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Cocoa flavanols protect endothelial function during prolonged sitting in healthy older adults

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Sitting time is high in older adults and has been shown to temporarily impair endothelial function and blood pressure (BP). Flavanols, plant-derived compounds, acutely enhance endothelial function and reduce BP in older adults. The aim of this study was to investigate whether acute ingestion of cocoa flavanols can improve peripheral endothelial function and BP during prolonged sitting in healthy older adults. In a randomised, double-blinded, within-subject, cross-over, placebo-controlled human study, 20 apparently healthy, older adults (age, 72.4 ± 5.0 years; 7 males, 13 females) consumed a high-flavanol (695 mg) and a low-flavanol (5.6 mg) cocoa beverage immediately before a 2-hour sitting bout. Flow-mediated dilation (FMD) of the superficial femoral (SFA; primary outcome) and brachial (BA) arteries, and BP, were assessed before and after sitting. Microvasculature haemodynamics were assessed in the gastrocnemius before, during, and after sitting. Sitting reduced both SFA FMD (Δ = −0.7%; p = 0.005) and BA FMD (Δ = −0.7%; p = 0.016) in the low-flavanol condition. The high-flavanol intervention prevented the decline in both SFA and BA FMD following sitting, with FMD measures remaining similar to pre-sitting (p > 0.3). Sitting increased both systolic (Δ = 6.1 mm Hg, p = 0.001) and diastolic BP (Δ = 2.6 mm Hg, p = 0.001), with no benefit from flavanol intake. Sitting increased muscle oxygenation resting levels (p < 0.001) and haemoglobin content (p < 0.001), and decreased muscle oxygen consumption during SFA occlusion (p < 0.001). Flavanols had no effect on the muscle microvasculature. These findings indicate that flavanol-rich foods may be efficacious nutritional strategies to counteract sitting-induced endothelial impairments during prolonged sitting in older adults, but do not alleviate sitting-induced increases in BP.

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Introduction

Sedentary behaviour is defined as any waking behaviour with an energy expenditure of less than 1.5 metabolic equivalents of task (also known as MET), while in a sitting, reclining or lying posture.[1] Sedentarism is more prevalent in older adults compared to all other age groups.[2] Older individuals are estimated to spend more than 10 hours in their waking day in sedentary activities,[3] which can include sitting bouts lasting up to 5.4 hours per day.[4] Greater sedentary time was found to be associated with a significantly increased risk of cardiovascular diseases (CVD) and all-cause mortality by 30% and 49%, respectively.[5,6] Critically, for every extra hour per day spent sedentary, there is an increased risk of CVD and all-cause mortality of 5% and 11%, respectively.[5,7] Among older adults, sedentary time has additionally been found to be positively associated with hypertension and metabolic disorders:[8,9] specifically, higher systolic and diastolic blood pressure (BP), higher triglycerides, decreased levels of high-density lipoprotein cholesterol, and increased glycated haemoglobin (HbA1c).[8,10–12] Interestingly, moderate-to-vigorous physical activity (MVPA) can reduce CVD risk and mortality,[13,14] yet, sedentary time's impact on CVD risk/mortality is still apparent among individuals who are physically active.[7,15] Therefore, this is an increasing public health concern given that trends indicate the number of older adults to rise more than twofold worldwide between 2021 and 2050.[16]

Numerous experimental studies have shown that even just one isolated episode of prolonged sitting (from 1 to 6 hours) has detrimental effects on human endothelial function, as measured by flow-mediated dilation (FMD).[17,18] A recent meta-analysis, which included young, middle-aged and older adults, estimated that sitting-induced declines in lower-limb FMD can reach up to 2.5%.[18] However, most of the available literature has demonstrated sitting-induced endothelial/vascular dysfunction in younger healthy adults (*e.g.*, ref. 19–21), with only a limited number of studies focused on older adults. For example, Climie *et al.*[22] have shown that 5 hours of uninterrupted sitting reduced FMD in the lower-limb superficial femoral artery (SFA) in sedentary overweight/obese adults,[22] whilst a more recent study reported no differences in SFA FMD following 7 hours of uninterrupted sitting in older adults with type 2 diabetes.[23] In addition, prolonged sitting has been shown to impact BP, with a recent

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study demonstrating that 8 hours of sitting can induce increases in both systolic (10 ± 1 mm Hg) and diastolic BP (5 ± 1 mm Hg) in overweight/obese older adults with type 2 diabetes.[24] Alarmingly, these values reach a clinically relevant threshold associated with an increased risk of atherosclerotic CVD and stroke.[25,26] This suggests that, if such behaviour persists, the impact of uninterrupted sitting periods in older adults is likely to contribute and/or aggravate long-term health issues.

Recent randomised controlled trials have shown that lowering sedentary time, for a duration of approximately 3 to 4 months, has induced meaningful reductions in systolic BP (down ~3.5 mm Hg) and lower-limb endothelial function (as measured by FMD).[27,28] Physical activity, particularly walking breaks and simple resistance-based activities, has also been shown to ameliorate the effects of sitting on SFA FMD[22,23,27] and BP[24,29,30] in late middle-aged and older adults. On the other hand, diet has been shown to be protective against cardiovascular disease (*e.g.*, Mediterranean or DASH diets),[31,32] but has been poorly explored as a strategy to counteract the negative effects of sitting/sedentarism on vascular health. Flavonoid-rich foods are typically consumed in high amounts in cardioprotective diets, with evidence from both observational and RCTs showing flavonoids to improve biomarkers of cardiovascular health, particularly upper-limb brachial artery (BA) FMD and BP both acutely and chronically.[33–42] In particular, flavanols, a subgroup of flavonoids present in unprocessed cocoa, tea, berries, grapes and apples,[43] have been extensively shown to act quickly within the vasculature, improving both brachial FMD[39,44–46] and lower-limb common femoral artery (CFA) FMD[47] within 2 hours of intake in older adults. As such, flavanol-rich foods may be an effective dietary strategy to help minimise the detrimental effects of prolonged sitting on endothelial function and BP in old age, particularly on occasions during which frequent breaks from sitting may not be viable in this population. Importantly, reducing sedentary behaviour remains a primary public health goal. However, when this is not possible due to occupational, environmental, or physical limitations, complementary dietary strategies may be able to minimise sitting-induced vascular deficits.

Therefore, the aim of the current study was to examine whether acute consumption of cocoa flavanols prior to a 2-hour bout of uninterrupted sitting can be beneficial in improving endothelial function (as measured by FMD) in the upper-limb BA and lower-limb SFA in older adults, as well as downstream muscle microvascular function. We further investigated whether cocoa flavanols could prevent sitting-induced increases in BP in older adults. We hypothesised that cocoa flavanols would be effective at rescuing upper- and lower-limb FMD and improve BP during uninterrupted sitting in older age.

Materials and methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN_19-0851B). Informed written consent was obtained from all participants before enrolment in the study. The study was retrospectively registered at clinicaltrials.gov (NCT07265869).

Participants

Twenty healthy older adults (aged ≥65 years old, males or females) were recruited from the University of Birmingham (Birmingham, England) and the surrounding community, and throughout 'the Birmingham 1000 Elders group'. Prior to participation in the study, all participants provided a signed informed consent form and completed a general health and lifestyle questionnaire. Excluding criteria included a history or symptoms of cardiovascular, renal, pulmonary, metabolic, or neurologic disease, hypertension (blood pressure higher than 140/90 mm Hg, based on recent guidelines[48]), diabetes mellitus, anaemia, asthma, immune conditions, or high cholesterol. In addition, smokers, individuals who were on weight-reducing diets or taking anticoagulants, or had recently undergone prolonged bed-rest periods, were also excluded from the study.

Study design

A randomised, double-blinded, counterbalanced, placebo-controlled, within-subject, cross-over intervention study (Fig. 1) was conducted.

All participants attended two experimental visits separated by a minimum washout period of seven days, in line with previous studies.[49–51] Prior to the experimental visits, participants were asked to fast for at least 12 hours. Participants were also instructed to refrain from caffeine, alcohol and polyphenol-containing foods/beverages (a detailed list of foods/beverages to avoid and that are allowed to consume was provided beforehand), and any form of physical activity for at least 24 hours. Although polyphenol metabolites from some sources can remain in circulation for up to 80 hours after ingestion,[52] the majority of metabolites are excreted within the first 24 hours.[53] The two experimental visits started in the early morning (between 08:00 and 10:30). Upon arrival at the laboratory, anthropometric characteristics were recorded using a telescopic measuring rod (Seca 220, Seca, Hamburg, Germany) and a flat scale (Seca 880, Seca, Hamburg, Germany) for height and weight, respectively. Participants were invited to rest in a supine position for approximately 15 min. To assess compliance with the 24-hour dietary restrictions, a 24-hour Dietary Recall Questionnaire was performed verbally at the start of each experimental visit. The baseline measures preceding sitting/flavanol intervention were measured in the following sequence: (i) upper arm systolic and diastolic blood pressure, (ii) heart rate, (iii) SFA FMD, (iv) gastrocnemius (medial head) oxygenation during post-occlusive reactive hyperaemia in concomitance with SFA FMD (assessed *via* near-infrared spectroscopy; NIRS) and (v) BA FMD. Subsequently, the participant sat for 2 hours on a comfortable armchair while consuming (within 10 min) the high- or low-flavanol cocoa beverage (ran-

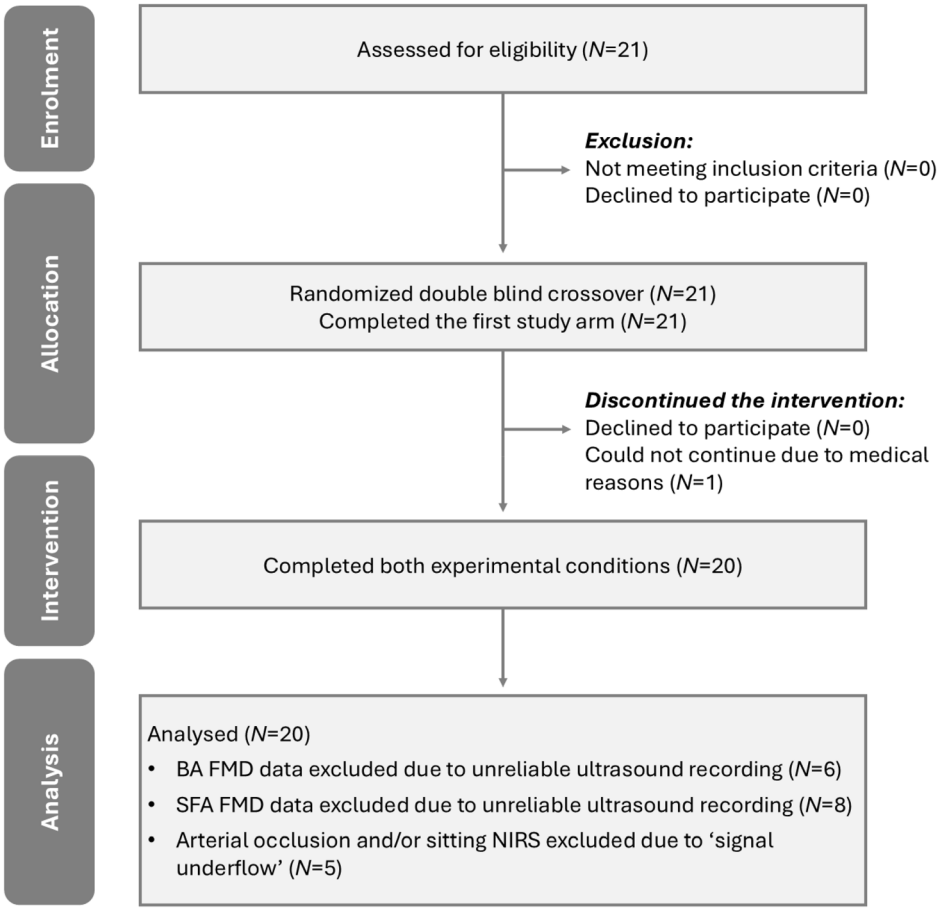

Fig. 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram for the intervention study. BA, brachial artery; FMD, flow-mediated dilation; NIRS, near-infrared spectroscopy; SFA, superficial femoral artery.

domly assigned, counterbalanced and double-blinded). In addition, medial gastrocnemius oxygenation (assessed *via* NIRS) was taken during the sitting trial (Fig. 2).

While sitting, the participants were asked to keep their lower limbs parallel and bent at approx. 90° with both feet in a neutral position with the plantar surface on the floor, while having the trunk resting on the backrest. Participants were only permitted to move their arms/hands to do light activities such as computer typing. Two hours after the commencement of the sitting/flavanol intervention, the aforementioned primary and secondary outcome measures were reassessed (Fig. 2). At the end of each experimental visit, participants were asked to complete a Feasibility Questionnaire to assess their comfort level during each FMD protocol. Experimental visits were performed in a quiet, darkened and temperature-controlled laboratory (22–24 °C), as recommended in guidelines for FMD procedures.[54,55] The study adopted a 2-hour sitting period, as this duration is adequate to induce measurable declines in FMD following prolonged sitting[21] and coincides with the peak bioavailability of flavanol metabolites in circulation, which generally occurs around 2 hours post-ingestion.[46] Our investigation focused on SFA FMD as the designated primary outcome measure. Complementary secondary outcome measures included: BA FMD, BP, heart rate, and NIRS-related measures.

Habitual diet and physical activity

Habitual diet and physical activity were assessed according to the following procedure: briefly, participants were asked to wear a wrist-worn tri-axial accelerometer (GENEActiv, version 1.1, ActivInsights Ltd, Kimbolton, England) for 7 consecutive days and to complete a Food Frequency Questionnaire where they were asked to recall their diet in the previous year. Physical activity data were collected at a frequency of 85.7 Hz, converted into a 60-second epoch and analysed using the GENEActiv software (GENEActiv, version 3.3, ActivInsights Ltd, Kimbolton, England). Habitual diet was assessed using the European Prospective Investigation into Diet and Cancer (EPIC) Norfolk Food Frequency Questionnaire (FFQ),[56] which comprises 131 food products to select the intake frequency on a 9-point scale. Nutrient and food group data was estimated using the FFQ EPIC Tool for Analysis (FETA):[57] energy (kcal), fat (g), saturated fat (g), carbohydrate (g), sugars (g), fibre (g), protein (g), total flavonoids (mg), and portions of fruit and vegetables (calculated as 1 portion corresponding to 80 g, from National Health Service [NHS] guidelines[58]) are reported.

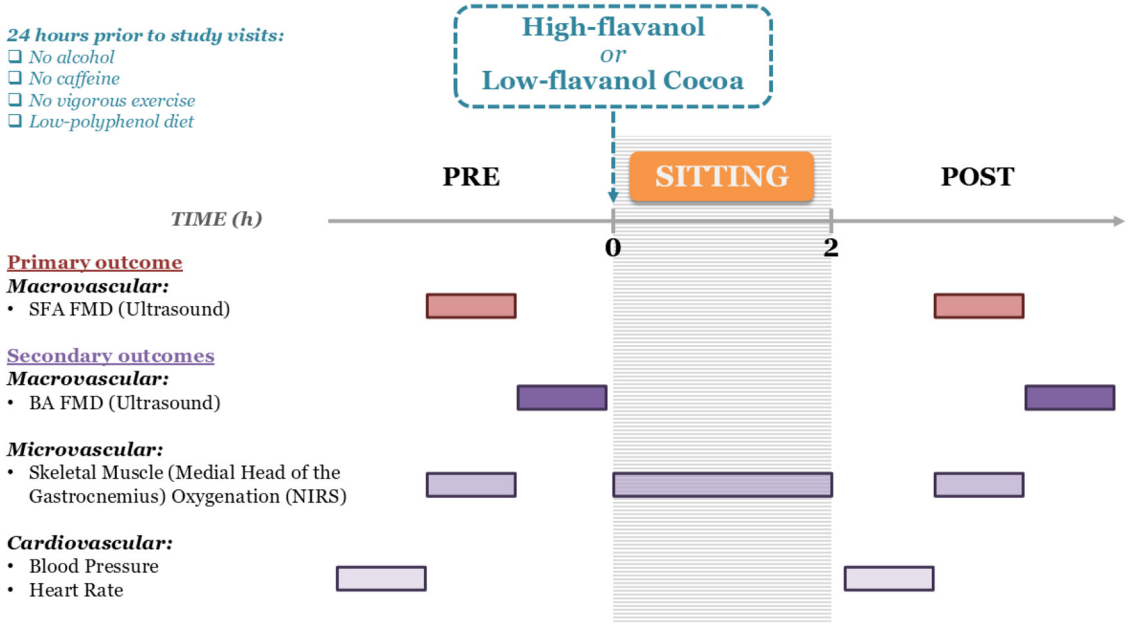

Fig. 2 Experimental study design featuring primary and secondary outcome measures assessed before and after a 2-hour sitting trial. BA, brachial artery; FMD, flow-mediated dilation; NIRS, near-infrared spectroscopy; SFA, superficial femoral artery.

High- and low-flavanol interventions

Cocoa flavanol beverages were prepared as previously described.[49,59] Briefly, 12 g of cocoa powder was dissolved in 350 mL of 'Buxton' still natural mineral water (nitrate: <0.1 mg L^{-1}). The two cocoa powders used are commercially available (Barry Callebaut AG, Zurich, Switzerland). The low-flavanol cocoa powder was a fat-reduced alkalized cocoa powder containing less than 6 mg of (−)-epicatechin, and 5.6 mg of total flavanols per beverage. The high-flavanol cocoa powder was a fat-reduced natural cocoa powder containing 150 mg of (−)-epicatechin, and 695 mg of total flavanols per beverage. Concentrations of macronutrient content and flavonoid compounds are reported in Table 1.

Table 1 Nutritional composition of the high- and low-flavanol cocoa powders (12 g per individual dose)

	Low flavanol	High flavanol
Total polyphenols[a]	260.0	1246.8
Total flavanols (mg)	5.6	695.0
Procyanidins (dimers-decamers; mg)	ND	459.6
(−)-Epicatechin (mg)	<6	150.0
(−) and (+)-Catechin (mg)	<6	85.4
Theobromine (mg)	278.4	262.8
Caffeine (mg)	22.2	27.6
Fat (g)	1.3	1.7
Carbohydrates (g)	1.2	2.7
Protein (g)	2.7	2.7
Fibre (g)	4.0	1.8
Energy (kcal)	36.6	41.4

[a] Concentrations expressed as mg of gallic acid equivalents per 12 g of cocoa powder.

The two cocoa-based flavanol interventions were matched for all micro- and macronutrients, including methylxanthines (caffeine and theobromine). Cocoa powder concentrations for flavanol monomers, procyanidins, and methylxanthines were measured by high-performance liquid chromatography, as described in previous studies.[60,61] Total polyphenol concentrations were estimated by a Folin–Ciocalteu reagent calorimetric assay, as described previously.[62] The dose of flavanol monomers used in the present study was chosen based on previous research demonstrating its effectiveness in modulating endothelial function and is consistent with that used in our prior studies.[49,51,59] To ensure double-blindness, cocoa beverages were indistinguishable in texture, aroma, and taste, and they were provided in an opaque container (covered on top) with a dark-coloured opaque straw. Cocoa interventions were unblinded after completion of all data analyses.

Flow-mediated dilation of the brachial and superficial femoral arteries

Endothelial-dependent vasodilation of the SFA and BA was assessed using established FMD protocol guidelines.[55] Briefly, artery diameter and blood velocity of the SFA and BA were non-invasively assessed by means of a high-resolution duplex ultrasound device (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA) with a linear array transducer (4.5 MHz) (Terason 15L4 Smart Mark, Teratech Corporation, Burlington, MA, USA) attached to an adjustable stereotactic probe-holding tool (FMD-probe-holder-xyz, Quipu S.r.l., Pisa, Italy). The pulse-wave Doppler signal was corrected at an insonation angle of 60°. The right SFA was located and scanned longitudinally 10–20 cm distal to the inguinal crease. A manual blood

pressure cuff was wrapped around the distal end of the right thigh (3–4 cm proximal to the patella), distal to the imaged artery. The right BA was located and scanned longitudinally between 5 and 10 cm proximal to the antecubital fossa. A manual blood pressure cuff was wrapped around the right forearm (~2 cm distal to the antecubital fossa), distal to the imaged artery. Artery diameter and blood velocity were continuously recorded for 1 min (baseline), 5 min during which the cuff was inflated and maintained at a pressure of 220 mm Hg, and 5 minutes following the rapid cuff deflation. The total duration of the FMD protocol was 11 min. All the FMD protocols were performed by a trained and experienced PhD student (AD; first author), with inter-day coefficients of variation (CV) for arterial diameter (mm) of 2.2% and 2.8% for BA and SFA, respectively, and for FMD (%), 10.9% and 8.3% for BA and SFA, respectively, in healthy young adults.

Measurements of arterial diameter and blood velocity were analysed offline by means of an automated edge-detection software (Cardiovascular Suite, version 3.4.0, Quipu S.r.l., Pisa, Italy). All video recordings were analysed by the same researcher (AD) who performed the FMD measurements. The blood velocity calculated by the software (*i.e.*, time-averaged maximum velocity) was adjusted to reflect time-averaged mean velocity as described in previous research,[63,64] based on the following formula: Time-averaged mean velocity = time-averaged maximum velocity/2. The resulting blood velocity was used to calculate blood flow using the following formula: Blood flow = [blood velocity × π (baseline diameter/2)^2] × 60. Baseline and peak diameters were calculated as the average diameter recorded during the minute preceding cuff inflation and the largest diameter observed following cuff deflation, respectively. Both parameters were then used to calculate the percent change in arterial diameter following the formula: FMD (%) = [(peak diameter − baseline diameter)/baseline diameter] × 100. In addition to the classic FMD (%) calculation, allometric scaling of FMD as proposed by Atkinson and Batterham[65] is also presented, in accordance with their previously published guidelines.[66] Shear rate, an adequate surrogate measure of shear stress,[67] was calculated as follows: 4 × baseline blood velocity/baseline diameter.

FMD data quality and exclusion criteria. FMD ultrasound recordings and analyses were evaluated using a set of predefined quality criteria, applied in the following order: (i) a clear baseline image with full visualization of the artery and stable region of interest (ROI) tracking; (ii) successful reacquisition of the artery image within 15 seconds following cuff deflation; (iii) reliable ROI detection of the intima layer of both arterial walls and stable tracking for at least 90 seconds post-deflation; (iv) consistent intima-to-intima diameter measurement during baseline and post-deflation phases. All the decisions were performed by the same investigator (AD) blinded to experimental condition and time point. Any more problematic cases were discussed with a second investigator (CR; corresponding author). The number of excluded recordings for each condition and time point is described in a detailed CONSORT flow diagram (SI, Fig. S1).

Comfort levels during FMD. Participants were asked to complete a Feasibility Questionnaire to assess their comfort level during the FMD procedure. This questionnaire comprises two closed-ended questions that ask participants to rate their perceived comfort level during SFA and BA FMD protocols on a scale from 1 to 5 (1-very uncomfortable, 2-uncomfortable, 3-neither uncomfortable nor comfortable, 4-comfortable, and 5-very comfortable) (data not shown).

Muscle microvasculature haemodynamics

Skeletal muscle microvascular haemodynamics were assessed in the leg using a NIRS device (NIRO-200NX, Hamamatsu Photonics KK, Shizuoka, Japan) during: (i) the 2-hour sitting trial, and (ii) the SFA FMD protocol, according to the following procedure: briefly, the NIRS probe was placed on the right medial gastrocnemius and covered with an opaque cover to block the external ambient light. Tissue oxygenation index (TOI) and normalised tissue haemoglobin index (nTHI) were the outcome measures used, derived *via* the spatially resolved spectroscopy (SRS) method.[68]

Reactive hyperaemia haemodynamics during SFA occlusion. Data of TOI (%) were filtered using a 5-second moving average in order to minimise the influence of artefacts. All TOI variables (*i.e.*, baseline, minimum, maximum, ΔTOI, overshoot, reperfusion magnitude, desaturation slope, and recovery slope), also depicted in Fig. 3, were determined as detailed below.

Sitting trial. Both TOI and nTHI were continuously measured during the 2-hour sitting trial. For analysis purposes, the following time points were considered: 0 min (start), 10 min, 60 min, and 120 min (end). For each time point, data were averaged over a 60-second period.

Blood pressure

Blood pressure was measured in the left upper arm in supine position (after more than 10 min of supine resting) using an automatic blood pressure (BP) monitor (Omron M3

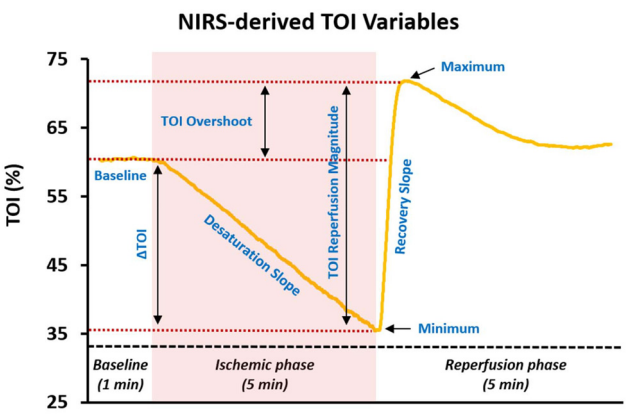

Fig. 3 Physiological response curve of TOI (%) for a representative participant measured during SFA FMD, including the NIRS-related variables. TOI, tissue oxygenation index.

[HEM-7131-E], OMRON HEALTHCARE Co., Ltd, Kyoto, Japan) to obtain systolic BP, diastolic BP, and heart rate. Within each time point, BP measurements were taken three consecutive times, which were then averaged.

Statistical analysis

All statistical analyses were performed using statistical software IBM SPSS Statistics for Windows, version 29 (IBM Corp., Armonk, New York, USA). Two-way repeated measures ANOVA with sitting time (0; 2 h) and dietary intervention (low flavanol; high flavanol) as within-subject variables was performed to analyse changes in SFA and BA FMD, arterial diameter, shear rate, blood flow, blood pressure and NIRS-related haemodynamic measures. To assess the impact of medication use for SFA FMD (N = 8, on medication; N = 4, free of medication) and BA FMD (N = 6, on medication; N = 8, free of medication), medication use was also added as a between-subjects factor in the analysis. Additionally, as a separate exploratory analysis, sedentary activity volume and vigorous activity volume (only habitual activity variables that had a considerable spread across the sample) were included as covariates in the model, to assess whether habitual levels of physical activity/sedentarism modulated the impact of flavanols on endothelial function during sitting. TOI and nTHI during sitting were analysed by a two-way repeated measures ANOVA with sitting time (0, 10, 60, 120 min) and dietary intervention (low flavanol; high flavanol) as within-subject variables. Paired-samples t-tests were used to assess differences between flavanol interventions (low flavanol; high flavanol) at baseline and to determine differences in comfort level ratings (data not shown) between arteries (SFA; BA) across the two experimental visits, and flavanol interventions (low flavanol; high flavanol). Normality of residuals was assessed using Shapiro–Wilk tests and Q–Q plots. For some outcome measures, the Shapiro–Wilk test indicated significant deviations from normality ($p < 0.05$), although Q–Q plots showed only mild departures and no substantial skew. To address this, data were normalised (Shapiro–Wilk test: $p > 0.05$) and statistical analysis in the normalised data confirmed previous statistical results in the raw data, confirming the ANOVA's robustness to moderate violations of normality.[69,70] Sample size estimation was performed using G*Power (version 3.1.9.3) and was based on acute changes in SFA FMD following the same dose of flavanols during sitting in 40 young healthy males.[51] This power analysis estimated that 20 participants were needed to determine a significant difference, of at least 1% FMD (SD = 1.2%), in SFA FMD between high and low flavanol post-sitting ($p < 0.05$; power of 95%, dz = 0.867). The data in the tables and figures are presented as mean ± SD. A p-value less than 0.05 was considered statistically significant. Non-usable data for BA FMD (N = 6/20) and SFA FMD (N = 8/20) were due to the inability to recover a reliable/stable B-mode image post-occlusion in some of the time points. For NIRS during SFA occlusion, the non-usable data (N = 5/20) was due to data files presenting high-amplitude, high-frequency artefacts and also included occasions in which 'signal underflow' was detected by the NIRS device, reflecting poor signal quality.

Results

Study participants

Participants' anthropometric characteristics, sedentary time, and physical activity levels are summarised in Table 2. All participants were older adults (age, 72.4 ± 5.0 years; N = 20) who were apparently healthy, with a healthy body mass index (BMI) (24.4 ± 3.6 kg m^{-2}). Seven-day averaged accelerometry data revealed that participants spent an average of 1.8 ± 0.6 hours per day engaging in light physical activity, and had a light physical activity volume of 263.6 ± 95.3 MET min. All participants recorded an average of more than 10 000 steps per day. Participants' medication use reflects that of the general older UK population, including medications for cardiovascular risk management, such as statins (N = 4), as well as treatments for hypothyroidism (*e.g.*, levothyroxine; N = 3), dyspepsia (*e.g.*, omeprazole; N = 1), and osteoporosis (*e.g.*, alendronic acid; N = 1).

Habitual dietary intake

Estimates of participants' daily dietary intake of macronutrients, total flavonoids and portions of fruits and vegetables are displayed in Table 3. The percentage of individuals who exceed or fail to meet the daily dietary recommendations (based on NHS guidelines[58,72]) are also summarised (Table 3). Participants consumed on average 863 mg per day of flavonoids, and 7.4 portions per day of fruit and vegetables. Further breakdown of flavonoid intake revealed that participants consumed approximately 154.9 mg per day of total flavanols, 54.0 mg per day of proanthocyanidin monomers, and 106.6 mg per day of polymers—with a degree of polymerisation of 2–10. Finally, participants consumed on average 60.8 g of fat, 97.1 g of sugars, and 17.9 g of fibre daily, which were

Table 2 Participants' baseline characteristics, sedentary time, and physical activity

	All participants
Anthropometric characteristics	
N	20
Age (year)	72.4 ± 5.0
Height (m)	1.67 ± 0.10
Weight (kg)	68.7 ± 15.6
BMI (kg m^{-2})	24.4 ± 3.6
Daily physical activity pattern	
N	19
Sedentary activity time (h)	9.8 ± 1.5
Light activity time (h)	1.8 ± 0.6
Moderate activity time (h)	2.5 ± 1.1
Vigorous activity time (h)	0.0 ± 0.1
Sedentary activity volume (estimated as MET min)	747.1 ± 138.7
Light activity volume (estimated as MET min)	263.6 ± 95.3
Moderate activity volume (estimated as MET min)	533.7 ± 243.6
Vigorous activity volume (estimated as MET min)	25.2 ± 35.3
Step count	10 651.8 ± 3 883.6

Recommendations for older adults (aerobic physical activity, per week) – at least 150–300 min of moderate intensity, or at least 75–150 min of vigorous intensity.[71] Data are presented as mean ± SD. BMI, body mass index; MET, metabolic equivalent of task.

Table 3 Estimated daily dietary intake of key nutrients, food sources and bioactives

	Sample average	% of participants over/ under the recommended daily intake
Energy (kcal)	1530.4 ± 439.8	N/A
Fat (g)	60.8 ± 23.8	31.6% over
Saturated fat (g)	21.2 ± 11.4	21.1% over
Carbohydrate (g)	175.3 ± 55.7	N/A
Sugars (g)	97.1 ± 44.1	100.0% over
Fibre (g)	17.9 ± 6.7	89.5% under
Protein (g)	70.7 ± 23.5	N/A
Total flavonoids (mg)	863.3 ± 430.1	N/A
Fruit and vegetables (g)	592.7 ± 328.6	N/A
Fruit and vegetables (portion)	7.4 ± 4.1	21.1% under

Recommendations for adults – fat: <70 g per day, saturated fat: <30 g per day (male) or <20 g per day (female), sugar: <30 g per day, fibre: >30 g per day, fruit and vegetables: >5 portions per day. *1 portion = 80 g (NHS guidelines[58]). Data are presented as mean ± SD (N = 19).

31.6% over, 100.0% over, and 89.5% under the recommended daily intake of fat, sugars, and fibre, respectively.

Vascular and haemodynamic measures

A summary of baseline (pre-intervention) resting vascular parameters—including arterial diameter, shear rate, FMD, blood pressure, and heart rate—during the low- and high-flavanol interventions is presented in Table 4. No significant differences were observed between the flavanol interventions for any parameter.

Resting blood flow and arterial diameter

Resting anterograde blood flow and arterial diameter of the BA and SFA are displayed in Fig. 4. In the BA, neither sitting nor flavanol intervention had an effect on anterograde blood flow (Fig. 4A). In the SFA, sitting resulted in a similar decline in anterograde blood flow for both flavanol interventions (p = 0.001; Fig. 4B). No differences in retrograde blood flow were detected in both arteries due to sitting or flavanol intervention (data not shown). Sitting had no impact on arterial diameter in the BA (Fig. 4C). In the SFA, however, sitting increased arterial diameter, but only following consumption of the low-flavanol cocoa (p = 0.003; Fig. 4D).

Flow-mediated dilation

Fig. 5 shows BA and SFA FMD as well as allometrically scaled FMD before and after 2 hours of sitting following either a low- or high-flavanol acute intervention.

As shown in Fig. 5, there was a significant interaction effect for both BA and SFA FMD responses ($p \leq 0.015$). Specifically, while sitting resulted in a decline in BA FMD (Δ = −0.7 ± 1.4%; $p < 0.05$; Fig. 5A) and SFA FMD (Δ = −0.7 ± 1.2%; $p < 0.01$; Fig. 5B) following consumption of the low-flavanol cocoa, after the high-flavanol cocoa there were no detectable changes in either the BA (Δ = 0.1 ± 1.3%; p = 0.375) or SFA (Δ = 0.0 ± 1.1%; p = 0.593) FMD response. After sitting, there was a higher FMD following the high-flavanol compared to the low-flavanol in the BA ($p < 0.001$), but not in the SFA (p = 0.164, η_p^2 = 0.168). No significant differences between the flavanol interventions were detected at baseline in both the BA and SFA.

Allometrically scaled BA FMD (Fig. 5C) outcomes showed the same pattern, with sitting-induced reductions following ingestion of the low-flavanol cocoa ($p < 0.005$), and no changes were observed in response to high-flavanol intake (p = 0.507). Higher FMD was reported after sitting following the high-flavanol compared to the low-flavanol cocoa ($p < 0.001$). For the SFA FMD (Fig. 5D), a significant effect of sitting was observed (p = 0.004), and a trend towards an interaction with flavanol intake (p = 0.055, η_p^2 = 0.090). Further *post hoc* analyses suggest a significant decline in SFA FMD only in the low-flavanol condition ($p < 0.001$), whilst FMD is maintained after

Table 4 Baseline vascular and haemodynamic characteristics of participants

	N	Low flavanol	High flavanol	p-Value
Brachial Artery				
Artery diameter (mm)	20	3.9 ± 0.7	3.9 ± 0.6	0.525
Anterograde shear rate (s^{-1})	20	92.0 ± 29.1	96.4 ± 34.9	0.540
Retrograde shear rate (s^{-1})	20	−28.7 ± 23.4	−28.8 ± 22.5	0.998
Anterograde blood flow (mL min^{-1})	20	31.9 ± 12.6	32.9 ± 14.9	0.530
Retrograde blood flow (mL min^{-1})	20	−9.6 ± 6.7	−10.1 ± 8.9	0.667
FMD (%)	15	3.4 ± 1.3	3.5 ± 1.4	0.258
Superficial femoral artery				
Artery diameter (mm)	19	6.5 ± 1.4	6.5 ± 1.6	0.415
Anterograde shear rate (s^{-1})	19	61.4 ± 24.9	62.5 ± 21.7	0.918
Retrograde shear rate (s^{-1})	19	−37.6 ± 20.6	−38.7 ± 21.1	0.798
Anterograde blood flow (mL min^{-1})	19	95.7 ± 42.8	95.2 ± 38.0	0.690
Retrograde blood flow (mL min^{-1})	19	−51.4 ± 20.2	−55.9 ± 23.5	0.183
FMD (%)	14	2.0 ± 1.2	1.7 ± 1.2	0.144
Blood pressure/heart rate				
Systolic BP (mm Hg)	20	128.8 ± 14.1	128.0 ± 14.1	0.798
Diastolic BP (mm Hg)	20	75.0 ± 7.4	74.0 ± 7.1	0.426
Heart rate (bpm)	20	62 ± 12	61 ± 12	0.535

Data are presented as mean ± SD. BP, blood pressure; FMD, flow-mediated dilation. Data analysed *via* paired-samples t-tests.

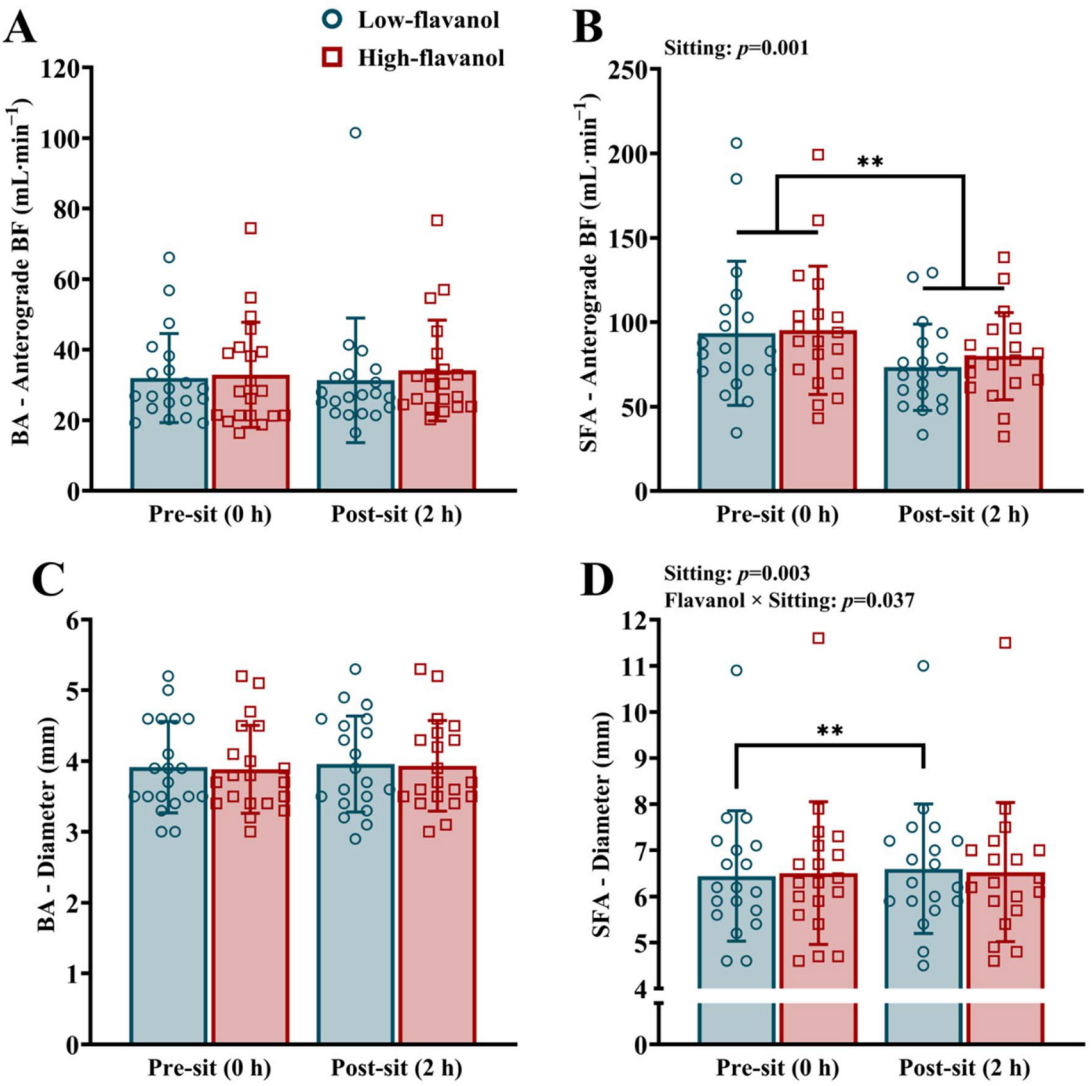

Fig. 4 Baseline anterograde blood flow and arterial diameter of the brachial (A and C) (N = 20) and superficial femoral arteries (B and D) (N = 19), before (0 h) and after 2 hours of sitting, following either a low- or high-flavanol intervention. Data are presented as mean ± SD. A significant main effect of sitting was observed in SFA anterograde BF (B) (p = 0.001), and SFA diameter (D) (p = 0.003); a significant interaction between flavanol intake and sitting time is shown in SFA diameter (D) (p = 0.037). **Denotes a significant difference (p < 0.01) between pre- and post-sitting. BA, brachial artery; BF, blood flow; SFA, superficial femoral artery. Two-way repeated measures ANOVA conducted. Bonferroni *post hoc* for significant interactions.

high-flavanol intake (p = 0.42). No differences between the flavanol interventions were detected at baseline in both the BA and SFA allometrically scaled FMD measures (both p > 0.05).

Participant habitual medication did not significantly affect the impact of sitting or flavanol intervention on endothelial function measures in the BA or SFA. However, individuals on medication had an overall lower BA FMD (p = 0.019). Additionally, sedentary activity volume or vigorous activity volume was included as a covariate in the statistical analysis, with no significant effects of the covariates observed for either BA FMD or SFA FMD, suggesting that habitual levels of vigorous physical activity/sedentarism does not affect benefits of flavanol interventions.

Leg muscle oxygenation haemodynamics during SFA occlusion

Microvascular tissue oxygenation in the medial gastrocnemius during rest, SFA occlusion (ischaemic period), and post-occlusion reactive hyperaemia are depicted in Fig. 6A. Parameters describing the microvascular haemodynamic response (as represented in Fig. 3) were estimated before and after sitting, following either a low- or high-flavanol intervention, and are summarised in Table 5.

During the reperfusion period (after the release of the cuff), sitting induced a reduction in reperfusion magnitude (minimum–maximum) (p = 0.006; Fig. 6B), with no significant effect on recovery slope (Fig. 6C), and overshoot (baseline–

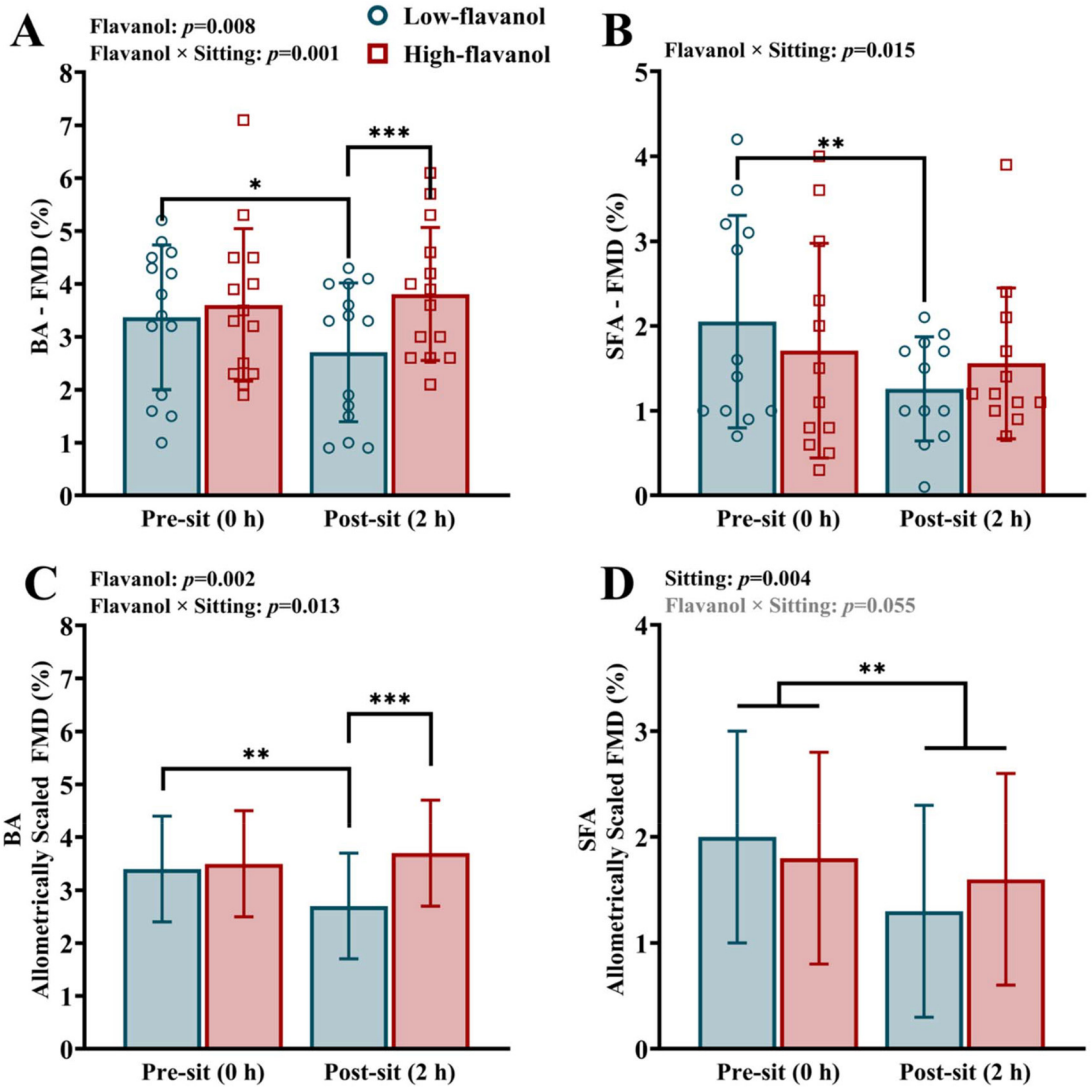

Fig. 5 Endothelial function, measured as flow-mediated dilation (FMD, %) of the brachial (A) (N = 14) and superficial femoral artery (B) (N = 12), and allometrically scaled FMD of the brachial (C) (N = 14) and superficial femoral artery (D) (N = 12), before (0 h) and after 2 hours of sitting, following either a low- or high-flavanol intervention. Data are presented as mean ± SD. Significant main effects were observed for flavanol in BA FMD (A) (p = 0.008) and BA allometrically scaled FMD (C) (p = 0.002), and for sitting in SFA allometrically scaled FMD (D) (p = 0.004); a significant interaction between flavanol intake and sitting time is shown in BA FMD (A) (p = 0.001), SFA FMD (B) (p = 0.015), and BA allometrically scaled FMD (C) (p = 0.013). ***Denotes significant difference (p < 0.001) between the flavanol interventions post-sitting. **Denotes a significant difference (p < 0.01) between pre- and post-sitting. *Denotes a significant difference (p < 0.05) between pre- and post-sitting. BA, brachial artery; FMD, flow-mediated dilation; SFA, superficial femoral artery. Two-way repeated measures ANOVA conducted. Bonferroni *post hoc* for significant interactions.

maximum; see Table 5). During the ischaemic period (when the cuff remains inflated), sitting resulted in a reduction in ΔTOI (baseline–minimum) (p < 0.001; Fig. 6D), a slower/reduced desaturation slope (p < 0.001; Fig. 6E), and an increase in minimum oxygenation (p = 0.006; see Table 5). Sitting did not induce any significant change in resting baseline oxygenation (Table 5). The high-flavanol intervention displayed a lower maximum compared to the low-flavanol (p = 0.018), but this difference was present before (0 h) and after sitting (2 h). Consuming flavanols prior to sitting did not affect any haemodynamic responses during the hyperaemic or occlusion phase (Table 5).

Leg muscle oxygenation haemodynamics during sitting

Oxygenation levels (TOI) and total haemoglobin content (nTHI) measured in the medial gastrocnemius during the 2-hour sitting trial are reported in Fig. 7 (A and B, respectively).

Sitting modulated both TOI (p < 0.001; Fig. 7A) and nTHI (p < 0.001; Fig. 7B) over the 2-hour period, regardless of the flavanols intervention. *Post hoc* analysis revealed a decline in TOI after 10 min of sitting (p = 0.035 *vs.* start [0]), whilst there was an increase from 10 min to 60 min (p = 0.002) and 120 min (p = 0.002 *vs.* 10 min). No significant differences were observed between start (0) and 60 min or 120 min. In nTHI, *post hoc*

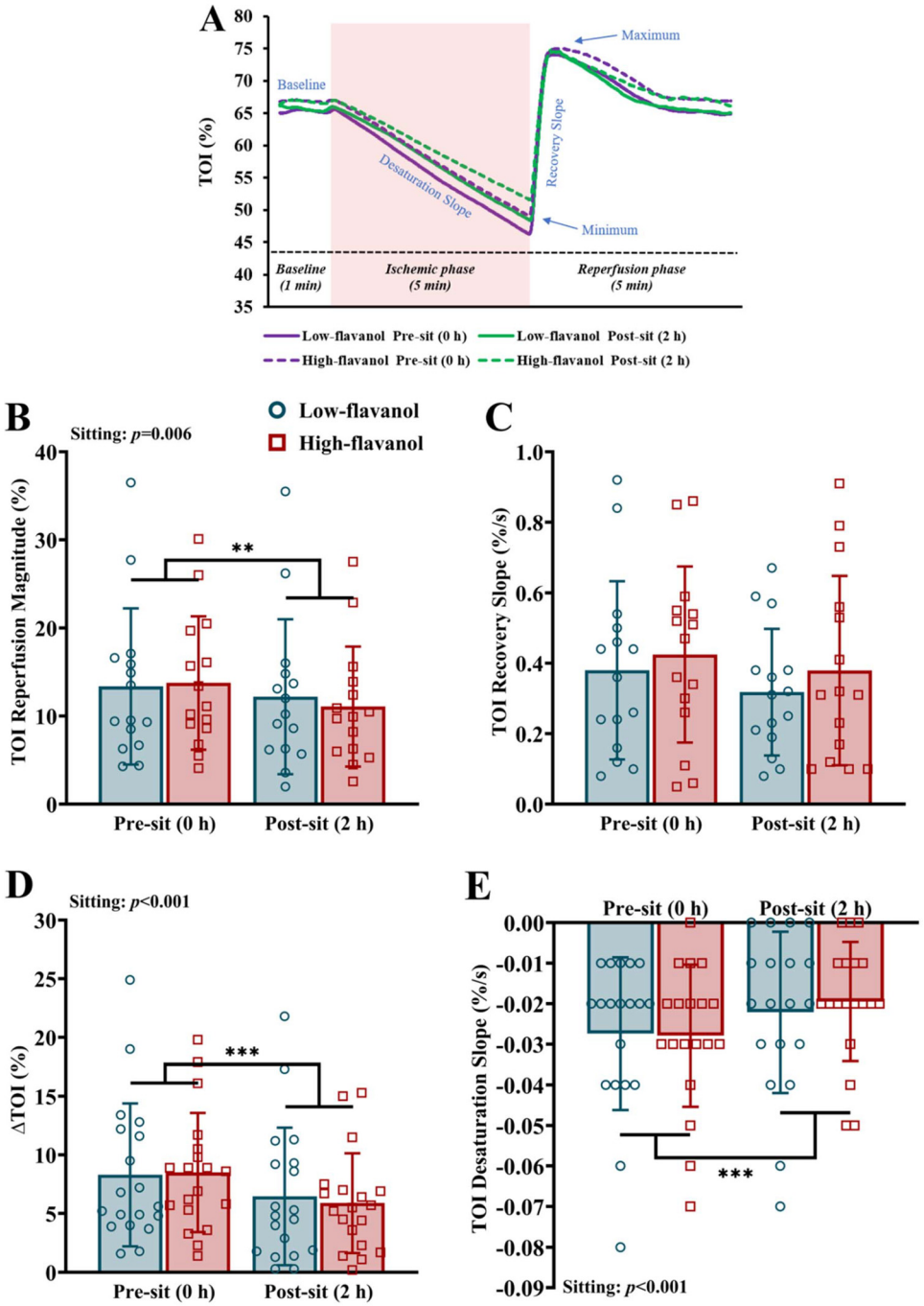

Fig. 6 Physiological response curves of TOI (%) for a representative participant measured during 5-minute SFA occlusion (FMD) (A) before and after the 2-hour sitting protocol following either a low- or high-flavanol intervention. This is followed by microvascular function measures of TOI in the medial gastrocnemius during SFA FMD. (B) TOI reperfusion magnitude (%) (minimum−maximum) (N = 15); (C) TOI recovery slope (% s^{-1}) (N = 15); (D) ΔTOI (%) (baseline−minimum) (N = 19); (E) TOI desaturation slope (% s^{-1}) (N = 19). Data are presented as mean ± SD. A significant main effect of sitting was observed in TOI reperfusion magnitude (B) (p = 0.006), ΔTOI (D) (p < 0.001), and TOI desaturation slope (E) (p < 0.001). ***Denotes significant difference (p < 0.001) between pre- and post-sitting. **Denotes significant difference (p < 0.01) between pre- and post-sitting. TOI, tissue oxygenation index. Two-way repeated measures ANOVA conducted.

analysis revealed increases from the start (0) at 10 min (p = 0.001), 60 min (p < 0.001), and at 120 min (p < 0.001). In addition, for TOI only, there was a main effect of flavanol intervention (p = 0.002), with the high-flavanol intervention displaying lower TOI during the sitting trial as compared to the low-flavanol.

Table 5 Measures of tissue oxygenation index in the medial gastrocnemius during FMD in the superficial femoral artery

		Low Flavanol		High Flavanol		
TOI Variable	N	Pre	Post	Pre	Post	Effect
Baseline (%)	20	70.2 ± 5.1	70.3 ± 4.8	69.5 ± 4.3	69.1 ± 4.9	NS
Minimum (%)	19	61.5 ± 9.5	63.6 ± 9.5	60.7 ± 8.0	62.9 ± 7.2	Sitting (p = 0.006)
Maximum (%)	15	73.4 ± 4.1	74.0 ± 3.5	72.5 ± 4.1	72.3 ± 3.2	Flavanol (p = 0.018)
ΔTOI (%)	19	8.3 ± 6.1	6.5 ± 5.8	8.5 ± 5.1	5.9 ± 4.2	Sitting (p < 0.001)
Overshoot (%)	15	3.9 ± 3.0	4.5 ± 3.3	3.8 ± 3.3	4.2 ± 3.4	NS
Reperfusion magnitude (%)	15	13.4 ± 8.9	12.2 ± 8.8	13.8 ± 7.6	11.1 ± 6.8	Sitting (p = 0.006)
Desaturation slope (% s^{-1})	19	−0.03 ± 0.02	−0.02 ± 0.02	−0.03 ± 0.02	−0.02 ± 0.01	Sitting (p < 0.001)
Recovery slope (% s^{-1})	15	0.38 ± 0.25	0.32 ± 0.18	0.43 ± 0.25	0.38 ± 0.27	NS

Data are presented as mean ± SD. TOI, tissue oxygenation index. Two-way repeated measures ANOVA conducted.

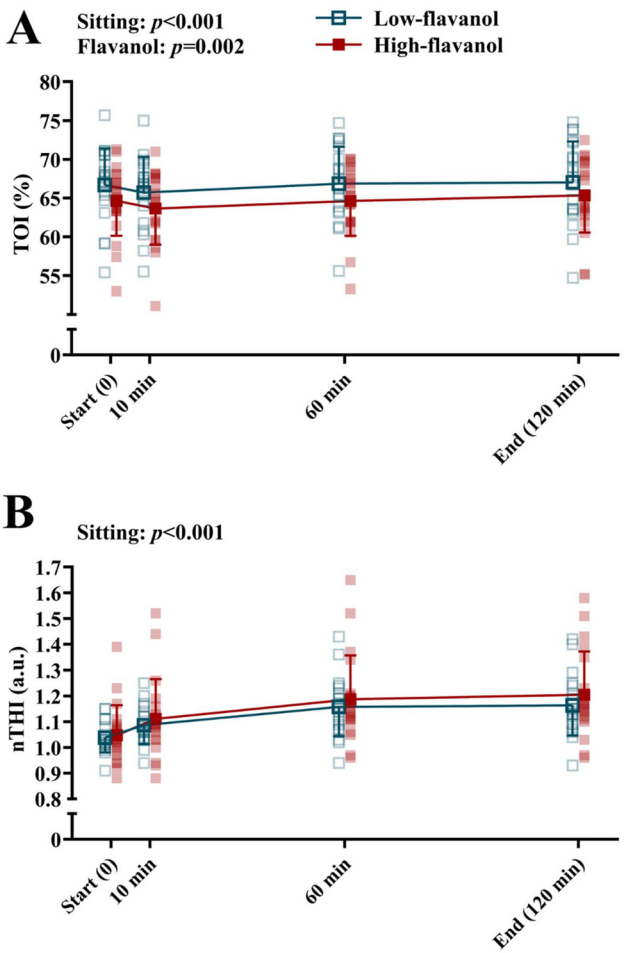

Fig. 7 Microvascular function measures in the medial gastrocnemius during the sitting trial. (A) TOI (%) (N = 20); (B) nTHI (a.u.) (N = 20). Data are presented as mean ± SD. Significant main effects were observed for flavanol in TOI (A) (p = 0.002), and for sitting in TOI (A) (p < 0.001) and nTHI (B) (p < 0.001). nTHI, normalised tissue haemoglobin index; TOI, tissue oxygenation index. Two-way repeated measures ANOVA conducted.

Blood pressure and heart rate

Sitting resulted in an increase in both systolic (low-flavanol: Δ = 5.2 ± 7.4 mm Hg; high-flavanol: Δ = 7.0 ± 10.1 mm Hg; p = 0.001; Fig. 8A) and diastolic BP (low-flavanol: Δ = 1.8 ± 3.5 mm Hg; high-flavanol: Δ = 3.5 ± 5.0 mm Hg; p = 0.001; Fig. 8B), and a decline in heart rate (low-flavanol: Δ = −5 ± 5 bpm; high-flavanol: Δ = −4 ± 4 bpm; p < 0.001; Fig. 8C). For both BP and HR, these changes were similar following both flavanol interventions (interaction effects: p > 0.05).

Discussion

In the current study, we showed for the first time that cocoa flavanols prevented sitting-induced declines in upper- and lower-limb FMD without affecting the microvasculature and blood pressure in apparently healthy, older adults. We further showed that 2 hours of uninterrupted sitting induced detrimental declines in BA and SFA FMD and reduced resting anterograde blood flow. Muscle microvascular reperfusion magnitude and rate of oxygen consumption (desaturation slope) were also impaired in the lower limbs, following sitting. Furthermore, the sitting intervention resulted in increases in both systolic and diastolic BP and declines in heart rate. This is the first study to suggest that dietary flavonoids may be protective in healthy older adults during prolonged sitting.

Impact of sitting on the macro and microvasculature

In the present study, we observed for the first time sitting-induced declines in endothelial function in the BA (0.7% FMD) in healthy older adults, in addition to impairments in the SFA (0.7% FMD). One previous study in late middle-aged overweight/obese adults reported no changes in BA and a larger sitting effect in SFA (1.5%) FMD.[22] The sample in the current study, was relatively healthy, which may contribute to these differences: for example, our sample had a healthier BMI (24.4 kg m^{-2}), was highly active and had a relatively heathy diet (>7 F&V portions per day). The BA FMD values of 0.7% are moderately close to the clinically relevant threshold of 1%, which has been found to be inversely associated with a risk of a future CVD event of at least 9%.[73] This may have clinical significance in this particular population, given that older adults can experience several bouts of prolonged sitting per day.[3,4] To date, only a limited number of other sitting studies have been conducted in older adults; specifically, with type 2 diabetes[23]

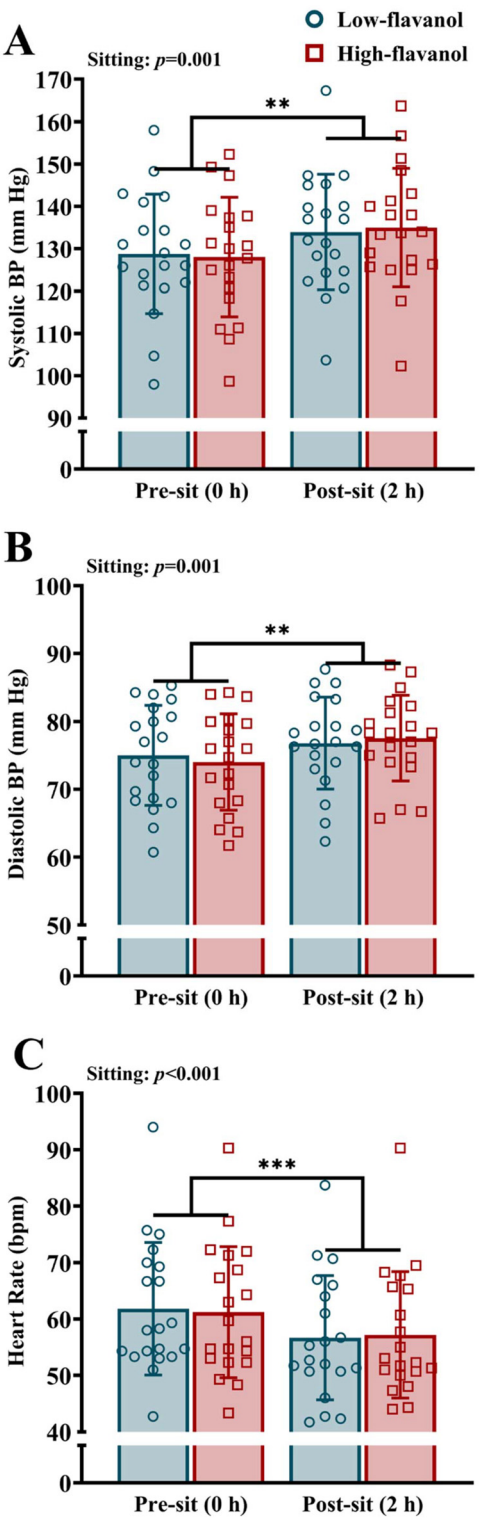

Fig. 8 Systolic (A) (N = 20) and diastolic blood pressure (B) (N = 20), and heart rate (C) (N = 20), before (0 h) and after 2 hours of sitting, following either a low- or high-flavanol intervention. Data are presented as mean ± SD. A significant main effect of sitting was observed in systolic BP (A) (p = 0.001), diastolic BP (B) (p = 0.001), and heart rate (C) (p < 0.001). ***Denotes a significant difference (p < 0.001) between pre- and post-sitting. **Denotes a significant difference (p < 0.01) between pre- and post-sitting. BP, blood pressure. Two-way repeated measures ANOVA conducted.

and increased cardiovascular risk.[27] As such our study adds to the body of evidence in a relatively healthy sample.

There were also reductions in anterograde blood flow and shear rate in SFA following the 2-hour sitting trial, with no significant changes in retrograde shear rate/blood flow. This has been extensively shown in young adults[21,74–77] but the data in older adults is more inconsistent, with some studies reporting either no significant changes,[23] increases[22] or decreases[27] in shear rate and/or blood flow in the SFA. This may be a reflection of a more heterogeneous population in comparison to a young group. Furthermore, only one other study assessed the retrograde component of blood flow,[27] whose modulation can be associated with endothelial dysfunction outcomes.[78] Similar to the current study, no changes in retrograde blood flow/shear rate were observed in upper and lower limbs after sitting, indicating that this is unlikely to be driving the negative effects of sitting on endothelial function. It is widely recognised that shear rate/stress plays a substantial role in the regulation of the vascular response in both conduit arteries and microvessels,[67,79] as it initiates a cascade that leads to increased vasodilation.[80,81] The FMD response, however, is not determined completely by shear rate/stress, as other aspects are involved as potential underlying mechanisms, which include increased hydrostatic pressure, blood viscosity, arterial bending, and modulation of metabolic and vascular factors such as insulin resistance, endothelin-1 (ET-1), and nitric oxide (NO) (as reviewed in Daniele *et al.*[17]).

The current study shows, for the first time, that sitting induces a decline in reperfusion magnitude in the lower limb microvasculature during reactive hyperaemia, suggesting impaired microvascular function. This was paralleled by a decline in ΔTOI (baseline–minimum, %) and a higher minimum oxygenation reached during occlusion, indicating reduced oxygen extraction during ischaemia.[82] Furthermore, the TOI desaturation slope was less steep after sitting in both flavanol interventions, indicating reduced muscle oxygen consumption during the occlusion phase of the FMD protocol, which is representative of impaired microcirculation.[83] Importantly, the TOI desaturation slope has been found to be an important predictor of mortality (*i.e.*, 0.70).[83] A recent study has found a negative correlation between TOI desaturation slope (tibial anterior muscle) and SFA FMD (r = −0.509, p = 0.044) in young healthy adults,[84] which is consistent with the observations of the current study.

The literature investigating microvascular function in response to prolonged sitting has mainly focused on younger populations, with little to no research in older populations. Similar to the present study, previous investigations in young adults show that exposure to sitting (2.5–3 hours) affects tissue oxygenation parameters, including reductions in baseline oxygenation and reoxygenation rate and slower/reduced desaturation rate.[85–89] Furthermore, the continuous recording of muscle oxygenation revealed an increase in oxygenation levels (TOI) and total haemoglobin content (nTHI) during sitting. This is generally in contrast with previous studies in young adults, showing typically a decline in TOI.[90,91] Future work is

needed to understand the underlying factors associated with these age differences.

Overall, these findings highlight that uninterrupted sitting impairs upper- and lower-limb macrovascular function, as well as leg microvascular function, given that reactive hyperaemia is a measure of peripheral microvascular function closely associated to cardiovascular risk factors, in healthy and at-risk populations.[92]

Impact of flavanols on the macro and microvasculature during sitting

Cocoa flavanols have shown efficacy in preventing declines in endothelial function in both BA and SFA, with levels post-sitting remaining similar to pre-sitting. The positive effects on the SFA seem less accentuated: this may be partially driven by the limited sample size (N = 12 for the SFA), which was under the sample size estimated (N = 20, Power = 95%). This was due to loss of data as implementing the SFA FMD protocol in this population is more challenging: this may be related to the elevated discomfort experienced during SFA FMD (data not shown). Interestingly, cocoa flavanols enhanced FMD without having an impact on shear rate, suggesting that improvements in endothelial function may be related to other mechanisms. Human studies have shown that flavanols—particularly, (−)-epicatechin—upregulate bioavailability and production of NO,[93] a potent vasodilator that largely mediates the FMD response,[46,94] whilst reducing concentrations of ET-1, a potent vasoconstrictor.[93] Furthermore, flavanols have been recently demonstrated to reduce oxidative stress, proinflammatory mediators, and lipid peroxidation in a dose-dependent manner,[95] which are all aspects that are deleterious to vasculature and particularly important in ageing.

To date, studies investigating acute cocoa flavanol intake in older adults mainly assessed FMD in the BA, with only one study measuring FMD in the lower-limb CFA. These studies typically showed improvements in FMD ranging from 1% to 4.3% in the BA,[34,39,44,47,96] and 2.8–2.9% in the CFA within 1–2 hours after flavanol ingestion.[47] Comparatively, the FMD differences observed between high- and low-flavanol interventions after 2 hours post-sitting in the present study were of smaller magnitude. This may be a consequence of sitting itself or related to differences in the conduit arteries' responsiveness. Importantly, flavanol-induced benefits in vascular function are, in some cases, comparable to other physical activity-based interventions that have been applied in older adults during sitting. For example, breaking prolonged sitting every 30 min (with 2 min light-intensity walking) induces a 1.1% increase in SFA FMD,[27] and resistance activities induce 0.4% to 3.1%[22,23] improvements in SFA FMD in older adults. Interestingly, the current study is the first to show benefits in endothelial function in the BA during sitting, not observed in the context of regular sitting breaks/resistance training activities.[22] As such, flavonoid-based nutritional approaches might be ideally used in combination with physical activity breaks to optimise protection of the vasculature from the deleterious effects of sitting in older adults.

Despite improvements in macrovascular function during sitting, flavanols had no impact on microvascular function (*e.g.*, desaturation slope, reperfusion slope) post-sitting or resting tissue oxygenation during sitting. The only significant differences detected between flavanol treatments during sitting (TOI) and post-sitting reactive hyperaemia (maximum TOI) were present also at baseline (0 h), suggesting a random difference in baseline physiology between visits, rather than an effect of flavanol intake. The lack of modulation of microvascular function by flavanols may indicate that different mechanisms may be at play. Whilst flavanols contribute to enhancements in macrovascular function through NO-mediated mechanisms, other vasoactive compounds seem to be more predominantly involved in microvascular function. For example, there is evidence to suggest that the NO contribution to peak reactive hyperaemia in the forearm and cutaneous microvasculature is limited, with other vasoactive factors, including prostaglandins and endothelium-derived hyperpolarising factors, playing a more prominent role.[97] Indeed, the literature investigating the acute effects of cocoa flavanols on microvascular function is scarce and particularly discrepant in older populations. For example, one study has reported an increase in haemoglobin oxygen saturation and blood flow in cutaneous tissue,[98] whilst other studies have generally reported no changes in microvascular parameters in cutaneous microvasculature, following intake of flavanols.[47,99] Similarly, in young populations, some studies report improvements in measures of the peripheral microcirculation, either cutaneous or muscular,[36,100] whilst others reported no significant effects during reactive hyperaemia in the forearm skeletal muscle.[101] It is possible that these inconsistencies are due to the methods used and location of assessment, so more well-controlled studies are needed to fully establish the potential benefits of cocoa flavanols on peripheral microcirculation. It is also possible that distinct timeframes of exposure to flavanols are needed to achieve benefits within the microvasculature, for example, chronic exposure. More studies are required to establish this.[102] Interestingly, previous studies using physical activity interventions during sitting—*e.g.*, leg fidgeting, foot elliptical exercise, and half squats plus calf raises—showed efficacy in attenuating sitting-induced microvascular dysfunction.[87–89] As such, dietary flavanols may be used in combination with physical-activity interventions in older adults to modulate both macro and microvascular function during sitting.

Blood pressure

We have observed sitting-induced increases in both systolic and diastolic BP, and declines in heart rate, following both flavanol interventions. Although the research investigating the impact of sitting in older populations is limited, these findings are in agreement with what other studies have reported. For example, some studies have shown that prolonged sitting (7–8 hours) significantly increased systolic and diastolic BP in early middle-aged overweight/obese hypertensive adults[103] and late middle-aged/older adults with type 2 diabetes.[24] The

observed elevations in BP from the current study may potentially be of clinical significance, considering the positive associations between high BP and CVD, CVD-related mortality, and all-cause mortality.[104–106] Furthermore, given that our study participants displayed resting systolic BP of approximately 128 mm Hg, the observed sitting-induced increase in systolic BP of >5 mm Hg would move participants towards a higher BP category (from 'normal' to 'high-normal'),[48] and may result in a significantly higher risk of major cardiovascular events and mortality if sitting is persistent.[107,108] Research indicates that cocoa flavanols can ameliorate BP as shown in a recent systematic review and meta-analysis of 91 RCTs reporting positive effects of flavanols in reducing systolic and diastolic BP of −1.46 mm Hg and −0.99 mm Hg, respectively.[41] Several studies have shown improvements in BP among older adults following short-term interventions (*e.g.*,[36,109,110]), however, less research has been conducted following acute interventions. Some experimental studies, but not all,[111] have shown that acute intake of cocoa flavanols reduces BP measures in middle-aged healthy adults and those with type 2 diabetes.[34,47] However, in the present study, acute flavanol intake did not prevent/attenuate sitting-induced increases in BP, suggesting that those benefits may not translate to prolonged sitting contexts in older adults. Further research is needed to establish the link between cocoa flavanols and BP in the context of sitting, particularly following chronic supplementation aimed at counteracting the impact of sedentary lifestyles with diets rich in flavanols.

Lifestyle: habitual diet and physical activity

The habitual diet of the study participants is moderately representative of the UK population. All participants exceeded the recommended daily intake of sugars as compared to 61.3% of the British population.[112] In addition, ~80% of the participants consumed at least 5 portions of fruit and vegetables per day, which is better than the UK average of 28%.[113] Importantly, participants consumed 870 mg per day of flavonoids, which is in line with reports in a recent large cross-sectional study (805.7 mg per day).[114] Given the current dose administered of 695 mg of flavanols, if this was to be maintained when combined with their habitual diet, it would translate into >1500 mg of flavonoids per day. This would have implications for health, given that doses of 1000–1200 mg per day, have been found to be associated with a 14% lower risk of atherosclerotic CVD (as compared to a lower daily intake).[115]

Participants were generally very active: all participants within our sample performed at least 150 minutes per week at moderate intensity, thus meeting the minimum recommendations for physical activity.[71] This is higher than the average prevalence in the UK, where only 37% of older adults, aged 65–74 years, are considered physically active.[116] In addition, participants took more than 10 000 steps per day, which is more than the amount of daily steps that healthy older adults generally perform (*i.e.*, 2000–9000 steps per day),[117] and above the number of steps that has been recently found to be associated with a progressively lower risk of mortality (*i.e.*, 6000–8000 steps per day).[118] However, habitual levels of physical activity or sedentary time do not seem to affect the impact of flavanols on endothelial function during sitting, in agreement with our previous data in young healthy males.[51]

In summary, the benefits of flavanols during sitting were demonstrated in a healthy and physically active group of older adults. Future studies should test the efficacy of dietary flavanols in sedentary older adults, more representative of the older adults in the UK.

Limitations

One limitation of the present study is related to the higher discomfort level associated with SFA FMD (*vs.* BA FMD), in agreement with previous research.[119] This led to worsening the quality of arterial images, especially post-occlusion, leading to higher exclusion of analysis and higher variability in this population (inter-day $CV_{SFA\ FMD}$ = 23.7%). This may have had negative implications for the statistical analysis (*e.g.*, reduced statistical power): based on the current data in older adults (η_p^2 = 0.168), a sample size of N = 12–19 (corresponding to power of 80–95%) is needed to detect a significant difference between high- and low-flavanol interventions 2 hours post sitting. Future studies looking to reduce variability in the SFA FMD should consider applying the cuff around the calf instead of the thigh, which is reported to be more comfortable.[119] Secondly, although this study included both males and females, it was not sufficiently powered to make comparisons between sexes for the primary and secondary outcome measures. Preliminary analysis including sex as a between-subjects factor indicated no significant impact of sex in the effect of flavanols on endothelial function during sitting. A balanced representation of sexes would also improve the generalizability of the results. Another important limitation is the inclusion of only apparently healthy older adults, as no formal clinical screening was performed. Therefore, the findings may not be generalisable to older individuals with existing or undiagnosed cardiovascular conditions or risk factors. Finally, the participants in this study were highly physically active for their age group, engaging in an average of 2.5 hours per day of moderate-intensity physical activity, which exceeds typical activity levels reported in older adults.[120] Therefore, the results may not be representative of more sedentary populations.

Conclusion

To the best of our knowledge, the current study is the first to investigate the acute impact of dietary flavanols on macro and microvascular function in response to 2 hours of uninterrupted prolonged sitting in apparently healthy, older adults. Findings showed that sitting induced adverse effects on BA and SFA FMD, resting physiological measures in the SFA, and measures of peripheral muscle oxygenation and BP. Consuming cocoa flavanols at the onset of sitting was effective at preventing sitting-induced declines in FMD across upper- and lower-limb conduit arteries, without affecting microvascu-

lar function and BP. Given that sedentary time is highly prevalent among older populations, and may increase their risk of CVD-related conditions, consuming flavanol-rich foods (*e.g.*, cocoa processed to minimize loss of flavanols, green tea, apples, berries) when sedentary may be an effective strategy to be used alone or in conjunction with other approaches (*e.g.*, regular walking breaks, intermittent leg fidgeting) to limit the detrimental effects of inactivity on vascular health.

Author contributions

Conceptualisation and funding acquisition: CR. Data collection and data analysis: AD. Writing of original draft: AD. Project administration, supervision, writing, review and editing: CR and SL.

Conflicts of interest

All authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author (CR), upon reasonable request.

Supplementary information (SI) is available. The SI includes Fig. S1, which presents the Consolidated Standards of Reporting Trials (CONSORT) flow diagram showing inclusion and exclusion of data points for the primary (femoral FMD) and secondary (brachial FMD) outcome measures. See DOI: https://doi.org/10.1039/d5fo02793d.

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