Protective effect of *Euterpe oleracea* Mart (açaí) extract on programmed changes in the adult rat offspring caused by maternal protein restriction during pregnancy

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**Keywords**

*Euterpe oleracea* Mart.; hypertension; low protein; oxidative stress; renal failure

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Received December 9, 2013
Accepted March 2, 2014
doi: 10.1111/jphp.12258

**Abstract**

**Objectives** This study examined the effect of açaí (*Euterpe oleracea* Mart.) seed extract (ASE) on cardiovascular and renal alterations in adult offspring, whose mothers were fed a low-protein (LP) diet during pregnancy.

**Methods** Four groups of rats were fed: control diet (20% protein); ASE (200 mg/kg per day); and LP (6% protein); LP + ASE (6% protein + ASE) during pregnancy. After weaning, all male offspring were fed a control diet and sacrificed at 4 months old. We evaluated the blood pressure, vascular function, serum and urinary parameters, plasma and kidney oxidative damage, and antioxidant activity and renal structural changes.

**Key findings** Hypertension and the reduced acetylcholine-induced vasodilation in the LP group were prevented by ASE. Serum levels of urea, creatinine and fractional excretion of sodium were increased in LP and reduced in LP + ASE. ASE improved nitrite levels and the superoxide dismutase and glutathione peroxidase activity in LP, with a corresponding decrease of malondialdehyde and protein carbonyl levels. Kidney volume and glomeruli number were reduced and glomerular volume was increased in LP. These renal alterations were prevented by ASE.

**Conclusions** Treatment of protein-restricted dams with ASE provides protection from later-life hypertension, oxidative stress, renal functional and structural changes, probably through a vasodilator and antioxidant activity.

**Introduction**

Numerous experimental and epidemiological studies have highlighted the association between small body size at birth and later cardiovascular disease and its biological risk factors.[1] This association is explained by the concept of ‘developmental programming’ that establishes a relationship between an adverse intra-uterine or early postnatal nutritional environment and permanent changes in the activity of functional systems, predisposing the development of diseases in later life.[2] There is growing evidence that maternal dietary composition and intake are important determinants of fetal growth and development, including aspects specific to reproduction and pregnancy outcomes, in both animal and human.[3] It has been shown that the experimental model of perinatal protein restriction in rats has several features in common with the observations in humans. In particular, offspring of rats that were protein restricted during pregnancy have lower than normal birth weight, develop hypertension in adulthood,[4] endothelial dysfunction,[5] oxidative stress[6] and have reduced nephron number.[7] These indicate that maternal undernutrition...
correlates with later disease, implying that fetal nutritional deprivation is a strong programming stimulus.

Over the years, natural products have been shown to be an important source of molecular diversity leading to drug discovery currently used in modern medicine. The plant *Euterpe oleracea* Mart. belongs to the family *Arecaceae* and is popular known as ‘açai’. It is widely diffused in the Amazon region of Brazil, and the fruit has always been an important food source for indigenous population. It is largely consumed in northern Brazil and has been considered one of the most important medicinal plants of the Amazon by its beneficial effect in the treatment of fever, pain, inflammation, and anaemia.[9,10]

Epidemiological studies have shown an inverse correlation between polyphenol-enriched diet and reduced risks of cardiovascular diseases.[11] Chemical studies have shown that fruits of *E. oleracea* Mart. are rich in anthocyanins (cyanidin 3-O-glucoside, and cyanidin 3-O-rutinoside), pro-anthocyanidins (polymerers) and other flavonoids such as epicatechin, catechin homoorientin, orientin, isovitexin and taxifolin deoxyhexose.[12,13]

Recently, we have reported that hydroalcoholic extract of açai seeds (ASE) has a potent endothelium-dependent vasodilator effect and induces nitric oxide (NO) release from endothelial cells in culture.[14] We also observed that chronic treatment with ASE prevents the development of hypertension, endothelial dysfunction, vascular structural changes and oxidative damage in experimental renovascular hypertension.[15,16] Thus, ASE presents an attractive pharmacological profile (efficacious antihypertensive and antioxidant properties without side effects), which suggests its use in pathological cardiovascular and renal conditions. However, there is no report about the activity of ASE on cardiovascular and renal disorders of adult offspring of an experimental model of developmental programming. Hence, this study aimed to test the hypothesis that the beneficial effects of ASE on cardiovascular control of dams would pass on to the male offspring as adults; that is, the cardiovascular and renal derangements in offspring caused by maternal protein deprivation is a strong programming stimulus.

Materials and Methods

Reagents and animals

2,4-Dinitrophenylhydrazine, naphthylenediamide dihydrochloride, acetylsalicylic acid (ACh), eosin, haematoxylin, nicotinamide adenine dinucleotide phosphate (NADPH), ‘noradrenaline’ (NE), nitroglycerin (NG), oxidized glutathione, reduced glutathione, paraplast plus, phosphoric acid, sodium nitrite, sulfanilamide and thiobarbituric acid were purchased from Sigma Chemical (St Louis, MO, USA). Ethanol, formalin and hydrogen peroxide were purchased from Vetec (Duque de Caxias, Brazil). The rats were obtained from the facilities of the Rio de Janeiro State University. All the experiments on animals were reviewed and approved by the Ethics Committee for Experimental Animals Use and Care (CEUA) of Instituto de Biologia Roberto Alcântara Gomes/Universidade do Estado do Rio de Janeiro (protocol: CEUA/021/2010). The CEUA follow guidelines from Intramural Animal Care and Use program of the National Institutes of Health.

Preparation of extract from *E. oleracea* Mart (açai)

*Euterpe oleracea* Mart. fruits were obtained from the Amazon Bay (Pará State, Brazil) voucher specimen MG 205222 Goeldi Museum – Belém do Pará. Hydroalcoholic extracts were obtained from decoction of the seeds of the fruits, as previously described.[16] Typically 100 g of seeds yielded approximately 5 g of lyophilized extract. The content of polyphenols in ASE, measured by analysing for total phenol by Folin–Ciocalteu procedure,[17] was around 265 mg/g of extract. The protein chemical composition of the ASE, analysed according to the official methods of analysis,[18] was 0.4 g of protein/100 g of extract. Recently, an analysis of the composition of ASE was performed by HPLC (Figure 1).[19] Briefly, ASE was analysed on an RP-18 column (250 mm × 4 mm, 5 μm particles; Shimadzu, Kyoto, Japan), and elution was made with solvents A (0.2% (v/v) phosphoric acid) and B (82% (v/v) acetonitrile, 0.04% (v/v) phosphoric acid). Flow rate was 1 ml/min. DAD UV-vis absorption spectra were recorded online during HPLC analysis. The HPLC elution profile observed is strongly indicative of the presence of pro-anthocyanidins. The peak eluting at 37.2 min corresponds to catechin as confirmed by co-injection of a standard and by comparison of the ultraviolet absorption spectrum. The late elution (at 54.7 min) and UV spectrum of the main peak were consistent with the presence of polymeric pro-anthocyanidins.

Experimental groups

Virgin female Wistar rats aged 3 months were caged with one male rat at a proportion of 3 : 1. After mating, determined by vaginal smear, each female was placed in an individual cage. During the pregnancy period (21 days), the dams had free access to standard diet (American Institute of Nutrition (AIN) 93) or low-protein (LP) diet 6% protein. The control group corresponding to male offspring from
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Dams fed the standard diet and allowed access to water (control group: 20% protein) or ASE (ASE group, 200 mg/kg per day, intragastric gavage) during pregnancy. Two other groups were fed the LP diet with access to water (LP group: 6% protein) or ASE (LP + ASE group; 200 mg/kg per day, intragastric gavage) during pregnancy (Table 1). At birth, the number of male pups was set as six per litter, and dams were fed a control diet. After weaning, the animals were fed a normal diet and sacrificed at 4 months old. The body weight (BW) was recorded weekly from 1 day of age until animals were fully grown at 120 days old. The diets (Table 1) were balanced by Rhoster (Rhoster, São Paulo, Brazil) in accordance with the standard recommendations for rodents in the maintenance state of AIN (AIN-93G).[20]

### Arterial pressure measurement and vascular perfusion studies

Systolic blood pressure (SBP) was measured in conscious rats by using tail-cuff plethysmography (Letica 5000 device, Panlab, Cornellà, Spain).

The perfusion pressure of isolated mesenteric arterial beds (MABs) of the rats from the different groups was measured as previously described.[16] The dose-dependent responses to ACh (1–1000 pmol) and NG (1–1000 nmol) were performed in preconstricted MABs with NE (10–30 μmol/l), and the vasodilator effect of the drugs was expressed as the percentage decrease of the pressor effect of NE.[16]

### Determination of oxidative damage: malondialdehyde and carbonyl protein assay

The lipid membrane damage was determined by formation of products of lipid peroxidation (malondialdehyde – MDA) concentration from plasma and kidney homogenates using the thiobarbituric acid reactive substances method as previously described.[21] Protein carbonylation was determined according to the method described by Levine et al.[22]
Nitrite assay and superoxide dismutase, catalase and glutathione peroxidase activity

Nitrite levels in plasma and kidney homogenates were determined by a method based on the Griess reaction. Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline auto-oxidation as absorbance at 480 nm. Catalase (CAT) activity was measured in terms of the rate of decrease in hydrogen peroxide at 240 nm. Glutathione peroxidase (GPx) activity was measured by monitoring the oxidation of NADPH at 340 nm in the presence of hydrogen peroxide.

Serum and plasma assays

Blood was collected from the rats at 120 days old. The animals were fasted for 12 h, and blood samples were then collected by thoracic aorta puncture in anaesthetized animals. Serum albumin, creatinine and urea levels were measured by a colorimetric assay (Bioclín, Belo Horizonte, Brazil). Serum sodium and potassium concentrations were measured by flame photometry (Laborlife, Clinical Analysis, Rio de Janeiro, Brazil). Plasma renin level was measured by radioimmunoassay (Active Renin IRMA kit, Beckman Coulter, Brea, CA, USA).

Urine measurement

Rats were placed individually in plastic metabolic cages, and urine was collected for 24 h. Urinary protein excretion was measured by the Bradford method and urinary sodium concentrations by flame photometry. Renal clearance was calculated by a standard formula (C_cr = urinary creatinine × urinary flow/serum creatinine). Fractional sodium excretion was calculated by a standard formula (FENa' = urinary Na' × serum creatinine × 100/serum Na' × urinary creatinine).

Light microscopy and stereology

The left kidneys were longitudinally divided in two halves, which were embedded face down in Paraplast plus (Sigma, São Paulo, Brazil), then serially sectioned at a nominal thickness of 5 μm and stained with H&E. Both cortex and medulla volumes were estimated according to Cavalieri’s method, glomeruli volume density (Vv(glm)) by the point-counting method, and fractionator method was used to estimate the glomeruli number in a slice (starting with a random number for each 10th section). The total number of glomeruli per kidney (N(glm)) was estimated considering the analysed fraction of the kidney corrected to the entire organ.

Statistical analyses

Data are shown as mean ± standard error of the mean. Statistical differences were evaluated by a two-way analysis of variance, with Bonferroni and Tukey post-hoc test. P-values less than or equal to 0.05 were accepted as statistically significant.

Results

Effect of ASE on body weight

At the end of gestation, there was no change in the maternal BW (g) among groups (control group: 256.4 ± 11.9, ASE: 256.6 ± 8.9, LP: 246.3 ± 8.1, LP + ASE: 255.3 ± 7.9). At 1 day of life, offspring BW of LP group was lower (P < 0.05)
compared with controls (Table 2). Treatment of LP-fed dams with ASE during pregnancy prevented ($P < 0.05$) offspring BW reduction (Table 2). At 120 days of age, there was no difference in offspring BW among groups.

**Effects of ASE on blood pressure and vascular function**

SBP was increased ($P < 0.05$) in LP group at 120 days of age compared with control groups. Treatment of LP-fed dams with ASE during pregnancy prevented SBP elevation (Figure 2). The reduced ACh-induced vasodilation ($P < 0.05$) in MAB from LP group was restored by treatment with ASE (Figure 2). Endothelium-independent response to NG was not different among groups (Figure 2).

**Effects of ASE on oxidative damage**

MDA and carbonyl protein levels in plasma and kidney samples were higher ($P < 0.05$) in the LP group than in the control one (Figure 3). Treatment with ASE reduced ($P < 0.05$) plasma and kidney protein carbonyl levels, but also plasma MDA levels in LP + ASE group. MDA levels from kidney homogenates of LP + ASE rats showed no significant difference from those of LP group.

**Effects of ASE on plasma and kidney nitrite content**

LP group showed lower plasma and kidney nitrite levels compared with the control groups ($P < 0.05$, Figure 3). ASE prevented kidney nitrite levels decrease ($P < 0.05$) with no change in reduced plasma nitrite levels.

**Effects of ASE on plasma and kidney antioxidant enzymes (SOD, CAT and GPx)**

SOD and GPx activity were lower in plasma of the LP group ($P < 0.05$, Figure 4) as compared with controls, and treatment with ASE recovered the enzymatic activity ($P < 0.05$, Figure 4). The decreased kidney SOD activity in the LP group ($P < 0.05$) was not restored by ASE. Treatment of control group with ASE increased kidney SOD activity ($P < 0.05$). Kidney CAT and GPx activity, as well as plasma CAT activity were not significantly different among groups.

**Effects of ASE on serum creatinine, albumin, urea and plasma renin levels**

Creatinine, urea and renin plasma levels were higher ($P < 0.05$) in the LP group compared with controls (Table 2). ASE treatment normalized creatinine, urea and renin plasma levels (Table 2). The reduced albumin concentrations ($P < 0.05$) in the LP group Ecompared with controls was prevented by ASE ($P < 0.05$; Table 2).
Effects of ASE on serum and urine parameters

Urinary protein excretion (proteinuria) and serum potassium were increased in the LP group compared with controls \((P < 0.05; \text{Table 2})\), and treatment with ASE did not alter these parameters. The increased \(\text{FENa}^+\) excretion in LP group \((P < 0.05)\) was normalized by ASE \((P < 0.05)\), and the serum sodium, renal clearance and urinary volume were not different among groups (Table 2).

Effects of ASE on kidney structure

Total glomeruli number and kidney volume were decreased in LP group compared with controls \((P < 0.05)\). The decrease in those parameters was reduced by ASE \((P < 0.05; \text{Figure 5})\). Glomerular volume was increased in the LP group compared with controls \((P < 0.05)\), and treatment with ASE normalized this parameter \((P < 0.05; \text{Figure 5})\).

Discussion

The metabolic programming can be defined as a uterine event that has permanent effects on the physiology, structure and metabolism of the individual.\[28\] Undernutrition during pregnancy impairs maternal haemodynamic adaptations, such as, systemic vasodilatation, increase in basal oxygen consumption and alveolar ventilation resulting in insufficient rise in cardiac output, plasma volume and
Several studies have shown that a protein restriction diet during pregnancy can 'programme' functional and structural alterations in adult offspring, such as hypertension, endothelial dysfunction and decreased nephron number. In this study, we demonstrated, for the first time, the preventive action of ASE on the cardiovascular and renal function, as well as renal structural changes observed in offspring from dams fed a LP diet during pregnancy.

Exposure to an LP diet during pregnancy was associated with an increase in SPB of adult offspring, which is in accordance with previous findings in the same experimental model. The mechanism involved in the development of hypertension observed in offspring from dams fed a LP diet is, at the moment, not established. Probably, oxidative stress, endothelial dysfunction and renin–angiotensin system may play a pathogenic role in programmed hypertension in adulthood. In this study, we found increased levels of renin in the LP group. Evidence has shown that renin and angiotensin converting enzyme (ACE) activity are elevated in rats exposed to an LP diet in the uterus, and brief administration of an ACE inhibitor lowered blood pressure in both neonates and young adults.

Treatment of LP-fed dams during pregnancy with ASE prevented the increase in renin plasma levels contributing for maintenance of normal blood pressure levels of adult offspring.

**Figure 4** Effects of açaí (*Euterpe oleracea* Mart.) seed extract (200 mg/kg per day) on (a and b) superoxide dismutase, (c and d) glutathione peroxidase and (e and f) catalase activity in the plasma and kidney of 120-day-old adult offspring of control or low-protein fed dams during pregnancy. Data are mean ± standard error of the mean, *n* = 8 for all groups. *Significantly different (*P* < 0.05) from control; #significantly different (*P* < 0.05) from açaí (*Euterpe oleracea* Mart.) seed extract; +significantly different (*P* < 0.05) from low protein.
offspring. The antihypertensive effect of ASE has previously been demonstrated by our group in different models of hypertension,\textsuperscript{[15,16]} but the present result suggests that the consumption of ASE early during pregnancy confers protection against the insult of a LP diet in adult offspring. This antihypertensive effect of ASE, rich in polyphenols, is likely to be mediated by endothelium-dependent vasodilation because our previous findings showed that ASE induces vasodilation of mesenteric arteries of the rat mediated by NO in combination with endothelium-dependent hyperpolarizing factor.\textsuperscript{[14]}

We found in this study a reduced endothelium-dependent vasodilation induced by ACh in the LP group, characterizing an endothelial dysfunction in this model. In line with this finding, a reduction of endothelial responsiveness to ACh\textsuperscript{[31,32]} has been demonstrated in mesenteric arteries from adult offspring whose dams were fed LP diet during pregnancy. Additionally, placental factors may

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{figure5.png}
  \caption{Photomicrographs of the renal cortex (400×). (a) Control, (b) açaí (Euterpe oleracea Mart.) seed extract, (c) low protein and (d) low protein + açaí (Euterpe oleracea Mart.) seed extract groups. Effects of açaí (Euterpe oleracea Mart.) seed extract (200 mg/kg per day) on (e) glomeruli number per area, (f) glomeruli number per kidney, (g) glomerular volume and (h) kidney volume in 120-day-old adult offspring of control or low-protein fed dams during pregnancy. Data are mean ± standard error of the mean, n = 5 for all groups. *Significantly different (P < 0.05) from control; #significantly different (P < 0.05) from açaí (Euterpe oleracea Mart.) seed extract; +significantly different (P < 0.05) from low protein.}
\end{figure}
induce endothelium-derived contracting factors, such as endothelin that has been demonstrated to induce increased vasoconstriction in resistance vessels from adult offspring of the same model.[32] These findings suggest that maternal undernutrition alone is at least detrimental to offspring endothelial function. There are a number of possible explanations for this, including an oxidative insult that could be induced in the neonatal period and perpetuated throughout adulthood,[33] the attenuation in vascular endothelial nitric oxide synthase (eNOS) or in antioxidant protection.[31] Metabolic changes, linked with the LP diet during pregnancy, can also influence the vascular function. These include a reduction in the availability of L-arginine, which could adversely influence endothelial NO synthesis.[34] Growing evidence suggests that the placental nitrergic system is involved in epigenetic fetal programming.[35] Although the arginine levels are not reduced in the plasma and kidney of adult offspring from a maternal caloric restriction rat model, the asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, is increased in plasma,[36] which could also induce endothelial dysfunction. In the same model, increased levels of ADMA are associated with oxidative stress and decreased renal neuronal NOS, resulting in chronic kidney disease progression.[36]

Recently, it was demonstrated that eNOS deficiency reduces uterine blood flow, spiral artery elongation and placental oxygenation in pregnant mice.[37] Furthermore, NO release induced by vascular endothelial growth factor is reduced in uterine arteries from protein-restricted dams, which may contribute to the decrease in uterine artery vasodilator potential,[37] demonstrating an important role of NO on fetal placental homeostasis.

There is evidence that polyphenols present in fruits are able to modulate the production of NO in vascular endothelium, contributing to the prevention of endothelial dysfunction.[38] We found that treatment with ASE prevented the endothelial dysfunction and increased the nitrite levels in adult offspring from LP-fed dams. The beneficial effects of ASE in dams fed LP diet may be related to increased bioavailability of NO in fetal-placental circulation during pregnancy, as our previous findings[14] showed that ASE induces NO production in cultured endothelial cells. The increased NO bioavailability could also counterbalance the increased vasoconstriction observed in resistance vessels from adult offspring of this model.[33]

Oxidative stress has been suggested as causative agent in human pregnancy-related disorders, such as embryonic resorption, recurrent pregnancy loss, pre-eclampsia and intra-uterine growth restriction. Nutritional deficiencies in protein and micronutrient antioxidant vitamins and trace minerals may impair cellular antioxidant capacities because proteins provide the amino acids needed for the synthesis of antioxidant enzymes.[39] Some studies indicate that maternal protein restriction during pregnancy and lactation induces oxidative damage in the offspring by increasing membrane lipid peroxidation and decreasing the antioxidant defence.[40] In this study, oxidative damage, assessed by MDA and protein carbonyl protein levels in plasma and kidney homogenates, was increased in the LP group. In accordance with previous findings in experimental model of maternal protein restriction,[41] the adult offspring of LP group showed a reduction in antioxidant enzyme activity (SOD and GPx) in kidney and plasma homogenates. Therefore, the increased MDA and carbonyl protein levels, associated with decreased nitrite levels and antioxidant activity in plasma and kidney may also contribute to decrease NO bioavailability, which can explain in part the endothelial dysfunction and early increase in blood pressure because it is largely known that NO contributes to the maintenance of SBP. We found that treatment with ASE during pregnancy reduced MDA and protein carbonyl levels, and increased antioxidant enzyme activity in the adult offspring, suggesting that its antioxidant action during developmental intrauterine period may be associated with the beneficial effects of ASE. The antioxidant action of ASE was previously demonstrated by our group in different experimental models of hypertension.[15,16]

Therefore, our results indicate that the beneficial effects of ASE in dams fed LP diet during pregnancy may pass to adult offspring and is probably associated with the improvement of the endothelial function in fetal-placental circulation. The increased NO production in endothelial cells[14] and antioxidant effects produced by ASE may increase NO bioavailability, leading to the improvement of uterine artery vasodilatation, blood flow oxygen and nutrients supply to the fetus.

LP diet in dams during pregnancy induces disturbances in the fetal placental homeostasis that can explain the reduction of BW,[7] glomeruli number and increase of the glomerular volume in the offspring.[12] These results are associated with altered renal parameters such as hipoalbunaemia, proteinuria, hypertension and increased levels of urea, creatinine and %FENa* of animals from the LP group. The exact mechanism involved in the reduced number of nephrons due to maternal protein restriction remains unclear, but increased evidence shows that apoptosis in the developing kidney and suppression of renin–angiotensin system in newborns could inhibit nephrogenesis, reducing the number of nephrons leading to a fall in the rate of total glomerular filtration.[43] Alterations in glomerular volume density could be mainly the result of an average glomerular volume, an event that has been proposed as a key stage in the pathogenesis of secondary renal lesions, including arterial hypertension, which represents a common final pathway for the eventual development of glomerulosclerosis.[42] Treatment with ASE
during pregnancy improved the renal structural changes, normalized BW, urea, creatinine, %FENa⁺ and serum albumin levels in the adult offspring, probably by its antioxidant action, contributing to improvement of nephrogenesis, renal function and protecting against the development of renal disease. Further studies should be carried out to elucidate the oxidant and renin–angiotensin system-regulating mechanisms by ASE.

Conclusion

In conclusion, this study provides further support for the early postnatal environment playing a critical role in programming later life hypertension, endothelial dysfunction, renal functional, and structural disorders and oxidative stress. These data emphasize the importance of a balanced diet during pregnancy and the consequences on chronic disease incidence if an unhealthy diet is consumed during this period. We suggest that the administration of ASE in rats fed an LP diet during pregnancy could contribute to uterine artery vasodilation, blood flow oxygen and nutrients supply to the fetus. These beneficial effects may be related to increased bioavailability of NO and to their antioxidant actions, contributing to the improvement of the parameters observed in this study. Thus, the administration of ASE may offer a promising natural and safe new trend for treatment and prevention of changes caused by maternal protein restriction.

Declarations

Conflicts of interest

Roberto Soares de Moura is inventor of a patent PCT/BR2007/000178 that may support the development of a product. The other authors state no conflict of interest.

Funding

This work was supported by the National Council of Scientific and Technological Development (CNPq, Protocol 473514/2011-7) and Rio de Janeiro State Research Agency (FAPERJ; Protocol E-26/102.920/2011).

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