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Limosilactobacillus fermentum prevents gut-kidney oxidative damage and the rise in blood pressure in male rat offspring exposed to a maternal high-fat diet

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Original Article

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Abstract

Oxidative stress along the gut-kidney axis is a risk factor for developing arterial hypertension in offspring from dams fed a high-fat diet. Considering the antioxidant capacity of probiotic strains, this study evaluated the effects of a daily multistrain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on blood pressure (BP), renal function, and oxidative stress and along the gut-kidney axis in male offspring from dams fed a high-fat high-cholesterol (HFHC) diet during pregnancy and lactation. Dams were fed a diet control or HFHC diet during pregnancy and lactation. At 100 days of age, part of the male offspring from dams fed a HFHC diet received *Limosilactobacillus fermentum* formulation for 4 weeks (HFHC + Lf) daily. After the 4-week intervention, BP (tail-cuff plethysmography) and urinary and biochemical variables were measured. In addition, malondialdehyde levels, enzymatic activities of superoxide dismutase, catalase, glutathione-S-transferase, and nonenzymatic antioxidant defense (thiols content) were measured in the colon and renal cortex. Male offspring from dams fed a HFHC had increased blood pressure, impaired renal function, and oxidative stress along the gut-kidney axis. Administration of *Limosilactobacillus fermentum* reduced systolic, diastolic, and mean blood pressure levels and alleviated renal function impairment and oxidative stress along the gut-kidney axis in male offspring from dams fed a HFHC diet. Administration of *Limosilactobacillus fermentum* formulation attenuated programmed hypertension in the HFHC group through oxidative stress modulation along the gut-kidney axis.

Introduction

Maternal high-fat diet (HFD) consumption is a risk factor for developing arterial hypertension in offspring through a complex mechanism involving sympathetic overactivity, enhanced peripheral chemosensitivity, baroreflex dysfunction, and impairment in kidney function.[1–3] Additionally, HFD intake during pregnancy and/or lactation has been shown to shift the composition of maternal–fetal gut microbiota, which has been linked to impaired gut barrier integrity and developmental programming of arterial hypertension.[4].

Oxidative stress plays a significant role in developing many diseases, being characterized by increased production of oxygen/nitrogen/sulfur reactive species and reduced tissue antioxidant capacity.[5] Increased renal oxidative stress and gut microbiota dysbiosis have been linked to the development of arterial hypertension and chronic kidney disease.[6–8] It has been previously demonstrated that offspring from dams fed a high-fat high-cholesterol diet during pregnancy and lactation developed arterial hypertension linked to renal dysfunction and increased oxidative stress along the gut-kidney axis at 90 days of age,[9] indicating that postweaning intervention targeting the gut microbiota could be a potential strategy to reprogramming arterial hypertension.

The administration of probiotics, an approach based on the administration of live nonpathogenic microorganisms that confer a health benefit to the host when administered in adequate amounts, has been considered a safe strategy capable of reducing oxidative stress.[10] Growing evidence has demonstrated that interventions targeting gut microbiota with probiotics have emerged as an innovative strategy to treat arterial hypertension,[11,12] and to protect cells from oxidative damage due to their antioxidant capacity.[13]

The administration of *Limosilactobacillus fermentum* 139, 263, and 296 has been shown to cause improvements in lipid profile and autonomic function in rat offspring from dams with

maternal dyslipidemia.[14] However, whether postweaning probiotic therapy with a multistrain formulation containing these *Limosilactobacillus fermentum* strains effectively reduces blood pressure, kidney dysfunction, and oxidative stress along the gut-kidney axis in the offspring later in life remains to be elucidated. These *Limosilactobacillus fermentum* strains have been previously characterized as having probiotic aptitudes and qualities to be translated into nutritional approaches.[14,15]

In the present study, the effects of a daily administration of a multistrain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on blood pressure, renal function, and oxidative stress along the gut-kidney axis in male offspring from dams fed with a high-fat high-cholesterol during pregnancy and lactation were evaluated.

Methods

Ethical aspects and experimental design

Wistar rats were used in this study. The animals were maintained in collective polypropylene cages under controlled temperature (22 ± 1 °C), humidity between 50 and 55 % 12 h light-dark cycle, and received water and diet *ad libitum*. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Paraíba (CEUA-UFPB protocol #9635020519; #9492260418) and followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and International Principles for Biomedical Research.

The female Wistar rats ($n = 8$) were maintained in polypropylene cages up to 90 days of age. Posteriorly, pregnant rats were allocated to a control group ($n = 4$) and received a diet prepared according to the American Institute of Nutrition – AIN-93G; or a HFHC group ($n = 4$) that received a diet (Table 1) purchased from Rhoster Company (Araçoiaba da Serra, São Paulo, Brazil) during pregnancy and lactation period as previously described.[1,16,17] After weaning (postnatal day 21), male rat offspring (CTL, $n = 16$ and HFHC, $n = 15$) were weighted, housed separately (3–4 per cage), and had free access to a commercial diet (Presence Purina, Paulínea, São Paulo, Brazil) and water *ad libitum* up to 100 days of age. At that age, the experimental groups were randomly formed with one to two rats from each litter. The CTL group was formed for six male offspring ($n = 6$) from the dams fed a CTL diet. The HFHC group was formed for five male offspring (n = 5) from the dams fed a HFHC diet. Similarly, HFHC + Lf group was formed for five male offspring ($n = 5$) from the dams fed a HFHC diet and received the multistrain *Limosilactobacillus fermentum* formulation (HFHC + Lf group) twice a day in a solution of approximately 3×10^9 CFU/mL by oral gavage for 4 weeks (Fig 1).

Table 1. Nutritional composition of control diet (CTL diet) and high fat and high cholesterol diet (HFHC diet)

	Diets	
Ingredients (for 100g)	CTL*	HFHC**
Corn starch	39.75	33.09
Dextrinized corn starch	13.20	15.50
Casein$^{\infty}$	20.00	19.86
Sucrose	10.00	6.00
Soybean oil	7.00	3.00
Animal fat (lard)	–	6.00
Sigma Cholesterol	–	1.00
Sigma Colic Acid	–	0.50
Cellulose	5.00	5.00
Mineral Mix 93M	3.50	3.50
Vitamin Mix	1.00	1.00
L-cystine	0.30	0.30
Choline bitartrate	0.25	0.25
t-BHQ#	0.014	0.014
Calories (kcal/g)	3.96	4.34
Carbohydrates (% kcal)	63.62	50.32
Proteins (% kcal)	20.47	18.58
Lipids ((% kcal)	15.92	31.11

Composition values obtained from previously established protocols.
*CTL diet adapted from Reeves; Nielsen; Fahey, (1993).
**HFHC diet according to Rhoster - Industry and Trade Ltda.
$^{\infty}$Casein had 85% purity (85 g protein for each 100 g casein).
#Tert-butylated hydroxytoluene.

Limosilactobacillus fermentum *strains*

Limosilactobacillus fermentum 139, 263, and 296 strains were gently provided by Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba (João Pessoa, Paraíba, Brazil). Stocks were stored at −20 °C in Mann, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (Sigma-Aldrich, St. Louis, USA; 20 mL/100 mL). The probiotic cell suspension was obtained from overnight cultures grown on MRS broth (HiMedia, Mumbai, India) under anaerobic conditions (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37 °C.[14,15] The mixed cell suspension with counts of approximately 9 log CFU/mL of each strain was obtained with a mixture of each probiotic strain suspension in a ratio of 1:1:1.

Urinary analysis

After the supplementation period, the male rats were individually acclimatized in metabolic cages for 3 days and on the 4th day, 24-h urine collection was done. The urinary volume was measured and freeze-stored (−20 °C). The urinary measurements of urea, creatinine, and total proteins were done with commercial kits following the protocols according to the manufacturer instructions (Bioclin, Belo Horizonte, Minas Gerais, Brazil). The creatinine clearance (CCr) was calculated with the formula: CCr = Urinary Creatinine × Urinary volume of 24h/plasmatic creatinine.[18]

Blood pressure and heart rate measurement

Blood pressure (BP) was recorded by tail-cuff plethysmography (V2.11 Plethysmography, Insight, Ribeirão Preto, São Paulo, Brazil). For 3 days, rats were encouraged to walk into the restraint tubes and acclimatized for approximately 1 h before experiments began. After the adaptation period, BP and heart rate were measured.[9] For each record, 15 cycles of inflation and deflation were done. Ten out of the 15 recorded values were used to calculate the average.

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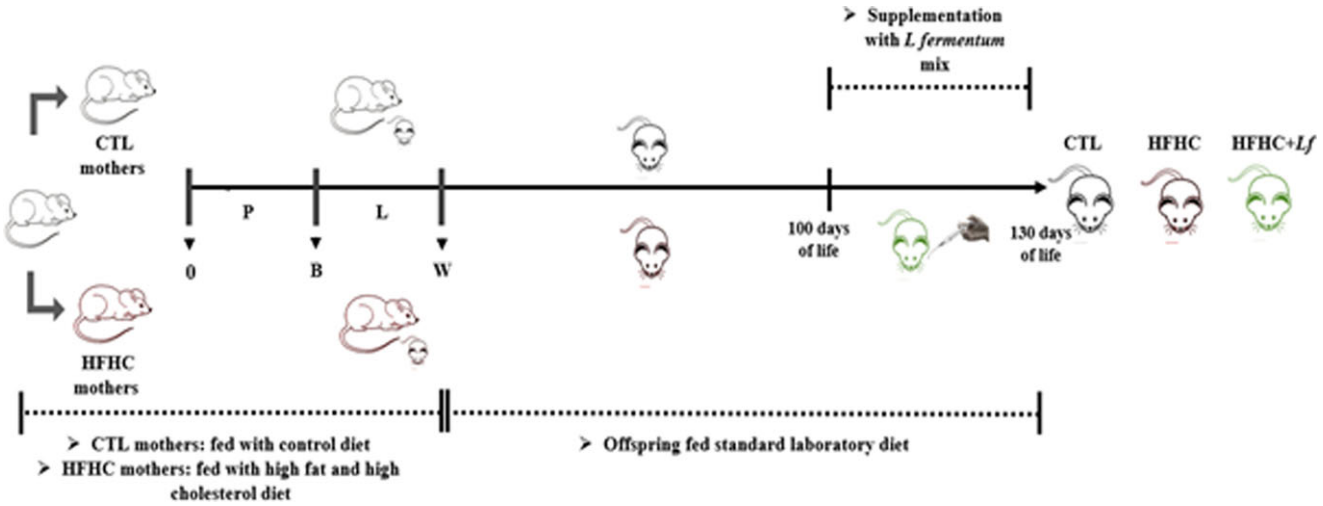

Fig. 1. Graphic design of the formation of experimental groups. B: Birth; CTL: Control; HFHC: High fat and high cholesterol; HFHC + Lf: High fat and high cholesterol diet and *Limosilactobacillus fermentum* formulation; L: Lactation; P: Pregnancy; W: weaning.

Euthanasia and blood collection

After urine collection and BP measurements, the rats were euthanized by decapitation, and blood was collected. To obtain the serum, the blood was centrifuged (58.136 g, 15 min, 4 °C). Serum measurements for creatinine concentration were done with enzymatic colorimetric kits (Bioclin).

Measurement of oxidative stress in colon and kidney

The right kidney and a portion of the colon were collected and freeze-stored (−80 °C) for further analysis. Renal cortex and colon tissues were homogenized in a cold buffer solution with 50 mm Tris and 1 mm EDTA (pH 7.4), 1 mm sodium orthovanadate, and 200 µg/mL phenylmethanesulfonylfluoride using an IKA RW 20 digital homogenizer, a pestle of potter-Elvehjem and glass tubes on ice. Homogenates were centrifuged (1,180 g, 10 min, 4 °C).[19] Protein contents were determined with Bradford protocol.[20]

Assessment of lipid peroxidation

An aliquot (0.3 mg/mL) of homogenate of tissues (renal cortex and colon) was used to quantify the production of malondialdehyde (MDA) in reaction with thiobarbituric acid (TBA, 100 °C). Sequential addition of 30% (v/v) of trichloroacetic acid and Tris −HCl (3 mm) was done to the sample, followed by centrifugation (2500x*g*, 10 min, 4 °C). TBA (0.8%, v/v) was added to the resulting supernatant, mixed, boiled for 15 min, and after cooling, the reaction was read at 535 nm on a spectrophotometer.

Assessment of superoxide dismutase (SOD) activity

Total superoxide dismutase (SOD) enzyme activity was determined according to Misra and Fridovich method. The tissue (renal cortex and colon) samples (0.3 mg/mL) were mixed with sodium carbonate buffer (0.05%, pH 10.2, 0.1 mmol/L EDTA, 37 °C), added of 30 mM/L of epinephrine (in 0.05% acetic acid). SOD activity was measured by the kinetics of epinephrine autooxidation inhibition for 1.5 min at 480 nm read on a spectrophotometer.[21]

Assessment of catalase (CAT) activity

Catalase activity was determined by the decomposition of H_2O_2 into O_2 and H_2O. A sample of tissue (renal cortex and colon) homogenate (0.3 mg/mL) in 50 mm phosphate buffer (pH 7.0) was added of 0.3M H_2O_2. Absorbance was measured at 240 nm for 1.5 min on a spectrophotometer.[22]

Assessment of glutathione S-transferase (GST) activity

A sample of tissue (renal cortex and colon) homogenate (0.3 mg/mL) was used to quantify GST activity, as previously described.[23] Phosphate buffer (0.1 M, pH 6.5 containing 1 mm EDTA), 1 mm 1-chloro-2,4-dinitrobenzene (CDNB), and 1 mm reduced glutathione (GSH) were added to tissue homogenate samples. Absorbance was measured at 340 nm for 1.5 min on a spectrophotometer.

Assessment of total thiols groups

Tissue (renal cortex and colon) homogenates samples (0.3 mg/mL) were incubated in extraction buffer (previously described) with 10 mm of 5,5'-dithiobis (2-nitrobenzoic acid) in a dark environment for 30 min. The absorbance of the reaction was measured at 412 nm on a spectrophotometer.[24]

Statistical analysis

Kolmogorov Smirnov test was used to assess the normality of data. The variables were reported as mean ± standard deviation and required the one-way analysis of variance (ANOVA) parametric test and Tukey post hoc test. For body weight measurements, statistical significance was evaluated with ANOVA (two-way test) with Bonferroni's post hoc test. Statistical analysis was done with the software Prism 5 (GraphPad Software, San Diego, CA, USA). The difference was considered significant when p was < 0.05.

Results

Body weight

Body weight at weaning up to 100 days of age was similar between groups ($p > 0.05$, Fig 2A). The body weight and weight gain at the

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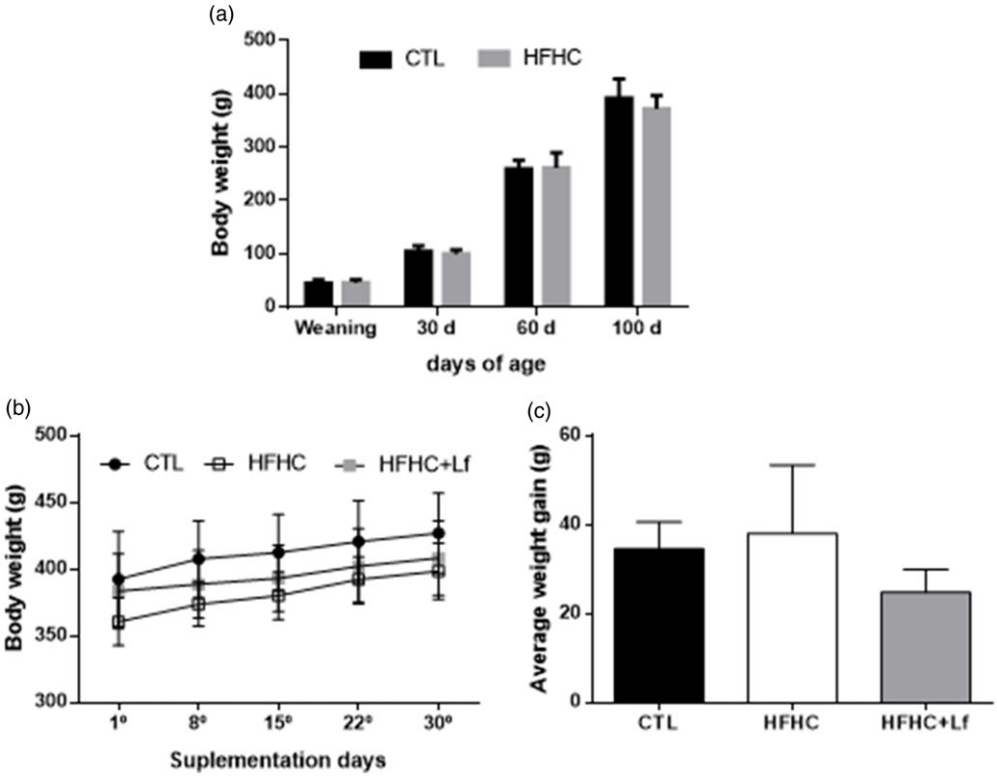

Fig. 2. Assessment of bodyweight at weaning up to 100 days of age and the effects of a multi-strain formulation with *Limosilactobacillus fermentum* 139, 263 296 on body weight in male offspring of dams fed a HFHC diet during pregnancy and lactation. Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test or ANOVA two-way with Bonferroni post-hoc test.

end of the protocol were similar among groups ($p > 0.05$, Fig 2B and C).

Blood pressure

Male offspring from dams fed a HFHC diet had increased SAP (CTL: 115 ± 6.5 *vs* HFHC: 127 ± 1.1mmHg, $p = 0.013$, Figure) and MAP (CTL: 89 ± 5.5 *vs* HFHC: 98 ± 3.8 mmHg, $p = 0.034$) when compared to the CTL group (Fig 3A and C). Administration of *Limosilactobacillus fermentum* formulation reduced SAP (HFHC: 127 ± 1.1 *vs* HFHC + Lf: 112 ± 4.2mmHg, $p = 0.004$), DAP (HFHC: 83 ± 5.3 *vs* HFHC + Lf: 74 ± 3.7mmHg, $p = 0.049$), and PAM (HFHC: 97 ± 1.9 *vs* HFHC + Lf: 87 ± 3.4 mmHg, $p = 0.015$) when compared to HFHC group (Fig 3A-C). HR was similar among groups ($p > 0.05$, Fig 3D).

Renal function

The HFHC group had a low urinary creatinine concentration compared to the CTL group ($p = 0.018$, Table 2). In contrast, the HFHC + Lf group had higher creatinine levels than the group exposed to HFHC diet ($p = 0.007$, Table 2). The urinary and serum creatinine values allowed to analyze creatinine clearance, an important indicator of renal function. There was a reduction in CCr in the HFHC group compared to the CTL group ($p = 0.048$, Table 2). On the other hand, administration of *Limosilactobacillus fermentum* formulation restored the renal function in male offspring from dams fed a HFHC diet ($p = 0.040$, Table 2). Serum levels of creatinine, urea, total proteins, and urinary volume were similar among groups ($p > 0.05$, Table 2).

Indicators of oxidative stress in the colon

The MDA levels, CAT activity, and thiols content in colonic mucosa were similar among groups ($p > 0.05$, Fig 4A, C, and E). HFHC group displayed reduced GST enzyme activity (CTL: 28.9 ± 8.1 *vs.* HFHC: 15.3 ± 2.8 U/mg protein, $p = 0.003$) compared to the CTL group (Fig 4D). Administration of a mixed *Limosilactobacillus fermentum* formulation increased antioxidant SOD activity (HFHC: 502 ± 75 *vs.* HFHC + Lf: 626 ± 70 U/mg protein, $p = 0.040$) in colon mucosa of male offspring exposed to maternal HFHC diet (Fig 4B).

Indicators of oxidative stress in the renal cortex

The GST activity and thiols content in the renal cortex were similar among groups ($p > 0.05$, Fig 5D and E). Male offspring from dams fed a HFHC diet had increased MDA levels (CTL: 0.12 ± 0.05 *vs.* HFHC: 0.25 ± 0.08 nmol/mg protein, $p = 0.023$) and reduced SOD (CTL: 399 ± 58 *vs.* HFHC: 309 ± 38 U/mg protein, $p = 0.051$) and CAT activities (CTL: 18.2 ± 2.9 *vs.* HFHC: 7.7 ± 2.7 U/mg protein, $p = 0.003$) in renal cortex when compared to CTL group (Fig 5A-C). Administration of *Limosilactobacillus fermentum* formulation restored the functional capacity of CAT activity in the renal cortex (HFHC: 7.7 ± 2.7 *vs.* HFHC + Lf: 15.2 ± 2.1 U/mg protein, $p = 0.005$, Fig 5C), besides tending an increase SOD activity (HFHC: 309 ± 38 *vs.* HFHC + Lf: 406 ± 75 U/mg protein, $p = 0.055$, Fig 5B) in male offspring from dams fed a HFHC diet.

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Table 2. Biochemical parameters of male offspring of dams fed a control diet (CTL) or high-fat high cholesterol diet (HFHC) during pregnancy and lactation and supplemented with a multi-strain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf)

	CTL	HFHC	HFHC + Lf
Urinary creatinine (mg/dL)	8.7 ± 3.1	3.5 ± 1.0*	10.4 ± 1.8†
Serum creatinine (mg/dL)	0.78 ± 0.19	0.80 ± 0.16	0.78 ± 0.10
Urea (g/24h)	400 ± 145	232 ± 89	370 ± 149
Total proteins (mg/24h)	15.3 ± 4.0	9.1 ± 5.0	16.5 ± 6.6
Urinary volume (mL)	7.0 ± 2.3	5.5 ± 1.2	7.2 ± 1.6
CCr (mL/min)	86.4 ± 43.5	25.2 ± 9.5*	82.9 ± 20.5†

CCr, Creatinine clearance.
*Shows significant difference compared to CTL.
† Shows significant difference compared to HFHC.

Discussion

The HFHC consumption during pregnancy has been shown to induce oxidative stress on the gut-kidney axis in rat offspring, renal dysfunction, and arterial hypertension later in life.[9] The results of the present study have demonstrated for the first time that administration of a multistrain *Limosilactobacillus fermentum* formulation reduced systolic, diastolic, and mean blood pressure levels and alleviated renal dysfunction and oxidative stress along the gut-kidney axis in male offspring from dams fed a HFHC diet during pregnancy and lactation.

Programmed arterial hypertension provoked by HFD consumption during pregnancy and/or lactation is complex and involves several impairments in key mechanisms related to blood pressure control.[1,25] It has been reported that gut inflammation and oxidative stress can compromise gut barrier integrity, favor LPS-translocation, and promote low-grade inflammation.[26] At the central nervous system, inflammation can lead to sympathetic overactivity and increased blood pressure.[27] On the other hand, the activation of the sympathetic nervous system induces increased gut permeability, low-grade inflammation, and alterations of gut microbiota composition, which in turn contribute to neuronal activity by releasing pathogenic bacterial metabolites into circulation.[28,29] Early studies have found increased gut damage, sympathetic hyperactivity, and enhanced blood pressure in offspring from dams fed a HFHC diet during pregnancy and lactation.[1,16]

Therapeutic strategies to alleviate the deleterious effects of maternal HFD consumption on blood pressure and cardiovascular function in offspring later in life are under investigation. Recent studies have suggested that interventions targeting the gut microbiota during pre-and postnatal periods could be important to prevent or reduce the risk of cardiovascular disorders in offspring later in life.[14,30,31] Dams fed a HFD and supplemented either with a prebiotic (long-chain inulin) or probiotic (*Lacticaseibacillus casei*)[32] or *Lactiplantibacillus plantarum* WJL[30] during pregnancy and lactation improved maternal gut microbiota diversity and protected male offspring against arterial hypertension and endothelial dysfunction.

Furthermore, it has been demonstrated that oral administration of *Limosilactobacillus fermentum* postweaning up to 90 days of age improved blood pressure and autonomic dysfunction in rat offspring exposed from dams fed a HFD.[14] This study has shown that administration of *Limosilactobacillus fermentum* in adult rats also

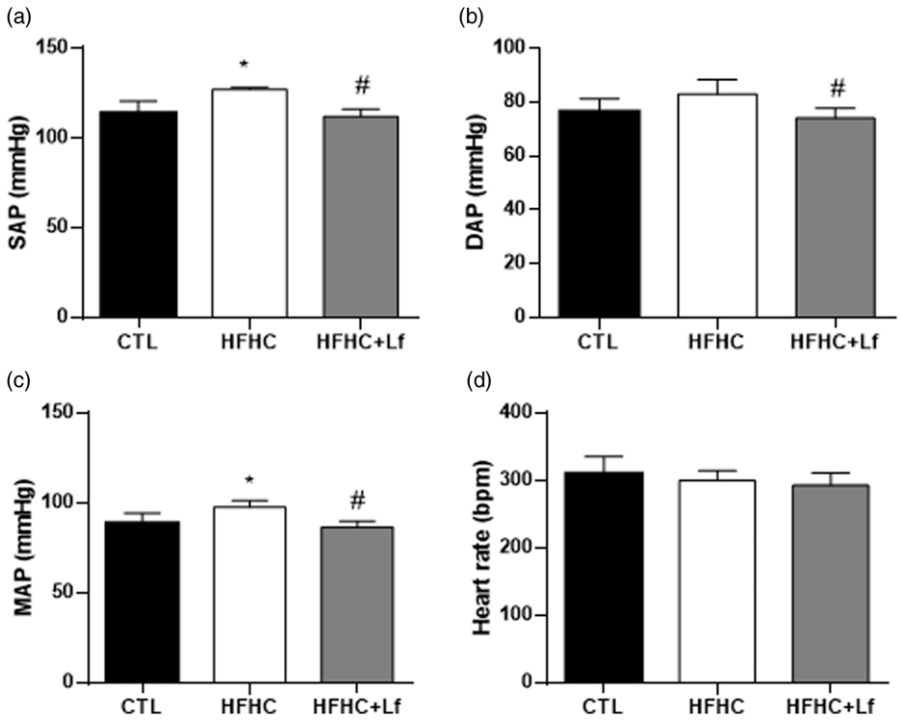

Fig. 3. Effects of a multi-strain formulation with *Limosilactobacillus fermentum* 139, 263, and 296 strains on blood pressure in male offspring of dams fed a HFHC diet during pregnancy and lactation. Assessment of systolic arterial pressure (SAP, A), diastolic arterial pressure (DAP, B), mean arterial pressure (MAP, C) and heart rate (D). Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test. *$p < 0.05$ indicates significant difference between HFHC or HFHC + Lf and CTL group; #$p < 0.05$ indicates significant difference between HFHC + Lf and HFD group.

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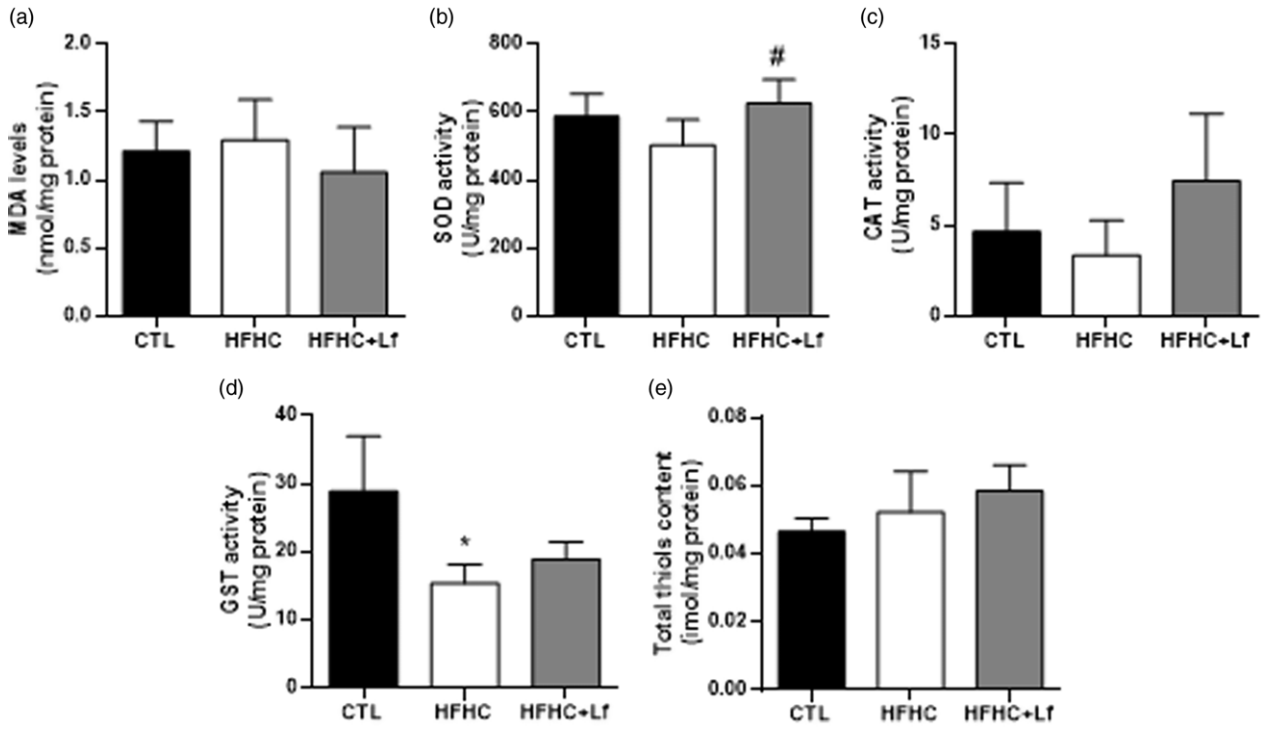

Fig. 4. Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on oxidative stress parameters in colon mucosa in male offspring of dams fed a HFHC diet during pregnancy and lactation. Assessment of malondialdehyde levels (MDA, A), superoxide dismutase activity (SOD, B), catalase activity (CAT, C), glutathione S-transferase activity (GST, D), and total thiols content (E) in the colon mucosa. Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test. *$p < 0.05$ indicates significant difference between HFHC or HFHC + Lf and CTL group; #$p < 0.05$ indicates significant difference between HFHC + Lf and HFD group.

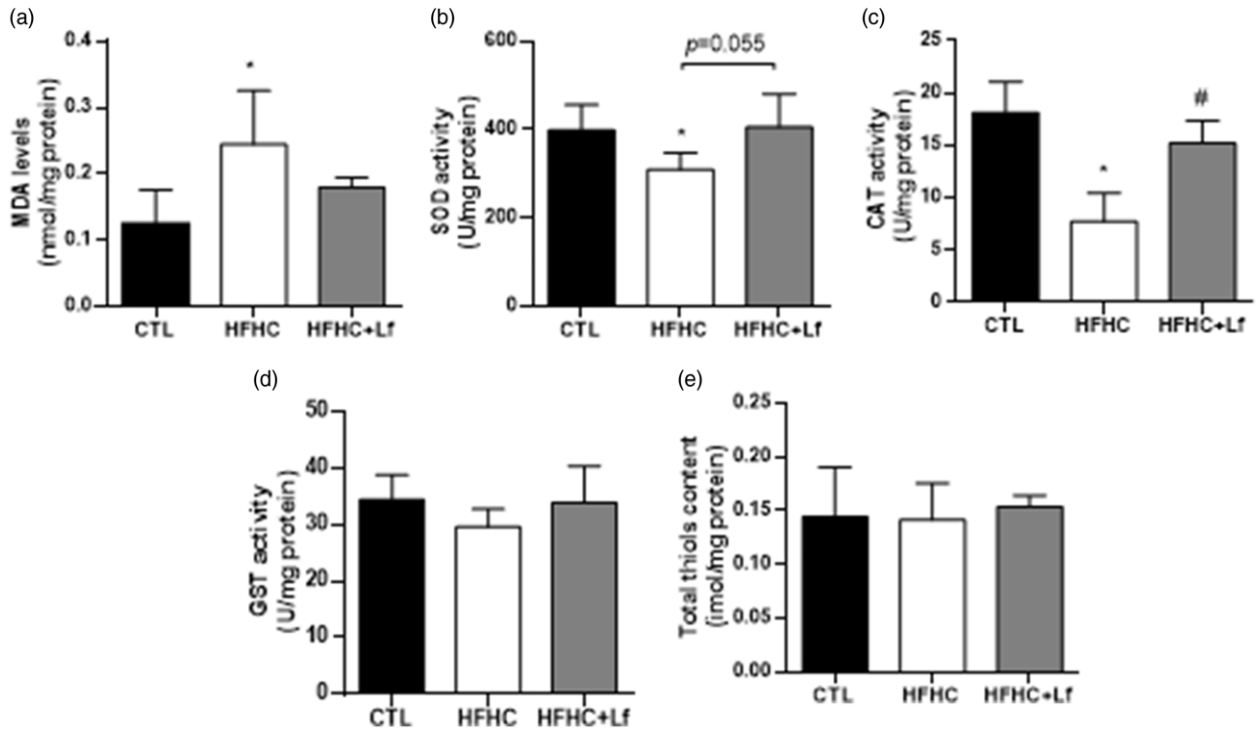

Fig. 5. Effects of a mixed formulation with *Limosilactobacillus fermentum* 139, 263, and 296 on oxidative stress parameters in renal cortex in male offspring of dams fed a HFHC diet during pregnancy and lactation. Assessment of malondialdehyde levels (MDA, A), superoxide dismutase activity (SOD, B), catalase activity (CAT, C), glutathione S-transferase activity (GST, D), and total thiols content (E) in the renal cortex. Groups: control (CTL); high fat and high cholesterol (HFHC); high fat and high cholesterol receiving *Limosilactobacillus fermentum* 139, 263, and 296 (HFHC + Lf). Data are presented as mean ± standard deviation and analyzed by ANOVA one-way test with Tukey as post-hoc test. *$p < 0.05$ indicates significant difference between HFHC or HFHC + Lf and CTL group; #$p < 0.05$ indicates significant difference between HFHC + Lf and HFD group.

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had a hypotensive effect in male offspring from dams fed a HFHC diet during pregnancy and lactation. These results suggest that targeting the gut microbiota on different developmental windows can exert a reprogramming strategy against arterial hypertension induced by maternal HFD consumption.

Although the underlying mechanisms by which *Limosilactobacillus fermentum* reduced blood pressure have not been assessed in the present study, some possible pathways related to probiotic-induced-hypotensive effects have been proposed.[33] First, it has been demonstrated that gut barrier integrity can be rescued by probiotic therapy, reducing LPS-translocation, inflammation, sympathetic activity, endothelial dysfunction, and blood pressure.[29,34] Second, short-chain fatty acids (SCFA), including acetate, propionate, and butyrate, are produced by specific gut microbes and can be enhanced during probiotic therapy.[35] An early study demonstrated that butyrate could lower arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors.[36] The propionate effect on blood pressure showed that propionate at low concentrations could activate Gpr41 and decrease blood pressure. In contrast, high concentrations can activate Olfr78 and increase blood pressure via renin signaling in the renal juxtaglomerular apparatus.[37,38] Third, the genus *Lactobacillus* has abundant gamma-aminobutyric acid (GABA)-producing species, including *Limosilactobacillus fermentum*.39 Although the effects of luminal GABA in the gut on blood pressure control are still controversial, there is evidence demonstrating that GABA can bind $GABA_B$ receptors and stimulate 5-HT release, attenuate sympathetic activity, and reduce blood pressure.[40]

In agreement with early studies,[9,41] maternal HFD diet consumption increased susceptibility to renal dysfunction and oxidative stress in kidney and colon mucosa in offspring later in life. Early investigations have demonstrated that administration of probiotic decrease renal dysfunction[42,43] and alleviate oxidative stress[13,44] in rats fed a HFD. For the first time, the results of this study have shown that the administration of a multistrain formulation with *Limosilactobacillus fermentum* with probiotic aptitudes,[14,15] improved renal function and antioxidant capacity along the gut-kidney axis in male offspring from dams fed a HFHD during pregnancy and lactation.

Although underlying mechanisms by which examined *Limosilactobacillus fermentum* formulation increased antioxidant capacity in colon and renal cortex were not explored in the present study, it has been reported MnSODs enzyme activity,[13] pseudocatalases,[45] and heme-dependent catalase[46] for some lactic acid bacteria. This becomes important since SOD has a key role in catalyzes of dismutation of superoxide anion in hydrogen peroxide, while CAT has a fundamental role in cellular detoxification of hydrogen peroxide, promoting a critical oxidative stress tolerance and antioxidant effect.[13] Second, the effects promoted by the administration of *Limosilactobacillus fermentum* formulation on renal function may be due to their ability to improve impermeability and immune function of the intestinal epithelium, preventing toxic compounds, such as lipopolysaccharides, from reaching the kidneys and causing their deleterious effects.[47] Lastly, short-chain fatty acid (acetate, propionate, and butyrate) generated from colonic bacterial fermentation can directly affect epigenome through histone posttranslational modifications.[48] Whether epigenetic pathways are involved in antioxidant capacity provoked by *Limosilactobacillus fermentum* formulation remains to be elucidated.

Although we have recently shown that *Limosilactobacillus fermentum* formulation alleviated gut microbiota impairment in male rats fed a HFD,[49] the lack of gut microbiota composition after *Limosilactobacillus fermentum* administration could be considered a bias for the results in this preclinical study.

In conclusion, administration of a multistrain containing three potentially probiotic *Limosilactobacillus fermentum* strains alleviated programmed hypertension, renal dysfunction, and enhanced antioxidant capacity along the gut-kidney axis in male offspring from dams fed a HFHC diet during pregnancy and lactation. These results indicate that gut targeting interventions could be a safe strategy for developmental reprogramming of arterial hypertension.

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Conflicts of interest. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (National Council for the Control of Animal Experimentation) and has been approved by the institutional committee of Federal University of Paraíba.

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