



ORIGINAL ARTICLE

The Effect Stool Transplantation as an Adjunct Treatment in Obesity in Rats

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Abstract

Introduction: Obesity is defined as a multifactorial metabolic syndrome, in which there's an excessive number of fat cells within the tissues. It's discussed that intestinal microbiota might have a relevant relation with obesity, since it's relevantly altered in obese patients.

Objectives: To assess the effect of stool transplantation (ST) in the condition of obesity and its outcomes in an experimental model of cafeteria diet by hepatic function test, lipid profile, total glycemia and histology.

Methods: 40 male *wistar* assigned in 5 groups: Control (CO), control with antibiotics (CO + ATB), obesity (CAF + ATB), stool transplantation (ATB + ST) and obesity with stool transplantation (CAF + ATB + ST). During the experiment, obesity induction groups received Cafeteria diet (CAF), whereas the remaining groups had normal diet *ad libitum*. After 3 months, daily ST was carried out for 8 weeks by gavage procedure. Animals were euthanized and mesenteric fat, aorta and liver were harvested for further analysis.

Results: It was observed that the CAF diet groups had a higher mesenteric fat weight compared to CO groups ($p = 0.0078$). Hepatic enzymes and free cholesterol also presented statistical significance comparing CO and CAF ($p < 0.0001$). There were no differences in laboratory tests between the groups that received ST and those that didn't.

The histopathological evaluation comparing CAF + ATB and CAF + ATB + ST showed that all groups presented steatosis and aortic lesion, but the ST groups had less signs of injury.

Conclusion: CAF resulted in an expected obese metabolic syndrome. In relation to the ST, there were no beneficial results regarding glycemia and triglycerides.

Keywords

Fecal microbiota transplantation, Obesity, Microbiota, Wistar rats

Abbreviations

CAF: Cafeteria diet; TGO: Glutamic-oxalacetic transaminases; TGP: Glutamic-pyruvic transaminase; CO: Control; CO + ATB: Antibiotic; ATB + ST: Stool transplant; CAF + ATB + ST: Obesity and stool transplantation

Introduction

Obesity is defined as an excessive accumulation of adipocytes in the tissues. Nowadays, it is recognized as a global epidemic and one of the biggest public health problems around the world [1-3].

Currently, it is estimated that there are more than 1 billion overweight people and at least 300 million of

them clinically obese [4]. Nowadays, it is known that the intestinal microbiota have an important role in relation to obesity, since it is intensely altered in obese individuals. Compared to thin, obese individuals have 10x more bacteria in the *Firmicutes* group compared to microorganisms in the *Bacteroidetes* group. This altered community structure is associated with a shift in function, resulting in increased energy harvest from ingested food [5].

Due to the clinical and social importance of obesity, and also because treatment for recurrent *Clostridium difficile* infection has been successful since 1958 with the fecal microbiota transplant, interest in applying therapy to other gastrointestinal diseases, including obesity [6,7].

Given this context, it is questioned whether the transplant of fecal microbiota, through stool transplantation, from thin patients to obese patients has a beneficial effect on the treatment and evolution of obesity.

Methodology

Experimental design

The procedures with the animals were in agreement with the one recommended by the *Comissão de Ética no Uso de Animais* (CEUA) of the *Faculdade Evangélica Mackenzie do Paraná* (FEMPAR), registered under the number 1558/2018.

Animals

Forty five-week-old male Wistar rats with an average weight of 300 g, from the Central Vivarium of the *Universidade Federal do Rio Grande do Sul* (UFRGS), were used in the research. The animals were kept in the vivarium of *Faculdade Evangélica Mackenzie do Paraná* in propylene cages of 47 × 34 × 18 cm, lined with shavings, in a controlled photoperiod of 12 hours light/dark (light from 7 am to 7 pm) and room temperature of 22 ± 2 °C. After 3 weeks of acclimatization with water and appropriate food ad libitum, the rats were identified and separated in the cages according to the experimental group, 4 rats were given per cages. The rats only had contact with others in the same group, thus not having access to the feces of rats from other groups so that they could eat.

Experimental groups

The rats were distributed in 5 groups with 8 animals each, named

- 1. Control (CO)** - Group fed standard commercial feed for 20 weeks.
- 2. Antibiotic Control (CO + ATB)** - Group fed standard commercial feed for 20 weeks. Received antibiotic for 3 days.
- 3. Obesity (CAF + ATB)** - Group subjected to obesity induction by the cafeteria diet method. Received antibiotic for 3 days.
- 4. Stool Transplant (ATB + ST)** - Group fed standard commercial feed for 20 weeks. Intragastric gavage was performed with feces preparation for 8 weeks, and 3 days before the group received antibiotics in the water.
- 5. Obesity and Stool Transplantation (CAF + ATB + ST)** - Group subjected to obesity induction by the cafeteria diet method. Intragastric gavage was performed with feces preparation for 8 weeks, and 3 days before the group received antibiotics in the water.

Experimental procedures

Experimental model of obesity: The model used to induce obesity was the Cafeteria diet (CAF) [8]. Thus, the animals were provided with normal diet *ad libitum* and a daily selection of grocery foods selected from a list of twelve items (Table 1), adapted from Almeida, et al. [9]. The diet was maintained for 20 weeks. The food was obtained commercially and the nutritional information (Table 2) of the food was obtained through the content indicated by the manufacturer about the product. The diet items were placed together with the regular food. The feed was changed daily for the animals not to get used to the same food and consequently reject it. There was good acceptance of the CAF by the animals, mainly sausage, hamburger, mortadela and wafer. It was observed daily when consuming food and almost the entire diet was consumed every day.

In addition, the animals had access to two bottles of 500 ml, one containing regular drinking water and the other containing 12% sucrose diluted in water, in order

Table 1: Feeding of cafeteria diet animals.

Sunday	Bread, Stuffed Cookie, Marshmallow, Mortadela and Regular Food
Monday	Wafer, Paçoca, Sausage, Brownie and Regular Food
Tuesday	Cheetos Cheese, Marshmallow, Hamburger, Chocolate and Regular Food
Wednesday	Bread, Mortadela, Paçoca, Wafer and Regular Food
Thursday	Stuffed Cookie, Sausage, Cheese Cheetos, Brownie and Regular Food
Friday	Bread, Mortadela, Marshmallow, Wafer and Regular Food
Saturday	Hamburger, Paçoca, Sausage, Chocolate and Regular Food

Source: Adapted from Almeida, et al. [9].

Table 2: Nutritional information of the cafeteria diet foods.

Bread	Per (g) 29	(kcal)	(g)	(g)	(g)	(g)	(g)	(g)	(mg)
		93	18	3.8	0.6	0.1	0	0.9	0.2
Stuffed Cookie	30	23	23	2.1	3.8	0.9	0	0.8	44
Marshmallow	20	65	16	0.9	0	0	0	0	6.4
Mortadela	40	88	2.7	4.8	6.4	2	0	0	562
Wafer	30	147	20	1.4	6.8	3.2	0	1.1	2.6
Paçoca	36	200	20	5.4	11.2	1.2	0	2	67.4
Sausage	100	292	3	12.6	26	8.4	0	0	1150
Brownie	60	292	33	3.9	16	3.3	0	1.8	106
Cheetos	6	30	4.1	0.4	1.2	0.3	0	0	23.8
Cheese Hamburger	80	225	3	15	15.5	51	0	1.3	465
Chocolate	25	138	14	1.8	8.1	4.3	0	0	22

Source: Information obtained from food packaging.

to mimic soft drinks. The bottles with water and sucrose were replaced daily until it reached 500 ml. The animals ingested about 250 ml of sucrose water per day. The effectiveness of the transplant was tested by comparing the groups.

Stool transplantation and vehicle administration:

The intestinal microbiota transplant by ST was performed during the last 8 weeks of the experiment. 200 mg of fresh feces from the CO group were collected and mixed with 5 ml of saline solution. A variation of 0.020 g was accepted between the pieces of feces. The solution was manually stirred and introduced via intragastric gavage in an amount of 200 μ L per day, according Zhou, et al. [10]. The animals in the control group received water in a volume of 5 ml from the 13th week by intragastric gavage to obtain the same level of stress as the other animals.

Euthanasia and obtaining tissue and blood samples:

After 20 weeks of experiment, the animals that were completely fast for 12 hours, were anesthetized with a mixture of Xilasina Hydrochloride 10 mg/kg of body weight and Ketamine Hydrochloride 90 mg/kg of body weight. Then, decapitation was performed with the collection of 4 ml of blood. Subsequently, after trichotomy and disinfection of the abdominal region, the following organs were harvested: liver, mesenteric fat and thoracic aorta.

Mesenteric fat was weighed using a precision scale and, together with the other organs, it was used for histopathological evaluations.

Tests from the blood sample: Liver integrity tests were performed: Glutamic-oxalacetic transaminase (TGO) and Glutamic-pyruvic transaminase (TGP). Total cholesterol, LDL, HDL and total blood glucose were also evaluated. Blood was collected during euthanasia.

Histological evaluation: To perform the histological process, the collected organs were fixated in 10%

formaldehyde dissolved in 0.1M PBS and pH 7.4. Then, fragments were removed for processing based on the conventional histological technique, which were included in paraplast, oriented so that the obtained cuts result in cross sections and stained with hematoxylin-eosin for the analysis of histopathological changes.

Statistical analysis: GraphPad Prism 7 was used for statistical analysis. Normality was analyzed by the Shapiro-Wilk test. The comparison of the average biochemical dosage data and animal weights was done using one-way ANOVA and the Tukey post-test and the variables were expressed as mean \pm standard deviation. The value of $p < 0.05$ was considered as a criterion of statistical significance.

Results

Body weight

For the animal's body weight analysis, a comparison between the average weekly weight gains of the animals before the beginning of the gavage was made. In doing so, a statistical difference was noticed between all groups on regular diet and those on high calorie diet all with $p < 0.0001$. Also, no difference was found when comparing the weights from control groups and the ones who received CAF.

Organ weight

After euthanasia, the liver and mesenteric fat were removed and weighed. There was statistical significance when comparing the weights of mesenteric fat (Table 3) of the groups CO and CAF + ATB ($p = 0.0078$) and CO + ATB and CAF + ATB ($p < 0.0001$).

There was no statistical significance between analyzes of mesenteric fat weight between groups CO and CO + ATB ($p = 0.4798$), CO and ST + ATB ($p = 0.5791$), CO + ATB and ST + ATB ($p = 0.9999$) and CO and CAF + ATB + ST ($p = 0.3779$).

Table 3: Arterial lesion classification on aortic microscopy.

Aortic lesion grade	CO		CO+ATB		ATB ST+ATB		CAF+ATB		CAF+ATB+ST	
	n	%	N	%	n	%	n	%	n	%
0	8	100	6	75	3	37.5	1	12.5	2	25
I			2	25	5	62.5	3	37.5	5	62.5
II							2	25	1	12.5
III							2	25		

Source: The author (2019).

Table 4: Presence of steatosis on hepatic microscopy.

Steatosis grade	CO		CO + ATB		ST + ATB		CAF + ATB		CAF + ATB + ST	
	n	%	n	%	N	%	n	%	n	%
0	4	50	5	62.5	5	62.5				
1	4	50	3	37.5	3	37.5	1	12.5	3	37.5
2							3	37.5	4	50
3							4	50	1	12.5

Source: The author (2019).

Table 5: Volume of adipocytes under microscopy of white adipose tissue.

Adipocytes volumes	CO		CO+ATB		ST+ATB		CAF+ATB		CAF+ATB+ST	
	N	%	n	%	n	%	n	%	n	%
Increased							5	62.5	4	50
Normal Decreased	8	100	8	100	8	100	3	37.5	4	50

Source: The author (2019).

There was no statistical significance when analyzing the weight of the liver between the groups.

Laboratory exams

After euthanasia, blood was collected from all animals and laboratory analysis was performed.

Regarding liver enzymes, there was statistical significance between the CO and CAF + ATB groups ($p < 0.0001$).

Regarding total cholesterol, HDL and LDL, statistical significance was observed between the CO and CAF + ATB groups ($p < 0.0001$).

Regarding fasting glucose and triglycerides, there was no statistical significance.

Among the groups of obese animals that received ST from those that did not, there was no statistical significance in the levels of cholesterol, triglycerides and fasting blood glucose.

Histological analysis

It was observed in microscopic liver analysis that 100% of the control group did not have steatosis or had grade 1 steatosis. The CAF + ATB group had 12.5% grade 0 or 1 steatosis. And the CAF + ATB + ST group showed 37.5% steatosis grades 0 or 1 (Table 4).

Regarding the aorta, it was observed that 100% of the animals in the CO group had no organ damage. The CAF + ATB group had 50% of the animals with grade II or III of aortic injury. While the CAF + ATB + ST group had 12.5% grade II or III injuries (Table 3).

Regarding mesenteric fat, it was seen that 100% of the animals in the CO group had no change in the volume of their adipocytes. The CAF + ATB group had 62.5% of adipocyte volumes increased. The CAF + ATB + ST group, in turn, had 50% (Table 5).

Discussion

In the context of obesity, insulin resistance is associated with inflammation in various tissues. It is currently suggested that visceral adipose tissue is a major source of inflammation due to the accumulation of inflammatory macrophages, natural killer cells and T cells [11].

In a study by de Bona Schraiber, et al. [12] in which obesity was induced, it was noted that the obese group showed an increase in mesenteric fat mass when compared to the CO group that was fed an adequate diet of macronutrients. This finding corroborates what was found in our study, in which the animals in the groups that did not receive a CAF had a lower weight of mesenteric fat mass than the animals in the group in which obesity was induced.

The expression and secretion of pro-inflammatory cytokines such as TNF-alpha are increased in obese animals and humans, positively correlating with an increase in the volume of adipocytes [13].

Amar, et al. [14] suggests that the alteration of the microbiota induced by a high fat diet is associated with the promotion of a translocation of gram negative bacteria through the intestinal mucosa for the circulation of the mesenteric adipose tissue, which could also contribute to the increase of inflammation a systemic and adipose tissue levels observed in obesity. This suggestion corroborates with the study findings, in which the increase in the volume of adipocytes in white adipose tissue was evidenced in the groups that received CAF when compared to the groups that did not.

Lai ZL, et al. [15] produced an experimental study in an animal model, in which it was evidenced that the ST from a rat with healthy practices, such as exercise and diet, administered in obese animals develop a good prognosis, to improve inflammation and obesity. It has also been seen that ST decreases the weight and size of fatty tissue adipocytes. This finding corroborates with that found in our study, in which the group of obese rats that received ST had a slight decrease in adipocyte volumes when compared to obese rats that did not receive ST.

Also according to Liu M, et al. [16], it was also noted that the ST suppressed the expression and the large number of proteins of the lipogenic genes in the liver, thus decreasing the steatosis picture, in addition to attenuating blood glucose levels. This finding partially corroborates our study, as it was found that transplanting feces reduces steatosis in animals that were subjected to a high calorie diet. However, when taking into account the glycemic level, there was no significant difference.

Garcia-Lezana, et al. [17] conducted a study with models of obesity induction with a diet rich in fructose and glucose. After a diet rich in fructose and glucose, the rate of hepatic triglycerides was increased and the rats developed histopathological NASH (concordance of steatosis, hepatocellular ballooning and lobular inflammation). They did not conclude that there were relevant intrahepatic changes in inflammation and fibrosis. In our study, the development of NASH was not observed within all of its criteria, only steatosis was clearly evidenced. Our study also found no inflammation and liver fibrosis, only steatosis.

Porras D, et al. [18] suggested the ST for modulation and treatment of NASH. Mice fed with a high calorie diet showed steatosis. With ST, a reduction in liver triglycerides was shown. This fact partially corroborates with the findings in our study. The rats in the high-calorie diet groups in our study had more steatosis than those who did not undergo a cafeteria diet. There was no statistical difference, however, in triglyceride levels.

According to the experimental study by Zhou, et al. [10], in rats that obtained a high caloric diet, an accumulation of lipids was observed (in comparison with the CO group, the other rats increased their lipid index by 4 and 17 times) which was significantly attenuated by treatment with ST, decreasing intrahepatic triglyceride and cholesterol levels. This fact partially corroborates with our study. There was a statistical difference in the levels of total cholesterol, HDL and LDL between the animals that were not submitted to a high calorie diet and the animals that were. However, no statistical significance was found in the levels of triglycerides and cholesterol among obese animals that received ST from those that did not.

According to the study by Gregory, et al. [19], the microbiota has a role in susceptibility to aortic atherosclerosis and can be a potential therapeutic treatment. Our study found that groups that received hypercaloric diet activity showed aortic lesions to a greater degree than animals that did not receive the CAF. At the same time, it was observed that the group that received ST after the induction of obesity had aortic lesions to a lesser extent than the group that only received the high-calorie diet.

The only variable between the CAF and CAF + ST groups was the fecal transplant, so the significant results between the two groups can be attributed to the transplant, even without an analysis having been made in this work to measure the microbiota.

Conclusion

The CAF diet have caused a clear metabolic obesity syndrome and altered all the laboratory tests. Regarding ST, it was found that there was no beneficial effect of the procedure in relation to glycemic indexes, triglycerides, total cholesterol and liver enzymes.

Regarding histology, the induction of obesity was accompanied by an increase in hepatic steatosis, aortic lesions and an increase in the volume of adipocytes in white adipose tissue. The ST seemed to mitigate the effects presented.

Limitation

The limitation of our study is due to the lack of biomolecular analysis, using the PCR technique, of the feces of the groups, as well as the non-dosage of laboratory inflammatory markers for lack of resources.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author Contributions

All authors have contributed equally to the work.

References

- James PT, Leach R, Kalamara E, Shayeghi M (2001) The worldwide obesity epidemic. *Obesity Research* 9: 228S-233S.
- Popkin BM, Doak CM (2009) The obesity epidemic is a worldwide phenomenon. *Nutrition Reviews* 56: 106-114.
- Bernhard F, Landgraf K, Kloting N, Berthold A, Buttner P, et al. (2012) Functional relevance of genes implicated by obesity genome-wide association study signals for human adipocyte biology. *Diabetologia* 56: 311-322.
- Waxman A (2004) WHO's global strategy on diet, physical activity and health. *Scandinavian Journal of Nutrition* 48: 58-60.
- Fujimura KE, Slusher NA, Cabana MD, Lynch SV (2010) Role of the gut microbiota in defining human health. *Expert Rev Anti Infect Ther* 8: 435-454.
- Smits LP, Bouter KEC, de Vos WM, Borody TJ, Nieuwdorp M (2013) Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* 145: 946-953.
- Li M, Liang P, Li Z, Wang Y, Zhang G, et al. (2015) Fecal microbiota transplantation and bacterial consortium transplantation have comparable effects on the re-establishment of mucosal barrier function in mice with intestinal dysbiosis. *Front Microbiol*.
- Gomez-Smith M, Karthikeyan S, Jeffers MS, Janik R, Thomason LA, et al. (2016) A physiological characterization of the Cafeteria diet model of metabolic syndrome in the rat. *Physiology & Behavior* 167: 382-391.
- Almeida FN, Almeida KN, Masi LN, Nachbar RT, Natali MRM, et al. (2008) A resposta do peso e da composicao corporal a inclusao da dieta de cafeteria e treinamento fisico aerobio em diferentes fases do desenvolvimento. *Ciencia, Cuidado e Saude*.
- Zhou D, Pan Q, Shen F, Cao H, Ding W, et al. (2017) Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep* 8: 7.
- Monteiro-Sepulveda M, Touch S, Mendes-Sa C, Andre S, Poitou C, et al. (2015) Jejunal T cell inflammation in human obesity correlates with decreased enterocyte insulin signaling. *Cell Metabolism* 22: 113-124.
- de Bona Schraiber R, de Mello AH, Garcez ML, de Bem Silveira G, Zacaron RP, et al. (2019) Diet-induced obesity causes hypothalamic neurochemistry alterations in Swiss mice. *Metab Brain Dis* 34: 565-573.
- Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB (2006) O tecido adiposo como centro regulador do metabolismo. *Arquivos Brasileiros de Endocrinologia & Metabologia* 50: 216-229.
- Amar J, Chabo C, Waget A, Klopp P, Vachoux C, et al. (2011) Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: Molecular mechanisms and probiotic treatment. *EMBO Mol Med* 3: 559-572.
- Lai Z-L, Tseng C-H, Ho HJ, Cheung CKY, Lin J-Y, et al. (2018) Fecal microbiota transplantation confers beneficial metabolic effects of diet and exercise on diet-induced obese mice. *Sci Rep* 23: 8.
- Liu M-T, Huang Y-J, Zhang T-Y, Tan L-B, Lu X-F, et al. (2019) Lingguizhugan decoction attenuates diet-induced obesity and hepatosteatosis via gut microbiota. *World J Gastroenterol* 25: 3590-3606.
- Garcia-Lezana T, Raurell I, Bravo M, Torres-Arauz M, Salcedo MT, et al. (2018) Restoration of a healthy intestinal microbiota normalizes portal hypertension in a rat model of nonalcoholic steatohepatitis. *Hepatology* 67: 1485-1498.
- Porras D, Nistal E, Martinez-Florez S, Olcoz JL, Jover R, et al. (2019) Functional interactions between gut microbiota transplantation, quercetin, and high-fat diet determine non-alcoholic fatty liver disease development in germ-free mice. *Mol Nutr Food Res* 63.
- Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, et al. (2015) Transmission of atherosclerosis susceptibility with gut microbial transplantation. *J Biol Chem* 290: 5647-5660.