

Changes in oxidative stress and lipoprotein in malnourished gerbils infected with *Giardia lamblia*

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ABSTRACT

Introduction: It is speculated that giardiasis, one of the most common parasites in the world, would produce metabolic changes that worsen the nutritional status of malnourished or immunocompromised patients. In this context, we seek to contribute to clarify this issue by evaluating the effects of infection on antioxidant defense and lipoprotein metabolism in gerbils previously malnourished. **Methods:** 32 gerbils were used, divided into four groups: Control (CT) and Control Infected (CTIn), which each received a 20% protein diet, Malnourished (MN) and Malnourished Infected (MNIn), which each received a 5% protein diet. CTIn and MNIn groups were inoculated with 1×10^6 trophozoites of *G. lamblia*, while the remaining groups were mock infected. Seven days post-infection, all groups were sacrificed and hepatic tissue collected for assessment of oxidative stress via dosing catalase and superoxide dismutase and serum was collected to evaluate the lipoprotein metabolism by Fast Protein Liquid Chromatography. In each fraction, cholesterol concentration was determined using commercial kits, and protein by absorbance difference. **Results:** Gerbils fed with a low protein diet had significantly lower body weight. Cholesterol and protein in the fractions were significantly lower in group MNIn compared to CT and Catalase activity was higher in group MNIn compared to control. **Conclusions:** Our results demonstrated that host response against giardiasis associated to malnutrition, led to an oxidative stress that produced changes on lipoprotein composition in the malnourished animals suggesting that the infection could influence on metabolic changes, worsening the nutritional status of infected animals.

Keywords: Giardiasis, Malnutrition, Metabolism, Superoxide, Catalase.

INTRODUCTION

Giardiasis caused by *Giardia lamblia*, is one of the most common parasitic diseases in the world, estimated to occur approximately 280 million annually symptomatic cases¹.

The infection tends to be self-limiting in individuals with competent immune system, while in immunosuppressed

or children in addition to causing diarrhea, weight loss, dehydration, abdominal discomfort, and steatorrhea², can lead to malabsorption of various nutrients, resulting from changes in the epithelium and intestinal barrier^{3,4}, aggravating the nutritional status of these patients.

Faced with the action of the parasite, the host organism has defense mechanisms, such as the production of chemokines, cytokines and reactive oxygen species (ROS). These ROS then exert direct cytotoxic activity on pathogens, contributing to the maintenance of the mucosal barrier⁵. In the case of infections by *G. lamblia*, the effect that ROS could play in defense against parasites, although it is still unclear, has been widely discussed, considering that this parasite has

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limited ability to detoxify and neutralize reactive oxygen species⁶.

However, some authors defend that this “peroxidative attack” is also involved in the pathogenesis of some enteric diseases, and in changes in the composition of lipoprotein particles^{7,8,9}, affecting the normal metabolism and subsequent distribution of both lipids and liposoluble vitamins to peripheral organs⁹. At the same time, studies show that changes in lipoprotein composition and structure turns the particles more susceptible to oxidation and amplify the effect of subsequent inflammatory events^{9,10,11}.

Although there is a considerable amount of studies evaluating the impact of giardiasis in parameters of nutritional status, such as weight gain, weight-to-height and height-age ratio¹², studies evaluating the impact of malnutrition on parameters related to oxidative stress and lipoprotein metabolism during giardiasis are rare.

Considering the above, this study aims to evaluate if the host production of reactive oxygen species against *G. lamblia* parasite could change lipid metabolism in gerbils previously malnourished.

MATERIALS AND METHODS

Experimental model and group divisions

32 female gerbils (*Meriones unguiculatus*), aged 4–6 weeks, were treated with Ivermectin 1% injectable solution, and metronidazole to ensure that they were free of any parasites and maintained under standard laboratory conditions with a 12:12 h light/dark cycle and controlled temperatures ($23 \pm 3^\circ\text{C}$), receiving *ad libitum* filtered water and a diet kept under refrigeration (ensuring that no other sources of infection were introduced to these animals throughout the course of the study). The animals were divided evenly according to body weight into four groups of eight animals each: Control (CT) and Control Infected (CTIn), with a diet containing 20% protein, and Malnourished (MN) and Malnourished Infected (MNIn), which received a 5% protein diet. Diets were prepared in the laboratory based on the American Institute of Nutrition (AIN-93G)¹³ standard diet, and hypoproteic diets had 15% of protein content substituted for corn starch, ensuring that the diet provided only 5% protein, maintaining the same caloric density than the control diet. The experiments were performed in compliance with the guidelines of the Institutional Animal Care and Committee on Ethics of Animal Experimentation (Ethics Committee on Animal Experiments - CETEA, national guidelines Law

11.794, dated October 8, 2008) from Universidade Federal de Minas Gerais (UFMG); protocol number 070/2010.

Cultures and growth conditions

Trophozoites from the Portland-1 isolate (ATCC 30888), Assemblage A, were kept in culture in TYI-S-33 medium modified by Keister¹⁴ and supplemented with bovine serum.

Experimental design and inoculation

The experiments were performed following the protocol of Gomes *et al.*¹⁵ with modifications. From the first day of the experiment (Day 0), groups MN and MNIn were maintained on a low-protein diet for four weeks. During the same period, the remaining groups received the control diet for the maintenance of nutritional status. Following this period (28 days), animals from groups CTIn and MNIn were infected orally by gavage with 1×10^6 trophozoites contained in 0.8 ml Phosphate Buffered Saline (PBS), while the CT and MN groups received 0.8 ml of PBS. Seven days post-inoculation, the animals were euthanized. The animals were weighed individually, each week, since the first day of the experiment (Day 0), using a balance (BL320H, Shimadzu).

General procedures

On the 7th day after inoculation, the gerbils were anesthetized [Ketamine (Agener União) 100 mg/kg and Xylazine (König) 12 mg/kg, i.p.] and blood was collected from the axillary artery. Blood samples were centrifuged at $12\,000 \times g$ (3500 rpm) for 10 min and the sera were used for biochemical analysis. Animals were euthanized and liver was removed, perfused with PBS and homogenized in phosphate buffer (50 mM) for determination of the antioxidant enzymes superoxide dismutase (SOD) and catalase.

Dosage of antioxidant enzyme catalase

Determination of catalase activity was based on the decrease in absorbance of hydrogen peroxide by this metabolization by catalase, as described by Nelson & Kieson¹⁶. The calculations were made by the difference of reading in the start and end time, divided by the sample volume (mL). The result was expressed by protein concentration (mg/mL) measured by the Bradford method¹⁷.

Dosage of antioxidant enzyme SOD

The dosage of SOD was based on its ability to clean the radical O₂⁻, decreasing the rate of auto-oxidation

of pirogallol, adapted to Dieterich *et al.*¹⁸. To calculate the result, it was considered that 1 unit (U) of SOD was able to auto-oxidation 50% of pirogallol of the standard. The result was expressed as unit per mg of SOD protein.

Lipoprotein analysis

Four samples from serum from each group (pooled serum of animals 1 and 2, pooled serum of animals 3 and 4, pooled serum of animals 5 and 6, and serum of animals 7 and 8) were used for lipoprotein determination. Serum lipoproteins were separated by FPLC analysis as described by Fazio *et al.*¹⁹. Cholesterol concentration was determined in each fraction using commercial kits (Cholesterol Liquiform, Labtest, Brazil). The protein levels in lipoprotein fractions were determined by ultraviolet (used the wavelength of 260 nm and 280 nm).

Statistical analysis

The data are expressed as the means \pm standard error. We used the Shapiro-Wilk test to verify the normality of the continuous sample and to compare the experimental groups and One way Analysis of Variance (ANOVA) followed by the Tukey test. All tests and analyses were performed in SPSS version 20.0, and a significance level of 5% ($p < 0.05$) was used.

RESULTS

The average weight of gerbils on the CT and CTIn diets was similar to the weight of the animals in the MNIn and MN groups ($p = 0.563$), at the beginning of the experiment. After four weeks, receiving specific diets, the weight of the animals in the MNIn and MN groups was significantly lower than the weight of the animals in groups CT ($p < 0.01$) and CTIn ($p < 0.01$) and remained so throughout the experiment. There were no differences in weight between the CT and CTIn groups or between the MN and MNIn groups.

The hepatic oxidative stress was evaluated by measuring the activity of SOD and Catalase. We found greater activity of the liver enzyme catalase in group MNIn compared to CT. There was no difference in the activity of hepatic SOD among the groups (Table 1).

The cholesterol distribution in circulating lipoproteins did not differ for the groups CT CTIN and MN. However, there was significant change in lipoprotein profile of MNIn group compared to CT, since the

Table 1 Evaluation of hepatic oxidative stress parameters of animals in groups CT, CTIn, MN and MNIn

Groups	Parameters	
	SOD	Catalase
CT	0,053 \pm 0,006	2,87 \pm 0,87
CTIn	0,044 \pm 0,007	3,86 \pm 1,18
MN	0,060 \pm 0,012	4,50 \pm 1,25
MNIn	0,048 \pm 0,009	6,79 \pm 0,74*

Values expressed as Means \pm Standard Error. * Different from the corresponding CT group, $p < 0,05$.

animals of group MNIn showed lower average content of cholesterol in lipoproteins (Figure 1).

Giardiasis and malnutrition led to a decrease in protein content in lipoprotein fractions (Figure 2).

Compared to the CT group, the distribution of proteins in circulating lipoproteins was lower in the CTIn group ($p < 0.05$), MN group ($p < 0.0001$) and in MNIn group ($p < 0.0001$). Groups MN and of MNIn showed a profile of protein distribution in circulating lipoproteins similar between groups ($p = 0.4$) but lower than the other groups ($p < 0.01$).

DISCUSSION

The inadequate supply of nutrients causes changes in anthropometric, metabolic, as well as intestinal morphology of individuals^{4,20}, decreasing the body's ability to combat pathogens²¹, as *G. lamblia*, worsening nutritional status, especially of children. However, the influence of nutritional deficit in the severity of giardiasis or by the infection on nutritional status of parasitized is still speculative²².

In this work, we observed that gerbils fed on a low protein diet had significantly lower body weight compared to those who received normal diet. This significant decrease in body weight as a result of ingestion of diets deficient in protein endorses the model used in addition to corroborate with the literature^{23, 24}.

It is also well known that protein malnutrition enhances hepatic triacylglycerol and cholesterol concentrations while decreasing hepatic protein and phospholipid contents and negatively influences on the composition and metabolism of lipoproteins in growing animals fed on protein-deficient diets. Bouziane, Belleville & Prost²⁵ showed that protein-deficient groups exhibited low concentrations of protein and triacylglycerol (in serum, very-low-density lipoprotein (VLDL), low-density lipoprotein-high-density lipoprotein, (LDL-HDL1) and HDL2-3), of cholesterol (in LDL-HDL1)

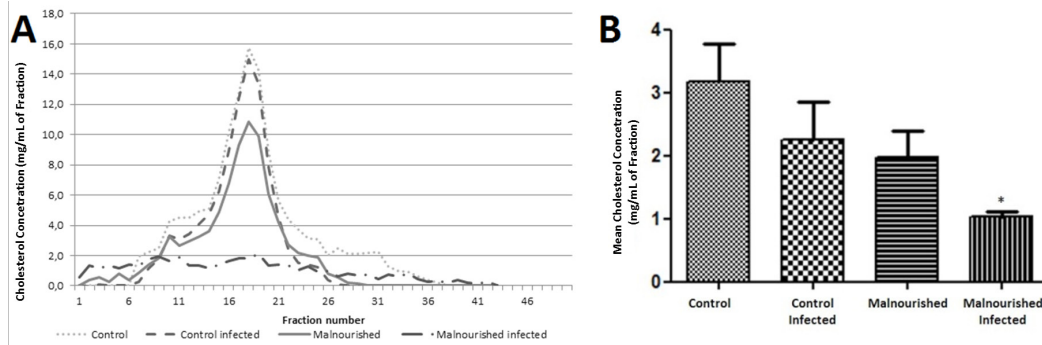


Figure 1. Cholesterol distribution on circulating lipoproteins in animal of groups CT, CTIn, MN and MNIn. A) Cholesterol profile found in lipoprotein fractions analyzed. B) Mean values of the sum of the cholesterol concentration found in the different experimental groups. It can be observed that malnutrition associated with giardiasis (MNIn group) resulted in lower levels of cholesterol in lipoprotein fractions in relation to animals of group CT. * $p < 0.05$ vs CT. Data shown is the means \pm SEM.

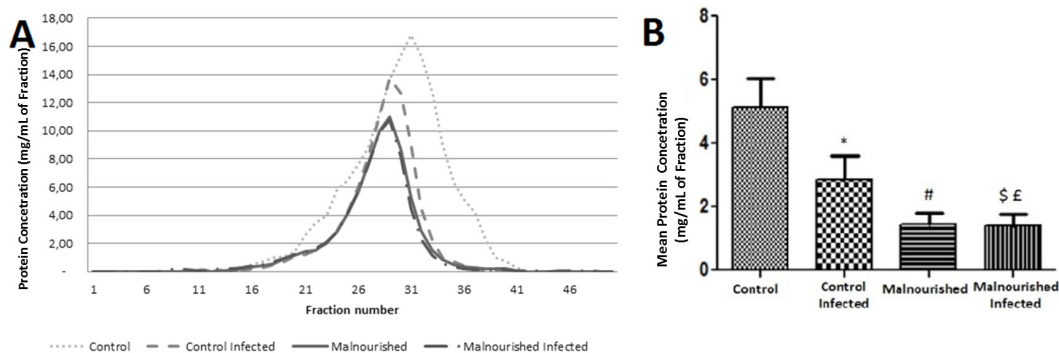


Figure 2. Protein distribution on circulating lipoproteins in animal of groups CT, CTIn, MN and MNIn. A) Protein profile found in lipoprotein fractions analyzed. B) Mean values of the sum of the protein concentration found in the different experimental groups. It can be observed that both giardiasis and malnutrition led to a decrease in protein content in lipoprotein fractions, and it was accentuated when both diseases were associated. * $p < 0.05$ vs CT, # $p < 0.0001$ vs CT, \$ $p < 0.0001$ vs CT; £ $p < 0.01$ vs CTIn. Data shown is the means \pm SEM.

and of phospholipids (in VLDL). The same authors in other paper found that VLDL-apolipoprotein concentrations were, in general, strongly reduced with protein malnutrition. Other authors showed that in humans, the malnutrition state led to quantitative and structural modifications of the triglyceride-rich lipoproteins, defined by their apolipoprotein composition, and was probably related to undercatabolism⁸.

Our study showed that malnourished animals showed changes in lipoprotein composition characterized by a reduction on cholesterol and protein levels. These animals when infected had an exacerbation of the catalase activity. Our data suggest that there was an increase in the oxidative stress, mainly in the lipoprotein oxidation. The production of reactive oxygen species is considered an important mechanism of host defense to anaerobic pathogens⁵, exerting direct cytotoxic activity on them. However, we guess that as in other intestinal infections^{7,8,9}, oxidative

stress due to both malnutrition as giardiasis may have led to increased oxidation of lipoprotein components in our model altering the composition of the same.

We also believe that this oxidative stress occurring in the lipoprotein particles induces an antioxidant response too, creating a “cycle of oxidative stress”, where ROS oxidize particles which in turn induce more oxidation, changing their composition. Thus, our data suggested that the mechanism by which changes in lipoprotein composition interfere in oxidative stress can be associated with antioxidant enzymes expression and activity.

These changes are very important, since these lipoprotein particles are responsible for the efficient transport of large amounts of lipids and liposoluble vitamins in the circulation and contribute to the maintenance of the structure and function of cell membranes. Thus, the increase in the oxidation of these particles, due to

malnutrition exacerbated by giardiasis, could exacerbate the physical and cognitive depletion affected by both diseases.

CONCLUSION

Based on what has been analyzed and discussed in this paper, we conclude that host response against giardiasis associated to malnutrition, led to an oxidative stress that produced changes on lipoprotein levels in the malnourished animals suggesting that the infection could influence on metabolic changes, worsening the nutritional status of infected animals. However, further studies should be conducted to clarify obscure points involving malnutrition, giardiasis and lipoprotein oxidation.

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Abbreviations

CT – Control
 CTIn – Control Infected
 MN – Malnourished
 MNIn – Malnourished Infected
 ROS – reactive oxygen species
 UFMG – Universidade Federal de Minas Gerais
 PBS – Phosphate Buffered Saline
 SOD – Superoxide dismutase
 FPLC – Fast Protein Liquid Chromatography
 SPSS – *Statistical Package for the Social Sciences*
 VLDL – Very Low Density Lipoprotein
 LDL – Low Density Lipoprotein
 HDL – High Density Lipoprotein

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