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

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Abstract

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 impact of reduced sitting-intervention on vascular and cerebrovascular function.

Methods This prospective study included 24 individuals with increased cardiovascular risk (65±5 years, 29.8±3.9 kg/m^2). Before and after 16-week reduced sitting, using a mobile-Health device with vibrotactile feedback, we examined: i. vascular function (flow-mediated dilation (FMD)), ii. cerebral blood flow (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-hour sitting with and without light-intensity physical activity breaks (every 30-minutes).

Results The first wave of participants showed no change in sedentary time (n=9, 10.3±0.5 to 10.2±0.5 hours/day, 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 hours/day, 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/s, P=0.02), without altering CA or CVMR. Before and after the 16-week intervention, 3-hour 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-/cerebrovascular risk. **Key words:** sedentary behavior, physical activity, vascular function, cardiovascular risk, cerebrovascular risk. **Trial registration** Study is registered at the Netherlands Trial Register (NTR6387) (https://www.trialregister.nl/trial/6215).

Introduction

Physical inactivity (*i.e.* lack of regular exercise) is strongly and independently related to the development of non-communicable diseases, such as cardiovascular and cerebrovascular diseases (1). In addition to physical inactivity, studies have revealed the detrimental impact of sedentary behavior; defined as any waking behavior in a sitting, reclining or lying posture with an energy expenditure below 1.5 metabolic equivalents (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 hours of uninterrupted sitting, and reported an attenuation of vascular function (9), resting cerebral blood flow velocity (10) and glucose tolerance (11). Interestingly, interrupting prolonged sitting by frequent walking breaks of 2-5 minutes prevented these deteriorations (9-11). Whilst acute short-term studies support the potential benefits of physical activity breaks, these effects may not simply translate to long-term changes. Whilst the acute changes is focused on a supra-normal 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.

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-week 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-week 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 impact of a sedentary bout may be equally present in participants with a less sedentary lifestyle. Therefore, we examined whether the 16-week intervention alters the acute (3-hour) impact of sedentary behavior, and the ability of physical activity breaks to prevent these effects. We hypothesize that the detrimental impact of 3-hour uninterrupted sitting, but also the protective effects of regular physical activity breaks, on vascular and cerebrovascular flow and function remain present after the 16-week reduced sitting intervention.

Methods

Participants

Individuals from the environment of Nijmegen, the Netherlands, aged ≥55 years with >40 hours per week of self-reported sedentary behavior were eligible for participation. Criteria for inclusion were the presence of one or more cardiovascular risk factors, consisting of BMI >28 kg/m^2, high blood pressure (SBP >160 mmHg, DBP >90 mmHg) and anti-hypertensive 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 3 clusters of 3 measurement days to our laboratory: a first cluster before a 16-week control period (T0), a second cluster after the 16-week control period (T1) and a third cluster after a 16-week intervention period (T2) (Figure 1). Measurements at T0 were performed as familiarization sessions for the participants and to minimize measurement variation in outcomes. On Day 1 and 2, peripheral vascular and cerebrovascular blood flow and function were assessed at baseline. Subsequently, in randomized cross-over order between Day 1 and 2, subjects underwent a 3-hour sitting trial without moving their lower extremities (SIT), and a 3-hour sitting trial with 2-minute light-intensity walking breaks at self-selected pace every 30 minutes (BREAKS). Immediately following the 3-hour 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-day period. The same set of measurements was repeated at T1 and T2.

Intervention. The 16-week reduced sitting intervention aimed to prevent prolonged sitting (>30 minutes) 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, 2M Engineering, Valkenswaard, the Netherlands) (see Figure, Supplemental Digital Content 2, Activ8Sit, a customized activity monitor, http://links.lww.com/MSS/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 wave 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 days (15). Data was processed using a validated analysis script in Matlab R2014b (The Mathworks Inc., Natick, MA, USA) (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-minute baseline period, a blood pressure cuff was inflated to supra-systolic pressure for 5 minutes (18). Ultrasound recording of the diameter and blood velocity resumed 30 seconds prior to deflation and continued for 5 minutes. 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). Cerebral blood flow velocity (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 pre-processed and analyzed using custom-written Matlab scripts (version 2014b, the MathWorks Inc.) as previously described by de Jong *et al.*(23).

CBFv was measured during 5 minutes 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 (3 periods of 2 minutes sitting and 1 minute standing) (23), as well as repeated sit-to-stand maneuvers (10 seconds sitting, 10 seconds standing) for 5 minutes to enhance hemodynamic fluctuations at 0.05 Hz (26). Using these fluctuations, cardiac

baroreflex sensitivity (BRS) was calculated using SBP and R-R intervals (23). In addition, cerebral autoregulation (CA) was computed via transfer function analysis, resulting in gain, normalized gain (nGain) phase and coherence (22). As 0.05Hz 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 wave 2.0 and 3.0 before and after the 16-week reduced sitting intervention, http://links.lww.com/MSS/C75; see 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).

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, USA). Mixed-models analyses for repeated measurements were performed to evaluate the effect of 16-week reduced sitting intervention on the outcomes (Intervention). In addition, mixed-models analysis were used to investigate the impact of 3-hour sitting (Acute), and whether SIT *versus* BREAKS modifies this effect (Acute*Breaks). To control for potential carry-over effects, the sequence of SIT and BREAKS was included in the model (Seq). Finally, we tested whether the acute impact of sedentary behavior and/or breaks changed after the reduced sitting intervention (Acute*Breaks*Intervention). Allometric modelling was used to correct for changes in baseline diameter on FMD (27). Pearson correlations were computed to correlate the 16-weeks' change in sedentary behavior with changes in vascular and cerebrovascular flow and

function. For these analyses data of wave 1.0 was also included, to evaluate this relation amongst 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-week familiarization period, because of the time burden of the measurements (n=4) or long-term illness (n=1). Twenty-five participants (65±5 years, 29.8±3.9 kg/m^2) performed the measurements before intervention, including the 3-hour 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-week reduced sitting

In wave 1.0, no changes in sedentary behavior characteristics were observed (see, Table, Supplemental Digital Content 6, 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 years, 29.7±4.6 kg/m^2, Table 1) significantly lowered sedentary time (10.2±0.3 to 9.2±0.3 hours/day, P<0.01, Figure 2A) and increased standing time (3.3±0.2 to 3.9±0.2 hours/day, P=0.03, Figure 2B), walking time (2.1±0.2 to 2.6±0.2 hours/day, P<0.01, Figure 2C), and step count (10,316±1,297 to 13,058±1,184 steps/day, P<0.01). No changes were observed in blood pressure, BMI, physical fitness, fasting glucose, insulin, HOMA-IR, 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-week reduced sitting intervention, http://links.lww.com/MSS/C79). Data of wave 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-week intervention (Figure 2D), but antegrade blood flow significantly increased (Table 2). In addition, resting SFA diameter significantly increased after intervention (Figure 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, Figure 2F, Table 2). Correcting for shear rate area-under-the-curve (SR_{auc}) 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, whilst 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, P=0.02) and CVCi (0.49±0.02 to 0.51±0.02 cm/s/mmHg, P=0.03) increased significantly after the 16-week intervention (Figure 2G,H, Table 2). There were no effects on CA, CVMR or BRS (Figure 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-week reduced sitting intervention, http://links.lww.com/MSS/C75). The increase in CA gain during slow sit-stands were 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 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 SR_{auc} corrected FMD showed a significant interaction effect across the 3-hour 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, whilst FMD improved when sitting was interrupted (Table 3). No differences were present in blood flow. The 16-week intervention (n=15) did not alter the acute impact of (un)interrupted sitting on flow or FMD (Table 2, Figure 3).

Cerebrovascular blood flow and function. Resting CBFv and CVCi did not change after the 3-hour period (Table 3). Pooled analysis of rest, hypocapnia and hypercapnia revealed significant interaction effects of the 3-hour 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-hour 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-hour impact of (un)interrupted sitting on CBFv, CVMR, CA, or BRS (Table 2).

Discussion

This study presents the following findings. First, the adjusted 16-week intervention resulted in a reduction in sedentary time of ~1 h/day. 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-hour uninterrupted sitting leads to a decline in peripheral vascular function and cerebral blood flow, but not cerebrovascular function, whilst these effects are prevented when sitting was interrupted by brief walking breaks. The 16-week 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 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 impact 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 co-developed 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 ~1hour/day after 16 week is important since a recent meta-analysis highlighted that only short-term (median 4 weeks), but not long-term (>3-months), mHealth-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-week intervention. One previous study, examining the long-term benefits of an 8-week 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-hour (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. Since 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 cerebral blood flow velocity with no changes in measures of function. Whilst no previous study has examined the long-term effects of reduced sitting, several studies investigated the impact of exercise on cerebrovascular function. Interestingly, trained men demonstrate higher cerebral blood flow compared to sedentary individuals (32), whilst 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-week reduced sitting intervention are in line with those found after exercise training. Even 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-weeks might relate to exposure to repeated increases in cerebral perfusion. Low-to-moderate intensity activities (*e.g.* walking), which increased across the 16-week 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 impact of sedentary behavior, we found that 3-hour uninterrupted sitting impairs SFA endothelial function and lowers cerebral blood flow in individuals with increased cardiovascular risk. Importantly, the 16-week intervention did not alter the acute impact 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-hour uninterrupted sitting extend previous work

in healthy individuals, in that 3-to-4h periods of uninterrupted sitting impacts (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 haemodynamic stimuli that occur during an exercise bout (31). Whilst the physical activity breaks as a consequence of lower sedentary time may contribute to vascular adaptations, also alternative pathways may 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 lab, 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. Whilst 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 to their healthy peers (40). This suggests that targeting sedentary behavior is relevant, especially since 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), whilst 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 impact. 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-week 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-week 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. Whilst 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 minimizes potential error and provides novel insight into the link between sedentary behavior and (cerebro)vascular function.

Perspectives

This study demonstrates the beneficial effects of a successful 16-week reduced sitting intervention on peripheral vascular function and cerebral blood flow in individuals with increased cardiovascular risk. These findings are in line with acute impacts 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 to 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 impact.

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Conflict of interest

PHGMW is scientific advisor of Khondrion, Nijmegen, The Netherlands. This SME had no involvement in the data collection, analysis and interpretation, writing of the manuscript, and in the 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 ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Authors' Contributions

YH, PW, CT, MH, JC and DT contributed to the conception or design of the work. YH, LT, DB, AH, KN, TE, PW, CT, MH, JC and DT contributed to the acquisition, analysis, or interpretation of data for the work. YH drafted the manuscript. LT, DB, AH, KN, TE, PW, CT, MH, JC and DT critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Figure titles and legend

Figure 1. Study design. Measurements were performed in 3 clusters of 3 measurement days: a first cluster before a 16-week familiarization period (T0), a second cluster after the 16-week familiarization period (T1) and a third cluster after a 16-week intervention period (T2). FMD: flow-mediated dilation, TCD: transcranial Doppler. TCD measurements included 5 minutes rest, hyperventilation, hypoventilation, slow sit-stand maneuvers and 0.05 Hz repeated sit-stand maneuvers.

Figure 2. Impact of 16-week 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-week reduced sitting intervention for wave 2.0 and 3.0 (n=15 for sedentary behavior and vascular function measures, n=14 for cerebrovascular measures). FMD: flow-mediated dilation, CBFv: cerebral blood flow velocity, CVCi: cerebrovascular conductance index, nGain: normalized gain.

Figure 3. Acute and long-term impact of reduced sitting on vascular structure and function. Average and individual values of flow, diameter and flow mediated dilation (FMD) before and after SIT and BREAKS at T1 and T2 for wave 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). Even though 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. Datapoints without connecting lines represent measurements with a missing baseline or follow-up point.

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Figure 1

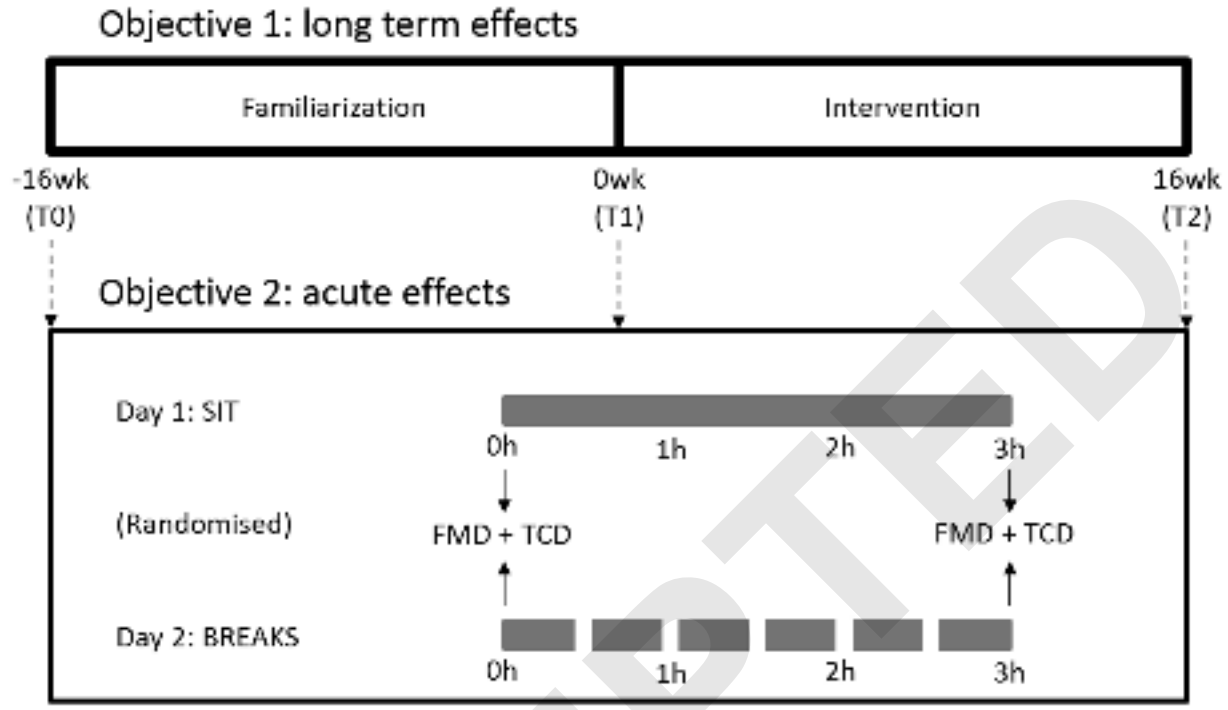

Figure 2

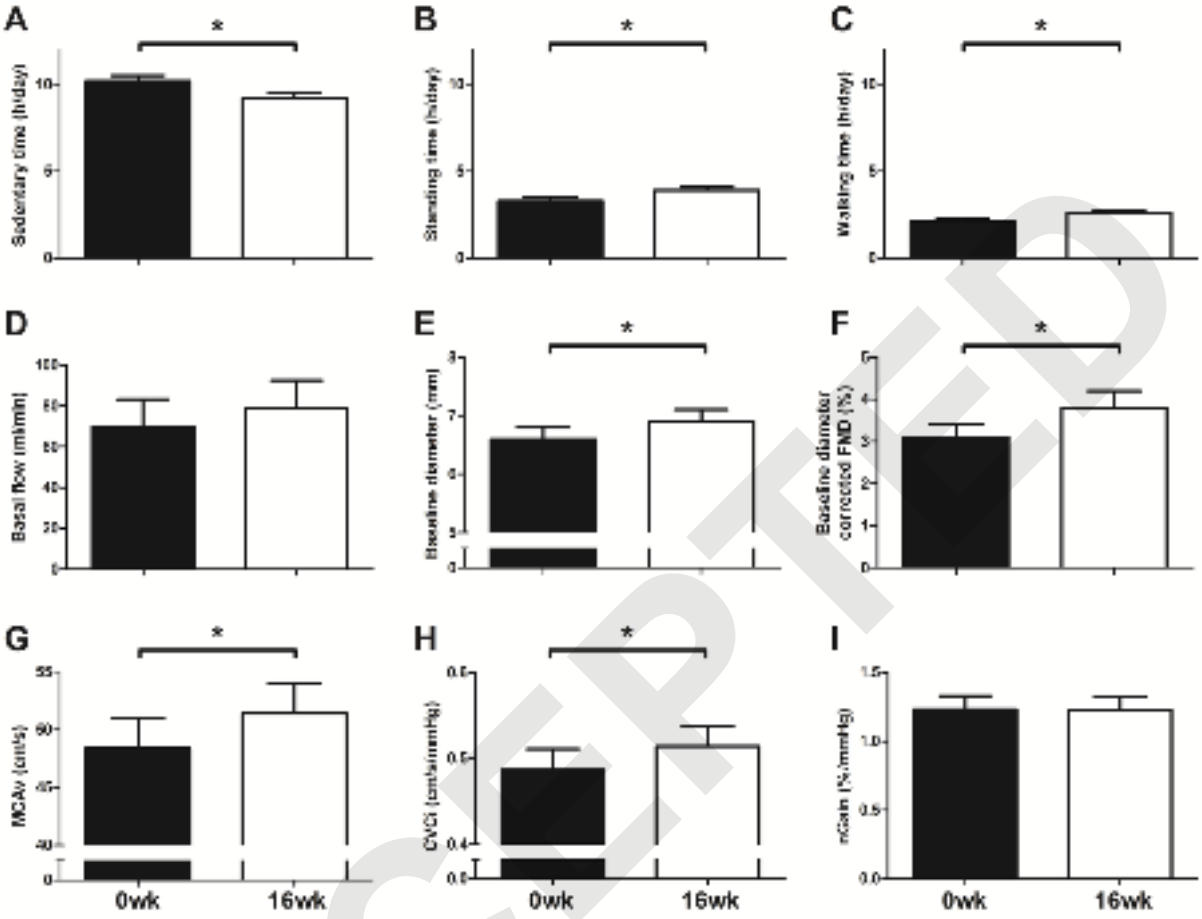

Figure 3

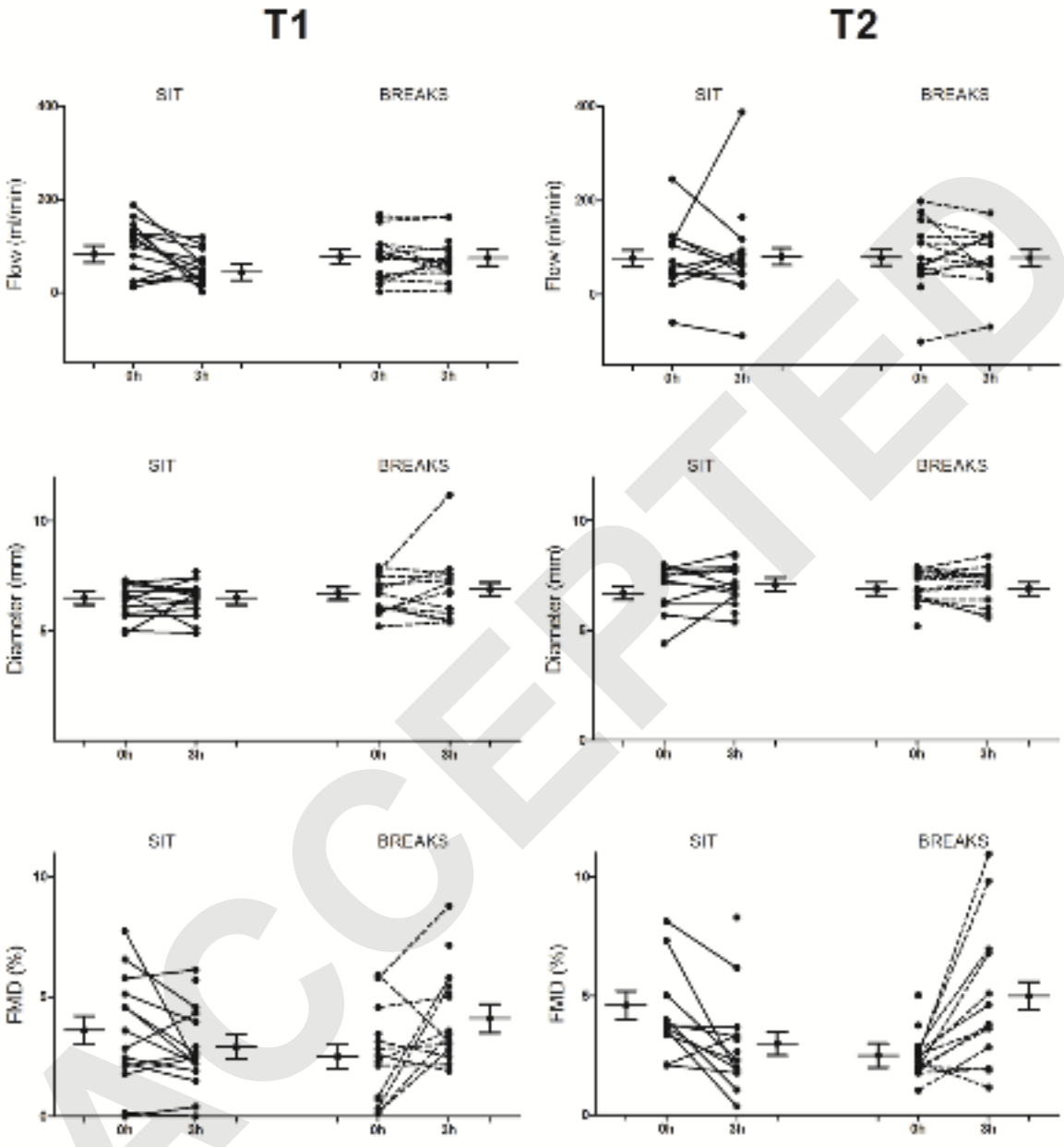

Table 1. Participant characteristics, before (T1) and after (T2) reduced-sitting intervention. Values represent mean±SD or median (IQR)

Baseline characteristics	Total (n=24)			Wave 1.0 (n=9)			Waves 2.0 and 3.0 (n=15)		
Sex (% male, n)	38 (9)			33 (3)			40 (6)		
Age (years)	65±5			66±5			64±5		
Current smoking (%, n)	8 (2)			0 (0)			13 (2)		
Hypertension (%, n)	67 (16)			44 (4)			80 (12)		
Intervention outcomes	T1	T2	P-value	T1	T2	P-value	T1	T2	P-value
SBP (mmHg)	134±13	135±16	0.63	128±11	134±7	0.06	138±13	136±20	0.46
DBP (mmHg)	81±9	83±9	0.03	79±7	83±7	<0.01	82±10	82±11	0.52
BMI (kg/m2)	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/L)	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/L)	n.a.	n.a.	n.a	n.a.	n.a.	n.a.	12 (8-21)	10 (5-21)	0.86
HOMA-IR (100/%S)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.2 (1.7-6.0)	3.2 (1.4-5.4)	0.43
Total cholesterol (mmol/L)	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/L)	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/L)	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/L)	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/L)	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 O2/ml/kg)	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*	n.a.	n.a.	n.a	n.a.	n.a.	n.a.	8.9±1.1	8.5±1.0	0.69
Peripheral vascular stiffness*	n.a.	n.a.	n.a	n.a.	n.a.	n.a.	11.9±1.9	10.5±1.7	0.48

*: only measured for waves 2.0 and 3.0.

Table 2. Vascular and cerebrovascular flow and function before (T1) and after (T2) the 16-week reduced sitting intervention. N=15 for vascular measurements, n=14 for cerebrovascular measurements. P-values represent the acute impact of 3-hour sitting (A), whether SIT *versus* BREAKS modifies this effect (A*B), the impact of the 16-weeks reduced sitting intervention (I) and whether the acute impact of sedentary behavior and/or breaks changed after the reduced sitting intervention (A*B*I). Data are reported as mean±SEM.

Intervention (I)	Pre-intervention				Post-intervention				**P-values**			
Breaks (B)	SIT		BREAKS		SIT		BREAKS					
Acute (A)	0h	3h	0h	3h	0h	3h	0h	3h	**A**	**A*B**	**I**	**A*B*I**
Peripheral vascular flow and function												
Blood flow patterns												
Basal flow (ml/min)	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)	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)	-80±23	-76±23	-71±23	-87±23	-97±24	-90±23	-88±23	-93±24	0.73	0.25	0.06	0.95
Flow-mediated dilation												
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 (mmHg)	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)	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/mmHg)	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 (mmHg)	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)	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/mmHg)	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
Cerebrovascular vasomotor reactivity (CVMR)												
MAP reactivity (unit)	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 (unit)	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 (unit)	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

0.05Hz repeated sit-stands

Intervention (I)	Pre-intervention				Post-intervention				**P-values**			
Breaks (B)	SIT		BREAKS		SIT		BREAKS					
Acute (A)	0h	3h	0h	3h	0h	3h	0h	3h	**A**	**A*B**	**I**	**A*B*I**
MAP (mmHg)	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)	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/mmHg)	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/mmHg)	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 (%/mmHg)	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 (degrees)	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 (unit)	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/mmHg)	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

Table 3. Acute impact of prolonged (3-hour) sitting (SIT) and interruptions in prolonged sitting (BREAKS) on vascular and cerebrovascular flow and function. Measured after finishing the 16-week familiarization period (T1), n=25 for vascular measurements and n=24 for cerebrovascular measurements. P-values represent the acute impact of 3-hour 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.

	Pre-intervention (T1)				**P-values**		
Breaks (B)	SIT		BREAKS				
Acute (A)	0h	3h	0h	3h	**A**	**A*B**	
Peripheral vascular flow and function							
Basal flow (ml/min)	93±13	61±12	86±13	84±13	0.08	0.12	
Antegrade flow (ml/min)	167±17	127±17	148±17	158±17	0.26	0.07	
Retrograde flow (ml/min)	-77±16	-65±16	-63±16	-74±16	0.99	0.17	
Flow-mediated dilation							
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 (mmHg)	97±1	100±1	97±1	100±1	**<0.01**	0.44	0.45
CBFv (cm/s)	49.6±2.4	47.3±2.4	46.3±2.4	47.7±2.4	0.76	0.20	0.55
CVCi (cm/s/mmHg)	0.51±0.03	0.47±0.03	0.48±0.03	0.47±0.03	0.12	0.27	0.52
Gain BRS	6.72±1.60	4.34±1.67	5.46±1.56	3.93±1.64	0.14	0.74	0.20
Pooled analysis							
MAP (mmHg)	98±1	100±1	100±1	100±1	0.16	0.40	
CBFv (cm/s)	50.5±2.2	47.8±2.2	46.3±2.2	46.9±2.2	0.21	0.05	
CVCi (cm/s/mmHg)	0.51±0.02	0.47±0.02	0.46±0.02	0.46±0.02	**0.03**	**0.04**	
Cerebrovascular vasomotor reactivity (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	
0.05Hz repeated sit-stands							
MAP (mmHg)	101±1	104±1	101±1	103±1	**<0.01**	0.42	
CBFv (cm/s)	44.3±2.3	42.1±2.4	42.0±2.2	43.2±2.3	0.74	0.27	

	Pre-intervention (T1)					P-values
Breaks (B)	SIT		BREAKS			
Acute (A)	0h	3h	0h	3h	**A**	**A*B**
CVCi (cm/s/mmHg)	0.44±0.02	0.41±0.02	0.42±0.2	0.42±0.2	0.27	0.21
Gain (cm/s/mmHg)	0.56±0.04	0.54±0.04	0.54±0.04	0.54±0.04	0.61	0.75
nGain (%/mmHg)	1.28±0.08	1.30±0.08	1.32±0.08	1.28±0.08	0.83	0.64
Phase (degrees)	49.8±3.2	48.9±3.3	48.4±3.1	48.2±3.3	0.81	0.86
Coherence (unit)	0.79±0.03	0.81±0.03	0.78±0.03	0.82±0.03	0.15	0.70
Gain BRS (ms/mmHg)	4.76±0.65	5.52±0.62	4.04±0.59	4.07±0.64	0.55	0.51

Supplemental methods, SDC 1

Participants

Individuals with an increased cardiovascular risk were recruited by local newspaper and internet advertisement in the direct environment of Nijmegen, the Netherlands. Individuals aged ≥55 years with >40 hours per week of self-reported sedentary behavior were eligible for participation. Criteria for inclusion were the presence of one or more cardiovascular risk factors, consisting of BMI >28 kg/m^2, high blood pressure (SBP >160 mmHg, DBP >90 mmHg) and anti-hypertensive 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 [1].

Study design

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

Intervention. The 16-week reduced sitting intervention aimed to prevent prolonged sitting time (>30 minutes) throughout the day and to promote low-intensity physical activity (*e.g.* walking, standing). Subjects received information regarding the purpose of the intervention and wore a customized activity monitor to objectively monitor sedentary behavior (Activ8sit, 2M Engineering, Valkenswaard, the Netherlands, Supplemental Figure 1). This pocket-worn device consists of an inclinometer and a tri-axial accelerometer, which allows for recognizing prolonged periods of sedentary behavior and physical activity patterns. Upon recording prolonged, uninterrupted sitting (*i.e.* 30-minutes), vibrotactile feedback was provided by the monitor to remind participants to replace sedentary behavior by low-intensity physical activity (*e.g.* walking, standing). Participants were able to review their physical activity patterns in a web-based environment. Regular phone meetings with a researcher were made to evaluate participation. To bypass previously identified problems when translating interventions to reduce sedentary behavior [2], we adopted an embedded pilot study-design, where input of participants was used to optimize the 16-week intervention for the subsequent groups of participants (*i.e.* waves). Following this approach, 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 was intensified to weekly meetings (phone or online) for subjects in wave 2.0 and 3.0 and a half-way group-meeting was organized to optimize the intervention and to further reduce sedentary behavior. Subsequently, participants were randomly assigned to wave 2.0 or 3.0. Intervention was performed in September 2017 to January 2018 (for wave 1.0, n=9), March to July 2018 (for wave 2.0, n=9), and October 2018 to February 2019 (for wave 3.0, n=8).

Measurements

Before each measurement day, participants were instructed to refrain from caffeine and alcohol intake at least 12 hours prior to the test. Moreover, participants were instructed to refrain from vigorous exercise 24 hours prior to the measurements. All vascular measurements were performed according to guidelines to assess peripheral vascular function [3].

Participant characteristics. Medical history, medication use and BMI were assessed in all participants. Capillary blood was used to measure fasting glucose levels. Fasting venous blood was collected in Lithium Heparin vacutainers and sample processing occurred within 30 minutes. Plasma was stored at -80°C until further use. Insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured in fasting lithium heparin plasma using standardized methods, and low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula. As an explorative outcome, HOMA-IR was calculated from glucose and insulin levels in wave 2.0 and 3.0. Blood pressure was measured twice by a manual sphygmomanometer after 5 minutes seated rest according to AHA guidelines [4]. The Astrand-Rhyming test was used as a submaximal cycling test to estimate physical fitness, expressed as estimated maximal oxygen consumption [5].

Physical activity patterns. A validated activity monitor (ActivPAL3 micro, PAL technologies, Glasgow, United Kingdom) was used to measure physical activity patterns [6]. As the ActivPAL combines a tri-axial accelerometer with an inclinometer, the ActivPAL is able to distinguish between sitting, standing and walking. The first day was excluded for data-analyses. ActivPAL data was processed using a validated analysis script in Matlab R2014b (The Mathworks Inc., Natick, MA, USA) [7]. Sedentary, standing, and walking time, sedentary breaks, number of sedentary bouts (>30 min), and steps per day were computed.

Peripheral vascular blood flow and function. Superficial femoral artery (SFA) flow-mediated dilation (FMD) was measured as a test of peripheral vascular function [8]. After a resting period of 10 minutes in the supine position, the right SFA was examined ~3 cm distal from the bifurcation using a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (T3000, T3300; Terason, Burlington, MA, USA). Continuous Doppler velocity assessment was simultaneously obtained using the lowest possible insonation angle (always $<60^{\circ}$). After a 1-minute resting period, a blood pressure cuff was inflated to supra-systolic pressure for 5 minutes. Recording of the diameter and blood velocity resumed 30 seconds prior to deflation and continued for 5 minutes. Analysis of SFA diameter, blood flow and shear rate was performed using custom-designed edge-detection and wall-tracking software [9,10]. Peak diameter after cuff deflation was automatically detected according to an algorithm as described elsewhere [11].

Cerebrovascular blood flow and function. Continuous blood pressure was measured in the middle finger of the right hand using photoplethysmography (Finapres Medical Systems, Amsterdam, the Netherlands). An arm sling was used to stabilize the hand at heart level. Heart rate was monitored using a three-lead electrocardiogram (BIOPAC Systems, Goleta, CA, USA). Cerebral blood flow velocity (CBFv) in the middle cerebral arteries was measured using transcranial Doppler ultrasonography. Once the left and right middle cerebral arteries were identified through the temporal windows by two 2-MHz probes (Multi-Dop, Compumedics DWL, Singen, Germany), the signal was optimized by adjusting gain, depth, angle and position, and the probes were fixed with a Spencer head frame (Spencer technologies, Seattle, WA, USA). A nasal cannula was used to monitor exhaled CO_2 by capnography (BIOPAC Systems). All signals were recorded at 200 Hz using a data acquisition system (MP150, BIOPAC Systems).

During 5 minutes sitting, resting CBFv was measured. Hypocapnia was induced by hyperventilating at a frequency of 0.5 Hz (1 second breathing in, 1 second breathing out) for 30 seconds. After 2 minutes rest, hypercapnia was induced by inhalation of a gas mixture with steadily increasing concentrations of CO_2 (30 seconds 3%, 30 seconds 4%, 4 minutes 5%). Using these values, cerebral vasomotor reactivity (CVMR), the change in CBFv to changes in arterial CO_2 concentration, could be determined [12]. CVMR was computed by the difference between maximal cerebrovascular conductance index (CVCi, *i.e.* the ratio of CBFv and mean arterial pressure) during hypercapnia and minimal CVCi during hypocapnia, divided by the mean CVCi during normocapnia. Cerebrovascular conductance index was used to account for confounding effects of CO_2 on blood pressure [13].

Repeated sit-to-stand maneuvers (10 seconds sitting, 10 seconds standing) for 5 minutes were used to enhance very low frequency hemodynamic fluctuations at 0.05 Hz [14]. Using these fluctuations, cerebral autoregulation (CA) could be computed via transfer function analysis, resulting in gain, normalized gain (nGain) phase and coherence as output. Gain is the damping of fluctuations in blood pressure in CBFv (lower gain indicates better CA). Phase represents the time shift in CBFv adaptations to blood pressure fluctuations (higher phase indicates active adaptation as seen in normal CA). Coherence is a measure for coherence between CBFv and blood pressure and serves as a measure of data quality [15]. These parameters were averaged over the very low-frequency (0.02–0.07 Hz), where CA is most active [15]. Next to CA, cardiac baroreflex sensitivity (BRS) was calculated by transfer function analysis using SBP and R-R interval as input [16]. Here, a higher gain indicates better BRS. Because the 0.05 Hz repeated sit-stand manoeuvers could influence the hemodynamic effects of prolonged sitting, as they require repeated active standing, we also analyzed CA and BRS using transfer function analysis in slow sit-stand maneuvers. These measurements were performed preceding the 0.05 Hz repeated sit-stand

manoeuvers, and consisted of a data segment of 9 minutes (3 periods of 2 minutes sitting and 1 minute standing) [16].

Beat-to-beat data of all cerebrovascular measurements were pre-processed and analyzed using custom-written Matlab scripts (version 2014b, the MathWorks Inc.) as previously described by de Jong *et al.* [16].

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Figure, SDC 2, Activ8sit, a customized activity monitor

A customized activity monitor (Activ8sit, 2M Engineering, Valkenswaard, the Netherlands) was developed for the 16-week reduced sitting intervention. See eMethods for detailed information.

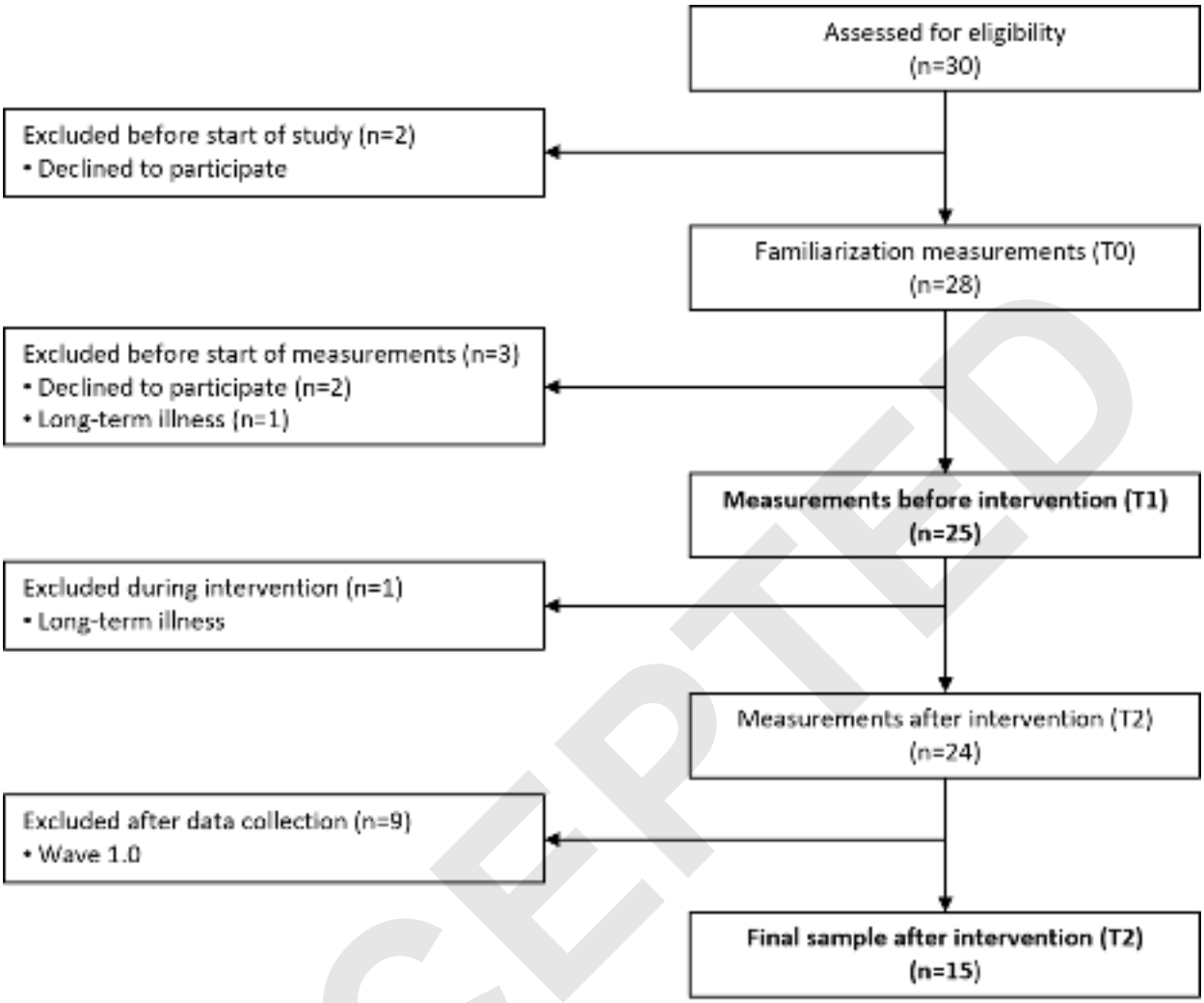
Table, SDC 3, Additional cerebrovascular flow and function measures of wave 2.0 and 3.0 before (T1) and after (T2) the 16-week reduced sitting intervention. N=15 for vascular measurements, n=14 for cerebrovascular measurements. P-values represent the acute impact of 3-hour sitting (A), whether SIT *versus* BREAKS modifies this effect (A*B), the impact of the 16-weeks reduced sitting intervention (I) and whether the acute impact of sedentary behavior and/or breaks changed after the reduced sitting intervention (A*B*I). Data are reported as mean±SEM.

Intervention (I)	**Pre-intervention**				**Post-intervention**				**P-values**			
Breaks (B)	SIT		BREAKS		SIT		BREAKS					
Acute (A)	0h	3h	0h	3h	0h	3h	0h	3h	**A**	**A*B**	**I**	**A*B*I**
Cerebrovascular flow and function												
Hypocapnia												
MAP (mmHg)	85±2	86±2	89±2	86±2	87±2	89±2	86±2	88±2	0.66	0.54	0.47	0.25
CBFv (cm/s)	35.7±2.2	34.4±2.1	31.9±2.1	32.6±2.1	35.5±2.2	36.1±2.1	35.5±2.1	34.7±2.1	0.82	0.87	**0.04**	0.34
CVCi (cm/s/mmHg)	0.40±0.02	0.40±0.02	0.36±0.02	0.38±0.02	0.41±0.02	0.40±0.02	0.41±0.02	0.39±0.02	0.87	0.84	0.84	0.39
Hypercapnia												
MAP (mmHg)	116±3	115±3	118±3	116±3	112±3	114±3	113±3	118±3	0.48	0.76	0.19	0.79
CBFv (cm/s)	65.5±5.2	67.5±5.2	59.1±5.1	60.3±5.1	66.7±5.2	67.2±5.1	65.3±5.0	66.0±5.0	0.54	0.93	0.10	0.89
CVCi (cm/s/mmHg)	0.57±0.04	0.60±0.04	0.51±0.04	0.52±0.04	0.59±0.04	0.58±0.04	0.58±0.04	0.56±0.04	0.82	0.70	0.17	0.99
Slow sit-stands												
MAP (mmHg)	100±2	105±2	100±2	102±2	101±2	104±2	102±2	104±2	**<0.01**	0.18	0.12	0.58
CBFv (cm/s)	45.5±2.9	46.1±2.9	42.9±2.8	44.2±3.0	47.3±2.8	47.4±2.9	45.9±2.7	47.8±2.8	0.43	0.62	0.05	0.82
CVCi (cm/s/mmHg)	0.46±0.03	0.44±0.03	0.43±0.03	0.44±0.03	0.47±0.03	0.46±0.03	0.45±0.03	0.46±0.03	0.79	0.42	0.16	0.94
Gain (cm/s/mmHg)	0.38±0.04	0.40±0.04	0.34±0.04	0.36±0.04	0.44±0.04	0.45±0.04	0.39±0.04	0.42±0.04	0.35	0.75	**<0.01**	0.83
nGain (%/mmHg)	0.85±0.10	0.87±0.10	0.82±0.10	0.83±0.11	0.99±0.10	0.99±0.10	0.87±0.10	0.89±0.1	0.79	0.91	**0.03**	0.86
Phase (degrees)	52±6	49±6	49±6	42±6	41±6	46±6	44±6	50±6	0.97	0.84	0.49	0.72
Coherence (unit)	0.62±0.04	0.58±0.04	0.54±0.04	0.6±0.05	0.66±0.04	0.64±0.04	0.55±0.04	0.63±0.04	0.40	0.05	0.14	0.98
Gain BRS (ms/mmHg)	3.2±0.5	3.6±0.5	3.3±0.4	2.7±0.5	3.2±0.5	3.4±0.4	3.3±0.4	3.8±0.4	0.63	0.45	0.30	0.18

Table SDC 4. Additional cerebrovascular flow and function measures of the acute impact of prolonged sitting (SIT) and interruptions in prolonged sitting (BREAKS). Measured after finishing the 16-week familiarization period (T1), n=25 for vascular measurements and n=24 for cerebrovascular measurements. P-values represent the acute impact of 3-hour 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.

	Pre-intervention (T1)				**P-values**	
Breaks (B)	SIT		BREAKS			
Acute (A)	0h	3h	0h	3h	**A**	**A*B**
Cerebrovascular flow and function						
Hypocapnia						
MAP (mmHg)	83±2	85±2	86±2	85±2	0.65	0.21
CBFv (cm/s)	36.0±1.5	33.0±1.5	32.1±1.5	32.0±1.5	0.07	0.08
CVCi (cm/s/mmHg)	0.43±0.02	0.39±0.02	0.38±0.02	0.38±0.02	0.09	0.07
Hypercapnia						
MAP (mmHg)	115±2	114±2	116±2	116±2	0.66	0.80
CBFv (cm/s)	66.8±3.5	64.0±3.6	60.6±3.4	61.4±3.4	0.59	0.33
CVCi (cm/s/mmHg)	0.58±0.03	0.56±0.03	0.52±0.03	0.53±0.03	0.92	0.55
Slow sit-stands						
MAP (mmHg)	100±1	103±1	99±1	102±1	**<0.01**	0.99
CBFv (cm/s)	46.0±2.3	43.0±2.3	42.0±2.2	45.5±2.4	0.87	**0.03**
CVCi (cm/s/mmHg)	0.46±0.02	0.42±0.2	0.43±0.2	0.45±0.02	0.47	**0.04**
Gain (cm/s/mmHg)	0.39±0.03	0.40±0.03	0.37±0.03	0.41±0.03	0.28	0.60
nGain (%/mmHg)	0.86±0.07	0.96±0.07	0.90±0.07	0.91±0.7	0.26	0.30
Phase (degrees)	48.7±3.8	44.1±3.8	45.9±3.6	43.7±3.9	0.24	0.67
Coherence (unit)	0.60±0.04	0.61±0.04	0.57±0.03	0.61±0.04	0.41	0.62
Gain BRS (ms/mmHg)	3.33±0.44	3.74±0.44	3.29±0.42	3.55±0.45	0.27	0.80

Figure, SDC 5: Flowchart of study participation



30 Individuals were assessed for eligibility. 25 Individuals participated in the acute sessions before intervention (T1; n=25 for vascular measurements and n=24 for cerebrovascular measurements) and 24 individuals fulfilled the 16-week reduced sitting intervention (T2; n=24 for vascular measurements and n=23 for cerebrovascular measurements). However, after exclusion of wave 1.0, the final intervention sample consisted of n=15 for vascular measurements and n=14 for cerebrovascular measurements.

Table, SDC 6, Sedentary behavior characteristics of wave 1.0 before (T1) and after (T2) 16-week reduced sitting intervention.

Sedentary behavior characteristics	T1	T2	P-value
Sedentary time (hours/day)	10.3±0.5	10.2±0.5	0.87
Standing time (hours/day)	3.5±0.3	3.6±0.3	0.53
Walking time (hours/day)	1.6±0.2	1.5±0.2	0.35
Steps (n/day)	7,342±771	6,803±771	0.46

N=9. Data are reported as mean±SEM.

Table, SDC 7, Vascular and cerebrovascular flow and function of wave 1.0 before (T1) and after (T2) 16-week reduced sitting intervention.

Intervention (I)	**Pre-intervention**				**Post-intervention**				**P-values**			
Breaks (B)	**SIT**		**BREAKS**		**SIT**		**BREAKS**					
Acute (A)	**0h**	**3h**	**0h**	**3h**	**0h**	**3h**	**0h**	**3h**	**A**	**A*B**	**I**	**A*B*I**
Peripheral vascular flow and function												
Blood flow patterns												
Basal flow (ml/min)	95±31	81±31	94±31	93±31	109±31	128±33	63±33	54±31	0.97	0.85	0.92	0.41
Antegrade flow (ml/min)	172±29	139±29	146±29	146±29	171±29	204±31	155±31	135±29	0.81	0.80	0.43	0.53
Retrograde flow (ml/min)	-77±16	-53±16	-53±16	-54±16	-62±16	-82±17	-91±17	-81±16	0.65	0.86	**<0.01**	**0.04**
Flow-mediated dilation												
Baseline diameter (mm)	7.1±0.4	6.9±0.4	6.8±0.4	6.7±0.4	6.9±0.4	6.8±0.4	7.0±0.4	6.8±0.4	0.22	0.82	0.96	0.78
Peak diameter (mm)	7.3±0.4	7.1±0.4	7.0±0.4	7.0±0.4	7.1±0.4	7.1±0.4	7.2±0.4	7.1±0.4	0.40	0.89	0.85	0.85
FMD (%)	2.6±0.6	3.1±0.6	3.0±0.6	3.9±0.6	3.0±0.6	4.0±0.6	3.1±0.7	3.9±0.6	0.05	0.88	0.44	0.89
SR_{auc} (10^3)	2.1±1.4	6.6±1.3	3.0±1.3	2.7±1.3	5.1±1.3	4.8±1.4	5.7±1.5	5.9±1.3	0.28	0.24	0.06	0.18
FMD corrected for diameter	2.7±0.6	3.1±0.5	2.9±0.5	3.9±0.5	3.0±0.5	4.3±0.6	3.1±0.6	3.5±0.5	0.08	0.79	0.44	0.61
FMD corrected for SR_{auc}	2.5±0.6	3.1±0.6	2.9±0.6	4.0±0.6	3.0±0.6	4.4±0.6	3.0±0.7	3.5±0.6	0.06	0.72	0.45	0.53
Cerebrovascular flow and function												
At rest												
MAP (mmHg)	95±2	98±2	95±2	100±2	99±2	101±2	100±2	102±2	**0.01**	0.85	**<0.01**	0.57
MCAv (cm/s)	53.6±4.5	43.1±4.6	46.2±4.5	49.0±4.6	47.8±4.5	54.0±4.5	52.0±4.5	49.3±4.5	0.47	0.46	0.05	**<0.01**
CVCi (cm/s/mmHg)	0.57±0.05	0.44±0.05	0.49±0.05	0.47±0.05	0.48±0.05	0.54±0.05	0.53±0.05	0.50±0.05	0.07	0.28	0.80	**<0.01**
Gain BRS	5.4±1.4	4.7±1.4	3.2±1.4	3.4±1.4	8.3±1.4	3.7±1.7	3.7±1.4	3.8±1.5	0.17	0.13	0.44	0.32
During hypocapnia												
MAP (mmHg)	80±3	85±3	84±3	86±3	89±3	89±3	90±3	91±3	0.30	0.76	**<0.01**	0.58
MCAv (cm/s)	37.1±2.9	30.5±3.0	32.9±2.9	31.9±2.9	34.6±2.9	36.8±2.9	37.1±2.9	33.1±2.9	**0.02**	0.89	**0.02**	**<0.01**
CVCi (cm/s/mmHg)	0.47±0.03	0.36±0.04	0.40±0.03	0.38±0.04	0.39±0.03	0.42±0.03	0.41±0.03	0.38±0.04	**<0.01**	0.65	0.90	**<0.01**
During hypercapnia												
MAP (mmHg)	114±3	112±3	114±3	116±3	118±3	123±3	121±3	119±3	0.68	0.83	**<0.01**	0.19
MCAv (cm/s)	67.8±6.4	54.5±6.7	61.3±6.2	62.3±6.3	67.0±6.2	72.3±6.2	73.7±6.2	70.3±6.3	0.25	0.53	**<0.01**	**0.01**
CVCi (cm/s/mmHg)	0.63±0.06	0.48±0.06	0.54±0.05	0.54±0.05	0.57±0.05	0.59±0.05	0.65±0.05	0.58±0.06	**0.02**	0.47	**0.03**	**0.01**
Pooled analysis												
MAP (mmHg)	96±2	98±2	98±2	101±2	102±2	104±2	104±2	104±2	0.05	0.81	**<0.01**	0.41
MCAv (cm/s)	52.7±4.2	43.1±4.3	46.8±4.2	47.7±4.2	49.8±4.2	54.4±4.2	54.3±4.2	51.1±4.2	0.09	0.52	**<0.01**	**<0.01**

Intervention (I)	**Pre-intervention**				**Post-intervention**				**P-values**			
Breaks (B)	**SIT**		**BREAKS**		**SIT**		**BREAKS**					
Acute (A)	**0h**	**3h**	**0h**	**3h**	**0h**	**3h**	**0h**	**3h**	**A**	**A*B**	**I**	**A*B*I**
CVCi (cm/s/mmHg)	0.56±0.04	0.43±0.04	0.48±0.04	0.46±0.04	0.48±0.04	0.51±0.04	0.53±0.04	0.49±0.04	**<0.01**	0.40	0.06	**<0.01**
Cerebrovascular vasomotor reactivity (CVMR)												
MAP reactivity	0.35±0.03	0.28±0.03	0.31±0.03	0.30±0.03	0.29±0.03	0.33±0.03	0.32±0.03	0.28±0.03	0.41	0.73	0.81	0.13
MCAv reactivity	0.60±0.06	0.56±0.06	0.61±0.05	0.66±0.06	0.68±0.05	0.65±0.05	0.71±0.05	0.71±0.05	0.85	0.33	**<0.01**	0.63
CVCi reactivity	0.33±0.06	0.28±0.06	0.30±0.04	0.36±0.05	0.32±0.05	0.32±0.04	0.43±0.05	0.42±0.05	0.92	0.45	0.10	0.37
Slow sit-stands												
MAP (mmHg)	98±2	100±2	97±2	102±2	105±2	105±2	104±2	106±2	**0.04**	0.38	**<0.01**	0.97
MCAv (cm/s)	49.9±4.7	42.3±4.5	42.5±4.2	49.0±4.3	45.6±4.3	50.8±4.3	49.3±4.3	44±4.2	0.87	0.63	0.42	**<0.01**
CVCi (cm/s/mmHg)	0.51±0.05	0.42±0.05	0.44±0.04	0.48±0.04	0.43±0.04	0.48±0.04	0.48±0.04	0.42±0.04	0.45	0.77	0.58	**<0.01**
Gain	0.42±0.06	0.41±0.06	0.41±0.05	0.45±0.05	0.36±0.05	0.43±0.05	0.39±0.05	0.35±0.05	0.62	0.68	0.17	0.15
nGain	0.87±0.10	0.95±0.09	0.97±0.08	0.93±0.09	0.79±0.09	0.84±0.09	0.77±0.09	0.77±0.08	0.62	0.38	**0.01**	0.71
Phase	45±7	38±7	42±6	45±6	42±6	50±6	37±6	42±6	0.66	0.69	0.91	0.41
Coherence	0.64±0.06	0.63±0.06	0.64±0.05	0.63±0.05	0.56±0.05	0.61±0.05	0.62±0.05	0.57±0.05	0.76	0.43	0.12	0.37
Gain BRS	3.3±1.4	3.1±1.4	2.3±1.3	3.0±1.3	4.4±1.3	3.0±1.3	3.9±1.3	3.1±1.3	0.54	0.55	0.28	0.94
0.05Hz repeated sit-stands												
MAP (mmHg)	99±2	102±2	98±2	102±2	104±2	107±3	106±2	108±2	0.05	0.94	**<0.01**	0.62
MCAv (cm/s)	49.3±4.6	42.1±4.6	42.0±4.6	42.1±4.5	46±4.6	47.2±5.1	49.5±4.5	46.4±4.8	0.32	0.72	0.13	0.20
CVCi (cm/s/mmHg)	0.50±0.05	0.41±0.05	0.43±0.05	0.42±0.05	0.45±0.05	0.44±0.05	0.47±0.05	0.44±0.05	0.15	0.62	0.64	0.29
Gain	0.59±0.08	0.56±0.08	0.56±0.08	0.57±0.08	0.6±0.08	0.56±0.09	0.62±0.08	0.51±0.08	0.25	0.85	0.98	0.50
nGain	1.16±0.12	1.34±0.12	1.33±0.12	1.38±0.11	1.31±0.12	1.20±0.13	1.22±0.11	1.05±0.12	0.82	0.41	0.07	0.76
Phase	49±4	46±4	48±4	49±4	49±4	52±5	47±4	45±5	0.89	0.98	0.98	0.41
Coherence	0.80±0.05	0.86±0.05	0.81±0.05	0.89±0.04	0.79±0.05	0.77±0.05	0.78±0.04	0.81±0.05	0.23	0.63	0.09	0.76
Gain BRS	3.4±1.0	4.7±1.0	3.7±1.0	4.3±1.0	6.1±1.0	3.0±1.2	2.8±1.0	4.1±1.1	0.94	0.11	0.97	**0.04**

n=9. Data are reported as mean±SEM.