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Major Article

Superimposing a high-fat diet on *Schistosoma mansoni* infection affects renin-angiotensin system components in the mouse kidney

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Abstract

Introduction: The levels of the full-length form of the (pro)renin receptor (PRR), a component of the renin-angiotensin system (RAS), may be reduced in the membranes of kidneys in renal diseases. This study aimed to investigate the RAS components in the kidneys of mice submitted to a combination of a high-fat diet and *Schistosoma mansoni* infection. **Methods:** Female BALB/c mice were maintained on a control or high-fat diet from 3 weeks of age. After 10 weeks on the designated diets, half the mice in each group were infected with *S. mansoni* cercariae. The blood and kidneys were harvested 8 weeks after infection. **Results:** The high-fat diet increased the number of eggs in the feces and the number of adult worms in the mesenteric bed. *Schistosoma mansoni* infection reduced the plasma levels of glucose, triglycerides, and HDL cholesterol in the control and high-fat diet groups. In mice on the control diet, *S. mansoni* infection resulted in increased expression of IL-6 in the kidneys; however, in mice on the high-fat diet, the levels of IL-6 were reduced and those of superoxide anions were increased. The RAS components evaluated were ACE2, renin, PRR, AT_1R, and AT_2R, and the levels of PRR were found to be reduced in the kidneys of infected mice on the high-fat diet. **Conclusions:** The finding regarding PRR is not yet clear. However, combining a high-fat diet and *S. mansoni* infection resulted in increased oxidative stress in the kidney that can aggravate hypertension as well as its associated complications.

Keywords: *Schistosoma mansoni*. Kidney. Renin-angiotensin system. (pro) renin receptor.

INTRODUCTION

Obesity associated with metabolic syndrome is a global public health burden[1], while schistosomiasis caused by *Schistosoma haematobium* and *S. mansoni* still affects countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia, and the Western Pacific[2,3]. Considering the high prevalence of overweight/obesity, this condition likely coexists with schistosomiasis[4,5]. The nutritional status of the host influences the *S. mansoni* cycle. For example, a host maintained on a low-protein diet elicits reproductive gland impairment and morphological changes in the worms[6,7], while a host maintained on a high-fat diet is beneficial for the life cycle of the parasite[8,9].

Renal function is impaired in 15% of patients with the hepatosplenic form of *S. mansoni*[10]. Retention of the antigen-antibody complex in the basolateral membrane of the glomerular capillary is normally responsible for the observed renal disorders. Furthermore, the resulting collateral circulation of the portal system, as well as immune/inflammatory factors, may also affect renal function[10]. In experimental models of *S. mansoni* infection, the levels of oxidative stress are increased

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in several organs, including the kidneys[11]. Angiotensin II, the most important component of the renin-angiotensin system (RAS), has been postulated to increase oxidative stress in the kidney through one of its receptors, AT_1R[12]; however, the opposite has also been observed, with increased oxidative stress having been shown to upregulate the levels of AT_1R and sodium transporters in the kidney[13]. In contrast, reduced levels of the (pro)renin receptor (PRR), one of the RAS components, in the renal membranes and increased levels of a soluble form of PRR (sPRR) in the urine are associated with kidney disease[14].

The RAS is overactive in adipose tissue of obese individuals, where it has pro-oxidative and pro-inflammatory roles[15], as well as in the kidney, where it has pro-oxidative and therefore pro-hypertensive effects[16]. Components of the RAS are present in liver fibrosis induced experimentally by *S. mansoni* infection, and AT_1R antagonists reduce the size of granuloma and the levels of inflammatory agents in granulomas[17].

The aim of the present study was to investigate whether obesity induced by a high-fat diet in combination with *S. mansoni* infection changes the RAS component profile and exacerbates oxidative stress and immune marker expression in the mouse kidney.

METHODS

Materials

Commercial kits for glucose, total cholesterol, HDL cholesterol, and triglyceride evaluation were purchased from Labtest (Lagoa Santa, Brazil). N,N′-dimethyl-9,9′-biacridinium dinitrate (lucigenin), β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (β-NADPH), 2-thiobarbituric acid (TBA), bovine serum albumin (BSA), Folin's phenol reagent, Tris, radioimmunoprecipitation assay (RIPA) buffer, protease inhibitor cocktail, and Ponceau S were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-angiotensin-converting enzyme 2 rabbit monoclonal antibody (ACE2, ab108252), anti-ATP6IP2/PRR rabbit polyclonal antibody (ab40790), and secondary anti-mouse IgG H&L rabbit polyclonal antibody (HRP-linked, ab6728) were purchased from Abcam (Cambridge, MA, USA). Mouse anti-renin monoclonal antibody (sc-133145), goat polyclonal anti-AT_1R antibody (sc-1173-G), rabbit polyclonal anti-AT_2R antibody (sc-9040), and mouse monoclonal anti-β-actin antibody (sc-47778) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Rabbit polyclonal anti-IL-6 antibody (IM-0468) and mouse monoclonal anti-TNFα antibody (IM-0406) were obtained from Rhea Biotech (Campinas, SP, Brazil). Donkey polyclonal secondary anti-goat IgG antibody (HRP-linked, 705-035-003) was obtained from Jackson Immunoresearch Laboratories, Inc. (Philadelphia, PA, USA). ECL™ Prime western blotting reagent and donkey whole secondary anti-rabbit IgG antibody (HRP-linked, NA934) were obtained from GE Healthcare Life Sciences (Little Chalfont, Buckinghamshire, UK).

Animals

All procedures using animals were carried out in accordance with the guidelines of the Brazilian Society of Laboratory Animal Sciences (SBCAL) and underwent ethical review and approval by the Committee on the Ethical Handling of Research Animals (CEUA) of the Aggeu Magalhães Research Center (Recife, PE, Brazil) (protocol number 65/2014). These guidelines strictly follow those contained in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications No 86-23).

Female BALB/c mice were obtained from the breeding facilities of the Aggeu Magalhães Research Center and maintained at 23 °C with a 12 h:12 h light-dark cycle. At three weeks of age, the animals were randomly distributed into either a control group maintained on a control diet (CD) or an experimental group maintained on a high-fat diet (HFD). Their body weight was assessed one week later and then weekly thereafter. After 10 weeks on the designated diets, half the mice in each group were infected (I) with *S. mansoni* cercariae to form the infected groups, ICD (n = 10) and IHFD (n = 10), and the non-infected groups, CD (n = 10) and HFD (n = 10). At 22 weeks of age, 60 d after infection, non-fasting mice were anesthetized with intramuscular administration of ketamine-xylazine (100–200 mg/kg + 5–16 mg/kg). Blood samples were collected by cardiac puncture. Both kidneys were collected. The mice were euthanized by exsanguination. Then, hepatic perfusion was performed using 0.9% saline and adult worms were recovered, identified according to gender, and counted in both the portal system and the mesenteric bed. Serum samples were separated using a refrigerated centrifuge and both kidneys were rapidly transferred to −80 °C. Serum samples were used to measure the metabolic parameters.

Diets

The control and high-fat diets were obtained from PragSoluções Biociências (Jaú, SP, Brazil). The macronutrient composition of the control diet was carbohydrates 73.9%, proteins 14.8%, and lipids 9.8%. The composition of the high-fat diet was carbohydrates 26.1%, proteins 14.4%, and lipids 57.6%. The diet components are shown in **Table 1**.

Cercariae and procedures for mouse infection

Biomphalaria glabrata snails exposed to *S. mansoni* (strain BH) were supplied by the Parasitology Laboratory at LIKA, Federal University of Pernambuco (Recife, Brazil). After 30 d of exposure to miracidia, the snails were placed in filtered tap water under light to induce cercarial emergence. The cercarial suspension (30 cercariae/50 µL distilled water) was maintained for 30 min in contact with the skin of anesthetized mice (ketamine-xylazine, 100–200 mg/kg + 5–16 mg/kg, IM) for percutaneous penetration. Infection was confirmed by counting *S. mansoni* eggs in the feces 45 d after infection. Two slides were prepared from each feces sample to count the number of eggs[18].

Analytical methods

The non-fasting levels of serum glucose, total cholesterol, HDL cholesterol, and triglycerides were measured using commercial kits.

Basal and NADPH oxidase-dependent superoxide anion levels in the kidneys

Frozen whole kidneys were thawed in an ice bath and homogenized in a tissue grinder tube with a Teflon pestle

TABLE 1: Diet components.

	Standard diet		High-fat diet	
Components*	g/kg	kcal	g/kg	kcal
Corn starch	415.0	1660	14.3	57.2
Soybean bran	305.0	1281	410.0	1722
Sucrose	80.0	320	80.0	320
Maltodextrin	70.0	280	70.0	280
Animal fat	0.0	0	302.0	2178
Soybean fatty acid	50.0	350	50.0	350
Cellulose microcrystals	31.7	0	25.4	0
L-cystine	1.8	7.2	1.8	7.2
Choline chloride	1.5	0	1.5	0
Butyl-hydroxytoluene	0.014	0	0.028	0
Mineral mix	35.0	0	35.0	0
Vitamin mix	10.0	40	10.0	40
Total	**1000**	**3938**	**1000**	**5494**

*According to PragSoluções Biociências.

(Kimble Chase, Rockwood, TN, USA) immersed in an ice bath and coupled to a rotor (IKA® RW20, Staufen, Germany) at 1200 rpm for 2 min. RIPA buffer supplemented with a protease inhibitor cocktail was used for homogenization of the tissue at a ratio of 1 g kidney to 7 mL of solution. Superoxide anion (O_2^-) generation was assessed using the lucigenin-enhanced chemiluminescence method, as previously described[19].

Renal RAS components and immune response markers

Aliquots of whole kidney homogenates (80 µg protein) were subjected to 10% SDS-PAGE and transferred to PVDF or nitrocellulose membranes. The membranes were incubated in 5% BSA to prevent non-specific binding. The membranes were immunoblotted with antibodies against ACE2 (1:1000), PRR (1:1000), renin (1:500), AT_1R (1:1000), AT_2R (1:500), β-actin (1:10000), IL-6 (1:500), and TNFα (1:1000). Each membrane was submitted to a maximum of two strips. After the membranes had been exposed to the corresponding secondary antibodies and sequentially incubated with an enhanced chemiluminescence western blotting detection reagent (ECL™ Prime), the blots were visualized with a chemiluminescence imaging system (ChemiDoc MP, BioRad, Hercules, CA, USA). Considering that PRR and β-actin have the same molecular weight, PRR expression was indexed by the total protein band in its respective molecular weight revealed with Ponceau S staining.

Statistical analysis

The data are expressed as means ± SEM. Two-way ANOVA followed by a Bonferroni test was applied for comparisons among groups. When there was no effect of diet or infection, a one-way ANOVA followed by the Student Newman Keuls test was applied. When two groups were compared, the Student's *t*-test was applied. GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for data analyses.

RESULTS

Parasitological data

The number of eggs/g feces, counted 45 d after infection, was higher in the IHFD than in the ICD group (314 ± 173 vs. 168 ± 91, respectively; $P < 0.05$). In accordance with this result, the number of female adult worms counted in saline perfused throughout the mesenteric bed after the mice were euthanized was higher in the IHFD than in the ICD group (12.20 ±1.62 vs. 9.30 ± 2.58, respectively; $P < 0.01$), as was the number of worm pairs (26. 40 ± 1.35 vs. 16.60 ± 4.50, respectively; $P < 0.001$). One week after the diet was implemented, the body weight was higher in the two groups on the high-fat diet (**Figure 1A**). However, in the last two weeks, the IHFD group showed striking weight loss, as shown by the body weight variation (**Figure 1B**) calculated as the last weight minus the weight one week after the diets were introduced.

Serum metabolic parameters

The serum metabolic parameters are shown in **Figure 2**. The high-fat diet did not affect the plasma glucose levels (**Figure 2A**), but led to lower plasma triglyceride levels (**Figure 2B**, HFD vs. CD) and higher plasma total cholesterol (Figure 2C, HFD vs. CD and IHFD vs. ICD) and HDL cholesterol (**Figure 2D**, HFD vs. CD) levels. *Schistosoma mansoni* infection reduced glycemia in both the control and high-fat diet groups (**Figure 2A**). *Schistosoma mansoni* infection reduced triglyceride levels in mice maintained on a control diet (Figure 2B, ICD vs. CD), and also resulted in reduced HDL cholesterol levels in mice fed both the control and high-fat diets (**Figure 2D**, ICD vs. CD and IHFD vs. HFD).

Immune response and oxidative stress markers in the kidneys

Figure 3 shows that the high-fat diet was associated with reduced levels of IL-6 in mice infected with *S. mansoni* (**Figure 3A**,

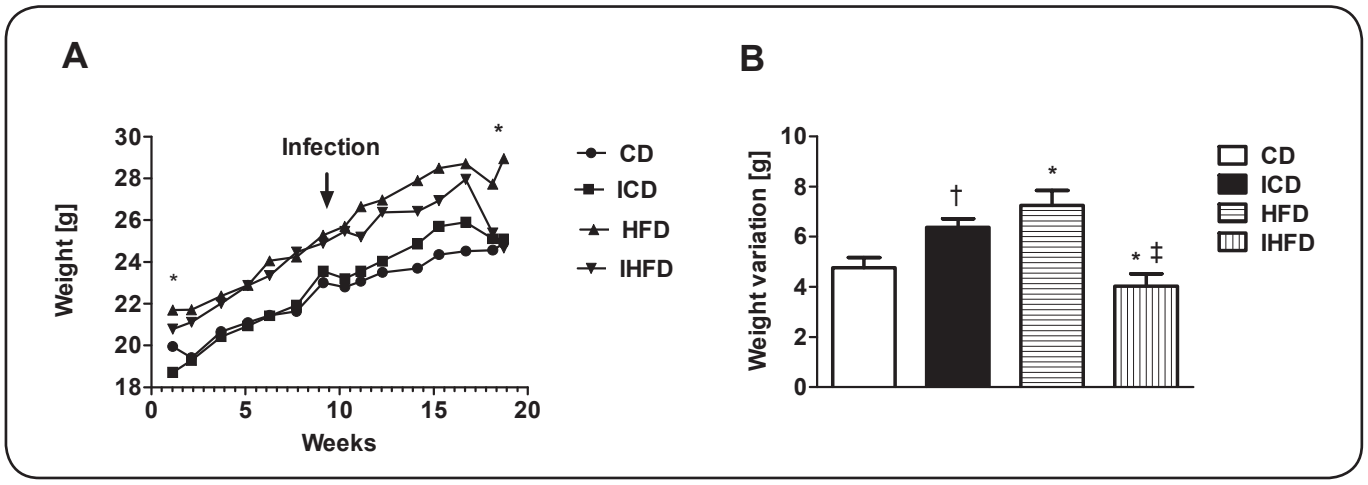

FIGURE 1: Body weight evolution of mice fed control (CD) and high-fat (HFD) diets and non-infected (CD and HFD) or infected (ICD and IHFD) with *Schistosoma mansoni* **(A)** and weight variation between the groups **(B)**. Control and high-fat diets were introduced at weaning (3 weeks of age). The first point in the curve represents body weight one week after the start of the diets. The infection was performed 10 weeks after the start of the diets. Body weight variation was calculated as the final body weight minus the body weight one week after the start of the diets. Data are expressed as means ± SEM. $P < 0.05$: * diet effect, † infection effect, ‡ combined effect of diet and infection.

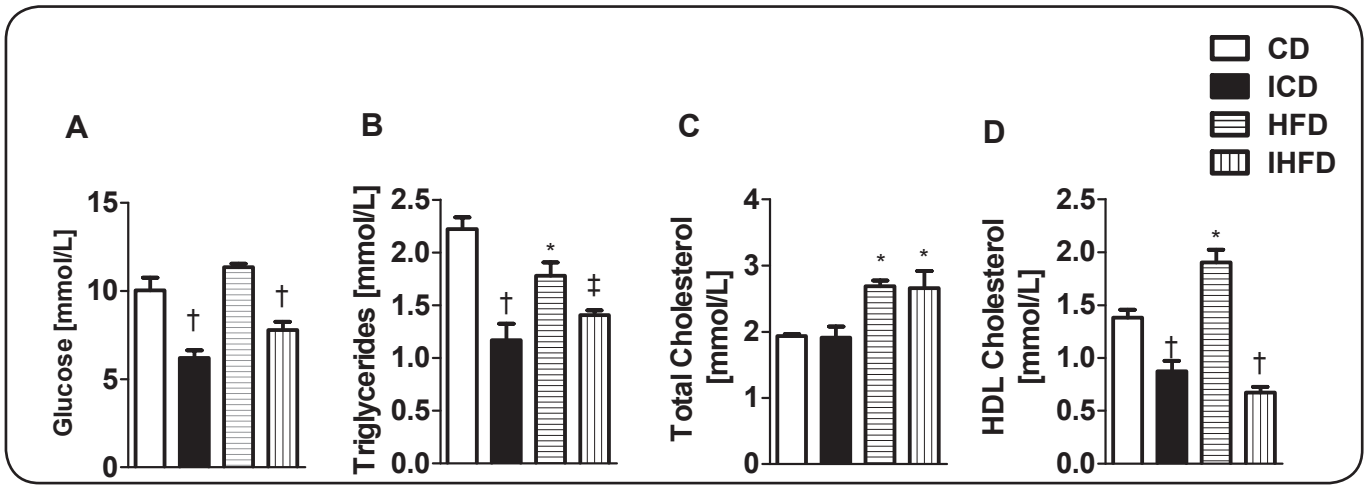

FIGURE 2: Non-fasting serum glucose **(A)**, triglyceride **(B)**, total cholesterol **(C)**, and HDL cholesterol **(D)** levels in non-infected mice maintained on a control diet (CD) or a high-fat diet (HFD), and *Schistosoma mansoni* infected mice maintained with similar diets (ICD and IHFD, respectively). Serum samples were obtained from the anesthetized mice by cardiac puncture after 19 weeks on the designated diets and 8 weeks after infection. The results are shown as the means ± SEM. $P < 0.05$: * diet effect, † infection effect (two-way ANOVA followed by the Bonferroni test). ‡ $P < 0.05$: diet and infection effect vs CD (one-way ANOVA followed by the Student Newman Keuls test).

IHFD vs. ICD), whereas it had no effect on renal TNF-α levels (**Figure 3B**). The high-fat diet led to increased basal superoxide anion levels (**Figure 3D**) as well as increased levels of NADPH-dependent superoxide anion generation (**Figure 3E**) in *S. mansoni*-infected mice (IHFD vs. ICD). *Schistosoma mansoni* infection increased IL-6 levels only in the mice maintained on the control diet (**Figure 3A**, ICD vs. CD) but did not affect TNF-α or superoxide anion levels. However, *S. mansoni* infection combined with the high-fat diet increased basal superoxide anion levels (**Figure 3D**, IHFD vs. CD).

Components of the RAS in the kidneys

The protein expression levels of the RAS components are shown in **Figure 4**. Among the proteins evaluated, namely, renin (**Figure 4A**), PRR (**Figure 4B**), ACE2 (**Figure 4C**), AT_1R (**Figure 4D**), and AT_2R (**Figure 4E**), the levels of PRR were decreased when the high-fat diet was combined with *S. mansoni* infection.

DISCUSSION

The main aim of the present study was to investigate the levels of RAS components in the kidneys of mice subjected to combined high-fat diet-induced obesity and *S. mansoni* infection. The higher number of eggs in the feces and the higher number of adult worms in the mesenteric bed of mice maintained on the high-fat diet indicate the vital role of host dietary lipids on *S. mansoni* development. Host dietary lipids increase the spermatozoa storage area in the male seminal vesicle and oocyte output in the female ovary at the adult stage[20] for egg

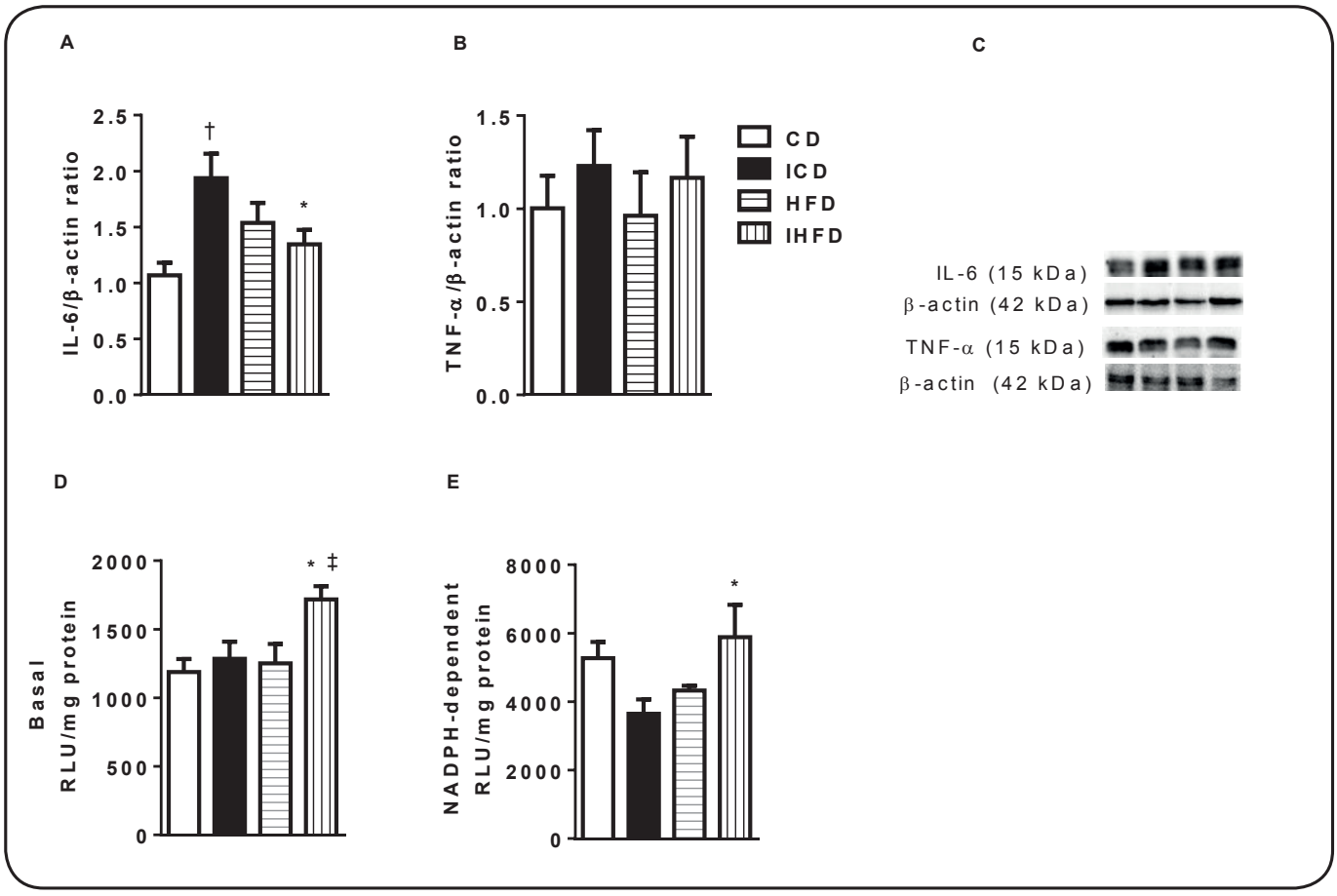

FIGURE 3: Immune response and oxidative stress markers in the kidney. The expression levels of IL-6 **(A)** and TNF-α **(B)** as markers of the immune response evaluated by western blotting. Representative images are in **(C)**. Superoxide anion levels measured by luminescence and expressed as relative light units (RLU) before **(D)** and after **(E)** NADPH addition. Th groups are described in **Figure 2**. The results are shown as the means ± SEM. $P < 0.05$: *diet effect (two-way ANOVA followed by the Bonferroni test). ‡ $P < 0.05$: diet and infection effect vs CD (one-way ANOVA followed by the Student Newman Keuls test).

production[21]. Furthermore, the schistosomula, the form that develops soon after cercariae access the vertebrate host dermis[22], uptakes cholesterol and lipids from the host for resistance against eosinophil-mediated cytotoxicity and complement-mediated damage[23].

The IHFD group that exhibited increased body weight after one week on the high-fat diet until two weeks before euthanasia showed an abrupt loss of body weight, likely because a high-fat diet in the acute phase of schistosomiasis, as in the present study, results in reduced villus length in the duodenum and an increased number of granulomas along the duodenum and jejunum[9], affecting mouse nutrition. In *S. mansoni*, oviposition begins at 42 d post-infection when the worms become adults[24]. Therefore, the higher number of eggs in the feces of the IHFD group 45 d post-infection suggests there was additional intestinal damage in the IFHD group compared to the HFD group that presented a slight loss of body weight; although its body weight was greater than that of the control group at the time of euthanasia.

In the vertebrate host, significant amounts of nutrients are provided by the schistosome integument. The integument has glucose[25] and amino acid[26] transporters in addition to water and electrolyte transporters[27]; thus, the serum glucose and triglyceride levels were reduced in both the ICD and IHFD groups. In human hosts, LDL is incorporated into the integument and gut of schistosomula either as a membrane constituent or as a strategy to evade the immune system[28]. There is consensus that the schistosome integument contains an antigenic protein anchored to glycosyl-phosphatidylinositol (GPI) which is shed by proteases or phospholipases[29] to interact with LDL particles in the human host. In contrast to humans, where approximately 75% of the plasma cholesterol is in the form of LDLs, approximately 80% of the mouse plasma cholesterol is in the form of HDLs[30]. Sprong et al.[31] proposed that, in mice, the lipoproteins bound to the GPI-antigen complex would be HDLs. Considering the consistent reductions in HDL observed in both infected groups, the present results support this hypothesis.

To survive *S. mansoni* infection, the mouse must present a balance between the Th1 and Th2 immune responses[32]. In *S. mansoni* infection, IL-6 suppresses Th1 cytokines and, therefore, Th2 cytokines would be expected to predominate in granulomas developed against the eggs[33]. A high-fat diet

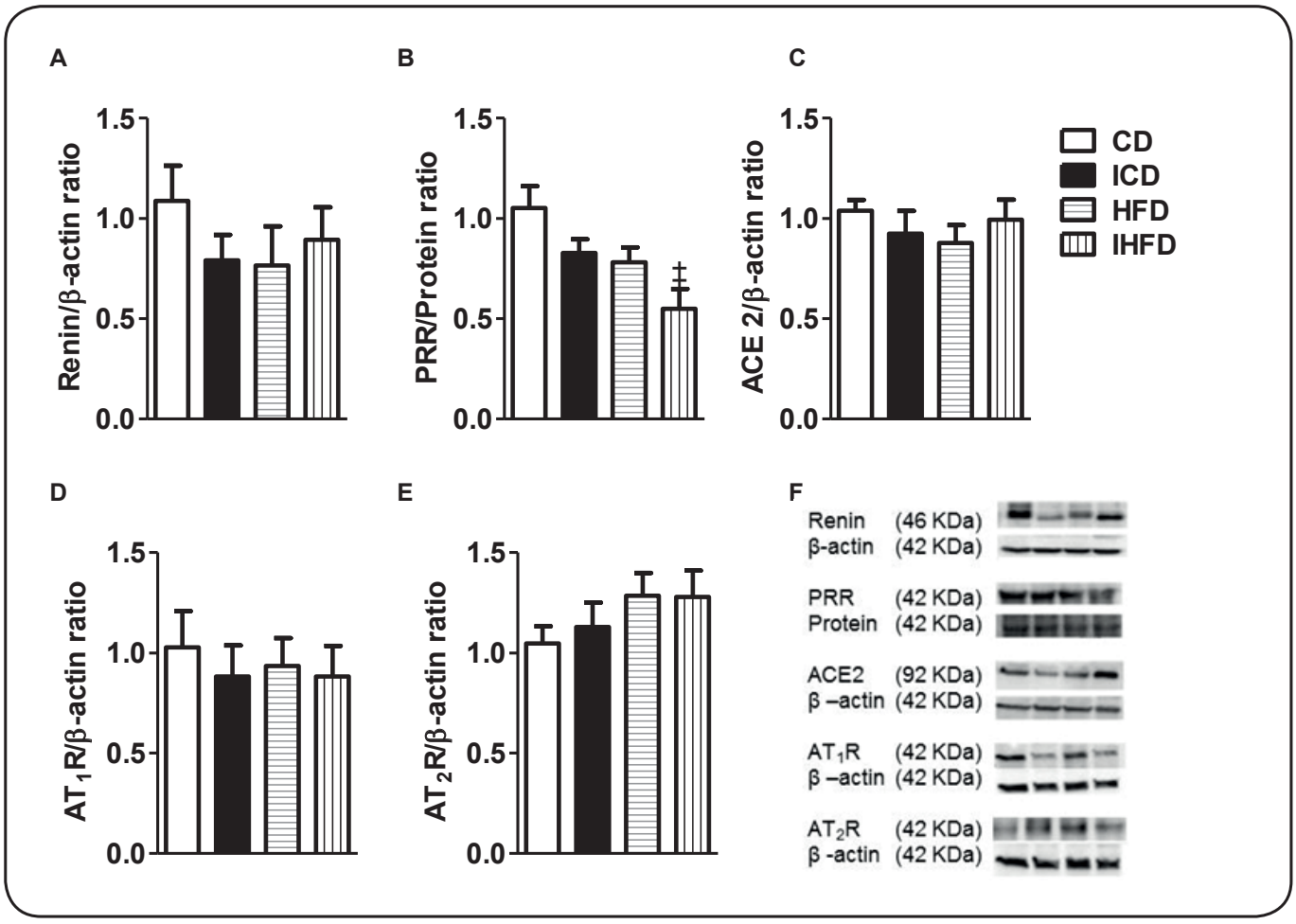

FIGURE 4: The protein expression of the RAS components in the kidney. The levels of renin **(A)**, ACE2 **(C)**, AT_1R **(D)**, and AT_2R **(E)** were indexed by β-actin and control values, while the levels of PRR **(B)** were indexed by the total protein in the band revealed with red Ponceau S staining and subsequently indexed to control values. Representative images are shown in panel **(F)**. The groups are described in **Figure 2**. The results are shown as the means ± SEM. Comparisons between the groups did not show any difference using two-way ANOVA followed by the Bonferroni test. ‡ $P < 0.05$: diet and infection effect vs CD (one-way ANOVA followed by the Student Newman Keuls test).

attenuates IL-6 production following an immune challenge, indicating an impaired acute innate immune response[34,35]. The reduced levels of IL-6 in the kidneys of the IHFD compared to the ICD group suggest that the high-fat diet attenuated the Th2 immune response[33], affecting granuloma development. Consequently, a higher number of eggs crossed the intestinal wall, as assessed by the larger number of eggs in the feces of the IHFD group. Although the levels of renal TNF-α were similar between the four groups, the higher superoxide anion levels in the IHFD group, both basal and NADPH oxidase-dependent, points to a suppressed Th2 immune response[36].

The increased superoxide anion levels in the kidneys of the IHFD group seems to be independent of the RAS components since the levels of AT_1R and AT_2R were unchanged and those of PRR, which is expected to increase oxidative stress, were reduced. The full-length PRR, the form investigated in the present study, is a 70–80 kDa transmembrane receptor that regulates various physiological and pathological processes[37] that are mediated by renin and prorenin. Reduced levels of the full-length form of PRR in the kidneys of the IHFD group may indicate proteolytic cleavage[38] that is known to occur in chronic kidney disease[14], metabolic syndrome[39], and hypertension[40]. Proteolytic cleavage of the full-length form of PRR gives rise to a 28 kDa soluble form of PRR (sPRR) the circulating levels of which are known to be increased along with a simultaneous reduction in the levels of full-length PRR in the kidneys[41].

In conclusion, the combination of a high-fat diet and *S. mansoni* infection led to a reduction in the renal levels of the full-length form of PRR, one component of the renin-angiotensin system, that was associated with reduced levels of IL-6 and an increased worm charge. The significance of the altered levels of the full-length form of PRR is not yet clear. However, combining a high-fat diet with *S. mansoni* infection led to increased oxidative stress in the kidney that can aggravate hypertension as well as its associated complications.

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