

Zinc Supplementation on Nutritional Status in Rats With Cholestatic Cirrhosis

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ABSTRACT

AIM: To evaluate the effect of a zinc supplement on the nutritional status of rats submitted to bile duct ligation.

METHODS: were constituted 4 groups with 12 animals within each group which were divided and called: (1) bile duct ligation-zinc supplemented diet (BDLZSD); (2) bile duct ligation-standard diet (BDL-SD); (3) sham operation-zinc supplemented diet (SHAM-ZSD); (4) sham operation-standard diet (SHAM-SD).

RESULTS: The mean body weight of rats in the SHAM-SD group was greater than the in the BDL-ZSD and BDL-SD groups ($p < 0.001$). Food intake was greater in the SHAM-SD and SHAM-ZSD groups ($p = 0.020$). The bile duct ligation groups demonstrated lower feed efficiencies than the sham-operation groups ($p < 0.005$). The mean fresh carcass weight of the bile duct ligation groups was lower than the mean fresh carcass weight of the sham-operation groups ($p < 0.001$). The mean body water content of the bile duct ligation groups was greater than both sham-operation groups ($p < 0.001$). The

shamoperation groups demonstrated greater mean dry carcass weights in compared with the bile duct ligation groups ($p < 0.001$). Mean body fat was lower in the bile duct ligation groups ($p < 0.008$). The total quantity of protein and nitrogen in the carcasses did not reveal any statistical differences between the groups ($p = 0.229$ and $p = 0.422$, respectively).

CONCLUSION: Zinc supplementation did not increase food intake in rats with bile duct ligation or prevent nutritional damage secondary to cholestatic cirrhosis.

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Key words: Nutritional status; Cholestatic cirrhosis; Zinc supplement, Liver, Children

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INTRODUCTION

Nutritional deficiencies are common in children suffering from chronic liver disease^[1], specially when the liver disease is cholestatic in origin and began during childhood^[2]. The mechanisms involved in the development of malnutrition in chronic hepatopathies suggest an interaction of various factors, including poor absorption and a deficient utilization of nutrients, hormonal disturbances, immunodepression, secondary tissue damage and anorexia^[3]. Anorexia and fat absorption deficiency are major problems for a child with cholestasis^[4]. Anorexia can be secondary to infections, secondary to discomfort from visceromegaly or a consequence of zinc deficiency^[5]. Additionally, reduced food intake by patients with liver disease may be the principal mechanism responsible for zinc deficiency^[6].

Zinc deficiency may cause anorexia and growth retardation in children without chronic hepatopathy^[7-14]. Hypozincemia has been observed in pediatric patients with liver disease^[15]. Experimental studies with cirrhotic rats revealed lower levels of serum and hepatic zinc compared to other animals were also cirrhotics, but with zinc supplementation^[16-18]. Thus, the aim of this study was to evaluate

Table 1 Body weight; fresh carcass weight, water content of carcasses, dry body weight, body fat and protein and nitrogen content of animals at the end of the study period.

	Bile duct ligation		Sham-operation		P value
	Zinc supplemented diet (n=11)	Standard diet (n=12)	Zinc supplemented diet (n=11)	Standard diet (n=12)	
Body weight (g)	182.6±21.5 ^a	176.2±27.0 ^a	207.6±23.2 ^b	212.3±24.8b	<0.001 ⁽¹⁾
Fresh carcass weight (g)	122.3±13.2 ^a	117.3±20.0 ^a	155.9±13.6 ^b	153.8±15.5b	<0.001 ⁽¹⁾
Carcass water content (%)	67.3±2.0 ^a	67.1±2.6 ^a	64.7±1.4 ^b	63.9±1.36b	<0.001 ⁽¹⁾
Dry body weight (g)	41.1 ^a (37.1 42.2)	39.6 ^a (31.6 46.4)	65.0 ^b (50.1 59.1)	55.9 ^b (54.4 58.2)	<0.001 ⁽²⁾
Body fat (g)	9.2±2.0 ^a	9.4±2.8 ^a	11.3±1.9 ^b	12.1±2.0b	0.008 ⁽¹⁾
Total body protein (g)	19.7±0.5	20.0±0.6	20.5±0.8	20.1±1.2	0.229 ⁽¹⁾
Body nitrogen (g)	3.1±0.1	3.1±0.1	3.2±0.1	3.1±0.2	0.422 ⁽¹⁾

⁽¹⁾ANOVA (mean±SD); ⁽²⁾ Kruskal-Wallis (median and P25 - P75). Multiple comparisons test: different letters in the same line: $p < 0.05$; Equal letters in the same line: $p > 0.05$.

Table 2 Food intake, feed efficiency, zinc intake, carcass zinc content and liver zinc content of the animals at the end of the study period.

	Bile duct ligation		Sham-operation		P value
	Zinc supplemented diet (n=11)	Standard diet (n=12)	Zinc supplemented diet (n=11)	Standard diet (n=12)	
Food intake (g)	353.9±13.1 ^{a,b}	321.4±43.6 ^{a,b}	384.7±58.1 ^{a,c}	380.2±57.3 ^{a,c}	0.020 ⁽¹⁾
Feed efficiency (g/g)	0.3±0.0 ^a	0.3±0.0 ^a	0.4±0.0 ^b	0.4±0.0 ^b	<0.005 ⁽¹⁾
Zinc intake (mg)	22.0 ^a (18.2;22.7)	9.0 ^b (8.0; 10.7)	22.0 ^a (19.5; 25.5)	11.0 ^b (9.5; 12.0)	<0.001 ⁽²⁾
Carcass zinc content (µg/g)	103.6 ^a (99.8 111.2)	106.4 ^a (101.9 114.5)	76.2 ^b (71.4 87.2)	73.0 ^b (71.0 80.2)	<0.001 ⁽²⁾
Liver zinc content (µg/g)	110.7 (26.3 7.9)	97.3 (25.7 7.75)	90.2 (33.4 9.6)	94.5 (37.0 10.6)	0.444

⁽¹⁾ANOVA (mean±SD); ⁽²⁾ Kruskal-Wallis (median and P25 - P75). Multiple comparisons test: different letters in the same line: $p < 0.05$; Equal letters in the same line: $p > 0.05$.

the effect zinc supplementation on diet intake, weight and body composition in recently weaned rats that were submitted to a bile duct ligation.

METHODS

Experimental design

The study was approved by the Research Ethics Committee of the Federal University of Sao Paulo, Paulista School of Medicine, Brazil. This was a prospective experimental study, using 48 male Wistar rats, aged 21 days with a body weight between 40 and 50g at the time of bile duct ligation in accordance with the surgical technique described by Cameron and Oakley (1932)⁽¹⁹⁾. The procedure was performed to produce cholestasis. The sham-operation animals were anesthetized and subjected to a laparotomy but no bile duct ligation was performed.

Diets

The animals received two diets: one with a standard quantity of zinc (30 mg zinc/kg - standard diet) and the other with zinc supplementation (60 mg zinc/kg - zinc supplemented diet). Four groups, each containing 12 animals, were created: (1) bile duct ligation-zinc supplemented diet (BDL-ZSD); (2) bile duct ligation-standard diet (BDL-SD); (3) sham-operation-zinc supplemented diet (SHAM-ZSD); (4) sham-operation-standard diet (SHAM-SD).

During 30-day experimental study period the animals received at the diet corresponding to their experimental group deionized water ad libitum. The nutritional composition of the diets utilized during the experiment followed that proposed for growth phases by the American Institute of Nutrition - AIN 93G⁽²⁰⁾.

Analytical procedures

Every three days, the animals were weighed in the morning up to the day they were sacrificed. The quantity of diet consumed each morning, and food intake was determined by the total quantity of feed consumed during the 30-day study period. Feed efficiency was calculated by dividing the weight gained by the quantity of food ingested during the 30 day study period

At the end of the study, the rats were sacrificed and evisceration then followed. The fresh carcasses were oven-dried at 120° C. When their dry weight was obtained, the carcasses were then pulverized. The carcass water content was determined by subtracting the dry carcass weight from the fresh carcass weight. Determination of the protein and nitrogen contents was performed by the Kjeldahl digestion process⁽²¹⁾. Body fat was determined by the continuous extraction method using a Soxhlet-type apparatus⁽²¹⁾. Body zinc content in the carcasses was determined by atomic absorption spectrophotometry.

Statistical analysis

Analysis of variance test (ANOVA) and the Kruskal-Wallis test were used for the statistical analysis of variables, when the test demonstrated a statistically significant difference, the analysis was complemented using a multiple comparison test⁽²²⁾ (Tukey Test or Dunn's Method). The Alpha error was fixed at 5%.

RESULTS

The animals weights at the start of the study were not different among the four groups ($p = 0.128$). One animal from the SHAM-ZSD group and one from the BDL-ZSD group, died five days before the end of the study period. These rats were excluded from the analysis.

At the end of the 30-day study period, the body weights of the SHAM-ZSD and SHAM-SD groups were higher than the bile duct ligation groups with cholestasis (Table 1). The statistical analysis revealed that the fresh carcass weights of the bile duct ligation groups were lower than those of the control groups. In regards to the water content of the carcasses, the medians of the bile duct ligation groups were higher than those for the SHAM-ZSD and SHAM-SD groups.

The dry body weights of the SHAM-ZSD ad SHAM-SD groups higher than those of BDL-ZSD and BDL-SD. Body fat was lower in the BDL-ZSD and BDL-SD groups. The average values for total protein and nitrogen did not demonstrated any significant differences among the groups (Table 1).

Table 2 reports, the food intake and feed efficiency. The food intake and feed efficiency were greater in the SHAM-ZSD and SHAM-SD

than in cholestatic groups. The results of zinc intake demonstrated a statistical difference between the groups that received a zinc-supplemented diet and the standard diet groups.

The zinc content in the carcasses was significantly higher in the BDL-ZSD and BDL-SD than that in SHAM-ZSD and SHAM-SD groups. The statistical analysis did not show a difference in the liver zinc content (Table 2).

DISCUSSION

Experimental models of hepatic cirrhosis in rats have been used to analyze the effect of zinc supplementation on biochemical and histopathology data^[16,17], but not nutritional status. Thus, our study is the first in the literature to evaluate the effect of zinc supplementation on food intake and body composition in rats with biliary cirrhosis resulting from bile duct ligation.

The technical surgery for bile duct ligation is efficient for promoting structural and functional alterations typical of cholestatic cirrhosis, as has been demonstrated in previous papers^[23-26]. The intervention period for zinc-supplementation was established based on a pilot study during which we verified that the frequency of deaths was higher after 30 days of bile duct ligation. These data were similar to other studies that used this experimental model for hepatic injury^[23,27]. The level of zinc supplementation (60 mg/kg of feed) was based on the recommendation for children with zinc deficiency^[28,29] that doubles the standard requirements for animals in a growth phase.

The body weight of cholestatic animals (with a standard diet or zinc-supplemented diet) was lower than the body weight of the sham-operation groups. Therefore, zinc supplementation did not prevent decreased weight gain secondary to hepatic cholestatic injury. The average weight of the fresh carcasses and the average weight of the dried carcasses for the cholestatic groups indicated that even at a lower weight, the carcasses more water, which is an expected result because chronic hepatic disease may lead to water retention.

As regards to body composition, the statistical analysis study did not reveal a difference among groups in regard to total body protein and nitrogen contents. However, body fat content was lower in the bile duct ligation groups, and may explain the lower body weight in these groups.

Rats in the bile duct ligation groups had more zinc in their carcasses than those in the control groups. This finding is a consequence of impaired zinc elimination via the bile duct^[24,25].

Few studies^[27,30] have evaluated the food intake of rats submitted to bile duct ligation. The statistical analysis revealed that the bile duct ligation group with a standard diet demonstrated a lower food intake compared with the sham groups. The food intake was also lower than the zinc-supplemented bile duct ligation group but the difference was not statistically significant. These findings suggest that the presence of anorexia in animals with a bile duct ligation was not completely prevented by zinc supplementation. Our results were not in accordance with data suggesting that zinc promotes an increase in appetite and food intake in humans^[8,9]. The feed efficiency was lower in the bile duct ligation groups suggesting that hepatic damage was associated with a lower incorporation of nutrients for the maintenance of nutritional status. Zinc supplementation did not have a positive effect on food incorporation (Table 2).

The animals with a bile duct ligation demonstrated a higher content of zinc in their carcasses compared to the sham operation groups. Zinc supplementation did not affect the carcasses zinc content. This result was not in accordance with the lower level of

zinc concentration found in pediatric patients with cirrhosis. This difference may suggest that serum zinc levels are not an adequate indicator of zinc nutritional status.

In conclusion, this experimental rat model demonstrated nutritional effects that are observed in cholestatic diseases. Zinc supplementation did not increase food intake in rats with bile duct ligation or prevent nutritional damage secondary to cholestatic cirrhosis.

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