Long-Term and Acute Benefits of Reduced Sitting on Vascular Flow and Function

YVONNE A. W. HARTMAN[1], LAURA C. M. TILLMANS[1], DAVID L. BENSCHOP[1], ASTRID N. L. HERMANS[1], KEVIN M. R. NIJSSEN[1], THIJS M. H. EIJSVOGELS[1], PETER H. G. M. WILLEMS[2], CEES J. TACK[3], MARIA T. E. HOPMAN[1], JURGEN A. H. R. CLAASSEN[4], and DICK H. J. THIJSSEN[1,5]

[1]Department of Physiology, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, THE NETHERLANDS; [2]Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, THE NETHERLANDS; [3]Department of Internal Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, THE NETHERLANDS; [4]Department of Geriatric Medicine, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, THE NETHERLANDS; and [5]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UNITED KINGDOM

ABSTRACT

HARTMAN, Y. A. W., L. C. M. TILLMANS, D. L. BENSCHOP, A. N. L. HERMANS, K. M. R. NIJSSEN, T. M. H. EIJSVOGELS, P. H. G. M. WILLEMS, C.J. TACK, M. T. E. HOPMAN, J. A. H. R. CLAASSEN, and D. H. J. THIJSSEN. Long-Term and Acute Benefits of Reduced Sitting on Vascular Flow and Function. *Med. Sci. Sports Exerc.*, Vol. 53, No. 2, pp. 341–350, 2021. **Purpose:** Sedentary behavior increases the risk for cardiovascular and cerebrovascular disease. To understand potential benefits and underlying mechanisms, we examined the acute and long-term effect of reduced sitting intervention on vascular and cerebrovascular function. **Methods:** This prospective study included 24 individuals with increased cardiovascular risk (65 ± 5 yr, 29.8 ± 3.9 $kg \cdot m^{-2}$). Before and after 16-wk reduced sitting, using a mobile health device with vibrotactile feedback, we examined (i) vascular function (flow-mediated dilation [FMD]), (ii) cerebral blood flow velocity (CBFv, transcranial Doppler), and (iii) cerebrovascular function (cerebral autoregulation [CA] and cerebral vasomotor reactivity [CVMR]). To better understand potential underlying mechanisms, before and after intervention, we evaluated the effects of 3 h sitting with and without light-intensity physical activity breaks (every 30 min). **Results:** The first wave of participants showed no change in sedentary time (n = 9, 10.3 ± 0.5 to 10.2 ± 0.5 $h \cdot d^{-1}$, P = 0.87). Upon intervention optimization by participants' feedback, the subsequent participants (n = 15) decreased sedentary time (10.2 ± 0.4 to 9.2 ± 0.3 $h \cdot d^{-1}$, $P < 0.01$). This resulted in significant increases in FMD (3.1% ± 0.3% to 3.8% ± 0.4%, P = 0.02) and CBFv (48.4 ± 2.6 to 51.4. ±2.6 $cm \cdot s^{-1}$, P = 0.02), without altering CA or CVMR. Before and after the 16-wk intervention, 3-h exposure to uninterrupted sitting decreased FMD and CBFv, whereas physical activity breaks prevented a decrease (both $P < 0.05$). CA and CVMR did not change ($P > 0.20$). **Conclusion:** Long-term reduction in sedentary behavior improves peripheral vascular function and cerebral blood flow and acutely prevents impaired vascular function and decreased cerebral blood flow. These results highlight the potential benefits of reducing sedentary behavior to acutely and chronically improve cardio- or cerebrovascular risk. **Key Words:** SEDENTARY BEHAVIOR, PHYSICAL ACTIVITY, VASCULAR FUNCTION, CARDIOVASCULAR RISK, CEREBROVASCULAR RISK

Physical inactivity (i.e., lack of regular exercise) is strongly and independently related to the development of noncommunicable diseases, such as cardiovascular and cerebrovascular diseases (1). In addition to physical inactivity, studies have revealed the detrimental effect of sedentary behavior, defined as any waking behavior in a sitting, reclining, or lying posture with an energy expenditure below 1.5 METs (2,3). High levels of sedentary time increase the risk for cardiovascular (4) and cerebrovascular (5) disorders (6,7). In addition to total sedentary time, lack of breaks in sedentary behavior might directly increase risk (8). Recent studies examined the immediate effect of a prolonged sedentary bout, i.e., 3 to 5 h of uninterrupted sitting, and reported an attenuation of vascular function (9), resting cerebral blood flow velocity (CBFv) (10), and glucose tolerance (11). Interestingly, interrupting prolonged sitting by frequent walking breaks of 2–5 min prevented these deteriorations (9–11). Although acute short-term studies support the potential benefits of physical activity breaks, these effects may not simply translate to long-term changes. Although the acute changes is focused on a supranormal amount of sedentary behavior, the longer-term intervention focused on improvements on daily basis in a sustainable manner. Therefore, long-term changes in sedentary behavior are required to unravel and understand the effects of sedentary behavior on cardiovascular and cerebrovascular outcomes.

Address for correspondence: Yvonne A. W. Hartman, MSc, Philips van Leijdenlaan 15, 6525 EZ Nijmegen, The Netherlands; E-mail: yvonne.hartman@radboudumc.nl.
Submitted for publication March 2020.
Accepted for publication June 2020.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/20/5302-0341/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000002462

Following up on our previous work (12), in which we demonstrated a shift in innate-immune function after reduced sitting, we examined the effect of a 16-wk intervention to reduce sedentary behavior on vascular and cerebrovascular function in individuals with increased cardiovascular risk. We hypothesize that the time spent in sedentary behavior can substantially be reduced in individuals with increased cardiovascular risk during a 16-wk intervention. Subsequently, we expect that these changes in sedentary behavior will result in improvements in vascular and cerebrovascular outcomes. Although lower levels of sedentary behavior are linked to reduced cardiovascular risk, the acute effect of a sedentary bout may be equally present in participants with a less sedentary lifestyle. Therefore, we examined whether the 16-wk intervention alters the acute (3-h) effect of sedentary behavior and the ability of physical activity breaks to prevent these effects. We hypothesize that the detrimental effect of 3 h uninterrupted sitting, but also the protective effects of regular physical activity breaks, on vascular and cerebrovascular flow and function remains present after the 16-wk reduced sitting intervention.

METHODS

Participants

Individuals from the environment of Nijmegen, the Netherlands, ≥55 yr old with >40 h·wk^{-1} of self-reported sedentary behavior were eligible for participation. Criteria for inclusion were the presence of one or more cardiovascular risk factors, consisting of body mass index >28 kg·m^{-2}, high blood pressure (systolic blood pressure, >160 mm Hg; diastolic blood pressure, >90 mm Hg), and antihypertensive medication use. Individuals were excluded if they were not able to perform light-intensity physical activity (i.e., standing and walking) or to provide informed consent. The study protocol was approved by the local ethics committee (CMO region Arnhem Nijmegen, the Netherlands) and registered at the Netherlands Trial Register (NTR6387). All individuals provided written informed consent. Measurements were performed between 2017 and 2019. A subset of this study answering a different research question was recently published elsewhere (12).

Study Design

Each subject reported in three clusters of three measurement days to our laboratory: a first cluster before a 16-wk control period (T0), a second cluster after the 16-wk control period (T1), and a third cluster after a 16-wk intervention period (T2) (Fig. 1). Measurements at T0 were performed as familiarization sessions for the participants and to minimize measurement variation in outcomes. On days 1 and 2, peripheral vascular and cerebrovascular blood flow and function were assessed at baseline. Subsequently, in a randomized crossover order between days 1 and 2, subjects underwent a 3-h sitting trial without moving their lower extremities (SIT) and a 3-h sitting trial with 2-min light-intensity walking breaks at self-selected pace every 30 min (BREAKS). Immediately after the 3-h period, peripheral vascular and cerebrovascular flow and function were assessed again. Finally, at day 3, baseline characteristics (13) and physical fitness were assessed (14). Physical activity monitors were mounted to assess physical activity and sedentary behavior characteristics across an 8-d period. The same set of measurements was repeated at T1 and T2.

Intervention. The 16-wk reduced sitting intervention aimed to prevent prolonged sitting (>30 min) throughout the day and to promote low-intensity physical activity (see Document, Supplemental Digital Content 1, Supplemental methods, http://links.lww.com/MSS/C73). Subjects received information regarding the purpose of the intervention and wore a customized activity monitor to objectively monitor sedentary behavior (Activ8sit, 2 M Engineering, Valkenswaard, the Netherlands) (see Figure, Supplemental Digital Content 2, Activ8Sit, a customized activity monitor, http://links.lww.com/MSS/

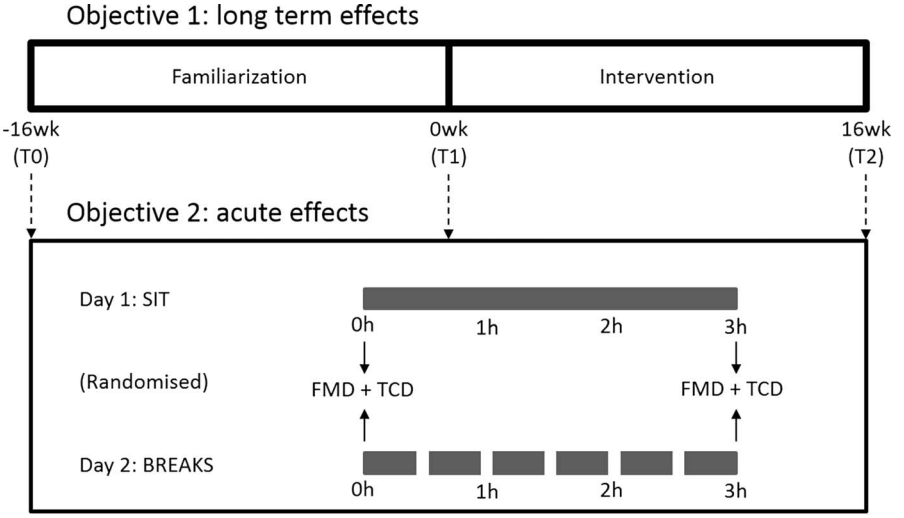

FIGURE 1—Study design. Measurements were performed in three clusters of three measurement days: a first cluster before a 16-wk familiarization period (T0), a second cluster after the 16-wk familiarization period (T1), and a third cluster after a 16-wk intervention period (T2). TCD, transcranial Doppler. TCD measurements included 5 min rest, hyperventilation, hypoventilation, slow sit–stand maneuvers, and 0.05 Hz repeated sit–stand maneuvers.

C74). Using an embedded pilot study design, the intervention was performed in three waves. Participants were assigned to wave 1.0 based on order of application. Based on feedback from the participants in wave 1.0, coaching and support were intensified to weekly meetings (phone or online) for subjects in waves 2.0 and 3.0. Subsequently, participants were randomly assigned to wave 2.0 or 3.0. Intervention was performed in September to January (wave 1.0, $n = 9$), March to July (wave 2.0, $n = 9$), and October to February (wave 3.0, $n = 8$).

Measurements

A detailed description of data collection and analysis is included in the supplemental material (see Document, Supplemental Digital Content 1, Supplemental methods, http://links.lww.com/MSS/C73).

Physical activity patterns. A validated activity monitor (ActivPAL3 micro, PAL technologies, Glasgow, United Kingdom) was used to measure physical activity patterns for 8 d (15). Data were processed using a validated analysis script in Matlab R2014b (Mathworks Inc., Natick, MA) (16).

Peripheral vascular blood flow and function. Superficial femoral artery (SFA) flow-mediated dilation (FMD) was measured as a test of peripheral vascular function (eMethods) (17). After a 1-min baseline period, a blood pressure cuff was inflated to suprasystolic pressure for 5 min (18). Ultrasound recording of the diameter and blood velocity resumed 30 s before deflation and continued for 5 min. Analysis of SFA diameter, blood flow, and shear rate was performed using custom-designed edge detection and wall-tracking software (19,20). No correction for viscosity was made. Peak diameter after cuff deflation was automatically detected (21).

Cerebrovascular blood flow and function. Continuous blood pressure was measured using photoplethysmography (Finapres Medical Systems, Amsterdam, the Netherlands). CBFv in the middle cerebral arteries was measured using two 2-MHz transcranial Doppler probes (Multi-Dop, Compumedics DWL, Singen, Germany; see Document, Supplemental Digital Content 1, Supplemental methods, http://links.lww.com/MSS/C73). CBFv measurements were tightly controlled and followed international recommendations (22). Beat-to-beat data were preprocessed and analyzed using custom-written Matlab scripts (version 2014b, MathWorks Inc.) as previously described by de Jong et al. (23).

CBFv was measured during 5 min sitting, hypocapnia, and hypercapnia. Cerebrovascular conductance index (CVCi, i.e., the ratio of CBFv and MAP) was used to account for confounding effects of CO_2 on blood pressure (24). The change in CBFv to changes in arterial CO_2 concentration, cerebral vasomotor reactivity (CVMR), was computed by the difference between maximal CVCi during hypercapnia and minimal CVCi during hypocapnia, divided by the mean CVCi during normocapnia (25). Data of rest, hypocapnia, and hypercapnia were pooled to evaluate the effect of SIT and Breaks.

We performed slow sit–stand maneuvers (three periods of 2 min sitting and 1 min standing) (23) as well as repeated sit-to-stand maneuvers (10 s sitting, 10 s standing) for 5 min to enhance hemodynamic fluctuations at 0.05 Hz (26). Using these fluctuations, cardiac baroreflex sensitivity (BRS) was calculated using systolic blood pressure and R-R intervals (23). In addition, cerebral autoregulation (CA) was computed via transfer function analysis, resulting in gain, normalized gain phase, and coherence (22). As 0.05 Hz, sit–stand maneuvers are the optimal protocol for CA analysis; the slow sit–stand maneuvers are reported in supplemental tables (see Table, Supplemental Digital Content 3, Additional cerebrovascular flow and function measures of waves 2.0 and 3.0 before and after the 16-wk reduced sitting intervention, http://links.lww.com/MSS/C75; see Table, Supplemental Digital Content 4, Additional cerebrovascular flow and function measures of the acute effect of prolonged sitting and interruptions in prolonged sitting, http://links.lww.com/MSS/C76).

Statistical Analysis

All data are presented as mean ± SEM for continuous variables, as number (percentage) for categorical variables and as median (interquartile range) for skewed distributed data, unless stated otherwise. All data were analyzed using SPSS version 23.0 (SPSS Inc., Chicago, IL). Mixed-models analyses for repeated measurements were performed to evaluate the effect of 16-wk reduced sitting intervention on the outcomes (intervention). In addition, mixed-models analyses were used to investigate the effect of 3 h sitting (acute), and whether SIT versus BREAKS modifies this effect (acute–breaks). To control for potential carryover effects, the sequence of SIT and BREAKS was included in the model (Seq). Finally, we tested whether the acute effect of sedentary behavior and/or breaks changed after the reduced sitting intervention (acute–breaks–intervention). Allometric modeling was used to correct for changes in baseline diameter on FMD (27). Pearson correlations were computed to correlate the 16-wk change in sedentary behavior with changes in vascular and cerebrovascular flow and function. For these analyses, data of wave 1.0 were also included to evaluate this relation among a larger range of changes in sedentary behavior (i.e., increase and decrease in sedentary behavior). P values <0.05 were considered statistically significant.

RESULTS

Five participants dropped out before and during the 16-wk familiarization period because of the time burden of the measurements ($n = 4$) or long-term illness ($n = 1$). Twenty-five participants (65 ± 5 yr, 29.8 ± 3.9 $kg \cdot m^{-2}$) performed the measurements before intervention, including the 3-h sitting trials. One participant dropped out because of long-term illness, resulting in 24 individuals who completed the intervention (Table 1; see Figure, Supplemental Digital Content 5, Flowchart of study participation, http://links.lww.com/MSS/C77).

Long-Term Effects: 16-wk Reduced Sitting

In wave 1.0, no changes in sedentary behavior characteristics were observed (see, Table, Supplemental Digital Content 6,

TABLE 1. Participant characteristics, before (T1) and after (T2) reduced sitting intervention.

Baseline Characteristics	Total (n = 24)	Wave 1.0 (n = 9)	Waves 2.0 and 3.0 (n = 15)
Sex (male), n (%)	9 (38)	3 (33)	6 (40)
Age (yr)	65 ± 5	66 ± 5	64 ± 5
Current smoking, n (%)	2 (8)	0 (0)	2 (13)
Hypertension, n (%)	16 (67)	4 (44)	12 (80)

Intervention Outcomes	T1	T2	P	T1	T2	P	T1	T2	P
SBP (mm Hg)	134 ± 13	135 ± 16	0.63	128 ± 11	134 ± 7	0.06	138 ± 13	136 ± 20	0.46
DBP (mm Hg)	81 ± 9	83 ± 9	0.03	79 ± 7	83 ± 7	<0.01	82 ± 10	82 ± 11	0.52
BMI ($kg \cdot m^{-2}$)	29.8 ± 3.9	29.9 ± 3.8	0.38	30.1 ± 2.5	30.5 ± 2.5	<0.01	29.7 ± 4.6	29.6 ± 4.4	0.83
Glucose ($mmol \cdot L^{-1}$)	6.0 (5.6–6.8)	5.9 (5.2–0.7)	0.10	6.8 (5.9–7.1)	6.0 (5.5–6.8)	0.16	5.8 (5.4–6.2)	5.7 (5.2–6.1)	0.59
Insulin ($mU \cdot L^{-1}$)	NA	NA	NA	NA	NA	NA	12 (8–21)	10 (5–21)	0.86
HOMA-IR (100/%S)	NA	NA	NA	NA	NA	NA	3.2 (1.7–6.0)	3.2 (1.4–5.4)	0.43
Total cholesterol ($mmol \cdot L^{-1}$)	5.1 ± 0.9	4.9 ± 0.8	0.15	4.9 ± 0.8	4.8 ± 0.6	0.65	5.2 ± 1.1	5.0 ± 0.9	0.14
HDL cholesterol ($mmol \cdot L^{-1}$)	1.3 ± 0.4	1.3 ± 0.3	0.74	1.3 ± 0.4	1.3 ± 0.3	0.43	1.3 ± 0.3	1.3 ± 0.3	0.82
LDL cholesterol ($mmol \cdot L^{-1}$)	2.9 ± 0.9	2.8 ± 0.8	0.28	2.7 ± 0.7	2.7 ± 0.6	0.72	3.0 ± 0.9	2.9 ± 0.8	0.12
Triglycerides ($mmol \cdot L^{-1}$)	1.8 ± 1.0	1.7 ± 0.9	0.35	2.0 ± 1.1	1.8 ± 0.9	0.57	1.7 ± 0.9	1.7 ± 0.9	0.42
Non-HDL cholesterol ($mmol \cdot L^{-1}$)	3.7 ± 0.9	3.6 ± 0.7	0.22	3.6 ± 0.8	3.6 ± 0.5	0.86	3.8 ± 0.9	3.6 ± 0.9	0.14
Estimated physical fitness ($mL\ O_2 \cdot mL^{-1} \cdot kg^{-1}$)	27.1 ± 6.4	26.9 ± 7.8	0.82	24.9 ± 5.2	22.3 ± 4.5	0.08	28.5 ± 6.8	29.7 ± 8.2	0.28
Central vascular stiffness[a]	NA	NA	NA	NA	NA	NA	8.9 ± 1.1	8.5 ± 1.0	0.69
Peripheral vascular stiffness[a]	NA	NA	NA	NA	NA	NA	11.9 ± 1.9	10.5 ± 1.7	0.48

Values are presented as mean ± SD or median (interquartile rage), unless otherwise indicated.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment for insulin resistance.
[a]Only measured for waves 2.0 and 3.0.

Sedentary behavior characteristics of wave 1.0, http://links.lww.com/MSS/C78). Based on feedback from participants, coaching and support were intensified. Participants in waves 2.0 and 3.0 (n = 15) (mean ± SD, 64 ± 5 yr, 29.7 ± 4.6 $kg \cdot m^{-2}$; Table 1) significantly lowered sedentary time (10.2 ± 0.3 to 9.2 ± 0.3 $h \cdot d^{-1}$, $P < 0.01$; Fig. 2A) and increased standing time (3.3 ± 0.2 to 3.9 ± 0.2 $h \cdot d^{-1}$, $P = 0.03$; Fig. 2B), walking time (2.1 ± 0.2 to 2.6 ± 0.2 $h \cdot d^{-1}$, $P < 0.01$; Fig. 2C), and step count (10,316 ± 1,297 to 13,058 ± 1,184 steps per day, $P < 0.01$). No changes were observed in blood pressure, body

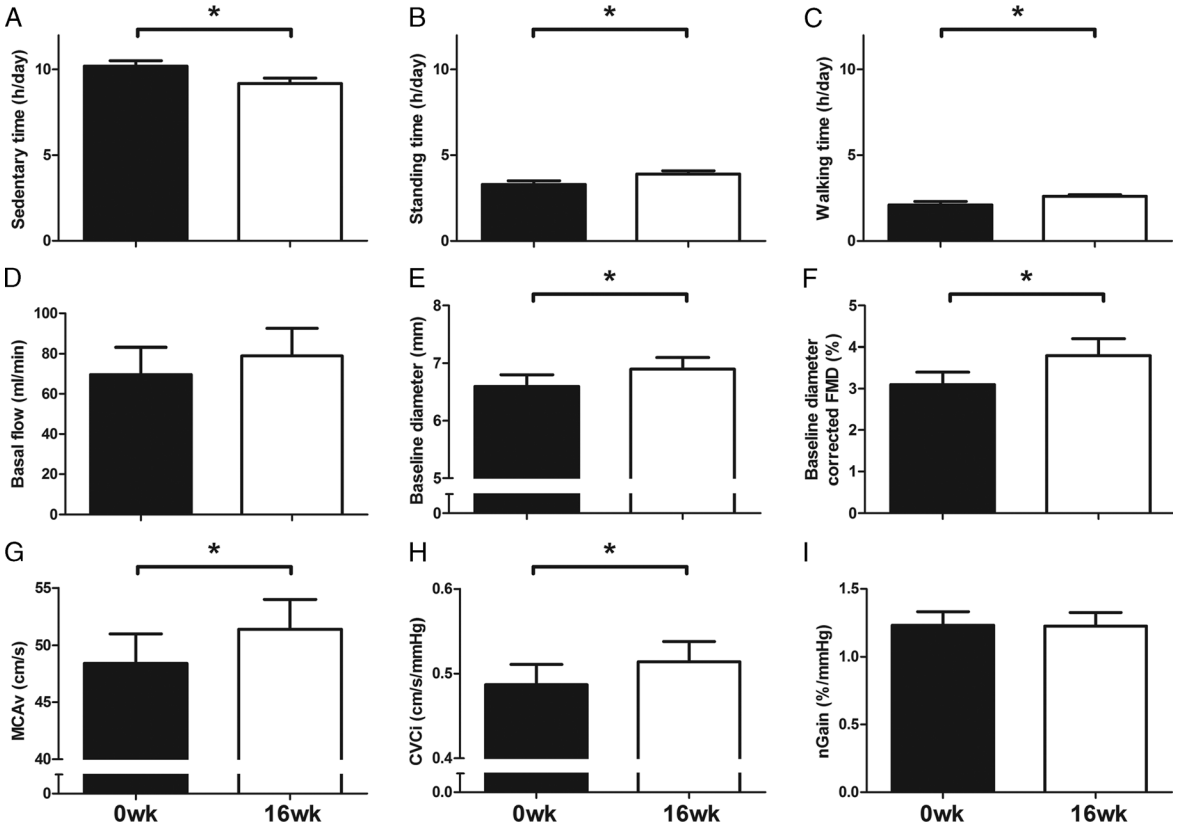

FIGURE 2—Effect of 16-wk reduced sitting intervention on sedentary behavior, vascular, and cerebrovascular outcomes. Average values ± SEM of sedentary behavior characteristics (A–C), vascular measures (D–F), and cerebrovascular measures (G–I) before (T1) and after (T2) 16-wk reduced sitting intervention for waves 2.0 and 3.0 (n = 15 for sedentary behavior and vascular function measures, n = 14 for cerebrovascular measures). nGain, normalized gain.

mass index, physical fitness, fasting glucose, insulin, homeostatic model assessment for insulin resistance, and blood lipids (Table 1). Given the absence of changes in sedentary behavior, data of wave 1.0 are reported in the supplemental content (see Table, Supplemental Digital Content 7, Vascular and cerebrovascular flow and function of wave 1.0 before and after 16-wk reduced sitting intervention, http://links.lww.com/MSS/C79). Data of waves 2.0 and 3.0 (n = 15) are presented below. Data of all participants were used to relate changes in sedentary behavior to primary outcomes.

Peripheral vascular blood flow and function. Mean SFA blood flow did not change significantly after the 16-wk intervention (Fig. 2D), but antegrade blood flow significantly increased (Table 2). In addition, resting SFA diameter significantly increased after intervention (Fig. 2E). A significant increase in FMD was found when corrected for baseline diameter (3.1% ± 0.3% to 3.8% ± 0.4%, P = 0.02; Fig. 2F, Table 2). Correcting for shear rate area under the curve resulted in a trend for an increase in FMD after the intervention (P = 0.08, Table 2). No significant correlation was found between changes in sedentary behavior and SFA blood flow or FMD (data not shown).

Cerebrovascular blood flow velocity and function. In one participant, we were unable to assess cerebrovascular blood flow due to technical difficulties, leaving 14 participants with valid data. Hypocapnia resulted in a significant decline in CBFv and CVCi, whereas hypercapnia significantly increased CBFv and CVCi (see Table, Supplemental Digital Content 3, Additional cerebrovascular flow and function measures of wave 2.0 and 3.0 before and after the 16-week reduced sitting intervention, http://links.lww.com/MSS/C75). Resting CBFv (48.4 ± 2.6 to 51.4 ± 2.6 cm·s^{-1}, P = 0.02) and CVCi (0.49 ± 0.02 to 0.51 ± 0.02 cm·s^{-1}·mm Hg^{-1}, P = 0.03) increased significantly after the 16-wk intervention (Fig. 2G, H; Table 2). There were no effects on CA, CVMR, or BRS (Fig. 2I; Table 2; Supplemental Digital Content 3, Additional cerebrovascular flow and function measures of wave 2.0 and 3.0 before and after the 16-wk reduced sitting intervention, http://links.lww.com/MSS/C75). The increase in CA gain during slow sit–stands was statistically significant (Supplemental Digital Content 3, Additional cerebrovascular flow and function measures of wave 2.0 and 3.0 before and after the 16-week reduced sitting intervention, http://links.lww.com/MSS/C75) but too small to represent a deterioration in CA (22). Combining all

TABLE 2. Vascular and cerebrovascular flow and function before (T1) and after (T2) the 16-wk reduced sitting intervention.

Intervention (I)	Preintervention				Postintervention							
Breaks (B)	Sit		Breaks		Sit		Breaks		P			
Acute (A)	0 h	3 h	0 h	3 h	0 h	3 h	0 h	3 h	A	A–B	I	A–B–I
Peripheral vascular flow and function												
Blood flow patterns												
Basal flow (mL·min^{-1})	83 ± 18	44 ± 17	77 ± 17	75 ± 18	77 ± 18	81 ± 18	79 ± 17	78 ± 18	0.31	0.39	0.32	0.34
Antegrade flow (mL·min^{-1})	160 ± 22	119 ± 22	148 ± 22	162 ± 22	176 ± 23	173 ± 22	167 ± 22	173 ± 23	0.62	0.18	**0.04**	0.57
Retrograde flow (mL·min^{-1})	−80 ± 23	−76 ± 23	−71 ± 23	−87 ± 23	−97 ± 24	−90 ± 23	−88 ± 23	−93 ± 24	0.73	0.25	0.06	0.95
FMD												
Baseline diameter (mm)	6.5 ± 0.3	6.5 ± 0.3	6.7 ± 0.3	6.9 ± 0.3	6.7 ± 0.3	7.1 ± 0.3	6.9 ± 0.3	6.9 ± 0.3	0.20	0.93	**0.02**	0.27
Peak diameter (mm)	6.7 ± 0.3	6.7 ± 0.3	6.8 ± 0.3	7.2 ± 0.3	7.0 ± 0.3	7.3 ± 0.3	7.1 ± 0.3	7.3 ± 0.3	0.11	0.42	**0.01**	0.36
FMD (%)	3.6 ± 0.6	2.9 ± 0.5	2.5 ± 0.5	4.1 ± 0.6	4.5 ± 0.6	3.0 ± 0.5	2.5 ± 0.5	5.0 ± 0.6	0.14	**<0.01**	0.14	0.57
SR_{auc} (10^3)	10.5 ± 1.2	6.9 ± 1.1	6.3 ± 1.2	8.0 ± 1.2	7.5 ± 1.3	4.5 ± 1.2	7.2 ± 1.1	6.4 ± 1.2	**0.05**	**0.01**	**0.04**	0.22
FMD corrected for diameter	3.3 ± 0.5	2.6 ± 0.5	2.4 ± 0.5	4.1 ± 0.5	4.4 ± 0.5	3.2 ± 0.5	2.6 ± 0.6	5.1 ± 0.5	0.06	**<0.01**	**0.02**	0.70
FMD corrected for SR_{auc}	3.3 ± 0.5	2.9 ± 0.5	2.6 ± 0.5	4.0 ± 0.5	4.4 ± 0.6	3.2 ± 0.5	2.5 ± 0.5	5.0 ± 0.5	0.08	**<0.01**	0.08	0.48
Cerebrovascular flow and function												
At rest												
MAP (mm Hg)	98 ± 2	101 ± 2	99 ± 2	96 ± 2	99 ± 2	101 ± 2	100 ± 2	100 ± 2	**<0.01**	0.05	0.40	0.99
CBFv (cm·s^{-1})	48.2 ± 3.1	50.2 ± 3.1	47.4 ± 3.1	47.9 ± 3.1	50.0 ± 3.2	52.8 ± 3.1	51.5 ± 3.1	51.4 ± 3.1	0.29	0.38	**0.02**	0.77
CVCi (cm·s^{-1}·mm Hg^{-1})	0.49 ± 0.03	0.50 ± 0.03	0.48 ± 0.03	0.48 ± 0.03	0.50 ± 0.03	0.52 ± 0.03	0.52 ± 0.03	0.51 ± 0.03	0.65	0.65	**0.03**	0.77
Gain BRS	7.0 ± 2.0	3.8 ± 2.2	6.5 ± 1.9	3.9 ± 2.1	4.0 ± 1.9	5.9 ± 2.0	5.5 ± 2.0	6.1 ± 1.9	0.45	0.89	0.95	0.66
Pooled analysis												
MAP (mm Hg)	100 ± 2	101 ± 2	102 ± 2	101 ± 2	99 ± 2	101 ± 2	99 ± 2	102 ± 2	0.15	0.48	0.77	0.31
CBFv (cm·s^{-1})	50.2 ± 3.2	50.4 ± 3.1	46.4 ± 3.1	46.9 ± 3.1	50.5 ± 3.2	51.9 ± 3.1	50.8 ± 3.1	50.4 ± 3.1	0.63	0.65	**<0.01**	0.55
CVCi (cm·s^{-1}·mm Hg^{-1})	0.49 ± 0.03	0.50 ± 0.03	0.45 ± 0.03	0.46 ± 0.03	0.50 ± 0.03	0.50 ± 0.03	0.50 ± 0.03	0.49 ± 0.03	0.79	0.67	**<0.01**	0.53
CVMR												
MAP reactivity (U)	0.28 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.30 ± 0.02	0.65	0.27	0.25	0.88
CBFv reactivity (U)	0.59 ± 0.04	0.62 ± 0.04	0.61 ± 0.04	0.60 ± 0.04	0.63 ± 0.04	0.60 ± 0.04	0.56 ± 0.03	0.59 ± 0.04	0.71	0.79	0.45	0.20
CVCi reactivity (U)	0.29 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.31 ± 0.03	0.36 ± 0.03	0.35 ± 0.03	0.30 ± 0.03	0.30 ± 0.03	0.92	0.47	0.68	0.31
0.05 Hz repeated sit–stands												
MAP (mm Hg)	102 ± 2	106 ± 2	102 ± 2	103 ± 2	104 ± 2	105 ± 2	102 ± 2	105 ± 2	**0.01**	0.72	0.43	0.32
CBFv (cm·s^{-1})	43.2 ± 2.7	44.9 ± 2.9	43.6 ± 2.6	45.2 ± 2.8	47.0 ± 2.7	47.0 ± 2.7	47.9 ± 2.7	48.4 ± 2.7	0.40	0.91	**<0.01**	0.90
CVCi (cm·s^{-1}·mm Hg^{-1})	0.42 ± 0.03	0.42 ± 0.03	0.43 ± 0.03	0.44 ± 0.03	0.46 ± 0.03	0.45 ± 0.03	0.47 ± 0.03	0.46 ± 0.03	0.83	0.89	**0.02**	0.78
Gain (cm·s^{-1}·mm Hg^{-1})	0.54 ± 0.05	0.54 ± 0.05	0.53 ± 0.04	0.52 ± 0.05	0.61 ± 0.05	0.53 ± 0.05	0.55 ± 0.05	0.56 ± 0.05	0.48	0.48	0.26	0.38
nGain (%·mm Hg^{-1})	1.29 ± 0.12	1.20 ± 0.13	1.26 ± 0.11	1.19 ± 0.12	1.36 ± 0.11	1.19 ± 0.12	1.18 ± 0.12	1.18 ± 0.11	0.12	0.40	0.93	0.47
Phase (°)	51 ± 4	52 ± 5	51 ± 4	47 ± 5	49 ± 4	52 ± 4	46 ± 4	51 ± 4	0.59	0.80	0.84	0.50
Coherence (U)	0.79 ± 0.04	0.84 ± 0.04	0.77 ± 0.04	0.80 ± 0.04	0.80 ± 0.04	0.79 ± 0.04	0.84 ± 0.04	0.84 ± 0.04	0.43	0.99	0.41	0.79
Gain BRS (ms·mm Hg^{-1})	6.4 ± 0.8	4.2 ± 0.9	4.1 ± 0.8	3.3 ± 0.9	4.9 ± 0.8	4.6 ± 0.8	4.2 ± 0.8	5.4 ± 0.8	0.30	0.14	0.60	0.95

n = 15 for vascular measurements; n = 14 for cerebrovascular measurements. P values represent the acute effect of 3 h sitting (A), whether sit versus breaks modifies this effect (A–B), the effect of the 16 wk reduced sitting intervention (I) and whether the acute effect of sedentary behavior and/or breaks changed after the reduced sitting intervention (A–B–I). Data are reported as mean ± SEM.

TABLE 3. Acute effect of prolonged (3-h) sitting (SIT) and interruptions in prolonged sitting (BREAKS) on vascular and cerebrovascular flow and function.

	Preintervention (T1)					
Breaks (B)	**Sit**		**Breaks**		***P***	
Acute (A)	**0 h**	**3 h**	**0 h**	**3 h**	**A**	**A–B**
Peripheral vascular flow and function						
Blood flow patterns						
Basal flow (mL·min^{-1})	93 ± 13	61 ± 12	86 ± 13	84 ± 13	0.08	0.12
Antegrade flow (mL·min^{-1})	167 ± 17	127 ± 17	148 ± 17	158 ± 17	0.26	0.07
Retrograde flow (mL·min^{-1})	−77 ± 16	−65 ± 16	−63 ± 16	−74 ± 16	0.99	0.17
FMD						
Baseline diameter (mm)	6.7 ± 0.2	6.6 ± 0.2	6.7 ± 0.2	6.8 ± 0.2	1.00	0.43
Peak diameter (mm)	7.0 ± 0.2	6.8 ± 0.2	6.9 ± 0.2	7.1 ± 0.2	0.81	0.24
FMD (%)	3.2 ± 0.4	3.0 ± 0.4	2.7 ± 0.4	4.1 ± 0.4	0.09	**0.01**
SR_{auc} (10^3)	7.6 ± 1.1	6.7 ± 1.1	5.2 ± 1.1	6.4 ± 1.1	0.90	0.22
FMD corrected for diameter	3.2 ± 0.4	2.9 ± 0.4	2.7 ± 0.4	4.2 ± 0.4	0.07	**<0.01**
FMD corrected for SR_{auc}	3.1 ± 0.4	2.9 ± 0.4	2.8 ± 0.4	4.1 ± 0.4	0.08	**0.03**
Cerebrovascular flow and function						
During rest						
MAP (mm Hg)	97 ± 1	100 ± 1	97 ± 1	100 ± 1	**<0.01**	0.44
CBFv (cm·s^{-1})	49.6 ± 2.4	47.3 ± 2.4	46.3 ± 2.4	47.7 ± 2.4	0.76	0.20
CVCi (cm·s^{-1}L·mm Hg^{-1})	0.51 ± 0.03	0.47 ± 0.03	0.48 ± 0.03	0.47 ± 0.03	0.12	0.27
Gain BRS	6.72 ± 1.60	4.34 ± 1.67	5.46 ± 1.56	3.93 ± 1.64	0.14	0.74
Pooled analysis						
MAP (mm Hg)	98 ± 1	100 ± 1	100 ± 1	100 ± 1	0.16	0.40
CBFv (cm·s^{-1})	50.5 ± 2.2	47.8 ± 2.2	46.3 ± 2.2	46.9 ± 2.2	0.21	0.05
CVCi (cm·s^{-1}·mm Hg^{-1})	0.51 ± 0.02	0.47 ± 0.02	0.46 ± 0.02	0.46 ± 0.02	**0.03**	**0.04**
CVMR						
MAP reactivity (unit)	0.32 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.31 ± 0.02	0.36	0.21
CBFv reactivity (unit)	0.59 ± 0.02	0.61 ± 0.03	0.61 ± 0.02	0.62 ± 0.02	0.40	0.97
CVCi reactivity (unit)	0.31 ± 0.03	0.32 ± 0.03	0.31 ± 0.02	0.33 ± 0.02	0.58	0.85
0.05 Hz repeated sit–stands						
MAP (mm Hg)	101 ± 1	104 ± 1	101 ± 1	103 ± 1	**<0.01**	0.42
CBFv (cm·s^{-1})	44.3 ± 2.3	42.1 ± 2.4	42.0 ± 2.2	43.2 ± 2.3	0.74	0.27
CVCi (cm·s^{-1}·mm Hg^{-1})	0.44 ± 0.02	0.41 ± 0.02	0.42 ± 0.2	0.42 ± 0.2	0.27	0.21
Gain (cm·s^{-1}·mm Hg^{-1})	0.56 ± 0.04	0.54 ± 0.04	0.54 ± 0.04	0.54 ± 0.04	0.61	0.75
nGain (%·mm Hg^{-1})	1.28 ± 0.08	1.30 ± 0.08	1.32 ± 0.08	1.28 ± 0.08	0.83	0.64
Phase (°)	49.8 ± 3.2	48.9 ± 3.3	48.4 ± 3.1	48.2 ± 3.3	0.81	0.86
Coherence (U)	0.79 ± 0.03	0.81 ± 0.03	0.78 ± 0.03	0.82 ± 0.03	0.15	0.70
Gain BRS (ms·mm Hg^{-1})	4.76 ± 0.65	5.52 ± 0.62	4.04 ± 0.59	4.07 ± 0.64	0.55	0.51

Measured after finishing the 16-wk familiarization period (T1), $n = 25$ for vascular measurements and $n = 24$ for cerebrovascular measurements. *P* values represent the acute effect of 3 h sitting (A) and whether sit versus breaks modifies this effect (A–B). Values are corrected for the sequence of sit and breaks measurement days. Data are reported as mean ± SEM.
SR_{auc}, shear rate area under the curve.

data ($n = 23$), a significant inverse correlation was found for the change in sedentary time and the change in resting CBFv ($r = -0.352$, $P = 0.02$) and CVCi ($r = -0.419$, $P < 0.01$).

Acute Effects: Prolonged Sitting versus Interrupting Sitting ($n = 24$)

Vascular blood flow and function. Uncorrected, diameter corrected, and shear rate area under the curve corrected FMD showed a significant interaction effect across the 3-h periods ($P = 0.01$, $P < 0.01$, and $P = 0.03$ respectively), with *post hoc* tests revealing a small decline in FMD after uninterrupted sitting, whereas FMD improved when sitting was interrupted (Table 3). No differences were present in blood flow. The 16-wk intervention ($n = 15$) did not alter the acute effect of (un)interrupted sitting on flow or FMD (Table 2, Fig. 3).

Cerebrovascular blood flow and function. Resting CBFv and CVCi did not change after the 3-h period (Table 3). Pooled analysis of rest, hypocapnia, and hypercapnia revealed significant interaction effects of the 3-h period of SIT or BREAKS on CBFv and CVCi ($P = 0.04$ and 0.05, respectively), with a decrease in CBFv and CVCi after uninterrupted sitting, which was prevented by physical activity breaks (Table 3). No changes were found for CVMR (Table 3). No differences of the 3-h periods were present in CA and BRS outcomes (Table 3; Table, Supplemental Digital Content 4, Additional cerebrovascular flow and function measures of the acute impact of prolonged sitting and interruptions in prolonged sitting, http://links.lww.com/MSS/C76). The intervention ($n = 14$) did not alter the acute, 3-h effect of (un)interrupted sitting on CBFv, CVMR, CA, or BRS (Table 2).

DISCUSSION

This study presents the following findings. First, the adjusted 16-wk intervention resulted in a reduction in sedentary time of ~1 h·d^{-1}. Second, the reduction in sedentary behavior was linked to a significant improvement in peripheral artery vascular structure and function, but also to an increase in cerebral blood flow, whereas no changes in cerebrovascular function were observed. Third, 3 h uninterrupted sitting leads to a decline in peripheral vascular function and cerebral blood flow, but not cerebrovascular function, although these effects are prevented when sitting was interrupted by brief walking breaks. The 16-wk intervention did not alter these acute responses. Altogether, these data indicate that in individuals with increased cardiovascular risk, acute and long-term reductions in sedentary behavior improve vascular function and increase cerebral

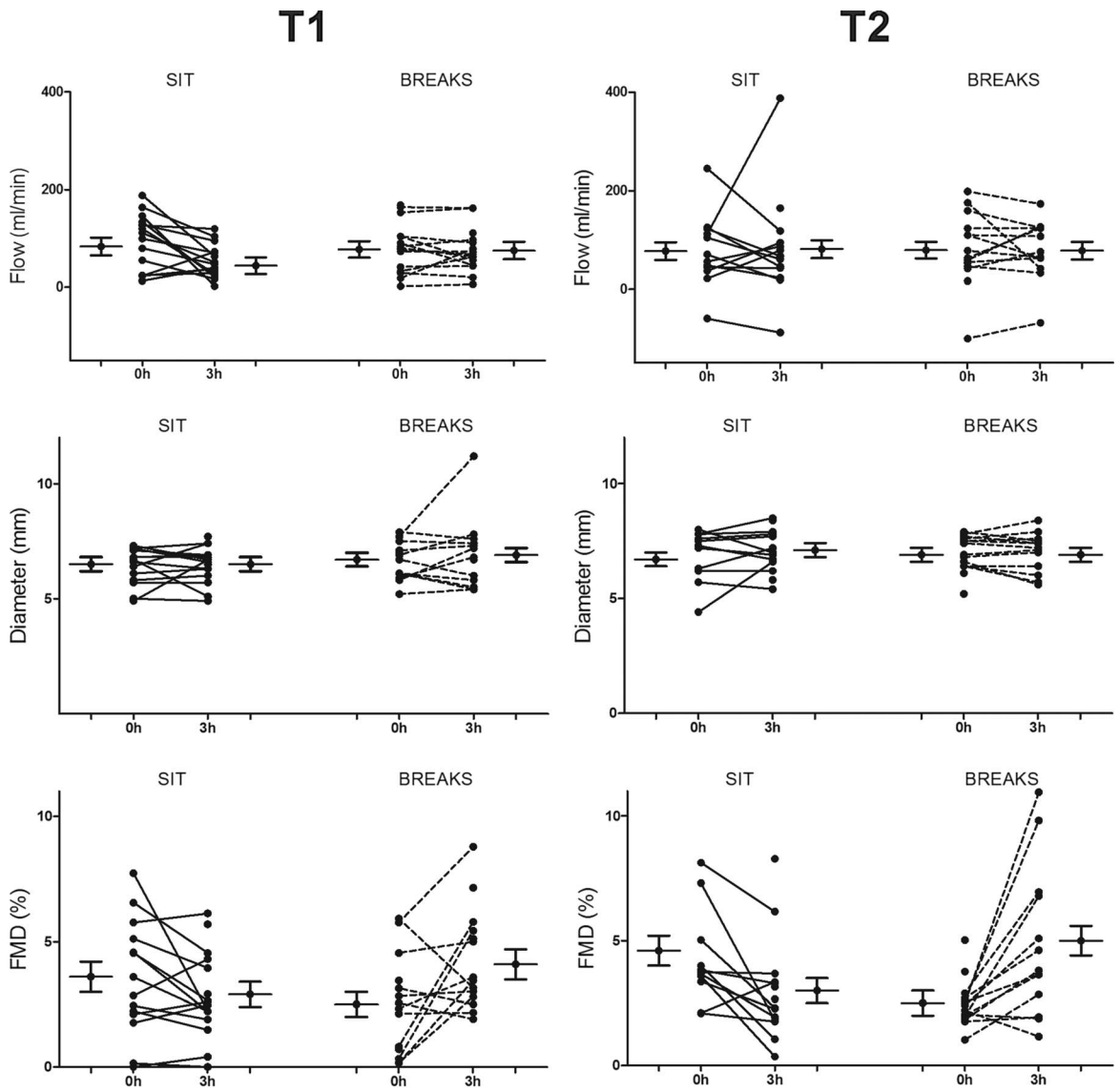

FIGURE 3—Acute and long-term effect of reduced sitting on vascular structure and function. Average and individual values of flow, diameter, and FMD before and after SIT and BREAKS at T1 and T2 for waves 2.0 and 3.0 (n = 15). No significant changes were present in flow. Diameter significantly increased from T1 to T2 (P = 0.02). Uncorrected FMD showed a significant difference in response to SIT or BREAKS (P < 0.01). Although no changes were present in uncorrected FMD from T1 to T2 (P = 0.14), correction for the larger diameter after intervention revealed a significant increase in FMD from T1 to T2 (P = 0.02). However, individual FMD data could not be corrected for diameter and therefore is not shown. Averages are reported as mean ± SEM. Data points without connecting lines represent measurements with a missing baseline or follow-up point.

perfusion. Accordingly, sedentary behavior is a potential target to prevent future cardiovascular or cerebrovascular disease in this group.

Studies that examined the acute effects of (un)interrupted sitting have highlighted the potential detrimental effect of sedentary behavior on (cerebro)vascular health but were unable to evaluate the causal link between long-term reduction in sedentary behavior and changes in (cerebro)vascular blood flow and function. One important factor limiting long-term studies is that most wearables cannot validly distinguish between standing and sedentary behavior and therefore are unable to provide feedback on sedentary behavior specifically. We codeveloped a pocket-based pedometer to provide direct and online feedback on sedentary behavior. Within our embedded pilot study, intervention optimization resulted in substantial improvements in sedentary behavior. The observation of a significant reduction of ~1 h·d^{-1} after 16 wk is important because a recent meta-analysis highlighted that only short-term (median 4 wk), but not long-term (>3 months), mHealt interventions were successful to decrease daily sedentary behavior (28). This stresses the difficulty of inducing long-term changes in sedentary behavior but also highlights the relevance of participants' feedback to ultimately successfully improve physical activity patterns.

We found that, when correcting for the increase in diameter, SFA endothelial function significantly improved after the 16-wk intervention. One previous study, examining the long-term benefits of an 8-wk intervention using a standing desk, found no significant improvement in endothelial

function (29). The key difference between both interventions is the focus on breaking up daily sedentary time versus lowering sedentary time at work. This suggests that regularly breaking up sitting, rather than decreasing total sedentary time, may be important. In line with the observations of 3-h (un) interrupted sitting in our study, the frequent exposure to increases in shear may represent key stimuli to explain our results (30). Importantly, regular exercise training and/or higher fitness may also increase shear stimuli and improve vascular function and structure (31) and should be carefully considered in relation to our intervention. Because physical fitness or engagement in exercise did not change, we can assign improvements in vascular function to our intervention that specifically focused on replacing sedentary behavior with light-intensity physical activities.

Related to the cerebrovascular system, we found an increase in CBFv with no changes in measures of function. Although no previous study has examined the long-term effects of reduced sitting, several studies investigated the effect of exercise on cerebrovascular function. Interestingly, trained men demonstrate higher cerebral blood flow compared with sedentary individuals (32), whereas 4-month exercise training increased hippocampal blood flow (33). Despite changes in blood flow, cross-sectional and intervention studies found no effect of exercise training on cerebrovascular function (34,35). This suggests that adaptations after the 16-wk reduced sitting intervention are in line with those found after exercise training. Even the presence of Alzheimer's disease may not markedly affect cerebrovascular function (23), highlighting the robustness of the cerebrovascular system to regulate fluctuations in cerebral blood flow. The higher blood flow velocity found after 16 wk might relate to exposure to repeated increases in cerebral perfusion. Low- to moderate-intensity activities (e.g., walking), which increased across the 16-wk intervention, could acutely enhance cerebral perfusion by ~10%–15% (36). In line with peripheral arteries (31), repeated exposure to these stimuli might explain the higher blood flow to the brain. Future work is required to better understand these adaptations in cerebral blood flow.

When evaluating the acute effect of sedentary behavior, we found that 3 h uninterrupted sitting impairs SFA endothelial function and lowers cerebral blood flow in individuals with increased cardiovascular risk. Importantly, the 16-wk intervention did not alter the acute effect of uninterrupted sitting. In other words, breaking up sedentary time remains an effective strategy to prevent the detrimental effect of prolonged sitting on vascular function and cerebrovascular blood flow. Our findings after 3 h uninterrupted sitting extend previous work in healthy individuals, in that 3- to 4-h periods of uninterrupted sitting affects (cerebro)vascular function (9,10). Physical activity breaks successfully prevented these effects in our subjects with *a priori* lower vascular function and cerebral perfusion, as is also in line with previous work in healthy individuals (9,10). Mechanisms explaining these effects might relate to the repeated exposure to shear stress experienced during physical activity breaks (9), which are linked to immediate changes in endothelial function (37). In fact, increasing vascular shear rate by heating prevented the decline in vascular function after prolonged sedentary behavior (38).

Future research is warranted to better understand and link the acute changes in shear stress to changes in vascular function, but also how the frequency, duration, and intensity of physical activity interruptions affect these responses.

One could speculate about potential mechanisms contributing to vascular adaptations after reduced sitting. During exercise training, vascular adaptations are evoked due to the repeated exposure to hemodynamic stimuli that occur during an exercise bout (31). Although the physical activity breaks as a consequence of lower sedentary time may contribute to vascular adaptations, alternative pathways may also be involved. We hypothesize that less sitting is associated with fewer potentially harmful triggers that are typically released with prolonged sitting bouts (e.g., cytokines). An alternative explanation relates to a recent observation from our laboratory, which revealed that small fluctuations in shear rate, without changing mean shear rate, benefits vascular function (39). Possibly, the frequent short activity bouts could result in such an accumulation of small beneficial fluctuations in shear rate. Although both exercise and reduced sitting strategies may improve vascular function and perfusion, different mechanisms might be involved (30). Specific mechanisms contributing to vascular benefits after reduced sitting are needed to be investigated in future research.

Clinical implications. We showed that high levels of sedentary behavior can substantially be reduced and are a feasible target for new interventions. It is important to realize that community-dwelling cardiovascular and dementia patients spend more time in sedentary behavior compared with their healthy peers (40). This suggests that targeting sedentary behavior is relevant, especially because these (clinical) groups typically do not meet guidelines for exercise training and performance of light-intensity activities is easy to perform (including in the home environment). Vascular dysfunction is linked to future cardiovascular disease (41), whereas lower cerebral blood flow is associated with cognitive decline and development of dementia (42). Therefore, our findings of increased peripheral vascular function and cerebrovascular blood flow might have potential clinical effect. A final consideration is that breaking up sedentary activity is one of the most important interventional approaches to avoid sedentary vascular dysfunction, as the 16-wk intervention did not alter the adverse effects of a single bout of prolonged sitting.

Limitations. A potential limitation is that we did not include a control group. However, subjects served as their own controls, as no changes in outcome parameters were found after a 16-wk familiarization period (data not shown). Another limitation of our study is the potential presence of a seasonal effect. However, all waves started in different seasons, thereby correcting for potential seasonality effects. As transcranial Doppler measures flow velocity instead of actual flow, and is unable to measure vessel diameter, we were unable to correct for a potential change in middle cerebral artery diameter. However, middle cerebral artery diameter stays

rather constant during mild stimuli (43), and therefore potential changes in vessel diameter unlikely explain our major findings. Finally, we included a relatively small sample size. Although this should be taken into consideration and limits widespread extrapolation, the close monitoring of the subjects across a prolonged period and the use of state-of-the-art technology minimize potential error and provide novel insight into the link between sedentary behavior and (cerebro) vascular function.

PERSPECTIVES

This study demonstrates the beneficial effects of a successful 16-wk reduced sitting intervention on peripheral vascular function and cerebral blood flow in individuals with increased cardiovascular risk. These findings are in line with acute effects of prolonged sitting on vascular function and cerebrovascular flow, highlighting the relevance of frequent interrupting sedentary periods. Given the role of vascular function and cerebral blood flow in the development of cardiovascular and cerebrovascular disease, our observations may have important clinical implications. Reducing sedentary behavior is an accessible intervention and therefore might be easier applicable, compared with exercise training, in clinical groups. Reducing sedentary behavior is a promising target to prevent future cardiovascular or cerebrovascular disease and should be further investigated to reveal its clinical effect.

Y. A. W. H. received an Internal Radboudumc PhD Studentship award. The funding organization had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

P. H. G. M. W. is a scientific advisor of Khondrion, Nijmegen, The Netherlands. This SME had no involvement in the collection, analysis, and interpretation of the data; writing of the manuscript; and decision to submit the manuscript for publication. All other authors declare that there is no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Authors' contributions: Y. A. W. H., P. H. G. M. W., C. J. T., M. T. E. H., J. A. H. R. C., and D. H. J. T. contributed to the conception or design of the work. Y. A. W. H., L. C. M. T., D. L. B., A. N. L. H., K. M. R. N., T. M. H. E., P. H. G. M. W., C. J. T., M. T. E. H., J. A. H. R. C., and D. H. J. T. contributed to the acquisition, analysis, or interpretation of data for the work. Y. A. W. H. drafted the manuscript. L. C. M. T., D. L. B., A. N. L. H., K. M. R. N., T. M. H. E., P. H. G. M. W., C. J. T., M. T. E. H., J. A. H. R. C., and D. H. J. T. critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Trial registration: This study is registered at the Netherlands Trial Register (NTR6387) (https://www.trialregister.nl/trial/6215).

REFERENCES

1. Lee IM, Shiroma EJ, Lobelo F, et al. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet*. 2012;380(9838):219–29.
2. van der Ploeg HP, Chey T, Korda RJ, Banks E, Bauman A. Sitting time and all-cause mortality risk in 222 497 Australian adults. *Arch Intern Med*. 2012;172(6):494–500.
3. Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN. Sedentary behavior, exercise, and cardiovascular health. *Circ Res*. 2019; 124(5):799–815.
4. Chomistek AK, Manson JE, Stefanick ML, et al. Relationship of sedentary behavior and physical activity to incident cardiovascular disease: results from the Women's Health Initiative. *J Am Coll Cardiol*. 2013;61(23):2346–54.
5. Falck RS, Davis JC, Liu-Ambrose T. What is the association between sedentary behaviour and cognitive function? A systematic review. *Br J Sports Med*. 2017;51(10):800–11.
6. van der Berg JD, Stehouwer CD, Bosma H, et al. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: the Maastricht Study. *Diabetologia*. 2016;59(4):709–18.
7. Manini TM, Lamonte MJ, Seguin RA, et al. Modifying effect of obesity on the association between sitting and incident diabetes in post-menopausal women. *Obesity (Silver Spring)*. 2014;22(4): 1133–41.
8. Healy GN, Dunstan DW, Salmon J, et al. Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care*. 2008; 31(4):661–6.
9. Thosar SS, Bielko SL, Mather KJ, Johnston JD, Wallace JP. Effect of prolonged sitting and breaks in sitting time on endothelial function. *Med Sci Sports Exerc*. 2015;47(4):843–9.
10. Carter SE, Draijer R, Holder SM, Brown L, Thijssen DHJ, Hopkins ND. Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting. *J Appl Physiol (1985)*. 2018;125(3):790–8.
11. Dunstan DW, Kingwell BA, Larsen R, et al. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care*. 2012;35(5):976–83.
12. Noz MP, Hartman YAW, Hopman MTE, et al. Sixteen-week physical activity intervention in subjects with increased cardiometabolic risk shifts innate immune function towards a less proinflammatory state. *J Am Heart Assoc*. 2019;8(21):e013764.
13. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH guidelines for the management of arterial hypertension. *Eur Heart J*. 2018; 39(33):3021–104.
14. Astrand PO, Ryhming I. A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. *J Appl Physiol*. 1954;7(2):218–21.
15. Lyden K, Kozey Keadle SL, Staudenmayer JW, Freedson PS. Validity of two wearable monitors to estimate breaks from sedentary time. *Med Sci Sports Exerc*. 2012;44(11):2243–52.
16. van der Berg JD, Willems PJ, van der Velde JH, et al. Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. *J Sports Sci*. 2016;34(19):1867–73.
17. Kooijman M, Thijssen DH, de Groot PC, et al. Flow-mediated dilatation in the superficial femoral artery is nitric oxide mediated in humans. *J Physiol*. 2008;586(4):1137–45.
18. Thijssen DHJ, Bruno RM, van Mil ACCM, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J*. 2019;40:2534–47.
19. Woodman RJ, Playford DA, Watts GF, et al. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol (1985)*. 2001;91(2):929–37.
20. Pyke KE, Dwyer EM, Tschakovsky ME. Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. *J Appl Physiol (1985)*. 2004;97(2):499–508.
21. Black MA, Cable NT, Thijssen DH, Green DJ. Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. *Am J Physiol Heart Circ Physiol*. 2009;297(3):H1109–16.

22. Claassen JA, Meel-van den Abeelen AS, Simpson DM, Panerai RB, International Cerebral Autoregulation Research Network (CARNet). Transfer function analysis of dynamic cerebral autoregulation: a white paper from the international cerebral autoregulation research network. *J Cereb Blood Flow Metab*. 2016;36(4): 665–80.
23. de Heus RAA, de Jong DLK, Sanders ML, et al. Dynamic regulation of cerebral blood flow in patients with Alzheimer disease. *Hypertension*. 2018;72(1):139–50.
24. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol (1985)*. 2007; 102(3):870–7.
25. Glodzik L, Randall C, Rusinek H, de Leon MJ. Cerebrovascular reactivity to carbon dioxide in Alzheimer's disease. *J Alzheimers Dis*. 2013;35(3):427–40.
26. Smirl JD, Hoffman K, Tzeng YC, Hansen A, Ainslie PN. Methodological comparison of active- and passive-driven oscillations in blood pressure; implications for the assessment of cerebral pressure-flow relationships. *J Appl Physiol (1985)*. 2015;119(5):487–501.
27. Atkinson G, Batterham AM, Thijssen DH, Green DJ. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens*. 2013;31(2):287–91.
28. Stephenson A, McDonough SM, Murphy MH, Nugent CD, Mair JL. Using computer, mobile and wearable technology enhanced interventions to reduce sedentary behaviour: a systematic review and meta-analysis. *Int J Behav Nutr Phys Act*. 2017;14(1):105.
29. E F Graves L, C Murphy R, Shepherd SO, Cabot J, Hopkins ND. Evaluation of sit–stand workstations in an office setting: a randomised controlled trial. *BMC Public Health*. 2015;15:1145.
30. Carter S, Hartman Y, Holder S, Thijssen DH, Hopkins ND. Sedentary behavior and cardiovascular disease risk: mediating mechanisms. *Exerc Sport Sci Rev*. 2017;45(2):80–6.
31. Green DJ, Hopman MT, Padilla J, Laughlin MH, Thijssen DH. Vascular adaptation to exercise in humans: role of hemodynamic stimuli. *Physiol Rev*. 2017;97(2):495–528.
32. Ainslie PN, Cotter JD, George KP, et al. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *J Physiol*. 2008;586(16):4005–10.
33. Burdette JH, Laurienti PJ, Espeland MA, et al. Using network science to evaluate exercise-associated brain changes in older adults. *Front Aging Neurosci*. 2010;2:23.
34. Aengevaeren VL, Claassen JA, Levine BD, Zhang R. Cardiac baroreflex function and dynamic cerebral autoregulation in elderly masters athletes. *J Appl Physiol (1985)*. 2013;114(2):195–202.
35. Tanne D, Freimark D, Poreh A, et al. Cognitive functions in severe congestive heart failure before and after an exercise training program. *Int J Cardiol*. 2005;103(2):145–9.
36. Braz ID, Fisher JP. The impact of age on cerebral perfusion, oxygenation and metabolism during exercise in humans. *J Physiol*. 2016; 594(16):4471–83.
37. Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, Green DJ. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension*. 2010;55(2):312–8.
38. Restaino RM, Walsh LK, Morishima T, et al. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. *Am J Physiol Heart Circ Physiol*. 2016;310(5):H648–53.
39. Holder SM, Dawson EA, Brislane A, Hisdal J, Green DJ, Thijssen DHJ. Fluctuation in shear rate, with unaltered mean shear rate, improves brachial artery flow-mediated dilation in healthy, young men. *J Appl Physiol (1985)*. 2019;126(6):1687–93.
40. Hartman YAW, Karssemeijer EGA, van Diepen LAM, Olde Rikkert MGM, Thijssen DHJ. Dementia patients are more sedentary and less physically active than age- and sex-matched cognitively healthy older adults. *Dement Geriatr Cogn Disord*. 2018;46(1–2):81–9.
41. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation*. 2007;115(10):1285–95.
42. Wolters FJ, Zonneveld HI, Hofman A, et al. Cerebral perfusion and the risk of dementia: a population-based study. *Circulation*. 2017; 136(8):719–28.
43. Verbree J, Bronzwaer AS, Ghariq E, et al. Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *J Appl Physiol (1985)*. 2014;117(10):1084–9.