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Evidence-Based Complementary and Alternative Medicine Effects of Capybara Oil on Cardiac Remodeling of C57bl/6 Mice

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Abstract

Cardiovascular diseases are the main cause of morbidity and mortality in the world, and obesity and the metabolic syndrome are risk factors for its development. One of the therapies to reduce cardiovascular risk is the use of polyunsaturated fatty acids. In Brazil, a source of such acid is the oil extracted from the fat of the capybara. The objective of this work is to study the effects of the capybara oil on lipid and glucose metabolism, as well as its effects on the adipose tissue and cardiac remodeling. We assessed the effects of capybara oil treatment on body mass, lipid and carbohydrate metabolism, systolic blood pressure, adipose tissue and cardiac remodeling, and performed an ultrastructural evaluation of the myocardium in C57Bl/6 mice treated with high-fat diet. Treatment with capybara oil reduced total cholesterol and triglyceride levels, systolic blood pressure, visceral and subcutaneous adipose tissue, and adipocyte diameter. In addition, cardiac remodeling was attenuated, preserving cardiomyocytes, increasing vascularization, reducing cardiomyocyte hypertrophy and the extracellular matrix, and preserving the morphological integrity of mitochondria. Capybara oil has several beneficial effects on the cardiovascular and metabolic system, and further studies are needed to better understand its role in the prevention or treatment of cardiovascular diseases.

Keywords

Capybara Oil, Cardiac Remodeling, Polyunsaturated Fatty Acids, Obesity, Metabolic Syndrome

1. Introduction

Overweight and obesity are real epidemics, with statistics showing that more than 2/3 of the US population is affected [1]. These are also considered major risk factors for cardiovascular diseases, which are the leading cause of death in the world [2] [3]. The concomitant occurrence of abdominal obesity and risk factors such as hyperglycemia, dyslipidemia, and hypertension are compatible with a metabolic syndrome [4] [5]. Obesity, especially abdominal obesity, is related to insulin resistance, hyperinsulinemia, hyperglycemia, and adipokine secretion, which can lead to endothelial dysfunction, altered lipid profile, systemic arterial hypertension, and vascular inflammation, all of which are favorable to the development of atherosclerosis, the main cause of cardiovascular mortality [4] [6] [7] [8].

In obese patients, the cardiac output is increased to meet a higher tissue demand, which is justified by a higher body mass [9]. This increase is mediated by an increase in blood volume, with a consequent increase in the filling pressures, as well as a greater activation of the sympathetic nervous system. All of these common changes in obese individuals trigger an increase in oxygen uptake and, consequently, increase tissue demand [10] [11]. The major factors leading to cardiac remodeling increase with increasing body mass index (BMI) [12]. The excessive accumulation of visceral adipose tissue in the pericardium, epicardium and liver results from an increase in blood volume and activation of a pro-atherogenic inflammatory pathway that increases cardiac output, wall stress and myocardial injury, leading to concentric left ventricular hypertrophy and systolic/diastolic dysfunction [13].

In order to reduce the impact of cardiovascular diseases, several interventions have been studied, one of which is the use of oils that are rich in polyunsaturated fatty acids, which, when used with approximately 0.2 to 1.0 gram of omega 3, was associated with a close to 50% decrease in mortality [14]. Meta-analyses of these trials have generally not identified any significant beneficial effects of omega-3 fatty acids in major cardiovascular events [15] [16]. However, some omega-3 effects are well established. For lipid metabolism, they showed a 25% - 30% decrease in triglycerides [17] [18] [19], 3% increase in HDL and 5% increase in LDL [20] [21] [22]. On blood pressure, they decreased the systolic blood pressure by 1.52 mmHg and the diastolic blood pressure by 0.99 mmHg [23].

There are several sources of polyunsaturated fatty acids, and the oil extracted from the capybara fat is not very commonly used but is widely available in Brazil. It contains 19.6% linoleic acid (LA, 18:2n-6) and 17.9% α-linoleic acid (ALA, 18:3n-3), and is rich in omega-3 [24]. Few articles have been published showing the benefits of this oil. In the first article, Fukushima demonstrated cholesterol reduction [24]. Several years after that, Marinho demonstrated its beneficial effects as a skin wound healer [25] and has recently described beneficial effects on liver steatosis [26].

An experimental model for the study of the metabolic syndrome and obesity

can be obtained by using a high-fat (HF) diet in C57Bl/6 mice [27] [28] [29]. Experimental studies have shown that obesity can lead to structural changes in the heart, such as ventricular hypertrophy, interstitial fibrosis, and intracellular accumulation of lipids [30] [31]. Therefore, the objective of this work is to study the effects of the capybara oil on lipid and glucose metabolism, as well as its effects on adipose tissue and cardiac remodeling. Considering that capybara oil is rich in polyunsaturated fatty acids and is widely available in Brazil, we would like to assess whether this source of polyunsaturated fatty acids has the potential to be used for the prevention of cardiovascular diseases.

2. Methods

2.1. Experimental Groups

All of the procedures were conducted in accordance with the conventional guidelines for animal experimentation [32]. All of the experimental protocols were approved by the animal ethics committee of the State University of Rio de Janeiro. The animals were housed under controlled conditions (temperature at 21˚C ± 2˚C, humidity at 60% ± 10% and a 12-h/12-h dark/light cycle) and had free access to food and water. The mineral and vitamin contents of the two diets were identical and were consistent with the American Institute of Nutrition's recommendation (AIN 93M) [33]. The mouse chow was prepared by Pragsoluções (Jaú, São Paulo, Brazil).

The choice of animal species and lineage also influences the results of studies. The most useful animal models for studying cardiovascular and metabolic diseases are small rodents. C57BL/6 mice emerge as a model for studying metabolic syndrome in rodents for comparisons with humans. This lineage presents genetic vulnerability and is greatly influenced by environmental factors in the development of obesity, insulin resistance, and type 2 diabetes [27] [34]. Other important factors include the gradual development of metabolic changes and the selective deposition of fat in the mesentery, consistent with the fact that visceral obesity is an independent risk factor for diabetes in humans [35] [36].

Capybara fat was donated by a private slaughter farm in Brazil that breed capybara in captivity as authorized by the Brazilian Institute of Environment and Renewable Natural Resources. The CO composition was previously described [24]. Oil was extracted using hydrothermal processing of the fat in a water bath [25].

At baseline, after one week of acclimatization, 32 three-month-old C57Bl/6 male mice were randomly divided and fed different diets during a 12-week period, which included a C diet (control chow; 10% lipids, n = 16) or a HF diet (60% lipids, n = 16). Both diets are detailed in **Table 1**. The 12-week period of HF diet administration aimed at inducing the main features of the metabolic syndrome. Before being divided into four groups, the homoscedasticity of variances was tested and all animals followed the normal distribution and had no differences concerning body mass, which guarantee that different groups started the experiment without any differences that could add bias to the study.

Table 1. Composition and energy content of the control and high fat diet/cal = 4184 J.

Content (g/kg)	Diet	
	C	HF
Casein (≥ 85% protein)	140.0	190.0
Cornstarch (g/kg)	620.7	250.7
Sucrose (g/kg)	100.0	100.0
Soybean oil (g/kg)	40.0	40.0
Lard (g/kg)		320.0
Fiber (g/kg)	50.0	50.0
Vitamin mix (g/kg)*	10.0	10.0
Mineral mix (g/kg)*	35.0	35.0
L-cystin (g/kg)	1.8	1.8
Choline (g/kg)	2.5	2.5
Antioxidant (g/kg)	0.008	0.008
Total mass (g)	1000	1000
Energy content (Kcal/kg)	3573	5404
Carbohydrates (%)	76	26
Protein (%)	14	14
Lipids (%)	10	60

Afterwards, the C group was randomly divided into 2 groups (n = 8 each) in order to begin treatment with capybara oil, and the HF group was randomly divided into 2 groups (n = 8 each) in order to begin treatment with capybara oil. Consequently, four groups were formed, as follows: C group (control chow during the whole experiment, 18-week period/n = 8); C + CO group (control chow during the whole experiment, 18-week period, plus capybara oil 1.5 g/kg/day, in the last 6 weeks/n = 8); HF group (high fat diet during the whole experiment, 18-week period/n = 8); HF + CO group (high fat diet, 18-week period, plus capybara oil 1.5 g/kg/day, in the last 6 weeks/n = 8). Treatments lasted 6 weeks, and capybara oil was given by orogastric gavage. Fresh chow was provided daily, and any remaining chow from the previous day was discarded. The food intake was evaluated daily (1 p.m.), and the body mass was measured weekly. Taking daily food consumption and BM into account, the capybara oil doses were corrected to match the same concentrations as indicated.

Capybara fat was donated by a private slaughter farm in Brazil that breeds capybara in captivity as authorized by the Brazilian Institute of Environment and Renewable Natural Resources. The CO composition was previously described

[24]. Oil was extracted using hydrothermal processing of the fat in a water bath [25].

Body Mass (BM) was measured every week during the experiment. Systolic blood pressure was measured in conscious mice (tail cuff plethysmography with heater, V 3.0, Insight, Ribeirão Preto, Brazil) at the beginning and end of the experiment.

To evaluate the glucose tolerance, an OGTT was performed after 12 weeks of diet intake (before treatment) and, again, six weeks after treatment. The animals fasted for 6 h and then received 25% glucose in sterile saline (0.9% NaCl) at a dose of 1 g/kg by orogastric gavage. Blood was collected through a little incision at the tip of the tail, and the plasma glucose concentration was measured (glucometer; Accu-Chek Active; RocheApplied Science, Brazil). The plasma glucose was assessed before glucose administration and 30, 60 and 120 min after glucose administration. The response was expressed as the area under the curve (AUC) (GraphPad Prism version 5.03; San Diego, CA, USA).

The animals were food-deprived for 6 h and then deeply anaesthetized with sodium pentobarbital (150 mg/kg i.p.). The blood was sampled by cardiac puncture at the right atrium and then centrifuged (120 ×g for 15 min) at room temperature. The total cholesterol (TC) and triglycerides (TG) in the serum were measured by a colorimetric enzymatic assay (Bioclin, Quibasa, Belo Horizonte, MG, Brazil).

2.2. Light Microscopy

Some ventricle fragments were put into the fixative for 48 h (freshly prepared 4% (w/v) formaldehyde in 0.1 M phosphate buffer pH 7.2). After embedding in Paraplast Plus (Sigma-Aldrich, St. Louis, MO, USA), the blocks were sectioned and stained with hematoxylin eosin.

2.3. Electron Microscopy

The hearts were carefully removed, sectioned and fixed in 2.5% glutaraldehyde (Riedel-de-Haen, Germany) in 0.1 M cacodylate buffer (pH 7.2). After fixation (at least 12 hours), the samples were rinsed three times (15 min each) in 0.1 M imidazole buffer (pH 7.5) and post-fixed in 2% osmium tetroxide (Sigma-Aldrich Louis, USA) in imidazole buffer for 30 minutes [37], then rinsed three times (15 min each) in 0.1 M imidazole buffer. After rinsing, the samples were dehydrated through a graded series of acetone (30%, 50%, 70%, 90% and twice in 100%) and then embedded in Epon (Embed-812). Semi-thin sections (1 μm) were cut, stained with toluidine blue (Vetec, Rio de Janeiro, Brazil), and observed with a light microscope (OlympusBX 53F, Tokyo, Japan). Ultra-thin sections (70 nm) were obtained with an ultramicrotome (Leica Ultracut-UCT, Leica Mikrosysteme Gmbh, Austria), counter stained with uranylacetate and lead citrate, and examined with a Zeiss EM 906 transmission electron microscope (Carl Zeiss, Oberköchen, Germany) at 80 Kv.

2.4. Stereology

The obtained images were analyzed with a test-system composed of 36 test-points (PT) in a frame with a known area (AT). The following parameters were analyzed: volume density, VV [structure] = Pp [structure]/PT (Pp stands for the number of points that hit the structure); numerical density per area, QA [structure] = number of profiles/AT; and cross-sectional area, A[structure] = (VV)/(2 × (QA)). The estimated structures were cardiomyocytes (cmy) and cardiac interstitium (focusing on the capillary and Connective Tissue (CT)) of the myocardium [38] [39].

2.5. Adipocyte Morphometry

After euthanasia, the retroperitoneal and epididymal (visceral fat) and inguinal (subcutaneous fat) fat pads were collected and weighed. Histological slices of the epididymal fat pad were prepared, and digital images were obtained (LC Evolution camera; Olympus BX 51 microscope and Media Cybernetics Image-Pro Plus version 7.0; TIFF format; 36-bit color; 1280 × 1024 pixels). The mean cross-sectional area of at least 50 adipocytes per mice was estimated [40].

2.6. Data Analysis

The values are shown as the means ± SEM. In all of the cases in which homoscedasticity among the variances was confirmed, data were analyzed using ANOVA followed by post-hoc Tukey's test. If homoscedasticity was not confirmed, the differences were analyzed using the Kruskal-Wallis test and the post-hoc Dunn's test. A p-value ≤0.05 was considered statistically significant (GraphPad Prism version 5.03 for Windows).

3. Results

3.1. The Body Mass

The body mass (BM) of the mice fed the HF diet for 12 weeks increased progressively compared to the animals receiving the control diet (C vs. HF $p \leq 0.0001$, CI 95% −12.12 to −3.73, **Figure 1(A)**). After six weeks of capybara oil treatment (18 weeks on the respective diet), the BM remained greater in the HF and HF + CO groups compared with the C and C + CO groups (C vs. HF $p \leq 0.0001$, CI 95% −12.05 to −4.31; C vs. HF + CO $p \leq 0.001$, CI 95% −10.27 to −2.53; C + CO vs. HF $p \leq 0.0001$, CI 95% −14.63 to −6.89; C + CO vs. HF + CO $p \leq 0.0001$, CI 95% −12.85 to −5.11, **Figure 1**).

3.2. Oral Glucose Tolerance Test (OGTT)

The mice from the HF group exhibited glucose intolerance after 12 weeks on the HF diet (pre-treatment, **Table 2**). Before the capybara oil treatment, OGTT AUC was greater for the HF mice than the C mice (16% greater, $p \leq 0.05$, CI 95% 27.03 to 205.5). This difference was not observed at the end of the experiment (18 weeks). The administration of capybara oil did not improve glucose intolerance.

Table 2. Carbohydrate metabolism, lipid profile and adipose tissue weight and morphology.

Parameters	Groups			
Pre-treatment	C	HF		
OGTT (AUC) initial	707.45 ± 26.89	823.72 ± 35.66[a]		
	C	C + CO	HF	HF + CO
OGTT (AUC) final	686.1 ± 7.10	638.3 ± 14.10	714.5 ± 6.99	725.6 ± 5.36
Serum total cholesterol	153.8 ± 3.55	143.9 ± 3.21	211.7 ± 8.32[a,b,d]	141.3 ± 7.29
Serum triglyceride	174.0 ± 3.68[c]	180.0 ± 2	186.0 ± 2.44[a,d]	164.6 ± 2.35[b,c]
Visceral fat (mg)/tibia(cm)	0.738 ± 0.067[b,c,d]	0.558 ± 0.046[a,c,d]	1.370 ± 0.149[a,b]	1.350 ± 0.118[a,b]
Subcutaneous fat (mg)/tibia (cm)	0.037 ± 0.004[b,c,d]	0.023 ± 0.003[a,c,d]	0.080 ± 0.012[a,b,d]	0.060 ± 0.007[a,b,c]
Adipocyte diameter (μm)	431 ± 16.67	360.5 ± 12.3[a,c,d]	548.85 ± 15.67[a,b]	556.14 ± 12.55[a,b]

Abbreviations: C: Control Group; C + CO: capybara oil + Control Diet; HF: High-Fat Diet; HF + CO: capybara oil + High-Fat Diet. Subcutaneous fat (inguinal fat), Visceral fat (epididymal plus retroperitoneal fat). Symbols indicate differences with: [a] C group; [b] C + CO group; [c] HF group; [d] HF + CO group. Visceral fat(mg)/tibia(cm): C vs. C + OC CI 95% 0.067 to 0.292; C vs. HF CI 95% −0.744 to −0.52; C vs. HF + OC CI 95% −0.727 to −0.497; C + OC vs. HF CI 95% −0.924 to −0.699; C + OC vs. HF + OC CI 95% −0.907 to −0.677; HF vs. HF + OC CI 95% −0.095 to 0.135. Subcutaneous fat (mg)/tibia (cm): C vs. HF CI 95% −0.051 to −0.035; C vs. HF + OC CI 95% −0.032 to −0.014; C + OC vs. HF CI 95% −0.065 to −0.049; C + OC vs. HF + OC CI 95% −0.046 to −0.028; HF vs. HF + OC CI 95% 0.011 to 0.029. Adipocyte diameter (μm): C vs. C + OC CI 95% 12.47 to 128.5; C vs. HF CI 95% −174.3 to −61.39; C vs. HF + OC CI 95% −185.2 to −65.04; C + OC vs. HF CI 95% −239.3 to −137.4; C + OC vs. HF + OC CI 95% −250.6 to −140.7; HF vs. HF + OC CI 95% −60.56 to 45.97.

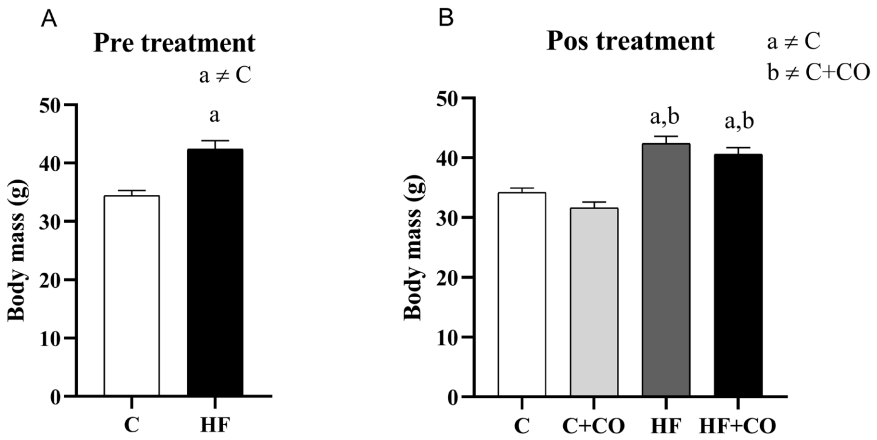

Figure 1. Initial—12 weeks (A) and final—18 weeks (B) body mass. Mice were fed the control chow (C) or a high-fat diet (HF) for 12 weeks (initial). The mice then received six weeks of capybara oil (OC) (final—18 weeks). The symbols indicate a difference compared with [a] the C group, [b] the C + CO group, [c] the HF group, and [d] the HF + CO group. The values are shown as the means ± SEM.

3.3. Blood Pressure

The mice from the HF group did not exhibit any increase in blood pressure after 12 weeks on the HF diet (pre treatment, **Figure 2**). At the end of the experiment, the other groups showed a reduction in blood pressure when compared to the HF group. The C group (−64 mmHg, $p \leq 0.0001$, CI 95% −74.39 to −53.61),

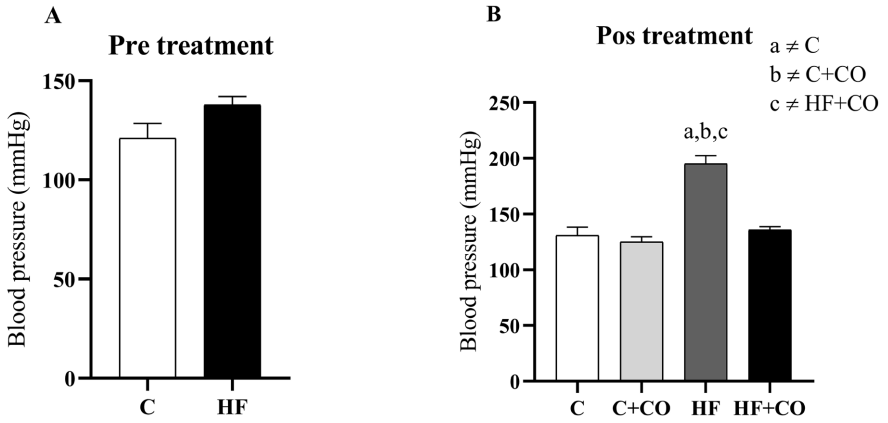

Figure 2. Blood pressure initial—12 weeks (A) and final—18 weeks (B). Pre- and Post-Treatment Period Systolic Blood Pressure. Measured by the tail cuff plethysmography method in mice in groups C (control), C + OC (control + capybara oil), HF (hyperlipid diet) and HF + OC (hyperlipid diet + capybara oil). No significant difference in the post-treatment period. Post-treatment: HF vs. C $p \leq 0.0001$; HF vs. C + OC $p \leq 0.0001$; HF vs. HF + OC $p \leq 0.001$. Abbreviations: C: Control Group; C + CO: capybara oil + Control Diet; HF: High-Fat Diet; HF + CO: capybara oil + High-Fat Diet. Symbols indicate differences with: [a] C group; [b] C + CO group; [c] HF + CO group.

C + CO group (−69.8 mmHg, $p \leq 0.0001$, CI 95% −80.19 to −59.41), and HF + CO group (−59 mmHg, $p \leq 0.001$, CI 95% −48.61 to −69.39). At the same time, groups treated with capybara oil showed a significant reduction in blood pressure when compared with the HF group and reached values that are similar to those in the C group (Figure 2).

3.4. Blood Biochemistry

Total cholesterol (TC) increased in the HF mice compared with the C group (57.9, $p \leq 0.0001$, CI 95% 82.28 to 33.52, Table 2), C + CO group (67.8, $p \leq 0.0001$, CI 95% 92.18 to 43.42, Table 2), and HF + CO group (70.4, $p \leq 0.0001$, CI 95% 46.02 to 94.78, Table 2). The decrease in total cholesterol seen in the HF + CO group when compared to the HF group shows a beneficial effect of the oil.

Serum triglycerides (TG) increased in the HF mice compared with the C group (12, $p \leq 0.05$, CI 95% 1.08 to 22.92, Table 2) and the HF + CO group (21.4, $p \leq 0.001$, CI 95% 10.48 to 32.32, Table 2). Triglyceride dosage was 15.4 greater in the C + CO group when compared to the HF + CO ($p \leq 0.01$, CI 95% 4.476 to 26.32, Table 2) group. The decrease in triglycerides seen in the HF + CO group when compared to the HF group shows a beneficial effect of the oil.

3.5. Adipose Tissue

The high-fat diet increased the visceral fat mass (retroperitoneal and epididymal fat pads, Table 2) which was weighed and its value corrected for tibia length. This fat mass was significantly greater in the HF group (+85%, $p \leq 0.0001$; +145%, $p \leq 0.0001$) than in the C and C + OC groups, respectively. There was an increase (+83%, $p \leq 0.0001$; +142%, $p \leq 0.0001$) in the HF + OC group, when

compared respectively to groups C and C + OC. There was a decrease in this fat (−24%, $p \leq 0.001$) in the C + OC group when compared to group C.

The inguinal fat was weighed and its value was corrected for post-euthanasia tibia length (Table 2). This fat mass was significantly greater in the HF group (+116%, $p \leq 0.0001$; +247%, $p \leq 0.0001$) than in the C and C + OC groups, respectively. Greater (62%, $p \leq 0.0001$; +160%, $p \leq 0.0001$) in the HF + OC group, when compared respectively to groups C and C + OC. There was a decrease in this fat (−38%, $p \leq 0.001$) in the C + OC group when compared to group C. Also, there was a decrease (−25%, $p \leq 0.0001$) in the HF + OC group when compared to the HF group.

Hypertrophied adipocytes were observed in the HF and HF + CO groups. The adipocyte diameter was 27% greater ($p \leq 0.001$) in the HF group than in the C group, and 29% greater ($p \leq 0.0001$) in the HF + CO group than in the C group (Table 2). The adipocyte diameter was 52% greater ($p \leq 0.0001$) in the HF group than the C + CO group, and 54% greater ($p \leq 0.0001$) in the HF + CO group than the C + CO group (Table 2). There was a 16% decrease in adipocyte diameter in the C + OC group when compared to the C group ($p \leq 0.05$).

3.6. Heart Mass

The HF group showed a significant increase (more than 25%, $p \leq 0.05$) in ventricular mass than the C group. The groups treated with capybara oil did not show a significant reduction in these values compared with the HF group and were not significantly different from the C group (Table 3). The ventricular mass values were normalized to the length of the tibia.

3.7. Ultrastructural Analysis

Figure 3 shows that the number of nuclei is markedly greater in group C (control) (Figure 3: image A) and group C + OC (Figure 3: image B) than in group HF (high fat) (Figure 3: image C) and group HF + OC (Figure 3: image D). Groups C and C + OC have more nuclei than the HF group.

3.8. Stereology Analysis

Cardiomyocyte density (QA [cmy]) in the HF group was significantly lower (approximately 22% less) than that of the C group ($p \leq 0.01$); the HF + CO group was lower (16% less) than that of the C group ($p \leq 0.05$) (Table 3). Animals treated with capybara oil (C + CO and HF + CO) showed no significant difference compared to the C group or HF group, respectively. The groups showed no significant difference in volume density of cardiomyocytes (Vv [cmy]) and volume density of the connective tissue (VV [ct]); however, when analyzing the sectional area of cardiomyocytes (A [cmy]), the HF group showed a significant increase (31% higher) when compared with the C group ($p \leq 0.0001$), suggesting hypertrophy of the cardiomyocytes. The C + CO groups were not significantly different from the C group (Table 3), but the sectional area of cardiomyocytes

Table 3. Heart mass and stereological analysis.

Data	C	C + CO	HF	HF + CO
Heart mass (g/cm)	0.008 ± 0.0003	0.0075 ± 0.0003	0.01 ± 0.0006[a,b]	0.01 ± 0.0006[a,b]
Myocardium				
QA cmy (1/mm²)	824 ± 30.89	886 ± 46.29	641 ± 22.05[a,b]	691.9 ± 35.86[a,b]
QA ima (1/mm²)	264.4 ± 21.22	282.1 ± 8.57	181.8 ± 6.97[a,b]	217.3 ± 10.55[b]
Vv cmy (%)	0.695 ± 0.025	0.691 ± 0.037	0.709 ± 0.024	0.651 ± 0.0076
Vv ima (%)	0.184 ± 0.024	0.187 ± 0.024	0.136 ± 0.0166[d]	0.207 ± 0.0087
Vv int (%)	0.12 ± 0.0097	0.122 ± 0.02	0.155 ± 0.0144	0.142 ± 0.014
A cmy (μm²)	422.3 ± 9.88	392.9 ± 22.89	554.3 ± 20.69[a,b,d]	475.2 ± 21.36[b,c]
Vv ima/cmy (%)	0.272 ± 0.045	0.28 ± 0.047	0.15 ± 0.028[d]	0.317 ± 0.012
Lv ima (mm/mm³)	528.7 ± 42.44	564.2 ± 17.14	363.6 ± 13.95[a,b]	434.6 ± 21.11[b]

Abbreviations: C: Control Group; C + CO: capybara oil + Control Diet; HF: High-Fat Diet; HF + CO: capybara oil + High-Fat Diet. Symbols indicate differences with: [a] C group; [b] C + CO group; [c] HF group; [d] HF + CO group. QA [Cmy]: Density of the cardiomyocytes; QA [ima]: Density of the intramyocardial arteries; Vv [Cmy]: Volume density of cardiomyocytes; Vv [ima]: Volume density of intramyocardial arteries; Vv [int]: Volume density of the connective tissue; A [Cmy]: Sectional area of cardiomyocytes; Vv [ima/cmy]: Volume density of intramyocardial arteries/cardiomyocytes; Lv [ima], length density intramyocardial arteries. Heart mass (g/cm): C vs. HF CI 95% −0.003752 to −0.000248; C vs. HF + OC CI 95% −0.003791 to −0.0002087; C + OC vs. HF CI 95% −0.004252 to −0.000748; C + OC vs. HF + OC CI 95% −0.004291 to −0.0007087. QA cmy (1/mm²): C vs. HF CI 95% 55.33 to 310.7; C vs. HF + OC CI 95% 4.433 to 259.8; C + OC vs. HF CI 95% 117.3 to 372.7; C + OC vs. HF + OC CI 95% 66.43 to 321.8. QA ima (1/mm²): C vs. HF CI 95% 34.76 to 130.4; C + OC vs. HF CI 95% 52.46 to 148.1; C OC vs. HF + OC CI 95% 16.96 to 112.6. A cmy (μm²): C vs. HF CI 95% −203.0 to −61.00; C + OC vs. HF CI 95% −232.4 to −90.40; C + OC vs. HF + OC CI 95% −153.3 to −11.30; HF vs. HF + OC CI 95% 8.101 to 150.1. Vv ima/cmy (%): HF vs. HF OC CI 95% −0.2985 to −0.03555. Lv ima (mm/mm³): C vs. HF CI 95% 69.41 to 260.8; C OC vs. HF CI 95% 104.9 to 296.3; C OC vs. HF OC CI 95% 33.91 to 225.3.

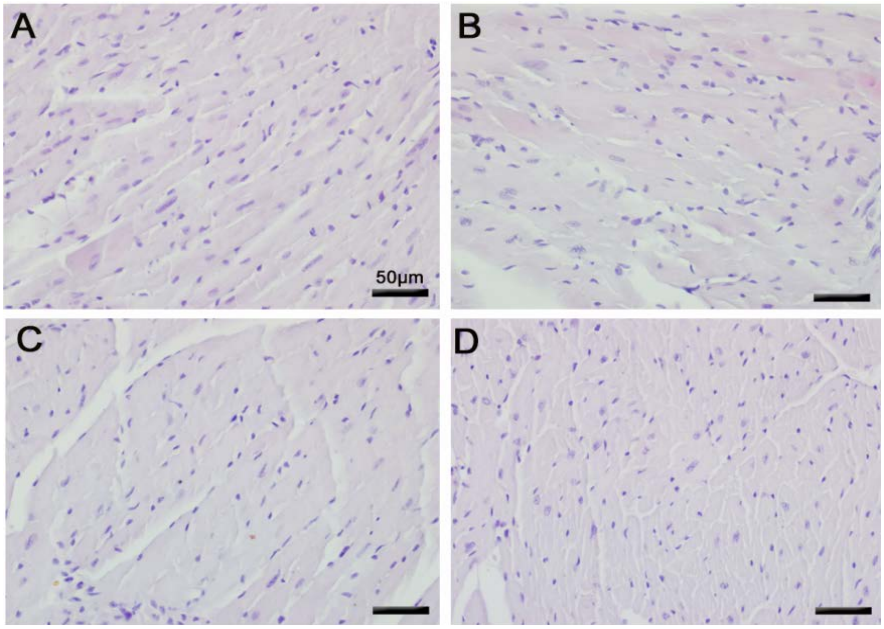

Figure 3. Photomicrographs of the myocardium (bar = 30 μm). (A) C group; (B) C + CO group; (C) HF group; (D) HF + CO group.

(A [cmy]) was lower (14% less) in the HF + CO group than the HF group ($p \leq 0.05$) (Table 3 and Figure 3), thus demonstrating a beneficial effect for capybara oil in reducing ventricular hypertrophy.

There was a 31% decrease in vessel density (QA ima) in the HF group compared to the C group ($p \leq 0.0001$). Another parameter evaluating vascularization is vessel length density (Lv ima), which was lower in the HF group when compared to C (31% lower, $p \leq 0.001$). Animals treated with capybara oil (C + CO and HF + CO) showed no significant differences compared to the C group or HF group, respectively, for these two parameters. But another result that evaluates vascularization—vessel volume density (Vv ima)—showed a 34% decrease in the HF group, when compared to the HF + OC group ($p \leq 0.05$). Another noteworthy result is volume density of the vessel/cardiomyocyte ratio (Vv ima/cmy), which showed an increase (111%) in the HF + CO group when compared to the HF group ($p \leq 0.01$). Thus, it demonstrates a beneficial effect of capybara oil in improving vascularization.

3.9. Electron Microscopy

Cardiomyocyte photomicrographs are shown in Figure 4. Figure 4(A) and Figure 4(B) refer to Group C, showing cardiomyocytes with a preserved ultrastructure, and with mitochondria (M) displaying several ridges and a euchromatic nucleus. Figure 4(C) and Figure 4(D) refer to Group C + OC with a euchromatic nucleus and the presence of few lipid inclusions (arrows) and several mitochondria (M). Figure 4(E) and Figure 4(F) refer to the HF Group showing several lipid droplets (arrows), mitochondria (M) with fewer ridges, and collagen fibrils in the extracellular matrix (*). Figure 4(G) and Figure 4(H) refer to the HF + O group and have numerous mitochondria (M).

4. Discussion

In our study, the group fed with the HF diet showed increased body mass, increased systolic blood pressure, altered lipid metabolism, elevated total cholesterol, elevated triglycerides, increased visceral and subcutaneous adipose tissue with adipocyte hypertrophy, which are similar findings to other articles [26] [29] [41] [42] [43] [44] [45]. These conditions play a crucial role in the development of heart disease, such as hypertension, left ventricular hypertrophy and interstitial fibrosis [46] [47] [48] [49] [50], so this experimental model is suitable for the proposed study.

Treatment with capybara oil was not able to promote weight loss in the treated mice. As to blood pressure, there was a significant decrease in the HF + OC group when compared to the HF group, and blood pressure in the HF group was 43% higher than the HF + OC group. Animal and observational studies in humans suggest that a decrease in blood pressure secondary to the intake of polyunsaturated fatty acids occurs due to a decrease in peripheral vascular resistance [51] [52]. Polyunsaturated fatty acids increase the production of nitric oxide, attenuate

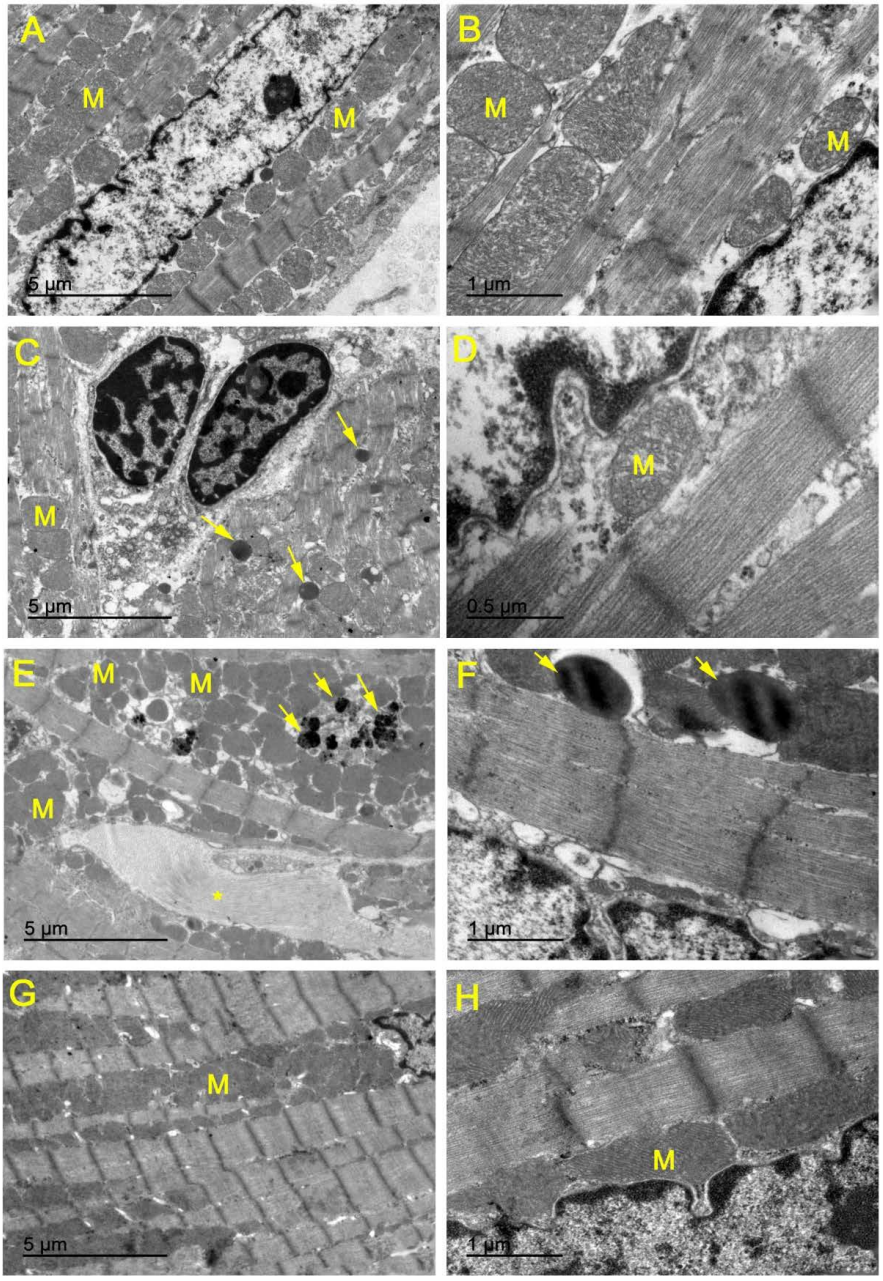

Figure 4. Left ventricular photomicrograph after 18 weeks ((A), (C), (E), (G) = bar 5 µm; (B), (F), (H) = bar 1 µm; (D) = bar 0.5 µm): (A) (B) group C. (C) (D) C + OC group. (E) (F) HF group. (G) (H) HF + OC group. Symbols: Mitochondria (M); arrows—lipid inclusions; (*) collagen fibrils in the extracellular matrix.

the peripheral vasoconstriction effect in response to angiotensin II and norepinephrine, improve vascular compliance and increase vasodilatory response [53] [54] [55] [56] [57]. These effects, either separately or added, lead to a decrease in peripheral vascular resistance and blood pressure. In studies with humans, the literature shows a reduction in systolic blood pressure of 1.52 mmHg and diastolic blood pressure of 0.99 mmHg with polyunsaturated fatty acids mmHg [23], data consistent with our study.

As to cholesterol, there was an increase in its dosage in the HF group when compared to the control group. When comparing the HF group to the HF + OC group, a 49% decrease in total cholesterol was seen in the group treated with capybara oil. A similar result was described by Fukushima *et al.* who used capybara oil [24]. In the serum triglyceride dosage, there was a 11.5% decrease in the HF + OC group when compared to the HF group. Polyunsaturated fatty acids have the characteristic of providing a decrease in triglycerides [17] [18] [19], similar to that found in our study using capybara oil. In studies with humans, the literature shows a reduction of 25% - 30% in triglycerides [17] [18] [19]; Increase of 3% in HDL; increase of 5% in LDL [20] [21] [22]. The reduction in triglycerides was consistent between our study and the literature.

Another parameter was heart mass, which showed a 25% and 33% increase in the HF and HF + OC groups, respectively, in relation to their respective control groups (C and C + OC). Treatment with capybara oil was not able to reduce heart mass in the HF + OC group. Studies with polyunsaturated fatty acid supplementation (fish oil and chia oil) showed similar results to those found in our study [48] [58] [59].

For adiposity, animals receiving a hyperlipid diet showed increased body fat. The HF and HF + OC groups showed a 85% and 142% increase in visceral fat, respectively, when compared to their respective control groups (C and C + OC), and a 116% and 160% increase in subcutaneous fat when compared to their respective controls (C and C + OC). These findings are similar to other studies developed in our laboratory [45] that demonstrate the metabolic imprint caused by a hyperlipid diet. Treatment with capybara oil was able to decrease visceral fat in the C + OC group by 24% compared to the C group. For subcutaneous fat, there was a 38% and 25% decrease in the C + OC and HF + OC groups, respectively, when compared to groups C and HF. A decrease in visceral fat has already been described by Poudyal *et al.* who used polyunsaturated fatty acids, with chia oil as the source [46].

As to adipocyte morphometry, a decrease was seen in the C and C + OC groups when compared to the HF and HF + OC groups, similar to other studies [27] [45] [60]. In addition, a relevant result was a 16% decrease in adipocyte morphometry for the C + OC group when compared to group C, demonstrating another beneficial effect of capybara oil. This is an important finding, since adipose tissue expresses several secretory proteins, including leptin, adiponectin, TNF-α, which are closely involved in the regulation of energy expenditure, lipid metabolism and insulin resistance [27]. There was a decrease in adipocyte diameter in the visceral fat in animals treated with capybara oil, demonstrating a possible beneficial effect of the capybara oil on remodeling the adipose tissue.

The stereological analysis showed a higher density of cardiomyocytes in the C and C + OC groups compared to the HF and HF + OC groups, but no differences were seen in the treated group when compared to its respective control. Decreased cardiomyocyte density is compatible with cardiomyocyte loss and is one

of the effects of cardiac remodeling. This finding is in accordance with the literature that shows that animals with metabolic syndrome have hypertrophy and cardiomyocyte loss [29] [61].

Myocardial vascularization was analyzed with several parameters. One of these parameters was the volume density of vessels (Vv vessels), with a 34% increase in the HF + OC group compared to the HF group. Another parameter that should be noted is volume density of the vessel/cardiomyocyte ratio (Vv vessel/cmi), which showed a 111% increase in the HF + OC group when compared to the HF group. Thus, these two parameters demonstrated the benefit of capybara oil for myocardial vascularization. The decreased vascularization found in the HF group was evidenced in several parameters and was compatible with a cardiac remodeling similar to that found in other experimental models [48] [58] [59] [62] [63] [64]. The benefit found with capybara oil in our study also occurred in other studies using non-lipid interventions [59] [62].

For ventricular hypertrophy, the area of cardiomyocytes was reviewed and showed a 31% and 21% increase in the HF and HF + OC groups when compared to the C and C + OC groups, respectively, which is compatible with other articles [48] [64] [65]. A 16% increase was seen in this parameter in the HF group when compared to the HF + OC group. Thus, capybara oil was able to promote a decrease in ventricular hypertrophy, a benefit found in our study which is similar to that found by Fernandes-Santos *et al.* who used medication interventions [58]. A possible explanation for this finding may be the decrease in systolic blood pressure in the group treated with capybara oil.

In experimental models of the metabolic syndrome, the literature shows the following findings in electron microscopy: cardiomyocyte loss, cardiomyocyte hypertrophy, and increased connective tissue, which promotes perivascular and interstitial fibrosis [29] [61] [65] [66] [67] [68] [69]. Our study shows increased connective tissue in the HF group, a finding that was not seen in the HF + OC group. This finding demonstrates the beneficial effect of capybara oil on reducing the connective tissue. A large accumulation of lipid inclusions was also seen in HF group animals which was not found in the other groups. This finding suggests decreased lipid uptake by cardiomyocytes in the HF + OC group, when compared to the HF group, showing another benefit of capybara oil. This finding shows that treatment with capybara oil was effective in decreasing the uptake of lipids by cardiomyocytes, showing yet another beneficial result of the treatment with capybara oil [30] [31].

Another important finding in this study was that the mitochondrial ultrastructure of cardiomyocytes changes in HF group animals. These animals had several mitochondrial lesions such as, for example, internal mitochondrial membrane distortion with loss of mitochondrial ridges. Previous studies showed the same findings in experimental models of the metabolic syndrome [29] [70]. Animals in the HF + OC group presented normal mitochondria, demonstrating that capybara oil had a protective mitochondrial effect. Gruber *et al.* described similar

results [48], showing yet another possible beneficial effect of capybara oil.

5. Conclusion

The capybara oil showed possible beneficial effects in this study, such as decreasing systolic blood pressure, improving lipid metabolism, and reducing and morphologically improving adipose tissue, findings that contribute to decreasing cardiovascular risk. Additionally, it provided improvements in various parameters of cardiac remodeling. Therefore, capybara oil has several possible beneficial effects on the cardiovascular and metabolic system, and further studies are needed to confirm these findings, as well as to better understand its role in the prevention or treatment of cardiovascular diseases.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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