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The probiotic *Lactobacillus fermentum* 296 attenuates cardiometabolic disorders in high fat diet-treated rats

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Abstract *Background and aim:* High-fat (HF) diet consumption has been associated with gut dysbiosis and increased risk of dyslipidemia, type 2 diabetes mellitus and hypertension. Probiotic administration has been suggested as a safe therapeutic strategy for the treatment of cardiometabolic disorders. This study was designed to assess the effects of probiotic *Lactobacillus (L.) fermentum* 296, a fruit-derived bacteria strain, against cardiometabolic disorders induced by HF diet.

Methods and results: Male Wistar rats were divided into control diet (CTL); HF diet; and HF diet treated with *Lactobacillus fermentum* 296 (HF + Lf 296). The *L. fermentum* 296 strain at 1×10^9 colony forming units (CFU)/ml were daily administered by oral gavage for 4 weeks. The results showed that rats fed with HF diet displayed insulin resistance, reduced *Lactobacillus* spp. counts in feces, serum lipids, and oxidative profile. Rats fed on HF diet also demonstrated augmented blood pressure associated with sympathetic hyperactivity and impaired baroreflex control. The administration of *L. fermentum* 296 for 4 weeks recovered fecal *Lactobacillus* sp. counts and alleviated hyperlipidemia, sympathetic hyperactivity, and reduced systolic blood pressure in HF rats without affecting baroreflex sensibility.

Conclusion: Our results suggest the ability of *L. fermentum* 296 improve biochemical and cardiovascular parameters altered in cardiometabolic disorders.

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Introduction

Increased fat and cholesterol intake contributes to the development of dyslipidemias, type 2 diabetes mellitus and hypertension [1] and has been considered a relevant risk factor for the expansion of cardiovascular disease and premature death worldwide [2–4]. An HF and cholesterol diet induces gut dysbiosis [5], chronic low-grade inflammation and increased blood reactive oxygen species [6,7]. These features may lead to impairment of the cardiac baroreflex control [8] and autonomic dysfunction, characterized by increased sympathetic nervous system activity and depressed parasympathetic activity [9,10].

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Growing evidence have suggested that targeting gut microbiota with probiotic intervention may be a safe therapeutic strategy to promote beneficial effects in the treatment and/or prevention of cardiometabolic disorders [11–13]. Probiotic administration has shown effective in improving insulin resistance [14], sympathetic hyperactivity [15], hypertension [16] as well as markers related to dyslipidemias [17]. Probiotics are defined as "non-pathogenic microorganisms that administrated in adequate amounts confer a health benefit on the host" [18,19]. Amongst the common probiotics used for therapeutic intervention, the genus *Lactobacillus* include species with well-known effects on the improvement of cardiometabolic disorders [11,13,15,20].

Recently, different *Lactobacillus* strains isolated from fruit processing by-products were identified using 16S rRNA gene sequence analysis [21] and characterized for a set of standard probiotic-related attributes *in vitro* [21–23]. Among these strains, *L. fermentum* 296 showed satisfactory performance in a series of safety and physiological functionality tests, such as adhesion, autoaggregation, coaggregation, antagonism against pathogens, besides the lack of hemolytic and mucolytic activity. Additionally, this strain showed good survival when challenged with simulated gastrointestinal conditions in distinct food matrices [22]. However, the effects of L. *fermentum* 296 on improving lipid–glucose profiles and cardiovascular parameters in hyperlipidemia conditions remain unknown. Therefore, this study evaluated the effects of the administration of this probiotic strain on cardiometabolic disorders induced by HF diet in male Wistar rats.

Methods

Tested probiotic strain and cell suspension preparation

The *L. fermentum* 296 strain was gently supplied by the Laboratory of Food Microbiology, Department of Nutrition, Federal University of Paraíba (João Pessoa, Brazil). This strain was previously identified using 16S rRNA gene sequence analysis [21]. Stocks were stored at −20 °C in de Mann, Rogosa and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (Sigma-Aldrich, St. Louis, USA; 20 mL/100 mL).

Preparation of L. fermentum 296

The bacterial suspension used in the current study was obtained from overnight cultures grown on MRS broth (HiMedia, Mumbai, India) and incubated anaerobically (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37 °C. Cells were harvested by centrifugation (4500×*g*, 15 min, 4 °C), washed twice with sterile saline solution, re-suspended and homogenized using a vortex (30 s) in sterile saline solution to obtain standard cell suspensions with optical density (OD) reading at 660 nm (OD_{660}) of 1.0, which provided viable counts of approximately 9 log CFU/mL.

Ethical aspects and animals

Male Wistar rats (*Rattus norvergicus*) at 90 days of age were maintained in collective polypropylene cages at controlled temperature (22 ± 1 °C), with humidity between 50 and 55%, with filtered water and diet *ad libitum*, in a 12-h light–dark cycle. All experimental procedures were submitted and approved by the institutional animal care and use committee of the Federal University of Paraíba (CEUA-UFPB protocol 6080240418) and followed the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and the International Principles for Biomedical Research Involving Animals. All efforts were made to reduce the number and suffering of animals. Interventions with oral gavage were carried during the experiment in all groups.

Experimental design

The rats were randomly assigned to three groups: i) control group (CTL, n = 8) that received a commercial diet (Presence Purina®, Paulínea, Brazil); ii) high-fat group (HF, n = 8) that received a high-fat (HF) diet from Rhoster® Company (Araçoiaba da Serra, São Paulo, Brazil) and iii) HF group that received *L. fermentum* 296 (HF + Lf 296, n = 8). In the CTL and HF groups, saline solution was administered as a placebo for 4 weeks. In the HF + Lf 296 group, *L. fermentum* 296 strain in a solution of approximately 1×10^9 CFU/mL was administered daily for 4 weeks. Administration of vehicle or *L. fermentum* 296 was performed by oral gavage. Diet and administration of vehicle or *L. fermentum* 296 were started at the same time. Body weight was weekly measured during all experiment using an appropriate scale (model AS-1000; Marte, Santa Rita MG, Brazil). After 4 weeks of treatment with saline or *L. fermentum* 296, biochemical measurements, glucose, and insulin tolerance tests, arterial pressure and heart rate records, spectral analysis of systolic arterial pressure and cardiac interval, baroreflex sensitivity, sympathetic-vagal balance and sympathetic vascular tone were evaluated in each group.

Glucose and insulin tolerance test

The oral glucose tolerance (OGTT) and insulin tolerance (ITT) tests were performed on rats fasted overnight. In the OGTT, an oral glucose-load (2 g/kg) was administered via gavage. Blood samples were taken from the tail veins before glucose administration and, subsequently, at 15, 30, 60, 90 and 120 min. The ITT was performed after 24 h of OGTT, following an intraperitoneal insulin injection (0.75 UI/kg body weight); blood glucose concentrations were measured before (0 min) and after (30, 60, 90 and 120 min) the peritoneal insulin injection. Measurements of blood glucose concentration were performed with an Accu-Check glucometer (Bayer®).

Assay for serum measurements

Serum measurements were performed 24 h after the glucose homeostasis experiments. The male rats were

fasted overnight, anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), and blood samples (approximately 2 mL) were collected by plexus retro-orbital disruption [24]. The blood was centrifuged at 3000 g, 25 °C, for 15 min and serum measurements of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides concentrations were performed using appropriate enzymatic colorimetric kits according to the manufacturer instructions (Bioclin, Belo Horizonte - Minas Gerais - Brasil).

The concentration of malondialdehyde (MDA), an end product of lipid peroxidation, was measured as an indicative of oxidative stress. Briefly, 400 μL of perchloric acid (7%) was added to 250 μL of serum, mixed and centrifuged at 600 g, 4 °C, during 20 min. The supernatant was collected, added to 400 μL of thiobarbituric acid (0.6%), heated at 100 °C during 1 h and read at 532 nm. In this assay, MDA reacts with thiobarbituric acid to produce a red−colored complex [24].

Determination of baseline cardiovascular parameters

At least 48 h after collecting blood samples, the rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) for insertion of polyethylene catheters into the femoral artery and femoral vein. The catheters were tunneled through the back of the neck and ketoprofen (5 mg/kg) was injected subcutaneously.

Rats underwent a period of surgical recovery for 24 h. After this period, rats were healthy and without clinical signs of pain or distress. Arterial pressure (AP) and heart rate (HR) were recorded in conscious animals connecting the arterial cannula to a pressure transducer (ML866/P, ADInstruments, Power Lab, Bella Vista, NSW, Australia), as previously described [25]. The pulsatile AP (PAP) and HR were recorded for 50−60 min under baseline conditions, and the values of the systolic AP (SAP), diastolic AP (DAP), mean AP (MAP) and HR were calculated offline by selection of 10 min for each animal (LabChartTM Pro, ADInstruments, Bella Vista, NSW, Australia).

Using the same period of 10 min of baseline AP and HR records, the spectral analysis in the frequency domain of SAP and pulse interval (IP) were assessed using appropriate computational software (CardioSeries-v.2.4; www.danielpenteado.com). The spectra of SAP were integrated into the LF (0.2−0.75 Hz) and the HF bands (0.75−3 Hz). In addition, the LF/HF ratio of the IP was used to assess the sympathovagal index. Lastly, the spontaneous baroreflex sensitivity (SBRS) was calculated using sequence method [24].

The pulse interval was exported and analyzed with Kubios HRV Standard software version 3.0.2 (The Biomedical Signal and Medical Imaging Analysis Group, Department of Applied Physics, University of Kuopio, Finland). The heart rate variability (HRV) was analyzed: 1) time-domain parameters: the standard deviation between the duration of RR intervals (SDNN) and square root mean squared differences of successive RR intervals (RMSSD), and 2) Nonlinear parameters (SD1 and SD2). Poincaré scatters plots were constructed and investigated as a nonlinear tool, including the transverse axes (SD1, an indicator of parasympathetic activity) and the longitudinal axes (SD2, an function of sympathetic and vagal activity) [26].

Assessment of baroreflex control, cardiac autonomic function, and sympathetic tone

After 60 min of AP and HR baseline recordings, the cardiovascular responses obtained to vasoactive drugs phenylephrine (8 mg/kg, i.v.) and sodium nitroprusside (25 mg/kg, i.v.) were used to assess the sensitivity of the baroreceptor, as previously described [27]. Reflex changes in HR produced by vasoactive drugs administration were quantified and plotted as changes in heart rate over changes in MAP (ΔHR/ΔMAP). In addition, the data were analyzed by linear regression using Prism 6 (GraphPad Software, Inc., San Diego, CA, USA) and the slope of linear regression provided baroreflex sensitivity for each animal.

After the cardiovascular parameters have returned to baseline values (approximately 1 h after baroreflex evaluation), intravenous bolus injection of the muscarinic receptor antagonist methylatropine (2 mg/kg i.v; Sigma-Aldrich, St Louis, MO, USA) and the b-adrenoceptor antagonist propranolol hydrochloride (4 mg/mL/kg, i.v.; Sigma-Aldrich, St. Louis, MO, USA) were used to assess cardiac autonomic function [27]. The interval of treatment between drugs was 20 min. The parasympathetic tone was evaluated by the change in HR caused by methylatropine (ΔHR = HR post-methyl − HR before-methyl), whereas the sympathetic tone was determined by the HR change after propranolol administration (ΔHR = HR post-propranolol − HR before-propranolol).

The contribution of the sympathetic vascular tone to the cardiovascular system in a group of rats was assessed by an intravenous injection of the ganglionic blocker (hexamethonium, 30 mg/kg, Sigma-Aldrich®, St Louis, MO, USA). The sympathetic tone was calculated by the changes in MAP (ΔMAP) [28]. At the end of the experiments, animals were euthanized with an overdose of ketamine (i.v.).

Enumeration of Lactobacillus spp. in feces

Fecal samples were homogenized in peptone water (100 mg peptone/mL) and serially diluted in the same diluent. Twenty μL aliquots of the respective dilutions were inoculated using a microdrop technique (30) in MRS agar, followed by incubation at 37 °C under anaerobic conditions (Anaerobic System Anaerogen, Oxoid Ltd., Basingstoke, Hampshire, UK). After an incubation period of 24−48 h, the number of colonies was counted, and the results were expressed as log CFU/g.

Statistical analysis

Results were expressed as mean ± SEM. Kolmogorov Smirnov test was used to assess the normality of data. Most of the variables required one-way ANOVA parametric test and Tukey post-test. In cases by which Kolmogorov Smirnov normality test failed, the Kruskal-Wallis nonparametric test,

and followed by Dunn multiple comparison test was used. Two-way ANOVA was used in body weight, food intake and curves of the glucose and insulin tolerance test. Statistical analysis was performed using the computational software Prism 6 (GraphPad Software, San Diego, CA). The difference was considered significant when $p < 0.05$.

Results

Body weight and biochemical measurements

The body weight gain was similar among groups during the experimental period (Table 1). At the end of the experiment, HF group exhibited reduced body weight when compared with CTL group (Table 1). The experimental protocol based on an HF diet intake was effective in developing dyslipidemia. The HF group presented increased serum levels of total cholesterol, LDL-cholesterol, and triglycerides when compared to the CTL group (Table 1). Administration of *L. fermentum* 296 for 4 weeks significantly reduced the serum levels of cholesterol, LDL, and triglycerides (Table 1), but did not improve the serum levels of HDL-cholesterol in HF group (Table 1).

Serum MDA concentrations were higher in rats fed on an HF diet when compared to the CTL group (Table 1). The *L. fermentum* 296 administration did not reduce the serum MDA concentrations in the HF group (Table 1).

Assessment of the glucose tolerance test (OGTT) and the insulin tolerance test (ITT)

Rats fed an HF diet for 4 weeks exhibited greater area under the curve (AUC) after oral glucose loading and insulin tolerance test in comparison to CTL group ($p < 0.05$, Fig. 1A,B). This result suggests that after consuming HF diet for 4 weeks, rats became glucose intolerant and insulin resistant. The administration of *L. fermentum* 296 for 4 weeks did not cause reversion of the disruption of glucose homeostasis induced by the HF diet (Fig. 1A,B).

Cardiovascular parameters

Representative baseline recordings of PAP, SAP, DAP, MAP, and HR from all groups are shown in Fig. 2A. SAP and MAP were increased in rats fed on an HF diet for 4 weeks when compared to CTL group ($p < 0.05$, Fig. 2B,D). Administration of *L. fermentum* 296 reduced SAP and HR in rats fed on an HF diet ($p < 0.05$, Fig. 2B,E).

A representative spectrum of SAP and cardiac interval from all groups are shown in Fig. 3A,B. The LF oscillations of SAP and LF/HF ratio and SD2/SD1 of the cardiac interval were increased in rats fed on an HF diet when compared to CTL group (Table 2). The HF oscillations of SAP and SDNN, RMSSD, SD1 and SD2 of the cardiac interval were similar between groups (Table 2). Administration of *L. fermentum* 296 reduced LF/HF ratio and SD2/SD1 of the cardiac interval in rats fed on an HF diet (Table 2).

Figure 4A shows the original tracings from one representative animal of each group during the baroreceptor reflex. Rats from the HF group exhibited a reduction in BRS when compared to CTL rats ($p < 0.05$, Fig. 4B–D). The administration of *L. fermentum* 296 for 4 weeks did not prevent the baroreflex impairment in rats fed on an HF diet (-2.7 ± 0.44 vs. -3.24 ± 0.35 bpm/mmHg, $p > 0.05$, Fig. 4B–D).

Original tracings from one representative animal from each group showing the changes in arterial pressure and HR in response to the administration of atropine (vagal tone) and propranolol (sympathetic drive) are illustrated in Fig. 5A. The vagal tone was reduced in HF group rats when compared to CTL rats (56.5 ± 7.1 *vs.* 102.8 ± 15.2 bpm, $p < 0.05$, Fig. 5C), while the cardiac sympathetic drive was increased in HF group when compared with the CTL group (-101 ± 9.3 *vs.* -52 ± 9.9 bpm, $p < 0.05$, Fig. 5D). In comparison to untreated HF group, administration of *L. fermentum* 296 for 4 weeks reduced the cardiac sympathetic tone (-60 ± 9.3 *vs.* -101 ± 9.3 bpm, $p < 0.05$, Fig. 5D), but the vagal tone of HF group rats was not recovered ($p > 0.05$, Fig. 5C). These results suggest that *L. fermentum* 296 could reduce dysautonomia in the HF group through the reduction of sympathetic tone. For this reason, the vasomotor sympathetic tone was evaluated using the pharmacological ganglionic blockage according to the representative recordings (Fig. 6A). The fall in MAP was larger in the HF group when compared to the CTL group ($p < 0.05$, Fig. 6B). Administration of *L. fermentum* 296 for 4 weeks in rats fed on an HF diet blunted the fall in blood pressure elicited by hexamethonium when compared to non-treated HF group ($p < 0.05$, Fig. 6B). This data suggest that administration of *L. fermentum* 296 for 4 weeks reduces the vasomotor sympathetic drive in rats fed on an HF diet.

Table 1 Body weight and biochemical parameters.

Parameters	CTL	HF	HF + Lf 296
Initial body weight (g)	319 ± 4.4	310 ± 7.1	324 ± 4.4
Final body weight (g)	$382 \pm 7.3^{\dagger}$	$340 \pm 8.1^{*\dagger}$	$357 \pm 7.3^{\dagger}$
Cholesterol (mg/dL)	53.7 ± 2.11	$161.9 \pm 18.19^{*}$	$111.5 \pm 10.0^{\#\Psi}$
LDL-cholesterol (mg/dL)	18.1 ± 2.35	$142.6 \pm 20.9^{*}$	$84.9 \pm 10.6^{\#\Psi}$
HDL-cholesterol (mg/dL)	22.4 ± 2.5	14.1 ± 1.7	16.3 ± 4.1
Triglycerides (mg/dL)	50.3 ± 7.3	$84.9 \pm 8.3^{*}$	$52.5 \pm 4.6^{\#}$
TBARS (nmol/mL)	7.4 ± 0.8	$15.4 \pm 1.6^{*}$	11.4 ± 0.7

Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *L. fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test or two-way ANOVA. $^{*}p < 0.05$ compared with the CTL group. $^{\#}p < 0.05$ compared with the HF group. $^{\Psi}p < 0.05$ compared with the CTL group. $\dagger p < 0.05$ compared with initial body weight.

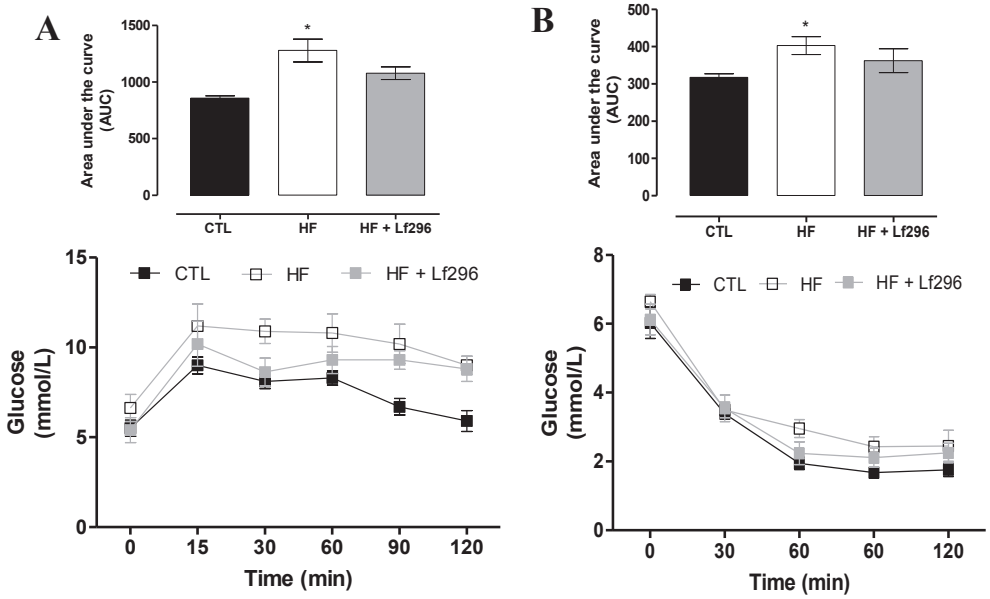

Figure 1 Effects of L. *fermentum* 296 treatment on glucose and insulin tolerance tests. Evaluation of area under curve (AUC) calculated from blood glucose values measured at different time point (0–120 min) during oral glucose (**A**) and insulin (**B**) tolerance tests in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with oral treatment of *L. fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. $^{*}p < 0.05$ compared with the CTL group.

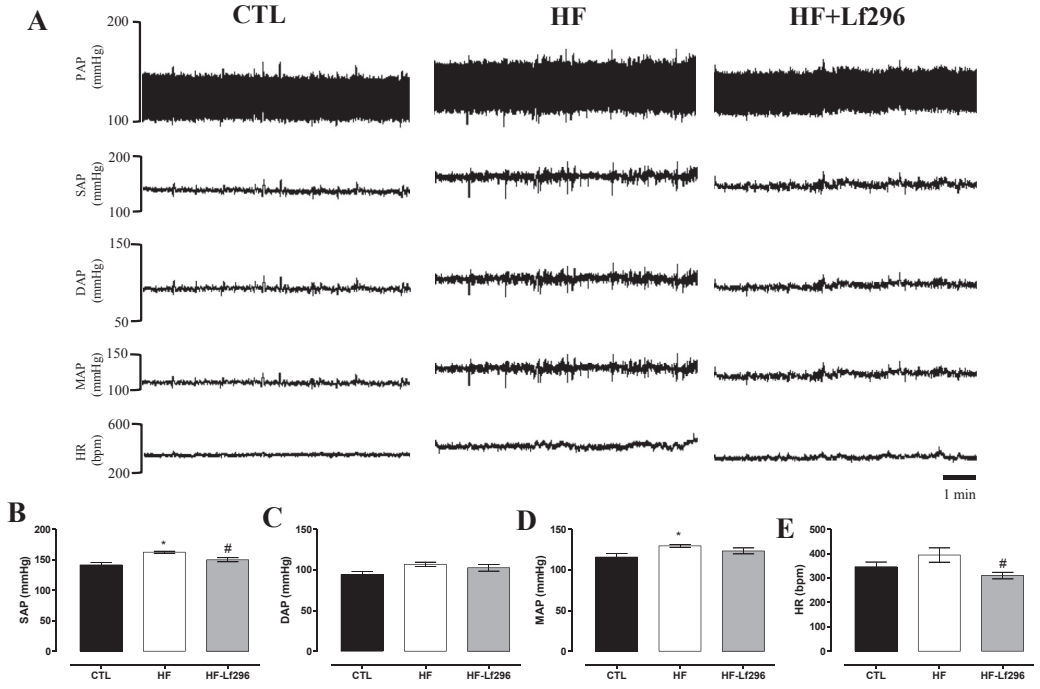

Figure 2 Effects of L. *fermentum* 296 treatment on resting blood pressure and heart rate. (**A**) Representative records of pulsatile blood pressure (PAP), systolic blood pressure (SAP), diastolic blood pressure (DAP), mean arterial pressure (MAP), and heart rate (HR). Evaluation of resting SAP (**B**), DAP (**C**), MAP (**D**) and HR (**E**) in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *L. fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. $^{*}p < 0.05$ compared with the CTL group. $\#p < 0.05$ compared with the HF group.

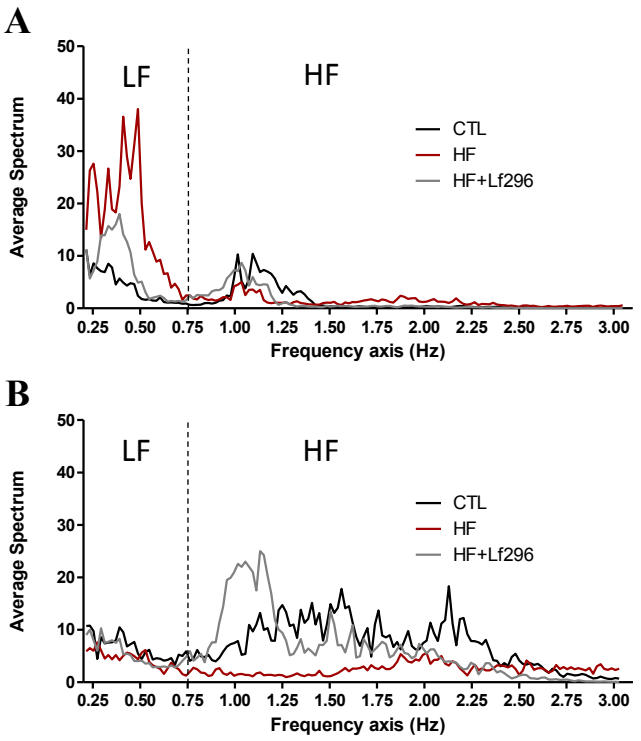

Figure 3 Representative spectra of the systolic arterial pressure (SAP, A) and cardiac interval (B) in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *L. fermetum* 296 (HF + Lf 296). Spectral power in the low frequency (0.25–0.75 Hz) band (LF); Spectral power in the high frequency (0.75–3.0 Hz) band (HF).

Lactobacillus spp. counts in feces

Consumption of HF diet reduced the counts of *Lactobacillus* spp. in feces when compared to the CTL group (Fig. 7). The population of *Lactobacillus* spp. in feces of HF group increased with administration of *L. fermentum* 296 for 4 weeks ($p < 0.05$, Fig. 7).

Table 2 Spectral analysis of systolic arterial pressure (SAP) and cardiac interval, spontaneous baroreflex sensitivity (SBRS) and heart rate variability measures between the three groups studied.

Parameters	CTL	HOF	HF + LG96
SAP			
LP band ($mmHg_2$)	4.0 ± 029	9.5 ± 2.1*	4.3 ± 0.83
HF band ($mmHg_2$)	2.9 ± 0.56	2.7 ± 0.74	2.3 ± 0.5
SBRS	1.8 ± 0.41	1.1 ± 026	1.88 ± 0.19
Cardiac interval			
LF'HF	0.17 ± 0.02	0.40 ± 0.04*	023 ± 0.03#
SDNN (ms)	424 ± 0.28	4.63 ± 0.51	4.65 ± 0.45
RMS SD (ms)	4.3 ± 025	4.3 ± 0.56	4.9 ± 0.61
SDÌ (ms)	3.07 ± 0.2	2.8 ± 0.5	3.47 ± 0.43
SD2 (ms)	4.8 ± 0.24	6.18 ± 0.62	5.6 ± 0.55
SD2/SD1	1.62 ± 0.06	2.30 ± 021*	1.67 ± 0.15#

Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of L. fermetum 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. *$p < 0.05$ compared with the CTL group. #$p < 0.05$ compared with the HF group.Ψ$p < 0.05$ compared with the CTL group. SDRR: standard deviation of NN; RMSSD: square root of the mean squared differences of successive RR intervals; LF: low frequency band; HF: high frequency band; SD: standard deviation of instantaneous RR interval variability.

Discussion

The results of this study revealed that the administration of *L. fermentum* 296 for 4 weeks increased the counts of fecal *Lactobacillus* spp. in rats, suggesting that *L. fermentum* 296 is able to modulate positively the population of *Lactobacillus* spp. in the gut and survive and colonize the gastrointestinal tract in adverse conditions such those imposed by the HF diet.

A growing number of clinical and experimental studies have demonstrated that probiotic administration can restore the microbial balance, gut permeability and insulin resistance in rats fed an HF diet [12,29,30]. Despite the reduction of approximately 15% in the glucose curves of OGTT and ITT, the administration of *L. fermentum* 296 for 4 weeks was not capable of restoring the glucose tolerance and insulin resistance in rats fed an HF diet.

Our study demonstrated that administration of the strain *L. fermentum* 296 successfully prevented the increase in the serum concentrations of TC, LDL-c, and triglycerides induced by HF diet consumption. Similarly, previous studies have demonstrated that administration of a single probiotic strain [11,31] or mixed probiotics formulation [32,33] exerted hypocholesterolemic and hypotriglyceridemic effects during HF diet consumption. Lactobacilli strain has been reported to bind to cholesterol in the intestine and enhance excretion of cholesterol in feces [34]. Indeed, colonic bacteria can produce propionate, a short chain fatty acid derived from the carbohydrate fermentative process, which acts inhibiting the hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme synthase enzyme and, consequently, reducing the hepatic cholesterol synthesis [35].

The etiology of hypertension in rats exposed to an HF diet includes a complex network involved in central and peripheral blood pressure control. Findings show that the hyperactivity of the sympathetic nervous system and impairment in arterial baroreceptor sensitivity plays a key role in establishment and maintenance of hypertension in rats fed on an HF diet [36,37]. Here we demonstrated that the intake of the HF diet for 4 weeks i) impaired cardiac baroreflex control, ii) provoked dysautonomia and iii) increased arterial blood pressure in male rats.

A growing body of evidence has demonstrated that gut dysbiosis may increase sympathetic activity [38] and exert a key role in the development of hypertension [39,40]. Studies show that strategies capable of recovering the community of commensal microbiota, such as probiotics [41], symbiotic [42] or fecal transplantation [38] may reduce sympathetic hyperactivity and blood pressure in hypertensive conditions [43–47]. We have demonstrated to the first time that the oral administration of the strain *L. fermentum* 296 for 4 weeks reduced sympathetic hyperactivity and systolic arterial pressure in male rats fed an HF diet.

Under baseline condition, the power of the LF band of SAP and LF/HF of the cardiac interval were significantly

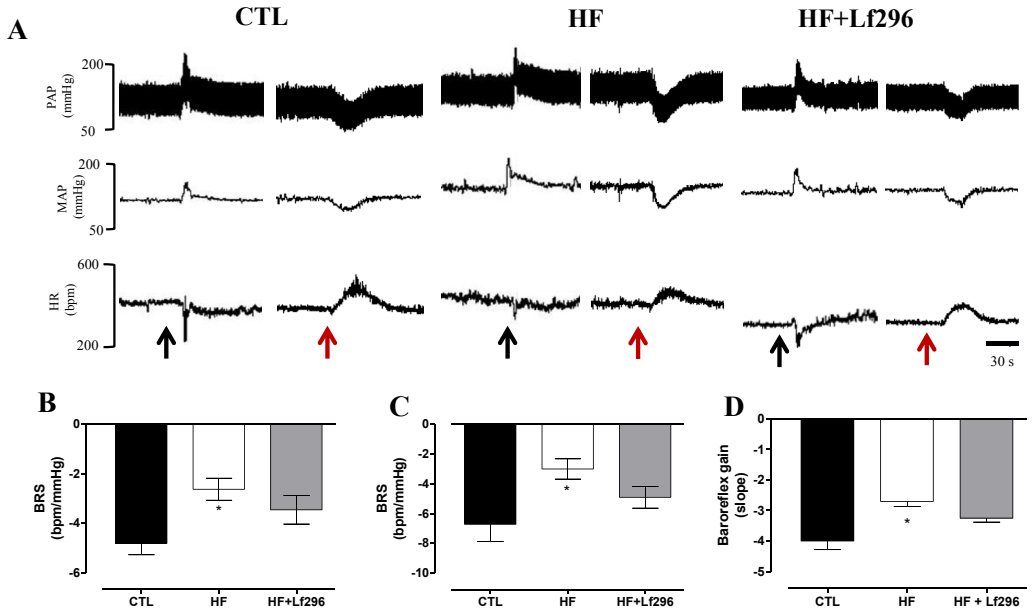

Figure 4 Effects of L. *fermentum* 296 treatment on baroreflex control. (**A**) Original tracings from one representative animal of each group showing changes in pulse arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in response to phenylephrine (8 μg/kg, i.v., black arrows) and sodium nitroprusside (25 μg/kg, i.v., red arrows). Evaluation of cardiac baroreflex during the administration of phenylephrine (**B**), sodium nitroprusside (**C**) and baroreflex gain (**D**) in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *L. fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. $^{*}p < 0.05$ compared with the CTL group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

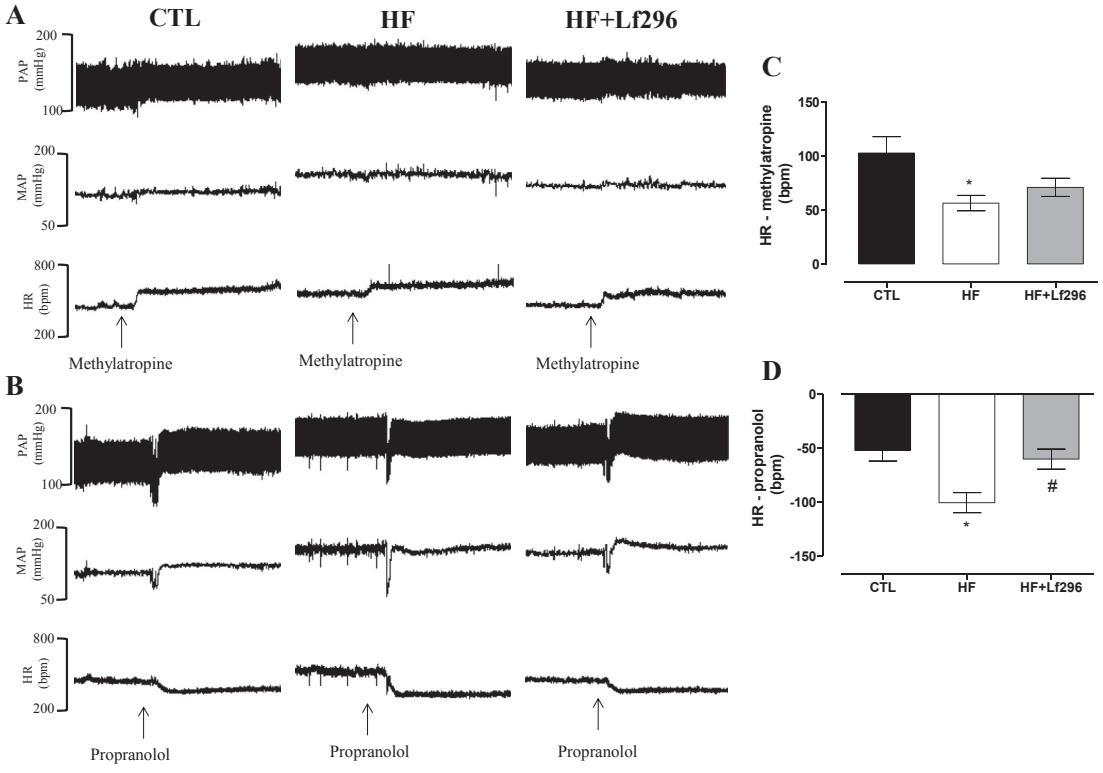

Figure 5 Effects of L. *fermentum* 296 treatment on cardiac autonomic modulation. (**A - B**) Original tracings from one representative animal of each group showing changes in pulse arterial pressure (PAP) mean arterial pressure (MAP) and heart rate (HR) in response to methylatropine (2 mg/kg i.v) and propranolol (4 mg/kg, i.v.). Evaluation of parasympathetic (**C**) and sympathetic (**D**) tone in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *Lactobacillus fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. The parasympathetic tone was analyzed by the Kruskal-Wallis test, and followed by Dunn post-test $^{*}p < 0.05$ compared with the CTL group. $\#p < 0.05$ compared with the HF group.

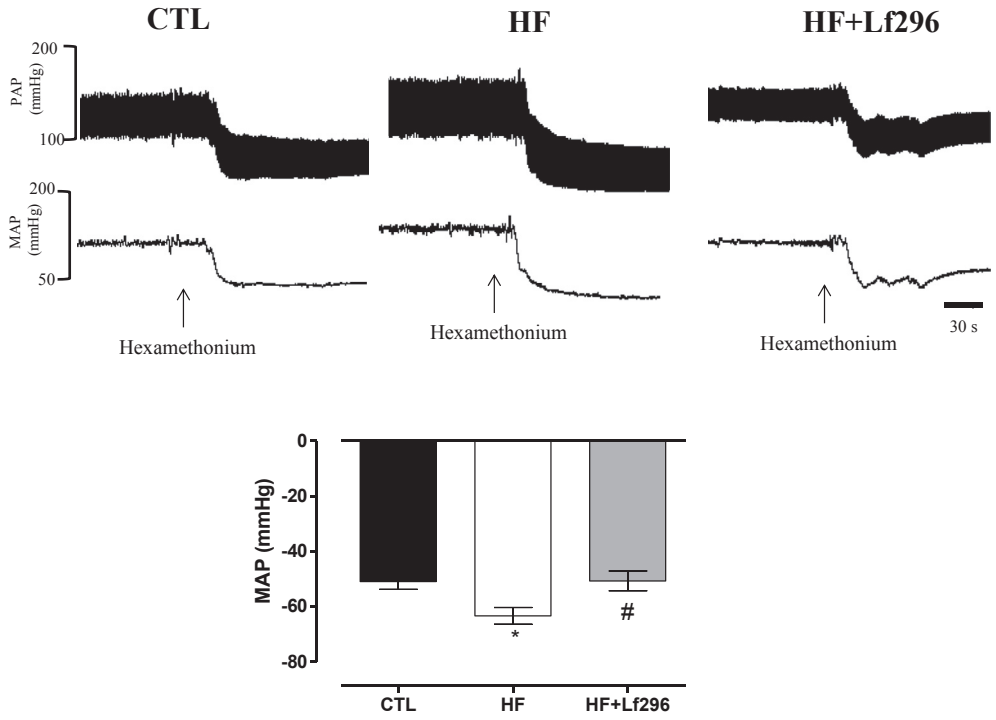

Figure 6 Effects of L. *fermentum* 296 treatment on sympathetic tone. (**A**) Original tracings from one representative animal of each group showing changes in pulse arterial pressure (PAP) and mean arterial pressure (MAP) in response to ganglionic blockade with hexamethonium (30 μg/kg, i.v.). Assessment of delta change of the MAP (**B**) after blockade with hexamethonium in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *L. fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. $^{*}p < 0.05$ compared with the CTL group. $\#p < 0.05$ compared with the HF group.

reduced in rats that received *L. fermentum* 296 when compared to non-treated HF group. This reveals that oral administration of *L. fermentum* 296 could reduce sympathetic hyperactivity in the HF group. The HRV reflects the oscillation in the time intervals between consecutive RR intervals and is extensively used to evaluated autonomic modulation of the cardiac function [26]. Although, in time-domain did not exhibit any meaningful differences among the study groups, in the nonlinear analysis, the oral administration of the strain *L. fermentum* 296 reduced SD2/SD1 in rats fed on an HF diet, demonstrating that oral administration of *L. fermentum* 296 could improve sympathovagal control in the HF group.

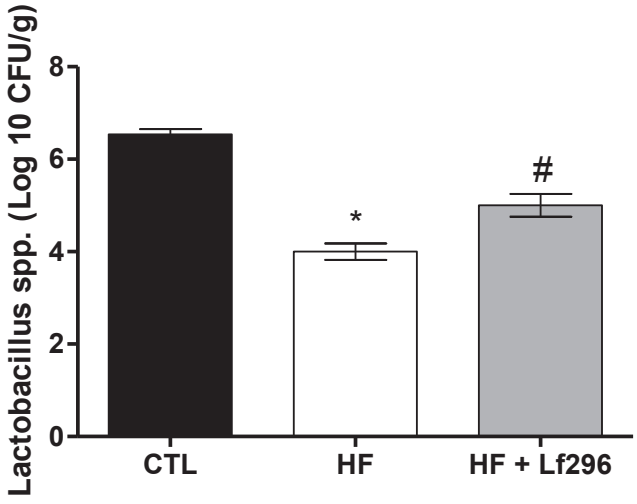

Figure 7 Effects of L. *fermentum* 296 treatment on fecal Lactobacillus counts. Assessment of fecal Lactobacillus counts in Wistar control rats (CTL), fed on a high fat diet (HF) and HF with the oral treatment of *L. fermetum* 296 (HF + Lf 296). Data are shown as mean ± SEM (n = 6–8 per group), analyzed by one-way ANOVA, and followed by Tukey post-test. $^{*}p < 0.05$ compared with the CTL group. $\#p < 0.05$ compared with the HF group.

Using a pharmacological approach, rats that received *L. fermentum* 296 for 4 weeks the fall in blood pressure elicited by hexamethonium was significantly reduced when compared to non-treated HF group. In addition, after propranolol administration, rats fed on an HF diet displayed an increase in cardiac sympathetic tone when compared with CTL rats and oral administration of *L. fermentum* 296 significantly reduced sympathetic cardiac tone in HF group. Taken together, pharmacological, spectral analysis of SAP and HRV, endorse that oral administration of *L. fermentum* 296 effectively reduced sympathetic hyperactivity in rats fed an HF diet.

One of the mechanisms proposed to explain sympathetic activation in hypertensive condition involves abnormalities in arterial baroreceptor sensitivity [48]. In this study, we observed that there was a reduction in baroreflex control in rats fed an HF diet and the oral administration of *L. fermentum* 296 was not able to restore baroreflex proper functionality in these animals. On the

other hand, a previous study demonstrated that the treatment with kefir for 8 weeks (a symbiotic matrix containing lactic acid bacteria and yeasts) recovered baroreflex sensitivity and attenuated hypertension in spontaneously hypertensive rats [42]. Our next step will be to test whether administration of *L. fermentum* for a time greater than 4 weeks could improve baroreflex functionality in rats fed an HF diet.

Although it has not yet been possible to establish the underlying mechanism by which *L. fermentum* 296 reduces sympathetic activity and blood pressure in rats fed an HF diet, the increased production of short-chain fatty acids (SCFAs), which modulate vasodilatation and induce hypotension, has been proposed as possible mechanism associated with the induction of these effects by acid-producing probiotics [43,49]. In addition, studies have demonstrated that *Lactobacillus* strains administration helps to reduce systolic arterial pressure by the decrease in vascular oxidative stress and vascular inflammation [41,50]. Lastly, the intestine is the most highly innervated peripheral organ with a significant number of motor fibers identified as sympathetic nerves [16]. The intraduodenal injection of *Lactobacillus johnsonii* La1 inhibited the adrenal sympathetic nerve and facilitated the gastric vagus nerve function in urethane-anesthetized rats. The inhibition of the adrenal sympathetic nerve was associated with decreased secretion of adrenaline, glucagon, and blood glucose levels [15].

In this way, studies will be carried by our laboratory to understand the underlying mechanisms by which *L. fermentum* 296 reduce sympathetic hyperactivity and systolic arterial pressure in rats fed an HF diet. In addition, future clinical studies should investigate the hypothesis that acute and chronic treatment with *L. fermentum 296* can dampen sympathetic nervous activity in patients with dyslipidemia or hypertension. If correct, this could be translated into new nutritional approaches to prevent or treat the cardiometabolic disease.

An important limitation of the study is that we evaluate the effects of *L. fermentum* 296 administration only in male rats. Further studies will be needed to know if the prevention of cardiometabolic disorders elicited by the *L. fermentum* 296 in male rats fed an HF diet happens in a similar way in females rats.

Conclusion

Administration of the potentially probiotic *L. fermentum* 296 strain for 4 weeks alleviates cholesterol and triglycerides serum levels in rats fed an HF diet. In addition, our study is the first to show that the administration of *L. fermentum* 296 effectively reduced sympathetic cardiovascular tone and systolic blood pressure in dyslipidemic rats, suggesting that *L. fermentum* 296 administration could become an alternative strategy to prevent diet-induced hypercholesterolemia and hypertriglyceridemia as well as their related cardiovascular complications.

Additional information

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this study.

Author contributions

RGSC and JLBA designed the research. RGSC, TMRA, MOLF, GAHF, and LACA conducted the experiments. RGSC and JLBA analyzed the data and performed the statistical analysis. RGSC and JLBA had primary responsibility for the final content. MM, JCC, VAB, ELS, and JLBA contributed to interpretation of the data and critically revised the manuscript. All authors read and approved the final manuscript.

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