Coconut oil supplementation and physical exercise improves baroreflex sensitivity and oxidative stress in hypertensive rats

Naiane F.B. Alves, Suênia K.P. Porpino, Matheus M.O. Monteiro, Enéas R.M. Gomes, and Valdir A. Braga

Abstract: The hypothesis that oral supplementation with virgin coconut oil (Cocos nucifera L.) and exercise training would improve impaired baroreflex sensitivity (BRS) and reduce oxidative stress in spontaneously hypertensive rats (SHR) was tested. Adult male SHR and Wistar Kyoto rats (WKY) were divided into 5 groups: WKY + saline (n = 8); SHR + saline (n = 8); SHR + coconut oil (2 mL·day⁻¹, n = 8); SHR + trained (n = 8); and SHR + trained + coconut oil (n = 8). Mean arterial pressure (MAP) was recorded and BRS was tested using phenylephrine (8 μg/kg, intravenous) and sodium nitroprusside (25 μg·kg⁻¹, intravenous). Oxidative stress was measured using dihydroethidium in heart and aorta. SHR + saline, SHR + coconut oil, and SHR + trained group showed higher MAP compared with WKY + saline (175 ± 6, 148 ± 7 vs. 113 ± 2 mm Hg; p < 0.05). SHR + coconut oil combined with exercise training improved BRS in SHR compared with SHR + saline group (−2.47 ± 0.3 vs. −1.39 ± 0.09 beats·min⁻¹·mm Hg⁻¹; p < 0.05). SHR + saline group showed higher superoxide levels when compared with WKY + saline (774 ± 31 vs. 634 ± 19 arbitrary units (AU), respectively; p < 0.05). SHR + trained + coconut oil group presented reduced oxidative stress compared with SHR + saline in heart (622 ± 16 vs. 774 ± 31 AU; p < 0.05). In aorta, coconut oil reduced oxidative stress in SHR compared with SHR + saline group (454 ± 33 vs. 689 ± 29 AU, p < 0.05). Oral supplementation with coconut oil combined with exercise training improved impaired BRS and reduced oxidative stress in SHR.

Key words: virgin coconut oil, swimming, hypertension, baroreflex, antioxidant.

Introduction

Several studies have shown that there is a strong association between hypertension and the formation of reactive oxygen species (ROS) (Botelho-Ono et al. 2011; Braga et al. 2011; Mei et al. 2014). The generation of ROS is involved in cardiovascular autonomic dysfunction and reduction in baroreflex sensitivity (Botelho-Ono et al. 2011; Guimarães et al. 2012; Nishi et al. 2013). Recently, we showed that increased plasma lipid peroxidation is associated with reduction in baroreflex sensitivity in renovascular hypertensive rats as well as in spontaneously hypertensive rats (Guimarães et al. 2012; Monteiro et al. 2012; Mendes-Junior et al. 2013). However, studies investigating the use of natural products as dietary supplements combined with exercise training in experimental models of hypertension focusing on baroreflex sensitivity are missing.

Antioxidants added to the diet combined with changes in lifestyle to add moderate exercise training to the patient’s daily routine may be an effective approach in the treatment of hypertension (Appel et al. 2006; Queiroz et al. 2013).

Virgin coconut oil (Cocos nucifera L.) possesses antioxidant properties because of its high contents of vitamin E and polyphenols (Nevin and Rajamohan 2006). In addition, the lauric acid is the most predominant medium chain fatty acid found in coconut oil.
improved baroreflex sensitivity and reduced oxidative stress in spontaneously hypertensive rats (SHR).

However, chronic physical training promotes adaptive responses through the activation of signal transduction pathways that stimulate increases in endogenous antioxidant systems such as superoxide dismutase (Choi and Cho 2014; Rech et al. 2014) catalase (Choi and Cho 2014) and glutathione peroxidase (Rech et al. 2014), thus being beneficial to the cardiovascular system. Therefore, the coconut oil combined with exercise training would have the potential to be used as adjuvant in the treatment of hypertension.

In the present study we tested the hypothesis that oral supplementation with coconut oil combined with exercise training would improve impaired baroreflex sensitivity and reduce oxidative stress in spontaneously hypertensive rats (SHR).

**Materials and methods**

**Animals**

We used forty adult male SHR and 24 Wistar Kyoto rats (WKY) (200–300 g) that were housed in a temperature-controlled room set to a 12-h light/12-h dark cycle with free access to standard rat chow (Labina; Purina, Paulinea, SP, Brazil) and water. All protocols were approved by the Animal Care and Use Committee of the Federal University of Paraiba (CEUA/CIbiotec no. 0205/12).

**Study design**

Animals were divided into 5 different groups: WKY + saline, SHR + saline, SHR + coconut oil (2 mL·day⁻¹), SHR + trained, and SHR + trained + coconut oil (2 mL·day⁻¹). The experimental protocol lasted 4 weeks and at the end, blood pressure, baroreflex sensitivity, lipid peroxidation, and superoxide levels were evaluated. Figure 1 shows the study design.

**Coconut oil supplementation and reagents**

SHR + coconut oil and SHR + trained + coconut oil groups were supplemented daily with virgin coconut oil in nature (2 mL·day⁻¹). This dose was based on Nandakumar et al. (2011). Virgin coconut oil was administered by gavage and saline solution was used as vehicle. Dihydroethidium (DHE) was from Invitrogen (Carlsbad, Calif., USA). Hematoxiline and eosine were from Sigma–Aldrich (St. Louis, Mo., USA). Ketamine and xylazine were from Vetnil (Curitiba, PR, Brazil). Phenylephrine hydrochloride (PHE) and sodium nitroprusside (SNP) were from Sigma–Aldrich.

**Exercise training protocols**

Rats were submitted to swimming training protocol as described by Lee et al. (2009). First, rats were adapted to the water without workload during 20 to 60 min, 5 days-week⁻¹ for 1 week. Then the exercise-trained rats were exercised 5 days-week⁻¹ during 4 weeks with 4% body weight workload attached to animal tail (Deminice et al. 2011). Body weight was measured every week. The tank used in this study had water at a temperature of 31 ± 1 ºC and had a depth of 50 cm.

**Blood pressure and heart rate recordings**

Forty-eight hours after treatment, animals were anesthetized with ketamine and xylazine (75 and 10 mg·kg⁻¹, intraperitoneal, respectively). Polyethylene tube (PE-10 connected to PE-50) was implanted in abdominal aorta through femoral artery for arterial pressure recordings. Other catheter was inserted into the femoral vein for drug injection. Then, both catheters were tunneled subcutaneously and exposed in dorsal region of the neck. Twenty-four hours after catheter implantation, blood pressure and heart rate measurements were performed using a pressure transducer coupled to an acquisition system (PowerLab; ADInstruments, Castle Hill, NSW, Australia) connected to a computer installed with LabChart 5.0 software (ADInstruments). The recording of blood pressure and heart rate were performed with the animal awake and freely moving (Braga 2010).

| Table 1. Effects of coconut oil supplementation, exercise training, and coconut oil supplementation combined with exercise training on body weight. |
|---------------------------------|-----------------|-----------------|-----------------|
| Initial body weight (g) | Final body weight (g) | Weight gain (%) |
| WKY + saline                 | 187±12          | 242±9⁺        | 31±4            |
| SHR + saline                 | 202±9          | 274±10⁺       | 35±4            |
| SHR + coconut oil            | 206±7          | 225±5⁺,†,‡    | 10±2⁺,†,‡       |
| SHR + trained                | 189±4          | 212±5⁺,†,‡    | 12±3⁺,†,‡       |
| SHR + trained + coconut oil  | 186±6          | 216±6⁺,†,‡    | 16±2⁺,†,‡       |

Note: Data are expressed as means ± SE. n = 8 for all groups. SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats.

⁺p < 0.05 compared with baseline data.

⁻p < 0.05 compared with WKY + saline group.

⁻⁻p < 0.05 compared with SHR + saline group.

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Thiobarbituric acid reactive substances (TBARS) assay

To evaluate lipid peroxidation, serum samples were collected to measure levels of malondialdehyde (MDA) determined by TBARS assay (Monteiro et al. 2012). After blood collections, the samples were centrifuged at 14 000 g to obtain serum. Then, 250 μL of serum was stored at 37 °C for 1 h, after which 400 μL of 35% perchloric acid was added, and the mixture was centrifuged (14 000 g/4 °C) for 20 min. Four hundred microlitres of supernatant was removed and mixed with 0.6% thiobarbituric acid and incubated at 60 °C for 1 h. Then, absorbance at 532 nm was measured. A standard curve was generated using 1,1,3,3-tetrametoxypropane. The results were expressed as nmol of MDA per millilitre for serum.

Determination of superoxide levels

The fluorescent DHE was used to evaluate the production of superoxide in heart and aorta tissues. Heart tissues were collected and immediately embedded in a cold solution of methanol (80%) and dimethyl sulfoxide (20%) at −80 °C for 5 days. Then, the samples were dehydrated and embedded in optimum cutting temper-
nature compound (OCT) (TissueTek, Sakura, Japan) and frozen at −80 °C. Unfixed frozen samples were cut into 10-μm thick sections in a cryostat and placed on chilled glass slides. Each tissue section was administered with DHE (5 mmol·L−1) and incubated overnight at 4 °C. For the aorta, the tissues were carefully dissected in the Krebs–Henseleit solution and cut into 2-mm rings. The samples were administered DHE for 30 min at 37 °C. After incubation, the aorta rings were washed in the Krebs solution and embedded in OCT (TissueTek, Sakura, Japan) and frozen at −80 °C. Fluorescent ROS indicator DHE was examined by fluorescent microscopy (Zeiss Axiovert 200M) and images were obtained using Axiovision 3.0 software. These methods are adapted from previous studies (Cheng et al. 2008; Kuroda et al. 2010).

Statistical analyses
Data are presented as means ± SE. Comparisons among groups were performed using 1-way ANOVA followed by Bonferroni post hoc test. All statistical analyses were performed using GraphPad Prism, version 5.0 (GraphPad Software). Statistical significance was defined as p < 0.05.

Results
Coconut oil combined with exercise training reduces body weight gain
There was a significant increase in body weight in all groups after 30 days of treatment compared with initial body weight. All groups started the experiment with similar body weight (p > 0.05), but at the end of 4 weeks, SHR + coconut oil, SHR + trained, and SHR + trained + coconut oil groups showed a lower body weight gain compared with WKY + saline (+10% ± 2%, +12% ± 3%, +16% ± 2% vs. +31% ± 4%, respectively; n = 8 for each group; p < 0.05) and SHR + saline (+10% ± 2%, +12% ± 3%, +16% ± 2% vs. +31% ± 4%, respectively; n = 8; p < 0.05) groups as shown in Table 1.

Coconut oil combined with exercise training reduces blood pressure in hypertensive rats
SHR + saline, SHR + coconut oil, and SHR + trained groups showed high blood pressure compared with WKY + saline (175 ± 6, 148 ± 6, 147 ± 7 vs. 113 ± 2 mm Hg, respectively; n = 8; p < 0.05). Thirty days of coconut oil supplementation combined with exer-

Fig. 3. Baroreflex sensitivity test. Representative tracings from a rat from each group (WKY + saline; SHR + saline; SHR + coconut oil; SHR + trained; and SHR + trained + coconut oil) showing the changes in PAP (mm Hg), MAP (mm Hg), and HR (beats·min⁻¹) in response to PHE (8 μg·kg⁻¹; open arrows) and SNP (25 μg·kg⁻¹; black arrows) (A). Baroreflex gain using vasoactive drugs (B). Spontaneous baroreflex gain (C). *, p < 0.05 when compared with WKY + saline group; †, p < 0.05 when compared with SHR + saline group. HR, heart rate; MAP, mean arterial pressure; PAP, pulse arterial pressure; PHE, phenylephrine; SHR, spontaneously hypertensive rats; SNP, sodium nitroprusside; WKY, Wistar Kyoto rats.
Coconut oil combined with exercise training improves baroreflex sensitivity in hypertensive rats

Representative tracings of changes in blood pressure and heart rate induced by the administration of PHE and SNP are shown in Fig. 3A. A decrease of baroreflex sensitivity in SHR + saline group compared with WKY + saline group was observed (−1.39 ± 0.09 vs. −2.75 ± 0.15 beats·min⁻¹·mm Hg⁻¹, respectively; n = 8; p < 0.05). Of note, only exercise training combined with coconut oil improved baroreflex sensitivity in SHR (−2.47 ± 0.3 vs. −1.39 ± 0.09 beats·min⁻¹·mm Hg⁻¹ respectively; n = 8; p < 0.05). In addition, SHR + trained and SHR + trained + coconut oil groups demonstrated an improvement in spontaneous baroreflex sensitivity compared with SHR + saline (−1.88 ± 0.25 and −1.97 ± 0.20 vs. −0.97 ± 0.12 beats·min⁻¹·mm Hg⁻¹, respectively; n = 8; p < 0.05).

Coconut oil combined with exercise training decreases lipid peroxidation

Serum MDA levels in SHR + saline group were higher compared with the WKY + saline group (27.5 ± 1.8 vs. 10.9 ± 0.6 nmol·L⁻¹, respectively; n = 8; p < 0.05). Furthermore, MDA levels decreased in SHR after coconut oil supplementation, training exercise, and combined treatment with coconut oil and exercise training compared with SHR + saline (12.1 ± 0.8, 19.12 ± 0.3 and 18.4 ± 1.2 vs. 27.5 ± 1.8 nmol·L⁻¹ respectively; n = 8; p < 0.05). Figure 4 shows serum MDA levels for all groups.

Coconut oil and exercise training reduces superoxide levels

SHR + saline group presented higher levels of superoxide when compared with WKY + saline in heart tissues (774 ± 31 vs. 634 ± 19 arbitrary units (AU), respectively; n = 8; p < 0.05). Only SHR + trained + coconut oil group reduced oxidative stress in heart tissues compared with SHR + saline (622 ± 16 vs. 774 ± 31 AU, respectively; n = 8; p < 0.05) as shown in Fig. 5A. SHR + saline group also presented higher superoxide levels when compared with WKY + saline in aorta tissues (689 ± 29 vs. 493 ± 26 AU, respectively; n = 8; p < 0.05) as shown in Fig. 5B. After treatment, coconut oil, exercise training and coconut oil combined with exercise training reduced oxidative stress in aorta when compared with SHR + saline group (454 ± 33, 498 ± 37 and 467 ± 38 vs. 689 ± 29 AU, respectively; n = 8, p < 0.05).

Discussion

In this study, we sought to investigate whether oral supplementation with virgin coconut oil combined with exercise training would improve impaired baroreflex sensitivity and reduce oxidative stress in spontaneously hypertensive rats. Several reports from our laboratory have demonstrated that antioxidants added in the diet, such as rutine (Mendes-Junior et al. 2013), lipoic acid (Queiroz et al. 2012) and quercetin (Monteiro et al. 2012), reduce ROS levels and restore baroreflex sensitivity in hypertensive rats. Nowadays, coconut oil has been used as dietary supplement because of its many benefits, including but not limited to weight loss (Assunção et al. 2009), anti-inflammatory (Intahphuak et al. 2010; Vysakh et al. 2014), antibacterial (Nevin and Rajamohan, 2004), and antioxidant activity (Vysakh et al. 2014). However, the combination of coconut oil supplementation and exercise training has not been investigated. Here we documented that coconut oil combined with exercise training reduces blood pressure, improves baroreflex sensitivity, and reduces oxidative stress in hypertensive rats.

Regarding weight loss, our data show that coconut oil reduces body weight. Similar to our results, previous studies demonstrated that after 16 weeks of coconut oil supplementation, the body weight was reduced in rats (Burneiko et al. 2006; Nurul-Iman et al. 2013). Coconut oil is commonly used in the treatment of obesity because of its high content of medium chain fatty acids, since such lipids are easily oxidized and are not normally stored in the adipose tissue, thus diminishing the basal metabolic rate. In addition, there is a clinical study documenting that supplementation with coconut oil reduces abdominal lipid accumulation, which could result in protection against cardiovascular diseases (Assunção et al. 2009). Under our experimental conditions, exercise training also improved weight loss compared with control group as expected and shown in Table 1. This is in agreement with several experimental studies reporting that moderate exercise promotes weight loss (Burneiko et al. 2006; Gauthier et al. 2003; Schrauwen and Westerterp 2000).

Regarding blood pressure, Nurul-Iman et al. (2013) observed a reduction in blood pressure of normotensive rats after 8 weeks of coconut oil supplementation. In this study, blood pressure was also reduced in hypertensive rats after coconut oil supplementation. The main mechanism underlying the reduction in blood pressure elicited by coconut oil can be attributed to its antioxidant activity, which will be discussed in detail below. In addition, several studies have demonstrated the effect of exercise training in reducing blood pressure in hypertensive rats (Horta et al. 2005; Soares et al. 2011; Abate et al. 2012; Rossi et al. 2013; Mizuno et al. 2014).

Our study suggests that hypertensive rats have lower baroreflex sensitivity compared with normotensives rats (Fig. 3A). Such decrease is associated with an increase of NADPH oxidase activity and generation of ROS (Botelho-Ono et al. 2011; Braga et al. 2011). Coconut oil combined with exercise training improved baroreflex sensitivity in hypertensive rats. Experimental studies demonstrated that antioxidants restore reductions in baroreflex sensitivity in...
hypertension (Costa et al. 2009; Braga et al. 2011). A previous study showed restoration of baroreflex sensitivity, following an increase in the parasympathetic component and reduction of oxidative stress after 4 weeks of training on a treadmill (Masson et al. 2014). The improvement in baroreflex sensitivity seen in our study could be explained by the fact that physical exercise promotes sympa-tho-inhibition via reduction of oxidative stress in the rostral ventrolateral medulla (RVLM) (Kishi et al. 2012). In addition, chronic exercise training has been associated with reduction in the brain renin–angiotensin system by blocking the angiotensin II type 1 receptor (AT1R) (Mousa et al. 2008). Costford et al. (2010) demonstrated that physical exercise regulates the redox balance by the NAD:NADH ratio and changes in mitochondrial function. Furthermore, others studies have showed that coconut oil increases nitric oxide availability probably by its polyphenols (Nurul-Iman et al. 2013, Arunima and Rajamohan 2013). One important limitation of our study is that the amount of coconut oil given via gavage (2 mL·day⁻¹) represents about 0.8% of total body weight, which would clearly be an excessive amount for potential use in humans. A recent study documented that virgin coconut oil has a content of approximately 84 mg·100 g⁻¹ of polyphenols, preventing lipid oxidation indicated by the decrease in MDA levels in liver, heart, and kidney (Arunima and Rajamohan 2013). Similarly, the results obtained in our study documented that serum MDA levels were reduced after coconut oil, training exercise, and combined treatment compared with hypertensive rats treated with saline. This effect could be occurred by increased nitric oxide levels by coconut oil supplementation and exercise training as previously...
described (Nurul-Iman et al. 2013, Mizuno et al. 2014). Of note, coconut oil alone had a better effect on reducing lipid peroxidation in SHR than when combined with exercise training. In fact, training alone was not able to reduce lipid peroxidation to the same extent as coconut oil alone. This could be explained by the limitations of using TBARS for determining lipid peroxidation. The TBARS test may work reasonably well when applied to defined systems, such as liposomes and microsomes, but its application to body fluids and tissue samples has been questioned (Gutteridge 1986). First, aldehydes other than MDA can form chromogens, with some absorbance at 532 nm, and many different aldehydes are formed in peroxidizing lipid (Kosugi et al. 1987). Second, TBARS tests rarely measure free MDA content of the lipid system but rather measure MDA generated by decomposition of lipid peroxides during the acid-heating stage of the test (Gutteridge 1986). In addition, several other compounds, including sugars, amino acids, and bilirubin are also reactive toward thiobarbituric acid. Finally, MDA does not just reflect lipid peroxidation but is also a byproduct of cytochrome oxidase activity in platelets during intense exercise training. Interestingly, either coconut oil or training reduced superoxide accumulation in the heart and aorta to the same extent. Those data are in agreement with Roque et al. (2013), who observed that aerobic exercise normalized superoxide anion production in hypertensive rats.

In conclusion, our data show that coconut oil supplementation combined with exercise training for 30 days promotes beneficial effects on the cardiovascular system of spontaneous hypertensive rats. Those effects include reduction in blood pressure, reduction in oxidative stress, and improvement of the baroreflex sensitivity. Taken together, our findings will open a new field of investigation to propose the use of coconut oil supplementation as an adjuvant treatment for hypertension in the future.

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