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EFFECTS OF CAPYBARA OIL ON PANCREATIC REMODELING OF C57BL/6 MICE FED A HIGH FAT DIET

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ABSTRACT

Considering the importance of the pancreatic role in the homeostasis of the organism, we evaluated whether the administration of capybara oil (CO) would have the ability to reverse or attenuate the alterations in the metabolism of carbohydrates and lipids and the pancreatic remodeling imposed by the consumption of a high-fat diet in C57BL/6 mice. Thirty-two, 3-month old male C57BL/6 mice received standard chow (SC, 10% of energy as lipids, n=16) or HFty diet (HF, 60% of energy as lipids, n=16). At week 12, the animals fed the High-Fat (HF) diet were then randomly assigned to one of the following groups: Standard chow (C group); Standard Chow + Capybara Oil (C +CO group); High-Fat diet (HF group); High-Fat diet + Capybara Oil (HF + CO group). Body mass (BM), plasmatic parameters, pancreas and adipose tissue structures were analyzed. All animals fed the high-fat diet gained more weight than animals in the SC group. Excess weight was not reversed by CO. No significant differences were observed between groups and their respective controls in carbohydrate metabolism. An increase in pancreatic islet diameter and immunostaining for insulin was observed in the HF group, while a reduction of this parameters was revealed in the CO-treated group. The HF diet fed groups demonstrated that pancreatic steatosis was significantly higher than the SC group. When comparing the HF group treated with CO against the untreated HF group, the group treated with capybara oil showed a reduction in pancreatic steatosis. CO promoted improvement in pancreatic remodeling, as it resulted in the normalization of biometric, morphological and ultrastructural parameters of the pancreas of animals submitted to a HF.

KEYWORDS: Pancreatic remodeling – Obesity – capybara oil.

INTRODUCTION

The hyperinsulinemia commonly observed in obesity is a result derived from insulin resistance, which is being found to be related to various factors; the accumulation of ectopic fat, the regulation of enzymes involved in lipogenesis, the oxidation of free fatty acids, thus making them available by storage in organs such as the pancreas, in the form of triglycerides. The consequence of this fatty infiltration is known as Non-Alcoholic Fatty Pancreas Disease (NAFPD), a complex disease having several metabolic pathways, where there is hypertrophy and hypersecretion of the pancreatic islet and the dysfunction and apoptosis of β-cells resulting in pancreatic exhaustion.[1, 2, 3]

Oil extracted from capybara fat is a lipid source for omega 3 fatty acids. Several studies have demonstrated its effects on lowering total cholesterol (TC) and low-density lipoprotein (LDL) in rats fed a high cholesterol diet, the effects upon wound healing and its ability to diminish liver steatosis.[4, 5, 6]

Although studies demonstrate a relationship between obesity with hyperinsulinemia, insulin resistance and

dyslipidemia, the evidence of pancreatic ultrastructure remodeling is still relatively limited. Considering the importance of the role of pancreatic islets in the body´s homeostasis, our objective was to evaluate whether the administration of capybara oil would enable the reversal or attenuate changes in carbohydrate metabolism and the lipid and pancreatic remodeling caused by the consumption of a high fat diet in C57BL/6 mice.

MATERIALS AND METHODS

Animals and diet

The Animal Ethics Committee of the University of the State of Rio de Janeiro (UERJ – Universidade do Estado de Rio de Janeiro) approved the present protocol (Number CEUA/015/2017). The procedures were conducted as according to the guidelines for animal experimentation.[7]

Thirty-two, 3-month-old male C57BL/6 mice were placed in an environment of controlled temperature (21o ± 1o C), humidity (60 ± 10%) and a 12-hour light/dark cycle (1:00 AM to 1:00 PM light) with food and water ad libitum ingestion. The mice received standard chow (SC, 10% of energy as lipids, n=16) or HFty diet (HF, 60% of energy as lipids, n=16). The diets are shown in detail in Table 1. At week 12 of a testing period lasting a full 18 week period, the animals fed the high-fat (HF) diet were then randomly assigned into one of the following groups:

a) Standard chow (SC group; 10% energy from fat, n=8);
b) Standard chow + Capybara Oil (SC group; 10% energy from fat, n=8);
c) High-fat diet (HF group; 60% energy from fat, n=8);
d) High-fat diet + Capybara Oil (HF + CO group; 60% energy from fat, n=8)

Upon placement into one of the above groups, treatment lasted six weeks with the mice receiving capybara oil by oral gavage.

The diets followed the American Institute of Nutrition recommendations (AIN-93M) (Reeves) and were manufactured by PragSolucoes (Jau, SP, Brazil). Body mass (BM) was evaluated once a week.

Table 1: Detailed diet composition.

Composition Ingredients g/kg	Groups	
	SC	HF
Casein	140,0	190,0
L-Cystein	1,8	1,8
Corn starch	620,7	250,69
Sucrose	100,0	100,0
Fiber	50,0	50,0
Soybean oil	40,0	40,0
Lard	----------	320,0
Vitamin mix*	10,0	10,0
Mineral mix*	35,0	35,0
Choline	2,5	2,5
Antioxidant	0,008	0,008
Energy		
Kcal/g	3,57	5,40
Protein, %	14,9	14,0
Lipid, %	9,4	60,0
Carbohydrate, %	75,7	26
*Vitamin and ineral mix were added in accordance with AIN-93M.		

Blood glucose test analysis

Oral glucose tolerance tests (OGTTs) were performed at week 12 during the pre-treatment period and at week 18 during the post-treatment period. OGTTs were performed using a glucose overload of 1.0 g/Kg, given after a six-hour fasting period through oral gavage. Blood glucose concentrations were measured prior to glucose administration (0 min) and then at interval 15, 30, 60 and 120 min post-administration. Blood samples taken from the tail vein were analyzed.

Euthanasia

At week 18 of testing, the mice were deeply anesthetized (IP sodium pentobarbital, 150 mg/kg) and killed by exsanguination. The serum was obtained via centrifugation (120 g for 15 min) at room temperature and kept at -20° C until analysis. The pancreas was immediately removed, weighed, fixed and prepared as described. Additional parts of the pancreas were quickly frozen for molecular evaluation. The left tibia was dissected and measured to correct for pancreas mass.

Plasma analysis

Blood lipids were also evaluated. Upon blood collection, plasma was separated from the blood by centrifugation at room temperature. Blood was centrifuged (120g for 15 minutes) and stored at j80-C until analysis. Total cholesterol and triglycerides were measured by a

kineticcolorimetric assay (Bioclin System II; Quibasa, Bioclin, Belo Horizonte, MG, Brazil). The Friedewald formula was used to calculate the low-density lipoprotein (LDL) Total cholesterol (TC), and triacylglycerol (TG) measures by using kinetic-colorimetric methods, as according to the manufacturer's instructions (Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil).

Immunostaining for Insulin

Immunostaining for insulin in paraffin sections was performed as described elsewhere. Digital images of stained slices were obtained (LC evolution camera, Olympus BX51 microscope; TIFF format, 36-bit color, 1028x1024 pixels), and Image Pro-Plus version 7.0 (Media Cybernetics, Silver Spring, Md) was used to determine the density of immunostaining. The results were then taken to calculate the insulin ratio.

Pancreas Stereology

Pancreatic Steatosis Volume Density (Vv[steaotosis]) - estimated by counting points of the ratio between the number of points that hit the pancreatic steatosis (Pp) and the total number of test points in a test system made up of 36 test points (PT): Vv[steaotosis] = Pp[steatosis] / PT (%).

β-Cell Volume Density (Vv[β Cell]) - was estimated by image analysis through the density threshold selection tool into the islet insulin-positive area, which was expressed as a percentage of the islet (Image Pro Plus version 7.0; Media Cybernetics).

Transmission Electron Microscopy

Routine methods to prepare pancreas specimens for transmission electron microscopy were used in at least 3 animals from each group. Small fragments of pancreas were fixated with 1.5% glutaraldehyde in 0.1 mol of cacodylate buffer, pH 7.4, post fixed with 1% osmium tetroxide in 0.1 mol of cacodylate buffer at 4-C, dehydrated through a graded series of acetone solutions, and embedded in epoxy resin. Semithin sections were made and helped to guide pyramid construction. (Important to highlight is that pyramids were made following the observation of the pancreatic islet to evaluate its ultrastructure together with surrounding acinar cells.) Ultra-thin sections were then cut on an ultracut microtome (Leica UltraCut ultramicrotome, Leica, Wetzlar, Germany), stained with uranyl acetate and lead citrate, examined, and then photographed using an electron microscope (Zeiss EM 906).

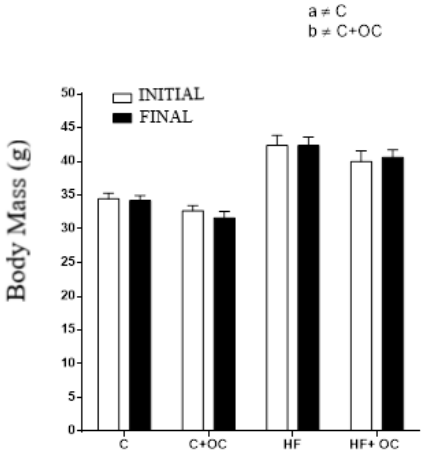
Data Analysis

Data are shown as mean T SEM. In the cases that homoscedasticity of the variances could be confirmed, comparisons among groups were made by 1-way analysis of variance followed by Tukey post hoc testing; otherwise, differences were tested with the Kruskal-Wallis test and the Dunn post hoc test. In every case, P is 0.05 and considered statistically significant. All analyses were performed using GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Body Mass

At baseline, all animals fed with the high-fat diet were heavier than SC animals and yet did not show any difference regarding body mass. Conversely, at the end of the experiment, the HF+CO (19% p ≤0,001) group did not present any difference in weight loss when compared with the untreated HF animals (24% HF p ≤0,0001) (Figure 1). It is worth mentioning that excess weight was not was seen to be corrected by Capybara oil.



Figue 1: Body Mass – pre-treatment (12 weeks) e post-treatment (18 weeks). Data for MC (grams) of mice from the C (Control) group, the C + CO (capybara oil treated Control group), and the HF (High Fat) and HF + CO (High Fat treated with capybara oil). Data were expressed as mean ± standard error of mean.

Fasting Glucose and Insulin

Hyperglycemia was found after 12 weeks of the high-fat diet in the HF group when compared with SC animals (Table 2; p≤0,05). At the end of week 18 (post-treatment period), a new OGTT was performed to verify whether the proposed CO treatment attenuated or reversed glucose intolerance. It was observed that there was no significant difference between the groups and their respective controls in the two analyses that were performed.

In regarding the pancreatic immunohistochemistry, the islets of the HF group showed increased immunostaining for insulin (+ 103%, p≤0,0001) when compared to group C results and a + 53.2% (p≤0,0001) increase when compared to the HF+CO group subsequently showing that capybara oil treatment promoted a reduction in insulin immunostaining (Figure 2 and Table 2).

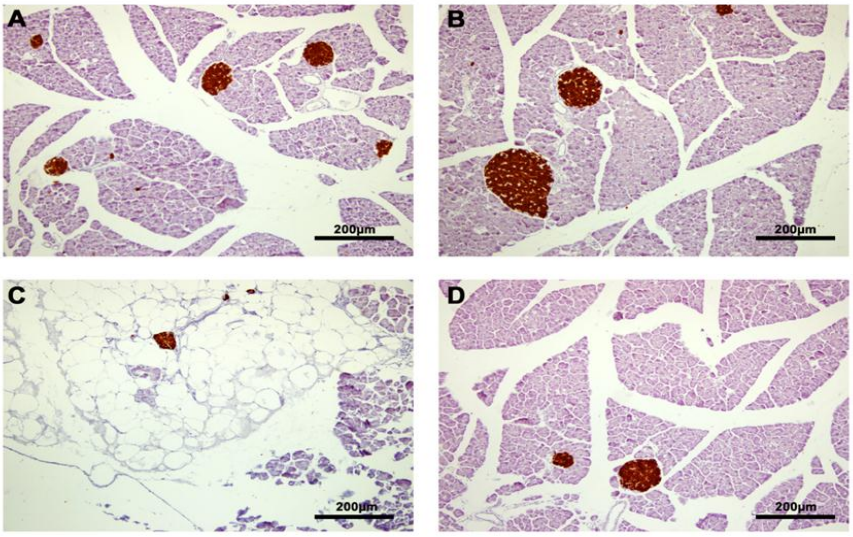


Figure 2: Immunostaining for pancreatic insulin in animals post treatment (week 18). A- Control group (C); B- Control group + capybara oil (C + CO); C- untreated high fat group (HF); D = high fat group treated with capybara oil (HF + CO). Same magnification for each slide image.

Table 3: Metabolic and pancreatic parameters of the experimental groups.

	Group C			Group COC		
	Mean	**SEM**	**SD**	**Mean**	**SEM**	**SD**
Cholesterol	150,27	2,76	6,18	141,56	2,8	6,2
Triglycerides	56,78	5,11	11,43	57,54	3,30	743
Adipocyte diameter(µm)	43,00	16,67	62,37	360,51*	12,30	55,03
Islet diameter(µm)	316,49	31,52	126,10	275,25	35,38	132,40
Insulin Imunostaining (%)	25,25	4,86	171	29,5	2,8	1,0
Pancreatic steatosis (%)	1,5	0,25	0,75	1,75	0,31	0,88
OGTT (PT)	707,45	26,89	120,26			
OGTT (T)	686,05	22,46	71,04	638,33	44,60	141,04
	Group HF			**Group HFOC**		
	Mean	**SEM**	**SD**	**Mean**	**SEM**	**SD**
Cholesterol	226,0*†	4,30	9,61	145,19‡	4,63	10,37
Triglycerides	88,77	2,67	5,98	76,64	3,67	8,22
Adipocyte diameter(µm)	548,84*†	15,67	75,17	556,14*†	1,27	54,70
Islet diameter(µm)	573,19*	61,63	222,20	308,65	35,68	142,70
Insulin Imunostaining (%)	51,87	3,72	1,31	33,87	2,85	1,0
Pancreatic steatosis (%)	10,25*	0,83	2,37	5,12*‡	0,51	1,45
OGTT (PT)	823,72*	35,6	159,5			
OGTT (T)	714,50	22,11	69,92	639,00	31,84	10,70

Abbreviations: *PT pretreatment, T treatment.*

**Significant difference with SC after treatment.*

† Significant difference with C+CO after treatment.

‡ Significant difference with HF after treatment.

§ Significant difference with HF+CO after treatment

Blood Biochemistry

In regarding serum cholesterol levels, the HF diet group showed a 33.5% increase when compared to the C group (p≤0,0001); 37.36% higher when compared to the C + CO group (p≤0,0001) and 35.75% higher when compared to the HF + CO group (p≤ 0.001) (Table 2).

In regarding serum triglyceride levels, the dosage was 36.03% in the HF group (p ≤ 0.005), 25.9% (p≤ 0.05) when compared to the C group. Dosage was 24.9% higher in the HF + CO group when compared to the C + CO group, with p ≤ 0.05 (Table 2).

Pancreas Stereology, Pancreatic Steatosis

Significant differences in pancreatic mass could not be found when compared to the other experimental groups. When considering the islet diameter, the HF group not treated with capybara oil showed an 81.3% increase compared to the control group (p≤0,0001) with this hypertrophy being prevented by the use of capybara oil (- 46, 3% in the HF + CO group p≤0.05) indicating an attenuation of this process.

Pancreatic steatosis was significantly higher in the HF group (583% higher compared to the control group, p≤0,0001) and in the HF + CO group (241.3% higher compared to the control group, p≤0,0001). It is worth mentioning that the HF group that received capybara oil presented a reduction of -50.1% (HF + CO p≤ 0.0001 when compared to the HF group) (table 2)

Pancreas Stereology, Pancreatic Steatosis

Significant differences in the pancreatic mass were not found when compared to the other experimental groups. Regarding islet diameter, the HF group not treated with capybara oil showed an 81.3% increase compared to the control group (p≤0,0001) and this hypertrophy was prevented by the use of capybara oil (- 46, 3% in the HF + CO group p≤0.05) indicating an attenuation of this process.

Pancreatic steatosis was significantly higher in the HF group (583% higher compared to the control group, p≤0,0001) and in the HF + CO group (241.3% higher compared to the control group, p≤0,0001). It is worth mentioning that the HF group receiving capybara oil presented a reduction of -50.1% (HF + CO p≤ 0.0001 when compared to the HF group) (Table 2).

Adipocyte Morphometry

Regarding adipocyte morphometry, a reduction of adipocyte diameter of 16% in group C + CO occured when compared to C group (p≤ 0.05). The HF group and the HF + CO group showed an increase of 52% and 54%, respectively, when compared to the C + CO group, both with p≤0,0001. Compared to C group, the groups HF and HF + CO presented an increase of 27% and 29%, respectively, both with p≤0,0001 (Table 2).

Electron Transmission Microscopy

Figure 3 shows electron micrographs of the exocrine pancreas. Untreated obese animals demonstrate abundant fat globules within the exocrine pancreas, characterizing NAFPD. Regarding the endocrine pancreas, numerous insulin granules were identified in untreated animals, in addition to a greater number of mitochondria. However, the ER was less developed than in the SC group or treated with capybara oil. In contrast, SC animals had numerous well-organized mitochondria, fewer secretion granules, and rare lipid droplets in the acinar cells, containing abundant ER and normal-looking insulin granules, suggesting a normal cytoarchitecture of the pancreatic islets. The capybara oil-treated groups resembled the cellular organization of the SC group, but showed slightly more lipid droplets within the acinar and islet cells as described.

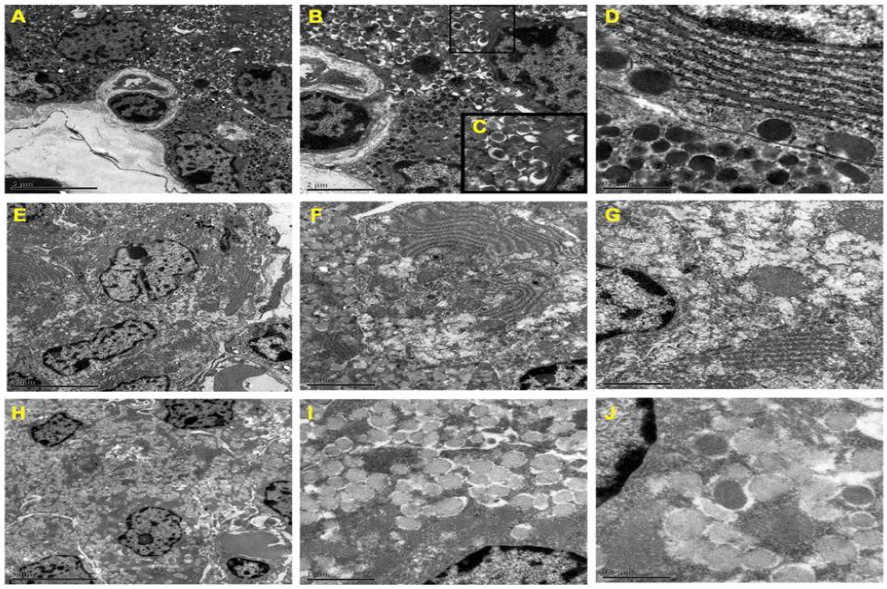


Figure 3: Electron micrograph of the pancreas of the animals at the end of the experiment (18 weeks) demonstrating beta cell ultrastructure. **A** - Insulin secretion granules with electrodense nucleus, and euchromatic nuclei are observed

in the Control group. (A- 5000x magnification). **B & C** - Euchromatic nuclei, insulin secretion granules with central electrodense nucleus and surrounded by a hyaline halo are observed in the C + CO group (B- 10000x increase; C- 40000x increase). **D** - Numerous insulin secreting granules. The majority of the electrodense granules appear to occupy most of the secretion granules, leaving the surrounding hyaline halo unclear or absent. The perinuclear space has a regular-looking granular endoplasmic reticulum in the C + CO group (40000x D-magnification). **E** - The β cells present ultrastructural disorganization with a significant decrease in insulin secretion granules accompanied by perinuclear degeneration in the HF group (E-5000x magnification). **F** - The perinuclear region of the β cell presents enlargement of the granular endoplasmic reticulum with disorganization of the cell membrane indicating reticular stress. The β cells have secretion granules containing little electrodense content and fusion of the granule membranes indicating intracytoplasmic degeneration in the HF group (F-10000x magnification). **G** - Intense perinuclear intracytoplasmic disorganization and fusion of secretory vesicles with loss of granular material, in addition to stress of the granular endoplasmic reticulum in the HF group (G - 10000x magnification). **H & I** - Showing β cells with euchromatic nucleus and presence of numerous secretory vesicles with little electrodense content in the HF + CO group (H- 5000x magnification, I- 20000x magnification). J- perinuclear portion of a β cell showing poorly electrode-secreting granules surrounded by a hyaline halo. In the HF + CO group (J- increase of 40000x).

DISCUSSION

The present model of metabolic syndrome based on the high fat diet mice is characterized by obesity, insulin resistance, dyslipidemia and pancreatic steatosis which is compatible with NAFPD. The high fat diet caused obesity and its comorbidities, such as dyslipidemia and insulin resistance in C57BL/6 mice. Other striking findings were adipocyte hypertrophy, and pancreatic islet hypertrophy concomitant with increased immunostaining for insulin in the islets of animals belonging to the HF group, thus indicating adverse morphological remodeling and impaired pancreatic tissue function. Every one of these changes were mitigated or reversed by using the capybara oil. This is the first report in the literature that capybara oil affects adipocyte size and improves pancreatic steatosis in a rodent MS model. In addition, normalization or attenuation of pancreatic islet size and function with the use of capybara oil has never been previously described.

The HF diet promoted weight gain and higher body fat accumulation. Excess visceral adipose tissue is a cornerstone of MS character development where adipose tissue fails to store postprandial lipids. Under this condition, excess free fatty acids are accumulated as ectopic fat in muscle, liver and pancreas. Weight loss was not significant in the HF + CO group, although presenting a lower body mass when compared to the HF group.[8, 9]

The adipose tissue undergoes continuous remodeling, which normally maintains the tissue's integrity, but it can get out of control and thereby lead to adipocyte death in association with macrophage recruitment and activation, and systemic insulin resistance. Through excessive caloric intake, the adipocytes suffer hypertrophy and hyperplasia, configuring a dynamic process of adipose tissue. However, this hypertrophy causes alteration of secreted products and, eventually, will negatively affect the remodeling of the adipose tissue. Changes in the pattern of adipocyte differentiation emerge as another mechanism by which capybara oil reverses insulin resistance, significantly improving pancreatic islet morphology.[10]

Lipid accumulation within the pancreatic exocrine system precedes steatosis and islet apoptosis and is thus considered an important marker for the prevention of pancreatic exhaustion. Other changes described in studies with animals fed a high-fat diet are alterations in cellular architecture, vesicular steatosis, enlargement of secretory granules, besides oxidative stress demonstrated by mitochondrial alterations. Our results offer relevant explanations for some mechanisms. Untreated HF animals had pancreatic steatosis (macro and microvesicular) concomitantly with pancreatic islet hypertrophy and hypersecretion. A significant point of our study was that pancreatic steatosis was effectively treated by using capybara oil as a pharmacological strategy according to previous studies developed in our laboratory. It may be justified by the decrease in free fatty acid (FFA) input parallel to the increase in output due to increased beta-oxidation.[11, 12, 13]

Experimental data reveal that mitochondrial dysfunction plays a crucial role in the genesis of NAFPD and NAFLD through mitochondrial DNA depletion, reduced respiratory chain activity, and impaired beta-oxidation. Our electron micrographs show fewer mitochondria with increased internal membrane lesions in HF animals, as previously described in the literature, whereas the treated groups showed more numerous mitochondria and preserved membranes with respect to cellular architecture.[14, 15]

Insulin resistance develops through a defect in the receptor or in the post-receptor of insulin altering the signaling for GLUT translocation into the membrane allowing glucose uptake and utilization by peripheral cells. As a result, islets hypertrophy and over-secrete insulin in an attempt to compensate for the reduced glucose uptake so as to normalize blood glucose until this process causes pancreatic exhaustion. The islet hypertrophy and hypersecretion observed in the HF group were efficiently treated in our study, seen as normal-sized pancreatic islets and a decrease in immunostained insulin expression in the treated groups. These findings allow us to conclude that capybara oil

was able to reverse the structural and functional damage caused by a diet of chronic high fat intake.[4, 16]

CONCLUSION

Non-pharmacological treatments may emerge as new strategies to reverse insulin resistance and control the economic impact that pancreatic changes in the current MS pandemic have been having. Unpublished observations such as the improvement of pancreatic islet function through the use of essential fatty acids have been relevant. The use of capybara oil is clinically useful since it resulted in the normalization of the biometric, morphologic and ultrastructural parameters of the pancreas in animals submitted to a high-fat diet. Further studies are needed to better clarify its mechanism of action and pleiotropic effects.

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