Roux-en-Y gastric bypass and inflammatory activity of the adipose tissue

Derivação gástrica em Y-de-Roux e a atividade inflamatória do tecido adiposo

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ABSTRACT

Objective: To evaluate the oxidative stress and inflammatory markers in obese patients before and after Roux-en-Y vertical banded gastroplasty. Methods: We studied 20 class III obese patients undergoing Roux-en-Y vertical banded gastroplasty, seven men and 13 women, mean age 39 years, and 20 non-obese subjects, nine males and 11 females, mean age 38 years. We determined the plasma levels of malondialdehyde, stress an index, total antioxidant capacity, catalase, reduced and oxidized glutathione and inflammatory markers (C reactive protein and á-1 acid glycoprotein). In the obese group, these parameters were determined before and 2, 6 and 12 months after gastroplasty. Results: Preoperatively, the obese group showed elevated levels of inflammatory markers of oxidative stress (malondialdehyde levels and stress index) and lower levels of indicators of antioxidant defense compared to the control group. Weight loss was accompanied by gradual reduction in the levels of malondialdehyde and stress index. We found an increased concentration of reduced glutathione and total antioxidant status and reduced levels of inflammatory markers. Conclusion: Weight loss improves the inflammatory state and oxidative stress levels.

Key words: Bariatric Surgery. Oxidative stress. Obesity. Biological markers.

INTRODUCTION

Obese individuals have elevated circulating levels of inflammatory cytokines such as interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα), both produced by adipose tissue1. These cytokines activate cells (phagocytes, fibroblasts, B lymphocytes and endothelial cells) that will release pro-oxidant molecules, mainly reactive oxygen species – ROS2.

Virtually all biomolecules (proteins, lipids and DNA) are susceptible to oxidation and, to protect itself, the organism has chemical and enzymatic mechanisms with antioxidant properties3. In situations where protection is inadequate and / or there is an excess of pro-oxidants, oxidative stress occurs4.

The reduction of adipose tissue decreases the secretion of inflammatory cytokines and the consequent drop in the level of oxidative stress, reducing the risk factors for obesity-related diseases5.

In this study we assessed inflammatory markers, indicators of oxidative stress and antioxidant defense in obese patients class III who underwent Roux-en-Y vertical banded gastroplasty (VBG). These parameters were monitored preoperatively and at two, six and 12 months after the procedure.

METHODS

This study is in line with the resolution 196/96 of the National Board of Health / National Committee for Ethics in Research and was approved by the Ethics Committee on Human Research of State University of Londrina (Opinion CEP 041/05).

Participants were divided into two groups: the obese group and the control group. The initial group was composed of 25 obese individuals. From this group five subjects were excluded: one had a postoperative infection, one required reoperation and three did not attend to collect blood samples in certain periods. In the non-obese group (control), two individuals were excluded due to change in the analyzed inflammatory parameters.

The groups participating in the study were as follows: the obese group consisted of 20 patients, seven

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men and 13 women, aged between 26 and 52 years and the control group consisted of 20 non-obese subjects, nine men and 11 women, aged between 27 and 53 years, who were not under any kind of medical treatment or drug therapy.

The collection of samples for measurement of markers of oxidative stress in the obese group was made in the preoperative period and at two, six and 12 months postoperatively. The collection of samples from the 20 individuals who formed the control group (not obese) was made in just one moment.

As an indicator of oxidation, we used the plasma concentration of malonic dialdehyde (MDA), a byproduct of lipid peroxidation. To evaluate the antioxidant defense, we used the SOD (superoxide dismutase), catalase, total and oxidized glutathione and the total antioxidant capacity by the plasma TRAP (Total Radical Antioxidant Parameter). The study protocol included the measurement of inflammatory parameters (C reactive protein – CRP – and alpha-1 acid glycoprotein – AGP). All exams were performed in duplicate, accepting the maximum coefficient of variation of 10% around the average.

For the calculation of stress index (SI), which indicates the oxidation state in erythrocytes, we used glutathione levels, according to the formula:

\[ SI = \frac{\text{GSSG (oxidized glutathione)}}{\text{GSH (reduced glutathione)}} \times \frac{1}{\text{GSSG}} \]

Results were expressed as mean and standard deviation and were presented in tables and figures. We performed the Shapiro-Wilk test for normality of the findings. For comparison of the groups we used the Student t test for unpaired samples. To check the intra-group variance the ANOVA test was used, with Bonferroni post hoc test when necessary. Correlation analysis was done by Pearson correlation test.

Significant differences were determined by p <0.05 and significant correlations (when re”0.45) were selected for analysis. We used the SPSS (Statistical Package for the Social Sciences) for Windows, version 13.0, for data analysis.

### RESULTS

The average values of the CRP inflammatory marker in the obese group before surgery were high compared to the control group (p<0.001) and was reduced gradually, being statistically significant at all moments assessed. After 12 months of operation, these values showed no difference from the control group. AGP showed a pattern similar to CRP, with a reduction of average values in the obese group at all time points assessed, and values in the preoperative higher than the control group (p<0.001) (Table 1).

Regarding the assessment of antioxidant defenses, catalase was not different between obese and control groups in the preoperative period and between different times. GSH and TRAP, which showed decreased values in the obese group preoperatively in the control group (p<0.001), were significantly increased after the operation, until after 12 months, when there were no significant differences in relation to the control group. SOD displayed increased activity in the obese group preoperatively compared to the control group (p<0.001) and this activity decreased during the evaluation period, but at 12 months it remained high when compared to the control group (p<0.001). Data are presented in table 2.

Markers of oxidative damage (MDA and SI) were significantly increased (p<0.001) in the obese group in the preoperative period in relation to the control group. The plasma MDA showed a statistically significant decline among all periods of the obese group. After 12 months, mean levels were still significantly higher (p<0.001) than those found in the control group. As for the MDA, although a significant reduction of the values has been observed in the obese group after 12 months, these values had remained high when compared to the control group (p<0.01). SI was preoperatively elevated in the obese group in relation to the control group, and after the operation it showed a significant reduction in the obese group in all periods. In this group, at 12 months after surgery the mean values were still high when compared to the control group (p<0.001). Data are presented in table 3.

### Table 1 - Results of determination of reactive Protein C and α-1 glycoprotein.

<table>
<thead>
<tr>
<th>TESTS</th>
<th>Control (a)</th>
<th>Preop (b)</th>
<th>2 months (c)</th>
<th>6 months (d)</th>
<th>12 months (e)</th>
<th>P Value between groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n=20)</td>
<td>Obese group (n=20)</td>
<td>Obese group (n=20)</td>
<td>Obese group (n=20)</td>
<td>Obese group (n=20)</td>
<td></td>
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<tr>
<td>CRP (mg/dL)</td>
<td>0.48 ± 0.24</td>
<td>1.30 ± 0.97</td>
<td>0.93 ± 0.42</td>
<td>0.64 ± 0.40</td>
<td>0.38 ± 0.26</td>
<td>* b/c** c/e*** a/b, b/d, b/e</td>
</tr>
<tr>
<td>AGP (mg/dL)</td>
<td>58 ± 14</td>
<td>126 ± 53</td>
<td>108 ± 34</td>
<td>84 ± 24</td>
<td>76 ± 18</td>
<td>* c/d** c/e *** a/b, a/e, b/d, b/e</td>
</tr>
</tbody>
</table>

Pre op = preoperative. * p < 0.05. ** p < 0.01. *** p < 0.001. CRP = C reactive protein. AGP = α-1 Glycoprotein.
DISCUSSION

The relationship between weight loss and reduction of CRP levels has been demonstrated. The hepatic synthesis of CRP is stimulated mainly by IL-6 and the adipocytes produce around 30% of circulating IL-6 of non-inflammatory origin. The production of IL-6 is directly related to the volume of adipose tissue, and weight loss caused by VBG leaves only a minor amount of circulating IL-6 with a consequent drop in hepatic synthesis of CRP, reducing the biological effects of this protein. Among these effects are its direct action on the vascular endothelium, by inducing the synthesis of adhesion molecules, and the increased generation and release of ROS by neutrophils.

Although the biological function of AGP remains unknown, it is an indicator that changes in the presence of processes that cause tissue damage (inflammation or infection) and whose hepatic synthesis is regulated by cytokines such as IL-1, IL-6 and other cytokines of the IL-6 family (such as leptin) and TNF-α. Studies involving AGP as an inflammatory marker show that it has been used in the monitoring and evaluation of recurrences in patients with cancer.

Regarding the inflammatory markers CRP and AGP, this study demonstrated a statistically significant difference between patients in the control group and obese group in the preoperative period. Two months after the operation, there was reduction in CRP levels and AGP and a positive correlation between these indicators (r = 0.837 and p < 0.001). The reduction in the levels of AGP was not significant in this period, whereas CRP has significant variation, probably because this protein is the earliest and most sensitive indicator of inflammation. The assessments in subsequent periods showed a gradual reduction of the mean values of CRP and AGP in the obese group.

AGP levels showed significant positive correlation with CRP levels in obese patients in the preoperative period (r = 0.809, p < 0.01) and 12 months after operation (r = 0.579 and p < 0.01), probably reflecting the fall in levels of cytokines due to the reduction of adipose tissue after VBG, suggesting the possibility of using this marker as an indicator of inflammatory status in obese patients.

Table 2 - Antioxidant defense markers.

<table>
<thead>
<tr>
<th>TESTS</th>
<th>Control (a)</th>
<th>Preop (b)</th>
<th>2 months (c)</th>
<th>6 months (d)</th>
<th>12 months (e)</th>
<th>P Value between groups</th>
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<tr>
<td></td>
<td>Control (n=20) Obese group (n=20) Obese group (n=20) Obese group (n=20) Obese group (n=20) Obese group (n=20)</td>
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<tr>
<td>CATALASE</td>
<td>12,3 ± 1,26</td>
<td>12,1 ± 1,19</td>
<td>12,7 ± 1,20</td>
<td>12,8 ± 1,30</td>
<td>13,2 ± 1,56</td>
<td>NS</td>
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<tr>
<td>(Δ/mgHb/min)</td>
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<tr>
<td>SOD</td>
<td>7,56 ± 0,79</td>
<td>10,70 ± 1,78</td>
<td>10,34 ± 1,10</td>
<td>9,74 ± 1,10</td>
<td>9,24 ± 1,27</td>
<td>* b/d, c/d** c/e*** a/b, a/e e b/e</td>
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<tr>
<td>(U/mgHb)</td>
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<tr>
<td>GSH</td>
<td>2728 ± 315</td>
<td>2002 ± 249</td>
<td>2276 ± 282</td>
<td>2598 ± 314</td>
<td>2833 ± 249</td>
<td>** b/d, e*** a/b, b/c, b/d, c/e, d/e c/e</td>
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<td>(mM/g de Hb)</td>
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<td>GSSG</td>
<td>127 ± 14</td>
<td>211 ± 35</td>
<td>181 ± 28</td>
<td>160 ± 24</td>
<td>148 ± 17</td>
<td>** c/e*** a/b, a/e, b/c, b/d, b/e e c/e</td>
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<td>(mM/g de Hb)</td>
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<tr>
<td>TRAP (plasma)</td>
<td>760,7 ± 64,3</td>
<td>585,5 ± 148,4</td>
<td>615,2 ± 111,6</td>
<td>717,5 ± 124,5</td>
<td>815,5 ± 149,4</td>
<td>** b/d, c/d*** a/b, b/e e c/e</td>
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<td>(nM Trolox®)</td>
<td></td>
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Pre op = preoperative * p < 0.05 < 0.01 *** p < 0.001
SOD = Superoxide dismutase, GSH = Reduced Glutathione, GSSG = Oxidized Glutathione, TRAP (Total Radical Antioxidant Parameter) = total antioxidant capacity.

Table 3 - Oxidative injury indicators.

<table>
<thead>
<tr>
<th>TESTS</th>
<th>Control (a)</th>
<th>Preop (b)</th>
<th>2 months (c)</th>
<th>6 months (d)</th>
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<th>P Value between groups</th>
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<td>Control (n=20) Obese group (n=20) Obese group (n=20) Obese group (n=20) Obese group (n=20) Obese group (n=20)</td>
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<tr>
<td>SI</td>
<td>0,049 ± 0,05</td>
<td>0,121±0,03</td>
<td>0,091±0,02</td>
<td>0,069±0,02</td>
<td>0,055±0,06</td>
<td>* d/e*** a/b, a/e, b/c, b/d, b/e, c/d e c/e</td>
</tr>
<tr>
<td>MDA (plasma)</td>
<td>4,99 ± 1,19</td>
<td>16,69 ± 8,23</td>
<td>15,04 ± 8,10</td>
<td>11,61 ± 5,45</td>
<td>9,10 ± 4,19</td>
<td>** b/d, c/e*** a/b, e a/e e b/e</td>
</tr>
<tr>
<td>(nmol/g prot)</td>
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</table>

Pre op = preoperative * p < 0.05 < 0.01 *** p < 0.001
MDA = malonic dialdehyde, SI = stress index.
The study participants started receiving vitamin supplements 60 days after the operation through the administration of one daily tablet of Centrum® multivitamin, which includes some antioxidants such as vitamin A (5,000 IU), vitamin E (30 IU) and vitamin C (60 mg). The roles of these antioxidants and their relationship to various diseases have been described by several authors. The big difference between dosages, methods, study groups and results do not allow us to conclude about the effect that these antioxidants may exert on the level of oxidative stress in patients with chronic diseases. Since the administration of multivitamin is part of the post-surgical therapy for obesity, its influence on the results of this study adds to the weight loss and changes in their diets.

There were no significant changes in the levels of the indicators of lipid peroxidation (plasma MDA) and activity of antioxidant enzymes SOD and catalase. The TRAP showed no statistical improvement. As for glutathione, there was a reduction in the levels of GSSG, increased GSH, reduced stress index and an inverse correlation between levels of oxidized glutathione and TRAP (r = -0.484, p <0.05), showing that the higher the level of TRAP, the smaller amount of oxidized glutathione. These observations reflect an improvement of antioxidant defense during this period.

The association between elevated levels of oxidative stress and obesity has been established in several studies involving animal and human models. Among the biomolecules that sustain the harmful effects of free radicals, lipids are the major class involved. The lipid oxidation process is called lipid peroxidation (LPO) and may occur in the cell membrane, in the free fatty acids or in lipoproteins such as LDL-cholesterol. Among the products generated by the LPO there is malondialdehyde (MDA), which can be used as a marker of oxidative injury. Beside this role, MDA has toxic effects on DNA. Despite its low reactivity at physiological pH, this molecule is capable of reacting with nucleic acids, altering the DNA and being possibly related to the development of several types of cancer. In this study we measured plasma MDA content as an indicator of oxidation of membrane lipids, free fatty acids or lipoproteins. The mean plasma levels of MDA in the obese group in the preoperative period were significantly elevated when compared to those found in the control group, showing that obese individuals have higher levels of lipoprotein oxidation.

Weight loss is accompanied by a reduction in ROS generation and a fall in MDA levels. Consistent with these data, after the operation we observed a significant reduction in the mean levels of plasma MDA, which were significantly higher preoperatively in the obese group than in the control group and decreased significantly, reflecting a fall in the indices oxidation of LDL cholesterol.

To assess the profile of antioxidant defenses, we analyzed the activity of SOD, catalase, oxidized and reduced glutathione levels and TRAP. We found a higher SOD activity in the obese group compared with the control group even 12 months after the operation. Although there was reduction in the SOD postoperative activity, these levels remained high and showed differences in relation to the control group in all periods. In the obese group, there was a positive correlation between weight and SOD (r = 0.444, p <0.05) in the preoperative period, which was not observed 12 months after the procedure. Increased SOD activity in the obese is related to conditions that cause increased synthesis of SOD induction by TNF-α, IL-1, IL-6, i.e., the presence of chronic inflammation and oxidative stress in obesity may result in increased generation of superoxide radicals, causing an adaptive response that leads to increased SOD activity. The weight loss coupled with a lower degree of synthesis and release of cytokines and ROS possibly decreased the inducing effect on SOD, which would explain this lack of correlation 12 months after VBG.

These data showed no postoperative change of catalase activity in the obese group, and that catalase activity was similar in the group of obese patients in the preoperative period in relation to the control group. Data from previous studies showed that catalase activity has a wide degree of variation. Nageswara et al. observed no change of catalase when assessing hypertension associated with metabolic syndrome components. In vitro studies indicated that increased levels of cytokines inhibit catalase activity in beta cells and that a low degree of oxidation can induce adaptive response by increasing the activity of this antioxidant enzyme in vascular cells. These differences between various studies may be partly explained on the basis of various animal models and interventions used, as well as by variations in gender, age, degree and duration of disease in which the catalase activity was evaluated.

Glutathione exists in the human body in two forms: reduced and oxidized. We used the values of reduced (GSH) and oxidized (GSSG) glutathione to calculate the stress index (SI) because this relationship can be a good biomarker of oxidative stress. Among obese patients, we found an inverse correlation between the levels of GSH and SI (r = -0.598, p <0.01), and direct correlation of GSSG with SI in the preoperative period (r = 0.778, p < 0.01). These correlations persisted twelve months after the operation (r = -0.671, p <0.01 and r = 0.541, p <0.05). Patients in the obese group had lower average GSH level in the preoperative period when compared with the control group, but 12 months after surgery these levels were not significantly different. The SI values suffered a significant drop in all periods in the obese group. The fall in reduced glutathione levels, as found in chronic renal, hypertensive, diabetic patients and obese participants in this study, may increase susceptibility to oxidative damage of these individuals and, to the contrary, increased levels of GSH, reduction of GSSG and decrease in SI, observed after the operation, possibly reducing the incidence of oxidative damage and related diseases.
TRAP provides a global estimation of antioxidant molecules present in biological fluids. Several experimental models show increased generation of ROS and decreased levels of TRAP\(^1\). The levels of TRAP in the obese group in the preoperative period were significantly reduced when compared to the control group. From the second month after VBG on, there was an increase of this variable, and after 12 months the patients had no difference in relation to controls. After VBG, the increase in TRAP possibly resulted from the sum of factors due to the decrease in weight, i.e., reducing levels of inflammatory markers, reduction in markers of oxidative injury and increased activity of markers related to antioxidant defense.

In conclusion: a) in the preoperative period obese people had higher indicators of oxidation and lower levels of antioxidant defense, i.e., they had a higher degree of oxidative stress; b) 12 months after the operation the levels of oxidative indicators showed a significant reduction; c) there was a significant reduction in the level of oxidative stress 12 months after the operation; d) the highest degree of antioxidant protection observed after the operation, combined with the reduction of inflammatory markers and oxidative damage, indicated lower oxidative stress level and strengthened the role of adipose tissue as an agent of chronic inflammation.

RESUMO

**Objetivo:** Avaliar os indicadores de estresse oxidativo e marcadores inflamatórios em obesos antes e depois da gastroplastia vertical com bandagem em Y-de-Roux. **Métodos:** Vinte obesos classe III foram submetidos à gastroplastia vertical com bandagem em Y-de-Roux, sendo sete homens e 13 mulheres, com idade média de 39 anos e 20 indivíduos não obesos, nove homens e 11 mulheres, média de idade de 38 anos. Foram determinados os níveis de malondialdeído no plasma, índice de estresse, capacidade antioxidante total, catalase e glutatona reduzida e oxidada, e marcadores inflamatórios (proteína C reativa e á-glicoproteína ácida). No grupo obeso, estes parâmetros foram determinados antes e 2, 6 e 12 meses após a gastroplastia vertical com bandagem em Y-de-Roux. **Resultados:** No pré-operatório, o grupo obeso apresentou níveis elevados de marcadores inflamatórios, de estresse oxidativo (níveis de malondialdeído e índice de estresse) e menores níveis de indicadores de defesa antioxidante em relação ao grupo controle. O emagrecimento foi acompanhado de redução progressiva do níveis de malondialdeído e do índice de estresse. Foi observado aumento da concentração de glutatona reduzida e da capacidade antioxidante total e redução dos níveis de marcadores inflamatórios. **Conclusão:** A redução do peso melhora o estado inflamatório e os níveis de estresse antioxidativo.


REFERENCES

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Valezi