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Research Brief

Effect of giardiasis combined with low-protein diet on intestinal absorption of glucose and electrolytes in gerbils

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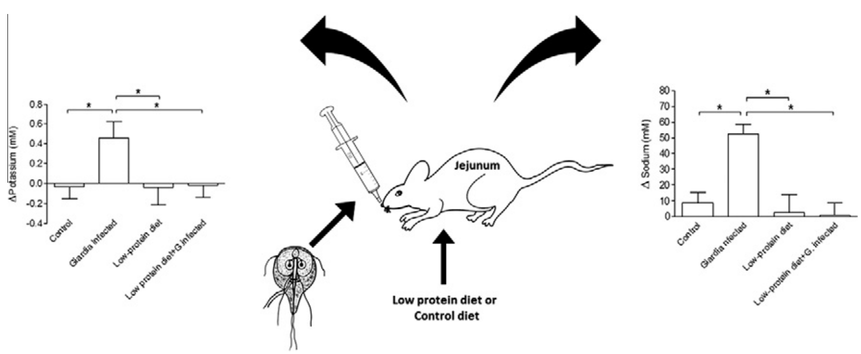
HIGHLIGHTS

- ▶ Gut absorption in giardiasis combined with low-protein diet was evaluated in gerbils.
- ▶ The glucose absorption was not significantly affected by diet or infection.
- ▶ Sodium and potassium uptake increased in infected group.
- ▶ Increased uptake suggests a compensatory mechanism to diarrhea caused by giardiasis.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies have shown that symptomatic infection by *Giardia lamblia* causes acute or chronic diarrhea, dehydration, abdominal pain and malabsorption, leading to undernutrition and weight loss. The aim of the present study was to evaluate the effects of giardiasis and its combination with a low-protein diet on the intestinal absorption of glucose and electrolytes in gerbils. The intestinal absorption of glucose, sodium and potassium was investigated in male gerbils weighing 46–64 g ($n \geqslant 5$). A Tyrode solution containing twice the glucose, sodium and potassium concentration (pH 7.4) was infused through the intestinal loops for 40 min. Glucose absorption was not significantly affected by diet and infection. However, there was a significant increase in sodium absorption in the *Giardia*-infected group (57.2 ± 6.1, $p < 0.05$) in comparison to the control, low-protein diet and low-protein diet + *Giardia*-infected groups (8.9 ± 6.5, 2.8 ± 11.1 and 0.8 ± 7.9, respectively; $p < 0.05$). Moreover, potassium was absorbed in the *Giardia*-infected group (0.45 ± 0.30), while the other groups exhibited potassium secretion. A low-protein diet and *Giardia* infection had no influence over glucose absorption. However, *Giardia* infection increased sodium and potassium uptake, suggesting a compensatory mechanism for maintaining homeostasis after likely hypernatremia and hypokalemia caused by the diarrhea that accompanies giardiasis.

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1. Introduction

Giardia lamblia (*syn.* duodenalis or intestinalis) is a noninvasive microorganism and the most common gastrointestinal parasite worldwide, causing infection of the small intestine, which leads to malabsorption and chronic diarrhea (Kim and Ravinder, 2010; Lane and Lloyd, 2002; Sullivan et al., 1990). Studies have been carried out with the aim of describing the mechanisms of diarrhea and pathological findings associated to giardiasis. An increase in enterocyte apoptosis is reported to be correlated with barrier dysfunction; moreover, Na/D-glucose malabsorption, chloride secretion, a reduction in villus height and an increase in intestinal barrier permeability have been reported, suggesting epithelial barrier dysfunction as a pathomechanism of diarrhea (Cotton et al., 2011; Troeger et al., 2007).

The attack on the intestinal mucosa by pathogens, such as *Giardia lamblia*, can lead to undernutrition (Gendrel et al., 2003; Lunn, 2000). The effects of giardiasis are associated with reduced cell mass in the lining of the gut, which has a number of consequences, such as malabsorption (Leitch et al., 1993; Wolfe, 1992). Moreover, the effects of giardiasis are more severe when the host has some degree of undernutrition, thereby creating a vicious cycle (Herrera, 2005; Hamadto et al., 1989; Solomons, 1982).

The gerbil is considered the best experimental model for the study of the development and pathogenesis of infection by *G. lamblia* due to its high degree of susceptibility to infection through the oral inoculation of cysts and trophozoites, with the considerable elimination of cysts in the stool, demonstrating the same pathophysiological alterations as those observed in humans (Amorim et al., 2010; Belosevic et al., 1983).

The purpose of the present study was to evaluate the effects of giardiasis and its combination with protein malnutrition on the absorption of glucose and electrolytes in gerbils.

2. Material and methods

2.1. Animals

Twenty-eight male gerbils (*Meriones unguiculatus*) weighing 46–64 g, with 4–6 week old, were housed in collective cages, with free access to filtered water and food available ad libitum. Before the experiments, the cages, containers of food and water were disinfected with chlorine. Every 3 days the feces of the animals were examined for the presence of intestinal parasites. Diet manipulation was done under suitable sanitary conditions, food was stored at −20 °C and removed just before of being offered.

The animals were maintained under standard laboratory conditions of a 12:12-h light/dark cycle and controlled temperature (23 ± 3 °C). All gerbils were fasted for 12 h prior to the surgical procedures, but water was offered ad libitum. All experiments complied with the International Principles of Animal Care and the study received approval from the local Ethics Committee on Animal Experimentation (CETEA/UFMG process No. 070/10).

2.2. Experimental design

The control diet (AIN-93G) (Reeves et al., 1993) contained 20% protein and the experimental diet contained 5% protein (low-protein diet). Casein was removed from the low-protein diet and replaced with corn starch in order to maintain the same energy density in both diets.

After a five-day adaptation period, the animals were randomly divided into four groups: Group 1 (control, $n = 7$) received the control diet; Group 2 (*Giardia*-infected, $n = 7$) received the control diet and was inoculated with *G. lamblia*; Group 3 (low-protein diet, $n = 7$) received the low-protein diet; Group 4 (low-protein diet + *Giardia*, $n = 7$) received the low-protein diet and was inoculated with *G. lamblia*.

2.3. Inoculation

Portland-1 (ATCC 30888) used for infection was maintained axenically at 37 °C in Diamond's modified TYI-S-33 medium supplemented with bovine bile and the duplication of cysteine (Araújo et al., 2008; Keister, 1983). On the 21st day after the introduction of the diets, Groups 2 and 4 were experimentally infected with 1×10^6 *G. lamblia* trophozoites in 1 mL of phosphate buffered saline by gavage. On the 3rd day after inoculation, a microscopic examination of the stools was performed to confirm infection.

2.4. General procedures

On the 15th day after inoculation, the gerbils were anesthetized [Ketamine (Agener União) 100 mg/kg and Xylazine (König) 12 mg/kg, i.p.]. Following the procedures of median xypho-pubic laparotomy, the small intestine after the duodenojejunal ligament until the end of the ileum was isolated, preserving the nerves and vascular pedicle. Two cannulae were then inserted into the extremities of the small intestinal loop – one for perfusion and the other for fluid drainage. The abdominal wall was then closed to prevent tissue dehydration. Both cannulae were exteriorized through the extremities of the abdominal suture. Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.36 mM $CaCl_2$, 0.49 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5 mM D-glucose) in a bottle connected to the catheter infusion pump was kept at 37 °C in a water bath. The Tyrode solution, pH 7.4 (buffered by HCO_3^-), was perfused at a rate of 0.25 ml min^{-1} for 15 min to equilibrate the fluids in order to reach a steady state within the intestinal lumen (Borges et al., 2003).

The animals were submitted to the Tyrode solution infusion containing twice the usual concentrations of glucose, sodium and potassium for the 40-min experiment under the same conditions described above. The effluents were collected separately in test tubes at 10-min intervals, maintained in ice and kept in a freezer at −20 °C for subsequent biochemical analysis.

2.5. Biochemical determinations

The glucose concentration of the effluent was determined using an enzymatic method based on glucose oxidase (Glucose PAP Liquiform, Labtest, Brazil) estimated from a standard glucose concentration curve. Effluent potassium and sodium ion concentrations were measured using flame photometry. The results were expressed by the difference between influx and efflux.

2.6. Statistical analysis

Split-plot analysis of variance (ANOVA) followed by the Student–Newman Keuls method were used for the statistical analysis, with the level of significance set at 5% ($p < 0.05$).

3. Results

Fig. 1 shows the effect of *G. lamblia* infection and diet on glucose absorption in the small intestine, for which no differences were found between groups.

However, there was a significant increase in sodium absorption in the *Giardia*-infected group (57.2 ± 6.1, $p < 0.05$) in comparison to the control, low-protein diet and low-protein diet + *Giardia*-infected groups (8.9 ± 6.5, 2.8 ± 11.1 and 0.8 ± 7.9, respectively; $p < 0.05$) (Fig. 2).