Effect of oral insulin and hyperlipidic diet on intestinal epithelium and adipose tissue

Efeito da insulina oral e da dieta hiperlipídica no epitélio intestinal e tecido adiposo

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RESUMO
O uso de insulina oral vem sendo estudado por seu potencial na reversão da hipertrofia intestinal característica de pacientes diabéticos e obesos. O presente estudo, avaliou a influência da insulina oral na hipertrofia e hiperplasia em adipócitos viscerais e alterações intestinais de ratos Wistar, tratados com dieta hiperlipidica e dieta padrão. Foram divididos 60 ratos em quatro grupos de animais, onde dois grupos foram tratados com ração hiperlipidica e dois grupos com dieta padrão; um grupo de cada tipo de ração recebeu todos os dias 20 UI de insulina oral. Durante 60 dias os animais foram pesados semanalmente e após este período eles foram eutanasiados e coletadas as gorduras e intestino foram realizadas. Houve influência positiva da insulina oral no peso dos animais, nos pesos das gorduras e na redução da hipertrofia dos adipócitos, possivelmente por alterações na microbiota e camada de muco do epitélio intestinal, propiciando um maior efeito incretínico; houve diferença no peso, altura e largura dos vilos intestinais relacionado possivelmente com a ação sensibilidade à insulina. Diante dos resultados encontrados podemos concluir que a insulina oral tem efeito positivo nas alterações intestinais e dos adipócitos em animais alimentados com dietas ricas em gordura.

Palavras-chave: insulina oral, gordura visceral, intestino delgado

ABSTRACT
The use of oral insulin has been studied for its potential in reversing the intestinal hypertrophy characteristic of diabetic and obese patients. The present study evaluated the influence of oral insulin on hypertrophy and hyperplasia in visceral adipocytes and gut alterations of Wistar rats treated with hyperlipidic diet and standard diet. Sixty rats were divided into four groups of animals, where two groups were treated with high fat diet and two groups with standard diet; one group of each type of feed received 20 UI of oral insulin every day. During 60 days the animals were weighed weekly and after this period they were euthanized and collected the fats and blood for biochemical tests. There was a positive influence of oral insulin on animal weight, fat weight and reduction of adipocyte hypertrophy, possibly due to changes in the microbiota and mucus layer of the intestinal epithelium, favoring a greater incretin effect; there was a difference in the weight, height and width of the intestinal villus, possibly related to the insulin sensitivity action. In view of the founded results, we can conclude that an oral insulin has a positive effect on intestinal and adipocyte changes in animals treated with high fat diet.

Keywords: oral insulin, visceral fat, small gut

1 INTRODUCTION

Diabetes and obesity are comorbidities that are strongly associated. Insulin is a peptide that has low bioavailability when administered orally due to its degradation in stomach pH. However, studies show that this hormone improves serum parameters and promotes morphologically changes in the intestinal epithelium [1,2].

The intestinal epithelium has a fundamental role in the development and progression of diabetes mellitus, especially when related to the hypercaloric diet, rich in carbohydrates and lipids [3].

The reversal of intestinal hypertrophy by degraded insulin products has led to correlations between the higher intestinal mass in diabetic and obese patients when compared to non-diabetic obese patients, and that intestinal changes may have a fundamental role in the evolution of the disease [4,5].

In addition, insulin is able to act by reducing the weight of adipose tissue [2]. The cause of the inflammation in adipocytes caused by diabetes is not yet fully defined. However, the expansion of adipose tissue caused by obesity triggers intrinsic factors capable of initiating an inflammatory response [6].

Obesity is one of the risk factors linked to peripheral insulin resistance; mainly visceral adipose tissue (including omentum and mesentery) serving not only as a fat storage site, but also as an endocrine organ, secreting various hormones such as adipokines, leptins and various inflammatory cytokines [7,8].

Thus, it is imperative in scientific research to understand the range of the physiopathological process to these diseases, as well as the gastrointestinal mechanisms participating in the insulin-tissue interaction. This understanding can enable a less invasive, painful and greater effective treatment. The oral route is preferred among all alternatives [9].

This study was developed to evaluate the histological changes of the small intestine, and adipocytes of Wistar rats, in two different diets with oral insulin treatment.

2 MATERIAL AND METHODS

2.1 HYPERLIPIDIC DIET

The commercial feed used in the high-fat diet was obtained using 600g of the conventional laboratory Nuvilab®, plus 200g of soy bran, 180g of condensed milk and 200g of bovine fat; adding 200ml of water to facilitate pelletizing. The drying process took place under refrigeration for 24 hours.

2.2 ANIMAL ASSAY

The experimental protocol was previously submitted for approval to the Ethics Committee on the Use of Animals (CEUA) of the State University of Ponta Grossa (UEPG), protocol number 044/2018.

48 male Wistar rats, with an average weight of 200g were separated into four groups: Group 1 = standard diet and water; Group 2 = standard diet and 20 I.U. (International Units) of regular Insulin; Group 3 = high fat diet and gavage with 0.2 ml of water and Group 4 = high fat diet and gavage with 20 I.U. of regular Insulin.

The animals were weighed weekly and the treatment occurred for 60 consecutive days. The administration of 20 IU of insulin once a day was performed orally. The animals in the control group received water in equal volume to the treated group.

After 60 days, before the eutanásia, and after 12 hours of fasting the blood glucose was measuared using a digital blood glucose meter Accu-Chek Active, then the tissues were collected.

2.3 HISTOLOGICAL AND ANALYSIS

After euthanasia, 10 cm of intestine fragments (duodenum, jejunum, and ileum) were removed, and the retroperitoneal, omental, and mesenteric fat were weighed on a precision scale and then stored in 10% formaldehyde for fixation.

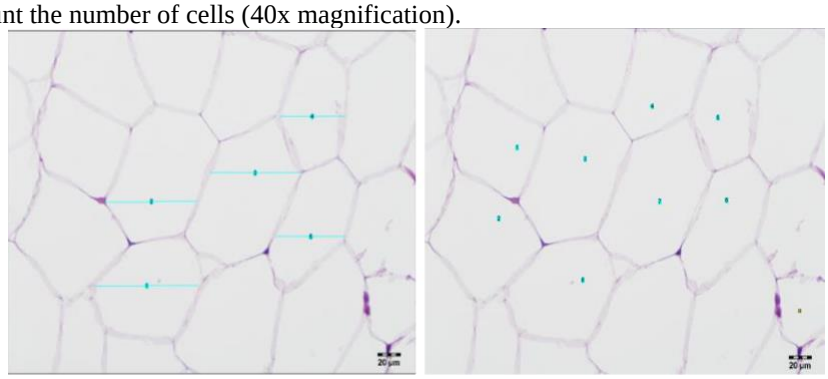
The material was processed, sectioned in a 3 µm-thick microtome, and stained with hematoxylin and eosin (HE). Then, the histological sections were photographed with an Olympus DP72 microscope using the CellSens Standard program. The analyses were performed in triplicate.

2.4 HISTOMORPHOMETRIC ANALYSIS OF FATS

The morphometric analysis of the count (hyperplasia) and measurement of the area (hypertrophy) of adipocytes (mesenteric, omental and retroperitoneal fat) were performed

using the ImageJ program (ImageJ 1.50i, Bethesda, MD, United States) as shown in Figure 1. All analysis were done in triplicate.

Figure 1. Photomicrophotographs of adipose tissue on the left to measure the area of the adipocyte and on the right to count the number of cells (40x magnification).

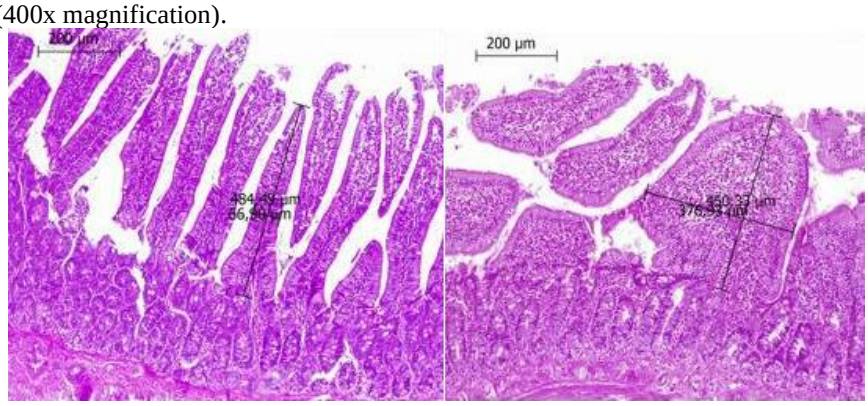


2.5 HISTOMORPHOMETRIC ANALYSIS OF THE GUT

In the analysis of intestinal tissue, portions of the duodenum, jejunum and ileum were performed. The fragments were routinely processed for histology in the same way as for fat fragments but with 3 µm thick cuts, and also stained by HE.

To evaluate the length and width of the villus (Figure 2), Image J software was used. The analysis was made in 3 samples, and the mean and standard deviation (SD) were calculated.

Figure 2. Photomicrophotographs of the intestinal epithelium showing the measurements of length and width of the villus (400x magnification).



2.6 STATISTICAL ANALYSIS

The statistical evaluation was performed using the Graphpad Prisma Version 7.0 software by analysis of variance (ANOVA) for multiple comparisons, followed by the Tukey test, with a 95% range of variance ($p \leq 0.05$).

3 RESULTS AND DISCUSSION

3.1 ANIMAL BODY WEIGHT

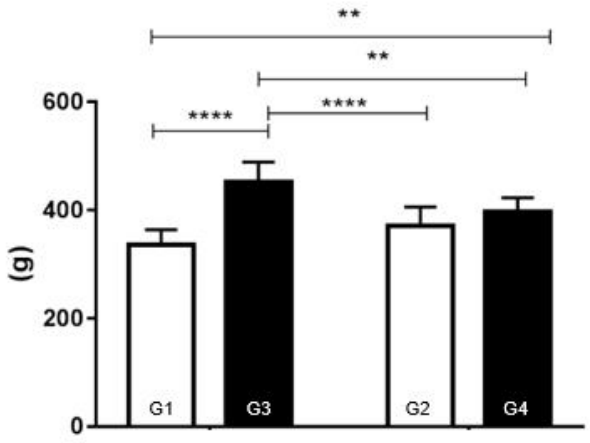
It is important to study obesity, because it has a great impact as a risk factor for the development of diabetes and cardiovascular diseases. After 60 days of experiment, the weight of the animals treated with hyperlipidic diet had a significant statistical significance ($p < 0.005$) when compared to the group that received the standard diet (Figure 3).

Weight gain was greater in the animals that received the hyperlipidic diet when compared to the animals that received the standard diet, a result that was also found in the study by Leopoldo [10], where the weight of the animals was higher in the Wistar rats that received a high-calorie diet and were sedentary.

However, oral insulin showed an important role in reducing weight only in group G4, showing that insulin had an indirect influence; even though it is not absorbed in its biologically active form, once it is denatured by stomach acid and pancreatic enzymes.

Air [11] also found a positive result when they administered insulin mimetic non-protein molecules to rats, the weight of animals and their visceral fats reduced only in groups that received hypercaloric diet together with the molecules, identifying their influence on lipid metabolism.

Figure 3. Weight of the animals after treatment with hyperlipidic diet or standard diets without (G1, G3) or addition of oral insulin (G2, G4). The data are the mean ± DPM. The symbols on the bars indicate differences between groups. ANOVA followed by post-hoc Tukey ** $p < 0.011$ and **** $p < 0.0001$.



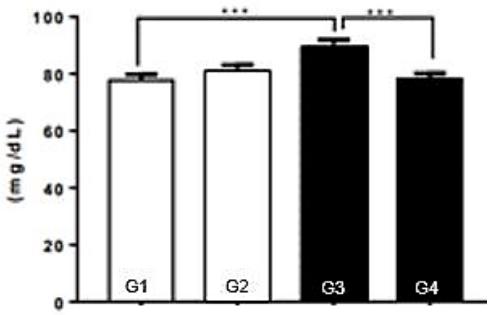
Fasting blood glucose

Figure 4 shows that there was an increase in fasting glycemia of animals in the G3 group when compared to the others. This result demonstrates that the hyperlipidic diet increases fasting glycemia, however, oral insulin administration can reverse this finding value.

This hypoglycemic effect of oral insulin, even if proteolyzed in TGI, is similar to the findings of other studies that revealed a hypoglycemic effect of oral insulin[12,13]. There are specific insulin receptors in the luminal and basolateral membrane of enterocytes, it is suggested that this route that the hypoglycemic action of insulin when administered orally occurs [14].

Oral insulin may have contributed to the animals overweight at the beginning of the treatment, before the hyperlipidic diet caused possible damage to the epithelial barrier and in the intestinal microbiome, which may lead to a loosening of the aggregating chains of the intestinal cells. This phenomenon may have favored the entry of the hormone molecule into the entering metabolism in the the intestinal lumen, an action explained by Do [15], causing a reduction in blood glucose, body weight and adipocyte hypertrophy.

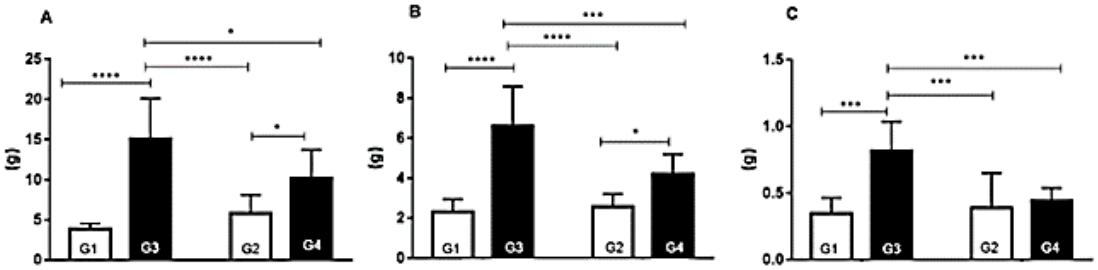
Figure 4. Effect of treatment with oral insulin on fasting blood glucose. There was no statistical difference between groups 1-2, 1-4 and 2-4. The bars represent the mean of the groups ± standard error of the mean. ANOVA followed by post-hoc Tukey * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.



Fat weight

The fat weight was statistically significant (p <0.05) in all groups when compared to the G1 group, which received standard diet and the G4 group, which received a hyperlipidic diet and oral insulin, with the G3 group which received hyperlipidic diet and oral insulin (Figure 5. It was also possible to observe a significant difference in retroperitoneal and mesenteric fats, between the G2 groups, which received a standard diet and oral insulin with the G4 group.

Figure 5. Weight of A-Retroperitoneal; B- Mesenteric and C- Omental. The lines represent the means ± standard error of the mean. Anova followed by post-hoc Tukey * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.



Adipocyte hyperplasia and hypertrophy

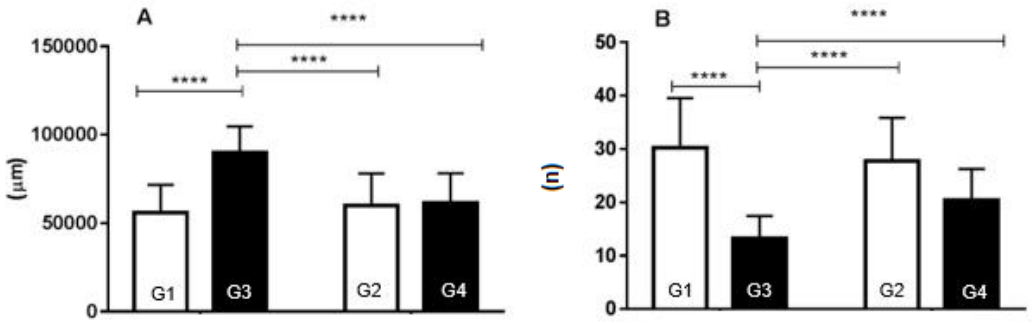
The hyperlipidic diet caused hypertrophy in the adipocytes, significantly increasing the total density of these cells, in the average sectional area and in their volume.

Mesenteric fat

Figure 6 presents the data of the mesenteric adipose tissue. In A, the diameter of the adipose cells is observed. Treatment with oral insulin was effective in causing a reduction in the diameter of the adipocyte in the G4 group when compared to the G3 group, a result of high statistical relevance (p <0.0001). There were no changes in the control group.

Figure XB shows the number of adipocytes per field. It was possible to observe a statistical difference between groups G1 and G3 that received a standard diet, and between groups G3 and G4 that received a hyperlipidic diet.

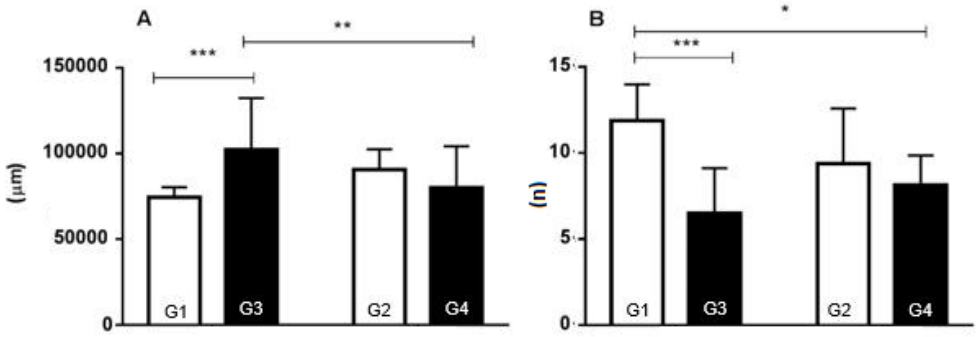
Figure 6: Effect of treatment with oral insulin on hyperplasia and hypertrophy of mesenteric adipose tissue. The data are the mean ± DPM. The symbols on the bars indicate differences between groups. A- Diameter of adipose cells; B- Number of fat cells. ANOVA followed by post-hoc Tukey. * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.



Retroperitoneal fat

Figure 7 shows hypertrophy and hyperplasia of the retroperitoneal fat. In Figure 7-A it is possible to notice that there is adipocyte hypertrophy in the G3 group, and that this hypertrophy was reduced by the use of oral insulin in the G4 group. Figure 7-B shows the number of fat cells observed per field. It is observed that the high-fat diet causes a decrease in the number of G3 and G4 cells when compared to groups that received a standard diet. The use of oral insulin showed no significant difference.

Figure 7: Effect of treatment with oral insulin on hyperplasia and hypertrophy of retroperitoneal adipose tissue. The data are the mean ± DPM. The symbols on the bars indicate differences between groups. A- Diameter of adipose cells; B- Number of fat cells. ANOVA followed by post-hoc Tukey. * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.

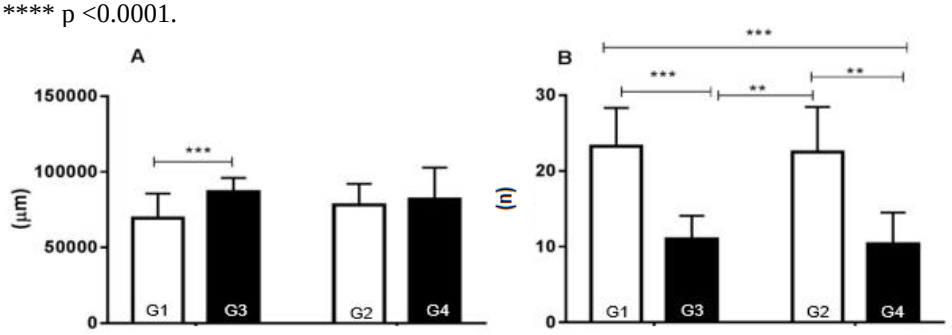


Omental fat

Figure 8-A shows the data of the hypertrophy of the omental adipose tissue. It was not possible to observe changes with oral insulin treatment in this parameter. Figure 8-B shows data on adipocyte numbers per field.

It is noted that the hyperlipidic diet causes a decrease in the number of adipocytes per field when compared to the control group, this decrease was present when the animals received treatment with oral insulin.

Figure 8: Effect of treatment with oral insulin on hyperplasia and hypertrophy of omental adipose tissue. The data are the mean ± DPM. The symbols on the bars indicate differences between groups. A- Diameter of adipose cells; B- Number of fat cells. ANOVA followed by post-hoc Tukey. * p <0.05; ** p <0.01; *** p <0.001; **** p <0.0001.



Visceral adipose tissue is directly involved with the development of insulin resistance, and there are a higher rate of lipolysis and a more pronounced hormonal function with the production of several adipokines such as Leptin, Adiponectin, and some directly linked to the inflammatory process such as resistin, IL -6, TNF-α; that justify more pronounced insulin resistance in obese individuals [16].

The animals in the groups that were fed with hyperlipidic diet, had a greater availability of fat in their intestines, which may have contributed to changes in their absorptive structure [17].

With the increase in intake and absorption of lipids in the groups that received hyperlipidic diet, we can see that there was a general increase in the diameter of the adipocytes, with a significant reduction in the same when compared to the rats that also received the diet associated with oral insulin. In a study by Higa [18] with mice that received two different diets, one being high in fat, it was demonstrated that there was hypertrophy of adipocytes and increased fat in the groups that received hypercaloric diet compared to the other groups.

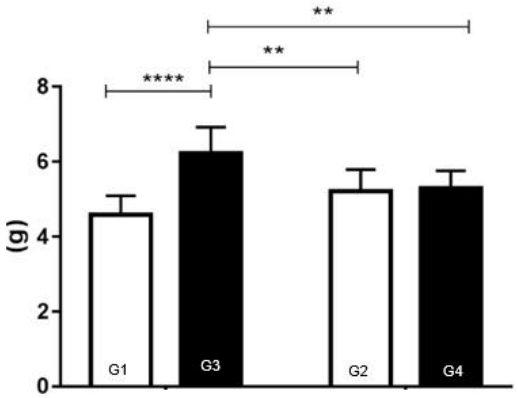
In order to accommodate the greater storage of lipids and anabolic strength due to hyperinsulinemia, an increase in the diameter and number of adipocytes is inevitable; however, this expansion reaches a limit in which this anabolic pressure can no longer be accommodated, due to space restriction, creating a stress that provides the inflammatory state characteristic of obesity [19].

Salviano [20] showed morphological changes and intense TNF-α labeling in the adipocytes of Wistar rats, demonstrating that the inflammatory response is a consequence of the increase in the size of the adipocyte. Obese people with few large adipocytes are more intolerant to glucose and hyperinsulinemic than those with the same degree of obesity and small adipocytes, but in greater numbers [21].

Intestinal weight

The intestinal weight of the animals treated with hyperlipidic diet was higher than the group of animals treated with a standard diet and oral insulin concomitant with the high fat diet reduces this increase (Figure 9).

Figure 9: Effect of treatment with oral insulin on the intestinal weight of rats fed a hyperlipidic diet. The data are the mean ± DPM. The symbols on the bars indicate differences between groups. ANOVA followed by post-hoc Tukey * p <0.05; ** p <0.01; *** p <0.01; **** p <0.0001.

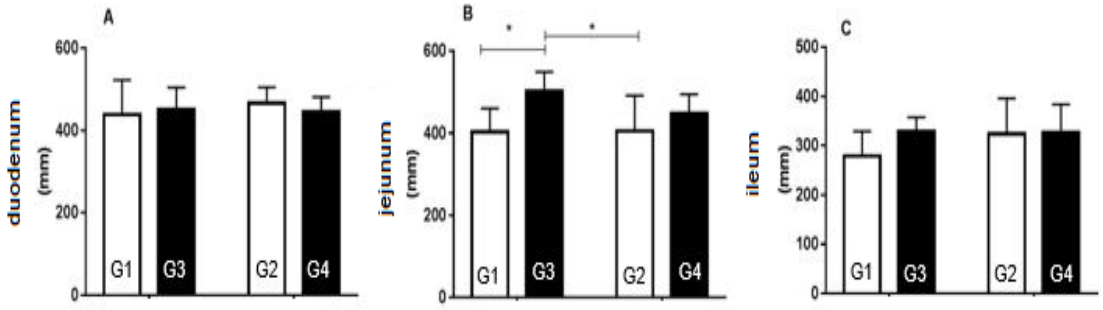


Insulin may have an effect on intestinal weight related to reduced insulin sensitivity. In non-diabetic patients the enteral mass of the small intestine decreases and insulin sensitization therapy indicates the return of the enteral mass to normal levels [22].

Villus length

Figure 10 shows the results of the villus height of the different parts of the intestine: A – villus of the duodenum; B - villus of the jejunum and C - villus of the ileum. For the duodenum, no changes in villus height were observed. For the jejunum there was no interaction between the variables, only the effect of the diet factor. The G3 group showed an increase in the height of the jejunal villus when compared to the control group. For animals that received oral insulin, the villus height of the jejunum was equal to that of animals that received a standard laboratory diet. There was also no difference in the villus height for the ileum. The hyperlipidic diet increased the villus height only in the jejunum.

Figure 10: Length of intestinal villus A - duodenum; B - jejunum and C-ileum. The data are the mean ± DPM. The symbols on the bars indicate differences between groups. ANOVA followed by post-hoc Tukey * p <0.05; ** p <0.01; *** p <0.001; **** p <0.001.



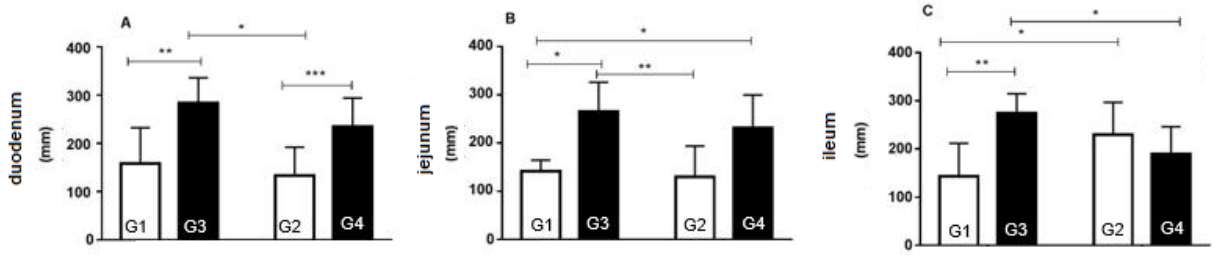
Villus width

Figure 11 shows the villus width data. For the duodenum, there was no interaction between the variables diet and treatment with oral insulin. It was possible to observe in the G3 group a significant increase in the villus width when compared to the control group.

For the jejunum, it was also observed that there was no interaction between the variables. The G3 group showed a significant increase in villus width when compared to its control group. For ileum, the result showed an interaction between the variables diet and treatment with oral insulin. The influence of the diet variable on the variance of the data can also be observed. Thus, in the ileum, oral insulin reduces the width of the villus when concomitant with the hyperlipidic diet and increases the villus width in a standard diet.

This phenomenon may be related to the anabolic effect of insulin and local intestinal action is suggested, which is not yet known in what way it can occur [13].

Figure 11: Width of intestinal villi A - duodenum; B - jejunum and C-ileum. The data are the mean ± DPM. The symbols on the bars indicate differences between groups. ANOVA followed by post-hoc Tukey * p <0.05; ** p <0.01; *** p <0.001; **** p <0.001.



The intestinal epithelium is a single layer of cells that lines the intestinal lumen, being the most proliferative tissue in the body [23]. The diabetic tends to have a thicker intestinal epithelium than the non-diabetic patients. According to this hypothesis, in the diabetic intestine, epithelial hypertrophy and / or hyperplasia occurs in the early stages of diabetes, especially in the proximal intestine.

The role of insulin in the intestinal epithelium is not completely understood and the results are still controversial. However, it was possible to observe a significant difference in villus width, demonstrating that although insulin undergoes gastric action, the metabolites appear to have an effect on the intestinal epithelium. Further studies are needed to elucidate the mechanism of action of this peptide in the intestine.

4 CONCLUSIONS

Based on the results obtained, it is concluded that oral insulin, despite being denatured, has positive effects in reducing weight and adipocyte hypertrophy when associated with a hyperlipidic diet, and may have a role in preventing metabolic syndrome and DM2. Several mechanisms may be involved in this result, such as microbiota and mucus production in the small intestine, which affect its epithelium, where nutrients are absorbed from the diet to enter the metabolism of visceral lipids and fats. Despite the findings, further studies are needed to assess the systemic effect of oral insulin for a longer period of time and to understand the role of the intestine in this mechanism.

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