

The outcome of acute schistosomiasis infection in adult mice with postnatal exposure to maternal malnutrition

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*Maternal malnutrition during the lactation period in early development may have long-term programming effects on adult offspring. We evaluated the combined effects of parasitological behaviour and histopathological features and malnutrition during lactation. Lactating mice and their pups were divided into a control group (fed a normal diet of 23% protein), a protein-restricted group (PR) (fed a diet containing 8% protein) and a caloric-restricted group (CR) (fed according to the PR group intake). At the age of 60 days, the offspring were infected with *Schistosoma mansoni cercariae* and killed at nine weeks post-infection. Food intake, body and liver masses, leptinaemia, corticosteronaemia, collagen morphometry and neogenesis and the cellular composition of liver granulomas were studied. PR offspring showed reduced weight gain and hypophagia, whereas CR offspring became overweight and developed hyperphagia. The pre-patent period was longer (45 days) in both programmed offspring as compared to controls (40 days). The PR-infected group had higher faecal and intestinal egg output and increased liver damage. The CR-infected group showed a lower number of liver granulomas, increased collagen neogenesis and a higher frequency of binucleate hepatocytes, suggesting a better modulation of the inflammatory response and increased liver regeneration. Taken together, our findings suggest that neonatal malnutrition of offspring during lactation affects the outcome of schistosomiasis in mice.*

Key words: *Schistosoma mansoni* - programming - lactation - host-parasite relationship - parasitology - histopathology

In developing countries, both malnutrition and parasite infections remain major public health concerns (WHO 2005, Atinmo et al. 2009). Epidemiological data have shown detrimental effects of malnutrition and infection on health, cognition and behaviour in preschool-aged children affected with concurrent morbidities (Stephenson et al. 2000, Crompton & Nesheim 2002, Muniz et al. 2002, Hughes & Kelly 2006, Casapia et al. 2007, Jardim-Botelho et al. 2008). Previous studies in Northeastern Brazil have demonstrated that schistosomiasis *mansoni* and malnutrition often overlap (Coutinho et al. 1997) and the infection exerts a negative effect on the anthropometric status of school-aged children, even during low-moderate levels of infection (Parraga et al. 1996, Assis et al. 1998).

Epidemiological, clinical and experimental data suggest that intrauterine undernutrition is closely associated with adulthood obesity and is related to detrimental metabolic sequelae (Godfrey & Barker 2000, Vickers et al. 2001), which has given rise to the concept of “developmental origins of health and disease”. This association is referred to as metabolic programming, which is defined as a biological phenomenon that determines the relationship between physical and chemical stimuli in critical periods of early life, such as gestation and/

or lactation, with future functional status (de Moura et al. 2008). Among children, protein-energy malnutrition is the most prevalent nutritional disorder and often occurs during gestation, lactation and the first two years of life (Desai et al. 2000). In fact, experimental data have reported that maternal malnutrition during the critical period of lactation may have deleterious effects during the adult life of the offspring, even if the animal had free access to a normal diet after weaning (Passos et al. 2000, 2004, Vicente et al. 2004, Fagundes et al. 2007, 2009, Moura et al. 2007, Lisboa et al. 2008).

Nutritional changes associated with lactation influence the host-parasite relationship of gastrointestinal roundworms *Heligmosomoides polygyrus* (Kristan 2002) and *Nippostrongylus brasiliensis* in the rat model (Normanton et al. 2007). Previous experimental studies have established that protein restriction post-weaning impairs the outcome of experimental schistosomiasis *mansoni* in adult mice (Akpom 1982, Rocha 1982, Coutinho et al. 1991, 2003, Ferreira & Coutinho 1999). However, no study has explored the joint effects of postnatal malnutrition on the outcome of schistosomiasis. Therefore, it is important to determine whether malnutrition during the lactation period only can affect adult offspring infected by *Schistosoma mansoni*. The present study used a murine model of nutritional programming in which lactating mice were submitted to a protein-restricted (PR) or caloric-restricted (CR) diet throughout lactation. Weaning pups received a normal diet. The effects of acute schistosomiasis in terms of weight gain, food intake, visceral fat mass, egg production in the stool, tissue egg count, worm burden and histopathology were evaluated.

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MATERIALS AND METHODS

Swiss Webster mice were housed in polypropylene boxes (40 × 33 cm) in a room with controlled temperature (25 ± 1°C) and humidity (60 ± 10%) and an artificial light/dark cycle (lights on from 07:00 am-19:00 pm). Two three-month-old, virgin, female mice were housed together with a male mouse for mating. After mating, each female was placed in an individual cage with free access to water and food until delivery.

Model of programming by maternal malnutrition during lactation - Mouse dams were randomly assigned to one of the following three groups: (i) control (C) (n = 10), allowed free access to a standard laboratory diet (23% protein), (ii) PR (n = 10), allowed free access to an isoenergetic, low-protein diet (8% protein) and (iii) CR (n = 10), received a standard laboratory diet in restricted quantities that were calculated according to the mean ingestion of the PR group (a pair feed group). Twenty-four hours after birth, litters were culled to six male pups per dam to maximise lactation performance.

Table I shows the composition of the diets (Reeves et al. 1993). The PR diet was produced in our laboratory, using the standard commercial diet for rodents (Nuvilab-Nuvital Nutrientes SA, PR, Brazil) by replacing parts of the protein with cornstarch (Lisboa et al. 2003, de Moura et al. 2007, Fagundes et al. 2009). The amount of cornstarch that was added compensated for the decrease in energy content due to protein reduction. Vitamin and mineral mixtures were formulated according to the AIN-93G recommendation for rodent diets (Reeves et al. 1993).

Neonatal malnutrition began at the time of the pup's birth (day 0) and ended at weaning (21 days). After weaning, offspring were fed a standard diet (23% protein) until they reached 120 days of age. We used two offspring randomly chosen from each litter per group (total of 20 pups/dam group).

Nutritional evaluation - Body weight and food consumption were monitored every four days from the time of weaning until the pups were 120 days old. The visceral fat mass (VFM) (mesenteric, epididymal and retroperitoneal adipose tissue) was excised and weighed for the evaluation of central adiposity. Fat mass was determined by carcass analysis as reported previously (Fagundes et al. 2007).

Blood samples were centrifuged (1,500 g/20 min/4°C) to obtain serum, which was frozen (-20°C) until assaying. Leptinaemia was determined with a specific radioimmunoassay kit (Linco Research Inc, Missouri, USA) that measures both rat and mouse leptin with a range of detection from 0.5-50 ng/mL. Measurements were performed in a single assay with an intra-assay variation of 2.9%. Corticosteronaemia was measured using a specific commercial RIA kit (ICN Biomedicals Inc, Aurora, OH, USA) with an assay sensitivity of 50 ng/mL and an intra assay variation coefficient of 7%.

Parasites and experimental infection protocol - An active life cycle of *S. mansoni* has been maintained at the Malacology Laboratory (Oswaldo Cruz Institute, Rio de Janeiro, Brazil) since 1985, using *Biomphalaria glabrata* snails and mice as intermediate and definitive hosts, respectively. Procedures for experimental infections have

TABLE I
Composition of the diets

	Control diet ^a (g/Kg)	Low-protein diet ^b (g/Kg)
Ingredients		
Soybean + wheat	230.0	80.0
Cornstarch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mix ^c	4.0	4.0
Mineral mix ^c	40.0	40.0
Macronutrient composition (%)		
Total energy (kJ/kg)	17,038.7	17,038.7
Protein	23	8
Carbohydrate	66	81
Fat	11	11

a: standard diet for rats (Nuvilab-Nuvital Nutrientes SA, PR, Brazil); *b*: the low-protein diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amount of the latter ingredient was calculated to make up for the decrease in energy content due to protein reduction; *c*: vitamin and mineral mixtures were formulated to meet the AIN-93G recommendation for rodent diet and contain the recommended amount of iodine (Reeves et al. 1993).

been described previously (Freire et al. 2003). At two months old, mouse pups were divided in six groups, according to diet: uninfected C, uninfected PR, uninfected CR, infected C (IC), infected PR (IPR) and infected CR (ICR). Infections consisted of 50 *S. mansoni* cercariae (BH strain) and were administered percutaneously. Ten animals were included in each group.

Parasitological studies - Faecal samples from infected groups were microscopically examined after six weeks to confirm that an infection was established. The mean number of eggs per gram was estimated from two fresh faecal samples, collected at two day intervals from six weeks until the time of euthanasia using the Kato-Katz smear technique (Katz et al. 1972). The pre-patent period was defined as the time when the first faecal eggs were found (Martinez et al. 2003). Infected mice were euthanized by cervical dislocation at 24 h after the last faecal examination. Parasite burden (infectivity) was calculated as the percentage of cercariae recovered from the portal system and mesenteric veins that had matured into adult worms (Freire et al. 2003).

Tissue egg load - To enumerate tissue eggs, the small intestine was digested in 4% potassium hydroxide at 56°C and centrifuged (900 g) for 5 min (Neves et al. 2007b). Aliquots of 100 µL were placed in duplicate on a glass slide and counted by light microscopy (100X) (Martinez et al. 2003).

Oogram pattern - Two segments (1 cm each) from different sites in the small intestine (proximal and distal sections) and large intestine of infected animals were

TABLE II
Biometrical parameters and serum leptin in schistosomiasis-infected adult male mice whose mothers were fed with control (C), caloric-restricted (CR) or protein-restricted (PR) diet during lactation

	Groups					
	C Uninfected	CR Uninfected	PR Uninfected	C Infected	CR Infected	PR Infected
Food intake (g)	9.30 ± 0.32	10.89 ± 0.30 ^b	6.59 ± 0.24 ^{b,c}	9.01 ± 0.33	9.70 ± 0.44 ^a	6.01 ± 0.39 ^{b,c}
Body weight (g)	33.80 ± 1.29	38.80 ± 0.55 ^b	27.30 ± 1.12 ^c	31.80 ± 1.29	35.20 ± 0.62 ^{a,b}	27.30 ± 1.12 ^{b,c}
Liver weight (g)	1.62 ± 0.04	1.60 ± 0.03	1.61 ± 0.05	2.14 ± 0.06 ^a	2.11 ± 0.07 ^a	1.87 ± 0.11 ^a
Visceral fat mass	1.70 ± 0.18	2.13 ± 0.40 ^c	0.993 ± 0.17	1.46 ± 0.22	1.28 ± 0.19 ^a	1.48 ± 0.31
Fat mass	8.30 ± 0.64	12.07 ± 1.06 ^b	14.13 ± 1.52 ^b	8.41 ± 0.81	5.56 ± 0.28 ^a	8.62 ± 0.51 ^a
Leptin	3.21 ± 0.43	6.92 ± 1.47 ^b	4.57 ± 1.37	3.35 ± 0.77	1.99 ± 0.35 ^a	4.24 ± 0.58
Corticosterone	54.1 ± 8.0	69.2 ± 8.9	62.1 ± 13.1	32.8 ± 6.3	90.3 ± 18.8 ^a	65.2 ± 13.5

a: infected vs. uninfected; b: vs. C ; c: vs. CR or PR. Data presented as means ± standard error of the mean (significant difference: $p < 0.05$).

removed (Machado e Silva et al. 1991). Segments were opened, washed and crushed between two glass slides to obtain a thin preparation. The percentage of immature, mature and dead *S. mansoni* eggs was determined by conventional light microscopy (Freire et al. 2003).

Histopathology and morphometry of *S. mansoni* granulomas - Liver, intestine and VFM were excised and weighed. Liver samples from all mice were fixed in 10% buffered formalin and processed for routine histopathological analysis. Five-micrometre sections were stained with haematoxylin and eosin, Masson's trichrome or Picrosirius for collagen plus polarisation microscopy (Lennert & Parwaresch 1978). Periocular reactions in the liver were classified according to Li Hsü et al. (1972) as modified by Lenzi et al. (1998). Reactions were classified as the pre-granulomatous stage (weakly reactive or non-reactive and exudative stages) or the granulomatous stage (exudative-productive, productive and involutinal granulomas). The area, perimeter and major and minor diameter of individual granulomas were measured by computed image analysis (Image Pro-Plus Media Cybernetics, US), as described previously (Costa-Silva et al. 2002).

Statistical analysis - Results are expressed as mean values ± standard error of the mean. The GraphPad Prism 4 programme (GraphPad Software, Inc, La Jolla, CA, USA) was used for statistical analyses and graphics. Body weight, food intake and parasitological results were analysed by one-way analysis of variance and Newman-Keuls multiple comparison tests. Other experimental data (infected vs. uninfected group) were analysed by Student's unpaired *t* test and differences were considered significant at $p < 0.05$.

Ethics - The use of the animals according to our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/232/2008), which based its analysis on the principles adopted and promulgated by Brazilian Law 11.794/2008 (Marques et al. 2009).

RESULTS

Effect of maternal malnutrition - At the end of lactation, mothers from the CR (30 ± 1.99 g) and PR (24.05 ± 0.38 g) groups had significantly ($p < 0.0001$) lower body mass compared to the C group (42.55 ± 2.01 g). There was also a significant difference in the weight of their offspring (C: 12.5 ± 0.18 g; CR: 11.3 ± 0.10 g; PR: 5.9 ± 0.12 g).

At 120-days-old (Table II), food intake was 17% higher in uninfected-CR pups compared to C pups ($p < 0.01$), but decreased in uninfected-PR pups (-29% vs. C; -39% vs. CR, $p < 0.001$). In uninfected animals, we observed that the PR offspring had a 19% reduction in body mass compared to C pups ($p < 0.01$), whereas the CR offspring had a 15% greater body mass compared to C pups ($p < 0.05$) and a 53% increase in VFM compared to the PR pups ($p < 0.05$). Compared to the C pups, the total body fat was 45% and 70% greater in CR ($p < 0.05$) and PR ($p < 0.001$) offspring, respectively, but only the CR offspring presented hyperleptinaemia (+115% vs. C).

Serum corticosterone and liver weight of uninfected C, CR and PR offspring was not significantly different (Table III).

Effect of infection - At the time of sacrifice (9 weeks post-infection), the infection resulted in a lower body mass (-9%, $p < 0.001$), lower food intake (-11%, $p < 0.05$) and lower VFM (-40%, $p < 0.05$) only in the infected-CR group compared to its respective uninfected group. The infection did not alter body mass, food intake or VFM in the PR group.

The IPR and ICR groups had a 39% and 54% decrease, respectively, in body fat mass compared to the CR and PR groups ($p < 0.001$). The ICR group showed lower levels of serum leptin (CR vs. ICR: -71%). As depicted in Table II, the IRC offspring showed higher serum corticosterone (+175%, $p < 0.05$) compared to the IC offspring.

The infection was associated with higher liver weight of all offspring compared to their respective C group (C vs. IC: +32%; CR vs. ICR: +32%, $p < 0.0001$; PR vs. IPR: +16%, $p < 0.05$) (Table II).

TABLE III
Parasitological parameters in schistosomiasis-infected adult male mice whose mothers were fed with control (C), caloric-restricted (CR) or protein-restricted (PR) diet during lactation

	Groups			p values
	C	CR	PR	
Pre-patent period (days)	40 ± 2	45 ± 2	45 ± 2	0.0010 ^a
Faecal egg count (total)	271.0 ± 69.0	172.0 ± 45.0	5424.0 ± 114.0	0.0140 ^a
Tissue egg load/section				
Proximal	1172.0 ± 290.0	319.0 ± 120.0	1130.0 ± 454.0	0.1227
Distal	1755.0 ± 286.0	962.0 ± 494.0	2029.0 ± 440.0	0.1923
Cecum	733.0 ± 246.0	183.0 ± 63.0	762.0 ± 177.0	0.0515 ^a
Egg developmental stages (oogram)				
Mature	39.0 ± 6.0	9.0 ± 1.0	62.0 ± 16.0	0.0123 ^a
Immature	221.0 ± 32.0	77.0 ± 15.0	300.0 ± 48.0	0.0004 ^a
Dead	9.0 ± 2.0	3.0 ± 1.0	26.0 ± 7.0	0.0058 ^a
Infectivity	50	52	77	0.4672

a: significant difference ($p < 0.05$). Data presented as means ± standard error of the mean.

Parasitological studies - As shown in Table III, schistosome eggs were first detectable in IC offspring and then simultaneously in ICR and IPR. IPR offspring eliminated a greater number of faecal eggs than the IC (211% increase) or ICR (600% increase) offspring. The infectivity and tissue egg load in the proximal and distal sections of the small intestine were not statistically different, despite differences in maternal diets. In the caecum section, the egg count was decreased by 75% in the ICR offspring compared to the IC offspring. Oogram evaluation revealed that the ICR offspring had a 65% decrease in the number of immature eggs compared to the IC offspring whereas the IPR offspring had a 288% increase compared to the ICR offspring. We observed a 561% increase in the number of mature eggs in the IPR offspring compared to the ICR offspring, as well as an increase in the number of dead eggs (167% increase vs. IC and 803% increase vs. ICR).

Histopathology and morphometry - Concerning the programming effect, the PR offspring exhibited normal liver structures in terms of aspects and trabecular architecture. However, CR offspring showed microvesicular steatosis.

The qualitative cellular composition of liver granulomas was similar among offspring, with accumulations of inflammatory cells (macrophages, eosinophils and lymphocytes) differing according to the type of granuloma evolution. In exudative granulomas, there were macrophages, eosinophils, neutrophils and lymphocytes (Table IV). However, exudative-productive granulomas were predominantly composed of fibroblasts and macrophages rather than lymphocytes, plasma cells and eosinophils (Table IV).

Morphometric analysis (Table IV) showed that mean area of exudative granulomas was 86% smaller in the ICR offspring and 82% larger in IPR offspring. However, exudative-productive granulomas were larger in both IPR (81% increase) and ICR (63% increase) offspring

compared to IC offspring. Collagen neogenesis was observed in some exudative granulomas from IC (14.93%), IPR (20.33%) and ICR (25.53%) groups.

Histopathological examination of C and PR offspring showed normal portal space and liver parenchyma (in terms of hepatocytes and sinusoids arrangement) (Figs 1A, 2A).

Regarding IC offspring, eggs elicited both monocyte and polymorphonuclear leucocyte migration (Fig. 1B). Fibrotic changes within the granulomas were highlighted by Masson's trichrome stain (data not shown). Photomicrographs of liver sections showed exudative granulomas with a central egg surrounded by a prominent disarranged cellular composition in the outer layer (Fig. 1C).

IPR offspring showed leucocyte infiltrate that was not associated with the presence of a schistosomal granuloma (Fig. 2B). Schistosome eggs elicited an intense acute inflammatory reaction that was characteristic of a pre-granulomatous type granuloma (data not shown) and exudative peri-ovular lesions surrounding a central schistosomal egg (Fig. 2C). At the cellular level, PR offspring presented pyknosis, karyorrhexis and karyolysis (data not shown).

Microvesicular steatosis was observed both in the liver parenchyma (Fig. 3A) and hepatocytes (data not shown) of ICR offspring. Intense leucocyte infiltrate with collagen neogenesis and exudative-productive granuloma with regular external contour was observed (Fig. 3B). Schistosomal granulomas showing a concentric arrangement of reticular fibres (Fig. 3C) and liver regeneration characterised by binucleate hepatocytes were also evident (data not shown).

DISCUSSION

Lactation is a critical period during the development of mammals and is important in the establishment of programming (Moura et al. 2008). Mice from CR and PR mothers had lower body weight during lactation. After

TABLE IV

Morphometry and cellular composition of liver granulomas in schistosomiasis-infected adult male mice offspring whose mothers were fed with control (C), caloric-restricted (CR) or protein-restricted (PR) diet during lactation

Granulomas	Groups					
	C		CR		PR	
	E (n = 60)	EP (n = 48)	E (n = 08)	EP (n = 122)	E (n = 303)	EP (n = 63)
	Morphometry					
Area of granuloma (μm^2)	42.25 \pm 74.52	59.50 \pm 66.27	5.94 \pm 5.94	96.76 \pm 14.16	76.97 \pm 14.37	108.02 \pm 97.72
Concentration of collagen (%)	-	14.93	-	25.53	-	20.33
	Cellular composition					
Macrophages	++	++	++	+++	+	++
Epithelioid cells	\pm	+	-	++	-	+
Giant cells	-	+	-	+	-	-
Neutrophils	+	+	+	+	+++	+
Eosinophils	++	+	++	+	+++	++
Mast cells	-	-	-	-	-	-
Fibroblasts	\pm	++	+	+++	-	++
Lymphocytes	++	+	++	+	++	+
Plasma cells	\pm	+	\pm	+	-	+
Pigment	+	+	+	+	+	+

E: exudative granulomas; EP: exudative-productive; grade of intensity: -: negative; \pm : rare; +: few; ++: much; +++: abundant.

weaning, even with an adequate supply of a commercial diet (23% protein) until 120 days of age, the programming of PR offspring resulted in a lower mean body weight, whereas CR programming resulted in overweight offspring. These findings corroborate our previous data using programmed rats (Passos et al. 2000, 2004, Vicente et al. 2004, Fagundes et al. 2007, 2009, Moura et al. 2007, Lisboa et al. 2008). These alterations of body mass can be explained by hypophagia (PR mice) and hyperphagia (CR mice), which is a different conclusion than can be drawn from the rat model, in which food intake was unchanged.

Adult CR offspring exhibited higher total and central fat. This agrees with several studies showing that early malnutrition programmes lead to the development of a thrifty phenotype (Petry et al. 2000, Ozanne & Hales 2002, Plagemann 2006). Interestingly, PR mice had higher total body fat, as previously detected in PR rats of the same programming model (Fagundes et al. 2007, 2009). Thus, it is possible that the lower body mass of this group is due to a loss of muscle mass. In addition, lower pituitary growth hormone mRNA has been reported in programmed PR offspring (de Moura et al. 2007). Because growth hormone increases protein synthesis in muscle, this finding may also explain the body composition changes of the programmed mice in the present study.

Because leptin is mainly produced by adipose tissue (Ahima 2005), CR offspring showed hyperleptinaemia due to increased adiposity. Hyperleptinaemia and increased food intake in CR mice suggests that these offspring developed central leptin resistance. In fact, rats pro-

grammed by maternal CR during lactation are resistant to the anorexigenic effect of leptin (Passos et al. 2004). PR offspring had no significant changes in serum leptin levels. Because these mice displayed a lower food intake, it is possible that they have an increased sensitivity to leptin.

Liver weight was not altered in uninfected CR and PR adult offspring. This was probably because the normal diet offered after weaning was effective at preventing liver weight changes. Histopathologic liver evaluation of uninfected PR offspring revealed that the planned structures were preserved, thereby maintaining the trabecular architecture. However, CR offspring showed microvesicular steatosis, which was probably associated with the higher VFM.

Fatty liver can ultimately lead to inflammation and scarring (Erhuma et al. 2007). Although this condition has multiple causes, fatty liver is a pathological condition in which triglyceride fats accumulate within hepatocytes. Studies in rodents have shown that the nutritional environment contributes to liver injury. Hepatocytes in fatty livers are vulnerable to insults (Carmiel-Haggai et al. 2005). Prolonged feeding of fat-enriched diets induces hepatic steatosis in rodents (Zhang et al. 1999, Rao et al. 2001, Picard et al. 2002, Buettner et al. 2007, Marques et al. 2010). During *S. mansoni* infection, immunocompromised mice fed either a normal diet (Doenhoff et al. 1981) or high-fat chow (Neves et al. 2007a) developed hepatic steatosis. Alternatively, PR throughout gestation and during the first half of lactation has caused higher rates of hepatic steatosis in six-month-old rats (Souza-Mello et al. 2007).

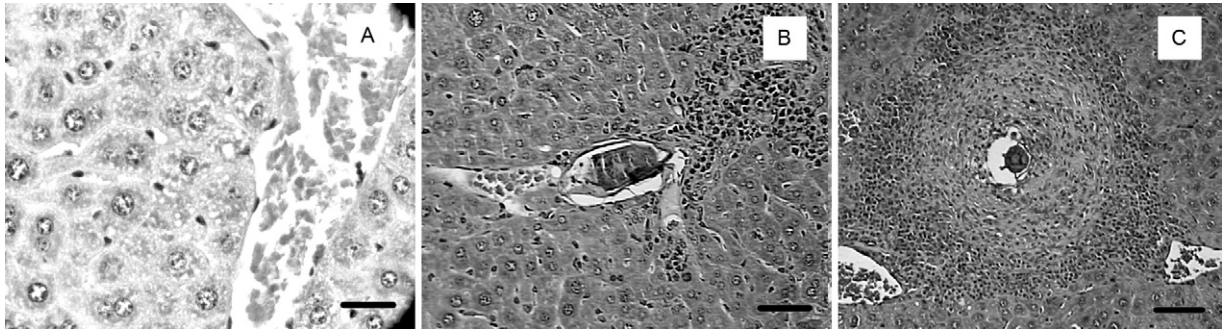


Fig. 1: histopathological analysis of liver sections from adult control offspring. A: portal vein and liver parenchyma are preserved (H&E) (Bar = 10 μ m); B: inflammatory cells are surrounding a schistosomal egg within the intravascular space (H&E) (Bar = 10 μ m); C: exudative granuloma within the portal tract (H&E) (Bar = 20 μ m).

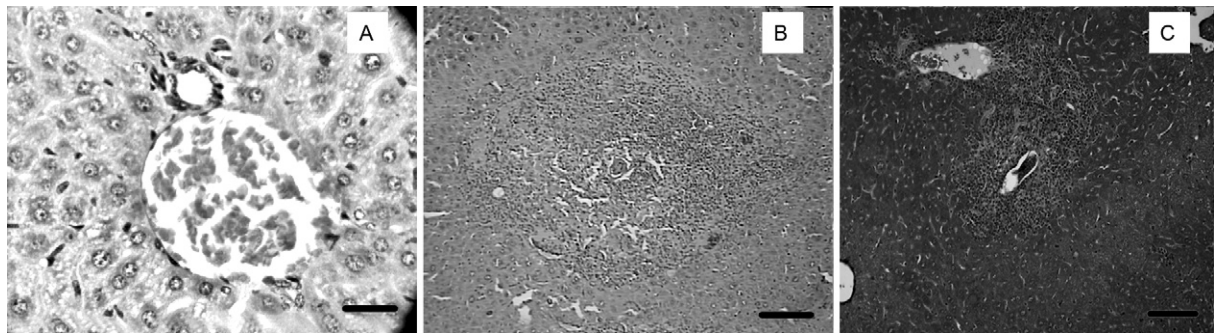


Fig. 2: histopathological analysis of liver sections from adult protein-restricted offspring. A: portal space and liver parenchyma (H&E) (Bar = 10 μ m); B: pre-granulomatous type granuloma characterized by intense acute inflammatory reaction (H&E) (Bar = 20 μ m); C: leukocyte migration encircling a central schistosomal egg (Masson's trichrome stain) (Bar = 20 μ m).

Here, for the first time, we evaluated the effects of *S. mansoni* infection in adult animals programmed by maternal undernutrition during lactation.

ICR offspring had lower body weight, food intake and total and VFM compared with uninfected CR. This suggests that infection influences the regulation of adiposity in obese mice. It has been shown that schistosomes are closely associated with lipid metabolism modulation (El Ridi et al. 2004, Tallima & El Ridi 2005, Alencar et al. 2009). IPR offspring presented only lower total fat when compared with non-IPR offspring. These findings are probably related to the increased lipolysis caused by inflammation due to the higher energy demand of this process (catabolic effects).

ICR offspring showed a reduction in fat and this was reflected by lower serum leptin. Ob/ob (leptin-deficient) mice had lower hepatic fibrosis after 12 weeks of infection with *S. mansoni* (Potter & Mezey 2002). ICR mice, which are probably hypoleptinaemic, likely have delayed development of hepatic fibrosis, despite the findings of the evaluation at nine weeks post-infection (acute phase). To confirm this possibility, other studies using a more advanced stage of infection are needed.

Some studies have shown that the host-parasite relationship can be modified by the nutritional status of the host. Thus, schistosome maturation (Kanuft & Warren

1969), worm burden and oviposition reduction (Akpom 1982, Rocha 1982, Magalhães et al. 1986, Neves et al. 2001, Oliveira et al. 2001), as well as the production of altered eggs, have been observed in nutritionally restricted mice (Akpom & Warren 1975). Our previous report showed that the pre-patent period and the kinetics of egg excretion were similar to well-fed controls (Simões et al. 2002).

The parasitological evaluations of adult offspring programmed by postnatal malnutrition showed that the pre-patent period was longer than 45 days. This result confirms studies with undernourished mice, which showed a pre-patent period of 43 days (Simões et al. 2002). At 55 days post-infection with *S. mansoni*, PR offspring had a higher number of eggs in their faeces. Studies have suggested that protein deficiency during lactation leads to a deficiency in the tissue and mechanical barriers in the collagen, which facilitates the inflammatory reaction caused by *S. mansoni* in the intestinal mucosa that is necessary for the elimination of eggs in faeces (Costa & Katz 1982, Costa et al. 1984, Lenzi et al. 1987, Machado e Silva et al. 1994). It is possible that programmed adult PR mice maintain the changes acquired during lactation and eliminate a greater number of eggs in the faeces as an adaptive strategy.

In the mesenteric vasculature, immature eggs require about five-six days for the embryo to differentiate and

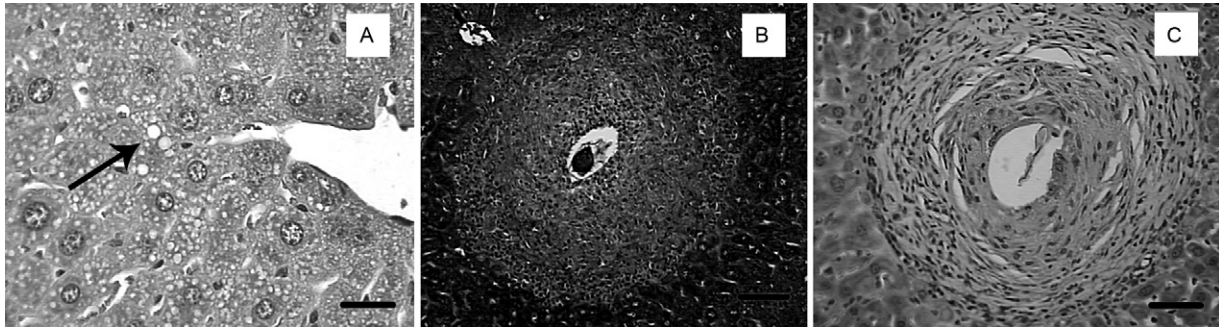


Fig. 3: photomicrographs of liver sections from adult caloric-restricted offspring. A: portal vein and microvesicular steatosis (arrow) and hepatocyte disclosing microvesicular steatosis (H&E) (Bar = 10 µm); B: an exudative-productive granuloma (H&E) (Bar = 20 µm); C: schistosomal granuloma disclosing concentric arrangement of collagen fibers (H&E) (Bar = 20 µm).

begin eliminating lytic and antigenic secretions through micropores in the eggshell (Andrade 2009). At this time, they cross the endothelial and mucosal barriers of the intestinal lumen, allowing mature eggs to reach the outside environment via the host's faeces. This process allows the eggs to exploit the host's cytokine production (Doenhoff et al. 1986, Lenzi et al. 1987, Brindley 2005).

In our study, all infected groups had higher numbers of immature eggs in the distal small intestine, which corroborates other studies (Costa & Katz 1982, Machado e Silva et al. 1991, Martinez et al. 2003). Adult PR offspring had a higher total number of eggs in their faeces and intestinal portions, as well as a higher number of eggs in all stages of development.

Regarding infectivity, adult worms recovered in the C, CR and PR offspring were localised in the mesenteric and portal-veins. This result showed similarity with other studies using malnourished animals (Akpom 1982, Rocha 1982, Ferreira & Coutinho 1999).

Schistosome eggs that do not pass through the intestinal mucosa are usually carried by the portal-vein blood flow to the liver, where they become trapped due to the insufficient diameter of the sinusoids (Pearce 2005). This process leads to inflammation, tissue eosinophilia, collagen deposition, fibrous expansion of the portal spaces and intra-hepatic portal-vein obstruction (Abath et al. 2006). Granuloma formation has been described as a two-stage process with a pre-granulomatous stage, characterised by a disorganised aggregation of cells, and a granulomatous stage, during which the cells become organised (Lenzi et al. 1998, 2006).

Infected offspring showed an increase in liver weight. This has been previously demonstrated in adult animals (Magalhães et al. 1986, Coutinho et al. 2003, Coutinho 2004) due to the inflammatory process caused by *S. mansoni* eggs (Mota & Sleight 1984, Sleight et al. 1986). Liver pathology around mature eggs depends on, among other factors, the host nutritional status (Coutinho et al. 1997, Oliveira et al. 2004). Liver injury was moderate when compared with wellnourished control mice (Coutinho et al. 1997, 2003). However, the effects of schistosome parasitism on lactation are unknown.

The quantitative assessment of liver granulomas in the programmed group showed that PR offspring had a higher total number of granulomas. Qualitative analysis of liver granulomas sorted by exudation showed that PR offspring had more granulomas of the pre-granulomatous stage, whereas CR offspring had more exudative-productive granulomas. Undernourished mice infected with *S. mansoni* were unable to produce periportal fibrosis in liver granuloma and it has been suggested that the nutritional status of the host contributes to the remodelling of liver granulomas during peri-ovular schistosomiasis (Coutinho 2004). One possible mechanistic explanation for the differences observed in liver morphology during *Schistosoma* infection concerns serum leptin levels. In the CR group, leptin is decreased with infection, whereas in the PR group there was no change. However, differences observed in the IPR offspring compared to the IC offspring could be related to differences in body weight.

IPR offspring, which showed lower body weight, had greater liver injury, as indicated by the increased number of areas altered by granulomatous exudative, as well as a decreased ability to produce collagen and higher inflammation due to the inability to modulate the inflammatory process. ICR offspring, which had higher adiposity but lower leptin levels, had a lower number of liver granulomas, increased production of collagen and several binucleate hepatocytes, thus providing better conditions for modulating the inflammatory response and regenerating the liver.

These findings indicate that the host-parasite relationship was more balanced. In conclusion, these observations suggest that protein or energy restriction during lactation has a prominent effect on the course of acute *S. mansoni* infection in adult offspring mice. It is known that leptin decreases the CRH-ACTH-corticosterone axis (Szücs et al. 2001) and cortisol decreases the inflammatory response necessary for granulomatous formation (Morales-Montor et al. 2001). In fact, ICR offspring displayed higher levels of serum corticosterone. Thus, ICR offspring that have inadequate levels of serum leptin may present a greater activation of the CRH-ACTH-corticosterone axis and, consequently, less-pronounced liver injury.

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