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Original paper

Hepatic ballooning degeneration: a new feature of the refeeding syndrome in rats

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Abstract

Aim of the study: Hepatic changes have been described during the refeeding syndrome due to increase in enzymes and hepatomegaly; however, they have not been properly described. Thus, the objective of this study was to investigate the hepatic histological characteristics and biochemical markers of hepatic steatosis in Wistar rats with refeeding syndrome.

Material and methods: Thirty male Wistar rats were allocated to one of three groups: C, F or R. The animals from group C received an AIN-93 diet for 96 hours, and were then sacrificed. Animals allocated to group F were fasted for 48 hours and sacrificed. Animals from group R were also fasted for 48 hours, but were refed for another 48 hours, with AIN-93. The liver, blood and epididymal and retroperitoneal fats were collected.

Results: Data obtained in groups F and R show the changes observed in refeeding syndrome, during starvation and refeeding. The serum glucose, magnesium, potassium and phosphorus, in group F, decreased. There was no evidence of hepatic steatosis. Hypophosphatemia, hypomagnesemia and hypokalemia were also observed in group R, confirming refeeding syndrome. The main histological characteristic, in this group, was the extensive presence of ballooning degeneration. This is the first article that has detected such change in liver structure, due to refeeding syndrome. The possible causes are: retention of sodium, causing whole body edema; and/or dysfunction of the sodium/potassium pump of the hepatocytes, as a result of hypophosphatemia.

Conclusions: This is the first description of an animal model of hepatic severe ballooning degeneration induced due to refeeding syndrome.

Key words: refeeding syndrome, hepatic steatosis, hydropic degeneration, ballooning degeneration, hypophosphatemia.

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Introduction

One of the first reports of the refeeding syndrome (RS) is from the 1940s, in malnourished patients and refeeding to general diet, who developed cardiovascular failure [1]. After World War II, 21% of Japanese prisoners with chronic malnutrition, died after refeeding with "appropriate diets" and supplementation of vitamins [2]. The term "refeeding syndrome", however, was first used in 1981 by Weinsier and Krumdieck [3].

Although this syndrome is now recognized, it is not diagnosed in all patients who present, because the symptoms are nonspecific. It is therefore associated with high morbidity and mortality [4]. The incidence of RS is approximately 48% in patients recovering the nutritional state [5], and 34% considering patients admitted to intensive care units [6]. Another situation with high prevalence of RS is anorexia nervosa [7, 8].

The common denominator for this syndrome that occurs is the presence of acute or chronic malnutrition associated with the refeeding. There was a high risk for the development of RS in patients with: marasmus, kwashiorkor, anorexia nervosa, chronic alcoholism, dysphagia, inflammatory bowel disease and

short bowel syndrome [9-12]. Studies have shown that in humans, even a short fast (duration 48 hours), followed by refeeding could induce RS [6, 13].

The basic physiological mechanism of RS begins with the metabolic abnormalities observed during fasting or negative energy balance. Under these conditions, there is a decrease in insulin and glucagon increased by stimulating glycolysis, gluconeogenesis, and lipolysis. The preferred source for energy is carbohydrates. However, when in an energy deficit state, there is depletion of glycogen, requiring the use of other nutrients for energy production. Under these conditions, the body begins to mobilize and use fat from the white adipose tissue and protein from the muscle tissue. Consequently, ketone bodies and free fatty acids start to replace glucose as the primary energy source [4, 10-12, 14, 15].

When the body, in the muscle and adipose tissue catabolism, is refeeding, numerous "dormant" enzymes become reactive, in a context of deficiency of nutrients and enzyme cofactors. It leads to a faster increase in blood insulin concentration, particularly when the refeeding is carried out with diets rich in carbohydrate. The cellular uptake of glucose increases. Micronutrients such as phosphorus, inorganic phosphate, magnesium and potassium which play an important role in metabolism are transported to the intracellular space. Water is also moved to the interior of cells. The transport of micronutrients to the intracellular space entails hypophosphatemia, hypomagnesemia and hypokalemia. These changes can lead to disastrous consequences for the organism, including death [9-12, 16, 17].

Other features of the RS, as well as mineral deficiency, include water imbalance, hyperglycemia and changes in lipid and hepatic metabolism. Hyperglycemia is a result of abundant supply of dietary carbohydrates, resulting in hyperinsulinemia, sodium retention and generalized edema [16, 18-22]. Hyperglycemia also affects lipid metabolism, leading to hypertriglyceridemia [10, 11, 16].

Although studies and case reports highlight the presence of liver changes suggested by imaging and changes in serum transaminases, no studies describe liver histological features in the short-term RS. The objective of this study was to investigate the histological characteristics and biochemical markers suggestive of fatty liver in animals subjected to fasting for 48 hours, and refeeding with a control diet for 48 hours.

Material and methods

For the experiment we used 30 male rats of the Wistar strain, weighing 250 grams. Animals were kept in a 12 h light/dark cycle, at room temperature of 22-25°C and with free access to water and a commercial diet for rodents – Nuvilab CR-1, Nuvital Nutrients S/A, Brazil. Every day the animals were weighed and the feed intake was noted.

All rats were handled in accordance with the recommendations of The Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 revised 1985) and the experimental procedure approved by the Ethics Committee on Animal Research of FMRP/USP with the Protocol for the Use of Animals in Experiments No. 182/2008.

Materials

The 30 animals were divided into three groups (10 animals/group): control group (C), fasted group (F) and refeeding group (R). Throughout the experiment two animals died (belonging to groups C and R). All animals underwent an adaptation period of approximately 10 days, so they could get used to individual cages and the animal room environment. During this period, the AIN-93 diet was offered ad libitum to growing animals [23].

The experiment lasting 96 hours, starting after the adaptation period, when the animals reached a body weight of approximately 350 grams. The group C (control) rats continued to receive the AIN-93 diet ad libitum for 96 hours, without any restrictions regarding the supply. The animals in groups F (fasted) and R (refeeding) were subjected to fasting for 48 hours, receiving in this period only water. The animals of group F were sacrificed after the fasting period. The animals of group R, after fasting for 48 hours were refed again with the AIN-93 diet, and then sacrificed.

We opted to use the AIN-93 diet in refeeding due to it containing a higher concentration of carbohydrate compared to other macronutrients. The diet contained 3.95 kcal/g and was composed of 63% carbohydrate (53% starch and 10% sucrose), 20% protein (casein), 7% lipids (soybean oil), 5% fiber (cellulose), 3.5% mix of minerals, 1% mix of vitamins, 0.3% L-cystine, 0.25% choline, and 0.0014% antioxidant BHT [23].

The diets were offered in individual stainless steel containers. The amount consumed was measured daily by weighing the vessel and immediately before sacrifice. The comparison of food consumption was only performed between C and R, whereas animals of group F were not refeeding. For comparison, we calculated the average energy intake (in calories) in the control group (group C). This average was then used to

compare the consumption in the group R in two days of refeeding separately.

The animals were weighed daily during the experiment, and immediately prior to euthanasia. At the end of the experiment the animals were decapitated and liver, epididymal fat, retroperitoneal adipose tissue and blood were collected for subsequent biochemical and histological analysis. For evaluation of liver weight, epididymal adipose tissue and the retroperitoneal adipose tissue were chosen by use of relative weight, which is the ratio of liver weight and adipose tissue, and body weight.

Biochemical analysis

The serum was analyzed for glucose, triglycerides, total cholesterol, magnesium, phosphorus, potassium and transaminases, alanine (ALT) and aspartate aminotransferase (AST). Potassium was measured using a commercial kit (Doles Reagents for Laboratory Medicine, Brazil). The remaining serum levels were measured using commercial kits (Labtest Diagnostica SA, Brazil).

Liver was quantified for total hepatic fat, cholesterol and triglycerides. For quantification of total hepatic fat we used the method proposed by Bligh & Dyer [24]. Liver concentrations of cholesterol and triglycerides were measured using commercial kits (Labtest Diagnostica SA, Brazil).

Histological analysis

Hematoxylin and eosin (H&E) staining method: Liver samples were fixed in 10% buffered formalin for twenty-four hours. After this period, they were removed from the formalin, processed and cut. The pieces were dehydrated by immersion in alcohols of increasing concentration for a predetermined time (70%, 80%, 90% and 100%). The subsequent step involved tissue immersion in three baths of xylene for a total period of 1 hour and thirty minutes. After this period, the samples were embedded in three paraffin baths of 60 minutes and included in paraffin blocks, submitted to microtomy. Histological sections of 4 microns thick were obtained and stained using the H&E method.

The histological sections of liver tissue stained by the H&E method were analyzed with a conventional light microscope, with emphasis on the detection of deposits and lipid degeneration of hepatocytes, ballooning, distribution and morphological location. The changes were semi-quantitatively evaluated, being considered: 0-5% (0), 5-33% (1†), 34-60% (2†), 61-100% (3†). We also determined the most prevalent type of hepatic steatosis (microvesicular, macrovesicular or mixed).

The morphological location of lipid deposits and degeneration of the ballooned hepatocytes were evaluated according to the acinar model proposed by Rappaport, distributed in the hepatic parenchyma in zones 1, 2 and 3 [25].

Sudan method: The evaluation of the presence of fat in the liver was performed by histological sections obtained from tissue previously frozen in liquid nitrogen and kept in a freezer at –80ºC, that were stained by the method of Sudan Herscheimer. The first cut was made with 5 mm thick, with the fabric still frozen, cryostat at –26°C (HM505E, Zeiss). Then, the sections were transferred to a glass slide, which were immersed in the respective solutions: ethanol 70°C (2 to 3 minutes), Sudan III (15 minutes), and Harris hematoxylin (2 to 3 minutes). The sections were then washed by dipping in water. Finally, we added 1 drop of Crystal/Mount (Biomeda) under the cuts and the coverslip was positioned. The fabrics were evaluated by conventional light microscopy (400×). This method stains the lipids in red and orange tones.

As the H&E, the degree of lipid deposition was also semi-quantitatively evaluated: 0-5% (0), 5-33% (1†), 34-60% (2†), 61-100% (3†). We also evaluated whether the lipid deposits were within the hepatocyte cytoplasm (intra-cytoplasmatic) or extra-cytoplasmatic.

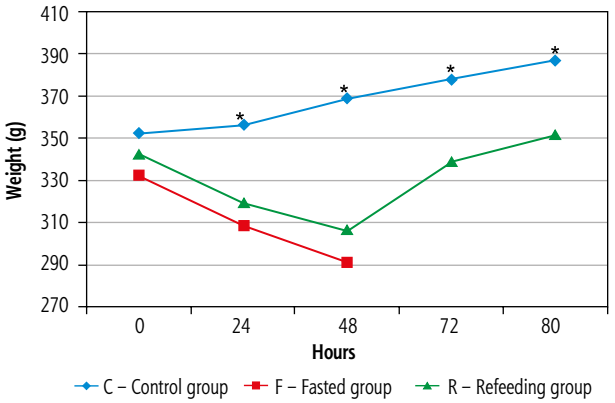
Statistical analysis

The statistical analysis for comparison between groups was the one-way variance analysis (Tukey test), considering $p < 0.05$ and using GraphPad InStat software, version 3.01.

Results

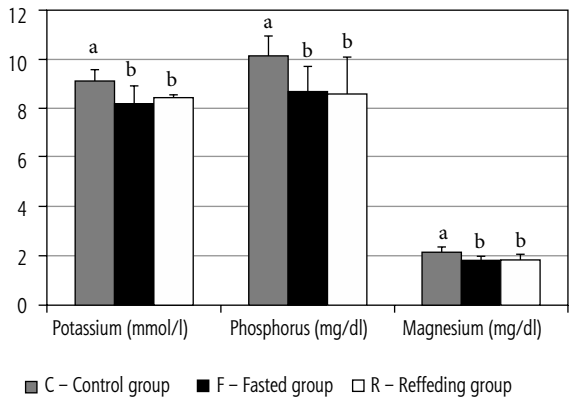
At the beginning of the experiment, after the adaptation period, the average weight of the animals between the groups was not statistically different. However, after the first 24 hours, there was a higher average weight in group C compared to groups F and R. This statistical difference was maintained until the end of the experiment. More detailed results relating to weight variations are presented in Figure 1.

The weight loss in group F was accompanied by depletion of fat mass. The relative weights of the retroperitoneal adipose tissue (0.0041 ±0.0021 g/g) and epididymal tissue (0.0058 ±0.0014 g/g) were in this group significantly lower compared to groups C (0.0119



Means $p < 0.05$

Fig. 1. Weight variation of animals during the fasted and refeeding period



Different letters mean $p < 0.05$

Fig. 2. Serum concentrations of potassium, phosphorus and magnesium

±0.0026 g/g, 0.0103 ±0.0019 g/g) and R (0.0089 ±0.0035 g/g, 0.0096 ±0.0032 g/g).

The average energy intake in group C was 100.7 ±7.7 kcal/animal/day throughout the experiment. There was a significant difference ($p < 0.001$) in energy intake refeeding on the first day, when the animals consumed on average 133.9 ±22.5 kcal in group R. However, no statistically significant difference in consumption was observed (97.4 ±13.1 kcal, $p = 0.536$) on the second day of refeeding.

The relative liver weight was significantly higher ($p < 0.001$) in group R (0.053 ±0.003 g/g) and lower in group F (0.032 ±0.001 g/g) compared to the control group (0.040 ±0.003 g/g). However, significant differences in total fat and liver triglyceride levels were found (Table 1). The quantification of hepatic cholesterol showed a significantly higher level in group F (Table 1).

Evaluation of serum lipid profile was very similar between the groups. There was only a significantly lower level of in triglycerides in group F compared to the others (Table 1).

The assessment of liver function was performed by measurement of liver transaminases. There was significantly ($p < 0.01$) higher serum ALT in group R (34.8 ±6.4 U/ml) compared to groups C (22.2 ±5.5 U/ml) and F (23.2 ±5.5 U/ml). However, the serum AST showed no significant difference between groups, the average for groups C, F and R being, respectively: 112.7 ±13.9 U/ml, 128.3 ±19.8 U/ml, 115.2 ±15.0 U/ml.

Serum glucose levels were significantly lower ($p < 0.001$) in group F (92.0 ±7.9 mg/dl) compared to groups C (155.7 ±15.4 mg/dl) and R (160.5 ±13.1 mg/dl).

To investigate the possibility of inducing RS we determined phosphorus, potassium and magnesium in serum (Fig. 2). For all trace elements there were significantly lower levels in groups F and R, compared to group C. Between groups R and F, however, there was no statistically significant difference.

The histopathological analysis of H&E staining (Table 2) showed hepatic architecture preserved in group C, in the presence of mild hepatic steatosis (1†), diffuse (zones 1, 2 and 3) and predominantly macrovesicular, in 29% of the animals. Diffuse ballooning degeneration was also observed (1†). The results of Sudan staining (Table 2) confirm the data obtained in the H&E method, with detection of hepatic fat and intracytoplasmic fat, but in a larger number of animals (58%). The classification of the lipid deposit did not

Table 1. Lipid determination in liver and serum of rats

Serum measures	Groups		
	C	F	R
Triglycerides (mg/dl)	159.8 ±48.1[a]	87.5 ±12.3[b]	115.1 ±36.6[a]
Total cholesterol (mg/dl)	78.1 ±6.9[a]	68.3 ±11.3[a]	62.7 ±12.1[a]
Liver fat total (mg/g tissue)	64.8 ±6.5[a]	54.0 ±11.7[a]	56.41 ±10.45[a]
Liver total cholesterol (mg/g tissue)	3.1 ±0.5[a]	3.8 ±0.5[b]	2.9 ±0.4[a]
Liver triglycerides (mg/g tissue)	13.4 ±4.2[a]	12.2 ±3.9[a]	14.4 ±1.7[a]

Data are given as means (SD). Values with different letters indicates statistical significance, p < 0.05. C – Control group, F – Fasted group, R – Refeeding group.

Table 2. Histological evaluation of liver tissue

			Groups		
			C	F	R
H&E	**Hepatic fat**				
	Cross score	0 (< 5%)	71%	100%	78%
		1 (6-33%)	29%	0%	22%
		2 (34-60%)	0%	0%	0%
		3 (61-100%)	0%	0%	0%
	Localization	Diffuse (zone 1, 2 and 3)	100%	100%	100%
	Type	Macrovesicular	71%	0%	22%
		Microvesicular	29%	90%	45%
		Mixed	0%	10%	33%
	Ballooning degeneration				
	Cross score	0 (< 5%)	71%	100%	0%
		1 (6-33%)	29%	0%	33%
		2 (34-60%)	0%	0%	11%
		3 (61-100%)	0%	0%	56%
	Localization	Diffuse (zone 1, 2 and 3)	100%	0%	100%
Sudan	**Hepatic fat**				
	Cross score	0 (< 5%)	43%	100%	0%
		1 (6-33%)	29%	0%	67%
		2 (34-60%)	29%	0%	33%
		3 (61-100%)	0%	0%	0%
	Localization	Intra-cytoplasmatic	58%	0%	100%
		Extra-cytoplasmatic	0%	0%	0%

C – Control group, F – Fasted group, R – Refeeding group.

exceed 2†. In animals of group F we observed preservation of liver tissue architecture, with no fat depot or liver degeneration, according to the H&E and Sudan methods.

The liver tissue of animals of group R showed an altered pattern compared to the control. It is the presence of discrete and diffuse steatosis in the liver parenchyma (zones 1, 2 and 3), in 22% of animals, ac-

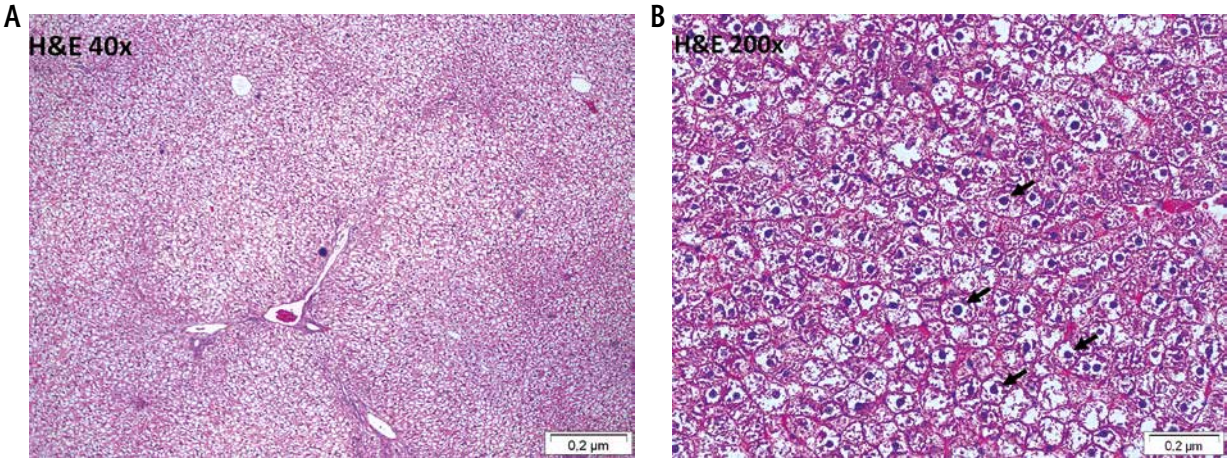


Fig. 3. Hepatic steatosis (**A**) and ballooning degeneration (**B**) in the livers of animals during the refeeding period

cording to the reading of H&E. It the livers we found microvesicular steatosis type in 45%, mixed in 33%, and 22% showed macrovesicular type. In this group, the most important result was the presence, in 100% of animals, of ballooning hepatocytes in the whole length of the liver tissue (zones 1, 2 and 3). Most animals (56%) had more than 60% of the liver tissue balloonized. The Sudan analysis showed the presence of liver discrete intracytoplasmic fat (1†) in 67% of animals, and 2† grade in only 33%. These results, shown in Figure 3, despite the presence of liver fat in this group, the more pronounced cell change was tissue ballooning degeneration type.

Discussion

After the fasting period, there was a weight loss of approximately 12.4% in group F and 10.4% in group R. In humans weight loss greater than 10% is considered severe and associated with a higher incidence of RS [4]. After the refeeding period, the animals of group R returned to the weight observed at the beginning of the experiment. This weight gain was a result of increased energy intake, as consuming a surplus of 33% of calories on the first day of refeeding. In animals, the increase in consumption after weight loss is a feature already described in the literature. One study demonstrates that caloric restriction induced prevention of non-alcoholic fatty liver disease (NAFLD) but this effect was lost with a 4-week return to *ad libitum* feeding in the hyperphagic Otsuka Long-Evans Tokushima Fatty (OLETF) rat. The development of NAFLD occurred despite only modest increases in body weight [26]. We verified in this study that excess calories after periods of fasting led to the presence of this syndrome in the reefed animals, which was found by other authors [10, 11].

Results of biochemical and histological analysis in the the groups F and R clearly illustrate a typical frame of RS induction in the fasting and nutrition period. The animals in group F showed a decrease in blood glucose and serum triglycerides and minerals involved in RS (magnesium, potassium and phosphorus). A reduction of blood glucose, the uptake of glucose by various tissues, and the absence of other carbohydrate intake and macronutrients are expected in the fasting period [15]. During this period, the animals were in catabolism, which can be assumed by the reduction in serum triglycerides, severe weight loss and decrease in epididymal and retroperitoneal adipose tissue. The reduction of liver weight in this context is explained by the loss of intracellular macromolecules, such as protein and glycogen [16]. The decrease in the concentration of minerals measured is also an important feature in periods of starvation or food restriction [10-12, 16, 17].

In this same group, fasted, results of liver triglycerides and total determination of fat are associated with the results observed in the histological analysis (H&E and Sudan), suggesting that there was no induction of hepatic steatosis. We observed only a slight increase in liver cholesterol concentrations. The liver actively participates in lipid metabolism via the beta-oxidation of fatty acids in mitochondria, by re-esterification of the fatty acids in triglycerides and cholesterol transport to peripheral tissues. This transport, however, is limited because it is carried out by lipoproteins. In weight loss situations very intense protein depletion and a decrease in lipoproteins carriers occur, which may cause accumulation of hepatic cholesterol [15].

In a study by Duarte *et al*. with Wistar rats, but with partial food restriction, similar results were obtained. In this study the animals in severe calorie restriction showed decreased body weight and adipose tissue. The relative liver weight was reduced and there was no increase of liver fat. The lipid profile of these animals, however, was altered, increasing the concentration of cholesterol [27]. It is important to note that the animals were not in full starvation, which could explain the difference in this outcome.

The animals that were refeeding exhibited the metabolic characteristics of the second phase of the RS, which corresponds to the renutrition period. The main aspect which explains the presence of RS is the reduction in serum phosphorus, potassium and magnesium. There was, therefore, cellular uptake of these micronutrients [10, 11, 14, 28].

In fact, hypophosphatemia is considered one of the most important pathophysiological mechanisms of RS [4, 9, 12, 26]. Crook *et al*., in a review article, which included clinical cases, hypophosphatemia was observed in 100% of cases of RS published [4]. Even some biochemical and histological characteristics presented by the animals of group R could be partly explained by hypophosphatemia.

In animals of group R we observed an increase of relative liver weight. When the liver lipid profile was observed, there was no evidence of a significant increase in hepatic fat content. Histological analysis by H&E and Sudan staining, however, showed the presence of hepatic steatosis, but at a lower level. Most animals (67%) were classified with only one † grade. The presence of mild hepatic steatosis in this group is the consequence of increased lipogenesis induced by hyperglycemia. Hyperglycemia provides the substrate for lipid synthesis and also stimulates the release of insulin, which is an anabolic hormone [4, 16].

Although fatty liver was observed in these animals, the most significant histological feature was the presence of massive degeneration of the ballooning profile throughout the extent of liver tissue. Most animals (56%) had more than 61% of the tissue affected by this degeneration. This degeneration is a type of hepatocyte ballooning, whose main characteristic is cellular edema. The water in the cell is input to the consequent impairment of cell volume regulation, which is incorporated by pump activity of the Na^+/K^+ membrane of hepatocytes. This pump uses ATP to maintain the levels of Na^+ and K^+ against the osmotic gradient. In malfunction conditions, the result is the entry of Na^+ and water to the intracellular space. The main causes of the malfunction of the Na^+/K^+ pump are: hypoxia; lack of substrate for ATP synthesis; oxidation and destruction of enzymes (ATPase), or infectious conditions as in the action of toxins.

In animals of group R, none of the above events, which could lead to malfunctioning of the pump, were observed. There was substrate, because the animals had food supply, and no enzymatic degradation was observed. However, the animals had hypophosphatemia. Knowing that phosphorus is one of the main components of ATP, in the hypophosphatemia state, a decrease of ATP can occur. In fact, the lack of ATP in patients with RS was considered a major cause of symptoms, including multiple organ failure [29, 30].

In addition to changes in the Na^+/K^+ the water retention observed during the RS could also contribute to the induction of hepatic ballooning degeneration. Studies have shown that the refeeding after periods of fasting with high-carbohydrate diets causes decreased urinary sodium excretion. Sodium retention occurs in the distal nephron, by the action of insulin, and leads to generalized edema [16, 18-22]. Therefore, pursuing RS with water retention and decreased pump activity of Na^+/K^+ in the liver leads to even greater ingress of water into the intracellular space. In this context, the increase in relative liver weight in group R was due to higher water content in this organ.

There are no studies reporting ballooning degeneration in liver tissue, resulting from the RS. This is the first study that this result was observed. However, there are studies that showed changes; for example, after an overnight fast, peroxisome proliferator-activated receptor β (PPAR-β) mRNA levels are dramatically down-regulated in liver and other organs, and are rapidly restored to normal levels upon refeeding, modulating fatty hepatic acid oxidation, which may result in hepatic steatosis [31].

The observed changes in hepatic animals of group R led to impaired liver function, since higher levels of ALT were observed in this group. Elevations in hepatic transaminases associated with the diagnosis of RS have already been described in the past [4]. Saito *et al.* described two different reports of clinical cases of RS patients (diagnosed by hypophosphatemia), which showed an increase in serum levels of liver transaminases. The authors suggested that the enzyme changes could be consequential to the induction of hepatic steatosis [32]. Korbonits *et al.* also detected elevated liver transaminases during refeeding of an individual who had remained fasted for 44 days [18]. The GOT and GPT concentrations gradually returned to normal levels, suggesting that hepatic RS changes are physiological and may be harmless. Therefore, the improvement in liver enzyme profiles could be a reflection of spare phosphate [18]. In rats fasted for 5 days and refeeding using total parenteral nutrition, with a high calorie solution with or without insulin administration the authors showed that plasma phosphate levels did not decrease in rats infused with the high calorie solution alone; but a 20% reduction compared to baseline was observed in rats administered insulin [33].

The authors showed that plasma phosphate levels did not decrease in rats infused with the high calorie solution alone; but a 20% reduction was observed compared to baseline when insulin was administered in rats [33].

Although hepatic steatosis has been considered the most important cause of serum transaminases changes, the results of this study suggest that the main change in hepatic RS is the ballooning degeneration. Therefore, increase in the serum SGPT observed in refeeding animals is a consequence of a number of liver disorders including hydropic changes as well as fatty liver already described. A longer-term study found only vacuoles after starvation and refeeding in the tissues of animals, and the severity of lesions gradually decreased during refeeding [34].

The results of this experimental study showed RS induction in Wistar rats, by fasting for 48 hours and refeeding for 48 hours, with an adequate diet. The presence of this syndrome has been explained by the occurrence of hypophosphatemia, hypokalemia and hypomagnesemia. The major change was observed in liver tissue, which resulted in impaired hepatic function. This, however, is the first description of this degeneration in the refeeding syndrome period. Further studies are therefore needed, so that all the pathophysiological mechanisms involved in the RS can be elucidated. In summary, this paper proposed one pathophysiological mechanism of the degeneration ballooning phenomena in the refeeding syndrome, which is the depletion of potassium, phosphorus and magnesium, with rises

in glucose, which leads to lipogenesis and hepatic steatosis, and the hyperglycemic state raises the sodium concentration, with occurrence of edema. The cited low phosphorus levels deplete the ATP and the combination of low ATP plus edema was responsible for hepatic ballooning degeneration. The complete understanding of these mechanisms could contribute to the adoption of preventive measures and more appropriate interventions in the refeeding period.

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Disclosure

The authors declare no conflict of interest.

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