# Effects of high-fat chow on heart tissue in acute and chronic experimental murine schistosomiasis mansoni

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SUMMARY

This study aimed to investigate myocardial injuries in mice infected with *Schistosoma mansoni* and fed a high-fat chow. Sections of myocardial tissue from *S. mansoni*-infected mice, and controls that had been killed 9 and 17 weeks post-infection, were stained with H&E and Picrosirius red. Histopathological examination, stereological design-based method (optical disector) and morphometry (vessels, cardiomyocytes and an amount of collagen) were used. Data were analysed using two-way ANOVA. Regardless of time of infection, myocardial tissue from the infected mice fed high-fat chow showed myocarditis characterized by a higher number of inflammatory foci, several areas displaying coagulation of cardiac fibres, a greater loss of cardiomyocytes and fibroblast proliferation than in the standard chow control. Comparing infected mice from acute and chronic infections, a higher cardiomyocyte hyperplasia ($P<0{\cdot}0001$) and higher amounts of collagen ($P<0{\cdot}05$) were observed than in standard chow control. In addition, all animals fed high-fat chow showed lower numerical density and total number of cardiomyocytes ($P<0{\cdot}05$), thicker vessel walls and narrowed luminal intramyocardial vessels ($P>0{\cdot}05$) than in the standard chow control. Altogether the data supported the view that a double burden has a synergistic deleterious effect on the myocardial tissue.

Key words: schistosomiasis mansoni, heart, high-fat chow, histopathology, stereology.

## INTRODUCTION

Schistosomiasis mansoni is one of the most prevalent neglected tropical water-borne diseases in populations living in low socio-economic and sanitary conditions (Gryseels *et al*. 2006). It is estimated that about 207 million people are infected worldwide, in which 120 million are symptomatic and 20 million have serious consequences (Chitsulo *et al*. 2000). Morbidity observed in schistosomiasis is linked to the intense granulomatous reaction to trapped eggs in host organs such as liver, intestine, lungs, brain and pancreas (Baptista and Andrade, 2005). Acute schistosomiasis is a clinical syndrome often seen in non-immune individuals 3 to 6 weeks after exposure during a bath in an endemic area (Epelboin *et al*. 2010). With ageing of infection, some patients may progress to develop the severe hepatosplenic form of the disease. Clinical presentation includes hepatosplenomegaly, periportal fibrosis, portal hypertension, ascites, oesophageal varices, collateral circulation and haematemesis (Gryseels *et al*. 2006; Lambertucci, 2010).

Even though cardiac involvement during acute schistosomiasis is uncommon, myocarditis associated with or without granuloma and right ventricular insufficiency has been reported (Chisty *et al*. 1999; Ramanampamonjy *et al*. 2007). Recently, electrocardiography showed myocarditis as a complication of acute schistosomiasis in non-immune travellers returning from endemic areas (Epelboin *et al*. 2010). Other image examinations showed that schistosomiasis-associated pulmonary arterial hypertension (PAH) also shows cardiac alterations, as right-side cardiomegaly, leading to a heart failure (Kolosionek *et al*. 2011).

Since the mid-1990s, a number of studies have used unbiased stereological methods to quantify heart morphology (Wulfsohn *et al*. 2004). Design-based stereology can provide fundamental information on the structural changes in the pathological heart (Medeiros *et al*. 2005). There is growing evidence that a high-fat chow contributes to increased

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myocardial injury. Previous work in rats fed lard and egg yolk showed cardiomyocyte hypertrophy, microcirculation rarefaction and myocardial ischaemia with consequent cardiomyocyte loss and interstitial fibrosis (Águila and Mandarim-de-Lacerda, 2001).

Considerable advances have been made in recent years in our knowledge on the role of dyslipidaemia associated with both acute and chronic liver and intestinal pathology in schistosomiasis by utilizing the stereological approach. These studies have shown lower hepatocyte and sinusoid numbers and higher liver steatosis (Neves *et al*. 2006), as well as a higher granulomatous reaction in the small intestine compared to mice fed a normal chow (Alencar *et al*. 2009). Morphometric studies also showed that the infected high-fat chow group had significant alterations in small intestine structure (De Barros Alencar *et al*. 2012). Much remains to be learned, however, about the burden of cardiac involvement in *S. mansoni*-infected mice fed a high fat chow. This study aimed to investigate myocardial injuries in these double burdens.

## MATERIALS AND METHODS

### *Experimental protocol*

The study design reported herein complies with the current laws regarding ethical procedures involving laboratory animals and was approved by Comissão de Ética de Uso de Animais-Fiocruz (L-0036/07), Rio de Janeiro, Brazil.

The animals, chow regimen, infection and biochemical analysis have been previously reported (Alencar *et al*. 2009). Briefly, female Swiss Webster mice were fed either a high-fat chow or a control chow for 6 months. Later, the mice were given 50 *S. mansoni* cercariae transcutaneously. Total serum cholesterol was assayed 1 day before experimental infection and 63 days post-infection. According to the chow regimen and infection (acute or chronic), mice were separated into 4 groups ($n = 5$ each/group) as follows: uninfected mice fed standard chow, uninfected mice fed high-fat chow, infected mice fed standard chow and infected mice fed high-fat chow.

Mice were euthanized on the 9th week (acute phase) and 17th week (chronic phase) post-infection to remove organs such as the heart. The heart was weighed and its volume determined by Scherle´s liquid displacement method (Scherle, 1970).

### *Histological procedures*

Hearts were fixed in modified Millonig's phosphate-buffered formalin, pH 7·4 for 48 h at room temperature prior to tissue procedures. Hearts were sectioned by the modified orthotopic method, separating the organ's middle region (Mandarim-de-Lacerda, 2003). Those fragments were embedded in paraffin and sectioned to 6$\mu$m thickness before staining with Haematoxylin-Eosin (vessels) or Picrosirius red (myocytes) (Rich and Whittaker, 2005) for general morphology, morphometry and stereology.

### *Histopathology and quantitative morphology*

The Haematoxylin-Eosin (H&E) stained-section from intact intramyocardial vessels found in the middle region heart cutting was further investigated. We used the Image Pro Plus – Media Cybernetics (USA) for measurement of total myocyte area ($\mu m^2$), average lumen diameter ($\mu$m) and mean wall thickness (left, right and bottom) ($\mu$m). Only cardiomyocytes (15–30 per animal) sectioned transversely, from the right ventricle and showing rounded shape were considered. Thus, we obtain a more uniform set of cardiomyocytes in all groups. The average cross-sectional areas obtained for each group were used as an indicator of cell size (Zornoff *et al*. 2006).

Picrosirius red-stained heart sections were observed under a polarized light microscope (Olympus BX51). Photomicrographs were analysed in Image Pro Plus software to quantify the percentage of non-fibril and fibril collagen in this tissue. The collagen percentage per area with dimensions $1360 \times 1024$ pixels was determined.

### *Stereological study*

The stereological study applied was the optical disector (Sterio, 1984). It was used to estimate the numerical density of cardiomyocytes (Nv[c]), determined by $Nv[c] = Q^{-}/t.A_T$ $1/mm^3$, where $Q^{-}$ is the number of cardiomyocyte nuclei seen in focus only in the look-up plane, t the thickness of the disector and $A_T$ the test area. The total number of cardiomyocyte nuclei per heart (N[c]) was estimated by the product of the numerical density of the cardiomyocyte nuclei by the previously measured volume of the heart (determined by Scherle's method) (Mandarim-de-Lacerda, 2003; Fernandes-Santos *et al*. 2009).

### *Statistical analysis*

All summary data for continuous variables were expressed as mean ± S.D. All analyses were performed with Instat software, in which groups were compared using variance analysis ANOVA. Values of $P \leqslant 0{\cdot}05$ were regarded as statistically significant (Zar, 1999).

## RESULTS

### *Histopathology study*

Histopathological examination of routine H&E cardiac sections revealed that infected control chow fed mice from the acute phase, compared to uninfected

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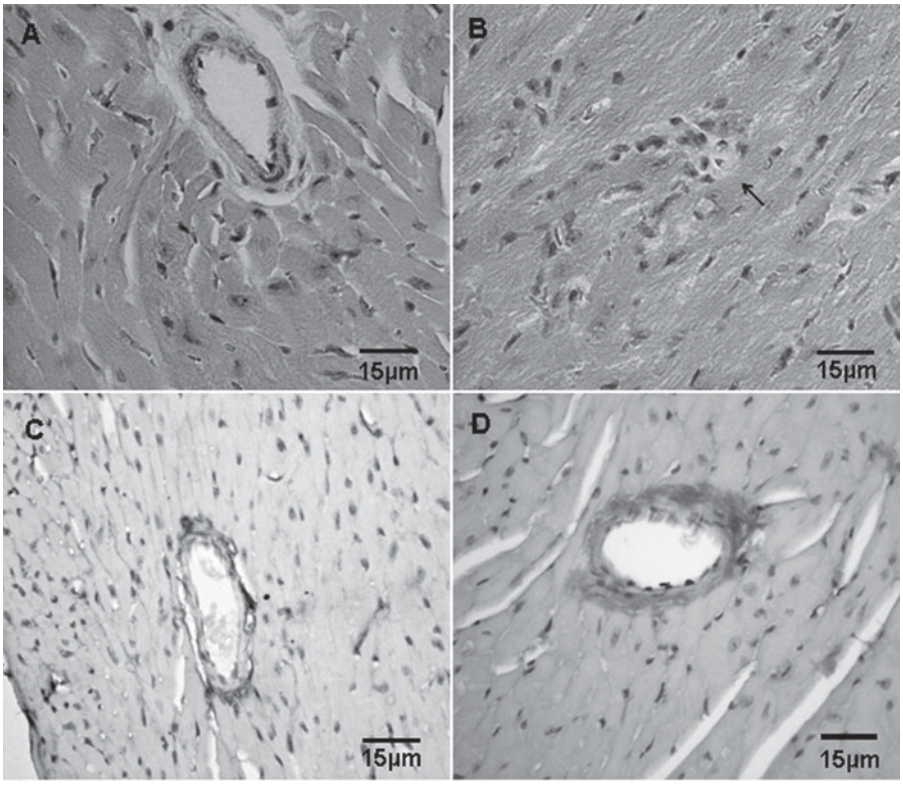


Fig. 1. Photomicrographs of the heart tissue in uninfected mice fed standard chow (A and C) and high-fat chow (B and D), stained with Haematoxylin-Eosin (A and B) and Picrosirius red (C and D). (A) Normal cardiac tissue of standard chow group (SC) with an intact blood-vessel. (B) High-fat group (HFC) with cardiac fibre loss and fibroblast proliferation (arrow). (C) Normal blood vessel with a thin collagen layer around of standard chow group. (D) Blood-vessel with a lot of collagen around of high-fat chow group. Scale bar = 15 $\mu$m.

exposed controls (Fig. 1A and C), showed myocarditis characterized by some inflammatory foci, disappearance of fibres and fibroblast proliferation (Fig. 2A and B). Animals from the chronic phase developed more severe inflammation and regenerative changes than those in the acute phase (Fig. 3A).

High-fat chow-fed mice showed cardiac fibre loss and fibroblast proliferation to perform local regeneration, but no inflammatory foci were detected. In addition, marked collagen deposition was observed surrounding intramyocardial vessels thicker than those analysed in uninfected standard chow-fed mice (Fig. 1B and D). All infected mice with high blood cholesterol from acute and chronic infection showed myocarditis characterized by a greater amount of inflammatory foci compared to standard chow-fed mice. Several areas displaying cardiomyocyte coagulation, extensive areas with fibre loss and fibroblast proliferation near blood vessels were identified in high-fat chow-fed mice. This group also presented inflammatory cells permeating the fibres (Fig. 2D-F and Fig. 3B). These animals contained a greater number of adipocytes deposited in tissue compared to their controls (Fig. 2C and Fig. 3C). A chronically high-fat infected mouse had an *S. mansoni* egg in its heart tissue, with extensive collagen deposition (Fig. 3D).

*Quantitative studies*

*Intramyocardial vessels*. High-fat fed infected mice from the acute phase (Table 1) displayed a narrower lumen diameter ($P = 0{\cdot}1895$) than those fed the standard chow. The medial vessel wall thickness was significantly thicker ($P = 0{\cdot}0046$) in infected mice than in controls, although in high-fat-fed infected mice it was 10·5% thicker than its control.

In the chronic phase of schistosomiasis (Table 2), lumen diameter ($P = 0{\cdot}8455$) and vessel wall thickness ($P = 0{\cdot}0573$) were not significantly different between infected groups and their controls, but the measurements were lower than those reported for the acute phase (Table 1). Even so, the blood vessel walls from infected animals were thicker than those of uninfected mice.

*Cardiomyocytes*. All high-fat chow-fed mice showed a significantly ($P < 0{\cdot}0001$) larger area than those fed a standard chow (Tables 1 and 2). In the acute phase (Table 1), the cardiomyocyte area from infected standard chow-fed mice was larger (+21·9%; $P < 0{\cdot}001$) than its control. Comparing infected high-fat chow-fed mice and their control, no significant difference was found. Uninfected high-fat chow-fed mice showed an area of cardiac cells that was

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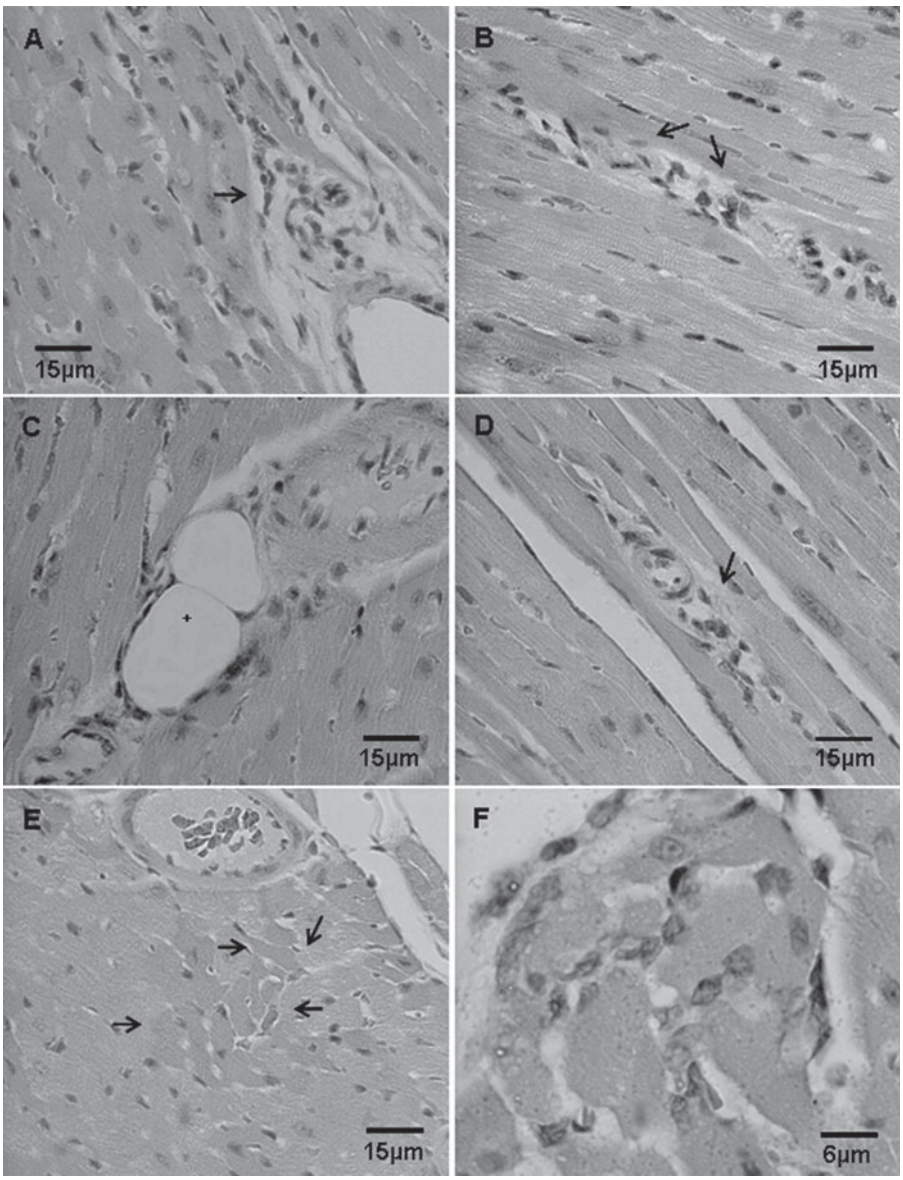


Fig. 2. Photomicrographs of the heart tissue in *Schistosoma mansoni*-infected mice fed standard chow (A and B) and high-fat chow (C-F) euthanized at acute phase, stained with Haematoxylin-Eosin. (A) Inflammatory focus (arrow) in standard chow group (ISCa). (B) Fibre loss and fibroblast alignment (arrows) in ISCa group. (C, D, E and F) High-fat chow group (IHFCa) with fat cell infiltration (+) (C); inflammatory focus (arrow) (D); coagulation of cardiac fibres (arrows) (E) and cardiac fibre loss and fibroblast proliferation (F). Scale bar = 15 $\mu$m (A-E) and 6 $\mu$m (F).

significantly (+40·7%; $P<0·001$) increased compared to that of those fed a standard chow diet. Infected high-fat chow-fed mice showed an area of cardiac cells that was significantly (+25·9%; $P<0·001$) increased compared to that of those fed a standard chow diet.

In the chronic phase (Table 2), it was observed that high-fat mice showed higher hyperplasia (+32%; $P<0·001$) than those fed on standard chow. Infected high-fat chow-fed mice showed a larger increased area (+29%; $P<0·001$) than the control.

*Collagen.* In the acute infection, infected standard chow-fed mice (+53%; $P<0·05$) and high-fat chow-fed mice (+38%; $P<0·01$) presented a higher amount of collagen per area than their controls. Uninfected high-fat chow-fed mice presented more collagen per area (+51%; $P<0·05$) than standard chow-fed mice, as well as infected high-fat chow-fed mice (+35%; $P<0·05$) compared to those infected and fed a control chow diet (Table 1).

In the chronic phase (Table 2), the amount of collagen in the cardiac tissue of all groups increased compared to the acute phase ($P=0·0024$). Infected standard chow-fed mice (+47%) and high-fat-fed mice (+2%) exhibited more collagen than the uninfected mice. Comparing the high-fat chow group to the control group, collagen was about 60% higher in uninfected and 25% higher in infected than in control chow-fed mice.

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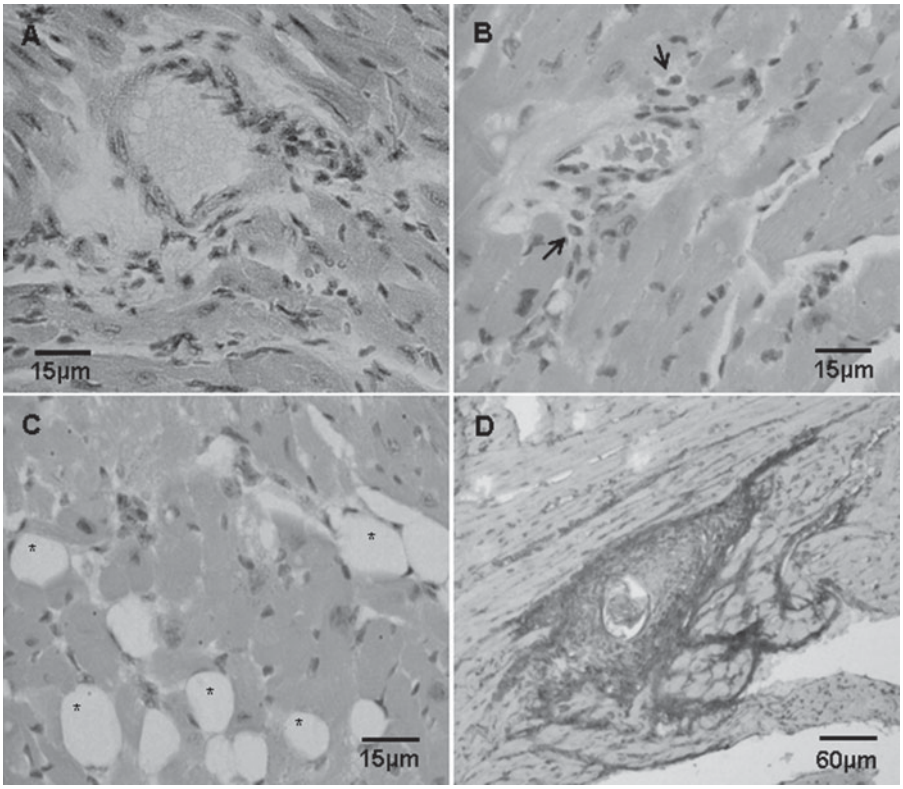


Fig. 3. Photomicrographs of the heart tissue in *Schistosoma mansoni*-infected mice fed standard chow (A) and high-fat chow (B-D) euthanized at chronic phase, stained with Haematoxylin-Eosin (A-C) and Picrosirius red (D). (A) Standard chow group (ISCc) with inflammatory cells, fibre loss and fibroblast proliferation. (B, C and D) High-fat chow group (IHFCc) with large inflammatory foci (B); more fat cells infiltrating the cardiac tissue (*) than in IHFCa (Fig. 2-C) (C); and presence of an egg of *S. mansoni* (D). Scale bar = 15 $\mu$m (A-C) and 60 $\mu$m (D).

*Stereological study*

All standard chow-fed mice from the acute infection showed a higher numerical density of cardiomyocytes (Nv[c]; $P<0{\cdot}0001$) and a higher total number of cardiomyocytes (N[c]; $P=0{\cdot}0104$) compared to animals fed high-fat chow (Table 1). Infected standard chow mice presented Nv[c] reduced ($-32{\cdot}6\%$; $P<0{\cdot}001$), whereas N[c] was ($-25{\cdot}6\%$; $P>0{\cdot}05$) reduced compared to control. Results from infected mice submitted to the high-fat chow were lower {(Nv[c] $-13{\cdot}7\%$; $P<0{\cdot}05$) and (N[c] $-2{\cdot}8\%$; $P>0{\cdot}05$)} than the uninfected control. Comparing infected high-fat chow-fed mice with standard chow-fed mice, we observed lower Nv[c] ($-28{\cdot}6\%$; $P<0{\cdot}001$) and N[c] ($-34{\cdot}8\%$; $P>0{\cdot}05$) compared to those from the control chow-fed mice.

In the chronic phase (Table 2), similar results to the previous phase were observed. Animals fed high-fat chow presented lower values of Nv[c] ($P=0{\cdot}0009$) and N[c] ($P=0{\cdot}0028$) compared to standard chow. Infected standard chow-fed mice presented lower Nv[c] ($-41{\cdot}9\%$; $P<0{\cdot}001$) and N[c] ($-41{\cdot}3\%$; $P<0{\cdot}001$) than the control group. Infected mice fed high-fat chow also showed Nv[c] reduced ($-21{\cdot}3\%$; $P>0{\cdot}05$) and N[c] decrease ($-13{\cdot}8\%$; $P>0{\cdot}05$) compared to uninfected mice. Comparing results from both chows, uninfected standard chow-fed mice presented higher N[c] ($+46\%$; $P<0{\cdot}001$) than the high-fat chow-fed group. Infected high-fat-fed mice presented reduced N[c] ($-20{\cdot}6\%$; $P<0{\cdot}001$) compared to those fed standard chow.

## DISCUSSION

Chow-induced models have proven beneficial for the evaluation of physiological changes that take place during the pathogenesis of diseases (Neves *et al*. 2006). Most cardiovascular research focuses on the knowledge of morphology and functionality of healthy versus sick hearts (Tang *et al*. 2009). Among other factors, poor nutritional conditions (Thone-Reineke *et al*. 2006) and fat-rich chows (Águila *et al*. 1998) are important causes of heart injury. This study was designed to answer some queries: do mice infected by *Schistosoma mansoni* display cardiac involvement? To answer that, histopathological, stereological and morphometric analyses were performed in acute and chronic murine schistosomiasis.

Our results clearly confirm cardiac involvement under experimental conditions. Even though *S. mansoni* adults and eggs are not commonly found in heart tissue, histopathological examination of cardiac sections revealed that infected mice fed

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Table 1. Quantitative data (mean ± S.D.) of acute schistosomiasis (euthanasia after 42 weeks of experiments and 9 weeks post-infection) and its controls in standard or high-fat chow-fed mice

(Nv[c], numerical density of cardiomyocytes; N[c], total number of cardiomyocytes; LD, lumen diameter; WT, wall thickness. (*) Significant differences between standard chow and high-fat chow; (♠) between high-fat chow and infected high-fat chow; (♦) between infected standard chow and infected high-fat chow; (◊) between standard chow and infected standard chow; (#) between all groups.)

| | Groups | | | | |
|---|---|---|---|---|---|
| Parameters | Standard chow | Infected standard chow | High-fat chow | Infected high-fat chow | *P* values |
| Vessels | | | | | |
| LD ($\mu$m) | 35·60 ± 22·42 | 37·74 ± 20·77 | 26·72 ± 18·43 | 29·12 ± 13·17 | 0·1895 |
| WT ($\mu$m) | 13·59 ± 5·28 | 18·68 ± 4·61 | 15·66 ± 3·73 | 17·50 ± 4·59 | 0·0046◊ |
| Myocytes | | | | | |
| Nv[c] ($\times 10^3$) | 3578 ± 145·35 | 2411 ± 107·4 | 1996·4 ± 106·08 | 1722 ± 148·6 | 0·0001# |
| N[c] ($\times 10^6$) | 646 ± 167·55 | 480 ± 170·39 | 322 ± 119·41 | 313 ± 90·98 | 0·0104* |
| Area ($\mu m^2$) | 430·28 ± 115·21 | 550·82 ± 137·43 | 742·92 ± 165·81 | 725·56 ± 190·31 | 0·0001◊*♦ |
| Collagen (%/area) | 0·60 ± 0·07 | 1·28 ± 0·19 | 1·23 ± 0·21 | 1·97 ± 0·55 | 0·0001◊*♦♠ |

Table 2. Quantitative data (mean ± S.D.) of chronic schistosomiasis (euthanasia after 50 weeks of experiment and 17 weeks post-infection) and its controls in standard or high-fat chow-fed mice

(Nv[c], numerical density of cardiomyocytes; N[c], total number of cardiomyocytes; LD, lumen diameter; WT, wall thickness. (*) Significant differences between standard chow and high-fat chow; (♠) between high-fat chow and infected high-fat chow; (♦) between infected standard chow and infected high-fat chow; (◊) between standard chow and infected standard chow; (#) between all groups.)

| | Groups | | | | |
|---|---|---|---|---|---|
| Parameters | Standard chow | Infected standard chow | High-fat chow | Infected high-fat chow | *P* values |
| Vessels | | | | | |
| LD ($\mu$m) | 30·88 ± 16·03 | 30·59 ± 18·63 | 29·26 ± 19·63 | 27·33 ± 12·26 | 0·8455 |
| WT ($\mu$m) | 13·68 ± 4·52 | 16·21 ± 6·14 | 14·80 ± 4·70 | 16·88 ± 4·51 | 0·0573 |
| Myocytes | | | | | |
| Nv[c] ($\times 10^3$) | 3226 ± 40·13 | 1874·2 ± 167·07 | 1744 ± 35·7 | 1373 ± 109 | 0·0009 |
| N[c] ($\times 10^6$) | 646 ± 7·76 | 379 ± 26·36 | 349 ± 7·05 | 301 ± 53·11 | 0·0028*◊♦♠ |
| Area ($\mu m^2$) | 494·31 ± 110·46 | 520·49 ± 137·91 | 727·32 ± 174·02 | 732·70 ± 151·26 | 0·001*♦ |
| Collagen (%/area) | 0·89 ± 0·36 | 1·67 ± 0·36 | 2·17 ± 0·52 | 2·21 ± 0·68 | 0·0024* |

control chow from both acute phase and chronic infection showed myocarditis. This was comprised of some inflammatory foci, disappearance of fibres, fibroblast proliferation and septal thickening of interstitial tissue among cardiomyocytes. Over the last several years, we have used design-based stereology that provides fundamental information on the structural changes in the pathological liver (Neves *et al*. 2006; Barros *et al*. 2009; Machado-Silva *et al*. 2010).

In this study, both lower numerical density and total number of cardiomyocytes were reduced in mice fed high-fat chow, compared with those fed normal chow. Furthermore, morphometry analysis confirmed that parasitized mice underwent cellular hypertrophy, whereas the wall thickness of intramyocardial vessels was increased. Even though heart damage during acute schistosomiasis mansoni is uncommon, myocarditis associated with or without granuloma and right ventricular insufficiency has been reported (Chisty *et al*. 1999; Ramanampamonjy *et al*. 2007). Recently, electrocardiography showed myocarditis as a complicating acute schistosomiasis in non-immune travellers returning from endemic areas (Epelboin *et al*. 2010). The mechanism to account for the myocarditis remains to be elicited.

In the present study, aged animals (around 12 months) had fewer cardiomyocytes than younger mice, suggesting that the ageing process facilitates cardiomyocyte apoptosis as seen by Águila *et al*. 1998. As the infection advances to chronicity, the host tries to delimit parasites or egg-induced cellular reactions (Borojevic, 1992), in which host responsiveness is diminished (Boros, 1989). Our study demonstrated that chronically-infected mice developed higher cardiac pathology than those in the acute phase. Previous studies showed that ageing

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of the infection leads to alterations in the hepatic vasculature due to the deposited eggs (Silva *et al*. 2006), which is associated with changes in the immunomodulation of liver granulomas (Alencar *et al*. 2009). In the current study, cardiomyocyte necrosis continues throughout the course of the disease, resulting in an accumulation of extracellular matrix (fibrosis). Previous studies have demonstrated human cases of endomyocardial fibrosis (Rashwan *et al*. 1995; Victor *et al*. 1996), a disorder characterized by collagen enhancement along the tissue and surrounding vessels.

Another issue that remains to be understood is the burden of cardiac involvement in *S. mansoni*-infected mice fed a high-fat chow diet. Feeding mice with a high saturated fat chow, as used in our study, increased cardiomyocyte apoptosis and adipocyte deposits within the heart tissue (Okere *et al*. 2006). The myocardium of spontaneously hypertensive rats displays morphological changes indicating tissue overload, such as myocyte loss, whereas the remainder undergo hypertrophy, reduced vascularization and ischaemia (perimysial and interstitial fibrosis) (Medeiros *et al*. 2005; Fernandes-Santos *et al*. 2009). Our results showed that high-fat chow caused cardiomyocyte loss, cellular hypertrophy, thickening of vessel walls, lumen diameter reduction and disruption of the cardiac tissue. Those data are consistent with the findings in rats (Águila *et al*. 1998; Águila and Mandarim-de-Lacerda, 2003) and obese mice (Pinheiro *et al*. 2007).

The heart injuries were worsened in all infected mice with high blood cholesterol from acute and chronic infection compared to mice fed standard chow. There was a greater amount of inflamed tissue, and reduction in the number and hypertrophy of cardiomyocytes. In addition, both necrosis events and cell death, resulting from fibre coagulation were increased. The increased number of fibroblasts that were permeating the coagulated and disappearing cardiomyocyte, led to increased collagen production throughout the tissue, characterizing ischaemic heart disease (Taube and Hutchins, 2008). Our quantitative morphology showed that mice from acute infection presented a higher collagen content and wall thickness than uninfected mice and those fed control chow. Fibroblast proliferation and collagen synthesis are essential to maintain the myocardium form and structure (Palaniyandi *et al*. 2009). Probably, excessive lipid accumulation in cardiomyocytes and infection may account for the cardiac disorder.

Though chronically-infected hosts have lower responsiveness than those from the acute infection, it may correspond to severe morbidity, given that enhanced collagen and fibrosis account for histopathological alterations (Boros, 1989; Borojevic, 1992). Furthermore, the long-term feeding of a high-fat chow has an effect on the outcome of chronic infection (Alencar *et al*. 2009). Microscopic examination from the chronically-infected high-fat chow group revealed severe cardiac damage due to myocarditis, extensive collagen content and fibrosis, which occurs at the site of the granulomatous inflammatory response and enhances the disease pathology (Boros, 1989). Quantification of numerical density and total number of cardiomyocytes showed significant reductions when comparing standard chow-fed mice euthanized at acute infection with those fed rich-lipid chow at chronic infection.

At this time, reparation of cardiac tissue is a strategy intended to promote the impairment in tissue structure, thereby improving associated functional derangements. Morphometry demonstrated that the collagen area was enhanced compared to control chow mice both at the acute and chronic infection phases. Otherwise, lumen diameter was reduced, while wall thickness was enhanced. Previous studies have demonstrated that adult worms significantly reduced atherogenesis in apolipoprotein E gene knockout mice (Doenhoff *et al*. 2002). The lumen vessel is bigger in infected, high-fat chow fed mice compared to uninfected mice. Recently, it was demonstrated that chronic exposure to schistosome eggs had no effect on atherosclerotic lesion development (La Flamme *et al*. 2007).

An egg was detected in the heart tissue. This notable feature provided by our data indicates that schistosome eggs can reach heart tissue without the associated hepatosplenomegaly and pulmonary hypertension (Warren, 1964; Lapa *et al*. 2009). These heart injuries may be associated with adult worms (Medeiros and Andrade, 1986) or toxic products from hepatic and intestinal granulomas (Rashwan *et al*. 1995; Carneiro *et al*. 2011) that reach cardiac tissue due to collateral circulation. Data from the literature suggest that *S. mansoni* eggs can be retained in successive thrombotic formations that reach the heart causing endomyocardial fibrosis in human patients (Carneiro *et al*. 2011). In conclusion, the collected data support the view that a double burden has a synergistic deleterious effect on myocardial tissue.

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