MASSIVE INTESTINAL RESECTION IN RATS FED UP ON GLUTAMINE: hepatic glycogen content valuation*

Ariney Costa de MIRANDA¹, Paulo Engler PINTO Jr.², Sidney Resende RIBEIRO¹, Samson Henrique BROMBERG¹, Fábio Pinatel LOPASSO² and Kiyoshi IRYA²

INTRODUCTION

Glutamine is classified as a nonessential amino acid since it can be readily synthesized by several tissues from alpha-keto-glutarate or glutamate¹⁴. Skeletal muscles, lungs, adipose tissues are capable to release it into the plasma, whereas bowel and kidneys absorb it¹⁸. Glutamine metabolic regulation occurs in the liver, especially on catabolism state.

Liver regulation property, as well as skeletal muscles one, make them able to absorb or release glutamine according to the body need⁸. Glutamine blood levels markedly fall, more during catabolic state than others amino acids, suggesting an important role in the breakdown proteins group moved after organic damage¹⁶.

Probably, glutamine blood levels increase is able to put down its endogenous synthesis and/or put up its catabolism²⁰. Its exogenous resource settles or prevents the free glutamine pool typical of critical state, being able to cause weight increased, positive muscle nitrogen balance and gastrointestinal cells hyperplasia. Glutamine is a conditionally essential amino acid, not required in normal conditions, so much important in catabolic states, though¹⁶.

Research reports glycogen synthesis enhance in hepatocytes, emphasizing composition and stimuli mechanisms from amino acids and glutamine⁹, ¹¹, ¹³. Hepatocyte volume enlarge stimulated by amino acids is related to hepatic glycogen formation¹¹. Glutamine use in intestinal resections and its outputs on hepatic glycogen synthesis has not been studied yet.

This study aimed to check weight evolution and glycogen content in hepatocytes of rats submitted to massive intestinal resection, which were fed on isoprotein and isocaloric polymeric diet via oral, with and without glutamine.

MATERIAL AND METHOD

Twenty five male, adults, Wistar inbreed, rats were operated, with the average weight of 341.9 g (296 to 450 g), originated from “Centro de Bioterismo”
of University of São Paulo Medical School, São Paulo, SP, Brazil, in adapt period of 3 days in the laboratory.

After 12 hours in fast at night, all animals were weighed and underwent anesthesia by 50% ketamina intraperitoneal injection (11.5 mg/100 g weight), in order to perform massive intestinal resection.

Surgical technique

Following abdominal epilation, rats were fixed in polystyrene board and submitted to abdominal antisepsis using polyvinylpyrrolidione. Surgical drape was fit and medium laparotomy of 3 cm was performed involving skin and subcutaneous tissue, then aponeurosis and peritoneum, using rib spreader to expose full intestine. Small intestine was measured over intestine counter-mesenteriale tenue using compasses and millimetre rules, where pylorus was the starting point. Then, resection was performed, allowing 25 cm of proximal small intestine left as remaining part.

Following principal mesenteric vessels and its branches ligation using 4-0 cotton thread, it was performed enterectomy with cecal portion resected in 3 cm besides ileocecal valve. Intestinal motion recovery was performed by entero-colon laterolateral anastomosis, with 6-0 nylon continuous suture. Five mL of warm saline (0.9% NaCl) solution was infused into peritoneal cavity, to postoperative moisturizing and abdominal muscle closure performed with a single interrupted 5-0 nylon suture.

Animals were randomly distributed into three groups, according to respective diet:

- G group: 10 rats undergone to massive intestinal resection with glutamine diet via oral.
- NG group: 10 rats undergone to massive intestinal resection without glutamine diet via oral.
- R group: 5 rats undergone to massive intestinal resection with standard diet from the laboratory without glutamine via oral.

Diets: Three isocaloric and isoprotein diets were used: a) polimeric diet composed by protein hydrolyzed - (calcium caseinate - 55.5 g), carbohydrate (maize amido - 162.5 g) and lipid (soil oil - 16.6 g). b) polimeric diet plus glutamine composed by protein hidrolyzed (calcium caseinate – 28 g and glutamine 25 g), carbohydrate (maize amido 162.5 g) and lipidum (soil oil - 16.6 g). c) standard diet from the laboratory, specific to rats and mice (Nuvilab®) which does not contain glutamine in its composition.

Animals were kept in cages with five animals in each group, separated by transparent and multidrilled partition which allowed visual and smelling contact among them. They were fed since the first postoperative day, with free water and diet and under daily clinical observation for 20 days. On the 20th postoperative day, the animal was put under a glass cone inhaling ethylic ether until death. Quickly, wide laparotomy was performed exposing innards, taking out the liver, preserved into recipient with 10% formalin solution.

Animals were weighed at postoperative and 20th postoperative day on electronic balance, before euthanasia. Hepatic fragments were fixed in formalin during 48 h fixation and included to paraffin in 5 µm thickness slices to histological analysis.

Slides were stained by periodic acid of Schiff (PAS) with diastasis. PAS stain evidences mucopolysaccharides presence. Mucine oxidation by PAS produces aldehyde which is strongly stained in purple by Schiff reagent. All polysaccharide and mucosubstances containing hexoses and desoxihexoses with branch glycol group stain in red by PAS (Figure 1).

Statistical analyses

Descriptive variances were presented in crosses (+), ranging from 1 to 3 (+, ++ or +++), with absolute and percentage frequency calculated to each group. As statistical pattern, it was applied arithmetic average and standard deviation and analysis of variance (ANOVA) procedures, intending to evaluate rats weight loss. Fisher’s exact test was employed to compare presence and semiquantification of cytoplasmatic glycogen in hepatocytes.

In all tests, it was fixed 0.05% as null hypothesis rejection level (significance level of 95%).

RESULTS

All animals lost weight within experiment time. Weight evolution average was 43.5% in G group, 49.2% in NG group and 37.1% in R group. Comparison of weight loss average expressed in percentage among groups achieved no statistical significance (Table 1).

In the glycogen content study into hepatocytes, using Schiff stain with diastasis, there was no significant difference among groups (P >0.05), in spite of G group has been presented larger pointing (++ and +++), related to NG and R groups (Table 2).
TABLE 1 – *G, **NG and ***R groups weight loss average compared, expressed in percentage, with their respective significance level

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight loss average %</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>G x NG</td>
<td>43.5 X 49.2</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>G x R</td>
<td>43.5 X 37.1</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>NG x R</td>
<td>49.2 X 37.1</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

ANOVA

*G – intestinal resection + oral diet with glutamine
**NG – intestinal resection + oral diet without glutamine
***R – intestinal resection + standard diet without glutamine

TABLE 2 – Comparison of glycogen accumulation into hepatocytes among *G, **NG and ***R and NG groups, expressed in percentage, semiquantified in +, ++ and ++++, studied by PAS differing on diastasis

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>NG</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>R</td>
<td>3</td>
<td>1</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

Fisher’s exact test P > 0.05

*G – intestinal resection + oral diet with glutamine
**NG – intestinal resection + oral diet without glutamine
***R – intestinal resection + standard diet without glutamine

DISCUSSION

Metabolic and nutritional changes following enterectomies depend on the resected segment (ileum and jejunum) and on presence of ileocecal valve\(^\text{(15)}\). It was noticed on pilot study of this work, where high death levels were present in groups undergone to intestinal extended resection associated to ileocecal valve removal.

Resection of 95% of small intestine in rats, preserving ileocecal valve was associated to significant weight loss in first 10 days, achieving stabilization, though\(^\text{(16)}\).

The postoperative observation time used in this study, to collect was 20 days, maximum period achieved with 75% resection of distal small intestine and ileocecal valve resection. Once ileocecal valve is an important factor on diarrhea control and consequent morbidity-mortality\(^\text{(14)}\).

Via oral as a way to administrate nutrients in experimental works, has been little used, mainly when catabolic state is tested, and when compared to via parenteral, perhaps because in the majority of clinical cases, this via is not available or what if, would not be so effective. On the other hand, via parenteral is widely used in malnourishment treatment, what lead to propose experimental models of parenteral nutrition in rats, although this via is not free of complications, because when used for a long time, it was associated to hepatic lipid accumulation, mainly triglycerides and cholesterol. As for its exclusive use in nutritional replacement, it was concluded very frequently and early, the hepatic steatosis onset\(^\text{(15, 21-22)}\).

Under catabolic state, systemic and intracellular amino acids levels fall, where glutamine plays the role of important factor that affects protein synthesis\(^\text{(17)}\).

Glutamine administration via oral may increase plasma levels until 46%, suggesting that a significant glutamine portion escapes from intestinal use, to be absorbed and metabolized by liver and kidney, returning to blood circulation. It may suggest via oral efficiency under this amino acid administration\(^\text{(18)}\).

In the current study, use of via oral to administrate nutrients containing glutamine did not allow notice specific alterations into the hepatic glycogen content, under absorption deficit. Diet consumption by rats was enough and similar among the three groups. It leads to understand that this choice has not influenced on diet consumption by the rats, enhanced by nutrients well utilization in animal models when related to this via, once there is no absolute contra-indication to its use.

In our research, all rats lost weight ranging widely from initial and final weight, after have being submitted to intestinal resection, reaching until 49.2% as weight loss average (NG group), with no chance to stabilization thanks to nutritional unbalance after continuous diarrhea.

Larger averages of weight loss appeared in G and NG groups. However, when three groups were compared, there were no statistically significant values. It leads us to confirm that glutamine addition did not carry to weight loss reduction in animals submitted to resection. Standard diet (R group) from the laboratory was more efficient, though, presenting smaller weight loss. That is not easy to explain these findings, once all diets were isocaloric and isoprotein. One probably explanation is based on the concept that glutamine added to isocaloric and isoprotein diet, required 50% reduction in other amino acids fractions, leading to protein synthesis damage. On the other hand, simply glutamine addition would implicate turn the hyperprotein diet into not comparable diets.

Histological analysis of cytoplasmatic granules in hepatocytes by PAS technique with diastasis showed that glycogen quantification did not differ statistically among the three groups. It may suppose that animals, after massive intestinal resection, used the majority of exogenous glutamine in the intestine, intending to improve its absorptive capacity, with less glycogen absorption and synthesis into hepatocyte, consequently.

Regulation of systemic levels of glucose occurs in the live through amino acids absorption following glycogen synthesis and storage (glyconeogenesis). Glycogen break down leads to glucose release, mainly in the period between the meals and in the beginning of extent fast, until its finish\(^\text{(21)}\). Glutamine plays a role as carbon resource to glycogenogenesis and ammonia for the urea cycle\(^\text{(7)}\).

Glutamine is a glycogenogenesis primor which is able to enhance the glucose homeostatic keeping in periods of high requesting and its glycogen tissue reserves are low\(^\text{(10)}\).

Glutamine hidrolisis occurs in the hepatocytes, by glutaminase action in order to provide substrates (ammonia and glutamate), used in the urea cycle and glycogenogenesis respectively. On opposite reaction, these substrates catalized by glutamino sintetase compose glutamine\(^\text{(15)}\).

In clinical catabolic states, it is known glucose and amino acids pick up increasing from blood circulation to the liver, as well as its storage on glycogen form. In isolated hepatocytes of rats, this aspect was studied from several substrates, with glucose in high concentration as the greatest responsible by this accumulation\(^\text{(21)}\). Significant increasing on the glucose absorption occurs when amino acids, as glutamine, are added, emphasizing the marked

Reference

Miranda AC, Pinto Jr PE, Ribeiro SR, Bromberg SH, Lopasso FP, Irya K. Massive intestinal resection in rats fed up on glutamine: hepatic glycogen content valuation
role in the glycogen synthesis regulation\(^{(10)}\). On rats undergone to fast for different periods of time, glutamine is the best substrate to hepatic glycogen compose after the third day, through the cellular volume enlarge and the glucogen synthetase enzyme which in its active form is responsible for the synthesis\(^{(11)}\).

Glucose formation mechanisms stimulated by amino acids in the liver are not completely known. Reports refer several hypotheses that should be more explained. In spite of experimental researches emphasize metabolism aspects without mention clinical situations which involve massive intestinal resection and its outcomes on organs as the liver.

In the present study, it was focused the glycogen content in the liver stimulated by glutamine via oral, evaluated by histological analysis.

CONCLUSION

It was concluded that glutamine did not contribute to reduce weight loss in animals submitted to intestinal resection. Glutamine feeding via oral was noticed as not providing glycogen accumulation in significant amount, in the hepatocytes, in rats submitted to massive intestinal resection.

REFERENCES