

# Consumption of a flavonoid-rich acai meal is associated with acute improvements in vascular function and a reduction in total oxidative status in healthy overweight men

Article

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### FMD Response to Acute Acai Consumption

1	Consumption of A Flavonoid -Rich Açai Meal is Associated with Acute Improvements
2	in Vascular Function and a Reduction in Oxidative Stress in Healthy Overweight Men.
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11	<sup>2</sup> Supported by the Saudi Arabian Ministry of Education. Kingdom of Saudi Arabia
12	Abbreviations used: AS, Açai smoothie; CVD, cardiovascular disease; DBP, diastolic
13	blood pressure; FMD, flow-mediated dilation; HR, heart rate; PS, control smoothie; SBP,
14	Systolic blood pressure; TOC, Total oxidant capacity.
15	
16	This trial is registered at Clinicaltrials.Gov as NCT02292329
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18	

20 Abstract

Background: Açai (Euterpe oleracea) is a polyphenol rich, Amazonian fruit which has been
suggested to have potential health benefits. There is however little direct evidence
demonstrating improvements in health markers arising from açai consumption in humans.

Objective: The objective of the present study was to investigate the effect of açai consumption on acute changes in vascular function, and on other cardiovascular and metabolic disease risk markers including postprandial plasma insulin, glucose and oxidative stress.

**Design:** Twenty-three healthy male volunteers, aged 30-65 y and with a body mass index 25-30 kg/m<sup>2</sup>, completed a randomized, control controlled, high fat challenge, double-blind crossover acute dietary intervention trial. The volunteers were randomized to consume either an açai based smoothie (AS) or a macronutrient matched control smoothie (PS) alongside a high fat breakfast meal challenge. The primary endpoint was the assessment of endothelial function in the brachial artery using flow-mediated dilatation (FMD).

Results: We observed that the acute consumption of an acai based smoothie containing 694 34 mg of total phenolics improved vascular function, as measured by FMD, with post prandial 35 increases from baseline of 1.4% at 2 hours, and 0.8% at 6 hours. There was also a 36 significantly lower incremental area under the curve (IAUC) for total peroxide oxidative 37 status after acai consumption relative to control. No significant changes were observed in 38 blood pressure, heart rate or the post-prandial glucose response. However, the first post-39 40 prandial insulin peak (after breakfast) and the IAUC for insulin were elevated for açai relative to control. 41

**Conclusions:** In this acute study in overweight men, açai consumption is associated with 43 improvements in vascular function which may lower risk of a cardiovascular event. Future 44 intervention studies, perhaps with a chronic design, in wider populations, and with other 45 biomarkers of disease risk are needed to fully elucidate açai benefits to health.

- 46 Key words Vascular Function, Açai (Euterpe oleracea), Flavonoids, Acute, FMD.

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#### 61 Introduction

62 Dietary practices which reduce the burden of cardiovascular diseases (CVD) are a desirable goal of public health programs. Prospective cohort studies strongly suggest that a diet high in 63 64 fruit and vegetables may protect against CVD (1, 2). There is however a need to identify individual plant foods with strong protective effects and also to better understand the 65 mechanisms involved in food mediated disease prevention (3). Polyphenols are widely 66 67 studied non-nutritive bioactive compounds found in plant foods; these compounds have a well characterized free radical scavenging ability in vitro which may decrease oxidative stress 68 in vivo (4). Polyphenols may also improve endothelial function, lower blood pressure (5, 6), 69 improve insulin sensitivity (7), decrease low-density lipoprotein level (LDL) (8), and 70 modulate inflammatory response (9). Dietary intervention with polyphenol rich berries 71 induce improvements in risk factors associated with metabolic syndrome, diabetes and 72 73 cardiovascular disease (10, 11).

74 The flow mediated dilation (FMD) method is a measure of arterial endothelial dysfunction 75 and a well-established early biomarker of cardiovascular disease risk (12). Dietary intervention with blueberry has been shown to reverse this endothelial dysfunction, at least in 76 77 an acute setting, where changes in the concentration of phenolic metabolites in plasma were associated with observed post-prandial improvements in FMD (13). This suggests that 78 polyphenols present in blueberry may mediate a benefical effect on FMD. Several other 79 80 dietary intervention studies with polyphenol rich foods, including for example cocoa (14), green tea (15) and grapes (16) report similar improvements in FMD when test foods are 81 consumed in an acute setting. 82

83 The açai fruit (Euterpe oleracea Mart., Arecaceae) is notable for its 84 very high concentration of flavonoids; this suggests potential health benefits from its 85 consumption may exist. The food media have picked up on this despite a paucity of supporting human intervention study data. Açai has subsequently found its way into the 86 global health food market (17, 18). In the UK consumption of açai remains very low; it is 87 88 available as a powdered supplement or as a minor constituent of foodstuffs such as in mixed berry drinks or yoghurts, often these are marketed with loose claims for health. Some 89 characterization of potential benefits from consuming açai is therefore necessary to better 90 inform the consumer and to establish appropriate dietary recommendations for the prevention 91 of disease (19). 92

To our knowledge, the effects of açai on arterial endothelial dysfunction have not been assessed. Here, we describe results from a double-blind control-controlled intervention trial with açai consumed as a smoothie blended with banana, as it is typically prepared in Brazil, and with FMD as the primary endpoint. We hypothesized that consuming açai, alongside a high fat breakfast intervention, would ameliorate the detrimental vascular response to that high fat intervention and induce improvements in other cardiovascular and metabolic disease risk markers in an acute setting.

#### 100 Subjects and Methods.

#### 101 Materials

Frozen açai pulp was kindly donated by the Sublime Foods Company Ltd (UK). Fresh banana was purchased locally. Flavonoid and phenolic acid standards were obtained from Sigma-Aldrich Co Ltd, UK or Extrasynthese, France. Water, methanol, and acetonitrile (HPLC grade) were purchased from Fisher Scientific. Glucose concentrations were quantified using an iLAB 600 biochemical analyzer (Instrumentation Laboratory) with enzymatic colorimetric and standard kits with appropriate sero-normal, low and high quality control standards supplied by Instrumentation Laboratories and Alpha Laboratories (IL Test TM 109 Glucose). Serum insulin was determined with an enzyme-immunoassay using reagents 110 supplied by Dako Cytomation and a GENios plate reader (Tecan Group). Blood tubes for 111 serum and plasma were supplied by Geriner BioOne Ltd (UK). All other chemicals and 112 reagents were obtained from Sigma-Aldrich Co Ltd or Fisher Scientific.

#### 113 Intervention study subjects

Twenty-three male volunteers were recruited from the University of Reading and surrounding 114 area through the Hugh Sinclair Unit of Human Nutrition volunteer database, and through 115 advertisements within the local community between September 2014 and January 2015. 116 Volunteers were required to be of good general health but with a BMI of 25-30 kg  $m^2$  which 117 is associated with a slight increase in risk of developing metabolic disease. The inclusion 118 criteria were as follows: male, aged 30-65 years, non-smokers, total cholesterol <8.0 119 mmol/ml. Volunteers were excluded if they suffered from diabetes, anemia, cardiovascular, 120 renal, gastrointestinal, hepatic disease or were being medicated for hyperlipidemia, 121 122 hypertension, inflammation or depression, if they were on a weight reduction program or 123 were taking any nutritional supplements. Of the first 37 men screened 24 were recruited and randomized onto the crossover study arms, 23 participants completed the study with one drop 124 out due to a change in his personal circumstances (Figure 1). 125

#### 126 Study design and treatments.

The study was registered as a clinical trial (Clinical trials.gov ID: NCT02292329) and was 127 conducted according the Declaration of Helsinki 128 to and 129 followed Good Clinical Practice (GCP). It was given a favorable ethical opinion for conduct by the University of Reading Research Ethics Committee (Ethics reference number 13/51). 130 The study was an acute randomized, controlled, double blind crossover study with an acai 131 132 smoothie (study treatment). The volunteers were randomized using a minimization program

(www.users.york.ac.uk) by the researcher to generate the randomization sequence (with discrimination for age and BMI) for the treatment arms. Açai is very rarely consumed in this format in the UK and it was unfamiliar to the participants enabling us to effectively blind them to the treatment arm.

Frozen açai pulp (150 g) was prepared in a smoothie with 50 g of banana and no other 137 additives. Analysis of the nutritional profile of both smoothies can be seen in Table 1. The 138 control smoothie was prepared from 50 g of banana and matched for fat by 1.5 g palmitic 139 acid and 8.5g sunflower oil (30% oleic acid, 60% linoleic acid and 10% palmitic acid, both 140 purchased from Sigma-Aldrich UK), carbohydrate (maltodextrin, Myprotein, UK) and fiber 141 142 (cellulose, Azelis, UK) and then blended with artificial food colors (Sensientflavors, UK) to create a dark color approximating that of the açai smoothie. 150 g of açai contains 8.4 g fat 143 (61.6% oleic acid, 12.6% linoleic acid and 25.7 % palmitic acid). The smoothies were 144 145 prepared fresh in the early morning on the day of the intervention.

146 Participants were given dietary advice directing them away from consuming polyphenol-rich 147 foods for at least 24-hours prior to the study visits and each volunteer was asked to consume a standard low flavonoid evening meal containing <15g fat and <7g of saturated fat and to 148 149 fast overnight (12 hours thereafter) prior to the intervention. A 24 hour dietary recall was also taken at each visit (Supplement table 1). The study visit began at 8am in the morning. 150 Subjects were rested in the unit for 30 minutes prior to measurements of baseline blood 151 pressure and FMD. Blood (18 ml) was then collected via a cannula inserted into the 152 antecubital vein of the forearm (Study procedure is shown in Supplement figure 1). 153 154 Volunteers were then provided with a high fat challenge breakfast (50g fat) (Supplement table 2) and asked to consume the smoothie with it, and within a ten minute total time frame. 155 An independent researcher, unaware of the study arm, collected blood samples at regular 156 157 intervals for 7 hours (1, 2, 3, 4, 5, 6, 7 hours) and performed vascular measurements at 2

hours, 4 hours and 6 hours. A second lunch-time standard moderate fat (14.2g fat) meal was provided after collection of the 4th hour blood samples (**Supplemental figure 1**). Urine samples were collected at baseline, 0-7 and 7-24 hours. Subjects were given a standard low polyphenol evening meal at the end of the visit day to ensure phenolics recovered in the 24 hour urines were from açai.

#### 163 Assessment of the polyphenol content of test foods.

Açai smoothies were prepared as described above. Samples were taken and freeze-164 dried. Flavonoids and phenolic acids were extracted using acidified methanol with 0.1% 165 formic acid for the anthocyanin compounds and methanol: water (80:20, v/v) for other 166 phenols. The polyphenols were characterized using a method previously validated by our 167 group (20). HPLC was performed using an Agilent 1100 series liquid chromatograph with a 168 quaternary pump and a photodiode array detector (Hewlett-Packard Agilent, Bracknell, UK). 169 A Nova Pak C18 4- $\mu$ m column (4.6 × 250 mm) (Waters, Elstree, UK) was used to separate 170 171 the phenolic constituents at a solvent flow rate of 0.4 mL/min and the column was allowed to equilibrate for 15 min between each injection. At least 50 µL of each sample or standard 172 solution was injected for each analysis. The mobile phase A was 95% HPLC water, 5% 173 174 methanol and 0.1% HCl; mobile phase B was 50% HPLC water, 50% acetonitrile and 0.1% HCl. The identification of phenolic compounds from açai was based on the mass spectra 175 fragmentation patterns and retention times in the UV spectra compared with standards 176 selected based on previous literature (17). The detection wavelengths were 254, 280, 320 and 177 520 nm. A standard curve was constructed to quantify the amount of each compound 178 identified in açai smoothie (20). 179

#### 180 Nitrite and nitrate in test foods.

Nitrite and Nitrate were assessed in aqueous extracts of test foods using the ENO-30, HPLC-181 based approach. Briefly, smoothies were prepared and mixed with distilled low nitrite/nitrate 182 water (50:50). Samples were centrifuged at 3000g for 10 min and the supernatant passed 183 through a 0.45µM syringe filter. 200 µl was collected and 10µl was immediately injected into 184 the ENO-30 system. Calibration curves for nitrite and nitrate were prepared using NaNO2 185 186 and NaNO3 standards in pure water. Carrier solution was prepared by using pure water (900 ml) with methanol (100 ml) and carrier powder (obtained from Eicom, Ireland). Mobile phase 187 188 A was prepared with pure water (450 mL), 100 mL of methanol, 12.5 mL of concentrated HCL (35-57%) and reactor A powder (obtained from Eicom, Ireland). Mobile phase B was 189 190 prepared from pure water (450 mL) and 100 mL of methanol and Reactor Powder B 191 (obtained from Eicom, Ireland), Solution A and B were mixed as 1:1. Separation was achieved using a NO-PAK Column size 4.6mm and 50mm and flow rate 100µl/min. Pump 192 pressures were A 300 µl/min and B 100 µl/min. 193

#### 194 Vascular function and measurements

#### 195 **FMD.**

196 Flow Mediated Dilation (FMD) of the brachial artery was the primary endpoint measure of the study and was measured according to standard guidelines (21) by using an ALT 197 Ultrasound HDI-15000 system (ATL Ultrasound, UK) in combination with a semi-automated 198 199 computerized analysis system (Brachial Analyzer; Medical Imaging Applications-IIc, IL, US). Briefly, after a 30 minute rest in the supine position in a quiet, temperature-controlled 200 201 room (22-24 °C) a baseline vascular measurement was taken. Vascular measurements were taken at 2, 4 and 6 h after the meal. The brachial artery was imaged longitudinally at 2-10 cm 202 proximal to the antecubital fossa. After baseline images were recorded over 60 seconds, a 203 204 blood pressure cuff placed around the forearm was inflated to 220 mm Hg. Then after 5 min

of occlusion, the blood pressure was rapidly released to allow reactive hyperemia, with image collection that continued for 5 min after release. A single researcher who was blinded to the intervention analyzed all image files. The peak diameter was defined as the largest diameter obtained after the cuff was released. The FMD percentage was calculated as the relative diameter change from the baseline comparison with the peak diastolic diameter.

210 **Blood pressure measurements.** 

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured in all subjects at baseline, 2h, 4h and 6h after the meal. Before measurement subjects were rested for 30 minutes and the mean of triplicate measurements was recorded with an OMRON-M6 automatic digital (HEM-7211-E8) Comfort Upper-Arm Blood Pressure Monitor.

#### 216 Biochemical analysis.

217 Plasma blood samples were collected in EDTA vacutainers (Greiner BioOne Ltd) and kept on ice until centrifugation at 1600 x g for 15 minutes at 4° C. Vacutainer rapid serum separator 218 tubes were used to collect blood and left at room temperature (RT) for 30 minutes to allow 219 for clot formation, then centrifuged at 1600 x g for 15 minutes at RT to 220 isolate serum. Urine samples were collected at different time points (0-7 and 7-24 hours) the 221 total volume of urine produced each time point was recorded and aliquots were prepared for 222 storage after centrifugation at 1600g for 10 minutes at 4°C. Blood and urine samples were 223 stored at -80°C until further analysis. Serum lipids were measured at baseline via the iLAB 224 225 600 and Low-density lipoprotein (LDL) was calculated from Friedewald equation. Total serum glucose and insulin were analyzed at all-time points (0, 1, 2, 3, 4, 5, 6, and 7h) using 226 an ILAB600 auto-Analyzer (Warrington, UK). Serum insulin concentrations at each time 227 point were analyzed using an enzyme linked immunosorbent assay (ELISA). 228

#### 229 **Total oxidant capacity (TOC)**

Total oxidant capacity in plasma was assessed as a measure of total peroxide levels according 230 to the method of Tatzber et al. (22). Briefly, 10 µL of standards (0, 0.125, 0.25, 0.5, 1mmol/L 231 232 of hydrogen peroxide (30%)) or plasma samples were added to the wells of a 96-well plate, followed by the addition of 200 µL of reaction mixture. The stock reaction mixture (prepared 233 fresh) contained 20ml reaction buffer (phosphate-citrate 0.05M, pH 5.0), 20 ml of substrate 234 solution (3,3',5,5'-Tetramethylbenzidine (TMB) solution at 1mg/ml in DMSO), and 20µl of 235 peroxidase solution (10mg/ml in phosphate buffer 0.1M, pH 6.0). The 96-well plate was 236 incubated for 20 mins on ice and the reaction was then stopped by the addition 2M sulfuric 237 acid. Absorbance was measured immediately at a wavelength of 450nm on a GENios plate 238 reader at room temperature with Magellan<sup>TM</sup> software. 239

#### 240 Statistical analysis and power calculations

Power calculations were based on the primary endpoint, change in FMD, from baseline at 2 hours, with a required sample size estimated based on the variance of repeated measurements in the control group and on control data. Based on previous acute studies of the effects flavonoid rich foods on FMD and aiming for statistically significant improvement in FMD of between 1.5 to 2%, with a baseline vasodilatation of 10%, 23 subjects were required to achieve a study power of 80 % with alpha at 0.05.

Results are expressed as mean, SEM. Data were checked for normal distribution. Two-factor repeated measures ANOVA was used to analyze the data for FMD, blood pressure, and 7 hours in postprandial glucose, insulin and total plasma oxidant capacity following the two treatments.

The incremental area under the curve (IAUC) over 7 hours was calculated for insulin, glucose and TOC using the trapezoidal method excluding the area below fasting level (23). The timings of the maximum concentration (MaxC) of serum glucose and insulin after each meal
were calculated (Tmax<sup>1-4h</sup> and Tmax<sup>4-7h</sup>) and used in the analysis by Paired samples t-test.
Significance was defined as P<0.05 with p-values represented in the figures as \*\*\*P<0.001,</li>
\*\*P<0.01, \*P<0.05. All statistics were performed using SPSS software (Version 18).</li>

257 **Results** 

#### 258 **Baseline characteristics of the study participants.**

The baseline characteristics of the study population are summarized in **Table 2**. The enrolled 259 subjects were men at slightly elevated risk of metabolic syndrome with a BMI in the 260 overweight category and a mean age of 46 (SEM 1.9 years). At screening the subjects had a 261 mean waist circumference of 97.6 cm (SEM 1.6 cm) and serum lipids within the normal 262 range (Table 2). The subjects were normoglycemic, test results for hemoglobin and for liver 263 264 function showed no evidence of ill health. Blood pressure (SBP and DBP) and heart rate were also within the acceptable healthy range. No side effects were reported by the study 265 participants. 266

#### 267 Polyphenol content of smoothies

The polyphenol and phenolic acid content of the acia smoothie was 694 mg (Table 1). Its 268 total anthocyanin content was 493 mg (principally cyanidin-3-O-glucoside, cyanidin-3-O-269 rutinoside, pelargonidin-3-O-glucoside and peonidin-3-O-rutinoside), the quercetin content 270 was 9.6 mg and gallic acid content 173.6 mg. Small amounts of some phenolic acids such as 271 caffeic acid, ferulic acid, vanillic acid, 3, 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid 272 and 2, 5-dihydroxybenzoic acid were also identified and quantified in the acai smoothie 273 274 (Table 1). The polyphenol content of the control smoothie was below the level of detection (< 10mg). 275

#### 276 Vascular function.

Flow mediated dilation (FMD) changed from baseline over the course of the study day (twoway ANOVA, time effect; P=0.028), peaking 2 hours after the test meal was consumed and again at 6 hours (Figure 2). Moreover, there was a highly significant treatment effect on FMD for the acai smoothie relative to control (two-way ANOVA, treatment effect; P<0.001). However the time by treatment interaction was not significant (P=0.2).

The primary endpoint for this study was the change from baseline in FMD at 2 hours, the 282 magnitude of increase in FMD from baseline after consumption of the acai smoothie was 283 1.4% (SEM 0.6%) (p=0.034, T-test), compared to only a 0.4% (SEM 0.6%) (p=0.52) 284 285 increase after consumption of the control. After 4 hours, the FMD measurements for the acai group had dropped to a 0.2% (SEM 0.5%) increase above baseline, whereas the control fell to 286 -0.5% (SEM 0.7%) below that of baseline levels. A second peak in FMD was observed at 6 h 287 288 hours post intervention with the acai smoothie to 0.8% (SEM 0.7%.), whilst the control remained slightly below baseline (-0.3% (SEM 0.5%). (Figure 2). 289

290 No significant differences in systolic blood pressure, diastolic blood pressure or heart rate 291 were observed between the treatment groups over the course of the study day (**Table 3**).

#### 292 Postprandial glucose and insulin response.

As expected, consumption of the breakfast resulted in a rapid rise in serum glucose concentration peaking on average at 1 hour (5.90 ±0.23 mmol/L with the açai smoothie and 5.28 ± 0.27 mmol/L with the control smoothie) and then returning to baseline between 2 to 4 hours (**Figure 3 A**). After consumption of the lunch, serum glucose concentrations again increased, reaching a higher maximum (7.49 ± 0.24 mmol/L with the açai smoothie and 6.75 ± 0.29 mmol/L with the control smoothie) than after breakfast. No significant differences in the incremental area under the curve for serum glucose were observed between the two treatments and no time by treatment interaction was observed (P=0.38) (Figure 3 B). The postprandial serum insulin response to the sequential mixed meals is shown in **Table 4**. Significant treatment (P=0.02) and time (P<0.001) effects were observed. The açai smoothie induced a significantly higher maximum insulin concentration (maxC <sup>1-4h</sup>) (P=0.009) than the control and significant differences were found in the IAUC (P=0.003). However, no time (h) x treatment interaction was observed (P=0.15).

#### **306** Total oxidant capacity.

The post-prandial total plasma oxidant capacity over seven hours following consumption of the açai smoothie or control is shown in (Figure 3 C). The IAUC over the 7 hour study period shows significantly lower total plasma oxidants for the açai smoothie relative to control (P=0.02) (Table 4). No significant differences were observed at any individual sampling time-point over this period and the time (h) x treatment interaction was P=0.68.

#### 312 **Discussion**

To our knowledge, this is the first human study demonstrating the effects of consuming açai, 313 with a high fat breakfast, on vascular function and other metabolic disease risk markers, in an 314 acute setting. We hypothesized that consuming acai would ameliorate the detrimental 315 vascular response to a high fat meal and induce improvements in other cardiovascular and 316 317 metabolic disease risk markers in an acute setting. Our study demonstrates that acai induces clinically meaningful improvement in vascular function (FMD) in overweight individuals (a 318 1.4% increase at 2 hours). At a population level, a 1% increase in FMD is associated with a 319 13% reduction in risk of a cardiovascular event (24). 320

We speculate that the polyphenols present in the açai were responsible for these changes in vascular function. No time by treatment effects were observed in FMD following

consumption of the acai smoothie versus the control smoothie over the six hour intervention 323 window. A comparison of the composition of the açai versus the control food revealed that 324 the control smoothie was matched to within 1% for energy and for fiber, to within 10% for 325 326 carbohydrate and to within 20% for total fat, the control had a slightly higher nitrate content but a lower nitrite content; the total content of nitrite/nitrate in the control was double that of 327 the açai smoothie. In contrast, the açai smoothie contained 694 mg of flavonoids and phenolic 328 329 acids versus less than 10 mg in the control smoothie. Our approach, using a whole food as opposed to purified phenolic isolates, does not allow us to fully assess whether it is the 330 331 polyphenols present in the food mediating the observed response, and we cannot completely exclude the possibility that other known or unknown compounds might be responsible for the 332 biological outcomes. Both acute and chronic dietary interventions with other plant 333 334 polyphenol rich foods such as blueberries (13), pomegranate juice (25), dark chocolate (6) and cocoa (14, 26, 27) induce similar changes in vascular function, and purified flavonoids 335 have also been shown to induce this effect in hypercholesteremic volunteers (28). We 336 337 observed peaks in FMD at 2 and 6 hours post consumption of the acai smoothie, Rodriguez et al. identified similar peaks in post-prandial FMD in healthy men fed blueberry at 2, and 6 338 hours, and this correlated with peaks in plasma concentrations of phenolic acid metabolites 339 and a reduction of neutrophil NADPH oxidase activity in that study (13). This time course 340 may reflect the emergence of phenolic metabolites in plasma following small intestinal 341 342 absorption and later, at 6 hours, the liberation of phenolic compounds during colonic fermentation (29-31) 343

The mechanism by which phenolics improve endothelial function is unclear, it is possible that it is related to their well-established antioxidant activity (32, 33). One hypothesis is that phenolics may reduce NADPH oxidase activity and that this may be linked to an increased level of nitric oxide via inhibition of superoxide production (34, 35). We observed no changes in blood pressure at any time point after açai smoothie intake. This is similar to another açai study that reported no effect on blood pressure after having 100g of açai pulp twice daily for one month (36) and in line with findings from previous acute interventions with polyphenol rich foods (13, 37). However, chronic, or longer term, interventions with polyphenol rich foods, fed to high risk groups, are often shown to be effective at lowering blood pressure (6, 25, 38-41).

No differences were observed in post-prandial glucose responses between the acai smoothie 354 and the carbohydrate and energy matched control, but a significantly higher insulin response 355 was observed for the açai compared to control. Zunino and others have argued that phenolics 356 357 can improve glucose control although the mechanism has not been elucidated (42), findings from studies of other polyphenol rich foods are not consistent when it comes to their effects 358 on plasma glucose and insulin (7, 8, 30, 43-50). Further work is clearly needed to understand 359 360 this elevated insulin response and to determine whether or not it may be clinically relevant to glucose control. 361

362 We also observed a reduction in total peroxide levels in plasma following consumption of the açai which we included as a measure of acute changes in plasma oxidant capacity. Again we 363 364 hypothesize that this is a function of phenolics emerging in plasma; previously the consumption of grape polyphenols induced similar effects in pre-and postmenopausal women 365 (50). In experimental models oxidative stress is strongly linked to endothelial function and to 366 cardiovascular disease (51, 52), Measures of plasma oxidant capacity are however widely 367 critiqued, and our observation therefore warrants follow up using different measures such as 368 369 the presence of oxidized LDL following a longer term intervention.

A limitation of the present study is that we did not determine and correlate directlypolyphenols and their metabolites in plasma or urine in tandem with our FMD measures and

nitric oxide as assessed by Rodriguez-Mateos in an intervention with blueberry (13). Our study was a short term acute intervention in overweight middle aged men, selected as a high risk group for cardiovascular disease. We cannot infer the long term effects of açai consumption on vascular function, or rule out physiological adaptations to the presence of high quantities of açai polyphenols in the diet which might off-set the observed short-term benefits reported here. Future studies might consider chronic study designs, and data are still needed on the effects of açai consumption in other groups.

This trial is the first well controlled acute intervention study in humans demonstrating actual benefits from consuming açai using a well validated biomarker of cardiovascular disease risk as an endpoint. The findings of this double-blind randomized crossover study are therefore timely and important and suggest that consuming açai as part of a balanced diet and a healthy lifestyle may improve cardiovascular health which strongly supports arguments for the health benefits of açai consumption.

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Compounds	Açai smoothie	Control smoothie (control)
Energy (Kcal)	154.5	155.3
Protein (g)	2.4	0.6
Fat (g)	8.5	10
Carbohydrate (g)	17.2	16
Fiber (g)	7.2	7.2
Fructose (g)	2.68	3.8
Glucose (g)	2.92	4.12
Total sugar (g)	8.4	11.2
Vitamin C (mg/L)	4.35	8.65
Total carotenoids (mg)	179.3	0
Nitrite (µM/L)	0.07	0.04
Nitrate (mM/L)	8.83	16.4
Anthocyanins (mg)	493	0
Chlorogenic acid (mg)	9.3	0
Caffeic acid(mg)	2.0	0
Syringic acid (mg)	2.7	0
Ferulic acid (mg)	0.6	0
Vanillic acid (mg)	0.2	0
Gallic acid (mg)	173.6	0
3,4-Dihydroxybenzoic acid (mg)	1.2	0
4-Hydroxybenzoic acid (mg)	0.8	0
2,5-Dihydroxybenzoic acid (mg)	0.4	0
Trans-cinnamic acid (mg)	0.4	0
Quercetin (mg)	9.6	0
Total phenols (mg)	694	<10

Table 1: Composition and Nutritional Profile of a 200g serving of the Intervention Foods<sup>1</sup>.

<sup>1</sup> The energy, protein, sugar, fat, fiber, vitamin C and sugar content were analyzed under contract by Campden BRI laboratories (UK). Identification and quantification of phenolic compounds in açai smoothie was assessed by HPLC.

Characteristics	Mean	SEMs
n	23	-
Age (years)	46	1.9
Body weight (kg)	88.8	2
BMI (kg/m <sup>2</sup> )	27.6	0.4
Waist circumference, cm	97.6	1.6
Body Fat (%)	26.3	2
Cholesterol (mmol/l)	5.1	0.2
Triglycerides (mmol/L)	1.3	0.1
HDL (mmol/L)	1.2	0.05
LDL (mmol/L)	3.2	0.1
Glucose, (mmol/L)	5.2	0.09
Creatinine (µmol/L)	95	1.8
Bilirubin (µmol/L)	11.7	0.7
Urea (µmol/L)	337	10
Haemoglobin (g/L)	148	1
SBP (mm Hg)	126	2
DBP (mm Hg)	75	2
Heart Rate (bpm)	63	2

Table 2: Baseline clinical characterization of study population<sup>1</sup>

<sup>1</sup>All values are means, SEMs (n = 23). Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

Maagumag	Timepoint							
Measures	Baseline	SEM	2 h	SEM	4 h	SEM	6 h	SEM
SBP (mm Hg)								
Açai smoothie	125.8	11.8	121.9	16	127	13.6	128.9	12.8
Control	127.9	12.5	125.7	11.3	127	13.6	127.4	10.9
<u>DBP (mm Hg)</u>								
Açai smoothie	73.7	8.9	72.1	9.8	74.1	9.3	74.3	10.4
Control	73.7	10	72.2	8	74.5	9.8	75.1	8.2
<u>Heart rate</u> (beats/min)								
Açai smoothie	59.1	8.8	61.2	9.9	57.2	8	61.9	10.2
Control	58.3	8.3	57.6	8	56.4	8.2	58	7.3

Table 3: Acute effects of açai polyphenols on blood pressure and heart rate (n=23)<sup>1</sup>.

<sup>1</sup>All values are mean, SEM (n=23). No significant differences were shown between baseline and post-intervention (2, 4, or 6 h) or between the açai smoothie and control treatments, P>0.05 for SBP, DBP and heart rate (repeated-measured and 2-factor repeated-measures ANOVA). There were no significant time x treatment effects for SBP, DBP or for HR (SBP =P=0. 6), (DBP P=0.9) HR P=0.7). DBP, diastolic blood pressure; SBP, systolic blood pressure; HR, heart rate.

24	Smoothies					
Measures	Control	SEM	Açai	SEM		
Glucose response						
maxC <sup>1-4h</sup> (mmol/L)	6.11	0.19	6.18	0.19		
Tmax <sup>1-4hours</sup> (hours)	1.61	0.15	1.30	0.13		
maxC <sup>4-7h</sup> (mmol/L)	7.57	0.27	7.95	0.24		
Tmax <sup>4-7hours</sup> (hours)	5.78	0.14	5.87	0.11		
IAUC (mmol/L ×hour)	2.35	1.13	3.42	1.10		
Insulin response						
maxC <sup>1-4h</sup> (pmol/L)	246.4	24.1	319 <sup>2</sup>	26.44		
Tmax <sup>1-4hours</sup> (hours)	1.57	0.12	1.22	0.09		
maxC <sup>4-7h</sup> (pmol/L)	302	29.8	323	26.4		
Tmax <sup>4-7hours</sup> (hours)	5.91	0.15	5.83	0.12		
IAUC (pmol/L ×hour)	733	86.3	907.7 <sup>3</sup>	76.5		
Oxidative capacity						
$maxC^{1-4h}$ (µmol/L)	310.3	20.7	310.8	19.5		
Tmax <sup>1-4hours</sup> (hours)	1.3	0.1	1.3	0.2		

Table 4: Postprandial measures of glucose and insulin responses and total oxidant capacity<sup>1</sup>.

<sup>1</sup> Values represent mean, SEM. Two-way repeated measures ANOVA were initially used to assess treatment, time and treatment by time interaction effects; when no differences were observed we performed paired samples t-tests to compare incremental area under the curve (IAUC) and observations at individual timepoints with the açai smoothie relative to control.

20.8

0.2

127.4

294.5

5.4

 $-165^4$ 

17.0

0.2

128.1

293.5

5.6

-30.5

Abbreviations; maxC, the maximum concentration; Tmax, the time to reach maxC; IAUC, incremental area under the curve.

<sup>2-3</sup> significantly different to control, P<0.01

<sup>4</sup>significantly different to control, P<0.05

 $maxC^{4-7h}$  (µmol/L)

Tmax<sup>4-7hours</sup> (hours)

IAUC ( $\mu$ mol/L ×hour)

#### **Figure legends**

Figure 1: Flow of the study participants through the intervention.

**Figure 2**: FMD after consumption of an açai smoothie (AS) containing 694 mg of polyphenols or a macronutrient matched control smoothie (PS) (n = 23). Data were analyzed using a 2- factor repeated measures ANOVA with time and treatment as the two factors [significant effect of time (P=0.03), treatment (P=<0.001)]. At baseline the treatment arms are not significantly different (P=0.31). There was no significant interaction of time x treatment (P=0.2) reflecting the similar, but much attenuated, pattern of FMD measures through the day in the control relative to treatment.

**Figure 3**: Mean, SEM for post-prandial serum glucose (A), insulin (B) and total plasma oxidant capacity (C) responses to a high-fat breakfast (50 g fat) and a standard lunch (14 g fat). The smoothies were consumed by volunteers with the breakfast meal only. The vertical line represents the timing of the lunch meal. Two-way repeated measures ANOVA was used to assess treatment, time and treatment by time interaction effect, and Paired samples t-test were used to compare incremental area under the curve (IAUC) between the açai smoothies relative to control.

- **A.** Glucose response: Treatment effect, P=0.14; Time effect, P<0.001; Time by Treatment interaction, P=0.38.
- B. Insulin response: Treatment effect, P=0.02; Time effect, P<0.001; Time by Treatment interaction, P=0.15.</p>
- **c.** Total plasma oxidant capacity: Treatment effect, P=0.85; Time effect, P=0.36; Time by Treatment interaction, P=0.68. Incremental area under the curve (IAUC), P=0.02.