Changes in the small intestine of *Schistosoma mansoni*-infected mice fed a high-fat diet

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SUMMARY

The consumption of a high-fat diet modifies both the morphology of the small intestine and experimentally tested effects of schistosomiasis mansoni. However, whether a schistosomiasis infection associated with a high-fat diet causes injury to the small intestine has never been investigated. Mice were fed either a high-fat or a standard-fat diet for 6 months and were then infected with *Schistosoma mansoni* cercariae. Physical characteristics of the intestinal tissue (mucosal thickness, small intestinal villi length and height, and abundance of goblet cells and enterocytes on the villous surface) and the distribution of granulomas along the intestinal segments and their developmental stage were measured at the time of sacrifice (9 or 17 weeks post-infection). The group fed a high-fat diet exhibited different granuloma stages, whereas the control group possessed only exudative granulomas. The chronically infected mice fed a high-fat diet exhibited higher granuloma and egg numbers than the acutely infected group. Exudative, exudative/exudative-productive and exudative-productive granulomas were present irrespective of diet. Computer-aided morphometric analysis confirmed that villus length, villus width, muscular height and submucosal height of the duodenal and jejunal segments were affected by diet and infection. In conclusion, a high-fat diet and infection had a significant impact on the small intestine morphology and morphometry among the animals tested.

Key words: *Schistosoma mansoni*, experimental infection, gut, cholesterol, histopathology, morphometry, morphology.

INTRODUCTION

Schistosome eggs are responsible for both the progression of the life cycle of the parasite and the tissue pathology observed during infection. After being released into the mesenteric vasculature, immature eggs require approximately 5–6 days to achieve embryo differentiation and to begin secreting lytic and antigenic effectors through micropores in the egg shell (Andrade, 2009). At this time, mature eggs migrate across endothelial and mucosal barriers to the intestinal lumen, where they can be excreted into the external environment in the host's feces (Doenhoff *et al*. 1986).

Animal studies indicate that the consumption of a high-fat diet modifies the morphology of the small intestine (Little *et al*. 2008), gastrointestinal transit (de Wit *et al*. 2008), lipid metabolism, the cell cycle and the inflammatory and immune responses (Neves *et al*. 2007). Previous studies have shown that mice fed a high-fat diet significantly increased both trapped eggs and faecal egg output during the acute (Neves *et al*. 2007) and chronic phases of schistosomiasis (Alencar *et al*. 2009). Moreover, human studies have demonstrated a positive correlation between egg load and organ pathology (Cheever *et al*. 1977).

Despite these interesting observations, whether a schistosomiasis infection associated with a high-fat diet causes injury to the small intestinal morphology of mice has not been investigated. This study compares the histological features of the small intestine in mice fed a high-fat or a standard diet during the acute and chronic phases of schistosomiasis mansoni.

MATERIALS AND METHODS

Animals and dietary regimen

Three-week-old female Swiss Webster mice were obtained from the Laboratory Animal Breeding Center (Oswaldo Cruz Foundation, Rio de Janeiro, Brazil). The mice were conventionally housed in polypropylene cages (40×33 cm) with screened covers made of stainless steel. The mice were maintained on a 12-h light-dark cycle with water provided *ad libitum* at a controlled temperature (21±1 °C) and humidity (60±10%). All procedures were approved

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by the local Commission for Ethics in the Use of Animals (L-0036/07; CEUA-FIOCRUZ).

Animals were assigned to one of 2 nutritional groups for a 6-month period: (a) high-fat diet (47% carbohydrate, 24% protein, 29% lipid; 5·7 kcal/g body weight/day), or (b) standard laboratory diet for mice (Nuvilab CR-1-NUVITAL Nutrients Ltda., Colombo, Paraná, Brazil; 12% fat, 28% protein, 60% carbohydrate; 4·6 kcal/g body weight/day) (Neves *et al*. 2006). The lipid proportions were 354·0 g/kg saturated fatty acids, 500·0 g/kg mono-unsaturated fatty acids and 146 g/kg polyunsaturated fatty acids (Neves *et al*. 2007). Body weight was recorded twice per week throughout the experiment. All mice were allowed to consume water and food pellets *ad libitum*.

Plasma cholesterol measurement

After the 6-month feeding period, blood was collected from food-deprived mice (overnight) by puncturing the retro-orbital sinus. Plasma was separated by low-speed centrifugation and stored at −20 °C until use. The total serum cholesterol was determined using a previously described colourimetric enzymatic method (Neves *et al*. 2006). Lipid analysis was performed 1 day prior to experimental infection and again at 17 weeks post-infection.

Mouse infection procedures

Thirty mice were subcutaneously infected with approximately 50 *Schistosoma mansoni* (BH strain) cercariae (Martinez *et al*. 2003) shed from laboratory-reared *Biomphalaria glabrata* snails provided by the Malacology Laboratory (Oswaldo Cruz Institute, Rio de Janeiro, Brazil) (Paraense *et al*. 1989).

The animals were divided into 6 groups of 5 mice as follows: SC (uninfected mice fed standard chow), HFC (uninfected mice fed high-fat chow), IHFCa (infected mice fed high-fat chow and euthanized during acute infection), IHFCc (infected mice fed high-fat chow and euthanized during chronic infection), ISCa (infected mice fed standard chow and euthanized during acute infection) and ISCc (infected mice fed standard chow and euthanized during chronic infection).

Tissue processing and histopathology

The entire small intestine was collected from each mouse euthanized at 9 (acute) or 17 (chronic) weeks post-infection by cervical dislocation. The intestine was divided into 3 segments (proximal, middle and distal) of equal length (1·0 cm long). The fragments were processed routinely for histological preparation, embedded in paraffin, sectioned at a thickness of 5 μm, and stained with haematoxylin (Proquimios Comércio e Indústria Ltda, Brazil) and eosin (Vetec Química Sima Ltda, Brazil).

The intestinal mucosal or submucosal distribution of eggs and gut wall lesions (duodenum and jejunum) were analysed by light microscopy. Representative images were captured with an Olympus BX50 microscope (Tokyo, Japan) with a Nikon Eclipse E200 camera Nikon DS-Fi1 and Image Pro Plus software (Media Cybernetics, US). Tissues were evaluated for egg responses according to the predominant components as follows: exudative (E), exudative/exudative-productive (E/E-P), exudative-productive (EP) and productive (P) and percentage of confluent granulomas, which was expressed as a percentage of the total number of granulomas counted for acutely and chronically infected mice. An exudative stage does not fit the fundamental requirement of the granuloma concept: they do not produce a chronic inflammatory reaction. In the intermediate, E/E-P stages, granulomas are rich in eosinophils and monocytes and are surrounded by a disorganized zone rich in fibroblasts. In EP and P granulomas, a random reticular scaffold or mesh intermingled with the cells begins to appear, defining clear zones within the granuloma as follows: an inner, internal or paucifibrillar zone, which consists of macrophages with or without epithelioid transformation and, occasionally, giant cells; the middle or paracentral zone, which is rich in fibroblasts with or without mast cells; and the outermost or external zone, which, in the more chronic phase of the infection, is rich in lymphocytes (T and B) and plasma cells (Li-Hsü *et al*. 1972; Lenzi *et al*. 1998).

Morphometric measurements of mucosal thickness, small intestinal villi length (distance between the top of the villus and the upper edge of the muscularis mucosae) and height (distance between the top of the villi and the opening of the glandular crypts) and the abundance of goblet cells and enterocytes on the villous surface were made on digitally captured images (Nikon Eclipse E200 camera Nikon DS-Fi1) using Image Pro Plus software (Media Cybernetics, US).

Statistical analysis

The data were statistically analysed with GraphPad InStat (version 3.01). Groups were compared using a Student's t-test, Kruskal-Wallis test and ANOVA (Tukey post-test). *P*-values $\leqslant 0·05$ were considered significant.

RESULTS

Weight gain

Body mass significantly increased ($P = 0·003$) in all mice by week 36, irrespective of diet. Mice fed the high-fat diet exhibited a higher body mass (HFC (58 ± 9 g) and IHFC (59 ± 12 g)) than those fed standard chow (SC (43 ± 2 g) and ISC (39 ± 4 g)).

Table 1. Morphometric parameters in the duodena of mice fed a high-fat or standard diet (mean ± S.D.) (μm)

Experimental group	Villus width	Villus length	Muscular height	Submucosa height
SC	105 ± 20	396 ± 82	49 ± 19	29 ± 10
HFC	110 ± 46	368 ± 85	49 ± 18	41 ± 23
ISCa	106 ± 27	382 ± 77*	63 ± 22	73 ± 20
IHFCa	84 ± 14	273 ± 108	51 ± 20	53 ± 30
ISCc	82 ± 31	250 ± 29	110 ± 63	142 ± 20**

Letters indicate significant differences between values in each column ($P < 0.05$). *Significant difference between ISCa and IHFCa; **significant difference between ISCc and IHFCc.
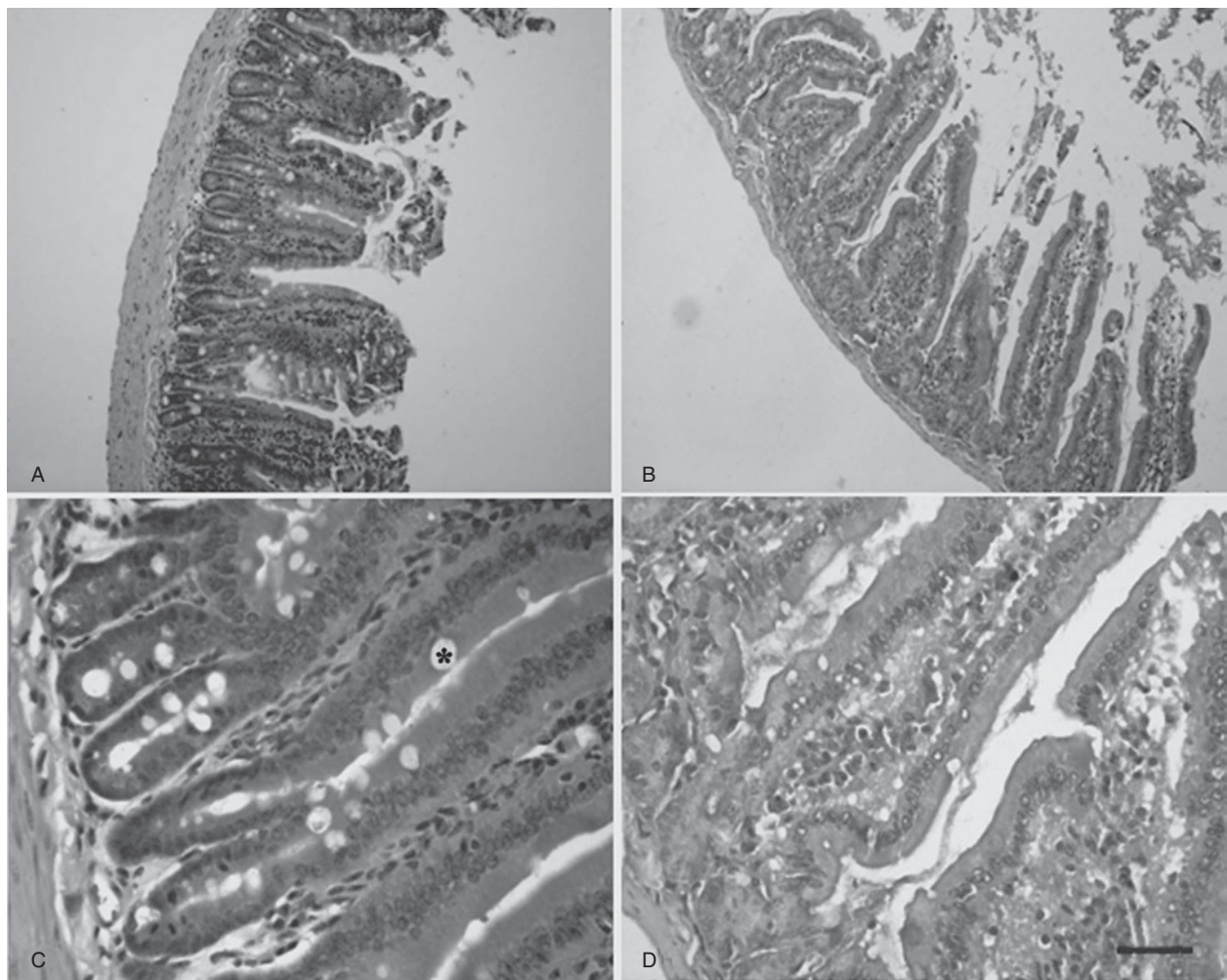

Fig. 1. Photomicrographs of normal intestines from mice fed a high-fat (A and C) or standard diet (B and D). (A) Transverse section of the duodenum demonstrating the reduction and widening of the villi; increase in muscular thickness caused by the increase in muscular fibres. The mucosa exhibits the increased size of goblet cells (*). (B) General features of normal jejunal villi. (C) Intestinal villi and hyperplasia of goblet cells (*). (D) Intestinal villi exhibiting normal appearance. Haematoxylin-eosin staining. (A and B = X10; C and D = X40).

Plasma cholesterol

SW mice fed a high-fat diet had significantly elevated plasma cholesterol before (HFC: 1·6–0·3; SC: 1·0–0·2; $P = 0.008$) and after infection (HFC: 1·2–0·1; IHFC: 1·2–0·3; SC: 0·7–0·2; ISC: 0·9–0·2; $P = 0.005$) compared with mice fed the standard diet.

Histoquantitative analysis

Changes in the small intestine of uninfected mice fed a high-fat diet. As shown in Table 1 and Fig. 1 (A,C), a high-fat diet substantially increased either the width of the duodenal villi (HFC, 110 ± 46 μm; SC, 105 ± 20 μm) or the height of the submucosa

Table 2. Morphometric parameters in the jejune of mice fed a high-fat or standard diet (mean ± S.D.) (μm)

Experimental group	Villus width	Villus length	Muscular height	Submucosa height
SC	77 ± 13	275 ± 60	49 ± 19	26 ± 7
HFC	84 ± 18	180 ± 38	49 ± 17	35 ± 6
ISCa	78 ± 12	269 ± 67	63 ± 22	59 ± 16
IHFCa	71 ± 12	334 ± 42	51 ± 20	58 ± 30
ISCc	68 ± 16	265 ± 38*	110 ± 63	122 ± 22

Letters indicate significant differences between values in each column ($P < 0.01$). *Significant difference between ISCc and IHFCc.

Table 3. Distribution (%) of schistosomal periovular and confluent granulomas in *Schistosoma mansoni*-infected SW mice fed a high-fat or standard diet during the acute and chronic phases of infection

(IHFCa, infected mice fed a high-fat diet euthanized during acute infection; IHFCc, infected mice fed a high-fat diet euthanized during chronic infection; ISCa, infected mice fed a standard diet euthanized during acute infection; ISCc, infected mice fed a standard diet euthanized during chronic infection; D, duodenum; J, jejunum; (—) absent.)

	Group							
	IHFCa		ISCa		IHFCc		ISCc	
Evolutionary stage	D	J	D	J	D	J	D	J
Exudative (E)	60	10	14	43	39	23	17	58
Exudative/exudative-productive (E/EP)	100	—	—	—	46	08	10	70
Exudative-productive (EP)	100	—	—	—	50	38	20	60
Confluent granuloma	81		19		68		32	

(HFC, 41 ± 23 μm; SC, 29 ± 10 μm). Villus length was reduced in mice fed a high-fat diet (HFC, 368 ± 85 μm) compared with the control (Fig. 1 B,D) (SC, 396 ± 82 μm). The muscular height was unchanged (HFC, 49 ± 18 μm; SC, 49 ± 19 μm).

Jejunal measurements are reported in Table 2. With respect to the jejunal segment, a high-fat diet induced a higher villus width (HFC, 84 ± 18 μm; SC, 77 ± 13 μm) and height of the submucosa (HFC, 35 ± 6 μm; SC, 26 ± 7 μm). Villus length was reduced in mice fed a high-fat diet (180 ± 38 μm) compared with those fed control chow (SC, 275 ± 60 μm). The muscular height was unchanged (HFC, 49 ± 17 μm; SC, 49 ± 19 μm). No statistical difference ($P > 0.05$) was observed between diet groups.

Histopathology

By week 9, mice fed a high-fat diet exhibited E, E/EP and EP granulomas, whereas in the control diet group, only E granulomas were found (Table 3). After week 17 of infection, mice fed a high-fat diet and control mice exhibited E, E/EP and EP ganulomas (Table 3; Fig. 3). Compared with acutely infected mice, chronically infected mice demonstrated a greater thickness of the mucosa and submucosa and shortened villi. The histological alterations were due to the presence of eggs and granulomas in the muscular, mucosal and submucosal layers (Fig. 2B, F).

The muscular layer was primarily uniform in appearance among all groups, whereas focal thickening was observed only in control diet-fed mice (Fig. 2B). Chronically infected mice fed a high-fat diet exhibited higher granuloma and egg numbers than the acutely infected group. The jejunal transverse histological section was within normal limits of muscular thickness and was only increased by periovular granuloma formation (Fig. 2D).

A comparison of the granuloma stage with diet revealed that they were more common in IHFCa and IHFCc than in ISCa and ISCc. Productive granulomas were not found (Table 3).

The percentage of granulomas in the duodenum was higher in the IHFC group compared with the ISC group, which had a greater abundance of granulomas in the jejune. The percentage of confluent granulomas was higher in the IHFC group, independent of the time of infection (Table 3). Eosinophils, macrophages and fibroblasts behave differently according to the evolutionary stage of the granuloma. Exudative granulomas exhibited a diverse cellular composition, consisting of a preponderance of eosinophils, a reduced number of neutrophils, few macrophages, epithelioid cells and giant cells (Fig. 3A). As the infection progressed to the chronic phase, there was less cellular diversity, irrespective of the group assayed. Fibroblasts, lymphocytes and macrophages were the predominant cells in both E/EP and EP granulomas.
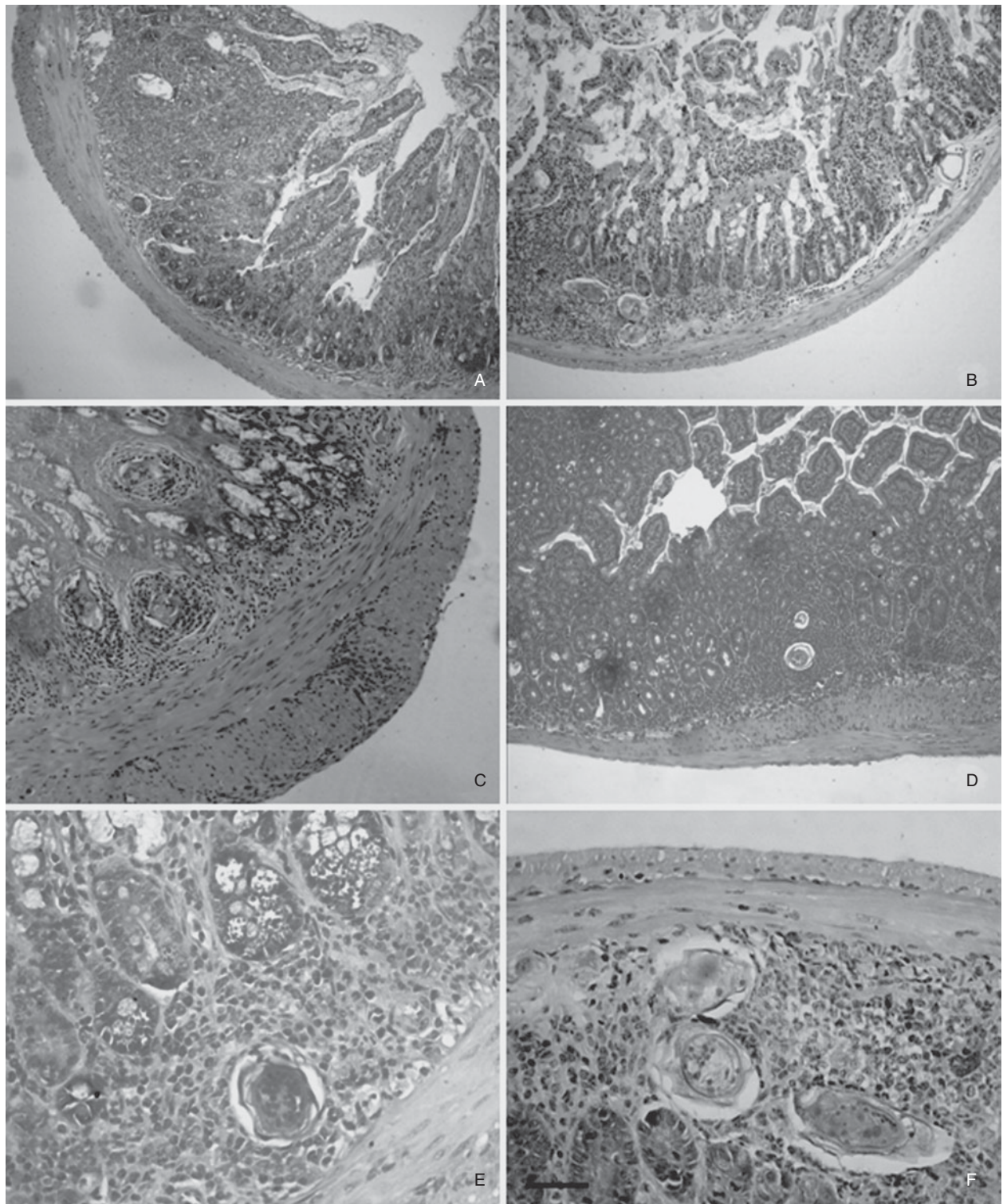
Fig. 2. Haematoxylin-eosin-stained sections of intestinal granulomas from acutely *Schistosoma mansoni*-infected mice fed a high-fat (A, C, E) or standard diet (B, D, F). (A) Transverse ileum section exhibits an increased muscle layer thickness caused by eggs within the mucosa and submucosa, polymorphonuclear infiltrates and hyperplasia of goblet cells (→). (B) Transverse duodenum section with thickening of the submucosal layer caused by schistosome eggs and mononuclear infiltrates. (C) Transverse section of jejune showing increased thickness of muscle layers, submucosa and mucosa due to the presence of lymphocytic and polymorphonuclear infiltrates; granulomas are present in the mucosa. (D) The histology of the transverse jejunal section was within normal limits of muscular thickness and only increased with periovular granuloma formation. (E) Detail of a granuloma of an egg in the mucosa with infiltrated polymorphonuclear cells. (F) Detail of an egg in the intestinal layer exhibiting infiltrated mononuclear and polymorphonuclear cells. (A, B, C, D=X10; E and F=X40).
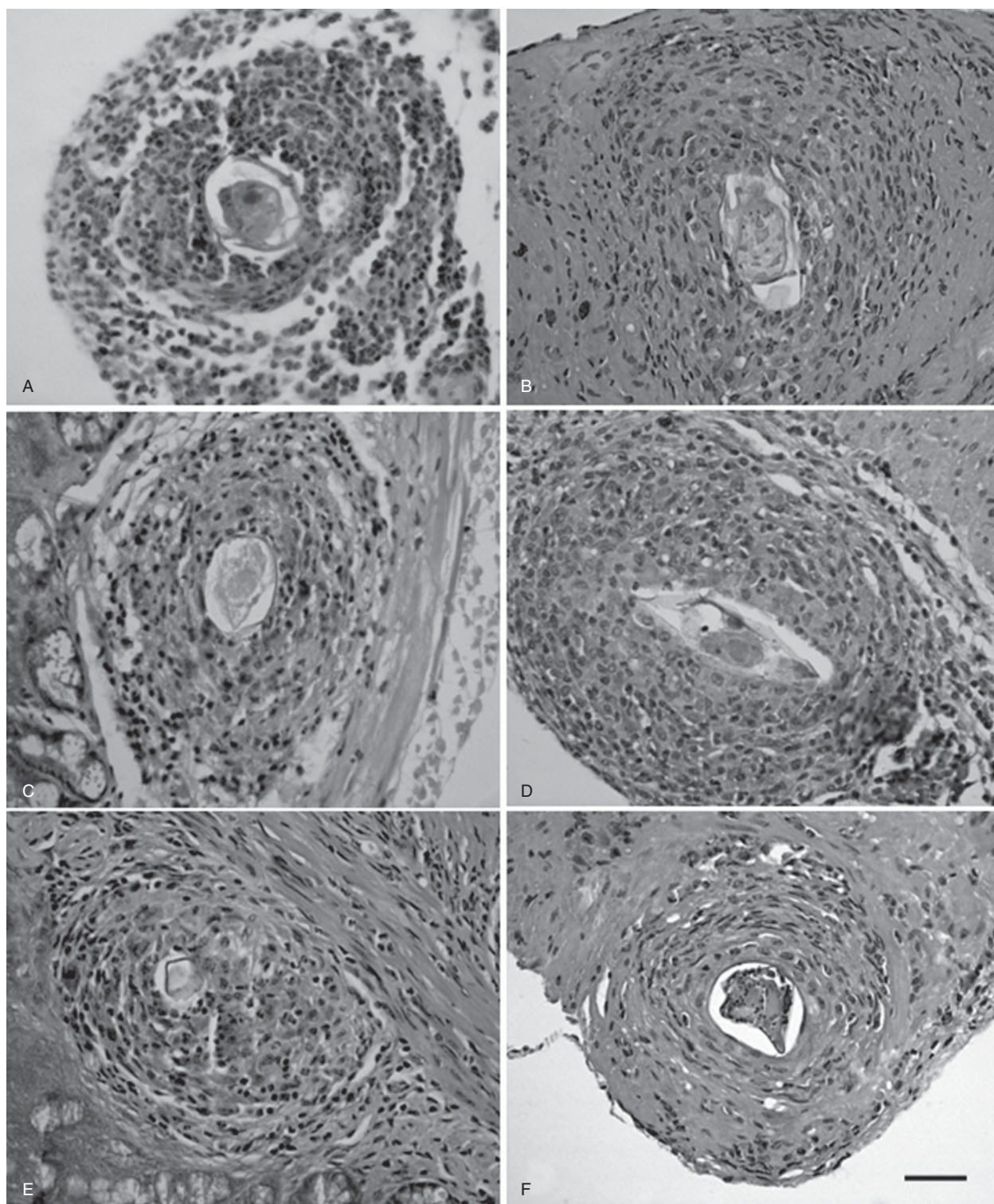
Fig. 3. Haematoxylin-eosin-stained sections of intestinal granulomas of chronically *Schistosoma mansoni*-infected mice fed a high-fat (A, C, E) or standard diet (B, D, F). Exudative granuloma located in the duodenal (A) and jejunal (B) mucosa; exudative/exudative-productive granuloma in the duodenal mucosa (C) and in the mucosa (D); exudative-productive in duodenum (E) and jejunum (F) mucosa (X40).

Changes in the small intestine of infected mice fed a high-fat diet. As demonstrated in Table 1, mice euthanized during acute infection exhibited a reduced duodenal villus width (IHFCa, $84 \pm 14\,\mu$m; ISCa, $106 \pm 27\,\mu$m; $P < 0.05$), villus length (IHFCa, $273 \pm 108\,\mu$m; ISCa, $382 \pm 77\,\mu$m), muscular height (IHFCa, $51 \pm 20\,\mu$m; ISCa, $63 \pm 22\,\mu$m) and height of the submucosa (IHFCa, $53 \pm 30\,\mu$m; ISCa, $73 \pm 20\,\mu$m). The villus length was reduced in mice fed a high-fat diet ($273 \pm 108\,\mu$m) compared with those that received control chow ($382 \pm 77\,\mu$m).

As indicated in Table 2, the jejunal villus width was slightly reduced (IHFCa, $71 \pm 12 \mu$m; ISCa, $78 \pm 12 \mu$m), the villus length was increased (IHFca, $334 \pm 42 \mu$m; ISCa, $269 \pm 67 \mu$m), the muscular height was reduced (IHFCa, $51 \pm 20 \mu$m; ISCa, $63 \pm 22 \mu$m) and the height of the submucosa was unchanged (IHFCa, $58 \pm 30 \mu$m; ISCa, $59 \pm 16 \mu$m).

During chronic infection, the duodenal villus width was slightly reduced (IHFCc, $76 \pm 9 \mu$m; ISCc, $82 \pm 31 \mu$m), the villus length was increased (IHFCc, $352 \pm 54 \mu$m; ISCc, $250 \pm 29 \mu$m), the muscular height was unaltered (IHFCc, $109 \pm 30 \mu$m; ISCc, $110 \pm 63 \mu$m) and the height of the submucosa was increased (IHFCc, $243 \pm 130 \mu$m; ISCc, $142 \pm 20 \mu$m; $P < 0.05$) (Table 1).

Regarding the jejunum, the villus width was reduced (IHFCc, $52 \pm 14 \mu$m; ISCa, $68 \pm 16 \mu$m; $P < 0.05$), the villus length was increased (IHFCc, $367 \pm 47 \mu$m; ISCc, $265 \pm 38 \mu$m), the muscular height was unaltered (IHFCc, $109 \pm 30 \mu$m; ISCc, $110 \pm 63 \mu$m) and the height of the submucosa was increased (IHFCc, $202 \pm 31 \mu$m; ISCc, $122 \pm 22 \mu$m; $P < 0.05$) (Table 2).

DISCUSSION

The morbidity observed in gastrointestinal schistosomiasis is linked to the deposition of eggs in the small intestine and their subsequent passage to the intestinal lumen, which largely depends on the host and its cellular immune response (Doenhoff *et al.* 1986; Andrade, 2009). This situation leads to a marked inflammation of the small intestine, which provokes structural and functional changes during both acute and chronic infection (Moreels *et al.* 2001).

Diet-induced models have proven beneficial for evaluating the physiological changes that take place during the pathogenesis of diseases (Neves *et al.* 2001). Most experimental studies on schistosomiasis have demonstrated that the host-parasite relationship may be modified by the host's nutritional status (Kanuft and Warren, 1969; Coutinho *et al.* 2002). This work aimed to evaluate whether a high-fat diet could affect the architecture of the intestine in *S. mansoni*-infected mice. The present findings confirm that the high-fat diet caused greater body weight and higher plasma cholesterol levels compared with mice fed a standard diet (Galloway *et al.* 2008; Stanley *et al.* 2009). Interestingly, *S. mansoni*-infected mice experienced a reduction in circulating cholesterol levels. Previous studies indicate that the anti-atherogenic effect of *S. mansoni* infection in mice was associated with the parasite-induced lowering of total blood cholesterol (Doenhoff *et al.* 2002). Studies in animal models provide evidence that schistosomes are closely associated with the modulation of lipid metabolism (Tallima and El-Ridi, 2005; Alencar *et al.* 2009), in which

cholesterol from the host's bloodstream is used for biological functions (Neves *et al.* 2006; Stanley *et al.* 2009).

The consumption of a high-fat diet modifies small intestinal morphology (Little *et al.* 2007, 2008), gastrointestinal transit, inflammation and the immune response (de Wit *et al.* 2008). In addition, the duodenum and proximal jejunum are the main sites for cholesterol absorption from the intestinal lumen (Balint *et al.* 1980). In this study, uninfected mice fed a high-fat diet exhibited similar changes in the duodenal and jejunal segments, where measurements of villus width and submucosa height were higher than in the control group; however, the villus length was reduced compared with the control. These alterations likely resulted from an inflammatory reaction (Xu *et al.* 2003; Weisberg *et al.* 2003), in which the accumulation of T-lymphocytes (Kintscher *et al.* 2008) could play an important role in the recruitment of macrophages (Nishimura *et al.* 2009) and in the regulation of the inflammatory response to bacterial antigen (Feuerer *et al.* 2009; Winer *et al.* 2009). The gut microbiota is implicated in a variety of host functions involving intestinal development and function, including digestion and absorption of carbohydrates, proteins and fats, immune modulation and gastrointestinal motility. Gut bacteria suppress the expression and release of fasting-induced adipose factor (Fiaf) from the small intestine, resulting in the increased activity of lipoprotein lipase (Backhed *et al.* 2004, 2007). It has been hypothesized that bacterial lipopolysaccharide (LPS) derived from gram-negative bacteria residing in the gut acts as a triggering factor linking inflammation to high-fat diet-induced metabolic syndrome. These results suggest that a high-fat diet increases endotoxaemia and affects the composition of the intestinal microbiota (Cani *et al.* 2007). In addition, bacterial interactions promote pro-inflammatory signalling in multiple cell types in the intestine (Ding *et al.* 2010). Studies in conventionally raised mice fed a high-fat diet indicate that changes in the intestinal microbiota and intestinal permeability may contribute to intestinal inflammation (Cani *et al.* 2007). In accordance with this, our results demonstrate an intestinal inflammatory response. It will be interesting to determine the composition of the gut microbiota in our diet-induced model in further studies.

An immunohistochemical analysis could help in characterizing the cellular composition and localization of the inflammatory response. Assessment of intestinal inflammation could be determined by immunofluorescence microscopy using antibodies against collagen I, collagen III, collagen IV, fibronectin, laminin and desmin (Silva *et al.* 2000). Alternatively, the infiltration of neutrophils (7/4), T lymphocytes (CD-3) and macrophages (F4/80) could be visualized by immunohistochemistry, and

the mRNA levels of markers of inflammation, including intercellular adhesion molecule-1 (ICAM-1), monocyte chemo-attractive protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2), could be measured. In addition, the levels of TNF-α, IL-6, IL-17A, and keratinocyte-derived cytokine (KC) could be assessed (Park *et al*. 2011).

A concomitant pathology could also compound the intestinal injury. Based on parasitological parameters, it has been previously demonstrated that the viability, maturity, and fecal excretion of eggs are much higher in mice fed a high-fat diet than those fed a standard diet during both acute (Neves *et al*. 2007) and chronic infections (Alencar *et al*. 2009).

Based on previous reports (Little *et al*. 2007; Kvietys *et al*. 1991), we expected that mice fed a high-fat diet could develop greater gut pathology than the control group. Although the role of granulomas in damaging tissue has been extensively studied (Domingo and Warren, 1969; Moreels *et al*. 2001; Stravitsky, 2004), researchers have also explored to some extent the tissue-protective potential of this inflammatory response. This observation supports the notion that a granulomatous response of diminished intensity is favourable to the host. Eggs deposited in tissues elicit an exudative response that can be characterized by a disorganized aggregation of cells as well as exudative-productive, productive, advanced or fibrotic-productive granulomas, which are morphologically organized (Lenzi *et al*. 1998).

Among the parameters examined, a difference in granuloma morphology was demonstrated according to each diet assayed and the time of infection. Mice fed a high-fat diet possessed granulomas at different stages of maturation, whereas the control group exhibited only exudative granulomas during the acute phase. Although modifications in the appearance of intestinal granulomas with time have not been previously reported (Silva *et al*. 2000), our results suggest differences that may be related to diet. Accordingly, previous results showed that eosinophils, macrophages and fibroblasts behave differently according to the evolutionary stage of liver granulomas in mice fed a high-fat or a standard diet (Neves *et al*. 2007). It is possible that the response induced by a high-fat diet may increase granuloma cell recruitment or be due to a higher intestinal egg load (Neves *et al*. 2007).

The current data show that egg and granuloma distribution within the gut wall confirm previous histological descriptions of acute (Borgers *et al*. 2000) and chronic infections (Holmes, 1990). In the acute phase, the jejunum segment exhibited more granulomas than the duodena of control mice, irrespective of the phase of infection. Evidence has been presented that adult worms have a realised niche (portion of the fundamental niche occupied within the host) for egg-laying (Holmes, 1990), which is the distal region of the small intestine in mice (de Lima and Katz, 1982; Martinez *et al*. 2003; Freire *et al*. 2003) and the large intestine in primate hosts (Nyindo and Farah, 1999). Previous studies hypothesize that the colonization of homoeotherms by vascular trematodes requires precision in egg laying near the conduit to facilitate the passage of the egg to the external environment (Platt and Brooks, 1997).

As the infection progressed to the chronic phase, reduced cellular diversity was observed, irrespective of the group assayed, which corresponds to the process of cellular organization (Lenzi *et al*. 1998). The data presented here show that mice fed a high-fat diet had higher granuloma and egg numbers than the acutely infected group. These findings raise some questions. First, animal (Rocha *et al*. 1995) and *in vitro* studies (Barth *et al*. 1996) have demonstrated that fecal egg excretion progressively declines during chronic infection. Although the mechanisms due to which the egg output declines are not well understood, some studies have correlated this decline with structural and functional changes in the host intestine (Moreels *et al*. 2001). In contrast, animal experiments have shown that older male and female schistosomes demonstrate age-induced morphological alterations in the reproductive system (Cheever *et al*. 1994; Machado-Silva *et al*. 2010). Second, previous studies have demonstrated that there is no tendency for egg output to decline among chronically infected mice fed a high-fat diet (Alencar *et al*. 2009). The most straightforward interpretation of this finding is that cholesterol may induce higher egg production among adult females, consistent with our previous report (Neves *et al*. 2007). Cholesterol represents a key node in cellular physiology because it is involved in membrane fluidity, the regulation of membrane traffic, signalling pathways and reproduction. *Caenorhabditis elegans* is a widely used model to study cholesterol distribution in living worms. The accumulation of lipids in oocytes and spermatozoa has been observed in this system, which suggests a possible role for cholesterol in sperm development (Matyash *et al*. 2001). Given that schistosomes do not synthesize cholesterol *de novo*, male and female worms could be maintained in medium containing radio-isotope labelled cholesterol (Rumjanek and Simpson, 1980). We suggest that an imaging assay based on fluorescence microscopy could be used to address the location of the labelled cholesterol (Matyash *et al*. 2001).

Considering the paucity of morphometric data on the mucosa of the small intestine of schistosomiasis-infected mice fed a high-fat diet, our histoquantitative results showed that villus, muscle and submucosa measurements were significantly modified. Concurrent infection with *S*. *mansoni* significantly altered the aforementioned anatomical sites mainly during chronic infection, irrespective of diet and the intestinal segment. Our results suggest that morphological alterations begin by week 9 and are aggravated

by week 17, consistent with other studies of intestinal pathology (Borgers *et al.* 2000; Couto *et al.* 2002; Siqueira *et al.* 2010). In this study, the damage to the intestinal tissue could be the result of inflammation (Trobojević-Stanković *et al.* 2010) due to the higher number of eggs trapped in the intestine in mice fed a high-fat diet (Neves *et al.* 2007, Alencar *et al.* 2009). In conclusion, a high-fat diet and schistosomiasis infection had a significant impact on the architecture of the small intestine.

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