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Original Article

Giardia lamblia infection induces changes in the biochemical profile of gerbils (*Meriones unguiculatus*)

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Abstract

Introduction: *Giardia lamblia* is a global parasite that infects both humans and animals. However, its pathogenic mechanisms remain poorly understood. Infected children, in particular, may exhibit deficits in physical and cognitive development. To better comprehend the metabolic changes associated with the disease, this study investigated the biochemical profile of gerbils experimentally infected with the Portland strain of *G. lamblia*

Methodology: Sixteen gerbils, equally divided by sex, were included in the experiment for 35 days, with blood samples collected every 7 days.

Results: blood glucose and insulin levels increased on the 21st day post-infection (DPI), suggesting persistent inflammation and increased insulin resistance. Additionally, there was a progressive decrease in total protein with a paradoxical increase in albumin, indicating a state of anabolism in response to infection. Alkaline phosphatase, AST, and ALT levels remained unchanged, suggesting that liver function was not affected by the infection. Cortisol levels increased from the 7th DPI onwards, while calcium, cholesterol, and triglyceride levels began to increase from the 21st DPI onwards.

Conclusions: These findings confirm the systemic inflammatory state induced by giardiasis and highlight significant metabolic alterations in experimentally infected animals. Further research is warranted to elucidate the pathophysiological mechanisms underlying these metabolic disturbances.

Key words: *Giardia lamblia*; biochemical profile; infectious disease; intestinal parasite.

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Introduction

Giardiasis is the most widespread parasitic infection in developed countries, resulting in at least 280 million symptomatic cases annually. However, prevalence varies considerably primarily due to local sanitation conditions [1]. *G. lamblia* inhabits the small intestine despite adverse conditions for colonization, such as periodic renewal of enterocytes, intestinal transit, and an unfavorable immune response to its adhesion. Upon intestinal colonization by the parasite, symptoms such as diarrhea and malabsorption occur. Its survival in the intestinal environment seems to be related to antigenic variation, which allows *G. lamblia* to alternate between specific surface antigens, thereby evading the host's immune response, and to cyst formation, which promotes the parasite's resistance to environmental changes [2].

Giardia follows a relatively straightforward life cycle involving two distinct phases in terms of structure and biochemical activity. The first phase is represented by the trophozoite, which resides in the host's intestine, while the second phase is the cyst, which can withstand various environmental conditions. Both cysts and trophozoites lack certain organelles common in eukaryotic cells, such as mitochondria, peroxisomes, and a distinct Golgi complex, along with specific components essential for oxidative phosphorylation [3]. This suggests that the parasite relies on the host's cellular mechanisms to survive. However, not all details of the mechanisms underlying giardiasis have been fully understood or clearly demonstrated [4]. Several studies indicate that factors related to the parasite (such as strain and infective load) and the host (such as diet, interactions with intestinal microbiota, gastric pH, bile salt concentration, immune response, nutritional status, and coinfections) play crucial roles in the pathogenesis of the infection [5-7].

Thus, interactions between the parasite and the host are fundamental for disease development. Different *Giardia* strains exhibit genetic variations that may be associated with their ability to induce different morphological changes in the intestine. Genetic characteristics of the parasite are significant determinants of the severity of infection in human giardiasis [8-9]. Some studies associate *Giardia* infection with chronic gastrointestinal disorders, such as irritable bowel syndrome, and pathological conditions outside the intestine, despite parasites being found only in the gastrointestinal tract [10-13]. The pathophysiology of giardiasis remains poorly understood. Data available in the literature are often controversial, highlighting the need for further studies on this parasite. Experimental models offer a valuable alternative for exploring the internal factors of a disease. Gerbils are one such model and are considered effective in reproducing human giardiasis [14-15]. In this study, the natural progression of infection with the Portland-1 strain of *G. lamblia* and its effects on the biochemical profile of experimentally infected gerbils were evaluated.

Methodology

Animals

Gerbils (*Meriones unguiculatus*) (4-6 weeks old, both sexes) were obtained from the Animal Facility of the Department of Parasitology at the Institute of Biological Sciences of UFMG and maintained according to standard protocols, under controlled temperature (23 ± 3ºC) and 12-hour photoperiods, with free access to filtered water and autoclaved commercial diet (calorie density of 3.95 kcal/g and 20% protein). Fecal samples were collected daily and macroscopically analyzed for seven days. Only pathogen-free gerbils were selected for experimentation. All procedures were approved and conducted following the guidelines of the Committee on Ethics in Animal Use of UFMG (CEUA, Protocol number 342/2015).

Giardia Strain, Culture, and Inoculum

Portland-1 strain of *G. lamblia* (ATCC® 30888TM) was used for animal infection. The axenic strain was maintained in a modified TYI-S-33 medium [16] at 37ºC. The animals were infected by gavage using a DELVO cannula Nº. 15, attached to a 1 mL syringe, containing 0.3 mL of 1×10^6 trophozoite suspension in a culture medium.

To confirm *Giardia* infection, animal stools were collected every two or three days diluted in saline, stained with Lugol, and qualitatively evaluated under an optical microscope at a magnification of 40 × to verify the presence of *Giardia* cysts. Individual body weight and food consumption per cage were recorded weekly.

Experimental Design

To evaluate the biochemical profile of animals with giardiasis, 16 gerbils (8 males and 8 females) were used. The animals were confined in individual cages, under controlled temperatures, with a 12-hour photoperiod, with free access to filtered water and an autoclaved diet. Blood samples were collected from the animals in microtubes containing EDTA, via caudal vein puncture, at time zero (D0) and every seven days, up to 35 days of infection (day 7 - D7; day 14 - D14; day 21 - D21; day 28 - D28; and day 35 - D35). After completing the experiments, all animals were euthanized (100 mg/kg ketamine and 12 mg/kg xylazine), and blood was collected from the inferior vena cava, followed by cervical dislocation.

Biochemical Profile

The blood of infected animals was centrifuged at 12000× g for 10 min and the sera were used for determination of glycemia, albuminemia, cholesterolemia, triglyceridemia, total proteins, the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), calcium, alkaline phosphatase, insulin, cortisol, and C-reactive protein. The assays were carried out in 96-well microplates, using 5 microliters of serum and 200 microliters of color reagent, observing the incubation time and wavelength of each analyte, following the manufacturer's protocol (Labtest Diagnóstica, Lagoa Santa, Brazil). Quantification was performed using a semi-automatic spectrophotometer LabQuest model (LabTest Diagnóstica, Lagoa Santa, Brazil).

Statistical Analysis

Kolmogorov-Smirnov test was used to verify normality and Grubbs' test was used to detect outliers. Data were analyzed using Prism 10 and compared using ordinary One-way ANOVA, with multiple comparisons test, comparing all data with control (D0). Graphs were plotted representing mean ± standard deviation and statistical difference, considered as $p < 0.05$, were represented as: * < 0.05; **< 0.01; ***< 0.001; **** < 0.0001; not significant were not represented.

Results

In the parasitological examination of feces, the animals showed *Giardia* cysts from the fourth day of infection, remaining positive until the 32^{nd} day. Throughout the experiment, no changes in stool consistency or animal weight were observed, suggesting that the strain used may have caused asymptomatic infection in the animals.

Blood samples from two groups of animals, eight males and eight females, were evaluated. The overall assessment of the 16 animals is presented below, without separating them by group, as no significant differences were observed between males and females regarding the evaluated analytes.

Food intake and body weight evolution

As shown in Table 1, there was a reduction in food consumption on days 14 and 35 of infection compared to day 7, and there was no change in body weight during the days of infection related to day zero.

Glucose and insulin concentrations

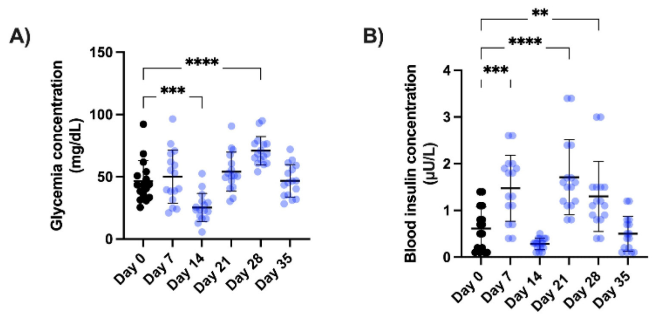
As can be seen in Figure 1A, compared to day 0, blood glucose was significantly lower on day 14 (25.31 + 2.85 mg/dL, $p < 0.001$) with a peak of increase on day 28 (70.90 + 2.85 mg/dL, $p < 0.001$).

Insulin concentration varied over time, peaked on the 7^{th} day (1.48 + 0.18 µU/L, $p < 0.001$), and increased again on the 21st (1.71 + 0.20 µU/L, $p < 0.0001$), and 28th (1.30 + 0.19 µU/L, $p < 0.01$) days, returning to baseline levels on the 35^{th} day (0.50 + 0.09 µU/L) (Figure 1B).

Cholesterol and triglycerides concentrations

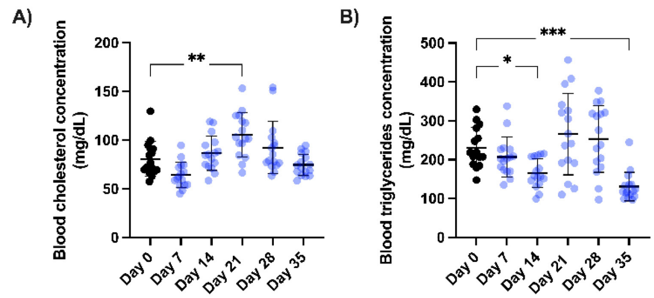
Compared to Day 0, a significant increase in blood cholesterol concentration was observed during day 21 (105.62 + 5.66 mg/dL, $p < 0.01$), but other days have a statistically non-significant change (Figure 2A). There was a significant reduction in triglyceride concentration on day 14 (165.72 + 9.35 mg/dL) ($p < 0.05$), an increase to values similar to day 0 on days 21 (265.73 + 26.17 mg/dL) and 28 (253.74 + 21.47 mg/dL) and a final significant reduction on day 35 (130.84 + 9.24 mg/dL, $p < 0.001$) (Figure 2B).

Figure 1. Graphics representing changes in blood glucose (a) and insulin concentration (b) before and after infection at days 7, 14, 21, 28, and 35. Black dots represent uninfected animals on day 0, and blue dots represent animals after infection.



Data represent mean ± S.E.M. Anova one-way, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure 2. Graphics representing changes in blood cholesterol (a) and triglycerides concentration (b) before and after infection at days 7, 14, 21, 28, and 35. Black dots represent uninfected animals on day 0, and blue dots represent animals after infection.



Data represent mean ± S.E.M. Anova one-way, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Table 1. Evolution of Food Intake and Body Weight of gerbils infected with *Giardia lamblia*.

Days of infection	Food intake (g/week)	Body weight (g/week)
0	Not measured	59.09 ± 1.35
7	333.5 ± 10.20	57.31 ± 0.83
14	292.4 ± 4.16*	56.92 ± 0.98
21	317.5 ± 5.99	60.09 ± 1.31
28	355.4 ± 9.28	Not measured
35	286.3 ±10.44***	57.14 ± 1.12

Data represented as mean ± S.E.M. Anova one-way, *$p < 0.05$, *** $p < 0.001$.

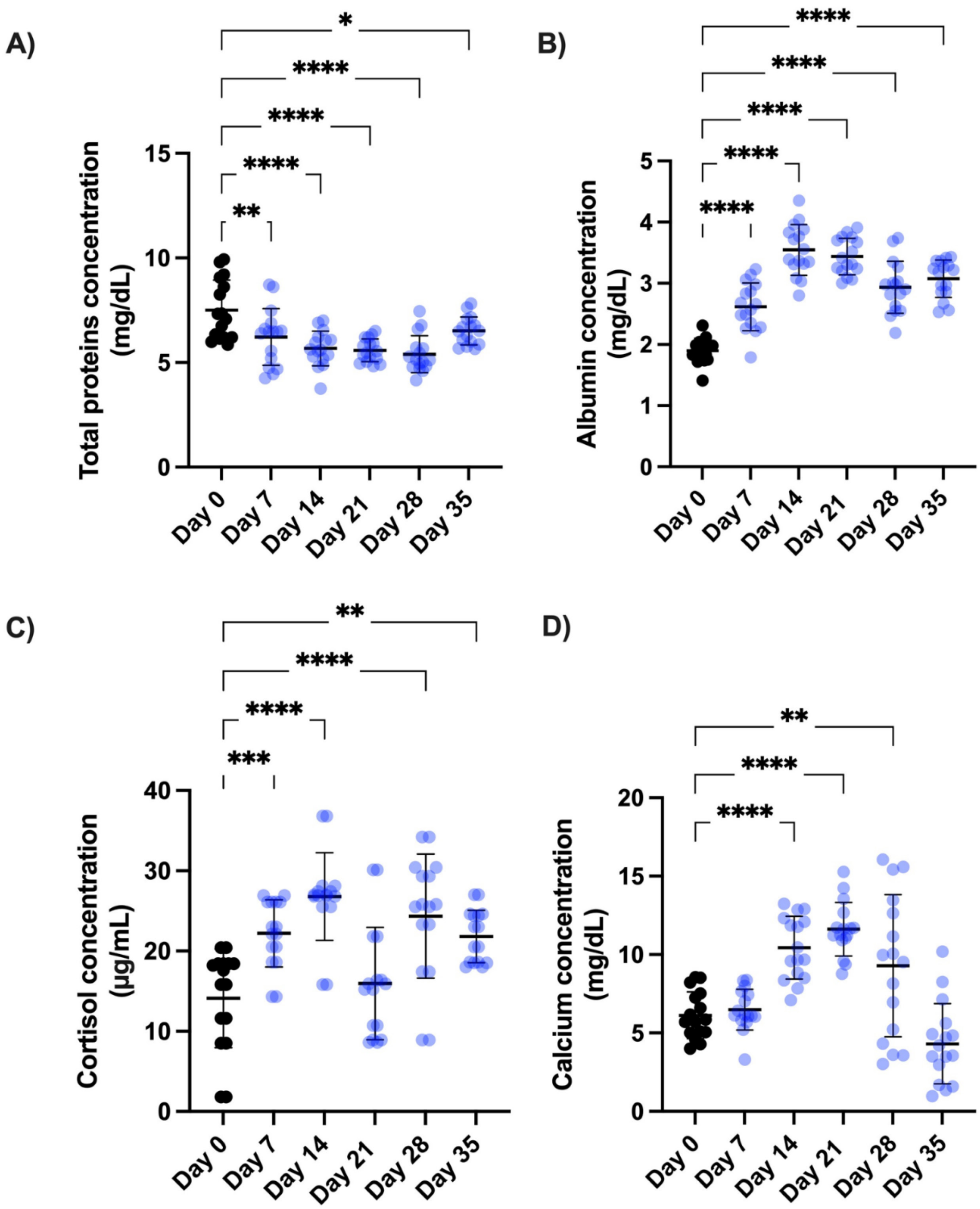
Proteins and calcium concentrations

When evaluating total proteins, a clear trend curve of significant decline in serum value from day 0 (7.51 + 0.36 mg/dL) to day 28 (5.40 + 0.22 mg/dL), with a little increase on day 35 (6.51 + 0.17 mg/dL), remaining significantly lower than basal levels all the experimental period (Figure 3a) despite the elevation of albumin concentration (Figure 3B).

Cortisol showed a significant increase from day 7 (22.20 + 1.05 µg/dL, $p < 0.001$) and kept increasing until day 14 (26.79 + 1.37 µg/dL, $p < 0.0001$). Subsequently, cortisol concentration decreased to baseline levels in day 21 (15.95 + 1.75 µg/dL), but values remained higher than in the control group during days 28 (24.35 + 1.94 µg/dL, $p < 0.0001$) and 35 (21.83 + 0.82 µg/dL, $p < 0.01$) (Figure 3C).

Serum calcium concentration increased on days 14 (10.44 + 0.50 mg/dL, $p < 0.0001$), 21 (10.44 + 0.50

Figure 3. Graphics representing changes in Blood total proteins (a), albumin (b), cortisol (c) and calcium (d) concentration before and after infection at days 7, 14, 21, 28, and 35. Black dots represent uninfected animals on day 0, and blue dots represent animals after infection.



Data represent mean ± S.E.M. Anova one-way, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

mg/dL, $p < 0.0001$), and 28 post-infection, returning to a value similar to day 0 on day 35 (4.32 + 0.64 mg/dL) (Figure 3D).

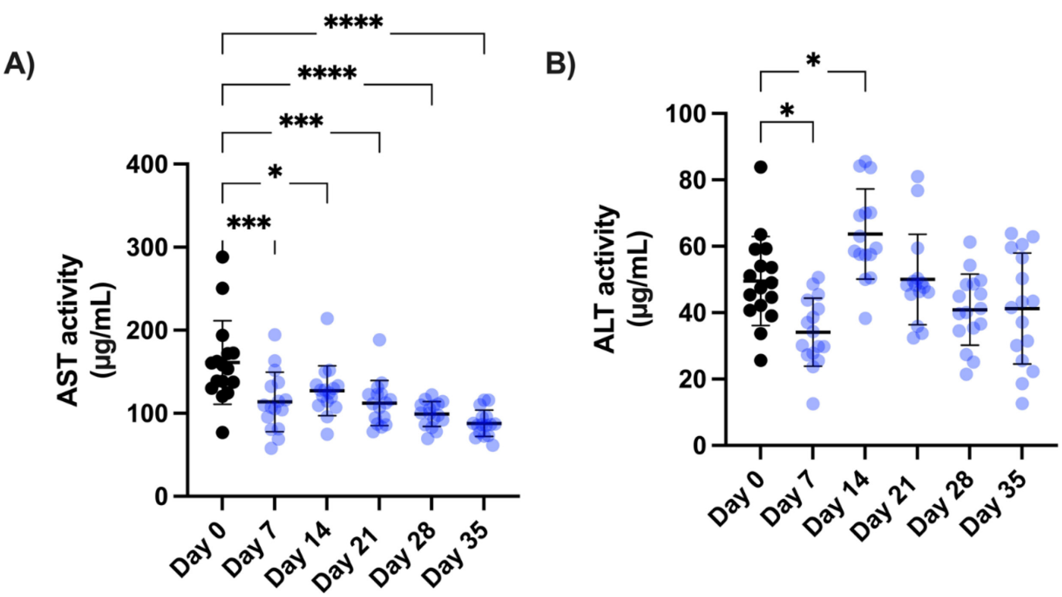
Serum enzymes activities

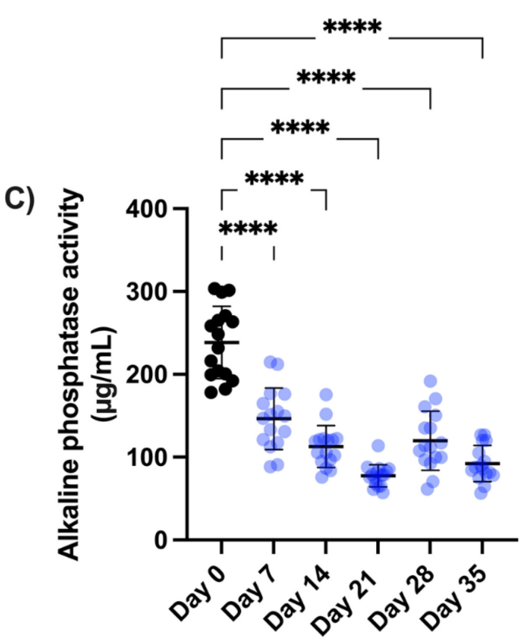
The AST enzyme activity presented a significant reduction throughout the experimental period compared to day 0 (Figure 4A).

The ALT enzyme activity showed a significant decrease in the first week (34.13 + 2.56 U/mL), followed by a significant increase on day 14 (61.87 + 3.75 U/mL), compared to day 0 (49.53 + 3.36 U/mL, $p < 0.05$), returning to values similar to day 0, from day 21 onwards (Figure 4B).

A significant decrease ($p < 0.0001$) in alkaline phosphatase activity was observed from day 0 (238.47 + 10.89 U/L) to day 21 (77.56 + 3.35 U/L), except on days 28 (119.80 + 8.91 U/L) and 35 (92.33 + 5.43 U/L), where there was a small increase again, albeit considerably smaller compared to the control group

Figure 4. Graphics representing changes in blood AST (a), ALT (b), Alkaline phosphatase (c) and table representing C-reactive protein analysis(d) before and after infection at days 7, 14, 21, 28, and 35. Black dots represent uninfected animals on day 0, and blue dots represent animals after infection.





D)

C-reactive Protein (mg/dL)	Mean	S.E.M.
Day 0	0.10	0.00
Day 7	0.10	0.00
Day 14	0.10	0.00
Day 21	0.10	0.00
Day 28	0.14	0.01
Day 35	0.11	0.01

Data represent mean ± S.E.M. Anova one-way, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

(Figure 4C).

C-reactive protein concentration could not be identified in the animals' serum (Figure 4D).

Discussion

G. lamblia is a complex parasite, and its pathophysiological mechanisms and clinical manifestations still have many gaps, signaling the need for expanded studies related to giardiasis in all aspects of the infection. The hematological profile of experimental giardiasis was first studied in a previous work [17], and the present study investigates the biochemical profile of the infection in the same experimental model. The main objective was to evaluate the progression of giardiasis in gerbils. The results were always compared to day 0, which was considered the control group.

It is well known that pancreatic beta cells respond rapidly to fluctuations in blood glucose levels by tightly regulating insulin secretion. *Giardia* metabolism is anaerobic, with glucose being the major carbohydrate source of energy. It is metabolized at different concentrations of acetate, ethanol, alanine, and CO_2 [3]. It is hypothesized that the interaction between Giardia metabolism and β-cells could induce a reduction in glucose levels and consequently decrease insulin secretion by the 14th day of the experiment.

A reduction in blood glucose was observed in animals on day 14 and an increase on day 28 post-infection. It is known that blood glucose tends to increase in inflammatory processes, whether infectious or not [16]. This mainly occurs due to increased insulin resistance, leading to a series of vascular, immune, and metabolic alterations [18]. Thus, the glycemic levels, which showed a significant increase on day 28 of infection and returned to baseline values after parasitological cure, suggest that giardiasis is responsible for an inflammatory process from this point of infection. These inferences are corroborated by insulin measurements, which peaked on the 7^{th}, 21^{st}, and 28^{th} days, followed by declines until the 35^{th} day. The infection appears to increase insulin resistance, thereby stimulating increased hormone secretion by the pancreas. With parasitological cure, insulin levels decrease to baseline values. It is possible that the infection caused enough inflammation to result in increased insulin resistance in the animals, which in turn stimulated the pancreas to secrete more insulin. With a parasitological cure, an improvement in the inflammatory process likely occurred, leading to a subsequent reduction in insulin levels to normal values.

Regarding cholesterol levels, a significant increase was observed on day 21, returning to baseline values from day 28 onwards. The relationship between *Giardia* and cholesterol levels is crucial for understanding the evolutionary stages, development, and establishment of the parasite [19]. As a "mucosal parasite" lacking well-developed mitochondria and Golgi complexes, *Giardia* develops unique metabolic pathways that allow it to survive and reproduce. It captures nutrients from the host but is unable to synthesize most of its lipids and cholesterol, thus acquiring them from the upper small intestine. Since membrane biogenesis in *Giardia* requires cholesterol, it appears evident that these parasites can uptake the lipids and cholesterol they need from lipoprotein particles present in the host [20] and internalize them via LDL receptor-mediated endocytosis [21]. Infection and inflammatory responses have been shown to affect levels of lipoproteins, including total cholesterol, in parasite-infected patients. Compared to uninfected children, those infected with *G. lamblia* showed changes in the lipoprotein profile, characterized by an increase in serum cholesterol and VLDL levels [22]. The acute phase response induced by infection and inflammation leads to multiple alterations in lipid and lipoprotein metabolism that initially protect the host from the harmful effects of bacteria, viruses, and parasites, but if prolonged, contribute to atherogenesis [23].

While all parasites metabolize cholesterol, the exact relationship with the pathogenic mechanism remains incompletely understood. Elevated levels of lipoproteins and total cholesterol have been observed in patients with parasitic infections [24]. A direct correlation between increased cholesterol levels and the development and establishment of infection has been noted with *Entamoeba histolytica* [25-26] and *Toxoplasma gondii* [27].

Triglycerides levels also exhibited variations, showing a significant reduction on day 14 compared to day 0. By days 21 and 28, these levels had increased to values similar to day 0, followed by a significant reduction by day 35 compared to day 0. The primary approach for lowering serum triglycerides involves managing lifestyle factors. Regarding diet adjustments, changes in macronutrient intake such as reduced fat or protein, low-carbohydrate diets, and caloric restriction appear to be effective strategies for lowering triglyceride levels [28]. Therefore, these results may be attributed to reduced food intake on days 14 and 35 post-infection.

In acute malaria, evaluation of plasma lipoprotein changes resulted in decreased HDL and LDL levels and modest triglyceride increase [29]. However, a study on ascariasis observed a decrease in total cholesterol, HDL, and triglyceride levels [30]. Concerning lipid profile results, the findings presented in this study align with glycemic and insulin alterations, indicating the occurrence of giardiasis-related inflammation from the second week of infection

Albumin exhibited an intriguing behavior pattern; its concentration significantly increased from the first week after infection, remaining considerably higher than on day 0. It was expected that the inflammatory response to giardiasis would result in a state of protein hypercatabolism, reflected by increased serum protein levels, which are closely linked to elevated immunoglobulin production. However, a reduction in total proteins was observed throughout the infection, despite the increase in albumin, which could be justified by a state of anabolism in response to infection. Increases in the protein synthesis rate have been described due to increased amino acid demands and energy expenditure directed to the synthesis of acute-phase proteins such as fibrinogen and albumin [31].

Cortisol, a potent anti-inflammatory hormone that promotes protein degradation and inhibits the anabolic process, followed an expected pattern during the infection. There was a significant increase from day 7 to day 14 post-infection, possibly indicating an active inflammatory phase due to the continued presence of *Giardia* in the animals. Subsequently, cortisol levels decreased but remained considerably higher than in the control group, without a clear explanation for this phenomenon, especially considering that the animals were likely in a phase of spontaneous recovery. A decrease in cortisol levels was observed on day 21, possibly related to changes induced by *Giardia* during the third week of infection.

Serum calcium levels were evaluated, and their behavior oscillated during the experiment period. From day 14 onwards, it became significantly higher, returning to values close to day 0 on day 35, but without a reasonable explanation, as the control of this ion is efficiently performed by calcitonin and parathyroid hormone. Generally, only deeper alterations from the perspective of these hormones would lead to significant and real fluctuations. However, as there was a significant increase in serum albumin, this may have influenced the measurement of total calcium value, as it binds to albumin for transport and storage.

Finally, in previous studies, hepatic steatosis has been observed in animals infected with *Giardia*, along with the presence of the parasite in trophozoite form in hepatic tissues such as bile ducts and the gallbladder [32-33]. Therefore, hepatic enzymes related to liver function (AST, ALT, alkaline phosphatase) and C-reactive protein were evaluated to establish patterns in giardiasis. The AST enzyme exhibited a significant decrease over time, maintaining levels similar to those observed on day 35. However, it was substantially lower compared to day 0, even after the parasitological cure of giardiasis. AST is an enzyme found in muscle, heart, and liver cells. Elevated levels may indicate pathologies in these specific cells, whereas a decrease is typically not associated with significant pathological conditions.

On the other hand, the ALT enzyme showed a significant decrease during the first week, followed by a notable increase in the second week, returning to values similar to day 0 after the third week of infection. The increase in ALT, an enzyme found mainly in liver cells, is associated with lesions in these cells when it exceeds three times the basal value. However, the observed increase was small, considered nonspecific, and possibly related to lesions in other organs.

Alkaline phosphatase levels showed a significant and progressive decrease at each evaluated time interval, except for day 28 and day 35, where there was an increase again but smaller compared to the control group. Alkaline phosphatase is an enzyme present in bile duct cells and found in various organs, mainly in bones, placenta, and intestines. Like AST and ALT, injury to these cells results in increased blood levels [34-35]. These results, combined with those of AST and ALT, suggest that liver functions were not affected by the infection. This indicates that despite the proximity of the trophozoites, neither liver functions nor hepatocytes were altered by the infection, suggesting that the observed hepatic steatosis, if caused by giardiasis, may have another generating mechanism.

Lastly, C-reactive protein (CRP) could not be detected in the animals' serum, even with the use of highly sensitive methods. This plasma protein is synthesized by the liver, and its physiological function involves binding to phosphocholine present on the surface of dead or injured cells or certain types of bacteria. This binding initiates their removal by activating the complement system and phagocytic cells, acting as an opsonin. CRP is an extremely sensitive marker of inflammation [36], with levels typically low in healthy individuals but capable of increasing significantly, up to a thousandfold, in response to infections or inflammatory stimuli [37]. Although previous studies have shown increased CRP levels in diarrheal infections caused by *G. lamblia* in humans [38], in this study, the undetectable presence of the protein may be related to the specific strain (Portland-1) used, possibly due to its ability to induce asymptomatic infection in animals.

Conclusions

In this model, the infection revealed a self-limited inflammatory process, with spontaneous resolution around the thirtieth day post-infection. Interestingly, despite the apparent absence of clinical symptoms, we observed elevations in blood glucose and insulin levels from the second week of infection, suggesting a potential role for *G. lamblia* in inducing insulin resistance. Levels of cortisol, calcium, cholesterol, and triglycerides also increased. These findings corroborate the inflammatory state generated by giardiasis and demonstrate that the infection, even asymptomatic, induced significant metabolic changes in the experimental animals, highlighting the importance of expanding studies of the pathophysiology of this disease to better understand its effects.

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Authors' Contributions

FF did conceptualization, validation, formal analysis, investigation, writing—original draft preparation. GM did data curation, writing—original draft preparation, review, and editing. DR, MC, and RC did methodology, investigation, and original draft preparation. MA and JS did conceptualization, methodology, validation, writing—original draft preparation and review, supervision, project administration, and funding acquisition.

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Conflict of interests

No conflict of interests is declared.

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