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EFFECT OF ACUTE BABASSU MESOCARP FLOUR SUPPLEMENTATION (*ORBIGNYA PHALERATA* MART.) ON AEROBIC CAPACITY, OXIDATIVE STRESS, AND MUSCLE DAMAGE IN RECREATIONAL RUNNERS: A RANDOMIZED, CROSSOVER, PLACEBO-CONTROLLED STUDY

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ABSTRACT

This study aimed to evaluate the effects of acute babassu mesocarp flour (BM) supplementation on aerobic capacity, oxidative stress, and muscle damage in recreational runners. 14 men (26,4±8,3 years) were randomly assigned and ingested one single dose of (BM) and maltodextrin (PLA) supplements and performed the 3200m running test. There was a 7-days washout period between each supplementation. Biochemical parameters such as serum glucose, malondialdehyde, creatine kinase, and lactate dehydrogenase were evaluated. There were no differences between the conditions for the time-trial performance (BM=12.3±1.5 vs PLA=12.2±1.3 s, t=1.743, p=0.612, *d*=0.10) and maximum heart rate (BM=106.7±9.8 vs PLA=111.6±15.3 bpm, t=-1.286, p=0.221, *d*=0.36). There was an increase in glucose, creatine kinase, lactate dehydrogenase, and malondialdehyde ($p<0.001$, $\eta^2<0.96$) in both procedures. However, there was no condition x time interaction ($p=0.469$, $\eta^2=0.04$). Babassu mesocarp flour supplementation did not alter aerobic capacity, serum glucose, oxidative stress and muscle damage markers in recreational runners.

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INTRODUCTION

Babassu mesocarp is a component of the babassu palm tree fruit (*Orbignya phalerata* Mart.) (Vinhal, Lima, & Barbosa, 2014) and is well known for its nutritional value due to its high-carbohydrate content and antioxidant components, such as polyphenols, as well as triterpenes, glycosylated triterpenes, tannins, saponins, and steroids (de Almeida et al., 2011). The medicinal potential of babassu mesocarp has been acknowledged through pre-clinical trials which show its biological activity due to its anti-inflammatory and immunomodulatory properties (Teixeira, Teixeira, Eiras, & Bezerra, 2013). Carbohydrate (CHO) intake is the most widespread nutritional strategy in order to improve physical performance and recovery of athletes, especially in aerobic sports, as it partially replenishes the glycogen storage during the exercise (Hawley & Leckey, 2015; Helge, 2017; Thomas, Erdman, & Burke, 2016). Stellingwerff and Cox (Stellingwerff & Cox, 2014) conducted a metanalyses of 61 studies to evaluate the efficacy of carbohydrate supplementation during physical exercises. They concluded that 82% of the studies demonstrated performance enhancement and longer time to exhaustion after CHO supplementation compared to placebo intake. Moreover, several studies point out the positive effects of the use of antioxidant as an ergogenic strategy, mostly due to the increase in the synthesis and bioavailability of nitric oxide (NO) (Freedman et al., 2001), which minimizes post-exercise muscle soreness (Howatson et al., 2009; Levers et al., 2016). Previous studies demonstrated additional benefits of

CHO intake associated with antioxidants, which may improve the recovery process after strenuous exercise (Carvalho et al., 2018; L. T. Toscano et al., 2015) Toscano et al. (2019) concluded that the supplementation with 10mL/kg/day of purple grape juice showed ergogenic effect in recreational runners, as well as increase in time to exhaustion and antioxidant activity. However, there were no alterations in the muscle damage and inflammation. Moreover, Nishizawa et al. (2011) verified that lychee fruit extract supplementation decreased inflammation and tissue damage induced by exercise. On the other hand, it did not improve physical performance in 5000m runners. Although there are studies verifying the long-term benefits of this supplementation, its effect on short-duration exercises needs to be better elucidated. In addition, it is believed that babassu mesocarp flour could be an important alternative for athletes as it is rich in CHO and polyphenols. Although the anti-inflammatory and antioxidant properties of the babassu mesocarp had already been tested, the ergogenic property of its acute supplementation has not been investigated yet. Thus, this study aimed to evaluate the effect of acute babassu mesocarp flour supplementation in the aerobic performance, oxidative stress, and muscle damage in recreational runners. We hypothesize that babassu mesocarp could be a strategy to optimize aerobic performance, decrease oxidative stress markers, and muscle damage.

MATERIALS AND METHODS

Participants: This is a randomized, cross-over, and placebo-controlled study. Fourteen recreational runners were randomly assigned (www.randomizer.org) to received two supplements: Babassu mesocarp flour (BM) and maltodextrin (placebo) (PLA). The sample size was calculated using the Gpower 3.1 software (Franz Faul, Universitat Kiel, Germany). The previous investigation by Tsintzas et al. (1996) was taken into consideration, which observed that the time to exhaustion in endurance runners after carbohydrate supplementation was 124.5 ± 8.4 min while with water ingestion was 109.6 ± 9.6 min, resulting in an effect size of 0.86. The minimum sample size of 10 participants was determined (α error = 0.05, statistical power (β error) = 0.90). The inclusion criteria for this study was at least one year of training (three uninterrupted months), five days a week (three running trainings a week), no chronic diseases, and no dietetic supplementation for the last three months. During the research period, the participants were asked to abstain from any food derived from babassu, foods high in antioxidant, and alcoholic drinks. No smokers we included in this study. Initially, thirty-seven recreational runners were recruited to participate in this study. However, only twenty met the inclusion criteria. The participants were randomly allocated to ingest BM and PLA. During the research period, four athletes decided to leave the study and two suffered an injury. Therefore, this study was carried out with fourteen participants (Figure 1). This study was approved by the ethics committee of the Universidade Federal do Piauí (UFPI), Teresina, PI, Brazil under protocol 2.445.726. All participants signed the consent form according to the resolution 196/96 of the National Health Council.

Experimental design: The participants were asked to refrain from physical training 48 hours and food intake 12 hours prior to the tests. All volunteers were anthropometrically evaluated (weight, height, bioimpedance) and underwent a nutritional evaluation. Afterwards, they had their blood collected for analysis of oxidative stress, muscle damage, and glycemia markers. After 30 minutes, the volunteers had breakfast (bread, cheese, and milk). There was an interval of two hours between breakfast and the supplementation. During this time, the subjects were instructed to complete a 24-hr dietary recall. 30 minutes before the 3200m run test, all volunteers ingested a dose of one of the supplements. Blood sample was again collected after the end of the exercise session (Figure 2). All participants performed the same procedures in two different moments, one for each supplement, according to the randomization order. There was a wash-out period of seven days between each moment.

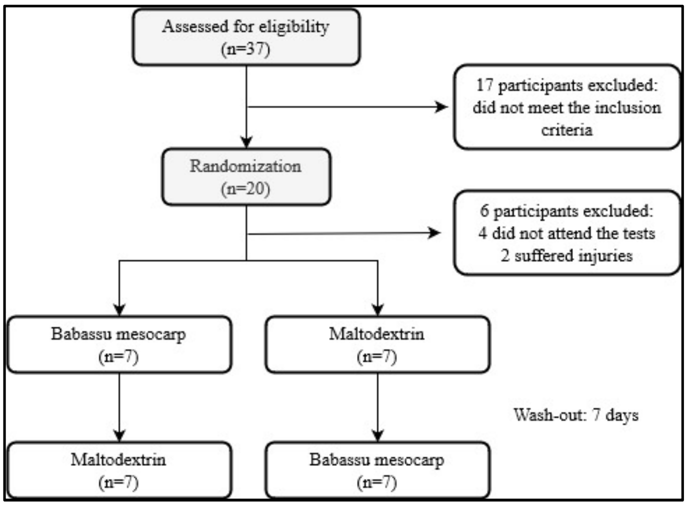

Figure 1. CONSORT Flow diagram

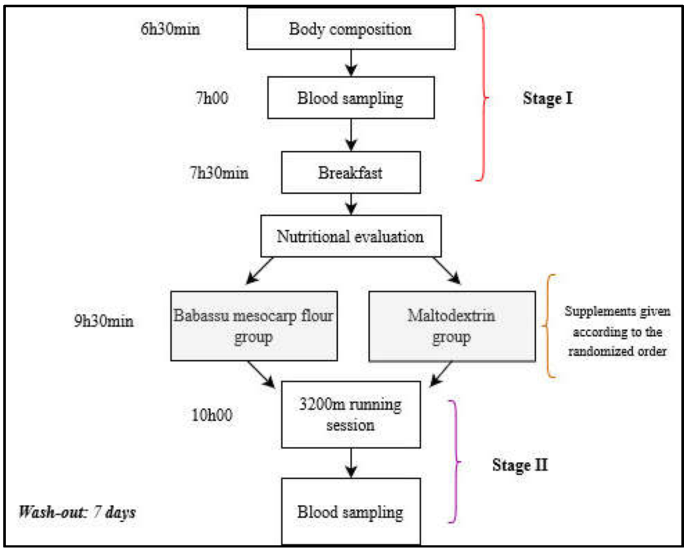

Figure 2. Flow diagram describing the design of the study

Supplementation protocol: All supplements were orally administered 30 minutes before the run test. The babassu mesocarp flour supplement was administrated in 0.4 g/Kg/day dosages, diluted in 0.5 liters of water, which is the quantity usually ingested by adults according to Silva et al. (2012). The control group took tasteless maltodextrin under isocaloric, isoglycidic, and isovolumetric administration. The supplement dosage was calculated according to the body weight of each athlete. The athletes were instructed to report any gastrointestinal discomfort after the supplementation at any moment during the tests. The babassu mesocarp flour (*Orbignya phalerata* Mart.) used in this study was provided by Babcoall, Teresina, PI, Brazil, which contains carbohydrate, protein, fibers (72%, 7%, 2,82%, respectively), and high levels of phenolics (558,87mg/dl) (Silva et al., 2012).

Nutritional evaluation: Dietary intake was evaluated via 24-hour dietary recall, which was conducted 3 times for each participant. Two of them referred to weekdays and one to the weekend. The average dietary intake was used to calculate the nutrients ingestion via software Avanutri Revolution, version 4.0 (Avanutri, Rio de Janeiro, Brazil). Body composition was analyzed using tetrapolar bioelectrical impedance analysis at 50 kHz using the device InBody® (model S20, Biospace Co Ltd, Seoul, South Korea) (NATIONAL INSTITUTE OF HEALTH, 1996) in a climatized room located at the Exercise Physiology Lab, Biophysics and Physiology Department, Universidade Federal do Piauí. All participants wore light clothes and were requested to urinate before the assessments. Moreover, they were asked to refrain from food and drink intake for 4 hours, intense exercise for 24 hours, and alcohol or caffeine intake for 48 hours before the tests.

Running performance evaluation: In order to evaluate the aerobic capacity, all participants completed the 3200m run test in an official running track at Universidade Federal do Piauí. All running sessions were performed under orientation and supervision of an experienced and trained Exercise Science professional. They were instructed to run as fast as possible and the running time was registered in minutes. The maximum heart rate was registered immediately after the testing using the heart rate monitor Polar V800 (Polar® Electro Oy, Kempele, Finland).

Data collection and biochemical analysis: Malondialdehyde (MDA) concentrations were determined via the production of thiobarbituric acid reactive substance (TBARS), according to the method described by Ohkawa et al. (1979). In order to calculate the corrected absorbance, which is proposed to minimize the interference of heme pigments and hemoglobin, the following formula was applied:

$$ABS = 1.22 \times [A532 - (0.56 \times A510) + (0.44 \times A560)]$$

Before the sampling processing, an analytical calibration curve was prepared using MDA as standard, in concentrations of 1, 5, 10, 25, and 50 nmol/mL. The results were displayed in nmol of MDA per mL of plasma. The plasmatic concentration of creatine kinase was kinetically determined according to the method proposed by the *International Federation of Clinical Chemistry and Laboratory Medicine* (UV-IFCC, 2002), using the commercial kit CK-NAC Liquiform (Labtest, Minas Gerais, Brazil). The plasmatic concentration of lactate dehydrogenase was kinetically determined via the Pyruvate-lactate method, using the commercial kit LDH Liquiform (Labtest, Minas Gerais, Brazil), following the manufacture's instruction. The absorbances were obtained in 340 nm (Labmax 240 premium analyzer, Labtest, Minas Gerais, Brazil). Blood glucose concentrations were determined via glucose oxidase enzymatic-colorimetric method (GOD-Trinder), using the commercial kit Glucose PAP Liquiform (Labtest, Minas Gerais, Brazil), in accordance with the manufacturer's instructions. The absorbances were obtained in 505 nm (Labmax 240 premium analyzer, Labtest, Minas Gerais, Brazil).

Statistical analysis: The data are presented as the mean ± standard deviation of the mean. Paired t-test was used to compare kilocalorie and macronutrients between trials. The effect of babassu mesocarp on the 3200m test performance and maximum heart rate (babassu mesocarp vs placebo) was compared using repeated measures ANOVA. Its effect on metabolic and inflammatory response was verified using two-way ANOVA for repeated measures [2 conditions (MB vs PLA) × time (Pre vs Post-exercise)], with Bonferroni post-hoc test. For all measured variables, the estimated sphericity was verified according to the Mauchly's W test and the Greenhouse–Geisser correction was used when necessary. The effect size for performance was calculated via Cohen's d, which describes the difference between the means normalized to the pooled standard deviation (SD) of the two groups (> 0.20 was considered small, > 0.50 moderate, and > 0.80 large) and eta-squared ($\eta 2$) was calculated for time and interaction when ANOVA was used (Cohen, 1988). The significance level was established at $p<0.05$. The statistical analysis was performed using the SPSS version 17.0 statistical software (SPSS, Inc., Chicago, IL, USA).

RESULTS

Baseline description of the subjects is presented on table 1. All athletes were eutrophic and presented optimal body fat percentage, good aerobic capacity, and normal biochemical parameters (Kreider et al., 2010; Mougios, 2007). They were familiar to 5 and 10km running events, thus they were classified as recreational athletes.

Table 1. Characterization of the participants

Variables	Mean ± standard deviation
Age (years)	26.4 ± 8.3
Body mass (Kg)	64.6 ± 7.6
BMI (Kg.m^2)	22.9 ± 2.2
% bf	12.9 ± 6.6
LM (Kg)	31.9 ± 4.2
VO_{2max} (mL.kg-1.min-1)	57.5 ± 5.9
Glucose	80.0 ± 8.3
CK (U/L)	164.2 ± 102.0
LDH (U/L)	289.4 ± 82.8
MDA (nmol/mL)	30.5 ± 8.9

The data are presented as mean ± standard deviation of the mean. %bf, body fat percentage; LM, lean mass; CK, creatine kinase; LDH, lactate dehydrogenase; MDA, malondialdehyde.

Table 2 shows the total average of kilocalorie, macronutrient, and micronutrient intakes for the three days considering the relative body and mass and general characteristics of the subjects. The results obtained from the dietary evaluation showed that the distribution of energy among the macronutrients was 47.14% carbohydrate, 17.43% protein, and 35.43% lipids. This represents 37,25 ± 11,3 Kcal/Kg/day of macronutrients (4,39 ± 2,1 g/Kg/day of carbohydrates, 1,6 ± 0,4 g/Kg/day of proteins, and 1,47 ± 0,8 g/Kg/day of lipids).

Table 2. Total kilocalorie and macronutrients intake

Variables	Mean ± standard deviation
Energy	2405 ± 721,6
Carbohydrates	283,43 ± 104,2
Protein	103,77 ± 57,6
Lipids	94,67 ± 53,2

The data are presented as mean ± standard deviation of the mean.

Regarding the time in seconds in the 3200m test, there was no significant difference between the conditions (BM= 12.3 ± 1.5 sec vs PLA= 12.2 ± 1.3 sec, F= 0.271, p= 0.612), with a trivial effect size (*d*= -0.10). Regarding the maximum heart rate in the 3200m test (BM = 106.7 ± 9.8 vs PLA= 111.6 ± 15.3 bpm), there was no significant difference between the conditions (F= 1.653, p= 0.221) and the effect size was small (*d*= 0.36).

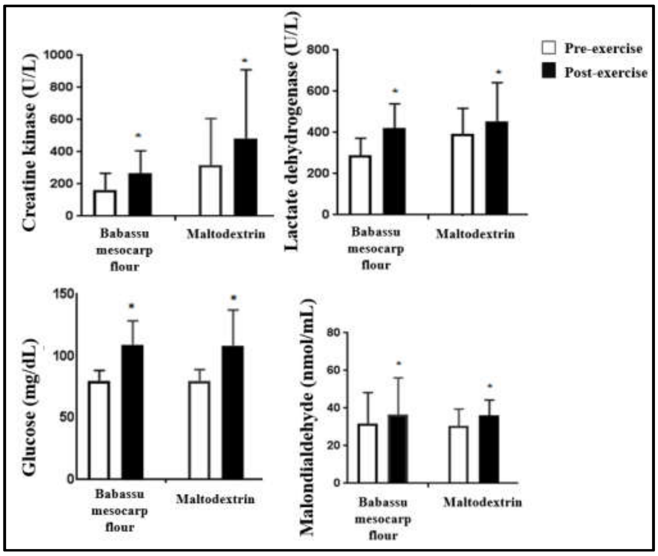

Figure 3. Comparison between the effect of babassu mesocarp flour and maltodextrin in the metabolic and inflammatory responses pre- and post-exercise. Data are presented as mean ± standard deviation of the mean. * = significant intragroup difference (p<0.05) compared to rest

Biochemical responses after the treatments are illustrated in figure 3. There was a significant increase of glucose across time (F= 52.231, p<0.001, η^2= 0.80). however, no condition x time interaction was observed (F= 0.003, p=0.959, η^2= 0.001). Creatine kinase levels significantly increased across time (F= 360.350, p<0.001, η^2= 0.96) and there was a tendency to a significant condition × time interaction (F= 4.350, p=0.057, η^2= 0.25). Similarly, there was a significant increase in lactate dehydrogenase after the test (F= 14.034, p=0.002, η^2= 0.52). However, no significant condition × time interaction was verified (F= 0.556, p= 0.469, η^2= 0.04). MDA was also affected across time (F= 13.628, p=0.003, η^2= 0.51) but there was no significant condition × time interaction (F=1.805, p=0.202, η^2= 0.12).

DISCUSSION

The major finding of this study was that the single-dose babassu mesocarp flour supplement taken 30 min before the 3200m run did not improve the aerobic capacity in recreational runners. Moreover, it did not affect the levels of muscular damage, oxidative stress, and glycemia markers. These findings contrast with the initial hypothesis that the babassu mesocarp flour supplementation would enhance the physical performance of runners due to an increase in CHO and antioxidant levels. To the best of our knowledge, until the present work there were no similar studies addressing babassu mesocarp flour supplementation and physical performance. Babassu mesocarp flour contains high levels of CHO(de Almeida et al., 2011), which enhances physical performance and accelerates recovery by replenishing the glycogen storage (Hawley & Leckey, 2015; Thomas et al., 2016). CHO supplementation also improves performance through attenuation of central fatigue and muscular damage, improvement of glycogen metabolism and resynthesis, and maintenance of CHO oxidation rate and muscle metabolite levels (Kreider et al., 2010). Furthermore, babassu mesocarp flour contains high levels of polyphenols, which can decrease oxidative stress and exercise induced muscle damage. In addition, it can reduce inflammation through several mechanisms, such as the inhibition of prostaglandins and proinflammatory cytokines (Lukaski, 2004). Several high-polyphenol foods have been investigated regarding their effect on physical performance. Cherry juice (Bell, Walshe, Davison, Stevenson, & Howatson, 2014; Connolly, McHugh, & Padilla-Zakour, 2006; Howatson et al., 2009), blueberry (McLeay et al., 2012), and pomegranate (Trombold, Barnes, Critchley, & Coyle, 2010; Trombold, Reinfeld, Casler, & Coyle, 2011)consumption improved strength recovery, and grape and appleintake improved endurance performance (Deley, Guillemet, Allaert, & Babault, 2017). Furthermore, polyphenols contain anti-inflammatory properties, which enhance vascular function through nitric oxide-mediated mechanisms. As a result, it is suggested that fruit-derived polyphenol supplementation can decrease muscle damage and improve physical performance through the attenuation of exercise-induced oxygen reactive species accumulation (Bowtell & Kelly, 2019).

In our study, the consumption of babassu mesocarp flour 30min before a short-duration aerobic exercise did not improve the physical performance. This may be related to the conversion of the bioavailable polyphenols into inactive forms in the stomach, intestine, or liver, which would impair their effect. The levels of hepatic and muscle glycogen, as well as the gastric emptying, may have also interfered in the efficacy of the supplementation (Skinner et al., 2013). In addition, one single dosage may have been insufficient to improve performance. Further studies should investigate the effect of babassu mesocarp flour supplementation in higher dosages and different exercise protocols (mid- and long-duration). Regarding the metabolic and inflammatory responses, there was no significant difference between babassu mesocarp and maltodextrin. Toscano et al. (2015) observed a reduction of oxidative stress and inflammation in recreational runners after grape juice supplementation. However, the significant difference was only observed after 28 weeks of supplementation. Thus, we recommend further studies investigating the chronic effect of babassu mesocarp flour supplementation. In spite of the lack of studies related to the ergogenic effects of babassu mesocarp, some studies verified its positive effect on the wound healing process (Bigelman, Chapman, Freese, Trilk, & Cureton, 2011), anti-inflammatory (Nascimento et al., 2006), antitumoral (Rennó et al., 2008), antithrombotic (Azevedo et al., 2007), and antimicrobial (Caetano et al., 2002) activities. Our study did not verify a metabolic response comparing babassu mesocarp and maltodextrin, which contrasts with the investigation by Toscano et al. (2015).

The limitation of the current study is the lack of analysis in the antioxidant enzymes, cytokines, and other oxidative stress markers. Further studies should investigate the effect of chronic babassu mesocarp flour supplement with different dosages in other exercise protocols. The acute supplementation of 0,4g/kg/day of babassu mesocarp flour did not improve 3200m test performance in recreational runners. Moreover, it did not alter blood glucose levels, muscle damage, and oxidative stress markers when compared to maltodextrin.

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