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# $I_1$-imidazoline receptor-mediated cardiovascular and metabolic effects in high-fat diet-induced metabolic syndrome in rats

Alessandro R. Nascimento[a,c,*], Fabiana Gomes[a], Marcus V. Machado[a], Cassiano Gonçalves-de-Albuquerque[b], Pascal Bousquet[c], Eduardo Tibiriçá[a]

[a] Laboratory of Cardiovascular Investigation, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil
[b] Laboratory of Immunopharmacology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil
[c] Faculty of Medicine of the University of Strasbourg, Strasbourg, France

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ABSTRACT

*Objectives:* The objective of this study was to investigate the effects of a new $I_1$-imidazoline receptor-selective pyrroline compound on the hemodynamic, metabolic and microvascular alterations in a high-fat diet (HFD)-induced model of metabolic syndrome in rats.
*Methods:* In total, twenty adult male Wistar rats were fed a high-fat diet (HFD, $n = 20$) for 20 weeks. Thereafter, the rats received a new pyrroline compound selective for I1-imidazoline receptors (LNP599; 10 mg/kg/day) or vehicle ($n = 10$/group) orally by gavage for 4 weeks. Functional microcirculation was assessed using intravital video microscopy, and structural microcirculation was evaluated using histochemical analysis.
*Results:* LNP599 induced concomitant reductions in the SBP, HR and plasma catecholamine levels. The animals treated with this new antihypertensive compound also presented an improvement in body weight and the metabolic parameters related to metabolic syndrome, such as the glucose and lipid profiles. These effects were accompanied by a reversal of the functional and structural capillary rarefaction in the skeletal muscle.
*Conclusions:* The modulation of the sympathetic nervous system by a selective agonist for $I_1$-imidazoline receptors improves the hemodynamic and metabolic parameters in an experimental model of metabolic syndrome. LNP599 can also contribute to the restoration of microcirculatory parameters.

## 1. Introduction

Since Bousquet and colleagues proposed that centrally acting clonidine-like antihypertensive compounds exert their pharmacological blood pressure effects by interacting not only with $\alpha_2$-adrenoceptors (A2Rs) but also with imidazoline receptors (imidazoline binding sites) (Bousquet et al., 1984), extensive biochemical and physiological studies have been performed to investigate the contribution of these receptors to metabolic and blood pressure homeostasis (Regunathan and Reis, 1996; Ernsberger et al., 1997). Many efforts have been made to identify the proteins bearing imidazoline-binding sites and the imidazoline-specific receptor protein structures. During this time, specific imidazoline receptors, $I_1$-imidazoline receptors (I1Rs) (Ernsberger et al., 1995), $I_2$-imidazoline receptors (I2Rs), and $I_3$-imidazoline receptors (I3Rs), have been characterized (Gongadze et al., 2008), and highly selective novel imidazoline agents have been developed without any activity on A2R (Dardonville and Rozas, 2004).

I1Rs in the rostral ventrolateral medulla (RVLM) are important for the sympathoinhibitory actions of clonidine-, rilmenidine-, and moxonidine-like antihypertensive drugs. The mechanism by which central antihypertensives lower blood pressure is a result of activation of both A2R and I1R in the RVLM (Ernsberger et al., 1990). A2R agonists directly inhibit presympathetic RVLM neurons, while I1R agonists increase the release of catecholamines in the RVLM. The catecholamines depress presympathetic RVLM neurons by activating A2Rs (Chan et al., 2007). Compared with clonidine, newer centrally acting antihypertensive drugs such as rilmenidine and moxonidine are more selective for I1-IR than for A2R, thus causing only a few $\alpha_2$-adrenoceptor

*Abbreviations:* A2R, $\alpha_2$-adrenergic receptor; FCD, functional capillary density; HFD, high-fat diet; HR, heart rate; I1R, $I_1$-imidazoline receptor; MetS, metabolic syndrome; NEFAs, non-esterified fatty acids; RVLM, rostral ventrolateral medulla; SBP, systolic blood pressure; SCD, structural capillary density; SHR, spontaneously hypertensive rats; SNS, sympathetic nervous system

* Corresponding author at: Laboratório de Investigação Cardiovascular, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, C.P. 926, 21045-900 Rio de Janeiro, Brazil.
*E-mail address:* alessandrorn1@gmail.com (A.R. Nascimento).

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mediated side effects (Feldman et al., 1990; Edwards et al., 2012).

Sympathetic hyperactivity is a recognized medical condition in the development of most of the metabolic syndrome (MetS) components, such as insulin resistance, hypertension, hyperlipidemia, and obesity (Grassi et al., 2005; Lindgren et al., 2006). Numerous reports have highlighted that antihypertensive treatment should not only aim for a reduction in blood pressure but also diminish sympathetic overactivity (Ernsberger et al., 1996; Kalil and Haynes, 2012). In this context, centrally acting antihypertensive drugs may be useful in the management of arterial hypertension because sympathetic overactivity can be effectively modulated by drugs that act directly on the site of origin, that is, the central nervous system. The mechanisms linking MetS to sympathetic activation are complex and still not completely understood. Accordingly, sympathetic activity modulation needs to be assessed in an experimental model of human-like MetS. Thus, the MetS model based on a diet enriched in lipids was shown to be useful for studies of sympathetic hyperactivity in the metabolic and microcirculatory disorders that characterize this syndrome (Dobrian et al., 2000).

The hypothesis underlying the present study was based upon the recent report from our group that demonstrated a link between the modulation of the central sympathetic nervous system (SNS) and its effects on hemodynamic and microcirculatory alterations in a rat model of high-fat diet-induced MetS (Nascimento et al., 2016). Our purpose for this work was to obtain additional information on the imidazoline receptor modulation of sympathetic hyperactivity with no known A2R side effects in a HFD-induced model of MetS in rats. Furthermore, we investigated the effects of a third-generation centrally acting antihypertensive compound on cardiometabolic parameters.

The present study reports that long-term treatment with this new I1R-selective compound results in the reversal of microvascular rarefaction in skeletal muscle and improves metabolic parameters in a human-like experimental model of MetS.

## 2. Materials and methods

### 2.1. Animals

All the experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animals and were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (protocol # LW-31/11). The experiments were performed in male Wistar Kyoto rats (WKY, Oswaldo Cruz Foundation Animal Facilities, Brazil) housed under controlled light (12:12 h light-dark cycle) and temperature (22 ± 1 °C) conditions.

### 2.2. Experimental protocol

At four weeks of age, the rats were fed a high-fat diet (HFD; $n = 20$) for 20 weeks. The HFD contained 14% protein, 56% carbohydrate, 30% lipid and salt supplementation (standard chow + corn starch + condensed milk + animal fat + 0.5% NaCl). The main fat source of the HFD was saturated fat (lard). The composition of the diet chow used in the present study is in agreement with the guidelines of the American Institute of Nutrition, as revised in 1993, for rats in this period of life.

After 20 weeks of HFD ingestion, the animals were divided into two groups of 10 animals each and treated for 28 days by gavage with vehicle (HFD + VEH, control group) or LNP599 (HFD + LNP, 10 mg/kg/day). We previously verified that this dosage of LNP599 normalizes arterial pressure in chronically treated hypertensive rats. To exclude a direct effect of calorie restriction, the HFD was maintained during the pharmacological treatment.

The total body weight and food intake of the animals were measured before and after the pharmacological treatment. At the end of the diet period, the visceral (retroperitoneal and mesenteric) and epididymal adipose tissue depots were dissected and weighed.

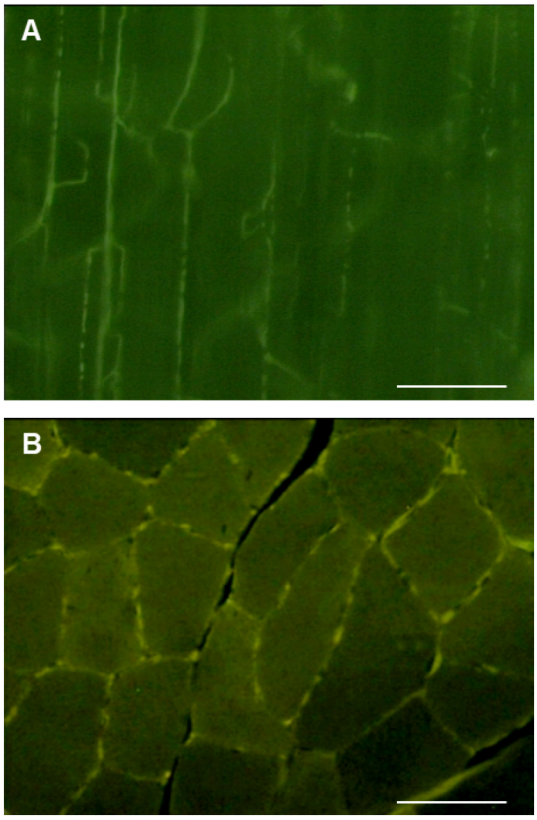


**Fig. 1.** Representative examples of microscopic images of the gracilis muscle microcirculation. (A) Intravital fluorescence videomicroscopy after IV injection of fluorescein isothiocyanate (FITC)-labeled dextran for functional capillary density and (B) transversal sections of the skeletal muscle stained with FITC-conjugated Griffonia simplicifolia lectin in the histologic evaluation of structural capillary density. Magnification 200×, bar = 100 mm.

### 2.3. Hemodynamic measurements

Systolic blood pressure (SBP) and heart rate (HR) were measured by a computerized tail-cuff plethysmography system (Visitech Blood Pressure Analysis System, model BP-2000, Apex, NC, USA) in conscious animals in the morning (8–12 AM). At least one week before arterial pressure recordings, the rats were acclimatized for 3 consecutive days using a prewarmed tail-cuff device. SBP was measured immediately before and on the last day of the antihypertensive treatment.

### 2.4. Intravital fluorescence video microscopy

In the morning of the terminal experiments, the animals were anesthetized with pentobarbital (75 mg/kg, i.p.); the anesthesia was complemented by i.v. injection of 5 mg/kg pentobarbital immediately before administration of the neuromuscular-blocking agent (pancuronium 1 mg/kg, i.v.). The rats were artificially ventilated with room air using a small animal ventilator (Ugo Basile model 7025, Varese, Italy). The right jugular vein was catheterized to permit injection of the anesthetic agents and fluorescent dye. The central temperature was monitored with a rectal probe, and the body temperature was maintained at 38 ± 0.5 °C with a homoeothermic blanket system (Harvard Apparatus, Boston, USA).

The gracilis muscle was exposed through an incision in the right thigh and covered with an oxygen-impermeable plastic wrap. The animals were then placed under an upright fixed-stage intravital microscope (Olympus BX51 WI, USA) coupled to a CCD digital video camera system (Optronics, Goleta, USA). Magnification with an Olympus objective (10×) was used in the experiments, resulting in a total

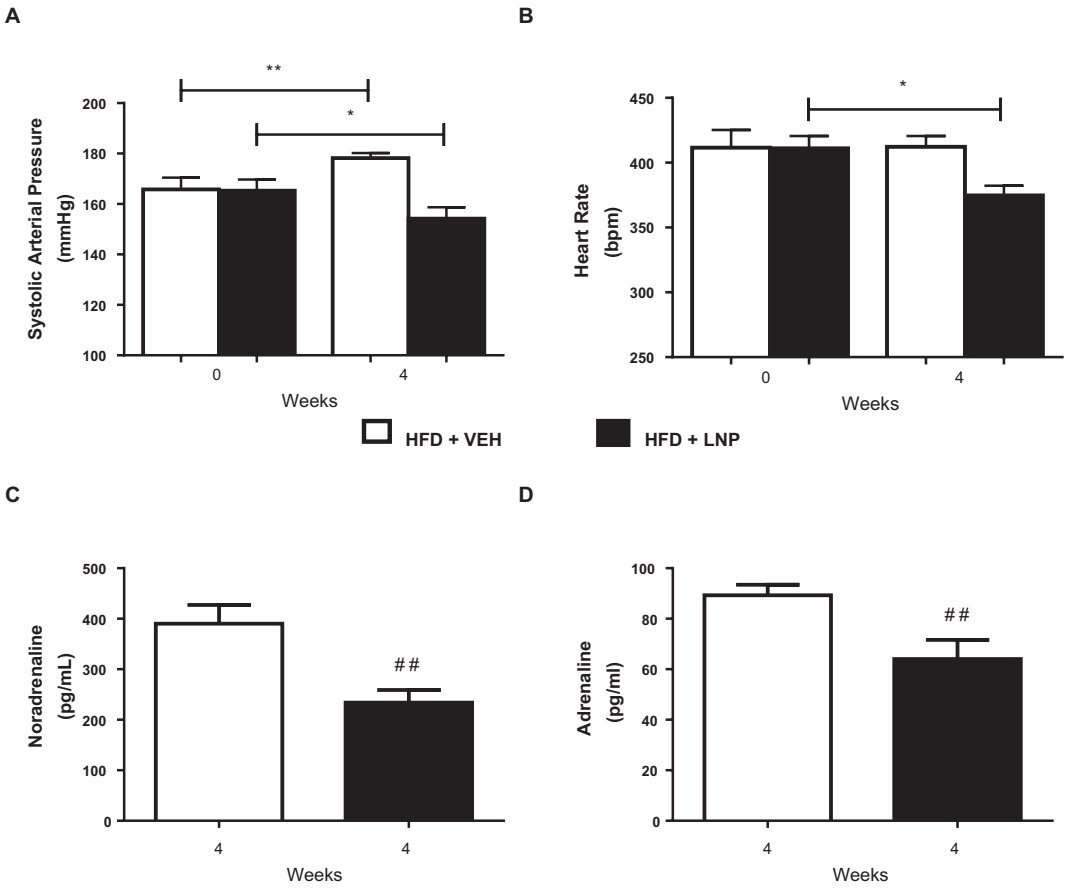


**Fig. 2.** Systolic arterial pressure (A) and heart rate (B) before and after four weeks of treatment. Noradrenaline (C) and adrenaline plasma levels (D) in the control (HFD + VEH, white columns) group treated with vehicle or LNP599 (HFD + LNP, 10 mg/kg/day, black columns) after 4 weeks of treatment.
$^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* before treatment.
$^{\#\#}p < 0.01$ *vs.* HFD + VEH after treatment.

magnification of 100× on the video monitor. After intravenous injection of 0.15 ml 5% fluorescein-isothiocyanate (FITC)-labeled dextran (molecular weight 150,000), microscopic images of the muscle (Fig. 1A) were successively obtained for online counting of capillaries using Saisam software (Microvision, Evry, France). Functional capillary density, which is considered the total number of spontaneously perfused capillaries per square millimeter of surface area (1 $mm^2$), was determined in random microscopic fields over a period of 4 min. After the experiment, the animals were sacrificed by pentobarbital overdose, and the gracilis muscle was immediately dissected and placed in 4% neutral-buffered paraformaldehyde for morphological analysis.

### 2.5. Histochemical analysis of skeletal muscle

Tissue samples were stained with FITC-conjugated *Griffonia simplicifolia* I lectin (Fig. 1B), and the structural capillary density (number of capillaries per $mm^2$) and structural fiber density (number of muscle fibers per $mm^2$) were identified and recorded with a fluorescence microscope (Olympus BX51 and Fluoview SV 300 scanning unit, Olympus, USA) as previously described (Sabino et al., 2008).

### 2.6. Evaluation of plasma catecholamines

Blood samples were collected using tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 3000 rpm for 15 min at 4 °C. The plasma was stored at −80 °C until the analysis. Noradrenaline and adrenaline levels in the plasma were measured by a reference high-performance liquid chromatography (HPLC) method.

### 2.7. Metabolic measurements

Plasma total cholesterol (TC) and triglyceride (TG) levels were directly measured using enzymatic methods (COLESTEROL Liquiform®, Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). Total plasma leptin levels were measured using a commercially available Leptin ELISA Kit (LDN, Nordhorn, Germany), and non-esterified fatty acid (NEFA) levels were measured using a NEFA Kit (Zen-Bio Inc., Research Triangle Park, NC, USA).

An oral glucose tolerance test (OGTT) was performed after a 6-hour fast to calculate the area under the curve (AUC). Blood glucose levels were measured at 0, 30, 60 and 120 min after the administration of a glucose overload (2.0 g/kg). A portable glucose monitor was used to measure the blood glucose levels in venous blood (OneTouch Ultra 2®, LifeScan Inc., Milpitas, CA, USA). Plasma insulin levels were measured by radioimmunoassay (ImmuChemTM, MP Biomedicals, Santa Ana, CA, USA). Evaluation of insulin resistance was accomplished using the Homeostasis Model of Assessment - Insulin Resistance (HOMA-IR), which was calculated using the following formula:

Fasting Glucose (mmol/l) × Fasting Insulin (μIU/ml)/22.5

### 2.8. Drugs

The following drugs were used: sodium pentobarbital, pancuronium bromide, FITC-labeled dextran, FITC-conjugated *Griffonia simplicifolia* I lectin, EDTA, glucose, (Sigma Chemical Co., St. Louis, USA) and LNP599 (Laboratoire de Neurobiologie et Pharmacologie

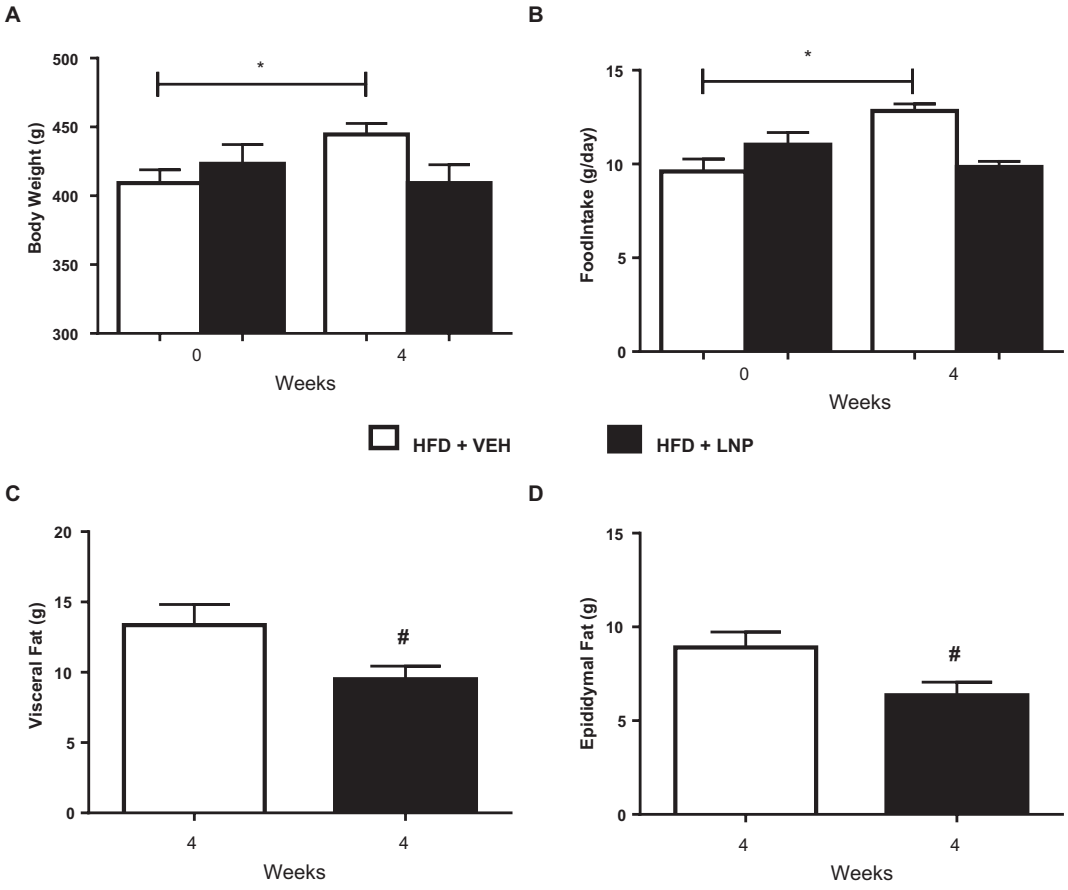


**Fig. 3.** Body weight (A) and food intake (B) before and after four weeks of treatment. Visceral (C) and epididymal fat (D) in the high-fat diet control group treated with vehicle (HFD + VEH, white columns) or LNP599 (HFD + LNP, 10 mg/kg/day, black columns) for 4 weeks.
*$p < 0.05$ *vs.* before treatment.
#$p < 0.05$ *vs.* HFD + VEH after treatment.

Cardiovasculaire, Strasbourg, France)

### 2.9. Statistical analysis

The results are expressed as the mean ± SEM for each group. The Shapiro-Wilk test was used to verify approximately normal statistic distributions. Comparisons between groups were made using Student's *t*-test or two-way ANOVA when appropriate. GraphPad Prism software (version 6.0, GraphPad Software, Inc., La Jolla, CA, USA). A $p$ value $< 0.05$ was considered as significant.

## 3. Results

### 3.1. Hemodynamic parameters, visceral fat depots, body weight and food intake

Systolic arterial pressure increased after four weeks of the high-fat diet and vehicle treatment (165 ± 4 mmHg *vs.* 178 ± 2 mm Hg, $p < 0.05$; Fig. 2A) without any heart rate alteration (411 ± 13 bpm *vs.* 412 ± 8 bpm, $p > 0.05$; Fig. 2B) in the control group (HFD + VEH). Antihypertensive treatment with LNP599 for four weeks caused a marked decrease in arterial pressure (165 ± 4 mm Hg *vs.* 154 ± 4 mm Hg, $p < 0.05$) and HR (411 ± 9 bpm *vs.* 374 ± 7 bpm, $p < 0.05$) compared with their values before treatment (Fig. 2A and B, respectively). The cardiovascular effects of this drug were accompanied by significant reductions in the plasma noradrenaline (390 ± 37 pg/ml *vs.* 233 ± 25 pg/ml, $p < 0.01$) and adrenaline (89 ± 4 pg/ml *vs.* 64 ± 7 pg/ml, $p < 0.01$) levels after 4 weeks of treatment (Fig. 2C and D, respectively).

The total body weight (409 ± 9 g *vs.* 444 ± 7 g, $p < 0.05$) and food intake (9 ± 0.6 g *vs.* 12 ± 0.3 g, $p < 0.05$) of the animals were increased by the HFD during the vehicle delivery period (Fig. 3A and B, respectively). In contrast, chronic treatment with LNP599 caused no significantly reduction in the body weight (423 ± 13 g *vs.* 409 ± 13 g, $p = 0.1$; Fig. 3A) and tended to prevent the increase in food intake (11 ± 0.6 g *vs.* 9 ± 0.2 g, $p = 0.2$; Fig. 3B). Finally, the prevention of increased food intake that was observed at the end of the treatment with LNP599 was associated with a reduction in the visceral (13 ± 1 g *vs.* 9 ± 0.9 g, $p < 0.05$) and epididymal (8 ± 0.8 g *vs.* 6 ± 0.7 g, $p < 0.05$) fat depots (Fig. 3C and D, respectively).

### 3.2. Metabolic measurements

The total cholesterol (199 ± 8 mg/dl *vs.* 160 ± 15 mg/dl, $p < 0.05$) and triglyceride (55 ± 7 mg/dl *vs.* 36 ± 3 mg/dl, $p < 0.05$) levels were significantly lowered by the LNP599 treatment (Fig. 4A and B, respectively). Pharmacological treatment did not affect the plasma leptin levels, probably because of the large dispersion of the values (1.3 ± 0.3 ng/ml *vs.* 1.0 ± 0.1 ng/ml, $p > 0.05$, Fig. 4C). However, LNP599 treatment markedly reduced the NEFA levels (1391 ± 70 µM *vs.* 1061 ± 44 µM, $p < 0.001$; Fig. 4D).

NEFAs, non-esterified fatty acids.
Values represent the mean ± SEM, $n = 10$ for each group.
#$p < 0.05$, ###$p < 0.001$ *vs.* HFD + VEH after treatment.

The fasting glucose concentrations were not different between the HFD + LNP group (100 ± 2 mg/dl *vs.* 95 ± 1 mg/dl, $p < 0.05$;

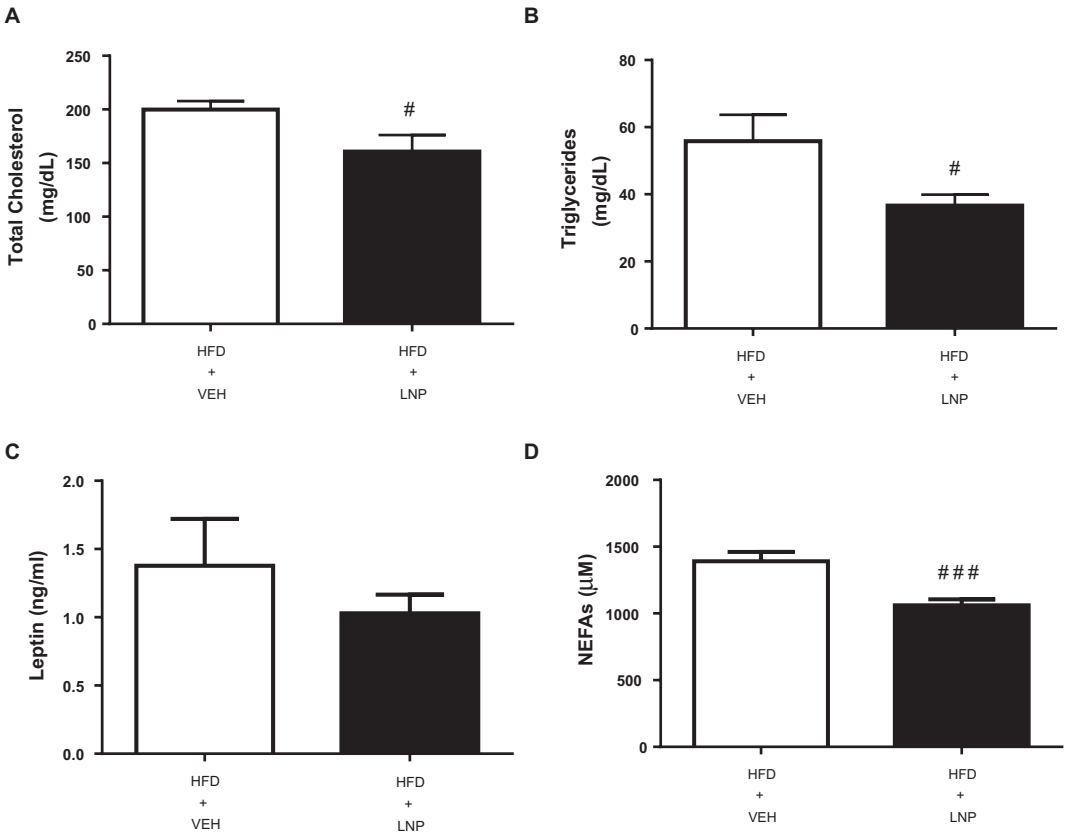


**Fig. 4.** Plasma levels of total cholesterol (A), triglycerides (B), leptin (C) and NEFAs (D) in the high-fat diet control group treated with vehicle (HFD + VEH, white columns) or LNP599 (HFD + LNP, 10 mg/kg/day, black columns) for 4 weeks.

Fig. 5A) and the control group (HFD + VEH). However, the glucose impairment was attenuated at 30 and 60 min of the oral glucose tolerance test after chronic treatment with LNP599 (Fig. 5A). This effect was confirmed by the reduction in the area under the curve for the oral glucose tolerance test (14,703 ± 314 mg/dl/min *vs.* 13,179 ± 260 mg/dl/min, $p < 0.001$; Fig. 5B). Pharmacological treatment with LNP599 did not alter the plasma insulin levels (58 ± 4 μIU/ml *vs.* 51 ± 7 μIU/ml, $p > 0.05$; Fig. 5C) or HOMA-IR values (14 ± 1 mg/dl/min *vs.* 12 ± 1 mg/dl/min, $p > 0.05$; Fig. 5D).

### 3.3. Microvascular parameters

The functional capillary density in skeletal muscle was markedly increased by pharmacological treatment with LNP599 (153 ± 8 capillaries/mm$^2$ *vs.* 241 ± 10 capillaries/mm$^2$, $p < 0.0001$; Fig. 6A). Finally, we also observed an important increase in the structural capillary density represented by the increase in the capillary-to-fiber ratio in skeletal muscle compared with that in the control group (1.5 ± 0.08 capillaries/fiber *vs.* 1.7 ± 0.03 capillaries/fiber, $p < 0.01$; Fig. 6B).

## 4. Discussion

In this work, we investigated the influence of a new $I_1$-imidazoline agonist that is known to modulate the central SNS on the cardiovascular and metabolic parameters in an experimental model of MetS induced by a high-fat diet. The main findings of the present study are as follows: (1) LNP599 significantly decreased the blood pressure and heart rate as a result of its sympathoinhibitory action in a rat model of MetS and (2) the modulation of the SNS, which was confirmed by a decrease in plasma catecholamine levels, improved the functional and structural microvascular alterations in this experimental model of MetS. In addition, (3) these results constitute the first demonstration of the effects of a selective $I_1$ imidazoline receptor agonist on the metabolic and microcirculatory parameters that are altered in MetS.

Based on structure-activity relationship data, we chose LNP599 [(3-chloro-2-methyl-phenyl)-(4-methyl-4,5-dihydro3Hpyrrol-2-yl)-amine-hydrochloride], a new pyrroline derivative with nanomolar affinity for I1Rs ($7.10^{-9}$ M) and no significant affinity for A2Rs ($> 10^{-5}$ M) and for a large set of receptors, transporters, and enzymes (Fellmann et al., 2013b). This compound was designed and synthesized to be an imidazoline-like drug acting exclusively on I1Rs and was selected because it can cross the blood-brain barrier and has good intestinal absorption, permitting both oral and intravenous delivery (Schann et al., 2012). LNP599 was used as a selective agonist of I1Rs; this feature is the main direct pharmacological effect of LNP599 that has been described to date.

Rats were used for the metabolic and hemodynamic effects observed in different studies after high-fat diet-induced MetS (Nascimento et al., 2013; Shiou et al., 2017; de Visser et al., 2017; Fellmann et al., 2013a; Palei et al., 2017). Animals with MetS were treated orally by gavage with LNP599 for 4 weeks. It is important to highlight that since we published the first study that included data concerning the characterization and validation of the experimental model of metabolic syndrome in rats after 20-week HFD diet in 2013 (Nascimento et al., 2013), we have published several additional articles (Estato et al., 2017; Machado et al., 2016; Machado et al., 2014; Nascimento et al., 2016; Pereira et al., 2017) that have confirmed the reproducibility of the mentioned animal model.

As predicted, the sympathoinhibitory effect of LNP599 produced an important reduction in arterial blood pressure and heart rate in the animals with MetS; this reduction was associated with a reduction in the plasma catecholamine levels after long-term treatment. The mechanism of the hypotensive action of centrally acting antihypertensive drugs is well established, but in this case, the effect is mediated

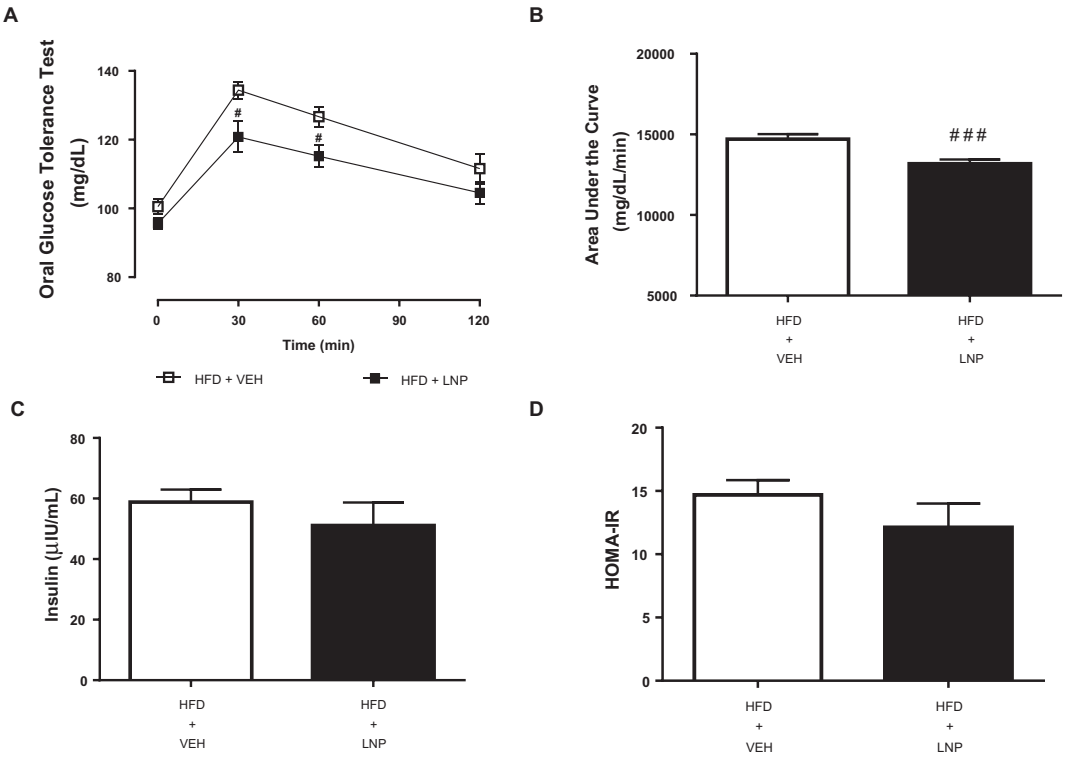


**Fig. 5.** Oral glucose tolerance test (A), area under the curve (AUC) of plasma glucose (B), insulin (C) and HOMA-IR (D) in the high-fat diet control group treated with vehicle (HFD + VEH) or LNP599 (HFD + LNP, 10 mg/kg/day) for 4 weeks.
HOMA-IR, homeostasis model assessment for insulin resistance.
Values represent the mean ± SEM, $n = 10$ for each group.
$^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ *vs.* HFD + VEH after treatment.

exclusively by I1Rs and is in principle insensitive to catecholamines. Sy et al. demonstrated that G-protein inwardly rectifying potassium (GIRK) channels are related to the hypotensive effect of drugs acting selectively on I1Rs in the central cardiovascular system. This effect, which was elicited by intracisternal injections of imidazoline-like drugs in anesthetized rabbits (Yoro Sy et al., 2008), seems to mainly be mediated by I1Rs located in the nucleus reticularis lateralis of the brainstem (Ernsberger and Shen, 1997).

Using the protocol of a high-fat diet to induce an experimental model of MetS, we succeeded in inducing a body composition alteration, which was improved by the treatment with LNP599. In this case, I1R modulation reduced the visceral and epididymal fat deposition with an effect on body weight. This effect was associated with the prevention of increased food intake during the treatment. The role of I1R in feeding behavior was already described by Chung et al. in 2012, when he demonstrated that rilmenidine – a centrally acting antihypertensive drug with less affinity for A2Rs than for I1Rs - decreased the food intake of STZ-diabetic mice in a dose-dependent manner through activation of I1Rs by lowering the hypothalamic NPY level (Chung et al., 2012). Thus, the alterations in the feeding behavior and body composition of the animals induced by LNP599 may have directly influenced the improvement in metabolic parameters, such as the total cholesterol and triglyceride plasma levels, observed in this study.

Several studies have suggested that sympathetic hyperactivity could be involved in the pathogenesis of MetS (Lambert et al., 2010; Lindgren et al., 2006; Straznicky et al., 2012a; Straznicky et al., 2012b). However, the cause of this overactivity is not clear, but it is commonly associated with certain adipokines (Smith and Minson, 2012). Leptin - a type of adipokine that is primarily secreted by white adipocytes - plays a role in physiologic functions that include reducing appetite, increasing energy expenditure, regulating glucose utilization, and improving insulin sensitivity (Kelesidis et al., 2010). It has been proposed that leptin concentration is positively correlated with adipose tissue as an independent marker of MetS. In our study, the plasma levels of leptin were reduced slightly but not significantly by LNP599. However, the level of plasma non-esterified fatty acids (NEFAs) – products from the breakdown of triglycerides ingested in the diet – was significantly reduced after long-term treatment with LNP599. In humans, circulating levels of NEFAs are increased in obesity, and augmented levels are inversely correlated with insulin sensitivity (Bruce et al., 1994; Perseghin et al., 1997). In contrast, in our study, the reduction in NEFAs was not reflected in an improvement in the insulin resistance of animals with MetS.

In a recent *in vivo* study performed with spontaneously hypertensive heart failure (SHHF), the same compound, LNP599, had favorable effects on blood pressure, body weight, lipids and the glucose profile (Fellmann et al., 2013b). Conversely, despite the similarities concerning the sympathomodulatory effects of LNP599 on cardiovascular parameters, the improvement in glucose metabolism was not clear in our study. This difference in insulin resistance and glucose intolerance seems to be dose dependent and could be better evoked in animals that have been treated with the present compound at a dosage of 20 mg/kg/day, as used by Felmann and collaborators in 2013 (Fellmann et al., 2013b). However, it is important to note that Fellmann et al. used SHHF, which is an experimental model of MetS that is not induced by diet.

We previously reported that first- and second-generation centrally acting antihypertensive drugs can reverse the functional and structural microcirculatory alterations associated with metabolic and hemodynamic modifications in rats used as an experimental model of HFD-induced MetS (Nascimento et al., 2016). In this context, in the present study, we confirmed that LNP599, a third-generation centrally acting antihypertensive drug, can also improve functional and structural capillary rarefaction. This effect is a result of precapillary sphincter and

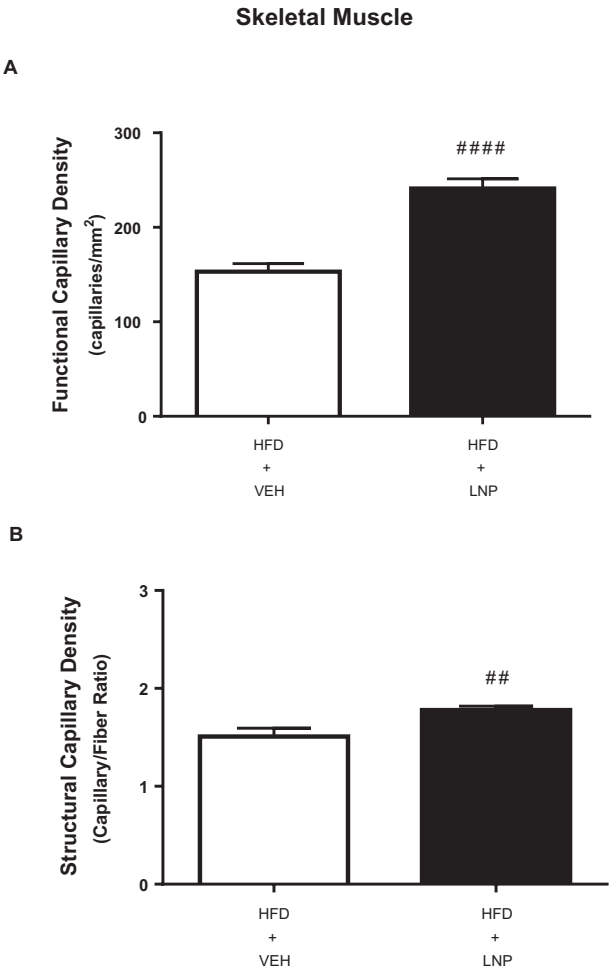


**Fig. 6.** Functional (A) and structural (B) capillary densities in the skeletal muscle of the high-fat diet control group treated with vehicle (HFD + VEH) or LNP599 (HFD + LNP, 10 mg/kg/day) for 4 weeks.
Values represent the mean ± SEM, n = 10 for each group.
$^{\#\#}p < 0.01$ and $^{\#\#\#\#}p < 0.0001$ *vs.* HFD + VEH after treatment.

arteriole vasodilation, followed by an increase in capillary recruitment due to central sympathetic inhibition. Our group already demonstrated the involvement of central sympathetic modulation in peripheral microcirculatory effects after the intracisternal administration of different centrally acting drugs into the central nervous system, which promoted vasodilation of the mesenteric microcirculation in SHR at doses that did not induce cardiovascular effects when administered systemically (Estato et al., 2004).

Regarding the limitations of this study, an important fact must be considered. We did not test the effects of central sympathetic inhibition with LNP599 in normal rats fed a standard diet. The present study focused essentially on the effect of the sympathetic hyperactivity modulation by LNP599 in a rodent model of metabolic syndrome. This experimental model presents an increase in the activity of the sympathetic tone that could most likely be modulated by centrally acting antihypertensive drugs. Consequently, we did not have tested over normal rats where the pharmacological effect could not be confirmed.

## 5. Conclusions

According to the results reported in the present study, we can suggest that the use of a well-tolerated, third-generation I1R-selective drug may be an appropriate and important strategy for improving metabolic parameters and hypertension in patients with MetS through the management of several of its components using only one drug.

## Competing interests

No conflicts of interest are declared by the authors.

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