.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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