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# DOES LACTOBACILLUS GASSERI BRING BENEFITS TO THE METABOLIC PROFILE OF WISTAR RATS FED A HYPERCALORIC DIET?

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## ABSTRACT

Obesity is considered a pandemic and is associated with innumerable risks and complications for health. *Lactobacillus gasseri* is a lactic acid probiotic bacterium that that may promote beneficial effects in rats and humans, such as improving the intestinal environment, reducing glycaemia and decreasing body weight and abdominal fat. In view of these effects, this study intended to evaluate the effect of *Lactobacillus gasseri* on the weight gain, visceral fat, and biochemical profile of Wistar rats fed a hypercaloric diet. Two experimental groups were used: G1 (control group treated with water and rat food *ad libitum,* and 0.5 mL of water using *gavage* route once a day) and G2 (treated with water and rat food *ad libitum*, and $10^9$ CFU (Colony Forming Units) of *Lactobacillus gasseri* using *gavage* route once a day). Our results showed that the animals presented similar body weight at the beginning and at the end of the experimental protocol. Percentage of weight gain and visceral fat was higher in G2. Both thoracic and abdominal circumference were higher in G2. No modifications in glycaemia, total cholesterol and triglycerides were found after the treatment. We may conclude that the supply *Lactobacillus gasseri* was not able to generate beneficial results on weight, adipose tissue and biochemical profile in the animals fed with hypercaloric diet.

**KEY WORDS:** *Lactobacillus gasseri*, obesity, lipids, glycaemia.

## INTRODUCTION

Obesity is already considered a pandemic, being the fastest growing non communicable disease in the world. In the United States, half of the population is overweight, in Spain and most European countries the percentage of obese people has increased from 20% to 40% every decade and, alarmingly, is also affecting the child population.[1-4]

This pathology is associated with innumerable risks and complications for health and comorbidities, whose main focus is on diabetes and cardiovascular disease.[5-7]

Some studies have shown the relationship between microorganisms of the intestinal microbiota and obesity, suggesting that the manipulation of diet and microbiota is a strategy for the treatment of obesity and its complications. [6-11]

Probiotics are living microorganisms that, in balance with the organism, bring benefits to the health. They may lead to inhibition of pathogen growth by modifying the composition of short chain fatty acids in the intestinal lumen, stimulation of mucin production of the colon, and potentiation of immune responses. [12-13]

*Lactobacillus gasseri* is a lactic acid probiotic bacterium that has been shown to exert beneficial effects in rats and humans, such as improvement of the intestinal environment[14], increase in the IgA production[15] and reduction of glycaemia.[16] There are also studies indicating that this microorganism may have the ability to promote weight loss and decrease abdominal fat. [17-18] These anti-obesity properties may be attributed to the increase of genes related to fatty acid oxidation and reduction of those related to its synthesis, increase of GLUT-4 expression, and reduction in the levels of leptin and insulin. [19]

In view of these effects, this study intended to evaluate the effect of *Lactobacillus gasseri* on the weight gain, visceral fat, and biochemical profile of Wistar rats fed a hypercaloric diet.

## METHODS

### Ethical principles

This experimental protocol was approved by the Animal Research Ethics Committee of the University of Marília (UNIMAR, Marília, SP, Brazil) with protocol number 13/2016. We have included in this study Wistar rats weighing approximately 110g to 130g, which were kept in the vivarium at UNIMAR. The rats were housed in collective cages under a dark/light cycle of 12 hours, room temperature of 22 ± 2°C, and relative air humidity of 60 ± 5%. During the experimental protocol, the rats were fed and watered *ad libitum*; *and* were cared for according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".

**Preparation of the supplemented rat food**

Rat food (500g) was crushed and mixed with condensed milk (395g). This mixture was molded into pellets that was dried in an air circulating oven at 65°C for about 8 hours, stored in polyethylene packaging, and refrigerated at 5°C until its utilization.

**Experimental Groups**

After acclimation to laboratory conditions (seven days), the animals were divided randomly in two experimental groups based on the model of Kang et al. [20]:

G1: control group treated with water and rat food *ad libitum*, and 0.5 mL of water using *gavage* route once a day,

G2: treated with water and rat food *ad libitum,* and $10^9$ CFU (Colony Forming Units) of *Lactobacillus gasseri* using *gavage* route once a day.

Consumption of water and food was evaluated 3 times a week and the body weight once a week.

After a period of 10 weeks, the animals were euthanized with a lethal intraperitoneal injection of 200 mg/Kg of thiopental. After death, blood samples were drawn from the vena cava to determine the biochemical profile (total cholesterol, triglycerides, and glycaemia that were measured in mg/dL).

**Anthropometric parameters**

After the euthanasia process, the weight and length of the animal were evaluated to find the Lee index = cube root of body weight (g) / nose-anus length (cm), and the percentage of weight gain. Results above 0.3 may indicate overweight. [21-22] Visceral fat was removed and weighed after incision and exposition of the abdominal region. Analysis of thoracic and abdominal circumference where also performed by measuring respectively the circumference immediately after the front and hind legs of the animals.

**Statistics**

T-Test and Mann-Whitney were performed for the statistical analysis and the variables were presented as mean and standard error mean, adopting a 5% level of significance.

## RESULTS

Our results show that the animals exhibited similar body weight at the beginning (p=0.4582) and at the end of the experimental protocol (p=0.0534). However, the percentage of weight gain and visceral fat was higher in G2 (treated group). We did not find significant differences in Lee Index (p=0.2468). Both thoracic and abdominal circumference were higher in G2 with significance for the abdominal circumference. In G2 we also observed higher consumption of water and food (Table 1).

**Table 1: Body weight, visceral fat, Lee Index and food and water consumption in the control group (G1) and in the treated group (G2).**

| Parameters | G1 (n=8) | G2 (n=8) | p-value |
|---|---|---|---|
| | Mean ± standard deviation | | |
| Initial weight (g) | 160.3±19.4 | 157.2±5.7 | 0.4582* |
| Final weight (g) | 382.7±41.4 | 416.3±36.2 | 0.0534** |
| Weight gain (%) | 241.4±41.5 | 264.1±20.4 | 0.0928** |
| Visceral fat (g) | 6.4±2.1 | 8.8±3.1 | 0.0425** |
| Lee Index | 287.2±4.3 | 287.9±12.6 | 0.2468* |
| Abdominal circumference (cm) | 16.4±1.0 | 17.4±0.8 | 0.0191** |
| Thoracic circumference (cm) | 17.9±0.9 | 14.9±0.6 | 0.4359** |
| Food consumption (g) | 424.0±83.9 | 468.3±86.9 | 0.0246** |
| Water consumption (mL) | 615.2±147.9 | 695.5±156.6 | 0.0228** |

*Mann-Whitney Test. **T-Test of independency. G1: control group. G2: treated group (*Lactobacillus gasseri*).

In figure 1 it is possible to observe that, from the second day of the experimental protocol, animals from the treated group exhibit superior values than the control group.

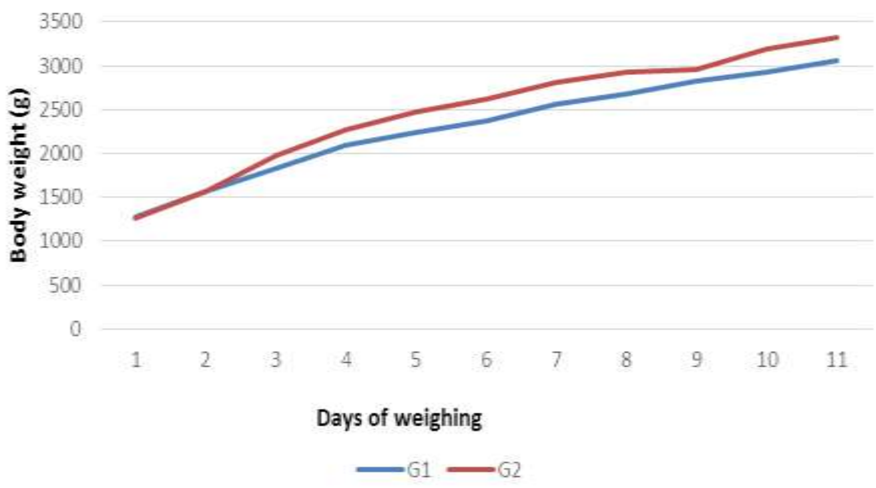


**Figure 1: Weight gain during the 10 weeks of the experimental protocol.**

Values of glycaemia, total cholesterol and triglycerides did not significantly differ (table 2).

**Table 2: Biochemical parameters of the control group (G1) and treated group (G2).**

| Parameters | G1 (n=8) | G2 (n=8) | p-value* |
|---|---|---|---|
| | Mean ± standard deviation | | |
| Glycaemia (mg/dL) | 147.3±27.2 | 142.8±19.5 | 0.3557 |
| Total cholesterol (md/dL) | 132.5±44.6 | 138.6±36.9 | 0.3847 |
| Triglycerides (mg/dL) | 209.9±70.1 | 176.5±36.1 | 0.1254 |

* T-Test of independency. G1: control group. G2: treated group (*Lactobacillus gasseri*).

## DISCUSSION

Sato et al.[23] found similar weight gain between animals receiving standardized diet plus skimmed milk powder and those who received this diet plus *Lactobacillus gasseri*. A double-blind, randomized, placebo-controlled study of overweight or obese humans demonstrated a small non-significant weight reduction over the twelve-week period among individuals who ingested this microorganism. [19] Kang et al. [20] found that animals with a hypercaloric diet receiving *Lactobacillus gasseri* had lower body weight gain than those who did not, although this variable was similar to that of animals fed a regular diet without administration of the microorganism. Hamad et al.[24] found that obese animals that received this microorganism did not present weight gain affected by diet, while eutrophic rats that received the microorganism had lower weight gain. This data resembles that of Shirouchi et al.[16] who observed that the animals that received *Lactobacillus gasseri* for four weeks had lower weight gain in comparison to the control.

In our study, it was possible to observe that there was no significant difference in the weight gain percentage of the animals of the two experimental groups, however at the end of the experimental protocol, animals from G2 presented significantly higher weight than G1. The Lee index of the animals of the two groups was similar in this study. However, Kadooka et al.[17] in a double-blind and parallel study observed a reduction in body mass index in humans that received different concentrations (106 and 107 FCU) of *Lactobacillus gasseri*, which did not occur in the control group. In our study, the visceral fat weight and the abdominal circumference of G2 were significantly higher when compared to G1. Hamad et al.[24] observed a decrease in the mass of adipose tissue in the animals that received *Lactobacillus gasseri*. Similarly, Kang et al.[20] found that the sucrose-rich hypercaloric diet induced an increase in the weight of the adipose tissue in the animals whereas administration of *Lactobacillus gasseri* suppressed this fact in addition to promoting the reduction the adipocyte size. A study by Kadooka et al. [17] in humans showed a significant reduction of visceral, subcutaneous and total fat, as well as the measurement of waist and hip circumferences through ingestion of *Lactobacillus gasseri*. On the other hand, similar to our findings, Sato et al.[23] found no effect of the diet containing this microoganism on the adipose tissue of the experimental animals as well as Jung et al.[19] on waist and hip circumference in humans.

Miyoshi et al.[25] found that *Lactobacillus gasseri* 2055 prevented body weight gain, fat accumulation and pro-inflammatory gene expression in the adipose tissue. They also found lower levels of triglyceride in the liver. Authors postulated that the improvement in the inflammatory state of the adipose would be linked to the anti-obesity effect of this microorganism.

Yonejima, Ushida, Mori[26] found that dietary supplementation with *Lactobacillus gasseri* reduced visceral fat weight and triglyceride in the liver of mice fed a high-fat diet.

Some studies have shown that this microorganism may improve the intestinal integrity, reducing the entry of inflammatory substances which may decrease inflammation in adipose tissue, thus, helping in the manage of obesity.[14, 26-28]

Shi et al.[29] studied the effects of Lactobacillus gasseri TMC0356 on the risk factors of metabolic syndrome and found improvement of glycaemia, insulin resistance, adiponectin levels and lipid profile.

Million et al.[4] performed a meta-analysis with different *Lactobacillus* species and the effects on the body weight and concluded that more research is needed to clarify the role of *Lactobacillus* species in the human energy harvest and weight regulation.

Other authors showed that the addition of *Lactobacillus gasseri* in a high sucrose diet promoted reduction of fasting and postprandial glycaemia and improved glucose tolerance in rats.[30-31] However, in an attempt to extend these analyzes, Kang et al.[20] repeated the experiment and observed that glycaemia did not change in the presence of *Lactobacillus gasseri* in animals with a hypercaloric diet, what is similar to our findings and similar to the findings of Sato et al.[23] and Shirouchi et al.[16] Jung et al.[19] observed an improvement in glucose tolerance, reduction in plasma insulin levels and decrease in blood glucose in humans.

In our study the administration of *Lactobacillus gasseri* had no effect on total cholesterol and triglycerides levels of the treated animals, as found by Sato et al.[23], Kang et al.[20] and Shirouchi et al.[16] in animals and Jung et al.[19] in humans. In contrast, Hamad et al.[24] found that mice fed this microorganism presented lower levels of total cholesterol, but with no effect on triglycerides.

## CONCLUSION

In our study the use of the microorganism *Lactobacillus gasseri* was not able to generate beneficial results on the body weight, adipose tissue and biochemical profile in the animals fed a hypercaloric diet. This suggests that the possible beneficial action of this probiotic is limited to the intake of a balanced and healthy diet.

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