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Basic nutritional investigation

Oral Ang-(1-7) treatment improves white adipose tissue remodeling and hypertension in rats with metabolic syndrome

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ABSTRACT

Objective: Angiotensin (Ang)-(1-7) has preventive effects on metabolic syndrome (MetS). The aim of this study was to evaluate the therapeutic effect of oral Ang-(1-7) on mean arterial pressure (MAP), insulin resistance (IR), inflammatory process, and remodeling of white adipose tissue (WAT) in rats with established MetS.
Methods: Rats were subjected to control (CT; AIN-93M) or high-fat (HF) diets for 13 wk to induce MetS and treated with Ang-(1-7) or vehicle (V) for the last 6 wk. At the end of 13 wk, MAP, biochemical and histological parameters, and uncoupling protein (UCP) and inflammatory gene expression were determined by quantitative reverse transcription polymerase chain reaction.
Results: HF-V rats showed increased visceral fat deposition, inflammatory cytokine expression, hyperplasia, and hypertrophy in retroperitoneal (WAT) and brown adipose tissue (BAT). Additionally, the gastrocnemius muscle reduced UCP-3 and increased the UCP-1 expression in BAT. HF-V also elevated levels of plasma insulin, glucose, homeostatic model assessment (HOMA) of IR and HOMA-β, and increased body mass, adiposity, and MAP. Ang-(1-7) treatment in rats with MetS [HF-Ang-(1-7)] reduced WAT area, number of adipocytes, and expression of proinflammatory adipokines in WAT and BAT and increased UCP-3 in gastrocnemius muscle and UCP-1 expression in BAT compared with the HF-V group. These events prevented body mass gain, reduced adiposity, and normalized fasting plasma glucose, insulin levels, HOMA-IR, HOMA-β, and MAP.
Conclusion: Data from the present study demonstrated that oral Ang-(1-7) treatment is effective in restoring biochemical parameters and hypertension in established MetS by improving hypertrophy and hyperplasia in WAT and inflammation in adipose tissue, and regulating metabolic processes in the gastrocnemius muscle and BAT.

Introduction

Metabolic syndrome (MetS) is a global health problem and it is characterized by the presence of at least three disturbances as insulin resistance (IR), hypertension, and central obesity [1]. White adipose tissue (WAT) has an energy reservoir function contributing as an important body metabolic regulator. Increased WAT deposit is considered an independent risk factor for IT [1,2]. Additionally, the increase in WAT is associated with the presence of low-grade inflammation and high levels of angiotensin (Ang) II, cyclooxygenase 2 (COX-2), and adipokines, including leptin, adiponectin, adpisin, resistin, and tumor necrosis factor (TNF)-α [2-4]. On the other hand, brown adipose tissue (BAT) has a thermogenic function by inducing lipid peroxidation through the action of uncoupling proteins (UCPs) and metabolizing lipids from WAT [2]. Adipose tissue (WAT and BAT), in response to changes in the body's energy status, undergoes

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dynamic adipocyte remodeling, including hyperplasia (increases in adipocyte number) or hypertrophy (enlargement of adipocyte area) that interferes with its functions.

Some studies have shown that blocking angiotensin-converting enzyme–Ang-I–angiotensin II receptor 1 (AT1R) axis of the renin–angiotensin system (RAS), by ACE inhibitors or AT1R antagonist, induces weight loss and decreased visceral obesity in animals fed a high-fat (HF) diet [5,6]. Other studies increasing the axis activity of ACE2–Ang-(1-7)–Mas receptor (MasR), or using transgenic (TGR) rats that express an Ang-(1-7) releasing fusion protein [7] improved glucose, lipid metabolism, attenuate inflammation, and decreases abdominal fat mass as preventive treatment in MetS [8] induced by a HF diet [7]. So, ACE2, Ang-(1-7), and MasR has counterregulatory effects to the ACE–Ang-II–AT1R axis [8]. However, studies showing Ang-(1-7) formulation as oral therapy correlating hypertrophy, hyperplasia, and inflammatory process of adipose tissue and cardiovascular parameters are lacking.

Thus, the present study evaluated the therapeutic effect of oral Ang-(1-7) formulation that was incorporated in the oligosaccharide cavity hydroxypropyl-β-cyclodextrin (HPβCD) that protects the heptapeptide from digestive enzymes [9], HPβCD/Ang-(1-7), in remodeling WAT associated with improvement of IR, inflammatory process, and increasing gene expression of UCP-1 in BAT and UCP-3 in the gastrocnemius muscle and mean arterial pressure (MAP) in rats with MetS induced by a HF diet.

Methods

Animals

The study used male Fischer rats, at 4 wk of age (50 ± 0.9 g, N = 40) from Animal Science Center (CCA/UFOP) of the Federal University of Ouro Preto (UFOP, Brazil). Animals were kept in individual cages under controlled temperature (25°C ± 1°C) and a 12-h/12-h light–dark cycle. Throughout the experiment, the animals had free access to water and diet. All procedures were performed in accordance with the Guidelines for Ethical Care of Experimental Animals. The protocol was approved by the Animal Ethics Committee of the Federal University of Ouro Preto (protocol no. 2011/31).

Experimental protocol

After weaning, rats were randomly subjected to a control diet (CT; AIN-93 M) or a HF diet (AIN 93M–37% lard) for 13 wk [10]. In week 7, in order to verify whether the animals presented disturbances characteristic of MetS, body mass (g), MAP (mm Hg), and heart rate (HR; bpm) were evaluated by digital tail plethysmography (Panlab, LE5001). Additionally, blood samples (12 h of fasting) were collected to evaluate blood glucose and oral glucose tolerance test (OGTT). OGTT was performed by assessing glycemia by gavage administration of 40% glucose solution (1 g/kg) to the animals after 10, 20, 30, 60, and 90 min by glucose analysis (glycosimeter, Accu-chek) and the area under the curve (AUC) was calculated using trapezoidal analysis [11]. Food intake and body weight of rats were calculated by the mean. In the last 6 wk of the diet, the animals were subdivided into four groups that received daily treatment by gavage with 40 μg/kg of Ang-(1-7) (CT-Ang-[1-7] or HF-Ang-[1-7]), or were treated with vehicle HPβCD without Ang-(1-7) (CT-V or HF-V). At the end of 13 wk, the animals were sacrificed for biometric and biochemical evaluation and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. Gastrocnemius muscle and inguinal, epididymal, and retroperitoneal fat and interscapular BAT were immediately collected, weighed, frozen instantaneously in liquid nitrogen, and stored at −80 or 10% of formaldehyde for further analysis. Adiposity index was calculated by the formula:

inguinal fat deposit + epididymal fat deposit

+ retroperitoneal fat deposit absolute weight/body mass of rat (g) × 100[12].

Cardiovascular parameters

MAP (mm Hg) and HR (bpm) were measured by digital tail plethysmography (Panlab, LE5001) in rats at weeks 7 and 13 of the diet.

Plasma analysis

At the end of 13 wk, after sacrifice by instant decapitation of the fasted animals at night, blood samples (2–3 mL) were collected. The samples were centrifuged (8000*g*, at 4°C for 6 min) to separate the plasma for determination of fasting glycemia (blood treated with Glistab anticoagulant containing EDTA and potassium fluoride). Analysis was performed using commercial kits (Labtest, Lagoa Santa, MG, Brazil) according to the instructions provided by the manufacturer at the Pilot Laboratory of Clinical Analyzes (LAPAC/UFOP). IR was calculated by the homeostasis model for IR (HOMA-IR):

fasting blood insulin in mU/L × fasting blood glucose in mmol/L/22.5

and a model for assessing homeostasis of the functional capacity of the β cells (HOMA-β):

20 × fasting blood insulin in mU/L/fasting blood glucose in mmol/L3:5 [13]

Leptin and insulin levels were determined by the enzyme-linked immunosorbent assay (ELISA) sandwich-type immunoassay method using the ultra-sensitive rat insulin ELISA Kit (Crystal Chem, Downers Grove, IL, USA) according to the manufacturer's instructions.

Analysis of gene expression by real-time qRT-PCR

In separated groups of rats, qRT-PCR was performed in retroperitoneal fat, BAT, and gastrocnemius muscle. The total RNA from retroperitoneal fat, BAT, and gastrocnemius muscle was isolated with TRI reagent (Sigma-Aldrich, Darmstadt, Germany), according to the manufacturer's protocol. All isolated RNA was quantified by spectrophotometry and the optical density was estimated from the 260/280 nm absorbance ratio. An RT reaction was performed using SuperScript III (Invitrogen Life Technologies, Carlsbad, California EUA) for first-strand cDNA synthesis. Real-time PCR was carried out following the generation of first-strand cDNA. A PCR for each sample was carried out in triplicate for all cDNAs and for the 18s ribosomal control, using SYBR Green PCR Master Mix (Applied Biosystems, Rockford, IL, USA). The analyzed genes are described in Table 1. The analyses were performed by a relative method for quantifying gene expression (comparative Cq, ΔCq), which allows one to quantify differences among samples in the level of expression of a specific target. The expression levels were normalized for the amount of the reference gene (18S rRNA) on each plate. The results were obtained with a formula that considers the amount of the target gene normalized to the calibrator gene, given by (2−ΔCq).

Histopathologic analysis

After sacrifice, the WAT (retroperitoneal fat) and BAT fragments were fixed in a solution composed of 20% dimethylsulfoxide and 80% methanol, cut crosswise (4 μm) processed in descending series of alcohols and included in blocks of paraffin and stained with hematoxylin and eosin for evaluation of hypertrophy and hyperplasia of

Table 1
RGD accession numbers and primer sequences of genes selected for qRT-PCR

Gene	Accession number (RGDID)	Primer sequence (5′–3′)
Rn18s	046237.1	F GTAAGTGCGGGTCATAAG R CCATCCAATCGGTAGTAGC
Leptin	3000	F GAACCTGTGAGGATGAGTG R CACTGGCTGACAGAACTATG
Resistin	628781	F CCAGAAGGCACAACCGTCAC R CCGCTGTCCAGTCTATGCTTCC
Adipsin	2498	F AGAGCAACCGCAGGGACACTTG R CCACGTAACCACAGCTTCGACC
TNF-α	3876	F GTGTCTGTGCCTCAGCCTCTTC R CCTCCTTGTTGGGACCGATC
COX-2	621872	F ATCTGGCTTCGGGAGCACAAC R TGGAACAGTCGCTCGTCATCC
Adiponectin	628748	F GCCGTTCTCTTCACCTACGACC R GGTCTCCCACCTCCAGATGG
Adiponectin receptor	207587.1	F GCCATGGAGAAGATGGAGGA R AGCACGTCGTACGGGATGA
UCP-1	3931	F CAAAGTCCGCCTTCAGATC R TGGTGATGGTCCCTAAGAC
UCP-3	3933	F CCCAAAGGAACGGACCACTC R GGGTTGAGCACAGGTCACTG

COX, cyclooxygenase; RGD, Rat Genome Database; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; TNF, tumor necrosis factor; UCP, uncoupled protein.
Primers used (*Forward and Reverse*).

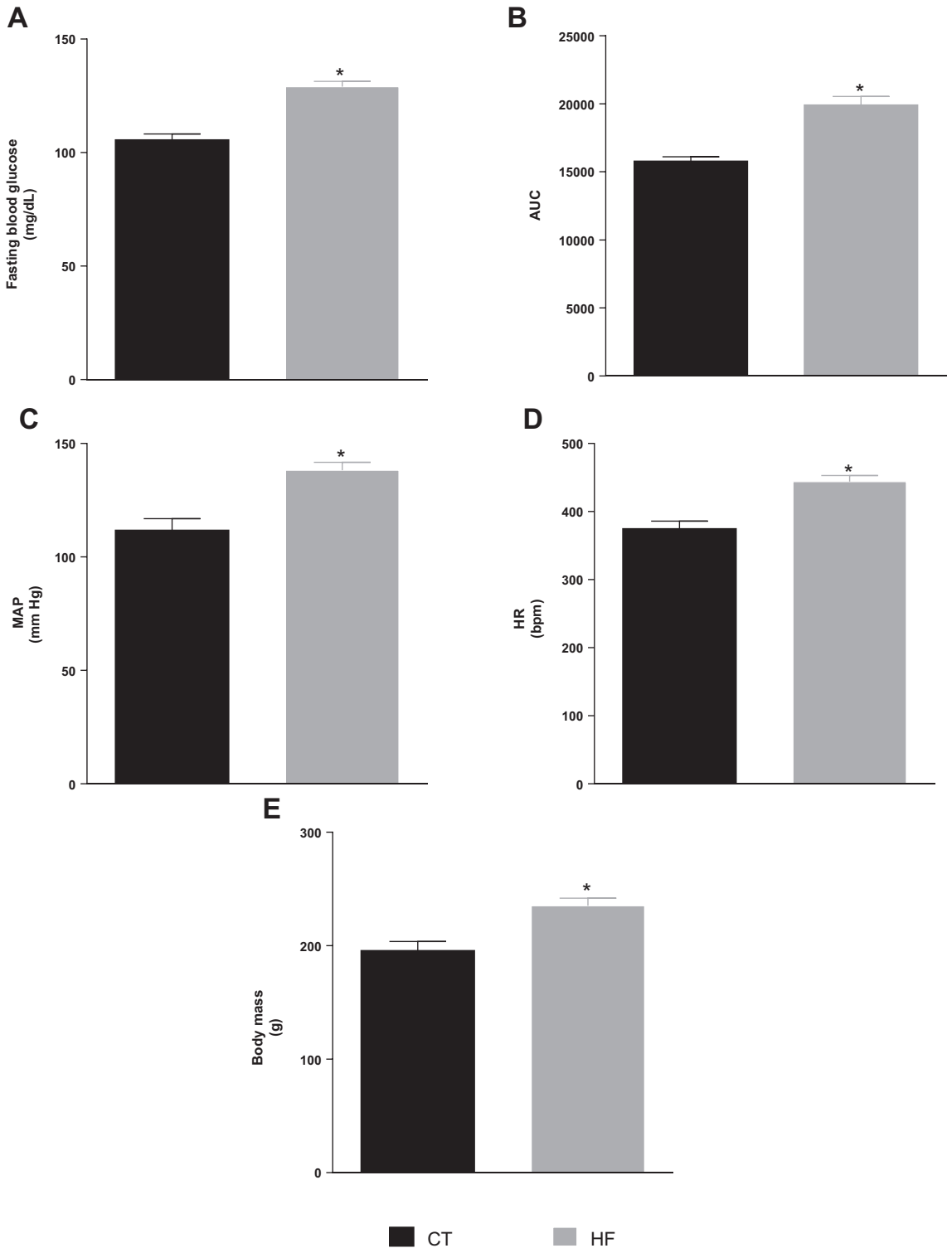

Fig. 1. Establishment of MetS in rats after 7 weeks of HF diet. Evaluations of fasting glucose (mg/dL; (**A**), AUC of blood glucose during OGTT (**B**), MAP (mm Hg) (**C**), heart rate (HR, bpm (**D**) and body mass (g) (**E**) of rats (n = 6–20) receiving HF or CT diet for 7 wk. Values are expressed as mean ± SEM and analyzed using unpaired Student's *t* test. $^{*}P < 0.05$ compared with CT-V rats. AUC, area under the curve; bpm, beats per minute; CT, control; CT-V, control group with vehicle; HF, high fat; HR, heart rate; MAP, mean arterial pressure; MetS, metabolic syndrome; OGTT, oral glucose tolerance test.

adipocytes (optical microscopy) [12]. Representative photomicrographs were obtained using a Leica MC170HO camera associated to Leica DM5000B microscopy using 40 × magnification. The number and area of the adipocytes were quantified in 30 random images (total area traveled equal to 35 000 μm^2) and multiplied by the mass of retroperitoneal fat deposit. The images were analyzed by software image J 1.45 (National Institutes of Health, Bethesda, Maryland, USA).

Table 2
Ang-(1-7) treatment restored biometric parameters on MetS rats*

Parameters	CT-V	CT-Ang-(1-7)	HF-V	HF-Ang-(1-7)
Food intake (g)	92 ± 4.3	82.1 ± 1.5	76.4 ± 3.4†	73.4 ± 1†
Caloric intake (kcal)	349.5 ± 16.4	312 ± 5.9	397.2 ± 17.5	381.9 ± 5.4
Brown fat (g/100 g rat mass)	0.122 ± 0.003	0.126 ± 0.009	0.155 ± 0.004	0.183 ± 0.019†
Inguinal fat (g/100 g rat mass)	2.12 ± 0.14	2.00 ± 0.15	3.38 ± 0.08†	2.85 ± 0.15†,‡
Retroperitoneal fat (g/100 g rat mass)	2.29 ± 0.23	1.72 ± 0.17	4.02 ± 0.20†	3.14 ± 0.25†
Epididymal fat (g/100 g rat mass)	1.55 ± 0.09	1.85 ± 0.11	3.13 ± 0.08†	2.52 ± 0.23†,‡
Gastrocnemius (g/100 g rat mass)	1.132 ± 0.03	1.182 ± 0.02	1.117 ± 0.04	1.244 ± 0.02†
Adiposity index§	7.43 ± 0.36	5.73 ± 0.43	10.86 ± 0.17†	8.95 ± 0.54†
Body mass (g)	307.2 ± 12.7	257.5 ± 6.7	386.5 ± 9.5†	344.7 ± 8.3†
Number of animals	8–10	9–10	7–10	7–10

Ang, angiotensin; CT, control; HF, high fat; MetS, metabolic syndrome.
Total food intake (kcal/body mass).
*Biometric parameters of rats subjected to HF or CT diets for 13 wk and treated with vehicle (V) or Ang-(1-7) for the last 6 wk of diet.
†$P < 0.05$ compared with CT-V group.
‡$P < 0.05$ compared with HF-V group (two-way analysis of variance followed by Bonferroni test), expressed as mean values ± SEM.
§Adiposity index: absolute mass (g) deposit of inguinal fat + retroperitoneal + epididymal / rat mass (g) × 100.

Statistical analysis

All statistical analyses were carried out with Prism 6.0 (San Diego, CA, USA). The results are expressed as means ± SEM. The data were analyzed for Kolmogorov–Smirnov normality and followed the standard normal distribution; they were subsequently assessed by two-way analysis of variance, followed by the Bonferroni post-test. The criterion for statistical significance was set at $P < 0.05$.

Results

After 7 wk, rats fed a HF diet displayed symptoms of MetS, including increased values of fasting blood glucose (mg/dL), AUC of blood glucose during OGTT, MAP (mm Hg), HR (bpm), and body mass (g) compared with CT rats (Fig. 1).

Ang-(1-7) treatment reduced inguinal, retroperitoneal, and epididymal fat mass, adiposity index, and body mass and increased gastrocnemius muscle compared with HF-V rats and increased brown fat deposition compared with CT-V rats. Animals that received the HF diet reduced food intake but there was no difference in caloric intake and body mass compared with CT-V rats (Table 2).

HF-V rats showed increased MAP and HR and IR as evidenced by higher concentrations of insulin, leptin, and glucose levels in plasma and increased HOMA-IR and HOMA-β levels compared with the CT-V group. Ang-(1-7) treatment in MetS rats reduced MAP and HR, fasting glucose, leptin, and HOMA-IR and HOMA-β levels compared with HF-V rats, and these parameters became similar to the CT-V rats (Table 3).

HF-V rats increased the ratio of visceral to subcutaneous fat and reduced the ratio of WAT to BAT mass compared with CT-V rats. Ang-(1-7) treated rats fed HF diet showed these ratios similar to CT-V animals (Fig. 2A, D). HF-V rats showed increased hyperplasia (adipocyte number) and hypertrophy (adipocyte area) in BAT and WAT adipocyte compared with CT-V rats (Fig. 2B, C, E, and F). However, Ang-(1-7) treatment decreased adipocyte hyperplasia in BAT and WAT compared with the HF-V rats (Fig. 2C, F). However, Ang-(1-7) was able to reduce the hypertrophy only in WAT adipocyte compared with HF-V rats (Fig. 2B, E).

HF-V rats showed increased mRNA expressions of leptin, resistin, adipsin, TNF-α, and COX-2 in retroperitoneal fat compared with CT-V rats, but this effect was reversed in Ang-(1-7)–treated rats (Fig. 3A–E). In contrast, Ang-(1-7) treatment had no effect on adiponectin levels associated with the HF diet (Fig. 3F). No difference was observed in adiponectin receptor 1 expression among the groups (Fig. 3G).

The HF-V rats showed an increase in leptin and TNF-α mRNA expression in BAT compared with the CT-V rats, and Ang-(1-7) treatment reversed this effect (Fig. 4A, B).

HF-V rats demonstrated an increase in UCP-1 expression in BAT, but a decrease in UCP-3 in gastrocnemius muscle. However, Ang-(1-7) treatment increased the expression of UCP-1 and UCP-3 in BAT and gastrocnemius muscle, respectively, compared with HF-V rats (Fig. 5A, B).

Discussion

The present study demonstrated that oral Ang-(1-7) treatment in the last 6 wk of a 13-wk HF diet induced improvement in

Table 3
Ang-(1-7) treatment restored insulin resistance, leptin, and cardiovascular parameters on MetS*

Parameters	CT-V	CT-Ang-(1-7)	HF-V	HF-Ang-(1-7)
Insulin (ng/mL)	1.06 ± 0.14	1.10 ± 0.29	2.08 ± 0.32†	1.18 ± 0.22
Fasting glucose (mg/dL)	111.6 ± 2.26	104.7 ± 4.91	128.0 ± 2.27†	114.6 ± 3.20‡
HOMA-IR§	7.03 ± 0.90	7.79 ± 2.07	17.73 ± 2.42†	8.60 ± 1.40‡
HOMA-β‖	164.6 ± 15.4	136.6 ± 29.7	314.4 ± 46.1†	180.2 ± 20.8‡
Leptin (ng/mL)	5.42 ± 1.3	4.6 ± 1	16.7 ± 2†	7.6 ± 0.8‡
MAP (mm Hg)	98.9 ± 3	108.6 ± 3.8	144.2 ± 8.7†	112.3 ± 5.9‡
HR (bpm)	374.8 ± 8.6	410.1 ± 11.5	412.4 ± 8.4†	381.6 ± 7.1
Number of animals	5–10	5–10	6–10	6–10

Ang, angiotensin; bpm, beats per minute; CT, control; HF, high fat; HOMA-β, homeostasis model assessment of the functional capacity of β cells; HOMA-IR, homeostatic model assessment of insulin resistance; HR, heart rate; MAP, mean arterial pressure; MetS, metabolic syndrome.
*Biochemical and cardiovascular parameters of rats subjected to HF or CT diets for 13 wk and treated with vehicle (V) or Ang-(1-7) for the last 6 wk of diet.
†$P < 0.05$ compared with CT-V group.
‡$P < 0.05$ compared with HF-V group (two-way analysis of variance followed by Bonferroni test) expressed as mean values ± SEM.
§HOMA-IR = fasting insulin (IJ) × (GJ) / 22.5.
‖HOMA-β = (20 × IJ) / (GJ − 3.5).

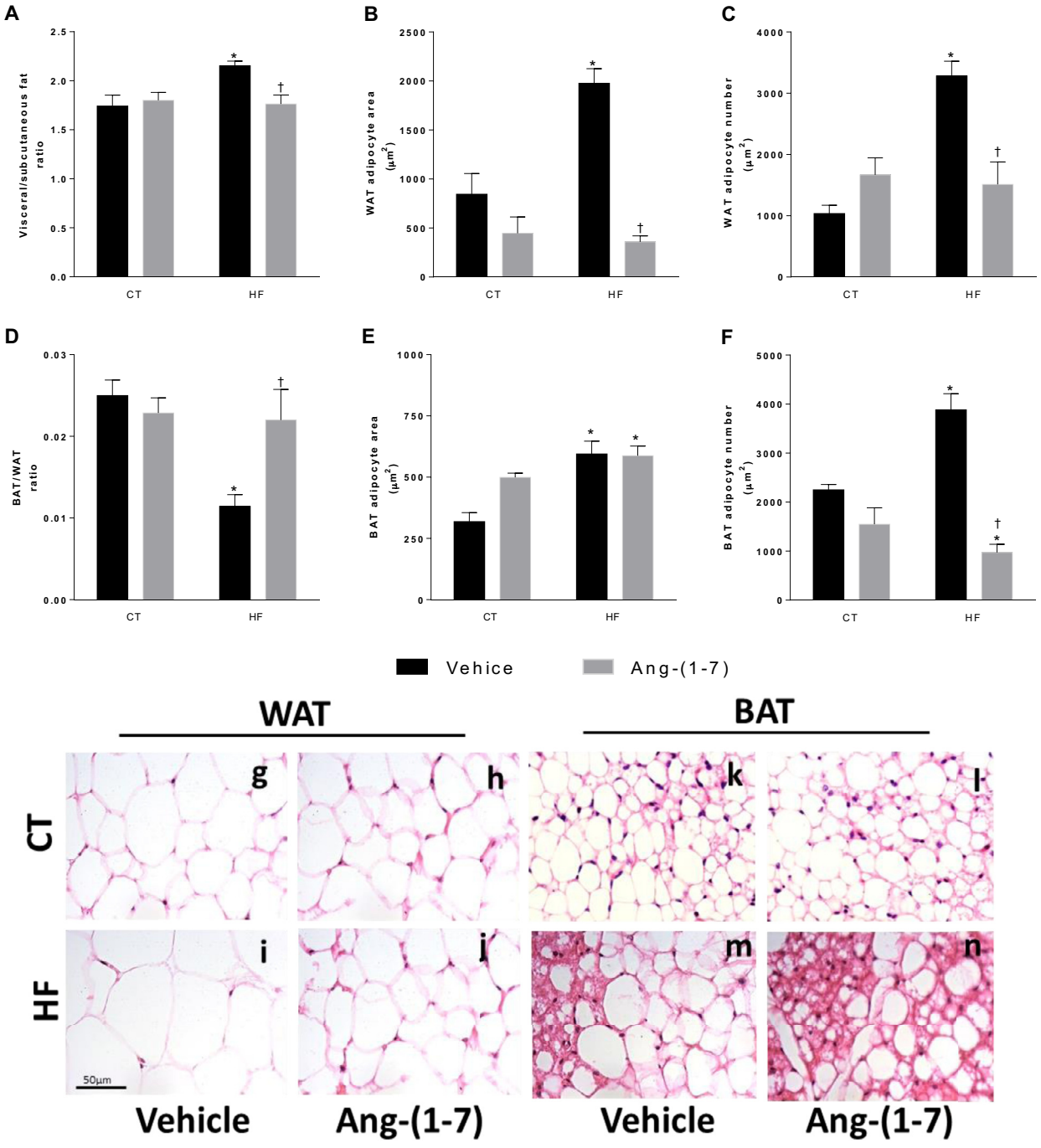

Fig. 2. Remodeling of WAT by Ang-(1-7) treatment. Visceral fat (retroperitoneal + epididymal)-to-subcutaneous fat (inguinal) fat ratio (**A**), BAT-to-WAT ratio (**B**), retroperitoneal adipocyte area (**C**), retroperitoneal adipocytes number (**D**), BAT adipocyte area (**E**), BAT adipocyte number (**F**) in rats (n = 4–8) receiving HF or CT for 13 wk and treated with vehicle (V) or Ang-(1-7) during the last 6 wk of the diet. Values are expressed as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$ compared with CT-V rats and $^{\dagger}P < 0.05$ compared to HF-V rats. Retroperitoneal fat histology (g-n) was performed by hematoxylin and eosin (HE) staining. Magnification × 440. Bar = 50 μm. Ang, angiotensin; ANOVA, analysis of variance; BAT, brown adipose tissue; CT, control; HF, high fat; WAT, white adipose tissue.

several MetS markers, including reduction in hypertension, body mass, visceral fat deposition, and adipocyte hypertrophy. It also reversed IR, in addition to normalizing the gene expression of inflammatory markers and reducing gene expression of adiponectin in the retroperitoneal fat deposit. Finally, Ang-(1-7) promoted increased mass and UCP-1 gene expression in BAT and gene expression in UCP-3 in the gastrocnemius muscle. Additionally, rats treated with Ang-(1-7), despite showing similar caloric intake, among other groups, demonstrated reduction in body and WAT mass and an elevation in BAT mass and expression of UCP-1 and UCP-3, suggesting an improvement in the global metabolism.

There is considerable evidence that fat deposition of subcutaneous (inguinal) and visceral (retroperitoneal and epidydimal) fat of WAT deposits have distinct functions [14]. Visceral WAT accumulation appears to play a more significant pathogenic role, whereas

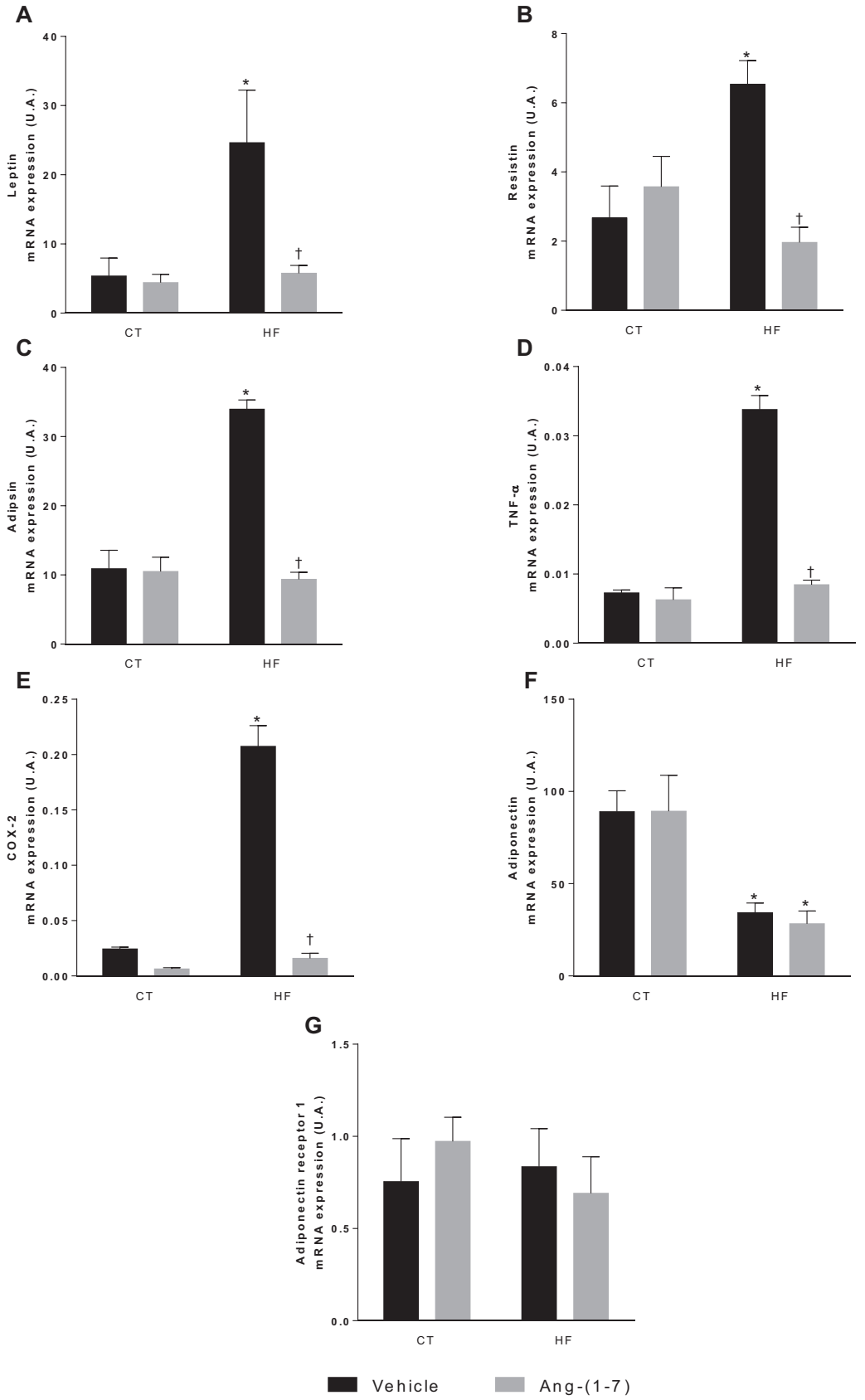

Fig. 3. Decreased inflammatory markers in WAT by Ang-(1-7) treatment. Evaluation of gene expression in mRNA levels of leptin (**A**), resistin (**B**), adpisin (**C**), TNF-α (**D**), COX-2 (**E**), adiponectin (**F**), and adiponectin receptor 1 (**G**) in retroperitoneal fat of rats (n = 4–6) receiving HF or CT diet for 13 wk and treated with vehicle (V) or Ang-(1-7) during the last 6 wk of the diet. Values are expressed as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$ compared with CT-V rats and $^{\dagger}P < 0.05$ compared with HF-V rats. Ang, angiotensin; ANOVA, analysis of variance; COX, cyclooxygenase; CT, control; HF, high fat; TNF, tumor necrosis factor; WAT, white adipose tissue.

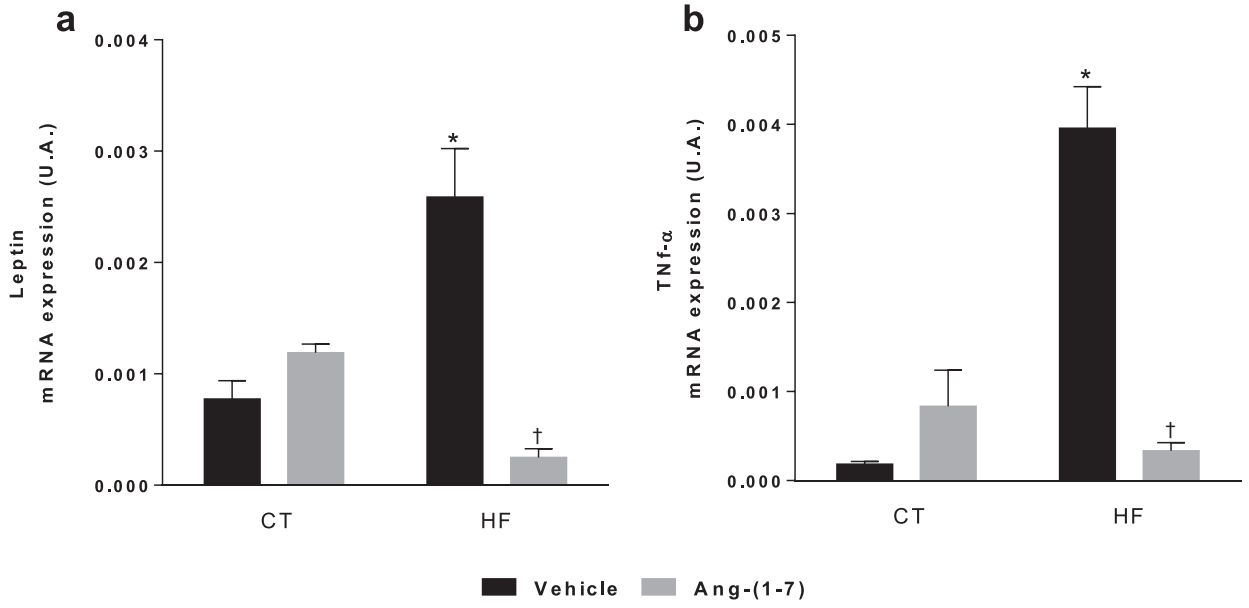

Fig. 4. Decreased inflammatory markers in BAT by Ang-(1-7) treatment. Evaluation of gene expression in mRNA levels of leptin (**A**), TNF-α (**B**) BAT of rats (n = 3–4) receiving HF or CT diet for 13 wk and treated with vehicle (V) or Ang-(1-7) during the last 6 wk of the diet. Values are expressed as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$ compared with CT-V rats and $^{\dagger}P < 0.05$ compared with HF-V rats. Ang, angiotensin; ANOVA, analysis of variance; BAT, brown adipose tissue; CT, control; HF, high fat; TNF, tumor necrosis factor.

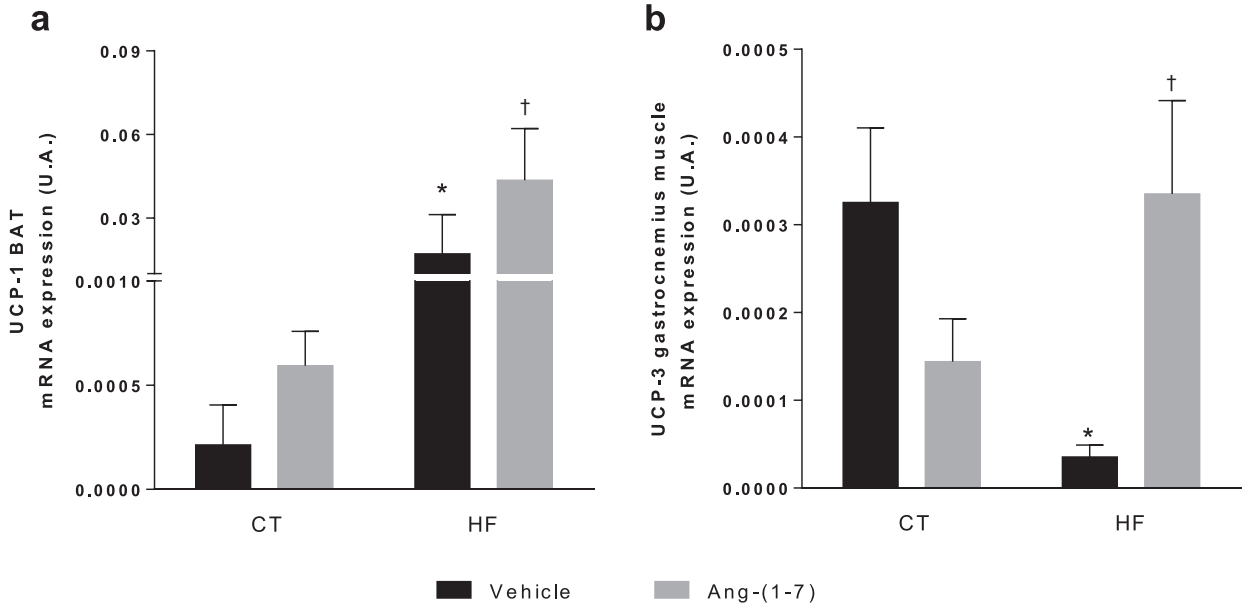

Fig. 5. Increased UCP by Ang-(1-7) treatment on BAT and gastrocnemius muscle. Evaluation of gene expression in mRNA levels of decoupled protein 1 (UCP-1) (**A**) in BAT, UCP-3 (**B**) in gastrocnemius muscle of rats (n = 7–9) receiving HF diet or CT for 13 wk and treated with vehicle (V) or Ang-(1-7) during the last 6 wk of the diet. Values are expressed as mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni post-test. $^{*}P < 0.05$ compared with CT-V rats and $^{\dagger}P < 0.05$ compared with HF-V rats. Ang, angiotensin; ANOVA, analysis of variance; BAT, brown adipose tissue; CT, control; HF, high fat; UCp, uncoupled protein.

subcutaneous WAT appears to be protective [2]. In the present study, HF-V rats showed increased inguinal, epididymal, and retroperitoneal fat compared with CT-V rats. However, oral Ang-(1-7)–treated MetS rats demonstrated decreased inguinal and epididymal fats compared with HF-V rats. Additionally, HF-Ang-(1-7) rats showed retroperitoneal fat deposition similar to the CT-V group. Therefore, we focus our studies preferentially on retroperitoneal fat deposition.

Reductions in WAT adipocyte mass can reduce the expression of proinflammatory cytokines and improves the insulin signaling pathway [2,3,6,15]. Leptin has been shown to promote lipid oxidation and mitochondrial biogenesis, thus accelerating energy expenditure in WAT and BAT through regulation of brain-derived factors [16–19]. However, in the MetS state, leptin resistance has been shown to contribute to an increase in proinflammatory cytokines [20]. High serum leptin levels probably occur owing to deregulation of the

adipocyte–insulin axis in pancreatic β cells in a state of hyperleptinemia that can contribute to IR [21]. The present data showed that oral Ang-(1-7) treatment was able to reduce body mass, adiposity, and retroperitoneal mass (hypertrophy and hyperplasia). Additionally, oral Ang-(1-7) treatment improved IR that was estimated using HOMA-IR of fasting insulin–glucose interactions, normalized insulin levels, and the β-cell functioning (HOMA-β). Accordingly, in the present study, HF-V rats showed increased plasma levels of leptin and gene expression of leptin and proinflammatory cytokines in WAT and BAT. However, oral Ang-(1-7) treatment decreased the expressions of leptin, resistin, adipsin, TNF-α, and COX-2 in retroperitoneal fat, leptin, and TNF-α in BAT of rats with MetS, indicating an important role for Ang-(1-7) in treating inflammatory process in MetS. On the other hand, adiponectin has been considered as adipokine that exhibits regulatory properties inhibiting proinflammatory transcriptional factors in MetS states [22]. Here, HF-V and HF-Ang-(1-7) groups showed similar reduction in adiponectin expression. The present data are in agreement with studies of prevention of MetS in epididymal deposition in rats or mice subjected to a HF diet [4,23] and a fructose-rich diet [24,25], showing that Ang-(1-7) treatment reduced the expression of inflammatory markers of interleukin-1β and COX-2 and decreased the activation of transcription factor of proinflammatory cytokines, nuclear factor-κB and ERK.

UCPs present in tissues of high metabolic demand, or involved in the metabolism balance such as WAT, BAT, and gastrocnemius represent possibilities to regulate metabolic disorders induced by a HF diet or sedentary lifestyle [12,26]. In the present study, treatment with Ang-(1-7) was able to increase the BAT-to-WAT ratio mass and UCP-1 expression in BAT. These processes probably occurred due to the regulation of metabolic processes such as thermogenesis, maintaining regular storage and mobilization of lipids, and preventing WAT fat accumulation in MetS rats. The present data are in agreement with Morimoto et al., who showed that Ang-(1-7) treatment increased UCP-1 expression in BAT of rats fed a HF diet [15]. Additionally, in the present study, the HF diet decreased the expression of UCP-3 in the gastrocnemius muscle and this effect was reversed by treatment with Ang-(1-7). This process suggests that treatment with Ang-(1-7) has beneficial effects on adaptive thermogenesis in response to variation in the HF dietary macronutrients [27–30]. Overall, the present data show that Ang-(1-7) increased UCP-3 expression in the gastrocnemius muscle and UCP-1 expression in BAT, having a protective effect against the MetS, and it could occur by normalizing the IR, hypertension, inflammatory process, and remodeling in WAT.

Arterial hypertension plays an important role in endothelial dysfunction in MetS as it is related to increased plasma glucose levels, dyslipidemia, low-grade inflammation, overactivity of the sympathetic nervous system [1,18], and the ACE–Ang II–AT1 axis [23,25]. In the present study, treatment with Ang-(1-7) improved MAP and HR in MetS rats that showed hypertension. The present data, using the oral Ang-(1-7) treatment, reinforced the counterregulatory actions of the ACE2–Ang-(1-7)–Mas axis in improving cardiovascular and metabolic parameters in rats with established MetS.

Conclusion

This study showed that oral Ang-(1-7) treatment in established MetS induced by HF diet reduced the mass of the visceral fat deposit and the hypertrophy in WAT, improving the inflammatory process, IR, and restoring cardiovascular parameters. Finally, oral Ang-(1-7) treatment increased BAT deposition, regulating metabolic processes by increasing UCP-1 gene expression in BAT and UCP-3 in the gastrocnemius. Thus, oral Ang-(1-7) treatment represents a potential therapeutic tool in MetS.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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