PARTIAL GASTRECTOMY ASSOCIATED TO ANTERIOR TRUNCAL VAGOTOMY: ALTERATIONS IN METABOLISM OF THE CALCIUM. EXPERIMENTAL STUDY IN RATS

INTRODUCTION

The partial or total gastrectomy is practically the main form of treatment of the gastric cancer. In the partial gastrectomy the gastrointestinal transit is recovered through anastomosis with the duodenum (Billroth I - BI) or jejunum (Billroth II - BII). In the total gastrectomy the main reconstruction is Roux-em-Y esophagojejunum anastomosis. The partial or total gastrectomy leads to a decrease in the secretion of hydrochloric acid (HCl) and of the intrinsic factor, reducing the digestive function of the stomach and the duodenum and the absorption processes are decreased.

The calcium is not absorbed in the lack of hydrochloric acid and the osteomalacia and osteoporosis may occurs; it is well recognized in patients that had gastric resection. The calcium absorption and metabolism of calcium in rats is reduced after gastrectomy. The sham operated animals showed higher diet consumption, weight gains, serum and urinary calcium, excretion of calcium in feces, apparent absorption of calcium and activity of the enzyme alkaline fosfatase as compared to the animals of the gastrectomized group. However, the concentration of the bone calcium was increased in the animals of the gastrectomized group. This research has as objective to evaluate the effects of the partial gastrectomy associated to anterior truncal vagotomy in the metabolism of calcium in rats.

METHODS

The experimental protocol was approved by the Committee of Ethics in Animal Experimentation (CEEA) of the State University of Campinas - UNICAMP (certificate number 839-1, 08/06/2005). The Sham operated group (10 animals) was submitted to the same surgical stress, where the abdominal cavity was maintained open for approximately 45 minutes, which is the duration of a gastrectomy. After 15 days of the procedure, the animals received the formulated experimental diet (Table 1) and denominated AIN-93M by eight weeks.

Trabalho realizado na Faculdade de Engenharia de Alimentos e Núcleo de Medicina e Cirurgia Experimental da Faculdade de Ciências Médicas da UNICAMP, Campinas, SP, Brasil.
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The animals were maintained in collective cages at room with controlled temperature (22 ± 1ºC), humidity (60-70%), cycle of 12 hours day-night (lights on at 7:00 am), with diet and deionized water ad libitum. The weight gain and the consumption of the diet of the animals were monitored three times a week, during eight weeks.

After 60 days from the beginning of the diet, were measured apparent absorption of the calcium, serum calcium, urinary and fecal calcium, activity of the enzyme alkaline fosfatase and calcium in the bone.

The serum and urinary calcium contents were measured by a commercial colorimetric method (Kit Laborlab, Guarulhos, Sao Paulo, Brazil) using a Spectrophotometer Beckman DU® at 560 nm.

The determinations of diet and fecal calcium were performed in an Optic Emission Spectrometer IRIS-AP (Thermo Jarrell Ash, Franklin - Massachusetts – USA) at the specialized Laboratory of Biominersals Chemical Analyses Ltda, Campinas, Sao Paulo, Brazil.

The collection of the feces of the animals, was performed in the metabolic cages at the 15th, 35th and 55th days of the experimental phase, for a three days periods.

The apparent absorption of the calcium was determined as followed: apparent absorption (mg/day) = ingestion of calcium (mg/day) - excretion of fecal calcium (mg/day).

The concentration of seric calcium (CO=7,20±0,34; GXT=6,10±0,44 mg/dL) in the animals of GXT group was reduced in 15,3% and urinary calcium (CO=134,11±9,27; GXT=94,68±9,62 mg/24h) of the GXT group was 29,4% lower as compared to the animals of the CO group (Figure 3 and Figure 4).

The excretion of calcium in feces of the GXT group was reduced in 6.4% (CO=61,13±1,81; GXT=57,21±2,23 mgCa/g of feces) as compared to the CO group (Figure 5).

The apparent absorption reduced in 41.0% (P<0,05) in the animals of the group GXT (CO=52,18±4,15; GXT=30,76±5,87 mg/day) as compared to the animals of the control group (CO) (Figure 6).

### RESULTS

The concentration of the diet (g/day) (Figure 1) as well as the weight gains of the gastrectomized rats (GXT) (Figure 2) were reduced (P<0,05).

The determinations of bone calcium were performed in an Optic Emission Spectrometer IRIS-AP (Thermo Jarrell Ash, Franklin - Massachusetts – USA) at the specialized Laboratory of Biominersals Chemical Analyses Ltda, Campinas, Sao Paulo, Brazil.

For statistical analysis, the data are expressed as mean ± standard error of the mean (SEM) and submitted to the variance analysis ANOVA (P<0,05).

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### TABLE 1 - Composition of experimental diet

| Ingredients                  | g/Kg |
|------------------------------|------|
| Cornstarch 1                 | 466  |
| Dextrinized cornstarch 1     | 155  |
| Casein 2                     | 140  |
| Soybean oil 3                | 40   |
| Cellulose 4                  | 50   |
| Sucrose 5                    | 100  |
| Mineral mixture 6            | 35   |
| Vitamin mixture 6            | 10   |
| L-Cystine 7                  | 1,8  |
| Choline bitartrate 8         | 2,5  |
| Tert-butylhydroquinone 8     | 0,008|

1 Cornstarch and dextrinized cornstarch (Corn Products Brazil - Ingredientes Industriais Ltda, Mogi Guaçu, Sao Paulo, Brazil).
2 Plury Chemistry Ltda, Diadema, Sao Paulo, fabricate pela Naarden Agro Products-Holland.
3 Mark Liza, Cargill do Brasil, Uberlandia, Minas Gerais, Brazil.
4 Mark Microcel, Blanver Farmoquimica Ltda, Cotia, Sao Paulo, Brazil.
5 Refinaria Uniao, Assis, Sao Paulo, Brazil.
6 Prepared according to the AIN-93M formulation (REEVES; NIELSON; FAHEY JR, 1993). Formulate for M. Cassab Comercio e Industry Ltda, Sao Paulo-Sao Paulo, Brazil.
7 Mark Synth C1027.01.AE; Diadema, Sao Paulo, Brazil.
8 Sigma Chemical Co., St. Louis, Mo, USA.

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The determination of the termolabil bone alkaline fosfatase, was firstly measured as total alkaline fosfatase by colorimetric method (Kit Laborlab, Guarulhos, Sao Paulo, Brazil) using a Spectrophotometer Beckman DU® - 70 with temperature control. The termolabil fraction inactived (56ºC during 15 min., water bath Dubnoff Tecnal Model TE-053 - Tecnal®), and the final result was obtained by the difference.

The determination of bone calcium was done in femur removed after the death of the animals, the muscle was removed and the femur was frozen. The bone was burned in muffle furnace (Fornitec Industria e Comercio Ltda, Sao Paulo, Sao Paulo, Brazil) at 600ºC to the obtain clear ashes.

The determinations of bone calcium were performed in an Optic Emission Spectrometer IRIS-AP (Thermo Jarrell Ash, Franklin - Massachusetts – USA) at the specialized Laboratory of Biominersals Chemical Analyses Ltda, Campinas, Sao Paulo, Brazil.

The consumption of the diet (g/day) (Figure 1) as well as the weight gains of the gastrectomized rats (GXT) (Figure 2) were reduced (P<0,05).

The concentration of seric calcium (CO=7,20±0,34; GXT=6,10±0,44 mg/dL) in the animals of GXT group was reduced in 15,3% and urinary calcium (CO=134,11±9,27; GXT=94,68±9,62 mg/24h) of the GXT group was 29,4% lower as compared to the animals of the CO group (Figure 3 and Figure 4).

The excretion of calcium in feces of the GXT group was reduced in 6.4% (CO=61,13±1,81; GXT=57,21±2,23 mgCa/g of feces) as compared to the CO group (Figure 5).

The apparent absorption reduced in 41.0% (P<0,05) in the animals of the group GXT (CO=52,18±4,15; GXT=30,76±5,87 mg/day) as compared to the animals of the control group (CO) (Figure 6).
Gastrectomy reduced the activity (16.5%) of the bone alkaline fosfatase (CO=134,61±8,78; GXT=112,36±6,64 U/L) (Figure 7). However the concentration of calcium in the bone in the animals of the group GXT increased (4.1%) (CO=344,17±5,54; GXT=358,98±3,87 mg/g of bone) as compared to the animals of the control group (CO) (Figure 8).

**DISCUSSION**

The reduced weight gain in the GXT animals was also verified by Ohta et al.\(^\text{12}\).

The reduced seric calcium of the animals of the GXT group suggests that the gastrectomy reduces the absorption of the calcium. These results were also verified by Axelson et al.\(^\text{1}\). These authors affirm that this is a similar process
in humans where the gastrectomy induces a progressive deficiency in the absorption in the calcium. They also suggest that the gastric mucosa may have a calcium tropic agent, the gastrocalcin, which stimulates the calcium absorption by the bone. Sakai et al. 17, also verified a reduced serum calcium in gastrectomized rats.

Zittel et al. 23 also verified reduced calcium in humans with partial and total gastrectomy, suggesting that this is due to a reduced release of calcium from the foods, the increased intestinal flow and to the removal of duodenum and jejunum. The sum of these factors leads to a reduced serum calcium.

The urinary calcium is used to evaluate the renal 17. The excretion of these mineral and is a good parameter to reach the balance between the absorption and the calcium lost from the bone 22.

The urinary calcium of the animals of GXT group was reduced (29.4%), this was also verified by Axelson et al. 1, however in only 20%. The reduced levels of calcium may be due to an increase tubular reabsorption when the serum calcium is reduced, an increased levels of PTH was observed, increasing the tubular reabsorption. Morohashi et al. 10 observed in gastrectomized rats an elevation of PTH, these was also verified by Zittel et al. 23 in humans. Then, these may occur in our experiments.

The intestinal absorption of calcium results from the active transport mainly in the duodenum and upper jejunum and the passive diffusion in the whole intestine, in the ileum and in the large intestine 19.

The fecal excretion and the absorption of calcium was lower in the gastrectomized animals as compared to the control, this suggests that these experimental protocol really causes depletion in the absorption of the mineral calcium. Some other studies showed that the total gastrectomy in rats reduces the calcium absorption 5,12. Zittel et al. 23 verified in humans with partial gastric resection and total gastrectomy these reduced absorption, they suggest that is due to the reduced release of calcium of the foods by the impaired digestion, to an increased intestinal flow and to the removal of the duodenum and upper jejunum. These summed effects impairs the calcium absorption after the gastrectomy.

The activity of the bone alcaline phosphatase was lower in gastrectomized animals as compared to the control group. The activity of enzime give us some information about the metabolism in the bone, where these increased activity indicates that process of formation of the bone is reduced.

Zittel et al. 23 demonstrated in humans that the activity of alcaline phosphatase showed alterations in the total gastrectomy. The BI or BII partial resections did not showed alteration in this enzime 23. In fact, this elevation may be due to an enhanced osteogenesis however it occurs also in the osteomalacia 22.

This research demonstrates that the concentration of calcium in the bone was higher in gastrectomized animals. The bone is the main storage site of calcium of the organism and the 60 days of depletion may be not enough to cause a sensitive reduction. After the gastric resection the content of calcium in the bone is reduced and became more pronounced during the time 14. These results were observed in other studies 4,12,15,16.

Some authors confirmed a correspondence of 30 days of the man’s life to one day of life of the rat 3,4,8,9.

CONCLUSION

The gastrectomized rat is a good experimental model for studies of the absorption of calcium, as only two months after the surgery were enough to modify statistically the metabolism in mineral calcium, however not changed the content of calcium in the bone.

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