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Associations of Sedentary Patterns with Cardiometabolic Biomarkers in Physically Active Young Males

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ABSTRACT

ZHENG, C., X. Y. TIAN, F. H. SUN, W. Y. HUANG, S. SHERIDAN, Y. WU, and S. H.-S. WONG. Associations of Sedentary Patterns with Cardiometabolic Biomarkers in Physically Active Young Males. *Med. Sci. Sports Exerc.*, Vol. 53, No. 4, pp. 838–844, 2021. **Purpose:** Sitting time (ST) is a serious global health issue and positively associated with cardiometabolic disease. The present study investigated associations between objectively measured ST, sedentary patterns, and cardiometabolic biomarkers in physically active young males. **Methods:** Cross-sectional analysis was completed in 94 males 18–35 yr of age. Total ST, prolonged sedentary bouts (≥30 min with no interruption), and sedentary breaks (transitions from sitting/lying to standing/stepping) were assessed using activPAL. Lipids, insulin, C-peptide, C-reactive protein (CRP), vascular cellular adhesion molecule-1, intercellular adhesion molecule 1, E-selectin, P-selectin, leptin, resistin, and adiponectin were measured using assay kits. The expression of specific proteins related to endothelial dysfunction was determined using quantitative real-time polymerase chain reaction. Associations between total ST, prolonged sedentary bouts, and sedentary breaks with cardiometabolic biomarkers and total ST and levels of gene expression were assessed using generalized linear models. **Results:** Total ST was significantly associated with triglycerides (B = 1.814), insulin (B = 2.117), homeostasis model assessment of insulin resistance (B = 0.071), and E-selectin (B = 2.052). Leptin (B = 0.086), E-selectin (B = 1.623), and P-selectin (B = 2.519) were significantly associated with prolonged sedentary bouts, whereas leptin (B = −0.017) and CRP (B = −0.016) were associated with sedentary breaks. After adjustment for moderate to vigorous physical activity, the associations between triglycerides (B = 2.048) and total ST, and between CRP (B = −0.016) and sedentary breaks, remained significant. E-selectin mRNA levels (B = 0.0002) were positively associated with ST with or without adjustment for moderate to vigorous physical activity. **Conclusions:** Total ST and prolonged sedentary bouts were positively associated with several cardiometabolic biomarkers, with interruptions in ST potentially contributing to reduced cardiometabolic risk in physically active young male adults. **Key Words:** SEDENTARY BREAKS, SITTING TIME, SEDENTARY BOUTS, ENDOTHELIAL DYSFUNCTION, ACTIVE ADULTS

The etiology of cardiometabolic diseases is complex, with excessive sedentary behavior (SB) often playing a deleterious role in the development of such diseases (1). To date, most studies examining the relationship between cardiovascular disease (CVD) and SB have focused primarily on traditional cardiometabolic biomarkers. Generally, each additional hour of SB is associated with higher blood pressure (2), fasting glucose (3), and fasting triglycerides (TG) (4). In addition to more traditional biomarkers, emerging novel biomarkers, including inflammatory markers and endothelium-specific biomarkers, also play a role in SB-induced cardiometabolic diseases (5). However, limited studies have examined the relationship between SB and these emerging biomarkers and have conflicting findings. No associations were found between accelerometer-assessed total SB and endothelium markers, including vascular cellular adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), L-selectin, and E-selectin, in adolescents (6), whereas E-selectin was positively associated with total SB in children (7). In adults, greater SB was associated with higher leptin (8); however, no associations between leptin and SB were reported in adolescents (6). Because to differences in participant characteristics and conflicting results from previous studies, both traditional and emerging novel biomarkers should be included in similar

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future studies to provide a more comprehensive understanding of the relationship between SB and cardiometabolic diseases.

In addition to total SB, prolonged sedentary bouts (long periods of uninterrupted SB) and sedentary breaks (sedentary bouts interrupted by standing or stepping) may also contribute to the development of cardiometabolic disease (2,9). One experimental study found that, in adults, both insulin and plasma glucose were significantly higher after 5 h of uninterrupted sitting (10). Similarly, positive associations between metabolic-related markers, including body mass index (BMI), waist circumference, body fat, diastolic blood pressure, and TG, and prolonged sedentary bouts have been observed in adults (2). In contrast to prolonged sedentary bouts, breaks in sedentary time were associated with a lower waist circumference and C-reactive protein (CRP) level in adults (4). Furthermore, Thosar et al. (11) found a significant decline in superficial femoral artery flow-mediated dilation (FMD) after 3 h of prolonged sedentary bouts, with declines in FMD prevented by walking 5 min every 30 min.

Although cardiometabolic diseases are more prevalent in older adults and obese individuals, the pathogenesis of these diseases begins in young adulthood (12,13). Therefore, young adults are an important population in which to examine relationships between SB and cardiometabolic diseases to potentially help reduce future cardiometabolic disease risk. The use of activPAL, the most accurate and sensitive accelerometer currently available to measure sitting time (ST), facilitates sedentary pattern analysis (14). To date, no research has examined the relationships between activPAL-assessed sedentary patterns and cardiometabolic biomarkers in young (Asian) males. Therefore, the present study aimed to investigate the association between objectively measured ST, sedentary patterns, and cardiometabolic biomarkers, especially those associated with endothelial dysfunction, in young male adults.

METHODS

Participants

A total of 104 participants were recruited based on their meeting the following inclusion criteria: 1) Chinese males, 2) 18 to 35 yr of age, 3) BMI under 24.9 $kg \cdot m^{-2}$, 4) blood pressure <140/90 mm Hg, 5) no tobacco smoking or alcohol drinking, and 6) no medical history of CVD or diabetes. One hundred male participants completed the entire study, with a subsample of 75 males agreeing to the collection of peripheral blood mononuclear cells (PBMC). Written consent was obtained from all participants, and assessments were performed between July 2018 and January 2019. This study was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee (CREC ref. no. 2018.314).

Study Design

The study was a cross-sectional study. Participants visited the laboratory twice. A trained research assistant collected anthropometric measurements, including body weight, height, waist circumference, and blood pressure, during the first visit on the first laboratory visit. Participants were instructed to wear the activPAL during the first visit for seven consecutive days and maintain their original lifestyle. The activPAL was taken off on the morning of the eighth day. On the ninth day, participants returned to the laboratory after a 10-h fast for blood sampling and to return the activPAL.

Objective Measurement of Sedentary Patterns and Moderate to Vigorous Physical Activity

Participants were asked to wear the activPAL™ micro (PAL Technologies, Glasgow, U.K.) on the front midline of the right thigh using transparent dressing for seven consecutive days for 24 $h \cdot d^{-1}$ without removal during water activity. The activPAL was fully waterproofed and sealed in a nitrile sleeve. The data were assessed using PALanalysis v8.0 (PAL Technologies) to obtain the raw 15-s epoch result files and the events file, which included all sedentary bouts. Participants recorded their bed and wake-up times, from which the time in bed was calculated. Subsequently, ST during waking hours was calculated as the total sitting/lying time minus the time in bed. A sedentary break was defined as a transition from sitting/lying to standing/stepping during waking times (2). A prolonged sedentary bout was defined as sedentary bouts ≥30 min with no interruption (2). Moderate to vigorous physical activity (MVPA) was defined as ≥5123 counts per minute and determined using the raw 15-s epoch result files. This cutoff point was previously validated in adults wearing the same activPAL model (15). The activPAL data were validated using the same rules as previously described by our research group. Briefly, a valid day had less than 240 min non–wear time (a period with ≥60 min of consecutive unbroken 0 counts) (16). Participants who provided four valid days (three weekdays and one weekend day) were included for further analysis (16). The daily objectively measured values of total ST, prolonged sedentary bouts, sedentary breaks, and MVPA were averaged to the number of valid days.

Blood Sample Measurements

Blood samples. Participants were asked to avoid alcohol, caffeine, and exercise 24 h before blood was taken. A registered nurse collected venous blood samples into EDTA and serum tubes in the morning (8:00 to 10:00 AM). The EDTA tubes were centrifuged (Heraeus™ Megafuge™ 8; Thermo Scientific™, Waltham, MA) for 10 min at 3500 rpm at 4°C to separate the plasma within 15 min. After clotting at room temperature for 30 min, the serum tubes were centrifuged using the same protocol. Serum and plasma were collected in Eppendorf tubes and stored at −80°C in an ultra-low-temperature freezer (Model MDFU52V; Sanyo™, Osaka, Japan).

PBMC isolation. PBMC were isolated from EDTA anticlotting blood following a standard protocol (17). Briefly, the blood was diluted in a conical tube to a 1:1 ratio with phosphate-buffered saline (1×, pH 7.4) (Sigma, St. Louis, MO), and Ficoll-Paque PLUS was used (GE Healthcare,

TABLE 1. List of primers.

Gene	Forward	Reverse
E-selectin	TGTGGGTCTGGGTAGGAACC	AGCTGTGTAGCATAGGGCAAG
P-selectin	ACTGCCAGAATCGCTACACAG	CACCCATGTCCATGTCTTATTGT
ICAM-1	TTGGGCATAGAGACCCCGTT	GCACATTGCTCAGTTCATACACC
CD44	CTGCCGCTTTGCAGGTGTA	CATTGTGGGCAAGGTGCTATT
PSGL-1	CCTGAGTCTACCACTGTGGAG	GCTGCTGAATCCGTGGACA
ESL-1	CCAAGATGACGGCCATCATTT	AGCCGAATACTGCCACATTTC
IL-6	CCTGAACCTTCCAAAGATGGC	TTCACCAGGCAAGTCTCCTCA
IL-1β	AGCTACGAATCTCCGACCAC	CGTTATCCCATGTGTCGAAGAA
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
GAPDH	CCACTCCTCCACCTTTGAC	ACCCTGTTGCTGTAGCCA

PSGL-1, P-selectin glycoprotein ligand-1; ESL-1, E-selectin ligand 1; TNF-α, tumor necrosis factor α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Uppsala, Sweden) to separate PBMC. All samples were centrifuged at 2000 rpm for 30 min at room temperature. PBMC were collected from the corresponding layer (between plasma and Ficoll-Paque) and washed twice using phosphate-buffered saline. Cells were tested for viability (>95%) using the trypan blue dye exclusion test. After washing, supernatants were aspirated, and the cell pellets were frozen at −80°C until quantitative real-time polymerase chain reaction (qPCR) analysis.

Biochemical measurements. Blood glucose was measured immediately after collection using Biosen-C (EKF Diagnostics, Wales, UK). Total cholesterol (TC), TG, and high-density lipoprotein cholesterol (HDL-C) were measured using a colorimetric kit (Stanbio, Wales, UK). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula: LDL-C = TC − HDL-C − (TG / 5) (18). Serum insulin and C-peptide were measured using an enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by multiplying glucose by insulin divided by 22.5 (19).

Cytokine multiplex assay. CRP, VCAM-1, ICAM-1, E-selectin, and P-selectin were tested using the customized LEGENDplex™ multianalyte flow assay kit (BioLegend, San Diego, CA). Leptin, resistin, and adiponectin were measured using the LEGENDplex™ multianalyte flow assay kit (human metabolic panel, BioLegend). Briefly, antibodies specific for the analytes were conjugated to different fluorescence-encoded beads. The beads were mixed with serum samples (diluted 50 times), incubated with shaking for 2 h at room temperature, washed, and incubated for 1 h with a cocktail of different biotinylated detection antibodies. Streptavidin-PE was added, the samples were incubated for 30 min, and the beads were washed and analyzed using BD LSRFortessa™ (BD Company, Franklin Lakes, NJ). The data were analyzed using LEGENDplex™ data analysis software (BioLegend).

qPCR. Total RNA from PBMC was extracted using Trizol (Sigma), and the total RNA was reverse transcribed using the cDNA Reverse Transcription Kit (Takara, Beijing, China). Following a standard protocol, qPCR analysis was performed in an Applied Biosystems ViiA7 (Thermo Scientific™) using SYBR PREMIX (Takara). The list of primers for qPCR is shown in Table 1. All reactions were performed in duplicate, and expression levels of the target mRNA were normalized to the glyceraldehyde-3-phosphate dehydrogenase expression level.

Statistical Analysis

All analyses were performed using SPSS for Windows, version 24 (IBM Corp., Armonk, NY). Descriptive statistical data for all participants were summarized and reported as mean ± SD and proportions of participants for continuous and categorical variables, respectively. The normality analyses were applied to all dependent variables. Associations between objectively measured total ST, prolonged sedentary bouts, and sedentary breaks with cardiometabolic biomarkers were assessed using generalized linear models. Specifically, a gamma distribution and log link were used for leptin, CRP, and VCAM-1, whereas a normal distribution and identity link were used for other dependent variables. Model 1 was adjusted for age, BMI, activPAL wear time, family history of diabetes, family history of CVD, and ST (sedentary breaks only). Model 2 was additionally adjusted for MVPA. Associations between total ST and levels of target mRNA expression were also determined using generalized linear models. The significance level was set at 0.05.

RESULTS

Ninety-four of the 100 participants (mean age ± SD, 21.7 ± 3.8 yr; mean BMI ± SD, 21.1 ± 1.8 $kg \cdot m^{-2}$) provided valid activPAL data and were included in the analysis. There were no differences in participant demographic characteristics between those who provided valid data and those who did not. The descriptive data of participants' characteristics and biomarkers are shown in Table 2. On average, 6.5 d of valid activPAL data were provided. Although participants accumulated 1.1 h of

TABLE 2. Participants' characteristics (n = 94).

	Mean ± SD or %
Individual variables	
Age (yr)	21.7 ± 3.8
Weight (kg)	62.2 ± 6.6
Height (cm)	171.6 ± 6.3
BMI ($kg \cdot m^{-2}$)	21.1 ± 1.8
Waist circumference (cm)	74.4 ± 5.3
Diastolic blood pressure (mm Hg)	71.1 ± 7.2
Systolic blood pressure (mm Hg)	109.1 ± 8.5
Family history of diabetes (%)	22.7
Family history of CVD (%)	36.1
Accelerometer-derived variables	
Wear time ($h \cdot d^{-1}$)	24.0 ± 0.1
ST ($h \cdot d^{-1}$)	10.8 ± 1.6
Prolonged sedentary bouts ($h \cdot d^{-1}$)	6.1 ± 1.9
Number of sedentary breaks (per day)	47.5 ± 13.0
MVPA ($h \cdot d^{-1}$)	1.1 ± 0.5
Biomarkers	
TC ($mg \cdot dL^{-1}$)	167.7 ± 26.5
TG ($mg \cdot dL^{-1}$)	115.8 ± 13.7
LDL-C ($mg \cdot dL^{-1}$)	92.8 ± 25.8
HDL-C ($mg \cdot dL^{-1}$)	51.7 ± 5.2
Plasma glucose ($mmol \cdot L^{-1}$)	4.8 ± 0.3
Insulin ($pmol \cdot L^{-1}$)	37.6 ± 17.1
C-peptide ($pmol \cdot L^{-1}$)	5.4 ± 1.7
HOMA-IR	1.2 ± 0.6
Leptin ($ng \cdot mL^{-1}$)	1.5 ± 1.4
Resistin ($ng \cdot mL^{-1}$)	1.5 ± 0.9
Adiponectin ($\mu g \cdot mL^{-1}$)	17.5 ± 10.1
CRP ($\mu g \cdot mL^{-1}$)	1.3 ± 1.1
E-selectin ($ng \cdot mL^{-1}$)	26.7 ± 14.3
P-selectin ($ng \cdot mL^{-1}$)	37.7 ± 24.6
VCAM-1 ($\mu g \cdot mL^{-1}$)	1.9 ± 1.2
ICAM-1 ($ng \cdot mL^{-1}$)	78.5 ± 41.9

MVPA per day and were therefore considered physically active, they also engaged in 10.8 h of ST per day.

Table 3 presents results from multivariate analyses investigating associations between total ST, prolonged sedentary bouts, and sedentary breaks with cardiometabolic biomarkers. TG was positively associated with ST, with each 1 h ST increment per day associated with a 1.814 mg·dL^{-1} (95% confidence interval [CI] = 0.107–3.520) and 2.048 mg·dL^{-1} (95% CI = 0.116–3.981) higher level of TG in models 1 and 2, respectively. In model 1, significant positive associations were detected between ST and both insulin (B = 2.117, 95% CI = 0.024–4.210) and HOMA-IR (B = 0.071, 95% CI = 0.003–0.138), whereas in model 2, these associations were attenuated after adjustment for MVPA. In model 1, E-selectin was positively associated with both ST (B = 2.052, 95% CI = 0.297–3.807) and prolonged sedentary bouts (B = 1.623, 95% CI = 0.156–3.090). Furthermore, prolonged sedentary bouts were correlated with higher levels of P-selectin (B = 2.519, 95% CI = 0.026–5.012). In model 1, leptin was positively correlated with prolonged sedentary bouts (B = 0.086, 95% CI = 0.001–0.171) but negatively associated with sedentary breaks (B = −0.017, 95% CI = −0.029 to −0.004). In addition, in model 1, CRP was negatively associated with sedentary breaks (B = −0.016, 95% CI = −0.029 to −0.004), which remained significant following MVPA adjustment.

Associations between the expression levels of several cardiometabolic-related target mRNA and ST are shown in Table 4. In model 1, a significant positive association was detected between ST and level of E-selectin mRNA expression (B = 0.0002, 95% CI = 0.0001–0.0003), with this association remaining significant (B = 0.0002, 95% CI = 0.0000–0.0003) after adjustment for MVPA in model 2. In addition, there was a trend (P = 0.06) of positive association between ST and interleukin 6 (IL-6) mRNA expression levels in model 2. As E-selectin mRNA expression was significantly associated with ST, three E-selectin ligands (P-selectin glycoprotein ligand 1, E-selectin ligand 1, and CD44) were further analyzed for their associations with total ST (Table 4). No significant associations were found between ST and other markers, including P-selectin glycoprotein ligand 1, E-selectin ligand 1, and CD44.

DISCUSSION

The present study is, to the best of our knowledge, the first to investigate relationships between objectively measured ST and sedentary patterns in physically active young male adults, using activPAL and cardiometabolic biomarkers. Traditional cardiometabolic biomarkers, including TG, insulin, and HOMA-IR, were positively associated with ST. Notably, circulating cell adhesion molecules such as E- and P-selectin correlated positively with prolonged sedentary bouts, whereas CRP and leptin were negatively associated with sedentary breaks. Moreover, E-selectin mRNA expression levels were positively associated with total ST.

Positive associations between objective ST and TG, independent of the time spent in MVPA, were consistent with a

TABLE 3. Associations of sedentary patterns with cadiometabolic biomarkers (n = 94).

		Total ST (h·d^{-1})	Prolonged Sedentary Bouts (h·d^{-1})	Sedentary Breaks (n per day)
Biomarkers	**Model**	***B* (95% CI)**	***B* (95% CI)**	***B* (95% CI)**
TC (mg·dL^{-1})	1	0.151 (–3.141 to 3.443)	–1.100 (–3.823 to 1.622)	0.057 (–0.356 to 0.469)
	2	–0.360 (–4.087 to 3.367)	–1.407 (–4.225 to 1.411)	0.075 (–0.340 to 0.489)
TG (mg·dL^{-1})	1	1.814 (0.107 to 3.520)*	0.141 (–1.307 to 1.589)	0.184 (–0.023 to 0.392)
	2	2.048 (0.116 to 3.981)*	0.039 (–1.463 to 1.541)	0.178 (–0.031 to 0.387)
LDL-C (mg·dL^{-1})	1	–0.413 (–3.627 to 2.801)	–0.839 (–3.502 to 1.824)	0.033 (–0.369 to 0.435)
	2	–0.705 (–4.348 to 2.939)	–0.974 (–3.737 to 1.789)	0.045 (–0.360 to 0.450)
HDL-C (mg·dL^{-1})	1	0.201 (–0.466 to 0.869)	–0.290 (–0.841 to 0.262)	–0.013 (–0.097 to 0.070)
	2	–0.065 (–0.813 to 0.684)	–0.441 (–1.003 to 0.121)	–0.006 (–0.089 to 0.077)
Plasma glucose (mmol·L^{-1})	1	0.004 (–0.031 to 0.038)	0.014 (–0.015 to 0.043)	0.001 (–0.003 to 0.005)
	2	–0.009 (–0.048 to 0.030)	0.009 (–0.020 to 0.039)	0.002 (–0.003 to 0.006)
Insulin (pmol·L^{-1})	1	2.117 (0.024 to 4.210)*	1.374 (–0.389 to 3.137)	0.211 (–0.047 to 0.470)
	2	1.398 (–0.963 to 3.758)	0.946 (–0.860 to 2.752)	0.233 (–0.025 to 0.491)
C-peptide (pmol·L^{-1})	1	0.100 (–0.109 to 0.310)	–0.007 (–0.183 to 0.169)	0.022 (–0.003 to 0.048)
	2	0.091 (–0.147 to 0.330)	–0.024 (–0.206 to 0.159)	0.023 (–0.004 to 0.049)
HOMA-IR	1	0.071 (0.003 to 0.138)*	0.049 (–0.008 to 0.105)	0.007 (–0.002 to 0.015)
	2	0.047 (–0.029 to 0.122)	0.035 (–0.023 to 0.093)	0.007 (–0.001 to 0.016)
Leptin (ng·mL^{-1})	1	0.057 (–0.040 to 0.155)	0.086 (0.001 to 0.171)*	–0.017 (–0.029 to –0.004)**
	2	–0.001 (–0.108 to 0.107)	0.060 (–0.024 to 0.144)	–0.012 (–0.026 to 0.001)
Resistin (ng·mL^{-1})	1	–0.001 (–0.112 to 0.110)	0.011 (–0.081 to 0.103)	0.006 (–0.008 to 0.020)
	2	0.069 (–0.055 to 0.194)	0.040 (–0.054 to 0.135)	0.004 (–0.010 to 0.018)
Adiponectin (µg·mL^{-1})	1	–0.443 (–1.621 to 0.734)	–0.480 (–1.451 to 0.490)	0.007 (–0.142 to 0.156)
	2	–0.812 (–2.157 to 0.533)	–0.616 (–1.626 to 0.394)	0.016 (–0.134 to 0.165)
CRP (µg·mL^{-1})	1	–0.043 (–0.148 to 0.063)	–0.011 (–0.103 to 0.080)	–0.016 (–0.029 to –0.004)**
	2	–0.054 (–0.170 to 0.062)	–0.014 (–0.110 to 0.081)	–0.016 (–0.029 to –0.004)**
E-selectin (ng·mL^{-1})	1	2.052 (0.297 to 3.807)*	1.623 (0.156 to 3.090)*	–0.032 (–0.257 to 0.193)
	2	1.961 (–0.059 to 3.981)	1.474 (–0.038 to 2.985)	–0.028 (–0.253 to 0.197)
P-selectin (ng·mL^{-1})	1	2.928 (–0.068 to 5.923)	2.519 (0.026 to 5.012)*	0.066 (–0.316 to 0.448)
	2	3.239 (–0.208 to 6.685)	2.471 (–0.106 to 5.047)	0.064 (–0.319 to 0.447)
VCAM-1 (µg·mL^{-1})	1	–0.003 (–0.069 to 0.064)	0.022 (–0.035 to 0.079)	0.001 (–0.008 to 0.010)
	2	0.025 (–0.048 to 0.098)	0.032 (–0.025 to 0.090)	0.000 (–0.008 to 0.009)
ICAM-1 (ng·mL^{-1})	1	0.165 (–5.044 to 5.375)	2.517 (–1.795 to 6.829)	–0.162 (–0.824 to 0.500)
	2	3.550 (–2.287 to 9.387)	3.828 (–0.503 to 8.160)	–0.214 (–0.862 to 0.434)

Model 1: adjusting for age, BMI, activPAL wear time, family history of diabetes, family history of CVD, and ST (sedentary breaks only). Model 2: adjusting for the above covariates and MVPA.
*P < 0.05.
**P < 0.01.

TABLE 4. Associations of total ST with mRNA expression levels (n = 75).

mRNA Expression Levels	B (95% CI) Model 1	Model 2
Cadiometabolic-related marker		
E-selectin	0.0002 (0.0001 to 0.0003)**	0.0002 (0.0000 to 0.0003)*
P-selectin	0.0034 (–0.0056 to 0.0124)	0.0026 (–0.0076 to 0.0128)
ICAM-1	–0.0000 (–0.0018 to 0.0018)	0.0005 (–0.0015 to 0.0025)
IL-6	0.0001 (–0.0000 to 0.0002)	0.0001 (–0.0000 to 0.0003)***
IL-1β	0.0014 (–0.0014 to 0.0042)	0.0010 (–0.0022 to 0.0041)
TNF-α	0.0002 (–0.0009 to 0.0013)	0.0002 (–0.0010 to 0.0014)
E-selectin ligands		
CD44	0.0134 (–0.0151 to 0.0419)	0.0190 (–0.0131 to 0.0511)
PSGL-1	–0.0033 (–0.0194 to 0.0127)	–0.0042 (–0.0223 to 0.0140)
ESL-1	–0.0020 (–0.0092 to 0.0052)	–0.0030 (–0.0111 to 0.0052)

Model 1: adjusting for age, BMI, activPAL wear time, family history of diabetes, and family history of CVD. Model 2: adjusting for the above covariates and MVPA. All the mRNA were normalized by the GAPDH expression level.
*P < 0.05.
**P < 0.01.
***P = 0.06.
TNF-α, tumor necrosis factor α; PSGL-1, P-selectin glycoprotein ligand-1; ESL-1, E-selectin ligand 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

large study conducted in the United States, involving 12,083 participants 18 to 74 yr of age, which found that prolonged ST was associated with increased TG, after adjustment for MVPA and confounding variables (20). Notably, although fasting insulin and HOMA-IR were positively associated with ST in the present study, no significant associations were found after adjusting for MVPA. Healy et al. (4) found that, in the general adult population, ST was associated with higher insulin levels and HOMA-IR even after adjusting for MVPA. However, no associations between ST and both fasting insulin and HOMA-IR were reported in adults with a family history of diabetes (21). Differences in the characteristics of participants, for example, age and family history, and PA levels may partially explain these inconsistent results. Specifically, participants in the current study engaged in more than 1 h of MVPA per day, whereas the MVPA median in the study by Healy et al. (4) was 0.34 $h \cdot d^{-1}$ (interquartile range 0.15, 0.61). This may explain why, in the current study, associations between ST and both fasting insulin and HOMA-IR were abolished after controlling for MVPA. Furthermore, observed TG findings may be explained by the plausible physiological mechanism that skeletal muscle inactivity may result in lipoprotein lipase activity suppression and plasma TG clearance (22). Higher insulin levels may be accounted for by a reduction in glucose transporter type 4, as associated with fewer skeletal muscle contractions (23). These results indicate the importance of muscle metabolism in maintaining glucose homeostasis and normal plasma lipid levels (24).

In the present study, significant associations were found between objectively measured ST and both prolonged sedentary bouts and E-selectin, as well as prolonged sedentary bouts and P-selectin, in healthy, physically active young males. E- and P-selectin are members of the selectin family and are expressed by endothelial cells; their roles relate to endothelial functions (25). These results are clinically significant because of the role played by these cell adhesion molecules in the early stages of atherosclerosis and other cardiovascular complications associated with metabolic syndrome, in which the endothelium expresses signals such as E- and P-selectin, to facilitate both immune cell adherence to, and transmigration through, the endothelium, causing an accumulation of immune cells in the vasculature, thus further amplifying inflammation. Interestingly, Martinez-Gomez et al. (6) did not find any associations between objectively measured ST and cell adhesion molecules, such as ICAM-1, VCAM-1, E-selectin, and L-selectin, in adolescents, although all of these factors positively correlated with TV viewing time. Similarly, in another study involving children, only ST was associated with E-selectin, whereas VCAM-1 was positively correlated with TV viewing time (7). Inconsistencies between the results of these studies may have occurred because of the differences in behaviors exhibited by the different populations examined. Specifically, children and adolescents engage in more PA and less ST compared with adults, with TV viewing time likely playing a more critical role in ST-induced negative health outcomes in the former demographic (26). A potential mechanism involving ST-induced CVD via endothelial dysfunction may provide further explanation. Briefly, under prolonged sitting conditions, shear rate and blood flow downregulation lead to endothelial dysfunction, with insulin resistance, inflammation, and reactive oxygen species production further contributing to vascular dysfunction (9). Another study also suggested that decreased insulin sensitivity and blood flow may result in endothelial dysfunction (27). Although existing evidence for associations between sedentary patterns and cellular adhesion molecules linked to endothelial function is limited, several experimental studies have directly examined the effects of prolonged sitting on vascular function in young adults. Briefly, endothelial function measured using FMD significantly declined after prolonged, uninterrupted sitting (11,28,29). This indicates that ST has a rapid and direct effect on endothelial function that may be harmful if accumulated over a long period of time.

Notably, CRP negatively correlated with sedentary breaks. This was an important finding because CRP is an acute-phase inflammatory marker and a novel therapeutic target for CVD (30). However, CRP in the present study was not associated with total ST or prolonged sedentary bouts, which is inconsistent with a previous study that found a positive association between CRP and ST in older adults (31). The negative relationship between PA and CRP may explain this result, as more sedentary breaks means less prolonged sedentary bouts

and more PA (32). Moreover, CRP was negatively associated with shorter sedentary bouts, for example, sedentary bouts of 1–4 min (33). Consistent with a previous study in a high type 2 diabetes–risk population (8), leptin was negatively associated with sedentary breaks, with the association disappearing after adjusting for MVPA. Leptin is involved in a complex neural circuit associated with controlling food intake. Therefore, it is reasonable to speculate that a high volume of prolonged sedentary bouts, which means less energy expenditure, would be positively associated with leptin (34). In addition to total ST, sedentary patterns such as sedentary bouts and sedentary breaks were also worth considering in this study because the relationship between total ST, sedentary patterns, and sedentary breaks with cardiometabolic biomarkers was variable. As sedentary breaks were assessed using activPAL in the current study, prolonged sedentary bouts interrupted by both standing and stepping were measured. However, it is not clear whether ST interrupted by different intensities of PA results in similar effects on cardiometabolic biomarkers. Specifically, interrupting ST with short bouts of light PA (LPA) or moderate PA (MPA) has been shown to lower both postprandial glucose and insulin levels (10), whereas interrupting ST with standing did not improve postprandial glycemia compared with LPA (35). Thus, reducing total ST and interrupting ST with LPA or MPA may be beneficial for cardiometabolic-related outcomes (36).

To gain further insight into the associations between ST and emerging cardiometabolic markers, the gene expression of these disease-related proteins was measured in PBMC. After prolonged sitting, blood flow and shear stress were reduced, and endothelial dysfunction was mediated by reduced shear stress (28,29). One previous study found that shear stress resulted in IL-6-modulated E-selectin expression in human endothelial cells (37). This may explain the positive association found between E-selectin mRNA level and total ST. Moreover, a positive association between IL-6 mRNA level and ST was also observed, which suggested that the elevated expression of IL-6-modulated E-selectin might be induced by a decreased shear stress during prolonged ST (37). In contrast to E-selectin protein level, positive associations between P-selectin mRNA level and ST remained significant after adjusting for MVPA. Plausibly, mRNA is not a direct indicator of protein level as there are additional control mechanisms besides transcription (38). Thus, high MVPA levels may only reduce ST-induced risk at protein, rather than mRNA, level. To the best of our knowledge, the current study is the first to examine associations between objective ST, cellular adhesion molecule gene expression, and inflammatory markers. Further research is necessary to determine associations between ST and endothelial function biomarker gene expression in different populations.

Findings that MVPA attenuated associations between ST and various cardiometabolic biomarkers are in line with a recently harmonized meta-analysis showing dose–response associations between accelerometry measured PA, ST, and all-cause mortality in middle-age and older adults (39). Similarly, another meta-analysis showed that, in adults, approximately 60–75 min of MVPA per day could eliminate increased mortality risks induced by high ST based on self-reported data (40). Collectively, this evidence highlights the importance of MVPA, especially for individuals who sit for a long time throughout the day (41). Similarly, the latest PA guidelines also recommended that ST should be interrupted with PA (42).

One of the strengths of our study is the use of activPAL, an objective measurement tool. This tool can differentiate between postures, such as sitting/lying, standing, and stepping, and is thus more accurate and sensitive for assessing sedentary patterns, compared with self-report or actigraphy, especially in free-living conditions (14). These measurements provided us with a more comprehensive examination of the relationships between ST, sedentary patterns, and cardiometabolic health. Moreover, the gene expression of statistically significant markers was measured to gain further insight into cellular events that may occur during prolonged sitting.

A limitation of this study was its cross-sectional design, making it difficult to determine causal relationships. Second, as only physically active young men were recruited, the results of this study may not be generalized. In females, different phases of the menstrual cycle may influence metabolic-related markers, such as LDL-C (43). Future studies should examine whether similar associations between ST and cardiometabolic health exist in females. Third, food intake, such as snacks, during ST was not considered in this study, a factor that may also relate to metabolic syndromes (44). Finally, no objective functional measurements of endothelial function were performed in the present study, such as FMD. Therefore, early microvascular dysfunction, such as structural changes, was not examined in the current study.

CONCLUSION

In summary, sedentary patterns, including total ST, prolonged sedentary bouts, and sedentary bouts, may play an important role in cardiovascular and metabolic diseases in physically active young males, through both traditional and novel biomarkers relating to endothelial dysfunction. The expression of genes related to inflammatory proteins and cellular adhesion molecules was positively associated with high ST. The findings from this study may have important public health implications and provide evidence for further intervention studies.

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