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High fat diet modulates inflammatory parameters in the heart and liver during acute *Trypanosoma cruzi* infection

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ABSTRACT

The high fat diet (HFD) can trigger metabolic and cardiovascular diseases. *Trypanosoma cruzi* infection induces progressive inflammatory manifestations capable to affect the structure and the function of important organs such as the heart and liver. Here we aimed to investigate the effects of a HFD on the immune response and matrix metalloproteinase (MMP) activities during acute infection with the *T. cruzi* strain VL-10. The VL-10 strain has cardiac tropism and causes myocarditis in mice. Male C57BL/6 mice were treated with either: (i) regular diet (Reg) or (ii) HFD for 8 weeks, after which mice in each group were infected with *T. cruzi*. Mice were euthanized on day 30 after infection, and the liver and heart were subjected to histology and zymography to determine MMP-2 activities and plasma levels of IL-10, TNF, CCL2, and CCL5. *T. cruzi*-infected HFD animals had higher parasitemia, LDL and total cholesterol levels. Regardless of diet, plasma levels of all inflammatory mediators and cardiac MMP-2 activity were elevated in infected mice in contrast with the low plasma levels of leptin. HFD animals presented micro- and macrovesicular hepatic steatosis, while cardiac leukocyte infiltration was mainly detected in *T. cruzi*-infected mice. Our findings suggested that a HFD promotes higher circulating *T. cruzi* load and cardiac and liver immunopathogenesis in an experimental model using the VL-10 strain of the *T. cruzi*.

1. Introduction

Trypanosoma cruzi is a digenetic flagellated protozoan that belongs to the order Kinetoplastida. *T. cruzi* undergoes various life form stages while infecting a wide range of vertebrate hosts [1,2]. The host inflammatory response is, at least in part, influenced by genetically distinct populations of this parasite [3–5]. Different strains of *T. cruzi* determine different histopathological changes in animal models, such as tissue tropism and type and intensity of injury [6]. The VL-10 strain has cardiac tropism and causes myocarditis in mice [7,8]. It is well known that *T. cruzi* can infect various tissues of the body, both in humans [9] and experimental animals [6,9], and thereby causes hepatomegaly, splenomegaly and cardiomegaly. Many cells types can be colonized by *T. cruzi* such as macrophages, Kupffer cells, adipocytes, cardiomyocytes, and hepatocytes [10,11]. Immunopathology also has great impact on the pathogenesis of the experimental Chagas' disease. In particular, the production of inflammatory mediators has highlighted the role of leukocyte activation and recruitment towards cardiac tissue during *T. cruzi* infection [12]. This immunopathogenesis leads to progressive fibrosis, alterations in the extracellular matrix, cellular destruction and consequently, myocyte remodeling [13]. Inflammation-induced remodeling of the heart is regulated by proteolytic enzymes such as the matrix metalloproteinases (MMPs). Enhanced cardiac activities of the closely related gelatinases, MMP-2 and MMP-9, have been demonstrated in both an experimental mouse model [14] and in human pathogenesis of cardiac disease following *T. cruzi* infection [15].

Immunopathogenesis related to *T. cruzi* infection appears to be linked to the host's nutritional status [16]. Inadequate dietary habits are

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prevalent in low-income populations [17]. High fat diets have been widely studied through the identification of adipose cells as potential sources of inflammatory mediators that interfere with inflammatory infiltration in glucose, insulin, and lipid homeostasis [18,19]. Previous studies have indicated that adipocytes are important target cells for *T. cruzi* [10]. Parasitic invasion and subsequent infection alters lipid and cholesterol levels, which contributes to host cell/tissue lipotoxicity and the pathophysiology of infection [20].

Inflammatory cell recruitment and the production of cytokines and chemokines drive the pathogenesis triggered by *T. cruzi* infection [21,22]. Thus, considering the influence of host diet on the course of infection [23,24], the present study aimed to evaluate the effects of a high fat diet on inflammatory soluble mediators and MMP-2 activity in the cardiac and liver tissues of C57BL/6 mice infected with the *T. cruzi* VL-10 strain.

2. Materials and methods

2.1. Parasites, animals, and experimental design

Blood trypomastigote forms of *T. cruzi* strain VL-10 were maintained through successive passages in Swiss mice at the Laboratory of Immunobiology of Inflammation at the Federal University of Ouro Preto (UFOP), Minas Gerais State, Brazil. The VL-10 strain was isolated from a patient in the chronic phase of Chagas disease in Minas Gerais State, Brazil [25]. Male 21-day-old C57BL/6 mice (n = 10 per group) weighing about 12 g were obtained from the animal facility (Center for Animal Science) at UFOP. Animals were housed in polypropylene cages with five mice per cage and were provided food and water *ad libitum*. Animal protocols were approved by the UFOP Ethics Committee in Animal Research (Protocol No. 2012/42).

Age- and weight-matched animals were selected and fed with either a predetermined diet (AIN-93M standard diet) [26], represented here as regular (Reg) diet, or with a high fat diet (HFD) containing 59% lard and 1% cholesterol (Table 1). The animals from this study were fed for eight weeks with specific diets and, then, infected or not with 5000 trypomastigote forms of the *T. cruzi* VL-10 strain. Mice were kept alive for more four weeks (infected or not) and finally euthanized, in a total period of 12 weeks. The diet was offered to the animals before and during the infection time till the day of euthanasia. Mice were divided into the following four treatment groups: (i) uninfected mice under Reg diet; (ii) uninfected mice under HFD; (iii) infected mice under Reg diet; and (iv) infected mice under HFD.

Table 1
Composition and energy content of experimental diets.

Nutrients (g/Kg)	Regular diet	High fat diet
Casein	140.0	140.0
Cornstarch	467.5	137.5
Sucrose	100.0	100.0
Maltodextrin	155.0	155.0
Soybean oil	40.0	40.0
Fiber (cellulose)	50.0	50.0
Mineral mix[1]	35.0	35.0
Vitamin mix[2]	10.0	10.0
Choline	2.5	2.5
Cholesterol	–	10.0
Lard	–	320.0
Energy Content (Kcal/Kg)	3800	5400
Carbohydrate (% energy)	76	26
Protein (% energy)	14	14
Fat (% calories)	10	60

[1]Mineral and [2]Vitamin mixture as recommended by the AIN-93M rodent diet. Reeves et al. [26].

2.2. Parasitemia

Parasitemia was determined daily by collecting fresh blood from the tail vein. After 4 weeks of infection, mice were weighed, fasted for 12 h, anesthetized with isoflurane, and euthanized by exsanguination. Blood was collected in polypropylene tubes containing heparin and centrifuged at 10,000*g* for 15 min at 4 °C. Plasma was then removed, and samples were stored at −80 °C. Heart and liver were washed with saline (0.9% NaCl solution), sliced into small pieces and stored in 10% buffered formalin for histopathological analysis and/or at −80 °C for immune, biochemical, and molecular assays.

2.3. Body weight, food intake, and Lee index assessment

Body weight and food intake was evaluated weekly. The Lee index, an indicator of increased percentage body fat in rodents, was calculated by dividing the cubic root of body weight (grams) by the naso-anal length (cm) [27,28].

2.4. Biochemical testing

Glucose and insulin tolerance. The oral glucose tolerance tests (OGTT) were performed, after an overnight fast by oral administration of 2 mg glucose/g body weight, using an *Accu-Chec®* Active blood glucometer [29]. For insulin tolerance tests (ITT) mice were fasted for 8 h before insulin administration (0.75 unit/kg of body weight, i.p.). The blood glucose level was monitored before the glucose and insulin administrations, at times 0, 30, 60, 90 and 120 min.

Cholesterol, triglycerides, and inflammatory mediators. Plasma concentrations of HDL, total cholesterol, and triglycerides were determined using commercial kits from Labtest (Lagoa Santa, MG, Brazil). Orbital venous sinus blood (0.5 mL) was collected during euthanasia and centrifuged at 1500*g* for 15 min at 4 °C. Samples were stored at −80 °C, and plasma levels of TNF, IL-10, leptin, CCL2, and CCL5 were measured using commercial ELISA Kits (Peprotech, Ribeirão Preto, SP) following the manufacturer's protocol. Measurements were performed in duplicate.

Liver lipid levels. Lipids were extracted from frozen livers using a chloroform/methanol solution (2:1, vol/vol) as previously described [30]. Total hepatic lipids were obtained by solvent evaporation and weighed.

2.5. Morphometric and histopathology analysis

Heart and liver tissue slices were fixed in 10% buffered formalin and embedded in paraffin. Tissues were cut into 4 μm thick sections, stained with hematoxylin and eosin (H&E), and subjected to microscopic analysis to assess both inflammation and the presence of amastigote nests. Twenty fields from each section were randomly chosen and analyzed. Images were obtained using a microscope equipped with a Leica DM 5000 B microchamber v. 2.4.0 R1 (Leica Application Suite, UK) and processed using Leica QWin (V3) image analyser software. Inflammation was evaluated based on a correlation index between the area (μm^2) of cellular nuclei in tissues from uninfected and those of infected animals and between the diet treatments.

A semi-quantitative scoring system was used to assess the severity of liver steatosis in ten microscopic fields as previously described [31]. Briefly, steatosis was graded from a scale of 1 to 3 based on the percentage of hepatocytes that contained fat. Samples were assigned grade 1 if < 33% of hepatocytes contained fat, grade 2 if between 33 and 66% of hepatocytes contained fat, and grade 3 if > 66% of hepatocytes contained fat.

2.6. Gelatin zymography

Preparation of tissue extracts. Frozen heart and liver tissues (−80 °C)