Dietary supplementation with camu-camu and continuous exercises in the treatment of obesity

Suplementação dietética com camu-camu e exercícios contínuos no tratamento da obesidade

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Objective
The goal of this study were to investigate the effects of continuous exercise with/or without the ingestion the Camu-camu pulp in a rat model of obesity.

Methods
Neonate male Wistar rats, receiving monosodium glutamate, subcutaneously were separated into four groups: sedentary group S (no treatment), exercise group E (continuous swimming training), Camu-camu group C (25mL of pulp of Camu-camu/day) and exercise and Camu-camu group EC (25mL of pulp of Camu-camu/day, continuous swimming). After 12 weeks, the animals were received euthanasia.
Results
The exercise program was conducted for five days for 12 weeks and the effects of supplementation with or without Camu-camu in obese rats were analyzed, showing that the relative levels of the variables cholesterol, triglycerides, glucose, High Density Lipoprotein and Low Density Lipoprotein and in all groups there was a significant reduction ($p<0.001$), except for the control group. Body weight and feed intake, epididymal and visceral fat deposits were not significantly different between the means of groups C and E, but these groups showed a significant difference when compared to the EC group ($p<0.001$).

Conclusion
The results demonstrate the effectiveness of continuous exercise and diet supplemented with Camu-camu fruit pulp to control obesity.

Keywords: Body weight. Diet, reducing. Obesity.

**INTRODUCTION**

The growth in the number of people with diseases associated to the weight excess make the experts consider obesity a disease with serious problems to public health [1]. The excess of adipose tissue with abdominal distribution, has been associated to the growth of incidence of several kinds of diseases especially the ones related to the metabolic syndrome [2].

Considering obesity is multifactorial, one of the strategies to be used would be the adoption of a heathy and balanced diet supplying the daily energy intake and make the individual adept regular physical activities [3,4]. Some studies show the use of physical activities as a cooperator in the treatment not only when the individual is exercising, but a series of adjetments after the activity, avoiding the unwanted body fat gains [4-6].

The Camu-camu (**Myrciaria dubia** H.B.K. McVaugh) is in the riversides of the Black River in Amazon and contains a remarkable content of ascorbic acid containing from 1,600 to 6,112
mg/100g of pulp [7] what makes it one of the richest in vitamin C if compared to other citric fruit and, in it nutritional composition, various antioxidanting components [8-10]. Some studies confirm the use of Camu-camu with antimicrobial [11] effects, antglycemic [12], antihypertensive [13], anticancer and avoids oxidative damages in hepatic and brain cells [14] contributing for quality life improvement [15].

In the present study, we demonstrated that the pulp of Camu-camu reduced body weight and epididymal and visceral fat, such as reduced fat deposited in the face, heart and liver, in addition to decrease the inflammatory proteins [16]. Several experimental studies have shown that exercise combined with continued dieting results in reduced adiposity, lipid profile and reduction of biochemical parameters in rats [17-19].

The aim of this study is to identify the potential effect of exercise combined with the pulp of Amazon fruit Camu-camu in reducing health risk factors associated with obesity and lipid profile.

M E T H O D S

This experiment was performed using 3-month-old (360.84±45.44g) male Wistar rats (Rattus norvegicus var. albinus) (n=32). The animals were obtained from the Instituto Nacional de Pesquisas da Amazônia (INPA, Amazon Research Institute). The procedures adopted with animals were performed in accordance with the criteria of the law nº11.794 of 2008 for use of animals for scientific purposes. Each animal was housed in an individual cage, with a temperature of 26±2ºC, and with a light-dark cycle of 12 hours. They received water and commercial rations ad libitum (Labina, São Paulo, Brazil) and contained 23g of protein (per 100g diet), 49g carbohydrate, 4g total fat, 5g fiber, 7g of ash, and 6g of vitamins (E and S); the diet groups (EC and C) received macerated feed and mixed with 25mL of Camu-camu pulp, daily for twelve weeks. The exercise program consisted of swimming in individual tanks filled with water and maintained at 28-32°C. Animals in groups (E and EC) swam for 30, 60 and 90min of the first, second and third days to adapt. The
swimming period was subsequently increased to 120 min/day. A weight of 5% of body weight was attached to the tail of each swimming rat. The exercise was conducted for five days a week for 12 weeks. Body weight, food intake, water, feces and body weight were recorded weekly.

At the end of the experiment, rats were weighed and anesthetized by overdose of choral hydrate i.p. The groups received euthanasia in the same period. Blood samples (5mL) were withdrawn and stored in tubes and centrifuged at 3,000 rpm for 5 minutes for further biochemical determinations. Immediately after sacrifice, an incision was made in the abdomen, and the following tissues were removed and weighed using a scalpel and forceps: white adipose Epididymal (EPI), Visceral (VIS). Levels of cholesterol, glucose, and triglyceride fractions were measured by enzymatic colorimetric method using commercial kits (Labtest Diagnóstica, MG, Brazil) by automation (Cobas mira Plus®, Roche). After precipitation of Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) by phosphotungstic acid/MgCl2, High Density Lipoprotein-cholesterol (HDL-c) in the supernatant was determined using a commercial kit (Labtest Diagnóstica, MG, Brazil). Low Density Lipoprotein-cholesterol (LDL-c) was calculated using a Friedewald formula.

Results are expressed as mean ± standard deviation. Comparisons between initial and final measures were statistically verified using paired t-tests. One-way Analysis of Variance (ANOVA) was used to compare data between groups. The multiple comparisons between groups as for the result of final to initial difference were done using Tukey test. The software used was Minitab 18.

**RESULTS**

The exercise program was conducted for five days for 12 weeks and the effects of the supplementation with/or without Camu-camu pulp in obese rats were analyzed, as shown in the Table 1. The relative levels of the variables cholesterol, triglycerides, glucose, HDL and LDL and p-value for the test of the difference between the final and initial means in all groups showed a significant reduction of cholesterol (p<0.001), except for the control group. Comparing the groups, the highest cholesterol reduction occurred in group E (Figure 1). For the triglyceride and glucose variables, there was also a significant reduction in all groups, and the group supplemented and exercised presented the highest glucose reduction while the Camu-camu group had the highest triglyceride indices, these variables were increased in the control group (Table 1). HDL showed a significant increase in all groups except the control group (Table 1). However, the group treated with Camu-camu had the highest HDL increase. There was no significant difference in LDL levels between groups except in the control group, but group E had the lowest reduction (p<0.001). The variables triglycerides, glucose, visceral fat, body weight and feed intake did not present significant differences between the means of groups C, E and EC, but presented a significant difference for group S (Table 2). For the cholesterol variable, we observed a significant mean difference of groups E, C and EC for group S, as well as group C for group EC (Table 2). There was no significant difference between groups C and E and between groups C and EC in the triglyceride variable. For epididymal fat deposits there was no significant difference between the means of groups C and E, but these groups showed a significant difference when compared with the EC group, the same responses were measured for epididymal fat.

**DISCUSSION**

Schoolars consider obesity as an incrising epidemic in several worldwide societies, the unbalanced feeding and the lack of physical activity practise are factors that contribute for it.
**Table 1.** Cholesterol, Triglycerides and Glucose of rats of different initial and final in groups.

<table>
<thead>
<tr>
<th>Variable</th>
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<td>E</td>
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<tr>
<td>Cholesterol (mg dL⁻¹)</td>
<td>-215.00 ± 31.2</td>
<td>&lt;0.001</td>
<td>-221.90 ± 44.60</td>
<td>&lt;0.001</td>
<td>-157.50 ± 60.20</td>
<td>&lt;0.001</td>
<td>53.70 ± 37.20</td>
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<tr>
<td>Triglycerides (mg dL⁻¹)</td>
<td>-124.10 ± 49.9</td>
<td>&lt;0.001</td>
<td>-119.80 ± 48.20</td>
<td>&lt;0.001</td>
<td>-100.80 ± 72.70</td>
<td>0.003</td>
<td>37.70 ± 52.80</td>
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<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>-17.90 ± 17.9</td>
<td>0.013</td>
<td>-11.96 ± 11.86</td>
<td>0.013</td>
<td>-30.58 ± 48.22</td>
<td>0.001</td>
<td>76.80 ± 44.00</td>
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<tr>
<td>HDL (mg dL⁻¹)</td>
<td>15.24 ± 17.9</td>
<td>0.001</td>
<td>7.95 ± 4.90</td>
<td>0.001</td>
<td>12.25 ± 4.66</td>
<td>&lt;0.001</td>
<td>-5.34 ± 8.87</td>
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<td>LDL (mg dL⁻¹)</td>
<td>-84.59 ± 17.9</td>
<td>&lt;0.001</td>
<td>-93.00 ± 6.09</td>
<td>&lt;0.001</td>
<td>-77.94 ± 7.14</td>
<td>&lt;0.001</td>
<td>-103.90 ± 8.10</td>
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Note: Statistical analysis was performed using the paired t-test for comparison of results initial and final in each group (p≤0.05). Camu-camu Group (C), Exercise Group (E), Exercise + Camu-camu Group (EC) and Sedentary Group (S).

**Table 2.** Cholesterol, Triglycerides, Glucose, Body Weight, feed intake, Visceral Fat and Epididymal Fat of rats of different initial and final in groups.

<table>
<thead>
<tr>
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<tr>
<td>Cholesterol (mg dL⁻¹)</td>
<td>-215.20 ± 31.20</td>
<td>B</td>
<td>C</td>
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<tr>
<td>Triglycerides (mg dL⁻¹)</td>
<td>-124.10 ± 49.90</td>
<td>B</td>
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<td>B</td>
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<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>-17.90 ± 17.90</td>
<td>B</td>
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<td>B</td>
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<tr>
<td>Body Weight (g)</td>
<td>-90.54 ± 23.74</td>
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<td>A</td>
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<tr>
<td>Feed intake (g)</td>
<td>-14.81 ± 5.30</td>
<td>A</td>
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<td>Visceral Fat (g)</td>
<td>-6.70 ± 2.40</td>
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<td>Epididymal Fat (g)</td>
<td>-19.75 ± 7.24</td>
<td>A</td>
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Note: Results are expressed as mean ± standard. Comparisons between initial and final measures were statistically verified using One-way ANOVA was used to compare data between groups. The multiple comparisons between groups as for the result of final to initial difference were done using Tukey test (p≤0.05) in which groups with the same letters are not significant. There was no deviation from the normality assumptions. Camu-camu Group (C), Exercise Group (E), Exercise + Camu-camu Group (EC) and Sedentary Group (S); ANOVA: Analysis of Variance.
Therefore, researches envolving physical activities and balanced diets are indispensable either on humans or experimental models [21-24]. Beside that, the caloric expenditure occasioned during and after the physical activity helps to reduce the adverse effects of the body fat gather specially the one placed in the abdominal wall, adding the balanced energy intake can be prescribed as a proposal to prevent obesity. Yet there is no agreement in literature about intensity, frequency and duration of the physical activity and what kind of diet promote different adaptations and effects when it comes to obesity [25,26].

This study showed that the group (EC) presented the largest final body weight reduction followed by groups C and E (Table 2). Physical exercise acts positively in the prevention and development of obesity, because it causes a series of physiological responses, resulting among other adaptations of lipid metabolism enzymes and the use of fats as energy source and, when associated with changes in diet, influence positively in almost all factors and risk of obesity. Demonstrating that both fruit and physical exercise protects against gains in body weight and adiposity. In addition, when the values of the visceral and epididymal fat deposits were added, both groups of rats that received the intervention in isolation, exercise or Camu-camu even without significant body weight reduction when compared with the group (EC) had values reduced in these deposits (Table 2). The exercise protocol was shown to act directly not only on body weight reduction, but on deposits.
of different fatty tissues such as visceral and epididymal, raising the hypothesis that these tissues may also contribute to the energy expenditure requested by the exercise. Findings found in this research corroborate similar results of swimming protocols used by other authors [26-29].

Demonstrating that both fruit and physical exercise protects against gains in body weight and adiposity. In addition, when the values of the visceral and epididymal fat deposits were added, both groups of rats that received the intervention in isolation, exercise or Camu-camu even without significant body weight reduction when compared with the group (EC) had values reduced in these deposits (Table 2). The light intensity of swimming exercises to moderate and stimulate the consumption of oxygen, stimulates the action of the lipase enzyme hormone sensitive providing preferably fatty acids, as a result of lowering the levels of adiposity [30].

Regarding the diet of the literature reports that the fruit of Camu-camu has anti-oxidative and anti-inflammatory properties which may have contributed to the reduction of fat deposits [31,32] since there was a decrease in body weight and its fat deposits reported in this study. Lipid profile markers were another marker of obesity risk investigated in our experiment. The isolated group that used Camu-camu showed the best results in the triglyceride and HDL levels, while the exercise group lowered cholesterol and LDL levels, results not found in the group that associated Camu-camu with exercise (Table 1). Researchers report results of biological assays on the action of Camu-camu (Myrciaria dubia H.B.K. McVaugh) on the reduction of lipid profile and lipid peroxidation, on triglyceride levels with decreased hypercholesterolemia in obese and diabetic rats [16,33,34] cholesterol reduction total cholesterol, LDL-c, triglycerides and modifications in the metabolic profile of adults with metabolic syndrome [35,36].

In an experimental model using monosodium glutamate, the association of Camu-camu with physical exercise was effective in reducing body weight. In addition, the values of the visceral and epididymal fat deposits, both groups of rats that received the isolated intervention of the exercise or Camu-camu had reduced values in these deposits. In the lipid profile markers the group supplemented with Camu-camu showed the best result in triglyceride and HDL levels, while the exercised group lowered cholesterol and LDL levels. In addition the group exercised
and Camu-camu was the one that best utilized the glucose levels. The results found in this experimental study are promising. However, further studies are needed to clarify the mechanisms of action found in these.

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