A single dose of beetroot juice improves endothelial function but not tissue oxygenation in pregnant women: a randomised clinical trial

Mônica Volino-Souza, Gustavo Vieira de Oliveira and Thiago Silveira Alvares*

Nutrition and Exercise Metabolism Research Group, Nutrition Institute, Universidade Federal do Rio de Janeiro, Macaé, 27979-000 Rio de Janeiro, Brazil

Abstract

Beetroot juice (BJ) consumption has been associated with improved cardiovascular health owing to an increase in NO bioconversion. This study evaluates the effect of BJ consumption on macrovascular endothelial function (flow-mediated dilation (FMD)) and muscle oxygen saturation (StO_2) parameters in pregnant women within a randomised, crossover, double-blind design in which twelve pregnant women consumed a single dose (140 ml) of BJ or placebo (PLA). Urinary nitrate was assessed before (T0) and 150 min after BJ/PLA consumption. FMD was used to evaluate macrovascular endothelial function, and near-IR spectroscopy was used to evaluate muscle StO_2 parameters during the occlusion and reperfusion phases, which were taken at baseline (PRE) and 120 and 140 min after BJ/PLA consumption, respectively. A significant increase in urinary nitrate was observed at 150 min after BJ consumption when compared with T0 (BJ: 0·20 (SD 0·13) v. T0: 0·02 (SD 0·00), $P = 0.000$) and PLA intervention (PLA: 0·02 (SD 0·00), $P = 0.001$). FMD improved after BJ consumption when compared with PRE (BJ: 11·00 (SD 1·67) v. PRE: 5·53 (SD 1·17), $P = 0.000$) and PLA (5·34 (SD 1·31), $P = 0.000$). No significant difference between PLA and PRE in FMD ($P = 1.000$) was observed. In StO_2 parameters, a difference was not observed after BJ consumption compared with PRE and PLA intervention. The data demonstrate that a single dose of 140 ml of BJ consumption improves macrovascular endothelial function, but not StO_2 parameters.

Key words: Dietary nitrate: Functional food: Vascular function: Microcirculation: Pregnancy

During pregnancy, a reduction in macrovascular endothelial function (measured by flow-mediated dilatation (FMD)) is associated with hypertensive syndromes, whereas an increase in FMD reduces the risk of CVD[1]. In addition, preserved microvascular endothelial function in pregnancy is necessary for adequate tissue oxygenation. For example, impairments in uteroplacental vascular function in pregnancy lead to increased vascular resistance and decreased blood flow across the placenta, which reduce the placental capacity for nutrient and gas exchange[2]. The low oxygen availability may compromise oxygen delivery to the fetus and favour the development of fetal growth restriction (FGR)[3]. The NO molecule is critically involved in maintaining uteroplacental blood flow in pregnancies as NO regulates vascular tonus. Thus, the reduction of NO bioavailability has been associated with FGR[2,4,5].

The regulation of vascular tonus varies according to anatomical location (conduit artery or resistance vessel) owing to different vascular morphologies[6]. Measuring FMD is a way of evaluating macrovascular endothelial function in the conduit artery (i.e. brachial artery), whereas microvascular function (microcirculation) may be evaluated by near-IR spectroscopy (NIRS), a technology that observes changes in tissue oxygen saturation (StO_2), allowing for the evaluation of tissue oxygen extraction (oxidative capacity) and microvascular function (tissue arterioles function) during the occlusion and reperfusion periods, respectively[7,8].

Beetroot has gained the attention of the scientific community owing to its beneficial effects on cardiovascular health. The function of beetroot consumption on the risk of CVD has been studied and clinical trials have shown that beetroot is linked to improvement at both the macro-circulatory and the micro-circulatory levels[7–13]. Beetroot has beneficial effects on cardiovascular health owing to the nitrate present in this food. Nitrate can be converted to nitrite in the mouth by action of commensal facultative anaerobic bacteria that express nitrate reductase enzymes. In the acidic stomach and with low oxygen availability, nitrite is rapidly converted to NO[8,13–15].

Effects of beetroot juice (BJ) consumption on macrovascular endothelial function have been studied, demonstrating improvements in endothelial function (by FMD) in elderly people with risk

factors for CVD (after ingestion of a single dose of beetroot-based nutritional gel containing approximately 12 mmol nitrate[9]); in healthy subjects after consuming 500 ml of BJ[10]; in treated and untreated hypertensive subjects after 28 d of BJ consumption containing approximately 6·4 mmol nitrate[11]; and in hypercholesterolaemic adult patients after 6 weeks of BJ consumption containing approximately 6 mmol nitrate[12]. The effects of BJ consumption at microcirculatory levels have also been evaluated by NIRS measurement. In healthy subjects, 6 d of ingestion of BJ containing approximately 0·07 mmol nitrate/kg body promoted an improvement in muscle oxygenation status during submaximal and maximal exercise in severe acute hypoxia[7]. Also in healthy subjects, 6 d of 500 ml of BJ consumption containing 11·2 mmol of nitrate promoted an improvement in indexes of muscle oxygenation during exercise[8].

Although evidence assessing the effect of BJ consumption on macrovascular function and StO_2 parameters exists, no study in pregnant women has been performed. Therefore, the present study investigated whether a single dose of BJ would improve FMD and StO_2 parameters in pregnant women. A single dose of BJ was hypothesised to result in increased NO bioconversion, consequently promoting beneficial changes in FMD and StO_2 parameters in pregnant women.

Methods

Subjects

This study was conducted in accordance with the guidelines set forth in the Declaration of Helsinki, and the research ethics committee of the Universidade Federal do Rio de Janeiro, Campus Macaé, Brazil (protocol no. 56732216.5.0000.5699), approved all procedures involving human subjects. The study was registered with the Clinical Trials Registry (NCT03315806). All pregnant women gave their written informed consent. A total of thirty-six pregnant women were recruited through announcements in flyers and advertisements during community events in the Macaé City, Rio de Janeiro Estate, Brazil; twenty-four recruits withdrew for personal reasons or were ineligible according to the inclusion criteria; and twelve recruits were randomised (Fig. 1). Pregnant women were selected if they were between 20 and 35 weeks of gestation and were over 18 years of age. Exclusion criteria included the presence of diabetes mellitus and gestational diabetes, hypertensive syndromes, HIV positive, liver or kidney disease, beetroot allergy, use of antioxidants and medication that alters endothelial function (angiotensin-converting enzyme inhibitor), use of antibiotics up to 2 weeks before the beginning of the study.

Study design

A randomised, double-blind, crossover and placebo (PLA)-controlled study was conducted from October 2016 to May 2017. All subjects reported to the Dynamic Vascular Laboratory of Federal University of Rio de Janeiro, Macaé City, Brazil, on three occasions, with at least a 1-week interval between visits. The first visit was used to register basal (PRE) macrovascular and microvascular function measurements, blood sample collection for the characterisation of pregnant women, followed by
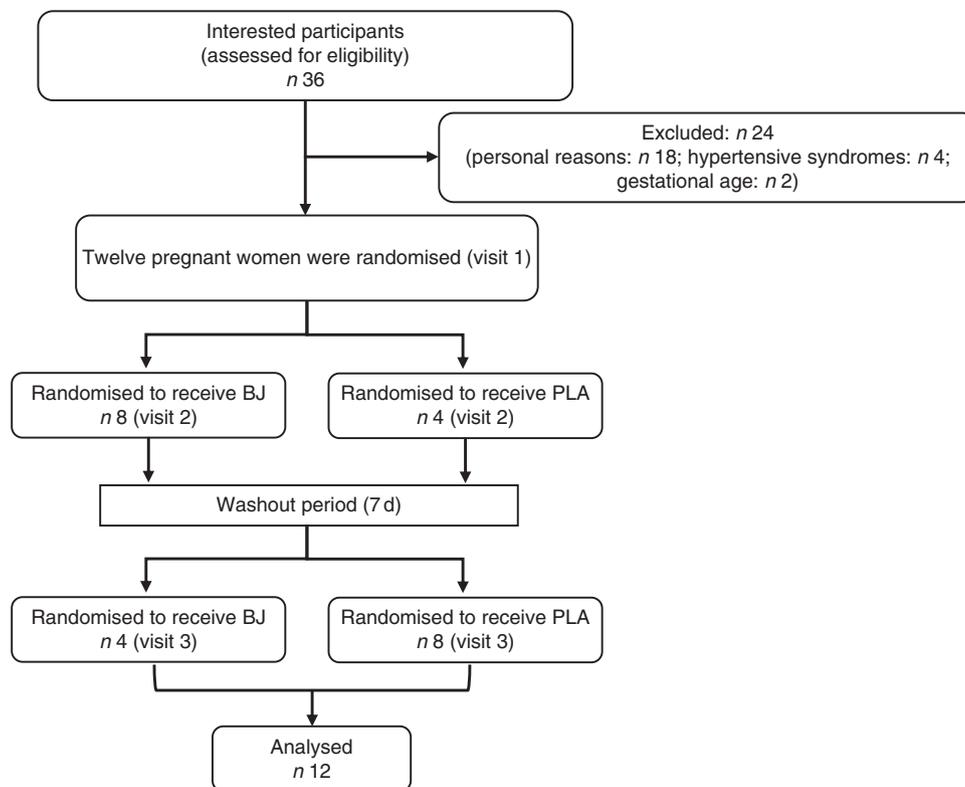
Fig. 1. Schematic of participant recruitment of the study. BJ, beetroot juice; PLA, placebo.
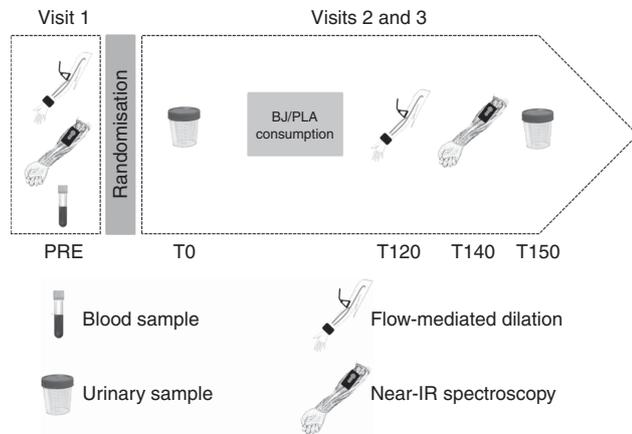
Fig. 2. Experimental design. BJ, beetroot juice; PLA, placebo.

randomisation (2:1 BJ:PLA). The BJ production requires several steps, including sanitation, centrifugation, pasteurisation and portioning. Production of small quantities of BJ for only one pregnant women was impracticable because it requires considerable time for production. In the randomisation 1:1, the remaining BJ would be lost as no preservatives were added, hence its short shelf life. Furthermore, it is important to point out that refrigerating (at 4°C) and freezing (at −20°C) the juice for 1 week could reduce the nitrate content by 15 and 14 %, respectively[16]. Thus, randomisation 2:1 (BJ:PLA) was more convenient in guaranteeing no nitrate losses. The participants were randomised and assigned by laboratory staff to ensure blind measurement. In the second and third visit, sample urine was drawn before (T0) BJ or PLA consumption. Afterward, macrovascular endothelial function, StO_2 parameters and urine samples were collected, respectively, at 120 min (T120), 140 min (T140) and 150 min (T150) after BJ or PLA consumption (Fig. 2). The three visits were held between 07.00 and 12.00 hours. The participants were instructed to fast for at least 8 h before each visit; to restrict carbonated mineral water; to avoid the intake of foods rich in nitrate and nitrite; to not use any mouthwash; to not consume coffee or teas; and not to exercise for at least 12 h before the visits.

Beetroot/placebo juice preparation

The beetroots (*Beta vulgaris* L.) were washed in tap water, sanitised with a chlorine solution and centrifuged (Model CE700; Black & Decker). The resulting juice was pasteurised, and 140 ml of juice was distributed in two bottles of dark colour. For preparation of the PLA (control nitrate-depleted juice), the nitrate was removed from BJ by using a specific anion-exchange resin A-520E (Purolite®). The contents of nitrate of the BJ and PLA consumed by the subjects of this study were 8·95 (SD 0·28) and 0·27 (SD 0·02) mM/140 ml, respectively.

Urinary nitrate

Nitrate analysis in urine was performed as previously described by Croitoru[17] by using a HPLC system. A volume of 100 µl of sample was added to 100 µl of acetonitrile (v/v) and centrifuged

(10 min at 14 000 rpm) for deproteinisation. Thereafter, 150 µl of supernatant was mixed with 150 µl of mobile phase (v/v) (5 mM tetrabutylammonium hydroxide) and the solution was mixed. Finally, 150 µl was injected in HPLC. Sodium nitrate was used for quantification, and the results were expressed as mmol/mmol creatinine for nitrate.

Macrovascular endothelial function analysis

The evaluation of the FMD was performed in a quiet air-conditioned room (constant temperature of 22–25°C) 120 min (T120) after BJ/PLA consumption. All examinations were conducted by the same investigator. Pregnant women rested quietly with an angle of 90° between the femur and the trunk. Endothelial function was assessed in accordance with Oliveira *et al.*[9]. FMD is calculated as the percentage dilated at the maximum peak brachial artery diameter after cuff deflation, relative to maximum baseline brachial artery diameter (BBD). All analyses were conducted by a single investigator who was blinded to the participant and supplementation order. CV was intraday 5·1 (SD 0·31) % and interday 5·1 (SD 0·12) %.

Oxygen saturation parameters analysis

Changes in tissue StO_2 were detected by a NIRS device (PortaMon; Artinis Medical Systems) that was placed on the flexor muscles of each subject's dominant forearm. After the StO_2 signals had been stable for 30 s, the cuff was rapidly inflated to suprasystolic levels (i.e. 250 mmHg), to obstruct blood flow to forearm muscles and a 5-min arterial occlusion was performed. The reperfusion phase after cuff release was recorded for more 2 min. During all tests, the NIRS system was connected to a personal computer via Bluetooth for data acquisition (10 Hz), analogue-to-digital conversion and subsequent analysis of the raw data (i.e. no filter was used) using native software (Oxysoft version 2.1.6; Artinis Medical Systems). The software calculates absolute changes in light absorption at the different wavelengths (750 and 850 nm) of oxyHb (O_2Hb) and deoxyHb (HHb) using the spatially resolved spectroscopy method. StO_2 was calculated as $(O_2Hb/O_2Hb + HHb) \times 100$. The following NIRS parameters were considered for statistical analysis: baseline StO_2 ($StO_{2\ base}$), which represents the average of 30 s of StO_2 before arterial occlusion; desaturation rate ($StO_{2\ slope\_1}$), which corresponds to the slope of StO_2 (the rate of change in StO_2 per time) during the arterial occlusion phase; minimum StO_2 ($StO_{2\ min}$), which corresponds to the lowest StO_2 value during the arterial occlusion phase; resaturation rate ($StO_{2\ slope\_2}$), which corresponds to the slope of StO_2 during the reperfusion phase; maximal StO_2 ($StO_{2\ max}$), which corresponds to the highest StO_2 value during the reperfusion phase; time to maximal StO_2 ($StO_{2\ tmax}$), which corresponds to the time to reach the $StO_{2\ max}$. CV was intraday 1·6 (SD 0·9) % for baseline, 2·0 (SD 1·6) % for occlusion and 1·6 (SD 1·1) % for reperfusion and interday 1·8 (SD 0·2) % for baseline, 2·9 (SD 0·7) % for occlusion and 1·5 (SD 0·1) % for reperfusion.

Statistical analysis

An *a priori* power analysis was conducted (G*Power version 3.0.1) for an *F* test (ANOVA, repeated measures, within factors). On the basis of statistical power $(1 − \beta)$ of 0·80, an effect size of 0·35 and an overall level of significance of 0·05, at least sixteen participants were needed to detect a statistical difference. Although this study was unable to achieve the sample size of sixteen participants, the effect size was taken into account in order to determine the magnitude of the BJ ingestion effect on endothelial function and muscle StO_2 parameters[18]. In addition, previous studies have observed statistical difference in StO_2 parameters with ≤12 volunteers after beetroot supplementation[8,9,19].

The normality and homogeneity of variances of the data were examined with the Shapiro–Wilk and Mauchly tests, respectively, and were violated only for $StO_{2\ base}$. To identify differences in the FMD and StO_2 parameters between PRE, PLA and BJ interventions, a repeated-measures one-way ANOVA was performed. When the assumption of normality and homogeneity of variances were violated, a Friedman test was used. To identify differences in urinary nitrate between BJ and PLA, a two-way ANOVA was used. In addition, ANOVA with repeated measurement was also performed to verify whether the order of BJ or PLA condition affected the results in this study. The effect size to observe the magnitude of the effect of BJ ingestion compared with PLA was calculated by Cohen's *d*, where a value <0·2 is considered trivial, 0·2–<0·5 is a small effect, 0·5–<0·8 is a moderate effect and ≥0·8 is a large effect. Statistical significance was set at the 0·05 level of confidence. All analyses were performed using a commercially available statistical package (IBM SPSS Statistics version 23 for Mac) and the results were expressed as means and standard deviations.

Results

Of the thirty-six assessed for eligibility, twelve (33 %) were randomised and completed the study (Fig. 1). Furthermore, the StO_2 data of one pregnant woman had to be excluded owing to technical reasons regarding the loss of NIRS signal. Therefore, only eleven volunteers were considered for analysis of this variable. The pregnant women were adult (27 (SD 6·51) years), sedentary, with a gestational age of 20–35 weeks, singleton pregnancies and the pregnant women did not use medications. The baseline volunteers' characteristics are shown in Table 1.

Urinary nitrate

T0 values of urinary nitrate (mmol/mmol creatinine) were not different between groups (PLA: 0·02 (SD 0·00) *v.* BJ: 0·02 (SD 0·00), *P* = 0·866). A significant main effect for time was observed (*P* = 0·001). *Post hoc* test revealed that urinary nitrate increased 10-fold in T150 after BJ consumption compared with T0 (T150: 0·20 (SD 0·13) *v.* T0: 0·02 (SD 0·00), *P* = 0·000), but not for PLA consumption compared with T0 (T150: 0·02 (SD 0·00)

v. T0: 0·02 (SD 0·00), *P* = 0·992). Furthermore, there was a significant treatment × time interaction effect for urinary nitrate (*P* = 0·001). *Post hoc* test revealed that urinary nitrate increased 10-fold in T150 after BJ consumption compared with PLA (*P* = 0·001). In addition, BJ and PLA have a large effect size (*d* = 1·95).

Endothelial function

The endothelial function parameters are shown in Fig. 3 and Table 2. FMD (%) increased 2-fold after BJ consumption (11·00 (SD 1·67)) compared with PLA (5·34 (SD 1·31)) and PRE (5·53 (SD 1·17)). BJ and PLA have a large effect size (*d* = 3·77). There were no changes on BBD and PDB after BJ compared with PRE and PLA. In addition, there was no effect on the supplementation order (BJ → PLA *v.* PLA → BJ) for FMD (*P* = 0·547), BBD (*P* = 0·673) and PDB (*P* = 0·658).

Microvascular function

The microvascular function parameters are shown in Fig. 4 and Table 3. There were no changes in $StO_{2\ base}$, $StO_{2\ slope\_1}$, $StO_{2\ min}$, $StO_{2\ slope\_2}$, $StO_{2\ max}$ and $StO_{2\ tmax}$ after BJ compared with PRE and PLA. In addition, there was no effect on the supplementation order (BJ → PLA *v.* PLA → BJ) for $StO_{2\ base}$ (*P* = 0·784), $StO_{2\ slope\_1}$ (*P* = 0·720), $StO_{2\ min}$ (*P* = 0·784), $StO_{2\ slope\_2}$ (*P* = 0·518), $StO_{2\ max}$ (*P* = 0·671) and $StO_{2\ tmax}$ (*P* = 0·386). BJ/PLA have a trivial effect size for $StO_{2\ base}$ (*d* = 0·29), $StO_{2\ slope\_1}$ (*d* = 0·03), $StO_{2\ min}$ (*d* = 0·04), $StO_{2\ slope\_2}$ (*d* = 0·04), $StO_{2\ max}$ (*d* = 0·06) and $StO_{2\ tmax}$ (*d* = 0·03).

Discussion

The aim of this study was to examine the effects of a single dose of BJ on macrovascular endothelial function FMD and StO_2

Table 1. Baseline demographic and clinical characteristics (Mean values and standard deviations)

Variables	Mean	SD
Demographics		
n	12	
Age (years)	27	6·51
Gestational age (weeks)	25·6	6·24
Body mass (kg)	79·14	20·20
Pre-pregnancy BMI (kg/m²)	25·41	4·81
BMI (kg/m²)	29·3	7·04
Forearm adipose tissue thickness (mm)	2·17	0·83
Biochemistry		
Total cholesterol (mmol/l)	6·52	1·04
Glucose (mmol/l)	4·23	0·54
HDL-cholesterol (mmol/l)	1·58	0·34
LDL-cholesterol (mmol/l)	4·12	0·87
TAG (mmol/l)	1·78	0·37
TAG/HDL-cholesterol (mmol/l)	1·15	0·28
Log(TAG/HDL-cholesterol) (mmol/l)	0·05	0·10
Clinical		
SBP (mmHg)	102·88	6·27
DBP (mmHg)	70·00	6·63
HR (beats per min)	82·5	8·04

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

parameters in pregnant women. This is the first investigation into the effects of the single dose of BJ on FMD and StO_2 parameters ($StO_{2\ base}$, $StO_{2\ min}$, $StO_{2\ slope\_1}$, $StO_{2\ max}$, $StO_{2\ slope\_2}$ and $StO_{2\ tmax}$) in pregnant women. The major finding of this study is that a single dose of a BJ improves FMD in pregnant women.

FMD is a non-invasive test of endothelial function, and changes in FMD values provide important prognostic information[20]. The FMD technique promotes increased blood flow and vasodilation owing to changes in shear stress, and this phenomenon is mediated by release of NO. Subjects with CVD have lower FMD values (endothelial dysfunction), whereas increased FMD decreases the risk of CVD[1]. Low FMD values in pregnancy are associated with health complications of the mother and fetus, such as pre-eclampsia and FGR. It has been shown that pregnant women who developed pre-eclampsia and FGR have shown FMD values of 3·5 and 6·2%, respectively, whereas pregnant women who did not develop pre-eclampsia and FGR presented FMD values of 8%[3]. The authors concluded that endothelial function is impaired in pregnant women who develop pre-eclampsia and FGR. In the present study, although the pregnant women did not develop pre-eclampsia and/or FGR, a low baseline FMD (5%) was observed, suggesting impaired endothelial function. After BJ consumption, the FMD values increased from 5 to 11% (meaning a 110% of increase), thereby reaching FMD values similar to pregnant women who did not develop pre-eclampsia and FGR[3].

The results of FMD in the present study are in accordance with previous findings[9,11,21]. Oliveira et al.[9] found improvements on FMD after ingestion of a single dose of beetroot-based nutritional gel rich in nitrate (containing approximately 12 mmol nitrate in 100 g) in the elderly with risk factors for CVD. Kapil et al.[11] demonstrated increased FMD after 28 d of ingestion of 250 ml of BJ (containing approximately 6·4 mmol of nitrate in 250 ml) in hypertensive subjects. Asgary et al.[21] observed improvements on FMD after 2 weeks of consumption of 250 ml of BJ and 250 g of cooked beet in hypertensive subjects. Therefore, the results of these studies suggest that the effect of a single dose of BJ on FMD may be the same regardless of the population studied.

Increase in urinary nitrate is in accordance with previous findings[9,22]. Baião et al.[22] demonstrated an increase in urinary nitrate concentration via bioconversion after ingestion of a single dose of 100 ml of BJ (containing approximately 1·6 mmol of nitrate). Oliveira et al.[9] showed an increase in urinary nitrate concentration after ingestion of a single dose of beetroot-based nutritional gel rich in nitrate (containing approximately 12 mmol nitrate in 100 g). Siervo et al.[23] showed an increase in nitrate concentration after ingestion of 140 ml of BJ (containing 12 mmol of nitrate) during 7 d.

StO_2 parameters during the arterial occlusion period ($StO_{2\ min}$ and $StO_{2\ slope\_1}$) provide information about tissue oxygen extraction[24]. Dipla et al.[25] evaluated recently StO_2 parameters during arterial occlusion in pregnant women with gestational
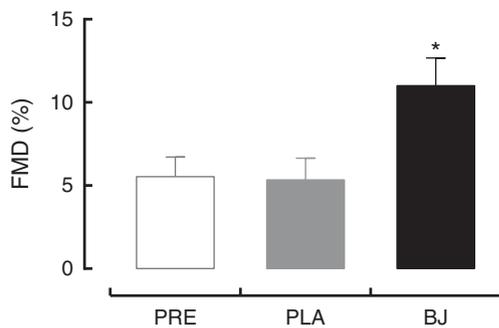
Fig. 3. Mean flow-mediated dilatation (FMD) after beetroot juice (BJ) and placebo (PLA) intervention. Values are means, with standard deviations represented by vertical bars. * Mean value was significantly different from baseline (PRE) and PLA ($P < 0.001$).
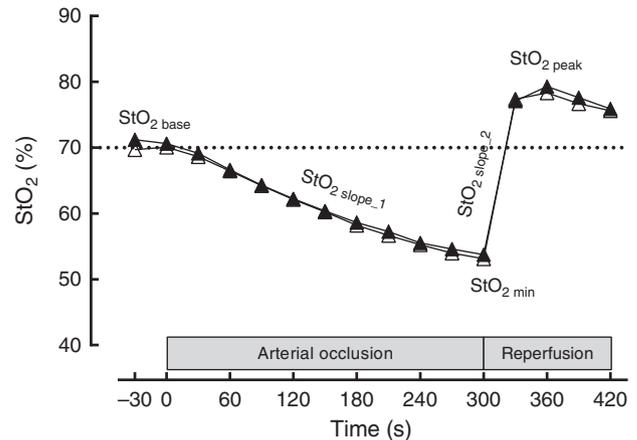
Fig. 4. Near-IR spectroscopy-derived profile of typical comportment of forearm muscle oxygen saturation (StO_2, %) and the parameters evaluated. $StO_{2\ base}$, baseline oxygen saturation; $StO_{2\ slope\_1}$, desaturation rate; $StO_{2\ slope\_2}$, resaturation rate; $StO_{2\ min}$, minimum oxygen saturation. —△—, Placebo; —▲—, beetroot juice.

Table 2. Macrovascular endothelial function parameters at baseline and after beetroot juice (BJ) and placebo (PLA) consumption
(Mean values and standard deviations)

	PRE		PLA		BJ	
	Mean	SD	Mean	SD	Mean	SD
FMD (%)	5·53	1·17	5·34	1·31	11·00***	1·67
BBD (mm)	3·39	0·63	3·34	0·50	3·28	0·66
PBD (mm)	3·56	0·66	3·52	0·53	3·58	0·81

PRE, baseline; FMD, flow-mediated dilation; BBD, maximum baseline brachial artery diameter; PBD, maximum peak brachial artery diameter.
*** Statistical difference compared with PRE and PLA ($P < 0.001$; one-way ANOVA followed by Bonferroni's post hoc test).

Table 3. Oxygen saturation (StO_2) parameters at baseline and after beetroot juice (BJ) and placebo (PLA) consumption*
(Mean values and standard deviations)

	PRE		PLA		BJ		
	Mean	SD	Mean	SD	Mean	SD	P
StO_2 base (%)	71·39	8·97	69·69	4·25	71·19	5·81	0·336
StO_2 slope−1 (%/s)	−0·52	0·30	−0·60	0·32	−0·58	0·29	0·908
StO_2 min (%)	54·10	13·03	52·83	12·99	53·37	12·41	0·973
StO_2 slope−2 (%/s)	1·03	0·65	1·03	0·55	1·06	0·72	0·993
StO_2 max (%)	78·96	3·04	79·63	1·18	80·15	3·56	0·985
StO_2 tmax (s)	49·28	17·84	43·06	8·43	43·43	11·68	0·475

PRE, baseline; StO_2 base, baseline StO_2; StO_2 slope−1, desaturation rate; StO_2 min, minimum StO_2; StO_2 slope−2, resaturation rate; StO_2 max, maximal StO_2; StO_2 tmax, time to maximal StO_2.
* One-way ANOVA followed by Bonferroni's *post hoc* test.

diabetes mellitus (GDM). In GDM, the StO_2 min value was greater when compared with the control group of pregnant women (approximately 55 % for GDM and 40 % for control), suggesting that the GDM group exhibited a reduced capacity for O_2 extraction at the skeletal muscle level. In the present study, pregnant women demonstrated an StO_2 min value near the GDM group in the Dipla *et al.*[25] study, suggesting that the pregnant women in the present study possibly presented an impaired capacity for O_2 extraction. It is already known that elevated blood glucose increases cell oxidative stress[26,27], which impairs oxygen extraction by tissues (mitochondrial dysfunction)[28]. In this study, the pregnant women presented normal blood glucose (<5·55 mmol/l); however, they presented elevated cholesterol (>5 mmol/l). Elevated blood cholesterol has also been shown to be associated with increased oxidative stress[29], which may impair oxygen extraction by tissues[26,27]. In addition, a significant correlation between StO_2 min and cholesterol (date not showed) was observed, which may explain the greater StO_2 min value in pregnant. It was expected that BJ consumption would improve StO_2 parameters during arterial occlusion owing to the effect of nitrate on mitochondrial function. The nitrate–nitrite–NO pathway has been shown to modulate O_2 consumption in mitochondria[30]. In complex IV of the electron transport chain, NO competes with O_2 for cytochrome *c* oxidase (COX), whereby NO binds to COX when oxygen is available[30]. However, no changes were observed in StO_2 parameters during arterial occlusion. Bioconversion of nitrite to NO requires approximately 50 % Hb-saturated oxygen[31]. The present study observed that approximately 53 % Hb was saturated with oxygen, which indicates that the environment was favourable for nitrite-to-NO bioconversion, although no significant difference was observed in muscle oxygen extraction.

During the reperfusion phase, StO_2 parameters (StO_2 max, StO_2 slope−2 and StO_2 tmax) reflect the ability of recruiting greater arteriole numbers[32]. After arterial disocclusion, there is an increase in blood flow in the ischaemic limb, which promotes an increase in vasoactive substances[33]. An improvement in StO_2 parameters after BJ consumption owing to vasodilator propriety of NO had been expected. However, there was no significant difference in StO_2 parameters after BJ consumption.

The StO_2 max and StO_2 slope−2 in the present study was near the control group (healthy pregnant) values in Dipla *et al.*[25] study, suggesting that pregnant women present a sufficient ability to recruit greater arteriole numbers. Hence, the integrity of microvascular reactivity in pregnant women in the present study may be a possible reason for the neglible effect on these parameters after BJ consumption. It was speculated that NO should be delivered only to the areas with impaired microvascular perfusion to avoid diffuse dilation in perfused capillaries. As microvascular reactivity is preserved in pregnant women of this study, the capillaries are sufficiently perfused and do not require increase in perfusion.

This study was unable to achieve a large sample size owing to physiological changes inherent in pregnancy, such as sea sickness and vomiting, as well as to the protocol of the study that requires periods of fasting. However, the strengths of the experimental study design are that all pregnant women were submitted to both BJ or PLA ingestion (crossover design), the muscle StO_2 measure presented good repeatability (CV approximately 3 %), the pregnancies were within the gestational age of 20–35 weeks, the study included adults (27 (SD 6·51) years) and the ingestion of the food containing nitrate and nitrite before all experimental visits was controlled. In addition, the BJ ingestion is a potent nutritional supplement, which has been demonstrated to provoke changes in muscle StO_2 parameters[34]. Therefore, the very small effect size reported in the present study suggests the weak magnitude of the BJ ingestion effect on muscle StO_2 parameters[35].

In addition, the differences among the responses observed in macro-circulatory and micro-circulatory levels after BJ consumption can be explained by cell heterogeneity. Differences in endothelial cells are reported at the level of morphology, function, gene expression and antigen composition[6]. Furthermore, endothelial cells exhibit differences depending on where the type of vessel or organ is located[36]. Therefore, NO action seems to be different in macrovasculature and microvasculature.

In summary, the effect of a single dose of BJ consumption, a source of dietary nitrate, on macrovascular endothelial function and StO_2 parameters was investigated. The results of the present study show that BJ improves macrovascular endothelial function, but not StO_2 parameters. Furthermore, BJ

consumption increases urinary nitrate. The beneficial effect on macrovascular endothelial function observed after BJ consumption may be an important nutritional intervention for pregnant women aiming to reduce the risk of developing CVD. However, future studies are necessary to address and evaluate the effects of BJ consumption in pregnancy with CVD.

Acknowledgements

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