Clinical Nutrition xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Impact of prolonged sitting and physical activity breaks on cognitive performance, perceivable benefits, and cardiometabolic health in overweight/obese adults: The role of meal composition

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ARTICLE INFO

Article history:
Received 25 June 2020
Accepted 1 October 2020

Keywords:
Meal composition
Sitting breaks
Cognitive performance
Vascular health
Metabolic health

SUMMARY

Background & aims: Physical activity (PA) breaks may effectively attenuate the detrimental impact of prolonged sitting on acute cognitive performance, perceivable benefits (e.g. mood), vascular function, and metabolic health. To date, the impact of meal composition on the effects of sedentary behavior and/or PA breaks on health has been scarcely studied. Therefore, our aim was to investigate whether meal composition alters how sedentary behavior and PA breaks affect these acute health outcomes.

Methods: A total of 24 overweight and obese, sedentary adults completed four conditions in randomized order in a cross-over design: [a] high-protein, low-fat breakfast (HPLF) + 4hrs uninterrupted sitting (SIT), [b] HPLF + 4hrs interrupted sitting (ACT; 5-min cycling every 30 min), [c] Western breakfast (WEST; higher in fats/simple sugars, lower in protein/fiber) + SIT, [d] WEST + ACT. WEST and HPLF were isocaloric. Linear mixed models were used to examine changes in cognitive performance (Test of Attentional Performance), perceivable benefits (Likert-scales, Profile of Mood States questionnaire), vascular health (carotid artery reactivity, blood pressure), and metabolic health (post-breakfast glucose, insulin, lipids).

Results: Independent of meal composition, we did not observe any effect of PA breaks on cognitive performance, vascular health and post-breakfast lipid responses. PA breaks delayed post-breakfast mood and vigor decrements, as well as increases in fatigue and sleepiness (all $p < 0.05$), but effects were independent of meal composition ($p > 0.05$). WEST resulted in higher post-breakfast glucose levels compared to HPLF ($p < 0.05$), while PA breaks did not impact this response ($p > 0.05$). PA breaks reduced post-breakfast insulin ($p < 0.05$), which did not differ between meals ($p > 0.05$).

Conclusions: The acute impact of PA breaks and/or prolonged sitting on cognitive performance, perceivable benefits, and vascular and metabolic health was not altered by the composition of a single meal in overweight/obese, sedentary adults. Possibly, breaking up prolonged sitting, rather than meal composition, is a more potent strategy to impact acute health outcomes, such as perceivable benefits and insulin levels.

1. Background

With the increasing prevalence of overweight and obesity in the Western world, physical inactivity is a great concern due to its

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https://doi.org/10.1016/j.clnu.2020.10.006

Please cite this article as: L. Wanders, I. Cuijpers, R.P.C. Kessels *et al.*, Impact of prolonged sitting and physical activity breaks on cognitive performance, perceivable benefits, and cardiometabolic health in overweight/obese adults: The role of meal composition, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2020.10.006

List of non-standardized abbreviations

HPLF	High-protein low-fat
WEST	Western
SIT	Uninterrupted sitting
ACT	Interrupted sitting

negative impact on health. A large part of the population spends a substantial part of the day sitting and being physically inactive [1]. Sedentary behavior is an important target for lifestyle interventions, as it may play a role in cognitive decline, brain atrophy and dementia [2–5], possibly independent of physical activity [5]. Moreover, prolonged sitting is an established independent risk factor for multiple adverse outcomes, including type 2 diabetes and cardiovascular disease [6,7].

Laboratory studies reinforce that breaking up sitting with short bouts of physical activity (PA) at low- to moderate-intensity may improve cognitive, mental, vascular and metabolic health [8–14]. Compared to prolonged sitting, PA breaks have been linked to improved cognitive performance [9,10], cerebral blood flow [12], mood [8,14], peripheral vascular function [13] and blood pressure (BP) [15]. In addition, PA breaks have been shown to lower postprandial glucose and insulin levels compared to uninterrupted sitting [11], with stronger effects being suggested with increasing BMI [16]. Whilst these observations support the beneficial impact of PA breaks, little work has focused on the potential of diet composition to interfere with these effects. While many previous studies provide standardized meals when investigating the effects of PA breaks on health outcomes, meal frequency and composition varies between studies, as well as the details provided about the meals. For instance, Dunstan et al., 2012, Wennberg et al., 2016, and Larsen et al., 2014, used a drink containing 763 kcal (50 g fat, 75 g carbohydrates), with or without a biscuit snack to investigate the effects of PA breaks on glucose [11,14], insulin [11,14], cognitive function [14], fatigue [14] and BP [14,15]. Bergouignan et al., 2016, provided breakfast and lunch with 15% protein, 55% carbohydrates and 30% carbohydrates looking at mood, fatigue, satiety and cognitive function [8]. Chrismas et al., 2019, provided a high glycemic index (GI) breakfast (570 ± 78 kcal, 19 ± 3 g fat, 80 ± 11 g carbohydrates, 17 ± 2 g protein) and moderate GI snack (285 ± 39 kcal, 14 ± 2 g fat, 35 ± 5 g carbohydrates, 6 ± 1 g protein), while Mullane et al., 2017, provided a 479 ± 18 kcal breakfast and 543 ± 10 kcal lunch with an unspecified but reportedly comparable macronutrient composition between participants, both focusing on cognitive function [9,10]. Carter et al., 2018, provided a 220 kcal cereal bar for breakfast (7 g fat, 34 g carbohydrates, 4 g protein) and a banana as snack looking at cerebral blood flow [12], while Thosar et al., 2015, performed assessments of vascular function in a fasted state [13].

Noticeably, macronutrient composition and food components such as flavonoids, contained for instance in some fruits, tea and cocoa, may acutely improve cognitive performance, vascular health [17,18] and metabolic health [17,19]. Since a majority of the day is spent sedentary *and* in a postprandial state, our research question is whether meal composition alters the impact of sedentary behavior and/or PA breaks on cognitive, mental, vascular and metabolic health. This information is especially relevant for overweight and obese individuals, as this group has a high risk for adverse health outcomes [20], and much of the previous research has focused on this group [9,11,14,15,21]. We hypothesized that meal composition and PA breaks interact to affect health outcomes. Specifically, we hypothesized that A) a Western meal would exaggerate decrements in cognitive performance, perceivable benefits (mood, sleepiness, hunger), vascular health and metabolic health upon prolonged sitting, and B) that a high-protein, low-fat meal, rich in flavanols would reduce these decrements in overweight and obese individuals.

2. Materials & methods

2.1. Participants

Twenty-four overweight or obese (age ≥45 years, BMI ≥25 and ≤35 kg/m^2) individuals were recruited for this study. We included men and women with a sedentary lifestyle (i.e. sitting >40 hours (hrs) per workweek, examined using the sedentary behavior questionnaire [22]), who did not meet the aerobic PA guidelines (i.e. 150 min of moderate-intensity PA per week [23]) and who were proficient in the Dutch language to enable cognitive assessment. We excluded individuals who were diagnosed with diabetes (type 1 and 2), cardiovascular disease, gastrointestinal disease/food allergies, epilepsy, cancer, mental disorders and cognitive decline (Montreal Cognitive Assessment [MoCA] score ≤25 [24]), and untreated or unstable thyroid disease, hypertension and depression. We also excluded individuals who were smokers, followed a strict diet, drank more than three consumptions of alcohol per day or were taking glucose/lipid lowering or anti-inflammatory medication. Pregnant or lactating women were excluded from participation, as well as people who simultaneously participated in other scientific research that required adherence to behavioral rules. Exclusion criteria were defined based on their potential to interfere with the outcomes or the intervention itself. Recruitment took place via flyers, local newspaper advertisements and *link2trials* [25]. All participants provided written informed consent before screening for eligibility. The study was approved by the Medical Ethical Committee (CMO, region Arnhem-Nijmegen, registration number NL64153.091.17), registered at the Dutch Trial Register (NTR, Trial NL6850 (NTR7028)) and conducted in compliance with the Declaration of Helsinki.

2.2. Study design

This randomized cross-over study consisted of one screening day followed by four study days. During the screening, the eligibility, demographic characteristics and lifestyle habits were examined. Participants were familiarized with the cognitive tests prior to the first assessment, in order to reduce practice effects. Thereafter, they received an activity monitor (activPAL3™ micro, PAL Technologies, Glasgow, UK), which was attached to the middle of the right thigh and worn for seven consecutive days. Prior to the first study day, participants filled in a food diary on three days of their choice (two weekdays, one weekend day), whereby one day had to be the day before the first study day. Participants were instructed to adhere to the following rules before each study day: 1) refrain from moderate/vigorous physical activity and vitamin/mineral supplements for 48hrs, 2) no consumption of alcohol or caffeine for 18hrs, 3) consumption of the same meals and foods on the day prior to each visit, and 4) report to the study days after an overnight fast.

The four study days consisted of a breakfast combined with 4hrs of: [a] uninterrupted sitting (SIT), or [b] sitting interrupted by PA breaks (ACT). Breakfast included: [a] high-protein/low-fat (HPLF), or [b] Western (WEST). This resulted in the following four conditions: [a] HPLF + SIT, [b] HPLF + ACT, [c] WEST + SIT [d] WEST + ACT (Fig. 1). The 24 participants were randomly allocated to one of 24 possible orders of combining the meal type with sitting

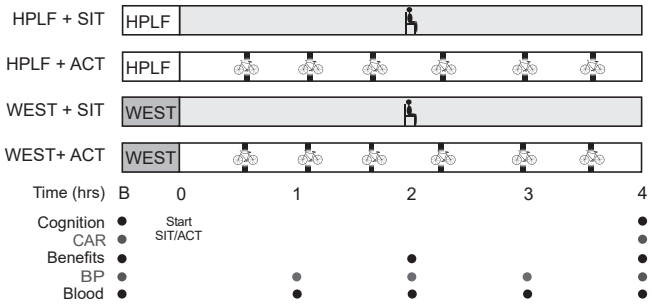

Fig. 1. Study design. Two different breakfasts were combined with 4hrs of prolonged or interrupted sitting, resulting in the four conditions shown in the figure. The time-points of the different measurements are indicated with black/grey dots. SIT, prolonged sitting; ACT, regular cycling breaks; HPLF, high-protein, low-fat breakfast; WEST, Western breakfast; B, baseline; 0, after meal consumption and before start sitting; CAR, carotid artery reactivity; BP, blood pressure; Benefits, perceivable benefits: mood, hunger, sleepiness.

condition. Resultantly, each participant completed the four conditions in a unique order (counterbalanced design).

Washout between visits varied between 1 and 4 weeks. Study days started between 8:00 and 9:00 am. Upon arrival, sleep duration of the prior night was recorded to potentially correct for differences, as sleep deprivation could affect cognitive performance [26]. To this end, participants where asked the question how many hours they slept the night before the study day. Cognitive performance and carotid artery reactivity were assessed before and after the intervention. Perceivable benefits were examined pre-meal, and 2 and 4hrs after the start of SIT/ACT. Blood pressure and blood samples were taken pre-meal and hourly after the start of SIT/ACT.

2.2.1. SIT/ACT

While seated, participants watched a nature documentary to standardize activities. Water was made available *ad libitum* and restroom breaks were permitted. Restrooms were located directly next to the study room, at a maximum of 10 m distance. During ACT, five-minute moderate-intensity (50–70% of maximum estimated heart rate [27]) cycling breaks were performed at 30-minute intervals. The fifth break was slightly delayed to ensure blood pressure was measured after at least 5 min of seated rest and not directly after a cycling bout. The total duration of both conditions, SIT and ACT, was 4hrs. In sum, participants cycled for 30 min during the 4hr ACT sessions. Cycling was chosen as the mode to break up sitting as it has previously been shown to provide the strongest benefits for cognitive function [9]. Moreover, it is a feasible and easily controlled method in the lab setting.

2.2.2. HPLF/WEST

HPLF consisted of 438 kcal, with 11% of the total energy coming from fat (3% saturated fat), 52% from carbohydrates and 31% from protein. The main carbohydrate sources of HPLF were whole meal bread and blueberries. The isocaloric WEST (439 kcal) had 39% of the total energy coming from fat (27% saturated fat), 45% from carbohydrates and 14% from protein. The main carbohydrate sources of WEST were white bread and strawberry jam. The detailed composition can be found in Table 1.

To allow for translatability to a real life setting, mixed meals were chosen with a non-extreme caloric content. Calories in the two breakfasts were comparable, to be able to draw conclusions on the effect of meal composition independent of the caloric content. WEST was chosen due to the negative effects of meals high in fats and refined sugars on several health outcomes [28–30]. HPLF was chosen as the comparison meal for the following reasons: [A] many of the negative effects of diet on vascular health and cognitive function are attributed to (saturated) fats increasing TAG levels, oxidative stress and inflammation, which can occur already after a single meal [30–34]. We therefore aimed to have a comparison meal low in (saturated) fats. [B] Glycemic index is thought to affect cognitive performance [35], wherefore the comparison meal has a lower GI than WEST. To focus on the type of carbohydrates (refined vs. complex), rather than amounts, carbohydrate content was kept comparable. [C] To keep the caloric content comparable while following the choices above, the control meal contains more protein, which is moreover beneficial for satiety [36] and may be beneficial for cognitive performance [35]. [D] Blueberries contain high levels of flavonoids, thought beneficial for cognitive function and vascular health [17,37].

2.3. Cognitive performance

Cognitive performance (attention and executive function) was assessed using the computer-based Test of Attentional Performance (TAP, version 2.3, Psytest, Herzogenrath, Germany) [38]. This test battery is sensitive and has been validated for use in biomedical research [39]. It has moreover been previously used in a study investigating the effects of breaking up sitting on cognitive function [40]. The investigated cognitive domains have been assessed in previous research on PA breaks [8–10,14,40,41] and also meal composition [35,42]. Participants completed the TAP alertness, mental flexibility and working memory subtests. A detailed description of the tests can be found on www.Psytest.net [38]. For alertness, the average of the individual median reaction times of phasic and intrinsic alertness was calculated in milliseconds. Participants completed three (non-verbal) mental flexibility tests (angular, round, alternating). The speed-accuracy tradeoff score was calculated for each of the three tests (% correct, divided by the median reaction times). The speed-accuracy tradeoff score of the alternating test was then divided by the average score of the angular and round tests to obtain the actual flexibility score. A higher flexibility score is indicative of a better executive function. Two levels of the working memory subtest were completed (level 1 and 2). For analysis, only level 2 was used to prevent a ceiling performance. For level 2, the percentages of errors and omissions were averaged. Thus, a higher score indicates worse performance.

2.4. Perceivable benefits (mood, hunger, sleepiness)

Mood was assessed with the short form of the Dutch version of the Profile of Mood States (POMS) questionnaire (original author: McNair et al., translated by: Wald & Mellenbergh, date version: 1990) [43]. This questionnaire consists of 32 items, each scored on a four-point Likert scale (1 = 'not at all', 4 = 'extremely'). Individual scores were summed to calculate five sub-scores: tension, depression, anger, vigor and fatigue. The total mood disturbance (TMD) score was used, calculated by adding the negative mood sub-scales tension, depression, anger and fatigue and subtracting the positive mood score vigor from the result (higher scores indicating a lower mood) [44]. The subscales fatigue and vigor were also analyzed separately. Hunger was assessed with a five-point Likert scale asking, 'how hungry are you at the moment?' (0 = 'not hungry at all', 5 = 'very hungry'). Sleepiness was evaluated with a Dutch translation of the Stanford Sleepiness Scale (SSS) [45], which is a 7-point Likert scale (1 = 'feeling active, vital, alert, or wide awake', 7 = 'no longer fighting sleep, sleep onset soon; having dream-like thoughts').

Table 1
Breakfast composition.

	Component	g	Energy (kcal)	Fat (g)	Sat. fat (g)	Carb. (g)	Protein (g)	Fiber (g)
HPLF	Skimmed milk	180	65	0.2	0.2	9.0	6.7	0.0
	Whole meal bread	70	154	1.0	0.1	25.9	7.7	4.2
	Diet margarine	10	27	3.0	0.6	0.1	0.1	0.0
	Sliced chicken breast	40	42	1.2	0.4	1.0	6.8	0.0
	Low fat yogurt	250	98	0.0	0.0	10.0	11.8	0.0
	Blueberries (fresh)	100	52	0.0	0.0	11.0	0.7	2.5
	Total	**650**	**438**	**5.4**	**1.3**	**57**	**33.8**	**6.7**
WEST	Whole milk	130	84	4.6	3.2	5.8	4.6	0.0
	White bread	70	172	0.6	0.1	35.0	6.3	1.0
	Butter	10	75	8.2	5.7	0.1	0.1	0.0
	Jam (strawberry)	15	37	0.0	0.0	9.0	0.1	0.2
	Cheese (48+)	20	71	5.8	4.0	0.0	4.8	0.0
	Total	**245**	**439**	**19.2**	**13.0**	**49.9**	**15.9**	**1.2**

HPLF, high-protein, low-fat breakfast; WEST, Western breakfast; sat, saturated; carb, carbohydrates; g, grams.

2.5. Vascular health (carotid artery reactivity, blood pressure)

Carotid artery reactivity (CAR) was assessed by measuring the diameter response of the common carotid artery during a Cold Pressor Test. Ultrasound (Terason uSmart 3300/T300, Terason, Burlington, Massachusetts, USA) with a linear probe was used to image the proximal 1.5 cm straight part of the left common carotid artery (B-mode, longitudinal). After a supine rest period of 15 min, a one-minute baseline recording of the artery was taken, after which the participant's hand was immersed up to the wrist in cold water ($\leq$4 °C) for three minutes, while recording continued. Blood pressure was measured twice before and after the test to confirm sympathetic stimulation. Data were processed blinded using wall-tracking and edge-detecting software and reviewed by an independent assessor. Baseline diameter was determined as the average diameter during the one-minute baseline recording. During the Cold Pressor Test, the average diameter was calculated for each 10-second interval. CAR% was calculated as the peak response (minimum or maximum, depending on the directionality) from baseline divided by the baseline diameter, in percentage (%). Directionality of the diameter response (dilation or constriction) was determined by subtracting the mean baseline diameter from the mean diameter during the Cold Pressor Test, with a positive value indicating dilation and a negative value indicating constriction.

All blood pressure measurements (Omron M6 Comfort, Omron healthcare Co., Ltd., Kyoto, Japan) were done after a seated rest for a minimum of five minutes. BP was measured twice at each time point and the average of the two measurements was used for analysis. Measurements were done on the side opposite to the venous catheter. During the screening, BP was measured in both arms to ensure that the measurements did not differ between arms.

2.6. Markers of metabolic health

After the cognitive tasks, a peripheral venous catheter was inserted for blood sampling. The first blood sample was taken in the fasted state. Samples were centrifuged at 3000 rpm for 10 min at 4 °C. After removal of the supernatant, samples were immediately frozen at −20 °C for a maximum of 4hrs and thereafter at −80 °C. Upon completion of the trial, samples were sent for blinded analysis to the Radboudumc Laboratory for Diagnostics and analyzed. Insulin (sandwich principle), glucose (UV test) and triglycerides (enzymatic, colorimetric method) were determined pre-meal and hourly after the start ACT/SIT. The timepoint of the first blood drawing (1 hr) was chosen to capture the effects of both the meal and the first activity break performed after 30 min. Cholesterol (enzymatic, colorimetric method), high-density lipoprotein (HDL, homogeneous enzymatic, colorimetric test) and low-density lipoprotein (LDL, calculated) were determined pre-meal, and 2 and 4hrs after the start of ACT/SIT.

2.7. Statistical analysis

All analyses and graphing were done using R version 3.6.2 (R core Team, 2019) [46]. Participant characteristics were described using the tableone [47] package. Data were analyzed using (repeated measures) linear mixed models with the lme4 package [48]. Linear mixed models were built with a random intercept for subjects and the fixed factors meal (HPLF/WEST), activity (SIT/ACT), time and their interaction. Where the interaction term activity × meal × time (model 1) was non-significant, it was removed from the model, with the final model then only containing the interaction effects for activity × time and meal × time (model 2). For all blood outcomes, the net incremental area under the curve (iAUC) was calculated according to the trapezoidal rule, with areas below the baseline level being subtracted. IAUCs were also analyzed with linear mixed models, as above, but without the fixed factor time. The assumptions of homogeneity of variances and normality of residuals were inspected visually. Data were log10 transformed when substantial deviations were observed. Potential differences in sleep duration the night prior to the study days were assessed with repeated measures ANOVA, using the ez R package [49]. Differences in the number of toilet breaks taken during the study days were analyzed with the Friedman test, due to non-normality. The one-week washout period was assumed sufficient to prevent carryover effects, which is supported by the results from the statistical comparison of baseline values (repeated measures ANOVA/Friedman test, Table S5). Alpha was set at 0.05 for all analyses.

3. Results

All 24 participants completed the study (Table 2). The mean age was 60 ± 8 years, the mean BMI 30 ± 3 kg/m^2 and 79% of the participants were female. Sleep duration during the night prior to the study days was not significantly different between study days (HPLF + SIT: 7.3 ± 1.1hrs, HPLF + ACT: 6.8 ± 1.6 hrs, WEST + SIT: 6.8 ± 1.5 hrs, WEST + ACT: 6.9 ± 1.4 hrs, p = 0.31). The number of restroom breaks taken during the study days was low (HPLF + SIT: 0 [0−1], HPLF + ACT: 1 [0−1], WEST + SIT: 0 [0−1], WEST + ACT: 0 [0−1]. During HPLF + SIT, 46% of the participants took a restroom break, 54% during HPLF + ACT, 33% during WEST + SIT, and 29% during WEST + ACT.

Meal composition did not alter the effects of PA breaks on any of the outcomes of cognitive performance, vascular function, blood pressure, perceivable benefits or metabolic health (see results from

Table 2
Participant characteristics.

Characteristic		Total (N = 24)
Age (years)		59.6 ± 8.1
Sex, female		19 (79)
Postmenopausal		15 (79)
BMI (kg/m^2)		30.2 ± 2.5
Antihypertensive medication		4 (17)
Systolic blood pressure* (mmHg)		128 ± 13
Diastolic blood pressure* (mmHg)		83 ± 7
Education level[a]	Average	8 (33)
	High	16 (67)
Employed		10 (42)
MoCA score		28.3 ± 1.2
Sleep duration[b] (hrs/day)		8.4 ± 0.8
Sitting time[b] (hrs/day)		10.2 ± 1.7
MVPA[b] (hrs/day)		0.9 [0.7–1.2]
Energy intake (kcal/day)		1724.5 [1546.9–2072.8]
Fat (g/day)		67.7 [60.4–78.7]
Saturated fat (g/day)		25.9 [19.2–30.3]
Protein (g/day)		77.4 ± 20.9
Carbohydrates (g/day)		190.4 ± 67.3

BMI, body mass index; MoCA, Montreal Cognitive Assessment; MVPA, moderate- to vigorous-intensity physical activity. *Average of two measurements on the right arm.

[a] Average education level based on the Dutch educational system: junior vocational training, high education level: senior vocational/academic training.

[b] Activity data as measured with the ActivPAL. Data are presented as mean ± SD (when normally distributed) and number (%), non-normal data as median [IQR].

model 1, Tables 3 and 4 and S1–S4). Therefore, the effects of PA-breaks and meal composition presented in the following sections are independent of each other (results model 2).

3.1. Cognitive performance and perceivable benefits

PA breaks and meal composition had no significant effects on the cognitive outcomes alertness, mental flexibility, and working memory (Table 3).

Sleepiness ($p < 0.001$), TMD ($p < 0.001$) and fatigue ($p = 0.01$) increased significantly across the 4hr intervention, and vigor scores decreased significantly ($p < 0.001$) (Fig. 2, Table S1). Compared to uninterrupted sitting, PA breaks resulted in less sleepiness, a better mood (lower TMD), less fatigue (all $p = 0.01$) and more vigor ($p = 0.04$) at 2hrs (Fig. 2, Table S1). Hunger scores were lower at 2hrs ($p < 0.001$), and returned to baseline at 4hrs ($p = 0.87$), but scores were not significantly altered by PA breaks. Meal composition did not have an effect on any of these perceivable benefits (Fig. 2, Table S1).

3.2. Vascular health

We did not find a significant effect of PA breaks or meal composition on CAR (Fig. 3, Table S2) or on changes in SBP and DBP across the 4hr intervention (Fig. 3, Table S2).

3.3. Metabolic health

Across the 4hr period, triglycerides (TG) increased significantly ($p < 0.001$), which was not significantly affected by PA breaks or meal composition (Table 4). Similarly, PA breaks and meal composition did not affect the changes in total cholesterol, HDL and LDL over time (Table 4). Supporting these observations, the iAUCs for cholesterol and LDL did not differ between conditions (Table S4). The iAUC for HDL was significantly higher after WEST compared to HPLF ($p = 0.03$, Table S4), without an impact of PA breaks.

Glucose and insulin showed the characteristic postprandial peak, which returned to baseline within 2hrs and 4hrs, respectively (Fig. 4). PA breaks did not significantly affect post-breakfast glucose levels. WEST resulted in significantly higher post-breakfast glucose levels 1hr post-meal compared to HPLF ($p = 0.049$) (Fig. 4A, Table S3). In parallel, the iAUC for glucose was significantly higher after WEST compared to HPLF ($p = 0.01$) (Fig. 4A1). Post-breakfast insulin levels were significantly lower 3hrs post-meal when interrupted with PA breaks ($p = 0.01$), with no effect of meal composition (Fig. 4B, Table S3). These results were reinforced by a significantly lower iAUC for insulin for the PA break interventions ($p = 0.004$), again without an effect of meal composition (Fig. 4B1).

4. Discussion

Since meal consumption is often followed by prolonged periods of sitting, either at home or during work, the purpose of this study was to determine whether meal composition alters the acute impact of sedentary behavior and PA breaks on cognitive performance, perceivable benefits, as well as vascular and metabolic health. First, we did not find any acute effects of sitting and/or PA breaks on cognitive performance or vascular function. In contrast, PA breaks delayed acute decrements in perceivable benefits (i.e. mood and sleepiness) during prolonged sitting and resulted in lower post-breakfast insulin levels. Second, we found that the Western meal induced higher post-breakfast glucose levels compared to the HPLF meal, independent of PA breaks, highlighting the distinct compositions of the meals. Third, and most importantly, meal composition did not alter the acute impact of sedentary behavior and/or PA breaks on cognitive performance, perceivable benefits, and vascular and metabolic health. Taken together, our results indicate that the composition of a single meal does not impact the acute effects of sedentary behavior and/or PA breaks on health outcomes in overweight and obese, sedentary individuals.

4.1. Cognition and perceivable benefits

In contrast to our hypothesis, we found no acute effect of PA breaks on attention and executive function and also no interaction

Table 3
Cognitive and vascular outcomes per condition and time.

Cognitive outcome	Time	HPLF + SIT	HPLF + ACT	WEST + SIT	WEST + ACT	P-value M1 A x M x T	P-value M2 A x T	M x T	T
Alertness, RT, ms	B	253.75 [236.12–298.00]	263.50 [236.62–294.00]	250.50 [240.50–279.00]	253.75 [236.62–297.75]	REF	REF	REF	REF
	4hrs	254.75 [244.50–294.50]	263.25 [244.12–303.25]	259.50 [248.38–289.88]	262.25 [245.62–326.00]	0.16	0.23	0.46	0.07
Working memory	B	3.63 [0.00–10.59]	3.33 [0.00–11.72]	8.63 [2.94–15.05]	3.33 [0.00–11.03]	REF	REF	REF	REF
2, error %	4hrs	3.63 [0.00–11.27]	6.67 [0.00–8.38]	10.00 [3.33–13.19]	5.29 [0.00–13.77]	0.30	0.71	0.83	0.95
Executive function:	B	0.81 ± 0.12	0.83 ± 0.10	0.84 ± 0.10	0.83 ± 0.10	REF	REF	REF	REF
flexibility score*	4hrs	0.83 ± 0.11	0.82 ± 0.11	0.83 ± 0.07	0.86 ± 0.10	0.095	0.93	0.65	0.46

RT, reaction time in milliseconds; SIT, uninterrupted sitting; ACT, interrupted sitting; HPLF, high-protein, low-fat breakfast; WEST, Western breakfast; B, baseline; A, activity; M, meal; T, time; x, interaction. *A higher flexibility score indicates better executive function. Normally distributed data are presented as mean ± SD, non-normal data as median [IQR]. M1, model 1: three way interaction activity x meal x time. M2, model 2: three way interaction was removed, due to non-significance.

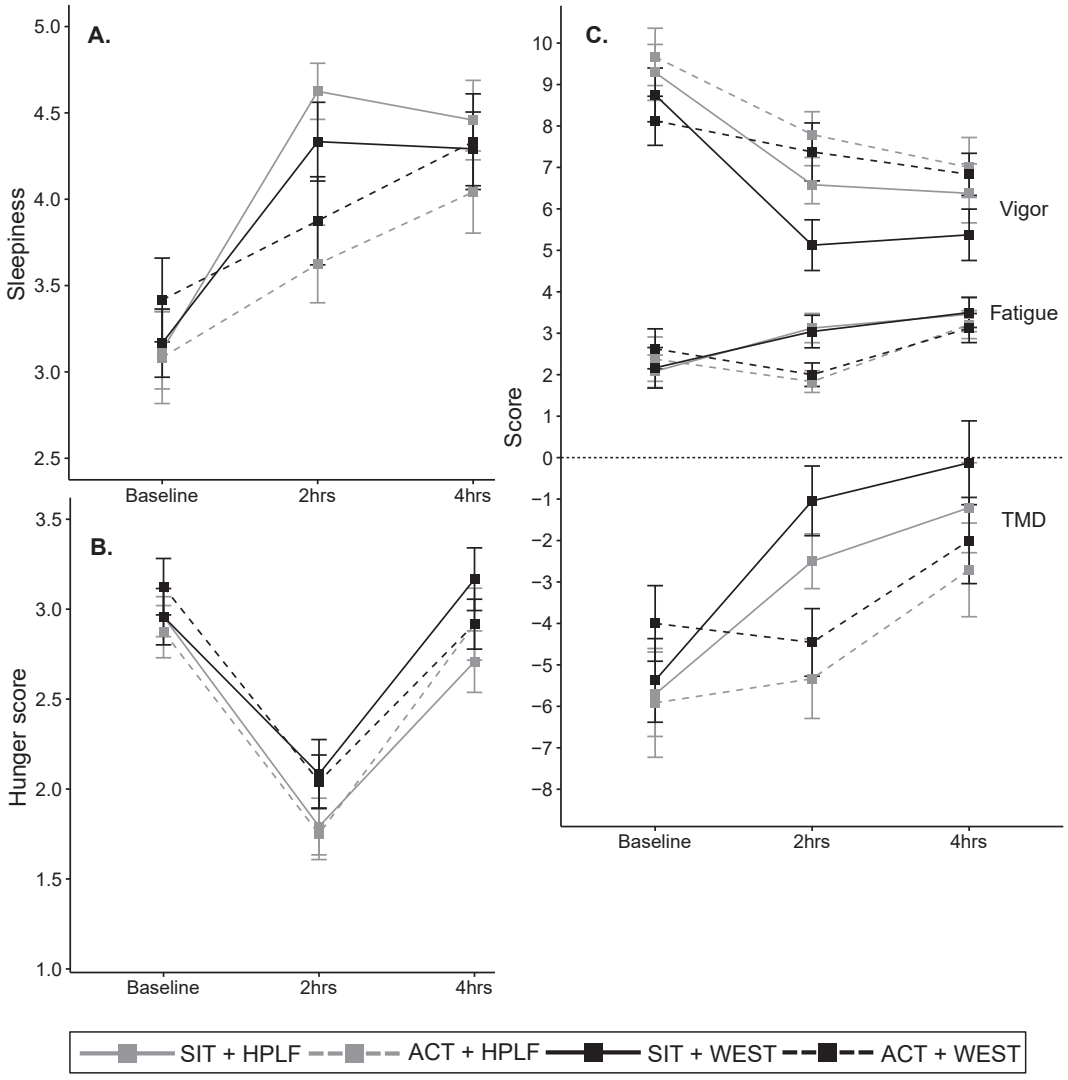

Fig. 2. Perceivable benefits over time: sleepiness (A), hunger (B) and mood (C) over time. Mean values are plotted, with error bars representing within-subject standard errors of the mean; TMD, total mood disturbance; SIT, uninterrupted sitting; ACT, interrupted sitting; HPLF, high-protein, low-fat breakfast; WEST, Western breakfast.

with meal composition. Previous studies have also examined executive function and attention [8–10,14,40,41], which are the domains most responsive to effects of physical activity [50–52]. They have moreover been found sensitive to acute effects of PA breaks [9,10] and short-term changes in nutritional interventions [42]. Therefore, we expected to find an effect, if present, on these domains. We did not expect effects on other domains. Although some previous studies show an acute effect of PA breaks on cognitive outcomes [9,10], a majority, including our work, shows that PA breaks do not acutely improve cognitive performance [8,14,40,41]. Since these previous studies used different meal compositions, our study adds the important novel observation that meal composition does likely not explain between-study differences in outcomes. In our study, meal composition alone, independent of PA breaks, did also not affect cognitive outcomes. This observation contrasts with previous literature, which suggests that in people with impaired glucose regulation, a meal with a low GI might benefit cognitive performance more than high GI [35]. The mean fasted glucose level in our study was 6 mmol/l, indicating impaired glucose regulation in our study population. Moreover, our Western meal was designed to have a higher GI compared to the HPLF meal, evident from the significantly higher glucose peak 1hr post-meal. However, 4hrs post-meal when cognitive performance was assessed, no differences were found in blood glucose levels between conditions, which may explain our null finding concerning cognition. Moreover, even though previous literature suggests that a low GI might be more beneficial for cognitive performance than a high GI meal, findings regarding attention and executive function are inconsistent and the small number of studies and varying methodology still make it difficult to draw solid conclusions on the effects of macronutrient composition [35].

One could argue that our study was underpowered given its explorative nature. However, compared to other studies in this field, our sample size is relatively large. For our primary outcome cognitive performance, most previous studies studied samples of less than 20 participants (range: 6–19) [9,10,14,41]. Only one study, which also did not find an effect of PA breaks on cognitive performance, reported a larger sample size ($n = 30$) [8]. Moreover, the crossover design of this study adds to its strength.

In line with the cognitive outcomes, meal composition did not alter the acute impact of sedentary behavior with or without PA breaks on perceivable benefits, including hunger, sleepiness and mood. Confirming our hypothesis, we found that sleepiness and mood outcomes worsened during the intervention, an effect that

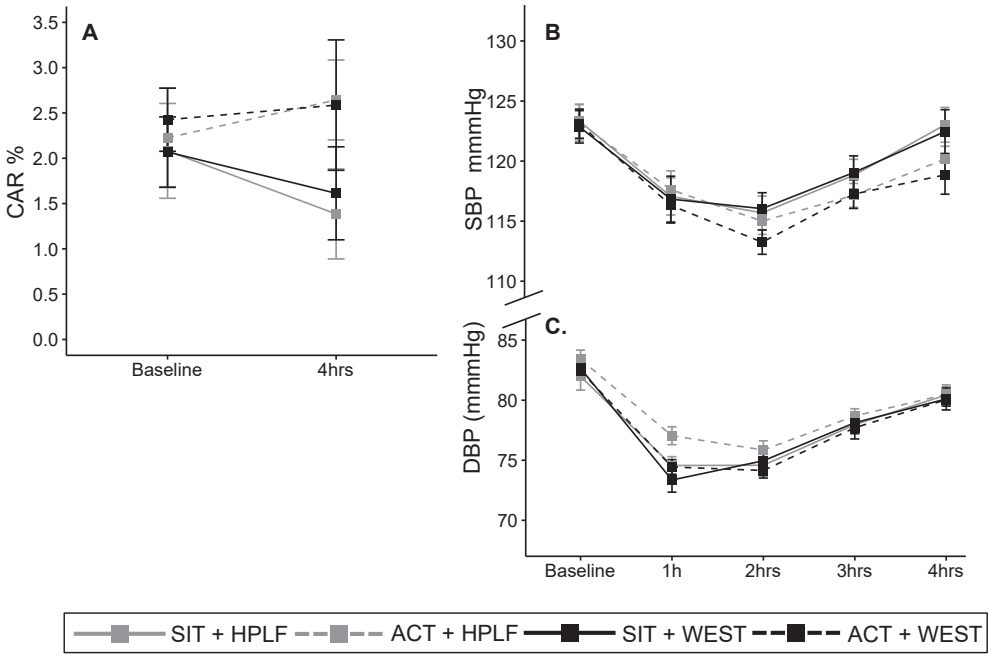

Fig. 3. Vascular parameters over time. Carotid artery reactivity (CAR) (A), systolic (B) and diastolic (C) blood pressure. Mean values are plotted, with error bars representing within-subject standard errors of the mean; SBP, systolic blood pressure; DBP, diastolic blood pressure; SIT, uninterrupted sitting; ACT, interrupted sitting; HPLF, high-protein, low-fat breakfast; WEST, Western breakfast. For CAR: n = 23: one subject was not eligible to undergo the carotid artery reactivity test; HPLF + ACT (baseline) and WEST + SIT (baseline): n = 22 due do measurement problems.

Table 4
Metabolic outcomes per condition over time.

Metabolic outcome	Time	HPLF + SIT	n	HPLF + ACT	n	WEST + SIT	n	WEST + ACT	n	P-value M1 A x M x T	P-value M2		
											A x T	M x T	T
TG, mmol/l	B	1.41 ± 0.62	18	1.49 ± 0.67	19	1.64 ± 0.96	18	1.47 ± 0.65	17	REF	REF	REF	REF
	1hr	1.55 ± 0.67	18	1.69 ± 0.75	18	1.81 ± 0.98	18	1.60 ± 0.71	16	0.70	0.87	1.00	0.12
	2hrs	1.63 ± 0.73	18	1.81 ± 0.83	18	2.03 ± 1.11	18	1.84 ± 0.87	15	0.59	0.79	0.25	**<0.001**
	3hrs	1.86 ± 0.75	17	1.92 ± 0.82	19	2.24 ± 1.11	17	1.89 ± 0.93	16	0.47	0.80	0.52	**<0.001**
	4hrs	2.00 ± 0.78	16	2.04 ± 0.84	18	2.28 ± 1.11	16	1.95 ± 0.86	16	0.51	0.73	0.93	**<0.001**
CH, mmol/l	B	5.15 ± 0.66	18	5.28 ± 0.77	19	5.24 ± 0.81	18	5.15 ± 0.81	17	REF	REF	REF	REF
	2hrs	5.03 ± 0.59	18	5.20 ± 0.73	18	5.22 ± 0.78	18	5.07 ± 0.78	15	0.62	0.95	0.56	0.62
	4hrs	5.13 ± 0.60	16	5.28 ± 0.76	18	5.47 ± 0.77	16	5.17 ± 0.82	16	0.62	0.97	0.44	0.30
HDL, mmol/l	B	1.40 ± 0.38	18	1.40 ± 0.40	19	1.38 ± 0.32	18	1.36 ± 0.39	17	REF	REF	REF	REF
	2hrs	1.34 ± 0.37	18	1.36 ± 0.39	18	1.35 ± 0.33	18	1.34 ± 0.40	15	0.91	0.83	0.41	0.21
	4hrs	1.32 ± 0.39	16	1.35 ± 0.40	18	1.37 ± 0.33	16	1.33 ± 0.40	16	0.81	0.67	0.29	0.20
LDL, mmol/l	B	3.11 ± 0.54	18	3.22 ± 0.63	19	3.13 ± 0.58	18	3.13 ± 0.60	17	REF	REF	REF	REF
	2hrs	2.96 ± 0.50	18	3.03 ± 0.60	18	2.98 ± 0.56	17	2.92 ± 0.56	15	0.93	0.69	0.87	**0.03**
	4hrs	2.89 ± 0.52	16	3.01 ± 0.61	18	3.11 ± 0.60	15	2.96 ± 0.57	16	0.76	0.93	0.39	0.11

SIT, uninterrupted sitting; ACT, interrupted sitting; HPLF, high-protein, low-fat breakfast; WEST, Western breakfast; B, baseline; A, activity; M, meal; T, time; x, interaction; TG, triglycerides; CH, cholesterol; HDL, high-density lipoprotein, LDL, low-density lipoprotein. Normally distributed data, presented as mean ± SD. M1, model 1: three way interaction activity x meal x time. M2, model 2: three way interaction was removed, due to non-significance. Significant results were indicated in bold.

was delayed by regular PA breaks. Improved mood, when prolonged sitting is interrupted with PA breaks, is in line with previous literature [8,14] and may have important implications for the mental wellbeing of this population. Hunger on the other hand was unaffected by PA breaks, which could be beneficial in the management of overweight and obesity, and is in line with previous studies that reported unaffected appetite and *ad libitum* food intake when prolonged sitting is interrupted [53,54].

With these observations, it is important to emphasize that we standardized the activities during the sitting period. In most previous studies, participants were allowed to choose to read/work/watch a movie [8,10,14], activities which could affect cognitive performance and mood differently. Our standardized approach adds to the strength of our observations. Moreover, sleep duration during the night prior to the test days did not differ between study days, which is relevant as sleep deprivation is known to affect cognitive outcomes [55]. Future studies could aim to measure sleep parameters between study days objectively and also include measures of sleep quality.

4.2. Vascular and metabolic health

Meal composition did not alter how PA breaks affected acute vascular and metabolic outcomes. Several studies report lower SBP or DBP when prolonged sitting is interrupted [15,56,57], although this is not a universal finding [14]. We observed a trend towards a

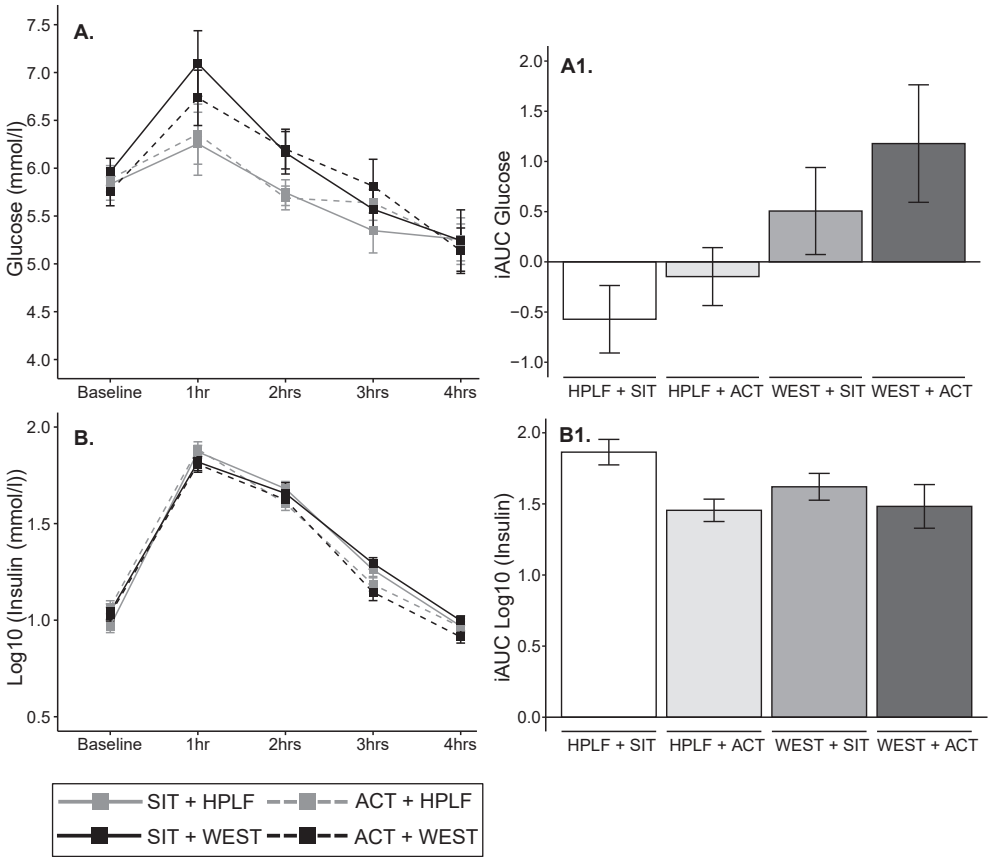

Fig. 4. Glucose (A) and log10 insulin (B) over time and incremental area under the curve (iAUC) (A1, B1). Mean values are plotted, with error bars representing within-subject standard errors of the mean; SIT, uninterrupted sitting; ACT, interrupted sitting; HPLF, high-protein, low-fat breakfast; WEST, Western breakfast.

lower SBP at 4hrs when sitting was interrupted. Although statistically not significant, carotid artery reactivity visibly decreased during prolonged sitting, while a slight increase was seen when sitting was interrupted with PA breaks. In the peripheral vasculature it is established that PA breaks can prevent decrements in vascular function induced by prolonged sitting [13]. In the central vasculature, cerebral blood flow has been reported to decrease after prolonged sitting, which was prevented by PA breaks [12]. In our study, we assessed carotid artery reactivity, which has been shown to be associated with cardiovascular risk factors and cardiovascular disease progression in patients with peripheral arterial disease [58,59]. As CAR is a risk predictor, it is possible that this measure does not change substantially in the short term. Therefore, we believe that the visible, albeit non-significant, effect of PA breaks and prolonged sitting on CAR might still be relevant. However, we acknowledge that longer term studies are needed to draw reliable conclusions on how PA breaks affect CAR.

One study suggests reduced cerebrovascular function after a high-fat meal in older individuals (≥60 years) but not in younger ones (≤35 years) [60]. However, this study did not include a control meal, which makes it difficult to establish whether these observations relate to the meal content or caloric intake. Remarkably, this study used a test meal more than 3 times higher in calories (1362 kcal) and more than 6 times higher in fat (130 g) compared to our meal. Our meal represents a typical breakfast caloric intake and fat percentage representative of a normal (Western) breakfast. It is likely that, unless extreme meal compositions are used, repeated exposure to a Western meal would be needed to negatively affect health.

In the periphery, hypertriglyceridemia after a high-fat meal is thought to stimulate oxidative stress, and thereby impair vascular function [32,61], while mechanisms in the central vasculature are less clear [60]. Although we did see a significant increase in TG during our intervention and also in LDL 2hrs post-meal, this increase did not differ between the meals. PA breaks did not lower TG levels in our study, which is in line with previous literature that suggests that PA breaks might only improve TG levels 12–16hrs post intervention [62] and after interventions lasting several days [21,62].

The observation that PA breaks did not attenuate postprandial glucose levels in our study contrasts previous work [16,62]. A potential explanation for this observation relates to the timing of postprandial blood analysis, as a previous study reported that PA breaks were effective in reducing 2hr, but not 4hr glucose iAUC [63]. Moreover, an additional blood drawing at 45 min could have been beneficial to capture the immediate effects of the first PA break. Additionally, more frequent blood sampling, specifically in the beginning of the trials, may provide a more detailed picture on the glucose and insulin dynamics post-breakfast. Nonetheless, PA breaks resulted in lower post-breakfast insulin levels compared to sitting, supporting the benefits of PA breaks on metabolic health reported in literature [16,62].

4.3. Mechanisms of interactions between diet and PA breaks

The absence of any interaction effect between meal composition and PA breaks on our outcomes contrasts with our hypothesis. Interestingly, a recent meta-analysis concluded that the effects of PA breaks on glucose, insulin and TG levels are not affected by the macronutrient composition of the test meal used in different studies [62]. Although this meta-analysis was unable to directly

compare the various macronutrient compositions, this observation supports our central conclusion.

It is possible that our study population might have already been used to the meal composition of the Western meal, as the percentages of calories from fat, protein and carbohydrates calculated from the food diaries were comparable to the composition of our Western meal. A single HPLF meal might not have been enough to elicit a strong positive effect on our health outcomes and exposure to multiple meals might have been required in our study population. Moreover, the meals used in this study might not have been extreme enough in composition and caloric content to see an effect in this population. Possibly, a single meal with a non-extreme composition does not acutely influence health in an overweight/obese population. It is important to highlight that our study was aimed at investigating the acute effects of meal composition and PA breaks. Results show the acute effects of a single meal only, combined with breaking up sitting for 4hrs. Both, the Western dietary pattern and sedentary behavior, have been linked to several adverse health outcomes [2–7,28–30]. Therefore, a longer-term diet change, combined with daily breaking up of prolonged sitting, could result in other findings. This remains interesting to investigate in future studies.

Moreover, while our study focused on outcomes commonly assessed in previous studies that investigate the effects of PA breaks, these do not provide strong insights into possible underlying mechanisms, which should be explored in future studies.

In addition to meal composition, the caloric content of a meal could be important. For cognitive performance, the caloric range of the meals reported in previous studies is comparable to ours and not extreme [8–10], wherefore it likely does not contribute to between-study differences in results. Regarding metabolic health, it has been shown that in healthy adults a reduction in energy intake could partly prevent reduced insulin action induced by prolonged sitting [64], indicating that caloric intake might play a role. However, in that study, PA breaks were still more effective than energy reduction in counteracting the negative effects of prolonged sitting [64]. In line with this, previous studies on the effects of PA breaks on glucose levels have used a wide variety of calories [11,65,66], suggesting that PA breaks are still effective in improving health outcomes after meals of varying energy content. This is in line with results for peripheral vascular function, where it was found that PA breaks can still improve endothelial dysfunction after a high-fat meal (1332 kcal, 71 g fat), compared to prolonged sitting [67]. Yet, as no control meal was included in that study, effects of prolonged sitting and the meal cannot be separated. One study reported that PA breaks were effective in reducing postprandial glucose iAUC after high and standard energy meals [68]. However, as this previous study was performed in adolescents, findings cannot be generalized to our population and should still be investigated.

PA-breaks in this study were achieved by cycling on a stationary bike. While cycling is feasible in a laboratory based setting or in the presence of an office desk bike, walking might be a more ubiquitously feasible means to break up prolonged sitting, as it does not require any equipment. Walking breaks have also been shown to improve cognitive function [9,10], mood, fatigue [8], peripheral vascular function [13] and metabolic outcomes [11].

Conclusion and clinical implication

In conclusion, we found that the composition of a single meal did not affect the acute impact of 4hrs of sitting with or without PA breaks on cognitive outcomes, perceivable benefits, and vascular and metabolic health in overweight and obese individuals. Whilst PA breaks were effective in delaying acute decrements in mood and sleepiness, but also improved post-breakfast insulin levels, these benefits of PA breaks were independent of the meal. Future research is warranted to investigate whether this holds true in longer-term interventions and, importantly, whether results are altered when using meals higher in calories.

Author statement

Lisa Wanders: conceptualization, methodology, validation, formal analysis, investigation, writing - original draft, visualization. Iris Cuijpers: formal analysis, investigation, writing - review & editing. Roy P. C. Kessels: conceptualization, methodology, resources, writing - review & editing. Ondine van de Rest: conceptualization, methodology, writing - review & editing. Maria T. E. Hopman: conceptualization, writing - review & editing, supervision. Dick H. J. Thijssen: conceptualization, methodology, validation, writing - review & editing, supervision.

Conflict of interest statement and funding sources

The project is organized by and executed under the auspices of TiFN, a public - private partnership on precompetitive research in food and nutrition. The authors have declared that no competing interests exist in the writing of this publication. Funding for this research was obtained from the Netherlands Organization for Scientific Research [grant number ALWTF.2016.032].

Acknowledgements

We would like to thank T.R.J. Landman, L. Janssen and V.L. Aengevaeren for their medical advice and help in placing the catheters for the blood drawings. Moreover, we would like to thank our participants for their involvement and cooperation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.10.006.

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