University of Reading

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. ORCID: https://orcid.org/0000-0003-2931-7274 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: 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

University of Reading

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

Consumption of A Flavonoid -Rich Açai Meal is Associated with Acute Improvements in Vascular Function and a Reduction in Oxidative Stress in Healthy Overweight Men.

[1-2] Randah M. Alqurashi, [1]Laura A Galante, [1]Ian R. Rowland, [1]Jeremy PE. Spencer, [1]Daniel M. Commane.

Alqurashi, Galante, Rowland, Spencer, Commane,

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

[2]Supported by the Saudi Arabian Ministry of Education. Kingdom of Saudi Arabia

Abbreviations used: AS, Açai smoothie; CVD, cardiovascular disease; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HR, heart rate; PS, control smoothie; SBP, Systolic blood pressure; TOC, Total oxidant capacity.

This trial is registered at Clinicaltrials.Gov as NCT02292329

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^2, 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 mg of total phenolics improved vascular function, as measured by FMD, with post prandial increases from baseline of 1.4% at 2 hours, and 0.8% at 6 hours. There was also a significantly lower incremental area under the curve (IAUC) for total peroxide oxidative status after açai consumption relative to control. No significant changes were observed in blood pressure, heart rate or the post-prandial glucose response. However, the first post-prandial insulin peak (after breakfast) and the IAUC for insulin were elevated for açai relative to control.

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

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

Introduction

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 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 mechanisms involved in food mediated disease prevention (3). Polyphenols are widely 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 *in vivo* (4). Polyphenols may also improve endothelial function, lower blood pressure (5, 6), improve insulin sensitivity (7), decrease low-density lipoprotein level (LDL) (8), and modulate inflammatory response (9). Dietary intervention with polyphenol rich berries induce improvements in risk factors associated with metabolic syndrome, diabetes and cardiovascular disease (10, 11).

The flow mediated dilation (FMD) method is a measure of arterial endothelial dysfunction 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 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 polyphenols present in blueberry may mediate a benefical effect on FMD. Several other 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 consumed in an acute setting.

The açai fruit (Euterpe oleracea Mart., Arecaceae) is notable for its very high concentration of flavonoids; this suggests potential health benefits from its

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 global health food market (17, 18). In the UK consumption of açai remains very low; it is 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 characterization of potential benefits from consuming açai is therefore necessary to better inform the consumer and to establish appropriate dietary recommendations for the prevention of disease (19) .

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.

Subjects and Methods.

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

Glucose). Serum insulin was determined with an enzyme-immunoassay using reagents supplied by Dako Cytomation and a GENios plate reader (Tecan Group). Blood tubes for serum and plasma were supplied by Geriner BioOne Ltd (UK). All other chemicals and reagents were obtained from Sigma-Aldrich Co Ltd or Fisher Scientific.

Intervention study subjects

Twenty-three male volunteers were recruited from the University of Reading and surrounding area through the Hugh Sinclair Unit of Human Nutrition volunteer database, and through advertisements within the local community between September 2014 and January 2015. Volunteers were required to be of good general health but with a BMI of 25-30 kg m^2 which is associated with a slight increase in risk of developing metabolic disease. The inclusion criteria were as follows: male, aged 30-65 years, non-smokers, total cholesterol <8.0 mmol/ml. Volunteers were excluded if they suffered from diabetes, anemia, cardiovascular, renal, gastrointestinal, hepatic disease or were being medicated for hyperlipidemia, hypertension, inflammation or depression, if they were on a weight reduction program or 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 out due to a change in his personal circumstances (**Figure 1**).

Study design and treatments.

The study was registered as a clinical trial (Clinical trials.gov ID: NCT02292329) and was conducted according to the Declaration of Helsinki and 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). The study was an acute randomized, controlled, double blind crossover study with an açai 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 additives. Analysis of the nutritional profile of both smoothies can be seen in **Table 1**. The control smoothie was prepared from 50 g of banana and matched for fat by 1.5 g palmitic acid and 8.5g sunflower oil (30% oleic acid, 60% linoleic acid and 10% palmitic acid, both purchased from Sigma-Aldrich UK), carbohydrate (maltodextrin, Myprotein, UK) and fiber (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 (61.6% oleic acid, 12.6% linoleic acid and 25.7 % palmitic acid). The smoothies were prepared fresh in the early morning on the day of the intervention.

Participants were given dietary advice directing them away from consuming polyphenol-rich 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 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. Subjects were rested in the unit for 30 minutes prior to measurements of baseline blood pressure and FMD. Blood (18 ml) was then collected via a cannula inserted into the antecubital vein of the forearm (Study procedure is shown in **Supplement figure 1**). 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. An independent researcher, unaware of the study arm, collected blood samples at regular 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-µm column (4.6 × 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 µL 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).

Nitrite and nitrate in test foods.

Nitrite and Nitrate were assessed in aqueous extracts of test foods using the ENO-30, HPLC-based approach. Briefly, smoothies were prepared and mixed with distilled low nitrite/nitrate water (50:50). Samples were centrifuged at 3000g for 10 min and the supernatant passed through a 0.45µM syringe filter. 200 µl was collected and 10µl was immediately injected into the ENO-30 system. Calibration curves for nitrite and nitrate were prepared using NaNO2 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 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 prepared from pure water (450 mL) and 100 mL of methanol and Reactor Powder B (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 pressures were A 300 µl/min and B 100 µl/min.

Vascular function and measurements

FMD.

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 Ultrasound HDI-15000 system (ATL Ultrasound, UK) in combination with a semi-automated 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 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 proximal to the antecubital fossa. After baseline images were recorded over 60 seconds, a 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.

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.

Biochemical analysis.

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 4º C. Vacutainer rapid serum separator tubes were used to collect blood and left at room temperature (RT) for 30 minutes to allow for clot formation, then centrifuged at 1600 x g for 15 minutes at RT to isolate serum. Urine samples were collected at different time points (0-7 and 7-24 hours) the total volume of urine produced each time point was recorded and aliquots were prepared for storage after centrifugation at 1600g for 10 minutes at 4ºC. Blood and urine samples were stored at -80ºC until further analysis. Serum lipids were measured at baseline via the iLAB 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 an ILAB600 auto-Analyzer (Warrington, UK). Serum insulin concentrations at each time point were analyzed using an enzyme linked immunosorbent assay (ELISA).

Total oxidant capacity (TOC)

Total oxidant capacity in plasma was assessed as a measure of total peroxide levels according to the method of Tatzber et al. (22). Briefly, 10 µL of standards (0, 0.125, 0.25, 0.5, 1mmol/L 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 fresh) contained 20ml reaction buffer (phosphate-citrate 0.05M, pH 5.0), 20 ml of substrate solution (3,3',5,5'-Tetramethylbenzidine (TMB) solution at 1mg/ml in DMSO), and 20µl of peroxidase solution (10mg/ml in phosphate buffer 0.1M, pH 6.0). The 96-well plate was incubated for 20 mins on ice and the reaction was then stopped by the addition 2M sulfuric acid. Absorbance was measured immediately at a wavelength of 450nm on a GENios plate reader at room temperature with Magellan™ software.

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 ($Tmax^{1\text{-}4h}$ and $Tmax^{4\text{-}7h}$) 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 ***$P<0.001$, **$P<0.01$, *$P<0.05$. All statistics were performed using SPSS software (Version 18).

Results

Baseline characteristics of the study participants.

The baseline characteristics of the study population are summarized in **Table 2**. The enrolled subjects were men at slightly elevated risk of metabolic syndrome with a BMI in the overweight category and a mean age of 46 (SEM 1.9 years). At screening the subjects had a mean waist circumference of 97.6 cm (SEM 1.6 cm) and serum lipids within the normal range (**Table 2**). The subjects were normoglycemic, test results for hemoglobin and for liver 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 participants.

Polyphenol content of smoothies

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

Vascular function.

Flow mediated dilation (FMD) changed from baseline over the course of the study day (two-way 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 magnitude of increase in FMD from baseline after consumption of the açai smoothie was 1.4% (SEM 0.6%) (p=0.034, T-test), compared to only a 0.4% (SEM 0.6 %) (p=0.52) 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 -0.5% (SEM 0.7%) below that of baseline levels. A second peak in FMD was observed at 6 h 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**).

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

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 $^{1\text{-}4h}$) (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).

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.

Discussion

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

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 window. A comparison of the composition of the açai versus the control food revealed that the control smoothie was matched to within 1% for energy and for fiber, to within 10% for 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 the açai smoothie. In contrast, the açai smoothie contained 694 mg of flavonoids and phenolic 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 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 biological outcomes. Both acute and chronic dietary interventions with other plant 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 have also been shown to induce this effect in hypercholesteremic volunteers (28). We observed peaks in FMD at 2 and 6 hours post consumption of the açai smoothie, Rodriguez et al. identified similar peaks in post-prandial FMD in healthy men fed blueberry at 2, and 6 hours, and this correlated with peaks in plasma concentrations of phenolic acid metabolites and a reduction of neutrophil NADPH oxidase activity in that study (13). This time course may reflect the emergence of phenolic metabolites in plasma following small intestinal absorption and later, at 6 hours, the liberation of phenolic compounds during colonic fermentation (29-31)

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).

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

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.

Acknowledgments

We thank all of the subjects for their collaboration and gratefully acknowledge our appreciation to Rada G. Mihaylove and Karen Jenkins for cannulation of the study subjects, and Jan Luff for logistical help throughout the study. We also thank Mark Pardy for his assistance on study visits. The study was funded by the Saudi Arabian Ministry of Education (Saudi Arabia). All authors have read and approved the final manuscript. The authors have no other conflicts of interest to declare. DC and JS designed the research, RA and LG conducted the study, IR and DC reviewed the manuscript, RA analyzed the data and prepared the 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 Foods[1].

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

[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 population[1]

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

[1]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)[1].

Measures	Timepoint							
	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
DBP (mm Hg)								
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
Heart rate (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

[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 capacity[1].

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

[4]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.