Associations between time spent sitting and cancer-related biomarkers in postmenopausal women: an exploration of effect modifiers

Raheem J. Paxton · Su Yon Jung · Mara Z. Vitolins · Jenifer Fenton · Electra Paskett · Michael Pollak · Jennifer Hays-Grudo · Stephen D. Hursting · Shine Chang

Abstract

Purpose Despite evidence that prolonged periods of sitting may influence biological mediators of cancer development, few studies have considered these relationships in a cancer-specific context.

Methods This cross-sectional study included 755 postmenopausal women enrolled in an ancillary study of the Women's Health Initiative. Plasma levels of Insulin-like growth factor-I (IGF-I), IGF-binding protein-3, leptin, insulin, C-peptide, C-reactive protein (CRP), and Interleukin (IL)-6 were measured. The time spent sitting per day was categorized as quartiles (Qs). The relationships between sedentary time and biomarkers were modified by race, physical activity, and exogenous estrogen use.

Results IGF-I levels among African American (AA) women were higher than those of white women across the Qs of sedentary time. Likewise, IL-6 levels in AA women were higher than those in white women at Q3 and Q4 of sedentary time. IGFBP-3 levels were higher and insulin levels were lower across the Qs of sedentary time among women meeting guidelines for physical activity than women who were not. Additionally, CRP levels were higher among estrogen users than nonusers at Q1, Q2, and Q4 of sedentary time.

Conclusions These results suggest that relationship between time spent sitting and cancer-related biomarkers may not be simply linear, but differ in the context of effect modifiers.

Keywords Sedentary behavior · Cancer-relevant biomarkers · Effect modifier · Postmenopausal women

Introduction

Sedentary behavior comprises a distinct class of behaviors (e.g., sitting, reclining, lying down) characterized by low energy expenditure [1–1.5 metabolic equivalents (METs) of rest] and the absence of skeletal muscle movement [1]. Meta-analyses and review articles have indicated that sedentary behavior is associated with negative health consequences [2, 3]. In particular, prolonged periods of sedentary behavior are associated with metabolic

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syndrome, type 2 diabetes, cardiovascular disease, and premature mortality in adults [4–7]. More recently, studies have proposed a possible link between sedentary behavior and cancers of the colon or rectum, prostate, endometrium, and ovary [8]. Prolonged periods of sitting may be a significant potential health issue because many adults spend most of their waking hours engaged in some form of sedentary activity (i.e., sitting at work and sitting at home) [9].

Epidemiological studies have revealed associations between prolonged periods of sedentary time and increased risk for certain cancers [8], but studies to characterize the relationship between sedentary time and important mediators of tumorgenesis are limited [8]. Sedentary behavior may be indirectly related to cancer development via abdominal obesity. Abdominal obesity increases the risk for insulin resistance, chronic low-grade inflammation [i.e., higher levels of interleukin-6 (IL-6) and C-reactive protein (CRP)], and sex hormone levels—consequences known to contribute to tumorgenesis [10, 11]. In addition, higher levels of insulin are correlated with high levels of C-peptide and insulin growth factor (IGF)-1, markers of the IGF-axis [12, 13]. Thus, sedentary time may be indirectly related to cancer initiation and promotion via its association with risk factors that influence metabolic abnormalities [14–18].

The relationship between sedentary time and cancer-related biomarkers may differ by several risk factors. In a recent study, the relationship between sedentary time and cardiometabolic markers differed by race/ethnicity [19], whereby the effects of prolonged periods of sitting were not as severe in African American (AA) or Hispanic individuals. Previous studies have observed race/ethnicity differences in several cancer-related biomarkers [20, 21]. Knowing that the relationships of lifestyle factors and biomarkers may differ in various subgroups may suggest that certain populations are more or less vulnerable to the consequences of prolonged periods of sedentary time.

To date, most studies evaluating the associations between sedentary time and health outcomes have focused primarily on cardiometabolic markers; few studies have focused on associations between sedentary time and cancer-related biomarkers. In this primary analysis of secondary data from an Ancillary study of the Women's Health Initiative, we assessed the association between time spent sitting and several cancer-related biomarkers originally collected for other purposes. The aims of the current study were (1) to characterize the relationship between sedentary time and several cancer-related biomarkers in a population of postmenopausal women and (2) to determine whether these associations differ by socio-demographic and medical characteristics.

Materials and methods

Study population

The study included 825 postmenopausal women who were enrolled in an ancillary study of the Women's Health Initiative Observational Study (WHI-OS) at the WHI clinical centers located at Baylor College of Medicine in Houston, TX, and Wake Forest University School of Medicine in Winston-Salem, NC, and in Greensboro, NC, between February 1995 and July 1998. Women were eligible if they were 50–79 years old, postmenopausal, residing near the clinical center during the study period, and able to provide written consent. This ancillary study included non-Hispanic white (NHW) and AA women. Details of the rationale and design of the WHI have been reported elsewhere [22, 23]. Of 825 participants, 25 participants who had missing predictor variable data (i.e., sitting hours per day) were excluded. In addition, participants who did not have data for the following outcome variables were excluded: insulin ($n = 5$); leptin ($n = 4$); CRP ($n = 3$); IL-6 ($n = 4$); and C-peptide ($n = 4$). Moreover, 45 participants who had missing information on covariates either at baseline or the third annual visit (AV3) were excluded. After excluding outliers (C-peptide, $n = 3$), the following number of participants were included in this study: IGF-I, number of participants = 755; IGFBP-3 = 755; insulin = 750; leptin = 751; CRP = 752; IL-6 = 751; and C-peptide = 748. This study was approved by the Institutional Review Boards at The University of Texas MD Anderson Cancer Center, Baylor College of Medicine, and Wake Forest University School of Medicine.

Data collection

Data on demographic factors, medical and reproductive history, and lifestyle behaviors were collected from participants at clinic visits using self-administered questionnaires. Demographic variables included baseline age and variables measured at AV3 such as race, education level, and employment status (i.e., full-time, part-time, or

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unemployed). Medical history including prescriptions of medications for hypertension or diabetes and reproductive history including prescription use of female hormones were assessed at AV3.

Lifestyle variables that were evaluated at AV3 included cigarette smoking status, dietary intake, physical functioning, and physical activity. Dietary intake was assessed with a food frequency questionnaire; however, for the purpose of this study, dietary alcohol intake (g) per day and dietary energy intake (kcal) per day were the only variables used to approximate diet. Physical functioning was scored using the RAND 36-Item Health Survey 1.0 scoring rules by assigning 0, 50, or 100 to each of 10 questions and averaging on a range of 0–100, with 100 reflecting the highest level of function possible [24, 25]. Each physical activity (Strenuous-, moderate-, and low-intensity activities) was assigned a MET value (7, 4, and 3, respectively) according to its physical intensity [26, 27], and total MET·hours·week^{-1} was estimated by multiplying the MET level for the activity by the hours exercised per week and summing the values for all types of activities [26, 28, 29]. Total MET·hours·week^{-1} was classified into two MET groups as <10 and ≥ 10 METs·hours·week^{-1}. The cutoff point at 10 METs·hours·week^{-1} is consistent with current recommendations from the American College of Sports Medicine and the American Heart Association [28].

Anthropometric data including weight, height, and waist circumference were collected by trained staff at AV3 [22]. Data quality assurance procedures were utilized when reporting or data entry errors were detected; the errors were corrected or treated as missing data. Corrections for discrepancies between answers to main-sub questions or among relevant variables were made according to data quality assurance procedures.

Predictor variable

The predictor variable was time spent sitting. Time spent in sitting was evaluated at AV3 using the following question: "During a usual day and night, about how many hours do you spend sitting? Be sure to include the time you spend sitting at work, sitting at the table eating, driving or riding in a car or bus, and sitting up watching TV or talking." Response values were provided with eight categories of sitting hours from less than 4 h to 16 or more hours per day. Sitting hours per day were re-categorized as quartiles based on the distribution of the data: (Q1) <4 h, (Q2) 4– $<$ 6 h, (Q3) 6– $<$ 8 h, and (Q4) 8 or more hours per day.

Laboratory methods

For the purpose of this ancillary study, 3-ml fasting blood samples were collected at AV3 from each participant by trained phlebotomists, and processed at laboratories located at each of clinics; plasma aliquots were stored at −80 °C. The plasma samples were analyzed to determine the levels of the following seven biomarkers: total IGF-I, IGFBP-3, insulin, C-peptide, leptin, CRP, and IL-6. IGF-I and IGFBP-3 were analyzed using an enzyme-linked immunosorbent assay kit (Diagnostic Systems Laboratories, Webster, TX), with inter-assay coefficients of variation of 4.16 and 6.01 %, respectively. Sensitivities of the assays for IGF-I and IGFBP-3 were 0.03 and 0.04 ng/ml, respectively. Plasma levels of insulin, C-peptide, leptin, and IL-6 were determined via a multiplex, bead-based assay kit (Linco Diagnostic Services, St. Charles, MO). The detection limits of the assays for insulin, C-peptide, leptin, and IL-6 were 18, 9, 42 pM, and 14 pg/ml, respectively. In addition, CRP was measured using a monoclonal antibody sandwich enzyme-linked immunoassay kit (Linco Diagnostic Services, St. Charles, MO). The detection limit of the assay was 0.1 to 10 ng/ml. Inter-assay variation was determined using control samples provided with the kit and was <5 %.

Statistical analysis

Differences in the distributions of participants' characteristics by sitting hours per day were evaluated using an omnibus F-test from an analysis of variance (ANOVA) for continuous variables and a Chi squared test for categorical variables. If continuous variables were skewed or had outliers, the Kruskal–Wallis test was conducted. Multicollinearity was assessed by using the coefficient of multiple determination (R2), tolerance, and variance-inflation factor for each covariate using the remaining covariates as its predictors; no apparent multicollinearity was shown.

Differences in the seven biomarkers stratified by sitting hours per day were examined using ANOVA if the distribution of the biomarkers was normally distributed, and using the Kruskal–Wallis test, otherwise. Analysis of covariance (ANCOVA) was performed to determine whether mean levels of each biomarker were different across quartiles of sitting hours per day while accounting for covariates; adjusted means and P values were reported. To validate ANCOVA analysis, the homogeneity of regression slope (i.e., no interactions) assumption was tested using an interaction plot and running ANCOVA models with and without interaction terms between sitting hours per day and each of the covariates. Additionally, an assumption test for homogeneity of variance was conducted using a graphical method and a Levene's test. All statistical tests were two-tailed and statistical significance was determined at $p < 0.05$. All statistical analyses were conducted in R Version 2.15.1.

Results

Of 755 participants, 43 % reported sitting for <6 h per day [<4 h per day (14.0 %, $n = 106$); 4–< 6 h per day (28.9 %, $n = 218$)], while 57 % of women were sitting for 6 h or more per day (6– < 8 h/day, 24.4 %; ≥8 h/day, 32.7 %). Across the sitting hour groups, the median age was 61 years (range 50–79 years). The majority of participants were NHW (83.4 %), and more than half of the participants had more than a high school education (60.0 %), no full-time or part-time employment (66.9 %), a history of exogenous estrogen use (65.7 %), and no history of hypertension (54.2 %). The median physical functioning score was 85 (range 0–100). The median of BMI and waist circumference were 27.4 kg/m^2 (range 14.2–59.9 kg/m^2) and 84.5 cm (range 56.5–221.0 cm), respectively. Most participants (94.6 %) were nonsmokers and did not meet current guidelines for physical activity (≥10 MET·hours·week^{-1}, 28.9 % vs. <10 MET·hours·week^{-1}, 71.1 %).

Baseline characteristics according to quartiles of sitting hours per day were presented in Table 1. Participants who reported greater time spent sitting were more likely to be younger ($p = 0.01$), educated beyond high school ($p = 0.01$), and employed full- or part-time ($p < 0.001$) than participants who reported less time spent sitting. Additionally, longer hours spent sitting were positively related to higher BMI ($p < 0.001$) and larger waist circumference ($p < 0.001$) and negatively associated with physical functioning ($p = 0.01$).

Relationship between time spent sitting and cancer related biomarkers

To assess the relationship between each of the seven biomarkers and the quartiles of sitting hours per day, we first sought to determine whether the mean distribution of each biomarker was different for the groups of women stratified by sitting hours per day, followed by multivariate analysis to determine whether the relationship between adjusted means of each biomarker and sitting hours was different according to statistically selected covariates (i.e., modifier of the relationship between each biomarker and sitting hours). See supplemental Figure S1. The other variables investigated for the interactions were not significant.

IGF-I levels

IGF-I levels, in univariate analysis, were not significantly different across the quartiles of sitting hours, although mean levels decreased as time spent sitting increased (Table 2); however, in multivariate analysis, this relationship was different (Table 3a). In analyses stratified by race [effect size for interaction = 0.5; P for interaction = 0.03; confirmed by interaction plot (Supplemental Figure S1.A)], AA women had significantly different mean IGF-I levels ($p = 0.03$) across quartiles of time spent sitting whereas mean IGF-I levels were not significantly different in NHW women. Moreover, IGF-I levels among AA women were higher than those of NHW women (Fig. 1a1); in particular, adjusted mean IGF-I levels for Q1 (<4 h/day, $p = 0.02$) were higher for AA women than NHW women.

IL-6 levels

Geometric mean levels of IL-6 among AA women were not different significantly across the quartiles of sitting hours, while in NHW women, decreasing IL-6 levels were associated with increasing time spent sitting on the borderline significance (Table 3a, $p = 0.059$). Additionally, geometric mean levels of IL-6 among AA women at Q3 ($p = 0.001$) and Q4 of time spent sitting ($p = 0.04$) were higher than those among NHW women at Q3 and Q4 of time spent sitting, respectively (Fig.1a2).

IGFBP-3 levels

The relationship between IGFBP-3 and time spent sitting had an interaction [effect size for interaction = −87.5; P for interaction = 0.09; confirmed by interaction plot (Supplemental Figure S1.C)] with physical activity that was stratified as <10 MET·hours·week^{-1} versus ≥10 MET·hours·week^{-1}. Mean IGFBP-3 levels did not differ across the quartiles of sitting hours in women who did not meet guidelines for physical activity nor in women who met the guidelines (Table 3b); however, IGFBP-3 levels were generally higher among women who met guidelines than in women who did not across Qs of time spent sitting (Fig. 1b1). Specifically, at Q1of time spent sitting, IGFBP-3 levels were significantly higher in women who met guidelines ($p = 0.001$).

Insulin

Likewise, the association between insulin and sitting hours was modified by physical activity [effect size for interaction = −0.1; P for interaction = 0.13; verified by interaction plot (Supplemental Figure S1.D)]. Insulin levels did not differ in either group (women who did or did not meet guidelines of physical activity) across quartiles of time spent sitting. However, geometric mean levels of insulin were, in contrast with IGFBP-3 levels, higher in women who did not meet guidelines for physical activity than women who did; particularly, at Q4 of time spent sitting, higher insulin levels in women who did not meet guidelines reached borderline significance ($p = 0.07$) (Fig.1.b2).

Table 1 Characteristics of participants ($n = 755$) enrolled in the ancillary study of the Women's Health Initiative Observational Study at Baylor College of Medicine and Wake Forest University School of Medicine from February 1995 to July 1998

Variable	Sitting hours per day							
	Q1 (<4 hrs/day)		Q2 (4–<6 hrs/day)		Q3 (6–<8 hrs/day)		Q4 (≥8 hrs/day)	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Age in years, median (range)*	61.0	(51.0–78.0)	62.0	(52.0-79.0)	61.0	(50.0–78.0)	59.0	(50.0–79.0)
Race								
Black	14	(13.2)	47	(21.6)	24	(13.0)	40	(16.2)
White	92	(86.8)	171	(78.4)	160	(87.0)	207	(83.8)
Education*								
≤high school	57	(53.8)	91	(41.7)	69	(37.5)	85	(34.4)
>high school	49	(46.2)	127	(58.3)	115	(62.5)	162	(65.6)
Occupation*								
No	70	(66.0)	170	(78.0)	126	(68.5)	139	(56.3)
Yes	36	(34.0)	48	(22.0)	58	(31.5)	108	(43.7)
Exogenous estrogen use								
No	38	(35.8)	81	(37.2)	63	(34.2)	77	(31.2)
Yes	68	(64.2)	137	(62.8)	121	(65.8)	170	(68.8)
Hypertension								
No	64	(60.4)	118	(54.1)	97	(52.7)	130	(52.6)
Yes	42	(39.6)	100	(45.9)	87	(47.3)	117	(47.4)
Physical functioning†, median(range)*	85.0	(20.0–100.0)	85.0	(5.0–100.0)	85.0	(15.0–100.0)	80.0	(0.0–100.0)
BMI, kg/m^2,median (range)*	25.4	(17.3–51.1)	26.8	(14.2–59.9)	27.5	(16.4–47.1)	29.0	(16.4–57.8)
Waist, cm, median (range)*	80.0	(56.5–221.0)	83.0	(58.2–153.8)	85.3	(61.5–128.2)	90.0	(59.0–155.5)
METs·hour·week^{-1}								
<10	73	(68.9)	152	(69.7)	130	(70.7)	182	(73.7)
≥10	33	(31.1)	66	(30.3)	54	(29.3)	65	(26.3)
Smoking status								
No	104	(98.1)	203	(93.2)	174	(94.6)	233	(94.3)
Yes	2	(1.9)	15	(6.9)	10	(5.4)	14	(5.7)
Dietary alcohol, g, median (range)	0	(0–70.4)	0	(0–150.9)	0	(0–39.3)	0	(0–99.6)
Total calories, kcal, median (range)	1243.0	(361.2–2993.0)	1401.0	(165.7–4454.0)	1340.0	(229.0–3345.0)	1432.0	(301.5–3526.0)

BMI body mass index, *MET* metabolic equivalent

† Physical functioning was estimated based on the approach to scoring the Rand 36-Item Health Survey. Final score of physical functioning per participant was ranged from 0 to 100, with 100 representing the highest level of functioning possible [24, 25]

* $p < 0.05$ from Chi squared test or Kruskal–Wallis test

Leptin levels

Leptin levels, in univariate analysis, were positively related to the quartiles of sitting hours and strongly correlated with BMI ($\rho = 0.74$, $p < 0.001$), whereas the relationship between leptin levels and time spent sitting was not significant in multivariate analysis (Table 3c). When stratified by estrogen use status [effect size for interaction = −103.11; p for interaction = 0.01; verified by interaction plot (Supplemental Figure S1.E)], mean leptin levels among estrogen nonusers were higher than those among users; however, this relationship was non-significant (Fig. 1c1).

CRP levels

Similarly, in univariate analysis, CRP levels were overall proportionate to time spent sitting, and this relationship, after accounting for covariates, was not significant in either estrogen users or nonusers (Table 3c). After CRP levels were stratified by estrogen use status [effect size for interaction = −0.1; P for interaction = 0.13; verified by

Table 2 Mean distributions of the cancer-related biomarkers by quartiles of time spent sitting in participants enrolled in the ancillary study of the Women's Health Initiative Observational Study at Baylor College of Medicine and Wake Forest University School of Medicine from February 1995 to July 1998

Cytokine	Sitting hours per day			
	Q1 (<4 h/day)	Q2 (4– < 6 h/day)	Q3 (6– < 8 h/day)	Q4 (≥8 h/day)
IGF-I, ng/ml				
n (%)	106 (14.0)	218 (28.9)	184 (24.4)	247 (32.7)
Mean ± SD	154.8 ± 60.0	138.5 ± 52.9	144.1 ± 60.6	140.4 ± 53.7
IGFBP-3, ng/ml				
n (%)	106 (14.0)	218 (28.9)	184 (24.4)	247 (32.7)
Mean ± SD	4,997 ± 986.8	4,841 ± 1105.5	4,898 ± 1103.5	4,848 ± 1048.7
Insulin, pM*				
n (%)	105 (14.0)	216 (28.8)	184 (24.5)	245 (32.7)
Mean ± SD	73.9 ± 64.6	74.1 ± 117.5	63.2 ± 62.8	74.0 ± 73.1
C-peptide, pM				
n (%)	105 (14.0)	215 (28.7)	184 (24.6)	244 (32.6)
Mean ± SD	425.7 ± 251.9	434.9 ± 293.5	434.8 ± 252.6	458.7 ± 298.2
Leptin, pM*				
n (%)	105 (14.0)	217 (28.9)	184 (24.5)	245 (32.6)
Mean ± SD	1,043 ± 742.4	1,261 ± 1059.3	1,233 ± 952.7	1,453 ± 1087.4
CRP, ng/ml				
n (%)	105 (14.0)	217 (28.9)	184 (24.5)	246 (32.7)
Mean ± SD	10.6 ± 10.5	10.0 ± 11.7	11.5 ± 14.5	11.9 ± 13.6
IL-6, pg/ml				
n (%)	105 (14.0)	217 (28.9)	184 (24.5)	245 (32.6)
Mean ± SD	36.3 ± 60.6	96.5 ± 606.1	28.0 ± 66.9	69.5 ± 640.6

CRP C-reactive protein, *IGF-I* insulin-like growth factor-I, *IGFBP-3* IGF-binding protein-3, *IL-6* interlukin-6

* $p < 0.05$ from Kruskal–Wallis test

interaction plot (Supplemental Figure S1.F)], the relationship between geometric mean levels of CRP and estrogen use was different from the relationship between geometric mean leptin levels and estrogen use. CRP levels were higher in estrogen users than in nonusers throughout the quartiles of sitting hours except Q3 (Fig. 1c2).

C-peptide levels

Finally, adjusted means of C-peptide concentrations were not different across quartiles of time spent sitting (Table 3d), and participants who had a history of hypertension had higher mean CRP levels than those who did not have a history of hypertension (Fig. 1d); however, the small sample size precluded the detection of statistically significant results. Considering that biomarkers may respond to sedentary behavior differently in participants diagnosed with diabetes, we did sensitivity tests of each of biomarkers in participants with diabetes by comparing the model including those with diabetes with the main effect model; no apparent differences were identified.

Discussion

In this cross-sectional study, we observed that there were no clear linear pattern of relationship between sedentary time and the cancer related biomarkers; however, the associations appear to differ by race/ethnicity, level of physical activity, and exogenous estrogen use. Overall, these data paint a complicated pattern of relationships between sedentary behavior and the cancer-related biomarkers that deserves further attention.

Prolonged periods of sedentary behavior in our study was not significantly associated the concentrations of plasma markers associated with the IGF-axis including IGF-1, IGFBP-3, insulin, and C-peptide. These data differ from the results of previous studies that revealed significant and positive associations between time spent sitting and insulin levels or insulin resistance [15, 30, 31] but are similar to the results of others that revealed no relationships between time spent sitting and IGF-1, IGFBP3, and C-peptide [32, 33]. Mechanistically, prolonged periods of sitting alter pathways (protein and serine/threonine kinases) of the IGF axis by reducing the glucose transport and the activity of lipoprotein lipase. This cascade of events prevents the hydrolysis of triglyceride-rich lipoproteins and causes surges of insulin in the blood [34].

In this study, we found that many of the associations between sedentary time and markers of the IGF axis differed by race/ethnicity and physical activity. The high levels of IGF-1 observed among AA women are consistent with previous research [20] showing racial differences between AA and NHW women. The racial differences in

Table 3 Adjusted mean levels of the cancer-related biomarkers by quartiles of time spent sitting and selected covariate in participants enrolled in the ancillary study of the Women's Health Initiative Observational Study at Baylor College of Medicine and Wake Forest University School of Medicine from February 1995 to July 1998

a. Distributions of adjusted means of biomarkers (IGF-I and IL-6) by sitting hours stratified by race

Cytokine	Black					White				
	Sitting hours per day					Sitting hours per day				
	Q1	Q2	Q3	Q4	*p* value	Q1	Q2	Q3	Q4	*p* value
IGF-I, ng/ml										
n (%)	14 (11.2)	47 (37.6)	24 (19.2)	40 (32.0)		92 (14.6)	171 (27.1)	160 (25.4)	207 (32.9)	
Adjusted mean*	184.8	143.0	175.0	150.9	0.03	148.8	136.4	139.6	139.6	0.35
IL-6†, pg/ml										
n (%)	14 (11.2)	47 (37.6)	24 (19.2)	40 (32.0)		91 (14.5)	170 (27.2)	160 (25.6)	205 (32.7)	
Adjusted mean*	18.7	24.4	23.9	21.1	0.87	21.2	18.3	16.0	17.3	0.06

b. Distributions of adjusted means of biomarkers (IGFBP-3 and insulin) by sitting hours stratified by physical activity

Cytokine	METs·hour·week^{-1} < 10					METs·hour·week^{-1} ≥ 10				
	Sitting hours per day					Sitting hours per day				
	Q1	Q2	Q3	Q4	*p* value	Q1	Q2	Q3	Q4	*p* value
IGFBP=3, ng/ml										
n (%)	73 (13.6)	152 (28.3)	130 (24.2)	182 (33.9)		33 (15.1)	66 (30.3)	54 (24.8)	65 (29.8)	
Adjusted mean*	4791.4	4867.5	4889.7	4744.4	0.61	5438.0	4886.8	4910.2	5042.7	0.11
Insulin†, pM										
n (%)	73 (13.7)	150 (28.1)	130 (24.4)	180 (33.8)		32 (14.7)	66 (30.4)	54 (24.9)	65 (30.0)	
Adjusted mean*	62.1	55.5	53.9	61.7	0.12	60.8	51.8	51.9	49.7	0.40

C. Distributions of adjusted means of biomarkers (leptin and CRP) by sitting hours stratified by estrogen use

Cytokine	Exogenous estrogen nonusers					Exogenous estrogen users				
	Sitting hours per day					Sitting hours per day				
	Q1	Q2	Q3	Q4	*p* value	Q1	Q2	Q3	Q4	*p* value
Leptin, pM										
n (%)	38 (14.7)	81 (31.4)	63 (24.4)	76 (29.5)		67 (13.6)	136 (27.6)	121 (24.5)	169 (34.3)	
Adjusted mean*	1459.0	1529.9	1485.7	1405.3	0.85	1050.5	1261.6	1113.7	1239.6	0.16
CRP†, ng/ml										
n (%)	38 (14.7)	81 (31.3)	63 (24.3)	77 (29.7)		67 (13.6)	136 (27.6)	121 (24.5)	169 (34.3)	
Adjusted mean*	5.5	4.4	6.2	4.5	0.31	7.5	7.0	6.1	7.2	0.57

d. Distributions of adjusted means of C-peptide by sitting hours stratified by history of hypertension

Cytokine	History of hypertension—no					History of Hypertension—yes				
	Sitting hours per day					Sitting hours per day				
	Q1	Q2	Q3	Q4	*p* value	Q1	Q2	Q3	Q4	*p* value
Cytokine										
n (%)	64 (15.7)	118 (29.0)	97 (23.8)	128 (31.4)		41 (12.0)	97 (28.4)	87 (25.5)	116 (34.0)	
Adjusted mean*	425.9	423.1	426.5	385.7	0.50	448.4	470.9	444.2	512.8	0.36

Quartile cut-points: Q1 < 4 h/day; Q2 = 4– < 6 h/day; Q3 = 6– < 8 h/day; Q4 ≥ 8 h/day

CRP C-reactive protein, *IGF-I* insulin-like growth factor-I, *IGFBP-3* IGF-binding protein-3, *IL-6* interlukin-6, *MET* metabolic equivalent

† Geometric mean was produced

* Adjusted mean was obtained by accounting for age, education, occupation, race (not in Table a), physical activity (not in Table b), estrogen use (not in Table c), hypertension (not in Table d), physical functioning, body mass index, waist circumference, smoking, dietary alcohol intake per day, and total daily calories; further adjustment excluding body mass index and waist circumference did not significantly change the estimates

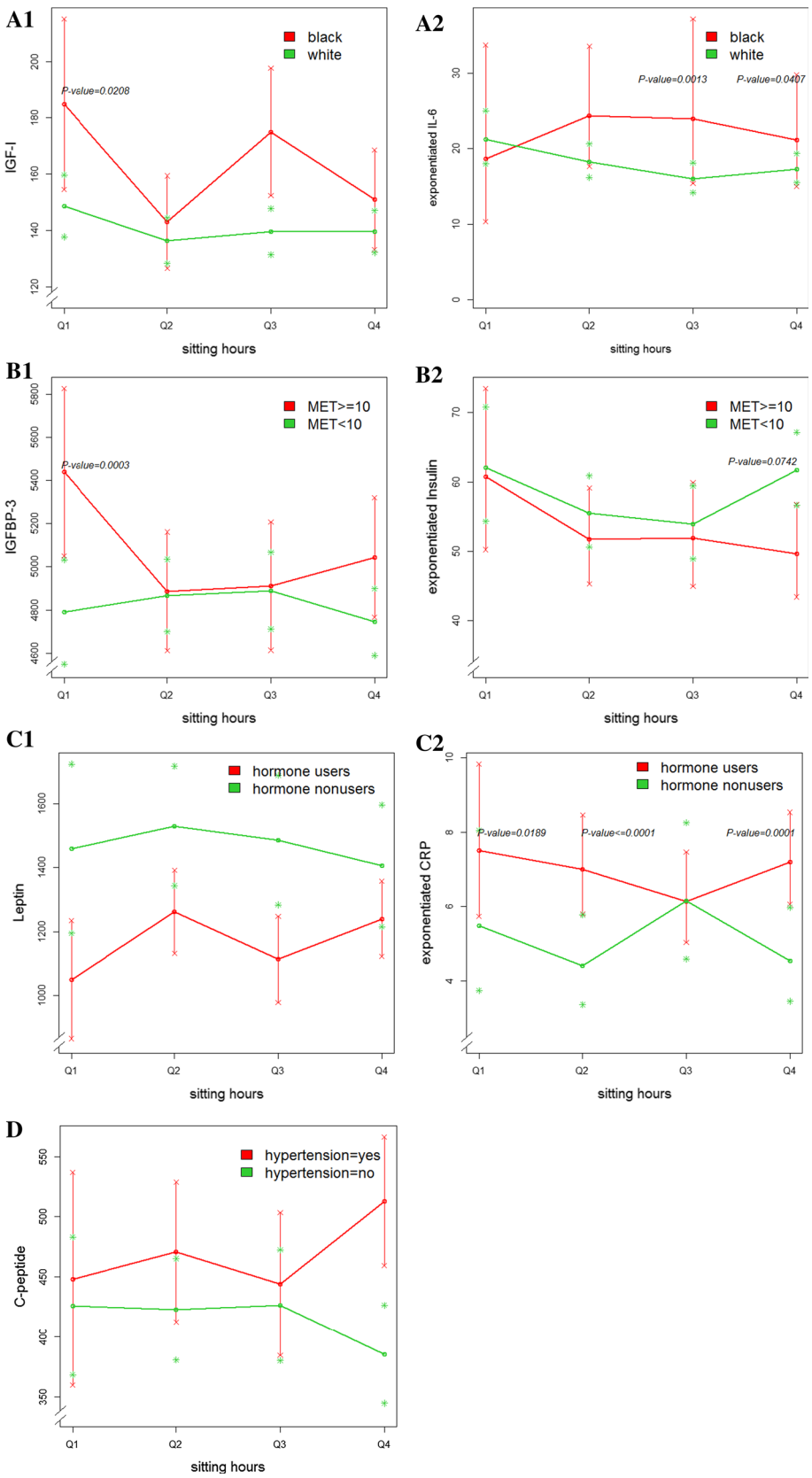

Fig. 1 Graphs presenting distributions of adjusted mean levels of the cancer-related biomarkers by quartiles of time spent sitting and selected covariate in participants enrolled in the ancillary study of the Women's Health Initiative Observational Study at Baylor College of Medicine and Wake Forest University School of Medicine from February 1995 to July 1998 (Note: Quartile cut-points: Q1 $<$ 4 h/day; Q2 $=$ 4– $<$ 6 h/day; Q3 $=$ 6– $<$ 8 h/day; Q4 $\geq$ 8 h/day; CRP, C-reactive protein; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF-binding protein-3; IL-6, interlukin-6; MET, metabolic equivalent) **a1** Distributions of adjusted mean levels of IGF-I by sitting hours stratified by race. **a2** Distributions of adjusted geometric mean levels of IL-6 by sitting hours stratified by race. **b1** Distributions of adjusted mean levels of IGFBP-3 by sitting hours stratified by physical activity. **b2** Distributions of adjusted geometric mean levels of insulin by sitting hours stratified by physical activity. **c1** Distributions of adjusted mean levels of leptin by sitting hours stratified by estrogen use. **c2** Distributions of adjusted geometric mean levels of CRP by sitting hours stratified by estrogen use. **d** Distributions of adjusted mean levels of C-peptide by sitting hours stratified by history of hypertension

IGF-1 as well as differences in IGFBP-3 by level of physical activity could be an artifact of higher rates of obesity and co-occurring risk factors among a high-risk population [20]. We are not the first to observe racial differences in the relationship between insulin and sitting time. Healy et al. [19]. observed clear and consistent linear patterns between sitting time and insulin, insulin resistance, triglycerides, and CRP among NHW women but not AA women. There may be other factors driving the relationship between sitting time and biological markers of cancer initiation and development that deserves attention. In particular, recent studies have suggested that interrupting or breaking up time spent sitting can counter the adverse consequences associated with prolonged sitting [35, 36]. It could be that AA women are more sedentary overall, more likely to watch television, and less likely to interrupt or break up prolonged periods of sitting. Television viewing has been shown to be the most hazardous form of sedentary behavior [5, 9, 14, 18].

Prolonged periods of sitting were not associated with higher serum levels of IL-6 or CRP in our study. Our findings differ with those of previous studies that revealed significant relationships between sitting time and inflammatory markers [30, 37]. Adjusting for both waist circumference and BMI could have attenuated the relationship between sitting time and IL-6 or CRP, as observed elsewhere [30]. Our analysis is unique in that we adjusted for known mediators as well as tested for interactions, analytic techniques that have not been commonly reported in the sedentary behavior literature. Similar results were observed by Allison et al. [37], who found that sedentary time was not associated with IL-6 and CRP but was associated with leptin and tumor necrosis factor-alpha independent of BMI and waist circumference. Here, sedentary time was not significantly associated with levels of leptin, despite evidence of a significant interaction effect between sedentary time and exogenous estrogen usage. The differential effect of sedentary behavior on various adipokines and both anti- and pro-inflammatory markers deserves further attention.

Although we did not observe any associations between sedentary time and the cancer-related biomarkers assessed herein, sedentary behavior is a probable cancer-related risk factor. Prolonged periods of sitting have been associated with abdominal obesity, insulin resistance, and diabetes [34, 38], all of which are known risk factors for cancer [39]. Thus, our working hypothesis was that time spent sitting contributes to higher levels of insulin in the blood, thereby suppressing levels of IGFBPs, leading to increased IGF-1 levels, and promoting the activation or deactivation of several protein kinases, ultimately resulting in uncontrolled cell growth [40]. In a laboratory study investigating the role that breaking up sedentary behavior may have on gene expression, Latouche et al. [36]. observed several genes involved in cell proliferation. Many of these genes (e.g., *DYNLL1* and *NNMT*) inhibit or activate pathways associated with cell proliferation, oxidative damage, and inflammation. Future studies are needed to replicate the findings of Latouche et al. [36]. as are studies of the differential muscle gene expression of naturalistic lifestyle behavior. Serum or plasma samples may not be sufficient to determine the effects of sedentary behavior at the cellular level or in the tumor microenvironment.

Overall, a number of strengths were associated with this study, including the measurement of several biomarkers and the comprehensive assessment of effect modifiers. Despite these strengths, the study also had a number of weaknesses. These data are cross-sectional and the sedentary behavior questionnaire consisted only of one item. Previous studies have indicated that the use of a global item to assess sedentary behavior may not be accurate, and objective measures of sedentary behavior may be a better correlate of biomarkers [8, 41, 42]. Our sample was modest in size, and the number of individuals available for subgroup analyses was too small for meaningful comparisons. However, it should be noted that sensitivity and collinearity analysis were conducted to validate the covariates included in our models. We acknowledge that with so many analyses, we might have a few false positive results, although the large sample size should reassure a bit against that issue. In any case, results should be carefully interpreted, especially when p-values are close to the assumed level of significance.

In sum, the associations between time spent sitting and cancer-related biomarkers were not linear, and no significant associations were found. Despite the non-significant associations, this study represents a unique contribution to the field. These data suggest that the relationship between sedentary behavior and cancer-related biomarkers may differ by important risk factors and more research is needed to unravel the probable indirect relationships that exist between sitting time and biological mediators of cancer development. Future studies should consider testing for interaction and other variables that may change the association between sitting time and various cancer-related biomarkers. Such analyses may yield a probable high-risk population and levels of behaviors that may interact to influence cancer initiation and development. In addition, future research is needed that utilize larger samples sizes of racial and ethnic minorities, objective assessments of sedentary behavior, and measurements of breaks in sedentary time.

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References

1. Tremblay MS, Colley RC, Saunders TJ, Healy GN, Owen N (2010) Physiological and health implications of a sedentary lifestyle. Appl Physiol, Nutr, Metab 35(6):725–740
2. Ford ES, Caspersen CJ (2012) Sedentary behaviour and cardiovascular disease: a review of prospective studies. Int J Epidemiol 41(5):1338–1353
3. Taylor WC (2011) Prolonged sitting and the risk of cardiovascular disease and mortality. Curr Cardiovasc Risk Rep 5:350–357
4. Wijndaele K, Brage S, Besson H et al (2011) Television viewing and incident cardiovascular disease: prospective associations and mediation analysis in the EPIC Norfolk Study. PLoS One 6(5):e20058
5. Wijndaele K, Brage S, Besson H et al (2011) Television viewing time independently predicts all-cause and cardiovascular mortality: the EPIC Norfolk study. Int J Epidemiol 40(1):150–159
6. Chang PC, Li TC, Wu MT et al (2008) Association between television viewing and the risk of metabolic syndrome in a community-based population. BMC Public Health 8:193
7. Katzmarzyk PT, Lee IM (2012) Sedentary behaviour and life expectancy in the USA: a cause-deleted life table analysis. BMJ Open 2(4):e000828
8. Lynch BM (2010) Sedentary behavior and cancer: a systematic review of the literature and proposed biological mechanisms. Cancer Epidemiol Biomarkers Prev 19(11):2691–2709
9. Owen N, Healy GN, Matthews CE, Dunstan DW (2010) Too much sitting: the population health science of sedentary behavior. Exerc Sport Sci Rev 38(3):105–113
10. Paxton RJ, King DW, Garcia-Prieto C et al (2013) Associations between body size and serum estradiol and sex hormone-binding globulin levels in premenopausal African American women. J Clin Endocrinol Metab 98(3):E485–E490
11. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008) Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 371(9612):569–578
12. Gunter MJ, Hoover DR, Yu H et al (2009) Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 101(1):48–60
13. Keinan-Boker L, Bueno De Mesquita HB, Kaaks R et al (2003) Circulating levels of insulin-like growth factor I, its binding proteins -1,-2, -3, C-peptide and risk of postmenopausal breast cancer. Int J Cancer 106(1):90–95
14. Wijndaele K, Healy GN, Dunstan DW et al (2010) Increased cardiometabolic risk is associated with increased TV viewing time. Med Sci Sports Exerc 42(8):1511–1518
15. Lynch BM, Friedenreich CM, Winkler EA et al (2011) Associations of objectively assessed physical activity and sedentary time with biomarkers of breast cancer risk in postmenopausal women: findings from NHANES (2003–2006). Breast Cancer Res Treat 130(1):183–194
16. Stamatakis E, Hamer M, Dunstan DW (2011) Screen-based entertainment time, all-cause mortality, and cardiovascular events: population-based study with ongoing mortality and hospital events follow-up. J Am Coll Cardiol 57(3):292–299
17. Sisson SB, Camhi SM, Church TS et al (2009) Leisure time sedentary behavior, occupational/domestic physical activity, and metabolic syndrome in U.S. men and women. Metab Syndr Relat Disord 7(6):529–536
18. Thorp AA, Healy GN, Owen N et al (2010) Deleterious associations of sitting time and television viewing time with cardiometabolic risk biomarkers: Australian Diabetes, Obesity and Lifestyle (AusDiab) study 2004–2005. Diabetes Care 33(2):327–334
19. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N (2011) Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. Eur Heart J 32(5):590–597
20. Fowke JH, Matthews CE, Yu H et al (2010) Racial differences in the association between body mass index and serum IGF1, IGF2, and IGFBP3. Endocr Relat Cancer 17(1):51–60
21. Pinheiro SP, Holmes MD, Pollak MN, Barbieri RL, Hankinson SE (2005) Racial differences in premenopausal endogenous hormones. Cancer Epidemiol Biomarkers Prev 14(9):2147–2153
22. The Women's Health Initiative Study Group (1998) Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Control Clin Trials 19(1):61–109
23. Langer P, Kocan A, Tajtakova M et al (2003) Possible effects of polychlorinated biphenyls and organochlorinated pesticides on the thyroid after long-term exposure to heavy environmental pollution. J Occup Environ Med 45(5):526–532
24. Hays RD, Sherbourne CD, Mazel RM (1993) The RAND 36-Item Health Survey 1.0. Health Econ 2(3):217–227
25. The RAND Medical Outcomes Study. Scoring Instructions for the 36-Item Short Form Survey (SF-36) (2009) http://www.rand.org/health/surveys_tools/mos/mos_core_36item_scoring.html; http://www.wsib.on.ca/files/Content/POCMTBIRand/MTBI_RAND.pdf
26. McTiernan A, Kooperberg C, White E et al (2003) Recreational physical activity and the risk of breast cancer in postmenopausal women: the Women's Health Initiative Cohort Study. JAMA 290(10):1331–1336
27. McTiernan A, Wu L, Chen C et al (2006) Relation of BMI and physical activity to sex hormones in postmenopausal women. Obesity (Silver Spring) 14(9):1662–1677
28. Haskell WL, Lee IM, Pate RR et al (2007) Physical activity and public health: updated recommendation for adults from the and

the American Heart Association. Med Sci Sports Exerc 39(8):1423–1434
29. Ainsworth BE, Haskell WL, Whitt MC et al (2000) Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 32(9 Suppl):S498–S504
30. Yates T, Khunti K, Wilmot EG et al (2012) Self-reported sitting time and markers of inflammation, insulin resistance, and adiposity. Am J Prev Med 42(1):1–7
31. Hansen AL, Wijndaele K, Owen N et al (2012) Adverse associations of increases in television viewing time with 5-year changes in glucose homoeostasis markers: the AusDiab study. Diabet Med 29(7):918–925
32. Chinapaw MJ, Yildirim M, Altenburg TM et al (2012) Objective and self-rated sedentary time and indicators of metabolic health in Dutch and Hungarian 10-12 year olds: the ENERGY-Project. PLoS One 7(5):e36657
33. Tworoger SS, Missmer SA, Eliassen AH, Barbieri RL, Dowsett M, Hankinson SE (2007) Physical activity and inactivity in relation to sex hormone, prolactin, and insulin-like growth factor concentrations in premenopausal women—exercise and premenopausal hormones. Cancer Causes Control 18(7):743–752
34. Hamilton MT, Hamilton DG, Zderic TW (2007) Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 56(11):2655–2667
35. Healy GN, Dunstan DW, Salmon J et al (2008) Breaks in sedentary time: beneficial associations with metabolic risk. Diabetes Care 31(4):661–666
36. Latouche C, Jowett JB, Carey AL et al (2013) Effects of breaking up prolonged sitting on skeletal muscle gene expression. J Appl Physiol 114(4):453–460
37. Allison MA, Jensky NE, Marshall SJ, Bertoni AG, Cushman M (2012) Sedentary behavior and adiposity-associated inflammation: the Multi-Ethnic Study of Atherosclerosis. Am J Prev Med 42(1):8–13
38. Grontved A, Hu FB (2011) Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis. JAMA 305(23):2448–2455
39. Hjalgrim H, Frisch M, Ekbom A, Kyvik KO, Melbye M, Green A (1997) Cancer and diabetes–a follow-up study of two population-based cohorts of diabetic patients. J Intern Med 241(6):471–475
40. Jalving M, Gietema JA, Lefrandt JD et al (2010) Metformin: taking away the candy for cancer? Eur J Cancer 46(13):2369–2380
41. Celis-Morales CA, Perez-Bravo F, Ibanez L, Salas C, Bailey ME, Gill JM (2012) Objective vs. self-reported physical activity and sedentary time: effects of measurement method on relationships with risk biomarkers. PLoS One 7(5):e36345
42. Healy GN, Wijndaele K, Dunstan DW et al (2008) Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). Diabetes Care 31(2):369–371