

*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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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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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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19

20 **Abstract**

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

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

28 **Design:** Twenty-three healthy male volunteers, aged 30-65 y and with a body mass index 25-  
29 30 kg/m<sup>2</sup>, completed a randomized, control controlled, high fat challenge, double-blind  
30 crossover acute dietary intervention trial. The volunteers were randomized to consume either  
31 an açai based smoothie (AS) or a macronutrient matched control smoothie (PS) alongside a  
32 high fat breakfast meal challenge. The primary endpoint was the assessment of endothelial  
33 function in the brachial artery using flow-mediated dilatation (FMD).

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

42 **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  
63 goal of public health programs. Prospective cohort studies strongly suggest that a diet high in  
64 fruit and vegetables may protect against CVD (1, 2). There is however a need to identify  
65 individual plant foods with strong protective effects and also to better understand the  
66 mechanisms involved in food mediated disease prevention (3). Polyphenols are widely  
67 studied non-nutritive bioactive compounds found in plant **foods**; these compounds have a  
68 well characterized free radical scavenging ability *in vitro* which may decrease oxidative stress  
69 *in vivo* (4). Polyphenols may also improve endothelial function, lower blood pressure (5, 6),  
70 improve insulin sensitivity (7), decrease low-density lipoprotein level (LDL) (8), and  
71 modulate inflammatory response (9). Dietary intervention with polyphenol rich berries  
72 induce improvements in risk factors associated with metabolic syndrome, **diabetes** and  
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  
76 intervention with blueberry has been shown to reverse this endothelial dysfunction, at least in  
77 an acute setting, where changes in the concentration of phenolic metabolites in plasma were  
78 associated with observed post-prandial improvements in FMD (13). This suggests that  
79 polyphenols present in blueberry may mediate a beneficial effect on FMD. Several other  
80 dietary intervention studies with polyphenol rich foods, including for example cocoa (14),  
81 green tea (15) and grapes (16) report similar improvements in FMD when test foods are  
82 consumed in an acute setting.

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

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

## 100 **Subjects and Methods.**

### 101 **Materials**

102 Frozen açai pulp was kindly donated by the Sublime Foods Company Ltd (UK). Fresh  
103 banana was purchased locally. Flavonoid and phenolic acid standards were obtained from  
104 Sigma-Aldrich Co Ltd, UK or Extrasynthese, France. Water, methanol, and acetonitrile  
105 (HPLC grade) were purchased from Fisher Scientific. Glucose concentrations were quantified  
106 using an iLAB 600 biochemical analyzer (Instrumentation Laboratory) with enzymatic  
107 colorimetric and standard kits with appropriate sero-normal, low and high quality control  
108 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**

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

### 126 **Study design and treatments.**

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

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

137 Frozen açai pulp (150 g) was prepared in a smoothie with 50 g of banana and no other  
138 additives. Analysis of the nutritional profile of both smoothies can be seen in **Table 1**. The  
139 control smoothie was prepared from 50 g of banana and matched for fat by 1.5 g palmitic  
140 acid and 8.5g sunflower oil (30% oleic acid, 60% linoleic acid and 10% palmitic acid, both  
141 purchased from Sigma-Aldrich UK), carbohydrate (maltodextrin, Myprotein, UK) and fiber  
142 (cellulose, Azelis, UK) and then blended with artificial food colors (Sensientflavors,UK) to  
143 create a dark color approximating that of the açai smoothie. 150 g of açai contains 8.4 g fat  
144 (61.6% oleic acid, 12.6% linoleic acid and 25.7 % palmitic acid). The smoothies were  
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  
148 a standard low flavonoid evening meal containing <15g fat and <7g of saturated fat and to  
149 fast overnight (12 hours thereafter) prior to the intervention. A 24 hour dietary recall was also  
150 taken at each visit (**Supplement table 1**). The study visit began at 8am in the morning.  
151 Subjects were rested in the unit for 30 minutes prior to measurements of baseline blood  
152 pressure and FMD. Blood (18 ml) was then collected via a cannula inserted into the  
153 antecubital vein of the forearm (Study procedure is shown in **Supplement figure 1**).  
154 Volunteers were then provided with a high fat challenge breakfast (50g fat) (**Supplement**  
155 **table 2**) and asked to consume the smoothie with it, and within a ten minute total time frame.  
156 An independent researcher, unaware of the study arm, collected blood samples at regular  
157 intervals for 7 hours (1, 2, 3, 4, 5, 6, 7 hours) and performed vascular measurements at 2

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

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

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

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

181 Nitrite and Nitrate were assessed in aqueous extracts of test foods using the ENO-30, HPLC-  
182 based approach. Briefly, smoothies were prepared and mixed with distilled low nitrite/nitrate  
183 water (50:50). Samples were centrifuged at 3000g for 10 min and the supernatant passed  
184 through a 0.45µM syringe filter. 200 µl was collected and 10µl was immediately injected into  
185 the ENO-30 system. Calibration curves for nitrite and nitrate were prepared using NaNO<sub>2</sub>  
186 and NaNO<sub>3</sub> standards in pure water. Carrier solution was prepared by using pure water (900  
187 ml) with methanol (100 ml) and carrier powder (obtained from Eicom, Ireland). Mobile phase  
188 A was prepared with pure water (450 mL), 100 mL of methanol, 12.5 mL of concentrated  
189 HCL (35-57%) and reactor A powder (obtained from Eicom, Ireland). Mobile phase B was  
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  
192 achieved using a NO-PAK Column size 4.6mm and 50mm and flow rate 100µl/min. Pump  
193 pressures were A 300 µl/min and B 100 µl/min.

## 194 **Vascular function and measurements**

### 195 **FMD.**

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

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

#### 210 **Blood pressure measurements.**

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

#### 216 **Biochemical analysis.**

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

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

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

## 240 **Statistical analysis and power calculations**

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

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

251 The incremental area under the curve (IAUC) over 7 hours was calculated for insulin, glucose  
252 and TOC using the trapezoidal method excluding the area below fasting level (23). The

253 timings of the maximum concentration (MaxC) of serum glucose and insulin after each meal  
254 were calculated ( $T_{max}^{1-4h}$  and  $T_{max}^{4-7h}$ ) and used in the analysis by Paired samples t-test.  
255 Significance was defined as  $P < 0.05$  with p-values represented in the figures as \*\*\* $P < 0.001$ ,  
256 \*\* $P < 0.01$ , \* $P < 0.05$ . All statistics were performed using SPSS software (Version 18).

## 257 **Results**

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

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

### 267 **Polyphenol content of smoothies**

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

276 **Vascular function.**

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

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

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.**

293 As expected, consumption of the breakfast resulted in a rapid rise in serum glucose  
294 concentration peaking on average at 1 hour ( $5.90 \pm 0.23$  mmol/L with the açai smoothie and  
295  $5.28 \pm 0.27$  mmol/L with the control smoothie) and then returning to baseline between 2 to 4  
296 hours (**Figure 3 A**). After consumption of the lunch, serum glucose concentrations again  
297 increased, reaching a higher maximum ( $7.49 \pm 0.24$  mmol/L with the açai smoothie and  $6.75$   
298  $\pm 0.29$  mmol/L with the control smoothie) than after breakfast. No significant differences in  
299 the incremental area under the curve for serum glucose were observed between the two

300 treatments and no time by treatment interaction was observed ( $P=0.38$ ) (**Figure 3 B**). The  
301 postprandial serum insulin response to the sequential mixed meals is shown in **Table 4**.  
302 Significant treatment ( $P=0.02$ ) and time ( $P<0.001$ ) effects were observed. The açai smoothie  
303 induced a significantly higher maximum insulin concentration ( $\text{maxC}^{1-4\text{h}}$ ) ( $P=0.009$ ) than the  
304 control and significant differences were found in the IAUC ( $P=0.003$ ). However, no time (h)  
305 x treatment interaction was observed ( $P=0.15$ ).

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

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

### 312 **Discussion**

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

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

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

344 The mechanism by which phenolics improve endothelial function is unclear, it is possible that  
345 it is related to their well-established antioxidant activity (32, 33). One hypothesis is that  
346 phenolics may reduce NADPH oxidase activity and that this may be linked to an increased  
347 level of nitric oxide via inhibition of superoxide production (34, 35).

348 We observed no changes in blood pressure at any time point after açai smoothie intake. This  
349 is similar to another açai study that reported no effect on blood pressure after having 100g of  
350 açai pulp twice daily for one month (36) and in line with findings from previous acute  
351 interventions with polyphenol rich foods (13, 37). However, chronic, or longer term,  
352 interventions with polyphenol rich foods, fed to high risk groups, are often shown to be  
353 effective at lowering blood pressure (6, 25, 38-41).

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

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

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

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

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

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393 manuscript.

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

Compounds	Açaí 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 ( $\mu$ M/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

<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.**

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

<b>Characteristics</b>	<b>Mean</b>	<b>SEMs</b>
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

<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.

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

Measures	Timepoint							
	Baseline	SEM	2 h	SEM	4 h	SEM	6 h	SEM
<b><u>SBP (mm Hg)</u></b>								
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
<b><u>DBP (mm Hg)</u></b>								
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
<b><u>Heart rate (beats/min)</u></b>								
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

<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.**

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

Measures	Smoothies			
	Control	SEM	Açai	SEM
<b><u>Glucose response</u></b>				
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
<b><u>Insulin response</u></b>				
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
<b><u>Oxidative capacity</u></b>				
maxC <sup>1-4h</sup> (μmol/L)	310.3	20.7	310.8	19.5
Tmax <sup>1-4hours</sup> (hours)	1.3	0.1	1.3	0.2
maxC <sup>4-7h</sup> (μmol/L)	293.5	20.8	294.5	17.0
Tmax <sup>4-7hours</sup> (hours)	5.6	0.2	5.4	0.2
IAUC (μmol/L ×hour)	-30.5	127.4	-165 <sup>4</sup>	128.1

<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.

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

## 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.
- 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.