

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

Accepted Version

Alqurashi, R. M., Galante, L. A., Rowland, I. R., Spencer, J. P. and Commane, D. M. (2016) 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. *American Journal of Clinical Nutrition*, 104 (5). pp. 1227-1235. ISSN 0002-9165 doi: <https://doi.org/10.3945/ajcn.115.128728> Available at <https://centaur.reading.ac.uk/67259/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

Published version at: <http://ajcn.nutrition.org/content/early/2016/09/28/ajcn.115.128728.full.pdf+html>

To link to this article DOI: <http://dx.doi.org/10.3945/ajcn.115.128728>

Publisher: American Society for Nutrition

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

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.**

3 ¹⁻² Randah M. Alqurashi, ¹Laura A Galante, ¹Ian R. Rowland, ¹Jeremy PE. Spencer, ¹Daniel
4 M. Commane.

5 Alqurashi, Galante, Rowland, Spencer, Commane,

6 ¹Hugh Sinclair Unit of Human Nutrition, Department of Food and Nutritional Sciences,
7 University of Reading, Reading RG6 6AP, U.K. Address reprint requests and
8 correspondence to, Daniel M. Commane, Department of Food and Nutritional Sciences,
9 University of Reading, Whiteknights PO Box 226, Reading RG6 6AP, United Kingdom.
10 Telephone; +44 (0) 118 378 7108 E-mail: d.m.commane@reading.ac.uk.

11 ²Supported 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

17

18

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/m², 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.

47

48

49

50

51

52

53

54

55

56

57

58

59

60

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 m² 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- μ 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 μ 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 NaNO₂
186 and NaNO₃ 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 μ 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 μ 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 μ 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 MagellanTM 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 ± 0.23 mmol/L with the açai smoothie and
295 5.28 ± 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 ± 0.24 mmol/L with the açai smoothie and 6.75
298 ± 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.

385 **Acknowledgments**

386 We thank all of the subjects for their collaboration and gratefully acknowledge our
387 appreciation to Rada G. Mihaylove and Karen Jenkins for cannulation of the study subjects,
388 and Jan Luff for logistical help throughout the study. We also thank Mark Parady for his
389 assistance on study visits. The study was funded by the Saudi Arabian Ministry of Education
390 (Saudi Arabia). All authors have read and approved the final manuscript. The authors have no
391 other conflicts of interest to declare. DC and JS designed the research, RA and LG conducted
392 the study, IR and DC reviewed the manuscript, RA analyzed the data and prepared the
393 manuscript.

References

1. 1- Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, Hu FB. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose- response metaanalysis of prospective cohort studies. *BMJ* 2014;349:g4490
- 2- Hartley L, Igbinedion E, Holmes J, Flowers N, Thorogood M, Clarke A, Stranges S, Hooper L, Rees K. Increased consumption of fruit and vegetables for the primary prevention of cardiovascular diseases. *Cochrane Database Syst Rev* 2013;6(6).
- 3- Woodside JV, Young IS, McKinley MC. Fruit and vegetable intake and risk of cardiovascular disease. *Proceedings of the Nutrition Society* 2013;72(04):399-406.
- 4- Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of botany* 2003;91(2):179-94.
- 5- Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim J- a, Quon MJ, Montagnani M. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. *American Journal of Physiology-Endocrinology and Metabolism* 2007;292(5):E1378-E87.
- 6- Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *The Journal of nutrition* 2008;138(9):1671-6.
- 7- Stull AJ, Cash KC, Johnson WD, Champagne CM, Cefalu WT. Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *The Journal of nutrition* 2010;140(10):1764-8.
- 8- Zunino SJ, Parelman MA, Freytag TL, Stephensen CB, Kelley DS, Mackey BE, Woodhouse LR, Bonnel EL. Effects of dietary strawberry powder on blood lipids and inflammatory markers in obese human subjects. *British Journal of Nutrition* 2012;108(05):900-9.
- 9- Mao T, Van de Water J, Keen C, Schmitz H, Gershwin M. Modulation of TNF- α secretion in peripheral blood mononuclear cells by cocoa flavanols and procyanidins. *Journal of Immunology Research* 2002;9(3):135-41.

- 10- Erlund I, Koli R, Alfthan G, Marniemi J, Puukka P, Mustonen P, Mattila P, Jula A. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *The American journal of clinical nutrition* 2008;87(2):323-31.
- 11- Simeonov S, Botushanov N, Karahanian E, Pavlova M, Husianitis H, Troev D. Effects of *Aronia melanocarpa* juice as part of the dietary regimen in patients with diabetes mellitus. *Folia medica* 2002;44(3):20-3.
- 12- Raitakari OT, Celermajer DS. Flow-mediated dilatation. *British Journal of Clinical Pharmacology*. 2000;50(5):397-404. doi:10.1046/j.1365-2125.2000.00277.x
- 13- Rodriguez-Mateos A, Rendeiro C, Bergillos-Meca T, Tabatabaee S, George TW, Heiss C, Spencer JP. Intake and time dependence of blueberry flavonoid-induced improvements in vascular function: a randomized, controlled, double-blind, crossover intervention study with mechanistic insights into biological activity. *The American journal of clinical nutrition* 2013;98(5):1179-91.
- 14- Heiss C, Kleinbongard P, Dejam A, Perré S, Schroeter H, Sies H, Kelm M. Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *Journal of the American College of Cardiology* 2005;46(7):1276-83.
- 15- Alexopoulos N, Vlachopoulos C, Aznaouridis K, Baou K, Vasiliadou C, Pietri P, Xaplanteris P, Stefanadi E, Stefanadis C. The acute effect of green tea consumption on endothelial function in healthy individuals. *European Journal of Cardiovascular Prevention & Rehabilitation* 2008;15(3):300-5.
- 16- Li S-H, Tian H-B, Zhao H-J, Chen L-H, Cui L-Q. The acute effects of grape polyphenols supplementation on endothelial function in adults: Meta-analyses of controlled trials. *PloS one* 2013;8(7).
- 17- Yamaguchi KK, Pereira LFR, Lamarão CV, Lima ES, da Veiga-Junior VF. Amazon acai: Chemistry and biological activities: A review. *Food chemistry* 2015;179:137-51.
- 18- Pacheco-Palencia LA, Mertens-Talcott S, Talcott ST. Chemical composition, antioxidant properties, and thermal stability of a phytochemical enriched oil from Açai (*Euterpe oleracea* Mart.). *Journal of agricultural and food chemistry* 2008;56(12):4631-6.
- 19- Heinrich M, Dhanji T, Casselman I. Açai (*Euterpe oleracea* Mart.)—A phytochemical and pharmacological assessment of the species' health claims. *Phytochemistry Letters* 2011;4(1):10-21.
- 20- Rodriguez-Mateos A, Cifuentes-Gomez T, Tabatabaee S, Lecras C, Spencer JP. Procyanidin, anthocyanin, and chlorogenic acid contents of highbush and lowbush blueberries. *Journal of agricultural and food chemistry* 2012;60(23):5772-8.

- 21- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *Journal of the American College of Cardiology* 2002;39(2):257-65.
- 22- Tatzber F, Griebenow S, Wonisch W, Winkler R. Dual method for the determination of peroxidase activity and total peroxides-iodide leads to a significant increase of peroxidase activity in human sera. *Analytical biochemistry* 2003;316(2):147-53.
- 23- Lunde MS, Hjellset VT, Holmboe-Ottesen G, Hostmark, AT. Variations in postprandial blood glucose responses and satiety after intake of three types of bread. *J Nutr Metab* 2011; 2011, 437587.
- 24- Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a metaanalysis. *Int J Cardiovasc Imaging* 2010;26:631–40.
- 25- Asgary S, Keshvari M, Sahebkar A, Hashemi M, Rafieian-Kopaei M. Clinical investigation of the acute effects of pomegranate juice on blood pressure and endothelial function in hypertensive individuals. *ARYA atherosclerosis* 2013;9(6):326.
- 26- Rodriguez-Mateos A, Hezel M, Aydin H, Kelm M, Lundberg JO, Weitzberg E, Spencer JP, Heiss C. Interactions between cocoa flavanols and inorganic nitrate: Additive effects on endothelial function at achievable dietary amounts. *Free Radical Biology and Medicine* 2015;80:121-8.
- 27- Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proceedings of the National Academy of Sciences of the United States of America* 2006;103(4):1024-9.
- 28- Zhu Y, Xia M, Yang Y, Liu F, Li Z, Hao Y, Mi M, Jin T, Ling W. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clinical chemistry* 2011;57(11):1524-33.
- 29- Wallace TC. Anthocyanins in cardiovascular disease. *Advances in Nutrition: An International Review Journal* 2011;2(1):1-7.
- 30- Aura AM, Martin-Lopez P, O'Leary KA, Williamson G, Oksman-Caldentey KM, Poutanen K, Santos-Buelga C. In vitro metabolism of anthocyanins by human gut microflora. *European journal of nutrition* 2005;44(3):133-42.

- 31- Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American journal of clinical nutrition* 2005;81(1):230S-42S.
- 32- Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. *Archives of Biochemistry and Biophysics* 2008;476(2):102-6.
- 33- Stoclet J-C, Chataigneau T, Ndiaye M, Oak M-H, El Bedoui J, Chataigneau M, Schini-Kerth VB. Vascular protection by dietary polyphenols. *European journal of pharmacology* 2004;500(1):299-313.
- 34- Steffen Y, Schewe T, Sies H. (–)-Epicatechin elevates nitric oxide in endothelial cells via inhibition of NADPH oxidase. *Biochemical and biophysical research communications* 2007;359(3):828-33.
- 35- Steffen Y, Gruber C, Schewe T, Sies H. Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. *Archives of Biochemistry and Biophysics* 2008;469(2):209-19.
- 36- Udani JK, Singh BB, Singh VJ, Barrett ML. Effects of Acai(*Euterpe oleracea* Mart.) berry preparation on metabolic parameters in a healthy overweight population: A pilot study. *Nutrition journal* 2011;10(45).
- 37- Jin Y, Alimbetov D, George T, Gordon M, Lovegrove JA. A randomised trial to investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects. *European journal of clinical nutrition* 2011;65(7):849-56.
- 38- Brown AL, Lane J, Coverly J, Stocks J, Jackson S, Stephen A, Bluck L, Coward A, Hendrickx H. Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: randomized controlled trial. *British journal of nutrition* 2009;101(06):886-94.
- 39- Basu A, Du M, Leyva MJ, Sanchez K, Betts NM, Wu M, Aston CE, Lyons TJ. Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *The Journal of nutrition* 2010;140(9):1582-7.
- 40- Almoosawi S, Fyfe L, Ho C, Al-Dujaili E. The effect of polyphenol-rich dark chocolate on fasting capillary whole blood glucose, total cholesterol, blood pressure and glucocorticoids in healthy overweight and obese subjects. *British journal of nutrition* 2010;103(06):842-50.

- 41- Draijer R, de Graaf Y, Slettenaar M, de Groot E, Wright CI. Consumption of a Polyphenol-Rich Grape-Wine Extract Lowers Ambulatory Blood Pressure in Mildly Hypertensive Subjects. *Nutrients* 2015;7(5):3138-53.
- 42- Zunino S. Type 2 diabetes and glycemic response to grapes or grape products. *The Journal of nutrition* 2009;139(9):1794S-800S.
- 43- Wang B, Liu K, Mi M, Wang J. Effect of fruit juice on glucose control and insulin sensitivity in adults: a meta-analysis of 12 randomized controlled trials. *PloS one* 2014;9(4)
- 44- Riso P, Klimis-Zacas D, Martini D, Campolo J, Vendrame S, Møller P, Loft S, Maria R, Porrini M. Effect of a wild blueberry (*Vaccinium angustifolium*) drink intervention on markers of oxidative stress, inflammation and endothelial function in humans with cardiovascular risk factors. *European Journal of Nutrition* 2013;52(3):949-61.
- 45- Lee I, Chan Y, Lin C, Lee W, Sheu W. Effect of cranberry extracts on lipid profiles in subjects with Type 2 diabetes. *Diabetic medicine* 2008;25(12):1473-7.
- 46- Chambers BK, Camire ME. Can cranberry supplementation benefit adults with type 2 diabetes? *Diabetes Care* 2003;26(9):2695-6.
- 47- Curtis PJ, Kroon PA, Hollands WJ, Walls R, Jenkins G, Kay CD, Cassidy A. Cardiovascular disease risk biomarkers and liver and kidney function are not altered in postmenopausal women after ingesting an elderberry extract rich in anthocyanins for 12 weeks. *The Journal of nutrition* 2009;139(12):2266-71.
- 48- Qin Y, Xia M, Ma J, Hao Y, Liu J, Mou H, Cao L, Ling W. Anthocyanin supplementation improves serum LDL-and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *The American journal of clinical nutrition* 2009;90(3):485-92.
- 49- Takikawa M, Inoue S, Horio F, Tsuda T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *The Journal of nutrition* 2010;140(3):527-33.
- 50- Zern TL, Wood RJ, Greene C, West KL, Liu Y, Aggarwal D, Shachter NS, Fernandez ML. Grape polyphenols exert a cardioprotective effect in pre-and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *The Journal of nutrition* 2005;135(8):1911-7.
- 51- Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circulation research* 2000;87(10):840-4.

- 52- Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;104(22):2673-8.

Table 1: Composition and Nutritional Profile of a 200g serving of the Intervention Foods¹.

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 (μ 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

¹ **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 population¹

Characteristics	Mean	SEMs
n	23	-
Age (years)	46	1.9
Body weight (kg)	88.8	2
BMI (kg/m ²)	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

¹All 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)¹.

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

¹ 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 capacity¹.

Measures	Smoothies			
	Control	SEM	Açai	SEM
<u>Glucose response</u>				
maxC ^{1-4h} (mmol/L)	6.11	0.19	6.18	0.19
Tmax ^{1-4hours} (hours)	1.61	0.15	1.30	0.13
maxC ^{4-7h} (mmol/L)	7.57	0.27	7.95	0.24
Tmax ^{4-7hours} (hours)	5.78	0.14	5.87	0.11
IAUC (mmol/L ×hour)	2.35	1.13	3.42	1.10
<u>Insulin response</u>				
maxC ^{1-4h} (pmol/L)	246.4	24.1	319 ²	26.44
Tmax ^{1-4hours} (hours)	1.57	0.12	1.22	0.09
maxC ^{4-7h} (pmol/L)	302	29.8	323	26.4
Tmax ^{4-7hours} (hours)	5.91	0.15	5.83	0.12
IAUC (pmol/L ×hour)	733	86.3	907.7 ³	76.5
<u>Oxidative capacity</u>				
maxC ^{1-4h} (μmol/L)	310.3	20.7	310.8	19.5
Tmax ^{1-4hours} (hours)	1.3	0.1	1.3	0.2
maxC ^{4-7h} (μmol/L)	293.5	20.8	294.5	17.0
Tmax ^{4-7hours} (hours)	5.6	0.2	5.4	0.2
IAUC (μmol/L ×hour)	-30.5	127.4	-165 ⁴	128.1

¹ 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.

²⁻³ significantly different to control, P<0.01

⁴significantly 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.