DOI: 10.1590/0100-6991e-20192238 Original Article

Dietary supplementation with Camu-Camu versus sleeve gastrectomy in Wistar rats weight control.

Análise dos efeitos da suplementação dietética com Camu-Camu comparada à gastrectomia vertical no controle de peso de ratos Wistar.

Hercules Magalhães Olivense do Carmo, TCBC-AC[1]; Feliph Miquéias Alcântara de Souza[1]; Ana Clara Leal Soares[1]; João Abner Marins Munhoz[1]; Francisco Glauco de Araújo Santos[1]; Nilton Ghiotti de Siqueira, TCBC-AC[1]; Romeu Paulo Martins Silva[1]

A B S T R A C T

Objective: to compare the effects of the hydroalcoholic extract from the peel of Camu-Camu, a fruit plant belonging to the Myrtaceae family, widely distributed in the Amazon basin, with those of sleeve gastrectomy, on the weight and glycemia of Wistar rats. **Methods:** twenty-four Wistar rats underwent obesity induction through a hyperlipid diet for eight weeks (fat period), and were randomized into three groups: Control Group (CG), Camu-Camu Group (CCG) and Bariatric Surgery Group (BSG). After this period, all animals returned to a normal diet and the intervention period began: CG did not undergo any intervention beyond diet change; CCG animals underwent gavage procedure for administration of Camu-Camu hydroalcoholic extract, 1g/kg/day, for four weeks; and the BSG was submitted to the surgical procedure of sleeve gastrectomy. We followed all animals for four weeks. **Results:** there was only one loss in BSG due to a gastric fistula. We observed significant variations in the animals' mean weight: the CG evolved with weight gain even after the withdraw of the hypercaloric diet, while the other two groups presented weight reduction. BSG presented a significant reduction of weight and BMI ($p<0.05$); CCG achieved a significant reduction only of the BMI ($p<0.05$). There were no statistically significant changes in the glycemic levels. **Conclusion:** in spite of reducing weight, the crude hydroalcoholic extract of the Camu-Camu peel was not able to be as efficient as sleeve gastrectomy in the control of body weight in Wistar rats.

Keywords: Obesity. Gastrectomy. Bariatric Surgery. Phytotherapy. Rats, Wistar.

INTRODUCTION

Obesity is a worldwide epidemic and represents a serious public health problem due to the association with several morbid states. It is estimated that obesity is the second most common cause of preventable death, second only to smoking[1-3]. Moderate weight loss, with an approximate reduction of 5% to 10% of the original weight, is associated with significant clinical improvement in the patients, with an increase in insulin production, a decrease in blood pressure, triglyceride levels and glycated hemoglobin. Weight loss over 15%, such as those produced by bariatric surgery, also lead to the remission of obesity-related diseases[1].

Bariatric surgery, when associated with a rigorous clinical and nutritional monitoring, is the most effective method to obtain long-term obesity control, which mainly benefits patients with comorbidities such as diabetes, hyperlipidemia, systemic arterial hypertension and sleep apnea, who have substantial improvement or even complete resolution of their diseases[3-8]. Dietary treatment, even associated with medication, induces on average a modest weight loss of up to 10% in a short period, but is often followed by weight regain.

In the search for less invasive measures for the treatment of obesity, there has been a great demand for natural products capable of leading

1 - Federal University of Acre, Health and Sports Science Center, Rio Branco, AC, Brazil.

to the discovery of new chemical entities and the development of starting materials for the synthesis of more specific and efficient drugs. Many Amazonian natural products have been used in experimental models for the identification of active principles with anorectic and hypoglycemic effects[9,10]. The *Myrciaria dubia* (HBK) McVaugh, known as Camu-Camu, is a fruit plant belonging to the *Myrtaceae* family, widely distributed in the Amazon basin. The concentration of vitamin C found in Camu-Camu generally corresponds to 40 times that of an orange and 55 times that of a lemon[11]. Its phytochemical components promote the improvement of the lipid profile, reduction of oxidative stress, reduction of plasma glucose, insulin and lipid levels, as well as anti-inflammatory, hepatoprotective, antimicrobial and antigenotoxic activities[11-15]. Sotero Solis *et al.*[16] verified antioxidant activity in the three components of Camu-Camu fruit, seed, pulp and peel, the best results being found in the latter.

The objective of the study was to compare the effects of Camu-Camu hydroalcoholic peel extract with those of sleeve gastrectomy on the weight and glycemia of Wistar rats. The use of peel extract in the present study guarantees the novelty of its use as a proposal of intervention for the treatment of obesity.

METHODS

We used twenty-four male Wistar rats, an albino line of the species *Rattus norvegicus domesticus*, obtained with the laboratory of the Federal University of Acre. In agreement with the model used by the Manual of Care and Procedures with Laboratory Animals of the FCF-IQ/USP Production and Experimentation Laboratory[17], we maintained controlled environmental conditions, with a temperature of 22°C, relative humidity of 55%, continuous aerial exhaust and light/darkness cicles of 12/12 hours.

After the rats completed eight weeks of life, we randomly allocated them into three groups: Control Group (CG), Camu-Camu Group (CCG) and Bariatric Surgery Group (BSG). All a received a hypercaloric fattening diet - fat and water *ad libitum* for eight weeks (fattening period)[18,19]. After this period, all groups returned to the normal diet (normal chow and water *ad libitum*) and the intervention period began, which lasted four weeks. During these four weeks, the CG did not undergo any intervention other than the dietary change itself (withdrawal of fattening chow and return to normal chow); CCG was submitted to gavage procedures for the administration of Camu-Camu hydroalcoholic peel extract at a dose of 1g/kg/day; and the BSG underwent sleeve gastrectomy (Figure 1).

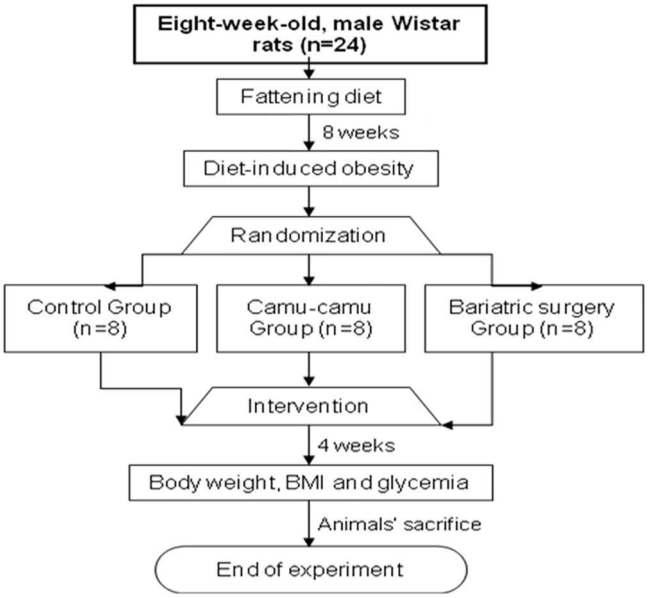

Figure 1. *Diagram showing the general study design. BMI: body mass index.*

We studied the following variables of interest: body weight, BMI and serum glucose. We performed the measurements at three moments: at the beginning of the study, immediately before the introduction of the fattening diet (rats at 8 weeks of age); immediately after the end of the fattening period (rats at 16 weeks of age); and immediately after the end of the intervention period (rats at 20 weeks of age) (Figure 2). At the end of the intervention period, all rats were submitted to euthanasia.

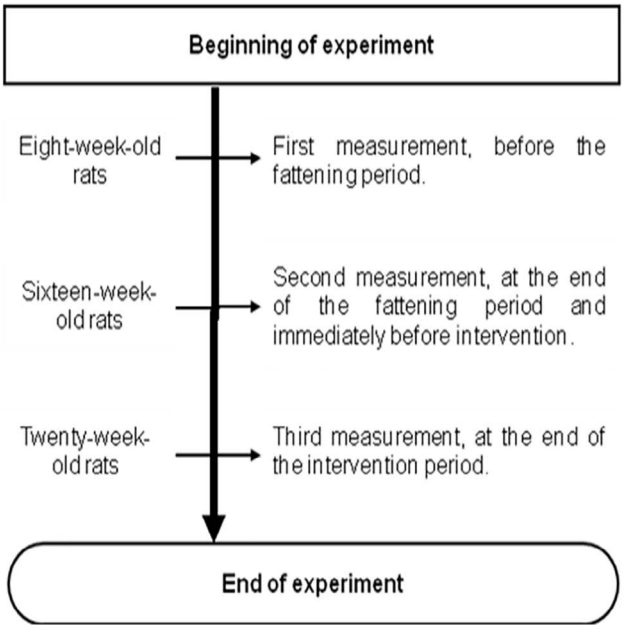

Figure 2. *Study time line showing the moment of each measurement of the parameters analyzed.*

Rats' body measurements and BMI

We recorded rats' body length in centimeters, the same pair of authors always performing the measurements, one for the immobilization of the animal and the other for the handling of the measurement tape. We defined as body length the distance between the tip of the nostril of the animal to the base of its tail.

We measured the animals' corporal weight in grams, using an Prix Lab analytical scale, model AS82220R2.

We performed all body weight measurements in duplicate, and when discrepant by any value, we considered the mean between the two numbers as the final value for the weight.

Blood samples

For glycemia analysis, each rat underwent a minimal incision at the end of its tail with the purpose of extracting a drop of blood to be processed by an Accutrend® Plus device (Roche).

Camu-Camu

We used approximately 70kg of *in natura* Camu-Camu fruit, which we purchased at the "Cooperativa Mista de Produtores de Açaí e Frutas Regionais de Codajás", Codajás, Amazonas". The formal identification of the *Myrciaria dubia* (HBK) McVaugh fruits was performed by Dr. Dionatas Ulises de Oliveira Meneguetti, registered in the Regional Council of Biology by the number of CRBio-6 n# 052581/06-D.

We washed the fruits and submerged them in 1% sodium hypochlorite solution (10 drops of solution to 1 liter of water) for 15 minutes and, after cleaning, we peeled them and discarded the pulps. We froze the peels at -80°C and lyophilized them with a Liotop type L101 Freeze Dryer, available at the Bionorte - Complexo UFAC Nanobiotechnology Laboratory.

We subjected the resulting lyophilization product to maceration with ethanolic solution (ethanol-water ratio 7:3) for 48 hours, repeating the whole process for three consecutive times. After this step, the solvent was evaporated resulting in a crude residue of the hydroalcoholic extract of the Camu-Camu fruit peel, which was again submitted to the lyophilization process for maximum water removal and retrieval of the extract in its final form.

Gavage procedure

The gavage process, started for the CCG group after the end of the fattening period, was performed daily to ensure correct dose administration of 1g of extract for each kg of mouse weight per day (1g/kg/day).

The eight CCG rats received the extract by gavage. The procedure was always performed by the same pair of authors: one to immobilize the animal and the other to perform the gavage, without changing the roles between them.

Sleeve gastrectomy

The rats were fasted for 12 hours before the sleeve gastrectomy surgical procedure. Thirty minutes before the procedure, they received antibiotic prophylaxis with ceftriaxone 50mg/kg intramuscularly. They were anesthetized with ketamine (100mg/kg) and xylazine (10mg/kg) intraperitoneally. During the procedure, we performed the resection of approximately 70% of the animal's stomach. Gastric wall synthesis was performed with PDS® II 6-0 (polydioxanone) in two planes and closure of the abdominal wall and skin, performed with catgut 3-0 and nylon 4-0, respectively. Each animal submitted to the procedure was isolated in a cage without other animals and received cleaning and exchange of surgical dressing daily. We used tramadol chlorhydrate (10mg/kg subcutaneously every 8/8 hours for 3 days) for analgesia.

Ethics in research

All the procedures applied during the experiment were in accordance with Law n# 11794/2008, which regulates the scientific use of animals in Brazil, and Normative Resolution n# 37, of January 27, 2018, of the Practical Euthanasia Guidelines of the National Council of Animal Experimentation - CONCEA.

The euthanasia procedure was carried out as recommended by the CONCEA Resolution n# 37, in a quiet and clean environment, in an individualized manner (away from other animals), and quickly.

We used the method of indirect hypoxia, inducing unconsciousness before ceasing motor activity. To do so, we placed each animal in a chamber containing 100% inhalation agent (isoflurane) to ensure rapid and painless death. After the procedure, we confirmed death with the following signs: absence of respiratory movement; absence of heartbeat; pale mucous membranes; and absent corneal reflex. Before being discarded, the animal remained on observation for ten minutes.

This study was approved by the Ethics Committee on Animal Use of UFAC, under process n# 23107.025548/2017-26 and protocol n# 54/2017.

Statistical analysis

We analyzed the data with the GraphPad Prism® software version 5.0 (GraphPad, United States). We assessed normality with the Shapiro-Wilk test. We presented data as mean and standard deviation for parametric variables. We used The Student t test to compare continuous intergroup endpoints with homogeneity of variances, in case of symmetric distribution, and heterogeneity of variance, in case of asymmetric distribution. We used One-way ANOVA to evaluate possible differences between groups over time (pre and post-dietary supplementation with the Camu-Camu extract). We set the statistically significant threshold (p) at 0.05.

RESULTS

Only one of the mice died 11 days after the surgical procedure due to complications of a gastric fistula.

The average weight in grams of the animals showed a significant variation between the groups (Figure 3A); CG maintained an upward curve of weight gain (CG2: 377.87±72g/CG3: 421.25±97g)

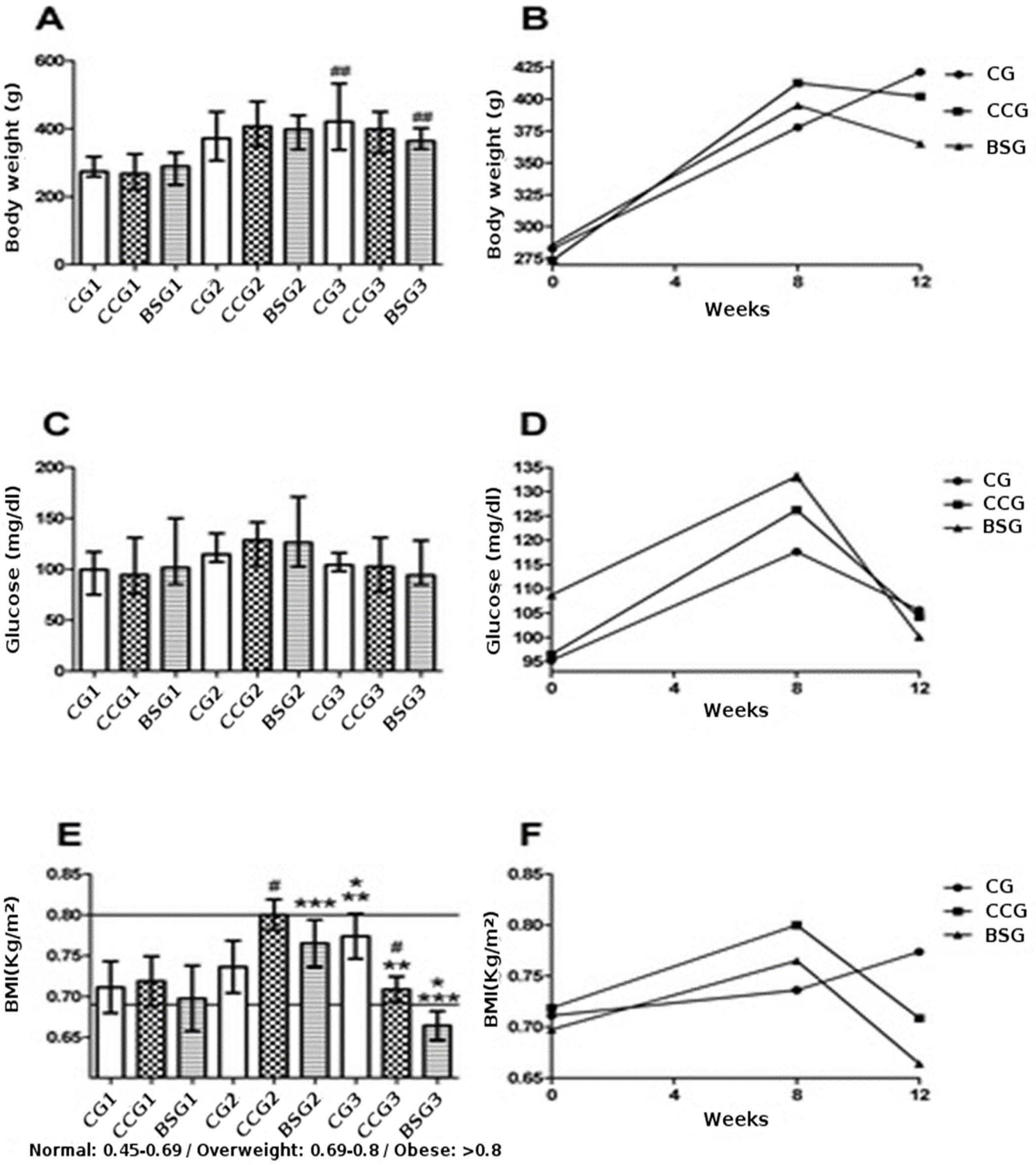

Figure 3. *Variation of body weight, blood glucose and BMI during the three moments of the experiment. The figures 1, 2 and 3 after the acronym for each group indicate the moment of measurement as quoted in the methodology. CG= Control Group; CCG= Camu-camu Group; BSG= Bariatric Surgery Group. (A) variation of body weight throughout the experiment. ## Statistically relevant difference with p=0.0313 (Student's t-test); (B) line graph showing the rats' body weight variation throughout the experiment; (C) variation of fasting glycemia; (D) tendency of variation of fasting glycemia; (E and F) variation of BMI throughout the experiment. The differences between the groups were analyzed by Student's t-test. # p=0.014; * p=0.8 (horizontal lines in Chart E).*

(Figure 3B) even after finishing the fattening period. The other two groups, CCG and BSG, presented a decrease in the mean weight (CCG2: 412.75±66g/ CCG3: 402±60g/BCG2: 395.12±50g/ BCG3: 365.28±31g), the difference between the end weight of CG and BSG groups being statistically significance by the Student's t test (p=0.0313).

Figures 3C and 3D show the results of biochemical analysis for the determination of blood glucose levels between the end of the fattening period and the end of the study. Although the BSG values were better than those of the other groups, this difference was not statistically significant.

The values found in the data analysis of the mean BMI of groups (Figures 3E and 3F) showed that the only intervention that could reverse the metabolic syndrome in rats was Sleeve Gastrectomy, $p<0.05$ in the Student's t test, corroborating the data found in the other measurements. The decrease in CCG BMI was also significant in relation to CG (p=0.0421).

DISCUSSION

The use of Camu-Camu as medicinal fruit by the Amazonian peoples aroused interest in determining its efficacy in the treatment of various diseases. Therefore, its use as a possible treatment of obesity is relevant. The hypothesis that Camu-Camu is efficient in the treatment of the metabolic syndrome has already been tested in a work with obese rats[20]. The experimental group treated with 25ml/day of pulp of the *M. dubia* fruit presented loss of body weight, with a decrease of 31.7% when compared to the control group. The treatment of obese rats with fruit resulted in a decrease in glycemia (23%), cholesterol (39.6%) and triglycerides (40.6%) compared with increases observed in controls: 19.4% glycemia, 60%cholesterol, and 44% triglycerides.

A recent study showed that dietary supplementation with Camu-Camu extract (200mg/kg) was able to prevent obesity in rats submitted to a hypercaloric/hyperlipidic diet[21]. These animals had an increase in resting metabolism, which, in turn, led to a lower accumulation of fat. An important factor in this study was the presence of a group that received the same dose of vitamin C found in the 200mg/kg of Camu-Camu extract (6.6mg/kg), but did not present good results in relation to weight loss. Therefore, there is great possibility that only vitamin C is not the reason for the anorectic effect of Camu-Camu.

The CCG of this study did not present results as expressive as those found in other studies[10]. However, the lower values of BMI observed in the animals submitted to Camu-Camu supplementation, even after only four weeks of intervention, indicate that the extract has the ability to regulate the inflammatory response induced by excess fat cells, proving to be a promising therapy for the treatment of obesity.

Our study showed that the crude hydroalcoholic extract of the Camu-Camu peel, although not as efficient as sleeve gastrectomy in the body weight control of Wistar rats, leads to a statistically significant reduction in the BMI of the animals after its administration for four weeks.

R E S U M O

Objetivo: *comparar os efeitos do extrato hidroalcoólico da casca do Camu-Camu, uma planta frutífera pertencente à família Myrtaceae amplamente distribuída na bacia amazônica, com os da gastrectomia vertical, sobre o peso e a glicemia de ratos Wistar.* **Métodos:** *vinte e quatro ratos Wistar foram submetidos à indução de obesidade através de dieta hiperlipídica por oito semanas (período de engorda), e randomizados em três grupos: Grupo Controle (GC), Grupo Camu-Camu (GCC) e Grupo Cirurgia Bariátrica (GCB). Após esse período, todos os animais retornaram a uma dieta normal e iniciou-se o período de intervenção: o GC não sofreu nenhuma intervenção além da mudança da dieta; o GCC foi submetido ao procedimento de gavagem para administração de extrato hidroalcoólico de Camu-Camu 1g/kg/dia por quatro semanas; e o GCB foi submetido ao procedimento cirúrgico de gastrectomia vertical. Todos os animais foram acompanhados por quatro semanas.* **Resultados:** *houve apenas uma perda no GCB devido à fístula gástrica. Observou-se variações significativas no peso médio dos animais: o GC evoluiu com aumento de peso mesmo após a retirada da dieta de engorda, enquanto os outros dois grupos apresentaram redução de peso. O GCB apresentou redução significativa do peso e do IMC (p<0,05); o GCC obteve redução significativa apenas do IMC (p<0,05). Não houve alterações estatisticamente significantes nos níveis glicêmicos.* **Conclusão:** *apesar de reduzir o peso, o extrato hidroalcoólico bruto da casca do Camu-Camu não foi capaz de se mostrar tão eficiente quanto a cirurgia de gastrectomia vertical no controle do peso corporal em ratos Wistar.*

Descritores: *Obesidade. Gastrectomia. Cirurgia Bariátrica. Fitoterapia. Ratos Wistar.*

REFERENCES

1. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. N Engl J Med [Internet]. 2017 Jan [cited]; 376(3):254-66. Available from: http://www.nejm.org/doi/10.1056/ NEJMra1514009
2. GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. Health effects of overweight and obesity in 195 countries over 25 Years. N Engl J Med [Internet]. 2017 Jul [cited]; 377(1):13-27. Available from: http://www.nejm.org/doi/10.1056/ NEJMoa 1614362
3. Tessier DJ, Eagon JC. Surgical management of morbid obesity. Curr Probl Surg. 2008;45(2):68-137.
4. Shah M, Simha V, Garg A. Review: long-term impact of bariatric surgery on body weight, comorbidities, and nutritional status. J Clin Endocrinol Metab. 2006;91(11):4223-31.
5. Brolin RE. Bariatric surgery and long-term control of morbid obesity. JAMA. 2002;288(22):2793-6.
6. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, et al. Bariatric surgery versus intensive medical therapy for diabetes - 5-year outcomes. N Engl J Med [Internet]. 2017 Feb [cited]; 376(7):641-51. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1600869
7. Buchwald H, Avidor Y, Braunswald E, Jensen MD, Pories W, Fahrbach K, et al. Bariatric surgery: a systematic review and meta-analysis. JAMA. 2004;292(14):1724-38. Erratum in: JAMA. 2005;293(14):1728.
8. Bose M, Oliván B, Teixeira J, Pi-Sunyer FX, Laferrère B. Do incretins play a role in the remission of type 2 diabetes after gastric bypass surgery: what are the evidence? Obes Surg. 2009;19(2):217-29.
9. Langley PC, Pergolizzi JV Jr, Taylor R Jr, Ridgway C. Antioxidant and associated capacities of Camu camu (Myrciaria dubia): a systematic review. J Altern Complement Med [Internet]. 2015 Jan [cited]; 21(1):8-14. Available from: http://online.liebertpub.com/doi/abs/10.1089/acm.2014.013
10. Arellano-Acuña E, Rojas-Zavaleta I, Paucar-Menacho LM. Camu-camu (Myrciaria dubia): Tropical fruit of excellent functional properties that help to improve the quality of life. Sci Agropecuaria [Internet]. 2016 Oct [cited]; 7(4):433-43. Available from: http://revistas.unitru.edu.pe/index.php /scientiaagrop / article/view/1266
11. Rodrigues RB, Menezes HC de, Cabral LMC, Dornier M, Reynes M. An amazonian fruit with a high potential as a natural source of vitamin C: the camu-camu (Myrciaria dubia). Fruits. 2001;56(5):345-54.

12. Zanatta CF, Mercadante AZ. Carotenoid composition from the Brazilian tropical fruit camu-camu (Myrciaria dubia). Food Chem. 2007;101(4):1526-32.
13. Inoue T, Komoda H, Uchida T, Node K. Tropical fruit camu-camu (Myrciaria dubia) has anti-oxidative and anti-inflammatory properties. J Cardiol. 2008;52(2):127-32.
14. Akachi T, Shiina Y, Kawaguchi T, Kawagishi H, Morita T, Sugiyama K. 1-methylmalate from camu-camu (Myrciaria dubia) suppressed D-galactosamine-induced liver injury in rats. Biosci Biotechnol Biochem [Internet]. 2010 Mar; 74(3):573-8. Available from: http://www.tandfonline.com/doi/full/10.1271/bbb.90775
15. De Souza Schmidt Gonçalves AE, Lellis-Santos C, Curi R, Lajolo FM, Genovese MI. Frozen pulp extracts of camu-camu (Myrciaria dubia McVaugh) attenuate the hyperlipidemia and lipid peroxidation of Type 1 diabetic rats. Food Res Int. 2014;64:1-8.
16. Sotero Solis V, Silva Doza L, García de Sotero D, Imán Correa S. Evaluación de la actividad antioxidante de la pulpa, cáscara y semilla del fruto del camu camu (Myrciaria dúbia H.B.K.). Rev Soc Quím Perú. 2009;75(3):293-9.
17. Neves SMP, Ong FMP, Rodrigues LD, Santos RA, Fontes RS, Santana RO. Manual de Cuidados e Procedimentos com Animais de Laboratório do Biotério de Produção e Experimentação da FCF-IQ/USP. São Paulo: FCF-IQ/USP; 2013.
18. Rosini TC, Silva ASR, Moraes C. Obesidade induzida por consumo de dieta: modelo em roedores para o estudo dos distúrbios relacionados com a obesidade. Rev Assoc Med Bras [Internet]. 2012;58(3):383-7. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0104423012705250
19. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Luvizotto RAM, Nogueira CR, et al. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in wistar rats. Arq Bras Endocrinol Metabol. 2008;52(6):968-74.
20. Nascimento OV, Boleti APA, Yuyama LKO, Lima ES. Effects of diet supplementation with Camu-camu (Myrciaria dubia HBK McVaugh) fruit in a rat model of diet-induced obesity. An Acad Bras Ciênc. 2013;85(1):355-63.
21. Anhê FF, Nachbar RT, Varin TV, Trottier J, Dudonné S, Le Barz M, et al. Treatment with camu camu (Myrciaria dubia) prevents obesity by altering the gut microbiota and increasing energy expenditure in diet-induced obese mice. Gut. 2018; pii: gutjnl-2017-315565.

Received in: 05/12/2019
Accepted for publication: 06/25/2019
Conflict of interest: none.
Source of funding: This study was partially funded by CNPq (National Council for Scientific and Technological Development) and CAPES (Federal Agency for Support and Evaluation of Post-Graduate Education).

Mailing address:
Hercules Magalhães Olivense do Carmo
E-mail: herculesdoc@uol.com.br