Interrupting Prolonged Sitting and Endothelial Function in Polycystic Ovary Syndrome

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ABSTRACT

TAYLOR, F. C., D. W. DUNSTAN, E. FLETCHER, M. K. TOWNSEND, R. N. LARSEN, K. RICKARDS, N. MANIAR, M. BUMAN, P. C. DEMPSEY, A. E. JOHAM, N. COHEN, N. OWEN, L. J. MORAN, and D. J. GREEN. Interrupting Prolonged Sitting and Endothelial Function in Polycystic Ovary Syndrome. *Med. Sci. Sports Exerc.*, Vol. 53, No. 3, pp. 479–486, 2020. **Purpose:** In healthy adults, the impairment of vascular function associated with prolonged sitting can be mitigated with intermittent brief bouts of activity. It is unknown whether these benefits extend to women with polycystic ovary syndrome (PCOS), in whom vascular function is typically impaired and sitting time is high. We examined the acute effect of regularly interrupting sitting time with brief simple resistance activities (SRA) on vascular function in PCOS. **Methods:** In a randomized crossover trial, 13 physically inactive women with PCOS (18–45 yr) completed two 3.5-h conditions: 1) uninterrupted sitting (SIT) and 2) sitting interrupted by 3-min bouts of SRA every 30 min. Femoral artery flow-mediated dilation (FMD), resting shear rate, and resting blood flow were measured at 0, 1, and 3.5 h. **Results:** Mean resting femoral shear rate, averaged across the 3.5 h, significantly increased in the SRA condition relative to the SIT condition (40.1 ± 6.1 vs 62.8 ± 6.1 s^{-1}, $P < 0.0001$). In addition, mean resting blood flow also significantly increased across the 3.5 h for SRA relative to SIT (45.0 ± 9.8 vs 72.8 ± 9.9 $mL \cdot min^{-1}$, $P < 0.0001$). There were no differences between conditions in the temporal change in femoral artery FMD across 3.5 h ($P_{time–condition} > 0.05$ for all). **Conclusion:** Frequently interrupting sitting with SRA acutely increased resting shear rate and blood flow in women with PCOS but did not alter FMD. With sedentary behavior increasing in prevalence, longer-term studies of similar interventions to reduce and break up sitting time are warranted. **Key Words:** ARTERIES, BLOOD FLOW, SEDENTARY BEHAVIOR

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in females of reproductive age, with a prevalence of 10% when using the broader Rotterdam criteria (1).

Traditional and novel cardiovascular risk factors associated with PCOS (endothelial dysfunction, dyslipidemia, oxidative stress, and inflammation) place women with PCOS at increased cardiovascular risk (2).

Growing evidence indicates that endothelial dysfunction is inherent in this population, irrespective of obesity and visceral adiposity (3,4). Endothelial dysfunction is an important early event in the progression of atherosclerotic cardiovascular disease, preceding obesity and diabetes, even in those considered otherwise healthy (5). Flow-mediated dilation (FMD) is a noninvasive approach to the assessment of endothelial function *in vivo* and has been widely used as a prognostic marker for the progression of cardiovascular disease risk (6). Despite this, there are limited data available on the effect of lifestyle modifications on endothelial function in women with PCOS. To date, only a small number of studies have investigated the effects of diet and exercise on endothelial function in PCOS, with mixed results (7–9).

Observational studies have shown that women with PCOS have increased sedentary time (10) and reduced physical activity (11) compared with women without PCOS. Further research

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is needed to examine whether prolonged sitting is associated with increased cardiovascular risk for women with PCOS (10,12). Recently, several experimental studies have reported that regular interruptions in prolonged sitting that involve simple resistance activities (SRA) can improve lower-limb endothelial function and arterial compliance, relative to prolonged sitting in healthy and overweight and obese populations (13–15). However, the effects of interrupting prolonged sitting time with brief activity bouts in women with PCOS are currently unknown. Interrupting prolonged sitting may provide an additional therapeutic option for lifestyle intervention in women with PCOS. This may also be a useful intervention for women with PCOS who are not overweight and obese but who still seek advice on lifestyle interventions. In light of this, the aim of the present study was to explore whether interrupting prolonged sitting every 30 min with brief activity interruptions is an effective strategy for improving endothelial function in women with PCOS. Based on our previous findings in overweight and obese populations (14), we hypothesized that, when compared with prolonged, uninterrupted sitting, regular active interruptions would improve endothelial function in women with PCOS.

METHODS

Participants

We recruited 14 women with PCOS (18–45 yr) via local community advertisement, social media, and doctor's clinics. PCOS was defined using the Rotterdam criteria (16), requiring the presence of two of the following three criteria: (i) oligoanovulation, (ii) hyperandrogenism (hirsutism or male pattern alopecia or high levels of testosterone), or (iii) polycystic ovaries on ultrasound (follicle number per ovary of ≥25 and/or an ovarian volume >10 mL). Exclusion criteria included the following: nonsedentary occupation (e.g., nurse), body mass index (BMI) ≥ 45 $kg \cdot m^{-2}$, pregnancy, self-reported regular engagement in moderate- to vigorous-intensity activity (≥150 $min \cdot wk^{-1}$), major acute or chronic illness that limited the ability to perform SRA, use of medications interacting with glucose or insulin metabolism (e.g., metformin), or reproductive (contraceptive pill) hormone production. No women reported being menopausal or perimenopausal.

Study Overview and Randomization

This study was a randomized crossover trial (ACTRN12618000239268) and took place at the Baker Heart and Diabetes Institute between August 2018 and February 2019 and was approved by the Alfred Human Research Ethics Committee (91-18). Potential participants were initially screened using an online eligibility survey, which asked about their general health and medical history. Eligible participants underwent further screening that included nonfasted blood tests for testosterone, sex hormone binding globulin, prolactin and thyroid-stimulating hormone at a local pathology clinic (Melbourne Pathology; Sonic Healthcare Ltd., Sydney, Australia), and/or polycystic ovary ultrasound (MIA, Victoria).

The order of experimental conditions (described below) was randomly assigned by computer generated random numbers (balanced block randomization). Participants were not aware of the condition order until the day of the first experimental visit.

Study Protocol

Familiarization visit. Participants provided written informed consent and attended a familiarization visit 4 to 6 d before their first experimental visit (Fig. 1), at which they were familiarized with the study procedures and measurements. Height, weight, neck, waist, and hip circumference measurements were taken by standard methods, in duplicate, to minimize error. Resting blood pressure (BP) was taken and participants also provided information about medical history and current medications. To minimize diet-induced variability, participants were provided with standardized meal packs for consumption the evening before testing. Consistent with previous investigations (14,17), using FoodWorks Software (FoodWorks Xryris, 2012), all meals were matched for 33.3% of estimated energy requirements (Schofield equation [18], 1.5 activity factor) with a target macronutrient profile of 12%–15% energy from protein, 30%–33% energy from fat, and 53%–55% energy from carbohydrate. Participants were instructed to eat their standardized evening meal between 1900 and 2100 h and to fast until the next morning. Participants were also instructed to avoid moderate to vigorous physical activity (exercise) for 48 h, and caffeine and alcohol for 24 h before each experimental condition. To objectively monitor daily activity levels in the 48 h before, participants wore an activPAL^3 triaxial physical activity monitor (PAL Technologies Ltd., Glasgow, Scotland).

Experimental conditions. On the experimental days, participants arrived at the laboratory between 0730 and 0800 h in a fasted state (>10 h). They were asked to record the day their most recent menstrual cycle commenced, and weight was remeasured. A peripheral intravenous catheter was inserted into the antecubital vein for blood sampling. Each experimental visit started with a 1-h "steady-state" period where blood samples were collected, BP measured, and femoral artery FMD recorded. At 0 h, participants were given a 75-g glucose drink to consume. A 75-g glucose drink was chosen to eliminate potential intra- and intersubject variability associated with chewing solid meals and meal consumption. Blood samples were then collected at half-hourly intervals up until the 3 h mark.

Participants were asked to remain seated in an upright chair, and minimize movement, for the duration of the visit. In the uninterrupted sitting (SIT) condition, participants were asked to remain seated for 3.5 h, only rising from the chair to visit the bathroom. This was replicated during the SRA condition, but sitting was interrupted every 30 min for 3 min of light-intensity body weight exercises (half squats, calf raises, and single knee raises with gluteal contractions). Each exercise was performed for 20 s and repeated three times in a sequential order, while mimicking a standardized, prepared video recording (17). The participant was then asked to return to the seated position. A 10-d washout period was observed between conditions.

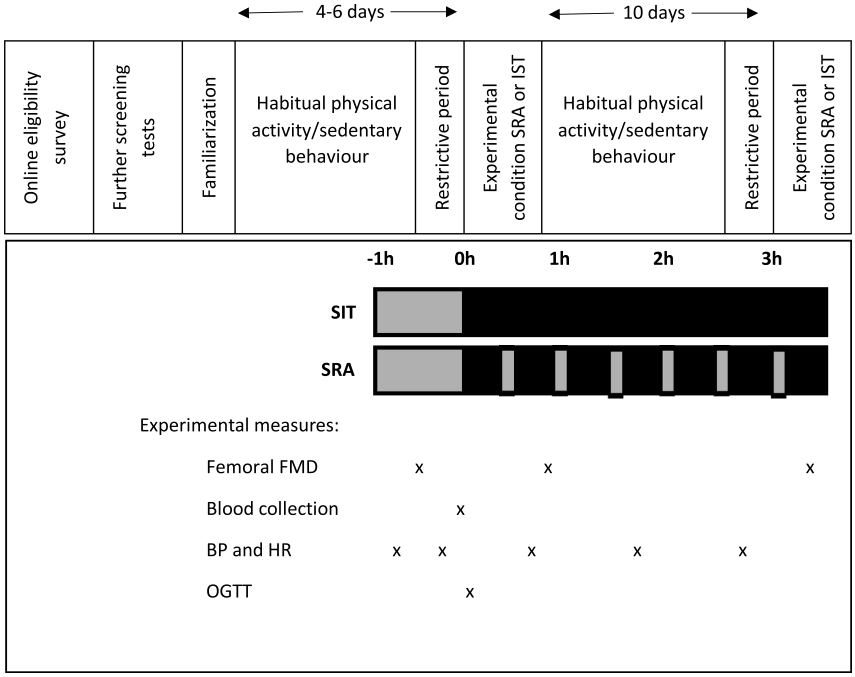

FIGURE 1—Study design and protocol. Participants were initially screened online, followed by further screening tests if eligible. Eligible participants then attended a familiarization visit followed by two experimental conditions in a random order. HR, heart rate; OGTT, oral glucose tolerance test; SIT, uninterrupted sitting condition; SRA, sitting interrupted by the simple resistance activities condition.

Measurements Arterial Function

All FMD measurements were completed in accordance with current evidence-based guidelines (6). Vascular function assessments were performed in a quiet, darkened, temperature-controlled (22°C–25°C) room in a seated position. Participants were left to equilibrate to the darkened room for ~15 min before assessment, and they were instructed to place both feet flat on the floor. The superficial femoral artery was measured in the right leg using a 10-MHz multifrequency linear array probe in conjunction with a high-resolution duplex ultrasound (Terason t3200; Teratech, Burlington, MA) machine at an insonation angle of 60°. A rapid inflation cuff (SC-12-D; D.E. Hokanson Inc., Bellevue, WA) was placed around the thigh, distal to the ultrasound probe. A 1-min recording of blood velocity and continuous resting vessel diameter was measured (live duplex mode) once an optimal image of the artery was obtained. The cuff was then inflated (~220 mm Hg) for 5 min. After 5 min of inflation, the cuff was released to induce reactive hyperemia, and continuous duplex ultrasound recording continued for a further 3 min to observe the postdeflation diameter and peak response. To avoid any transient effects of SRA that may have influenced the measurement, FMD measures occurred right before the SRA and 20 min after the previous activity bout. Placement of the probe was marked and recorded on the first scan at the first visit and replicated for corresponding vascular measurements.

One participant's FMD data were excluded from this analysis because we did not have a complete valid data set. One scanner performed analysis of femoral artery diameter and blood velocity using automated edge detection and wall tracking software (19). Analysis of ultrasound recordings was performed using LabVIEW (version 6.02; National Instruments, Austin, TX). This software has previously been demonstrated to significantly reduce observer error with an intraobserver CV of 6.7% (19). FMD was calculated as the percentage increase in peak diameter from the resting baseline diameter and was measured during the steady-state period (0 h), at 1 h, and at 3.5 h. Shear rate (s^{-1}), derived from blood velocity and diameter, was used as an assessment of shear stress on the artery wall. Shear rate area under the curve from time of cuff release to peak dilation was used to define shear stimulus (20). For this study, our between-visit reproducibility was 4.5%.

Resting BP

Seated resting brachial BP was measured at 5 points across the day, taken at hourly intervals in accordance with recommended guidelines. BP was measured 5 min after activity bouts and in triplicate, at 1-min intervals using an automated BP monitor (Dinamap Vital Signs Monitor 184465X, HEM-907; Omron, Kyoto, Japan) using an appropriately sized cuff (8). All measurements were repeated on the same arm between conditions. For analysis, an average of the three measurements was used.

Biochemical Analysis

To characterize baseline insulin resistance, fasting blood samples were collected during the steady-state period. Whole blood glucose levels were completed in duplicate via a point-of-care HemoCue glucose analyzer (HemoCue Glucose 201+ System, Canada AB) within 5 min of collection, using a modified dehydrogenase method and photometric detection.

For plasma equivalent results, the plasma conversion was made according to the International Federation of Clinical Chemistry using the factor of 1.11. Whole blood was also drawn into serum separator tubes and rested for 30 min before being centrifuged (2000 rpm for 15 min at 4°C). The serum fraction was then separated and stored at −80°C. Testosterone, sex hormone binding globulin, and serum insulin were determined using chemiluminescent microparticle immunoassay (Abbot Alinity) by an independent laboratory accredited by the National Association of Testing Authorities/The Royal College of Pathologists of Australasia (Alfred Pathology, Melbourne) according to manufacturer's instructions.

Statistical Analysis

All analyses were performed using R statistical programming language (version 3.6.1, 2019) (21). Based on previously published work (14), we anticipated an effect size of 3.28, and assuming >90% power and an alpha level = 0.05, we would require a sample size of 13. The primary outcome was FMD. We examined the within- and between-condition effects using generalized linear mixed models. Outcome variables were adjusted for age, BMI, day since commencement of last menstrual period, values at 0 h, and condition order. Additional adjustment for resting diameter and shear stimulus were used on FMD models (6). A condition–time interaction with *post hoc* comparisons was used to compare individual time points between conditions and within condition relative to 0 h. *Post hoc* comparisons between time points were adjusted for multiple comparisons using Šidák corrections. Descriptive data are presented as means ± SD, and output from mixed model analyses is presented as marginal mean ± SEM, where $P < 0.05$ was considered statistically significant.

RESULTS

Participant characteristics. Of the 14 participants randomized, 1 participant withdrew after the first visit because of personal reasons unrelated to the study, with 13 participants completing both experimental conditions (Fig. 2). The participant characteristics are presented in Table 1, and preexperimental

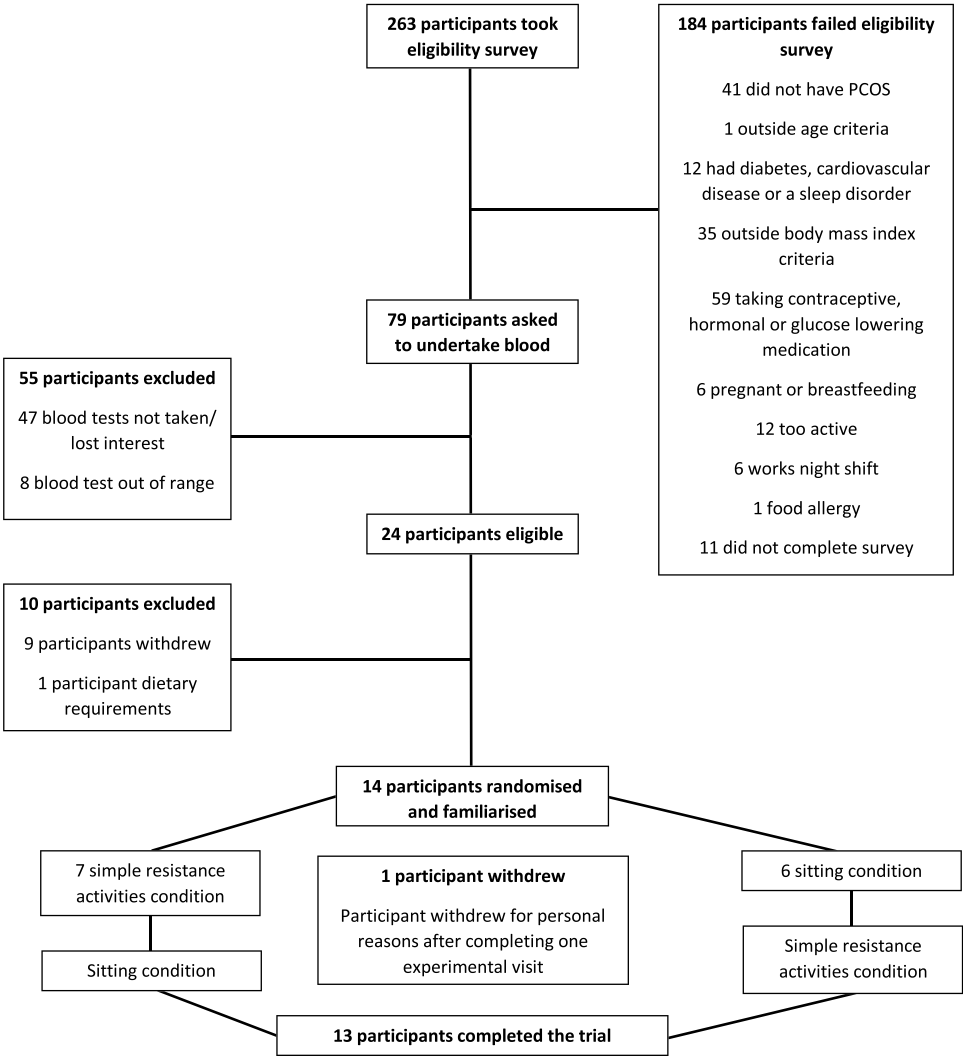

FIGURE 2—Consort standards of reporting trials (CONSORT) diagram.

TABLE 1. Participant characteristics.

	Participants (n = 13)
Clinical characteristics	
Age (yr)	32.2 ± 6.3
BMI (kg·m^{-2})	30.2 ± 5.3
Weight (kg)	81.5 ± 13.4
Waist circumference (cm)	98.5 ± 14.3
Waist-to-hip ratio	0.9 ± 0.1
SBP (mm Hg)	111 ± 10
DBP (mm Hg)	74 ± 10
Ferriman–Gallwey score	12.9 ± 8.1
Biochemical and metabolic parameters	
Testosterone (nmol·L^{-1})	1.5 ± 0.6
SHBG (nmol·L^{-1})	44.1 ± 29.6
FAI	4.0 ± 1.7
Fasting glucose (mmol·L^{-1})	4.7 ± 0.3
Fasting insulin (pmol)	51.6 ± 15.9
HOMA-IR	1.8 ± 0.5
HOMA2-IR	1.1 ± 0.3

Data are presented as mean ± SD. The Ferriman–Gallwey score is for hirsutism.
DBP, diastolic BP; FAI, free androgen index; HOMA-IR; homeostatic model assessment of insulin resistance; SBP, systolic BP; SHBG, sex hormone binding globulin.

period data are presented in the supplemental content (see Table, Supplemental Digital Content 1, Pre-experimental period, http://links.lww.com/MSS/C139). No differences in resting brachial systolic or diastolic BP, averaged across 3.5 h, were observed between the SIT and the SRA conditions, respectively (systolic BP: 105 ± 3 vs 106 ± 3 mm Hg, P = 0.668; diastolic BP: 68 ± 2 vs 67 ± 2 mm Hg, P = 0.701). There were also no differences in mean heart rate averaged over 3.5 h between SIT and SRA conditions, respectively (66 ± 3 vs 68 ± 4 bpm; P = 0.685).

FMD and hemodynamics. The data of 12 participants were analyzed for FMD. The hemodynamic and absolute (i.e., unadjusted) FMD data are presented in supplemental content [see Table, Supplemental Digital Content 2, Hemodynamic and absolute (i.e., unadjusted) flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C140]. Supplemental Digital Content 3 shows the adjusted data with statistical comparisons (see Table, Supplemental Digital Content 3, Hemodynamic and adjusted flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C141). No significant between-condition (7.31% ± 0.61% vs 7.40% ± 0.63%, P = 0.883) or within-condition (P > 0.438 for all; see Table, Supplemental Digital Content 3, Hemodynamic and adjusted flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C141) differences were observed for femoral artery FMD in SIT, relative to SRA condition. Femoral artery FMD averaged across 3.5 h was not significantly different between SIT and SRA conditions (7.66% ± 1.27% vs 7.50% ± 1.27%, P = 0.447; Fig. 3A). Femoral artery FMD change from baseline was not significant between conditions (P = 0.923), nor within conditions (P > 0.543 for all; Fig. 3B). Additional adjustment for resting diameter and shear stimulus did not change the interpretation of the results for SIT vs SRA.

Mean resting femoral shear rate, averaged across 3.5 h, was significantly higher in the SRA condition compared with SIT (62.8 ± 6.1 vs 40.1 ± 6.1 s^{-1}, P < 0.001). Mean resting femoral blood flow, averaged across 3.5 h, was significantly higher in the SRA condition compared with SIT (72.8 ± 9.9 vs 45.0 ± 9.8 mL·min^{-1}, P < 0.001). No significant differences were observed between conditions for baseline diameter (P = 0.597). Further analysis on common phenotypic differences did not yield any common factors in our participants.

DISCUSSION

To our knowledge, this is the first study to examine the acute effects of interrupting prolonged sitting time on endothelial function in women with PCOS. Femoral artery function (measured via FMD) remained relatively constant between the SIT and the SRA conditions across the 3.5-h trial duration, with minimal evidence of a consistent improvement in FMD

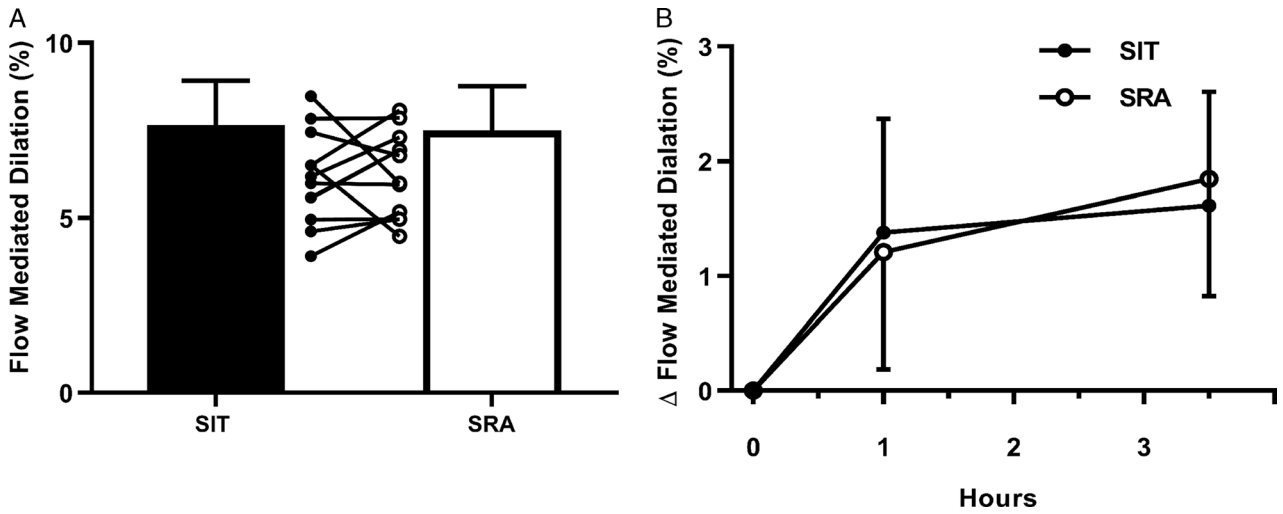

FIGURE 3—A, Mean femoral artery FMD over 3.5 h in the uninterrupted sitting (SIT) and sitting interrupted with SRA conditions. B, A time course of change from baseline of femoral artery flow-mediated (FMD) in the two conditions. Data are adjusted for values at 0 h, age, BMI, days since last period, and treatment order. Mean femoral artery FMD was additionally adjusted for resting diameter and shear stimulus. Data are marginal mean ± SE.

with SRA relative to SIT overall or at specific time points. However, statistically significant increases in both resting shear rate and blood flow were observed at 1 and 3.5 h in the SRA condition relative to the SIT.

Contrary to previous studies (13,14,22), vascular function did not decrease in our sample of women with PCOS after a bout of prolonged sitting. It is plausible that the effects of sitting on endothelial function were not as pronounced in our sample on the basis that we recruited women with PCOS who did not yet have clinically impaired vascular function. Women were recruited based on the Rotterdam criteria, which have been commonly used in observational studies examining endothelial function in PCOS (3). However, in many of these studies (23–26), women with PCOS had more severe insulin resistance and biochemical hyperandrogenism compared with our participants. For example, El-Kannishy et al. (2010) and Kravariti et al. (2005) observed testosterone measurements ranging from 2.50 to 2.95 $nmol \cdot L^{-1}$. By comparison, participants in our study reported testosterone levels of 1.5 ± 0.6 $nmol \cdot L^{-1}$ despite being of similar age and BMI. Moreover, the homeostatic model assessment of insulin resistance index for our participants more closely resembled that of the control (i.e., healthy) women in the aforementioned studies. El-Kannishy et al. (2010) and Kravariti et al. (2005) also reported lower baseline FMD, ranging from 3.50% to 4.13%, relative to our participants 6.6%. Indeed, current literature indicates that the effect of diet and exercise on endothelial function in PCOS has mixed results (7–9). It is possible this is due to a variance in the severity of PCOS. The reduced biochemical hyperandrogenism and insulin resistance severity may partly explain why our study did not demonstrate impairments in the SIT condition, or improvements in the SRA condition.

There was a small, albeit nonsignificant, increase in femoral FMD from baseline in both the SIT and the SRA conditions (see Table, Supplemental Digital Content 3, Hemodynamic and adjusted flow-mediated dilation data during 3.5 h of uninterrupted sitting and sitting interrupted with simple resistance activities, http://links.lww.com/MSS/C141). This is surprising, given that previous work has reported prolonged sitting to impair macrovascular function in both young, healthy (13,27), and older overweight/obese populations (14). However, these studies have been performed primarily in young, healthy men, with recent investigations into the effects of prolonged sitting in women reporting mixed results (22,27). Of relevance, and in line with our work, both these studies observed a subset of women who reported small or no changes in FMD after the SIT protocol. This supports previous suggestions that some young women may be more susceptible to sitting-induced endothelial dysfunction than others (22,27). Given the relationship between habitual activity and vascular function (28), sedentary behavior may, in part, explain the variance among women. Nevertheless, the lack of objectively monitored activity data and small sample sizes in studies to date makes it difficult to develop definitive inferences (22,27).

Although we did not observe a marked increase in FMD for the SRA condition, we did observe a significant increase in resting blood flow and shear rate at 1 and 3.5 h for SRA, compared with SIT. This is noteworthy because reduced leg vascular shear stress is likely the primary mediator of impaired endothelial function in lower extremity conduit arteries (29–31). Although the magnitude of increase in blood flow and, consequently, shear stress needed to induce a clinically meaningful improvement in vascular function is currently unknown, similar increases in shear stress have been reported in women with PCOS after a 12-wk exercise intervention (8). The increase in resting blood flow and shear rate occurred in the SRA condition despite no observed increase in FMD, which has also been reported in previous work (32). This could be partly due to the relatively "normal" baseline FMD in our subset of women with PCOS, making it harder to improve FMD, on average. However, given that in some women FMD improved, a more appropriately powered study may find different results. Nevertheless, shear stress has been recognized as an important physiological factor in maintaining endothelial health (29,31). At the same time, reduced blood flow and shear rate have been implicated in endothelial impairment, with the development of atherosclerotic lesions noted in arterial regions characterized by low shear stress (30). Given that the lower-limb vasculature is susceptible to atherosclerosis, and reduced shear is the primary mediator of impaired vascular function, SRA that promotes increased blood flow and shear stress may benefit leg endothelial function for women with PCOS over the long term.

No statistically significant changes in the mean systolic BP, diastolic BP, or heart rate measures were observed between conditions over the trial period. These results contrast those of previous studies that reported a systolic BP-lowering effect with short bouts of activity (33,34). This discrepancy may be related to the relatively low resting BP and/or methodological differences, including the shorter times between active breaks and BP measurements compared with previous studies (35,36).

The well-controlled randomized crossover design is a strength of this study because it provides control for person-specific factors and affords smaller sample sizes. Trial conditions were also strictly supervised and standardized, with restrictive periods before testing days (minimal variance in physical activity levels and diet) monitored through the use of weighed food records and objectively monitored activity data. We also adopted a seated posture for FMD measurements, rather than supine, for "steady state" and throughout the experiment, to reduce the influence of postural changes throughout the study.

The present trial also has limitations future studies could address. This study was performed in a laboratory-based setting. Although beneficial for establishing initial proof of concept, home- and/or work-based sitting reduction studies may more accurately reflect the effect of prolonged sitting on vascular function in a real-life setting. Similarly, given that food has the potential to influence postprandial responses, the use of standardized mixed meals in place of an oral glucose tolerance test may have changed the blood flow response and confounded the results. Further, this was an acute exposure study, and we only examined responses to sitting and breaking up sitting over a 3.5-h period. It is possible that longer-term exposures

may produce different results and assist in gaining a better understanding for the long-term cardiovascular health implications (14). Future research may also establish the efficacy and dose–response relationships associated with SRA breaks, and how different frequencies, intensities, and durations may be applied in free-living settings. The study is also limited by the small sample size, although our findings are in line with previous studies investigating FMD (%) in women (27), and we still observed a change in resting shear stress. Finally, PCOS in this study was defined by the more variable Rotterdam criteria. Given that the Rotterdam PCOS phenotype is associated with a less severe metabolic profile, compared with the classic National Institutes of Health PCOS subtype (oligomenorrhea and elevated testosterone), it is possible that the study may be underpowered to find a difference in non-NIH phenotypes (37). Unfortunately, it was not within the scope of this study to measure estrogen levels, which may have indicated the severity of PCOS in our participants.

In this sample of women with PCOS, we demonstrated that breaking up sitting increased resting blood flow and shear rate but did not alter FMD across 3.5 h when participants undertook brief periods of SRA. Given that women with PCOS report increased sedentary time compared with controls, and that high volumes of sitting contribute to increased risk for all-cause and CVD-related mortality, frequent brief bouts of SRA may provide an additional therapeutic target to maintain healthy vascular function via improved blood flow and shear rate. Future research should aim to examine the longer-term effects of sedentary behavior in women with differing presentations and severities of PCOS and the effectiveness of interventions on arterial function that aim to reduce and break up sitting.

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No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine. All authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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