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Chronic administration of grape-seed polyphenols attenuates the development of hypertension and improves other cardiometabolic risk factors associated with the metabolic syndrome in cafeteria diet-fed rats

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Abstract

The effects of grape-seed polyphenols against the development of hypertension and other cardiometabolic conditions associated with the metabolic syndrome (MetS) were studied in rats fed a high-fat, high-carbohydrate diet, known as the cafeteria (CAF) diet. Two groups of Wistar rats were fed standard (STD) or CAF diets for 12 weeks. The CAF diet-fed rats were administered different doses of a low-molecular-weight grape-seed polyphenol extract (LM-GSPE) (25, 100 and 200 mg/kg per d) or vehicle daily, and the STD diet-fed rats were administered LM-GSPE (100 mg/kg per d) or vehicle using ten animals per group. Body weight (BW), waist perimeter (WP) and systolic and diastolic blood pressures (BP) by the tail-cuff method were recorded weekly. The animals were housed in metabolic chambers every 2 weeks to estimate daily food and liquid intakes and to collect faeces and urine samples. The plasma lipid profile was analysed at time 0 and on the 4th, 7th, 10th and 12th weeks of the experiment. Moreover, plasma leptin was measured at the end of the experiment. Results demonstrated that LM-GSPE, when administered with the CAF diet, attenuated the increase in BP, BW, WP and improved lipid metabolism in these animals. However, although the 25- and 100-mg/kg per d doses were sufficient to produce beneficial effects on BP and lipid metabolism, a 200-mg/kg per d dose was necessary to have an effect on BW and WP. The present findings suggest that LM-GSPE is a good candidate for a BP-lowering agent that can also ameliorate other conditions associated with the MetS.

Key words: Body weight: Cafeteria diet: Flavanols: Lipid profile: Waist perimeter

The metabolic syndrome (MetS) constitutes an extended cluster of pathological conditions that include insulin resistance. The MetS is a risk factor for the development of diabetes mellitus and cardiovascular (CV) events. This syndrome is clinically diagnosed by the presence of at least three of the following components: high waist perimeter (WP), hypertriacylglycerolaemia, low HDL levels, high cholesterol levels, fasting hyperglycaemia and hypertension (HTN)[1]. Awareness of the adverse effects of the MetS is steadily increasing because of its expanding prevalence worldwide, and efforts are underway to prevent the development of this disease.

Rats fed a cafeteria (CAF) diet, which consists of free access to standard (STD) chow and water, while concurrently receiving highly palatable, energy-dense, unhealthy human food *ad libitum*, are considered a robust model of the human MetS[2]. This dietary model provides an exceptional tool to study obesity and the MetS, both being pandemic diseases among the Western population. The CAF diet-fed rats display increased body weight (BW), more abdominal fat, and develop hyperinsulinaemia, hyperglycaemia and hepatic steatosis[2–4]. The development of HTN in animals fed a CAF diet for 10 weeks has recently been reported by our group[5]. Thus, this animal model mimics classical human MetS, particularly because HTN is one of the most prevalent complications associated with the MetS in humans, more prevalent than obesity[6].

Grape seed, which is a by-product of the grape/wine industry, has been extensively investigated because of its high flavanol content[7]. It has been reported that grape-seed polyphenols could be particularly beneficial in the control of most metabolic disturbances observed in the MetS. Our research group has demonstrated that grape-seed polyphenol-rich extract, which is rich in monomeric flavanols and low-molecular-weight proanthocyanidins[7], exhibits antioxidant properties[8], improves lipid metabolism[9], limits adipogenesis[10], acts as an insulin-mimetic

Abbreviations: BP, blood pressure; BW, body weight; CAF, cafeteria; CAF200, CAF diet-fed rats administered daily with 200 mg/kg of LM-GSPE; DBP, diastolic BP; HTN, hypertension; LM-GSPE, low-molecular-weight grape-seed polyphenol extract; MetS, metabolic syndrome; STD, standard; SBP, systolic BP; TC, total cholesterol; WP, waist perimeter.

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agent[11] and alleviates inflammation[12]. In addition, the short-term antihypertensive properties of a single dose of a low-molecular-weight grape-seed polyphenol extract (LM-GSPE) have recently been demonstrated in spontaneously hypertensive rats and in hypertensive CAF diet-fed rats[5,7]. However, HTN and other cardiometabolic conditions are chronic pathologies that require chronic treatment.

Therefore, the objective of this study was to evaluate the effects of the long-term intake of LM-GSPE on the development of HTN and other main cardiometabolic risk factors associated with the MetS in an experimental animal model of the MetS – namely, CAF diet-fed rats.

Methods

Low-molecular-weight grape-seed polyphenol extract

The grape-seed extract used in the experiments was a low-molecular-weight polyphenol-rich extract obtained from white grape seeds, and was kindly provided by Les Dérives Résiniques et Terpéniques (Dax, France). Online Supplementary Table S1 shows the flavanol and phenolic acid contents of the grape-seed extract used in this study (taken from[13]).

Animal experimental procedures

In total, 6-week-old male Wistar rats, Crl:WI (*n* 60), were purchased from Charles River Laboratories (Barcelona, Spain) and were singularly housed in animal quarters at 22°C under 12 h light–12 h dark conditions. After quarantine and a training period of 2 weeks, the animals were divided into two dietary groups (Fig. 1). The STD diet-fed rats (*n* 20) were fed the STD Panlab A04 (Panlab) diet and tap water *ad libitum*, and the CAF diet-fed rats (*n* 40) had free access to a fresh CAF diet that consisted of bacon (10–12 g), sausages (8–12 g), biscuits with pâté (12–15 g), biscuits with cheese (10–12 g), ensaïmadas (pastries) or muffins (4–5 g), carrots (8–10 g), milk with sugar (220 g/l; 50 ml), water and the STD diet *ad libitum*. The STD chow had an energy content of 20 % protein, 4 % fat, 76 % carbohydrates and 0·3 % Na, whereas the CAF diet had an energy content of 14 % protein, 25 % fat, 61 % carbohydrates and 0·2 % Na. All animals were fed fresh food daily *ad libitum*. The different diets were maintained for 12 weeks. During the training period, the animals were trained to lick water containing 50 % low-fat condensed milk that was used as vehicle (1 ml). The STD diet-fed animals were administered vehicle or vehicle containing 100 mg/kg per d LM-GSPE (*n* 10 per group; STD and STD100, respectively) daily between 09.00 and 10.00 hours. The STD100 group was studied to evaluate the effect of LM-GSPE administration on the blood pressure (BP) of normotensive rats. The CAF diet-fed animals were administered vehicle or vehicle containing 25, 100 or 200 mg/kg per d of LM-GSPE (*n* 10 per group; CAF, CAF25, CAF100 and CAF200, respectively) daily between 09.00 and 10.00 hours.

BP, BW and WP were recorded weekly in all groups (Fig. 1). Systolic and diastolic BP (SBP and DBP) were recorded between 08.00 and 11.00 hours by the tail-cuff method[14], as described elsewhere[15]. To minimise stress-induced variations in BP, all measurements were made by one person in the same stress-free environment. After the quarantine period, BP was recorded as a training to acclimatise the animals to the procedure. WP was assessed on the largest zone of the abdomen of the vertically immobilised rat using a non-extensible measuring tape with an accuracy of 0·1 cm without applying pressure to the body.

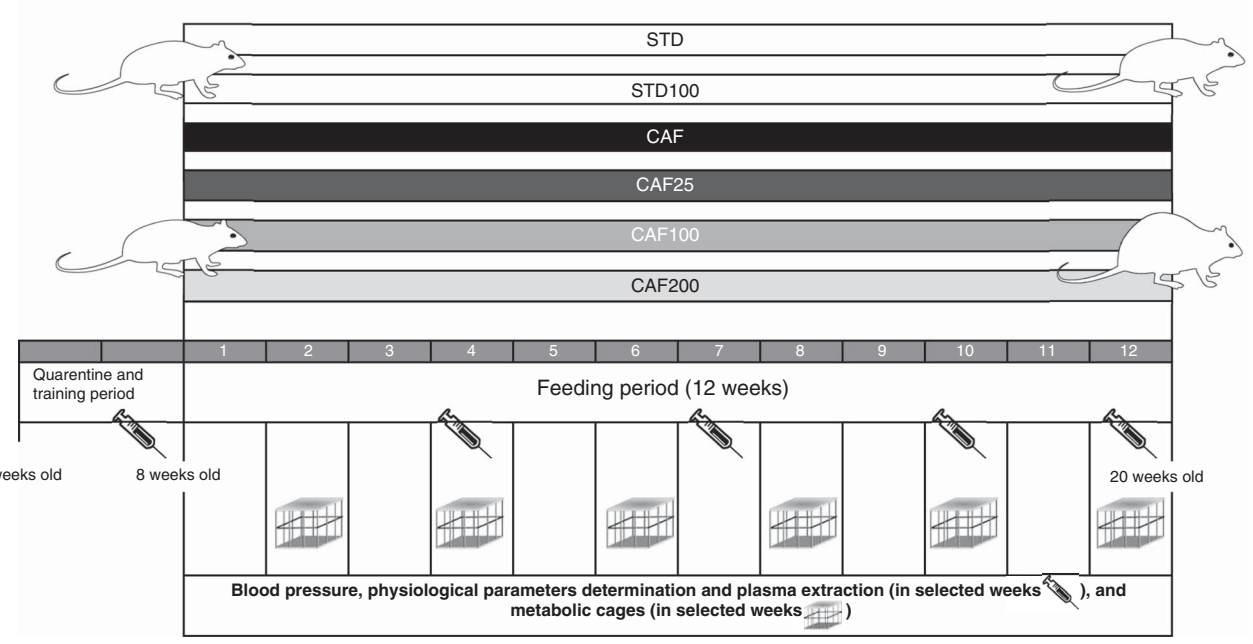

Fig. 1. Graphical representation of the experimental design used in this study. STD, standard diet-fed rats; STD100, STD administered with 100 mg/kg per d of low-molecular-weight grape-seed polyphenol extract (LM-GSPE); CAF, cafeteria diet-fed rats; CAF25, CAF administered with 25 mg/kg per d of LM-GSPE; CAF100, CAF administered with 100 mg/kg per d of LM-GSPE; CAF200, CAF administered with 200 mg/kg per d of LM-GSPE.

Initially and every 2 weeks thereafter, the animals were housed for 24 h in metabolic cages to estimate daily food and liquid intakes and to collect faeces and urine samples. The complete experimental design is schematised in Fig. 1. At the end of the experimental period, all rats were killed by decapitation after 6-h of fasting.

The animal protocol followed in this study was approved by the Bioethical Committee of the Universitat Rovira i Virgili (European Commission Directive 86/609 and Spanish Royal Decree 223/1988).

Biochemical assays

Plasma biochemical assays were performed using blood samples collected from the saphenous vein of all animals after 6 h of starvation at time 0 and on the 4th, 7th, 10th and 12th weeks of the experimental feeding period. Blood samples were collected into tubes containing lithium heparin as anticoagulant, and were centrifuged at 2500 ***g*** for 20 min at 4°C to obtain plasma samples, which were then divided into aliquots and stored frozen at −80°C until analysis.

Plasma total cholesterol (TC) and TAG concentrations were assayed using enzymatic colorimetric kits (cholesterol oxidase-peroxidase (CHOD-POD) method for TC and glycerol phosphate oxidase (GPO) method for TAG; QCA).

Plasma leptin concentrations were determined using the Rat Leptin ELISA kit, ninety-six-well plate (Millipore).

Statistical analysis

Data are expressed as mean values with their standard errors. The data were analysed by one-way ANOVA or two-way ANOVA (Tukey's test) using IBM SPSS Statistics 20.0.0 for Mac software. The Kolmogorov–Smirnov and the Levene's tests were used to examine the normality and equality, respectively, of variances in the data. The differences between the mean values were considered statistically significant when $P < 0{\cdot}05$.

Results

Blood pressure

Compared with the STD diet-fed rats, animals fed the CAF diet had increased BP (Fig. 2(A) and (B)), with significant increases in SBP and DBP beginning during the 2nd week of the diet. The increase in BP reached a maximum from the 6th to the 8th week after CAF diet intake. During this week, the SBP of the STD and the CAF rats differed by 18·3 % (Fig. 2(A)) and the DBP of these two groups differed by 14·2 % (Fig. 2(B)). Starting from the

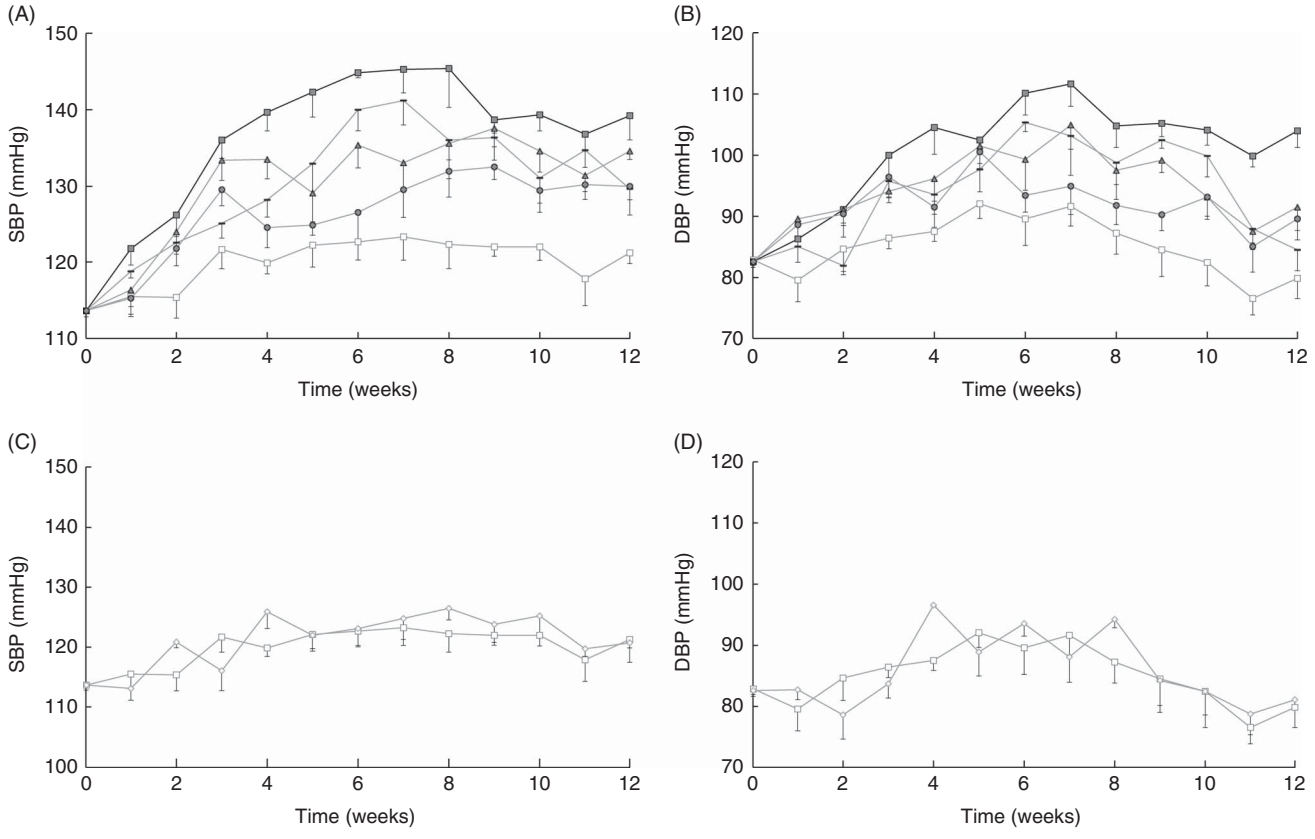

Fig. 2. Changes in (A) systolic blood pressure (SBP) and (B) diastolic blood pressure in the standard (STD) diet-fed rats and the cafeteria (CAF) diet-fed rats administered vehicle or different doses of low-molecular-weight grape-seed polyphenol extract (LM-GSPE) (25, 100 and 200 mg/kg per d: CAF25, CAF100 and CAF200, respectively; *n* 10 per group) for 12 weeks. Changes in (C) SBP and (D) DBP of the standard diet-fed rats administered vehicle (STD; *n* 10) or 100 mg/kg per d of LM-GSPE (STD100; *n* 10) for 12 weeks. Values are means (*n* 10 animals per group), with their standard errors. Mean values with unlike letters are significantly different for overall effect during the 12th week of the experiment (two-way ANOVA; $P < 0{\cdot}05$). A: —□—, STD (a); —■—, CAF (b); —▲—, CAF25 (c); —▬—, CAF100 (c, d); —●—, CAF200 (d); B: —□—, STD (a); —■—, CAF (b); —▲—, CAF25 (b); —▬—, CAF100 (c); —●—, CAF200 (c); C, D: —□—, STD (a), —◇—, STD100 (a).

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9th week of the diet, a mild decline in BP was observed in CAF diet-fed animals, but BP values were statistically significantly higher than those of the STD diet-fed rats ($P < 0{\cdot}001$).

The long-term administration of LM-GSPE produced an anti-hypertensive effect at all doses assayed, attenuating the rise in SBP (Fig. 2(A)) and DBP (Fig. 2(B)) caused by the intake of the CAF diet. Although the 100- and 200-mg/kg per d doses were the most effective against the CAF diet-induced increase in SBP, the SBP of the CAF25 group was also statistically lower than that of the CAF group (Fig. 2(A)). All assayed doses of the LM-GSPE were effective against the increase in DBP at the end of the study. However, during the course of the experiment, the 100- and 200-mg/kg per d doses were the most effective against the rise in DBP (Fig. 2(B)).

The STD100 rats showed similar SBP and DBP measures as the STD rats (Fig. 2(C) and (D)).

Body weight, waist perimeter and plasma leptin

BW and WP of all animals immediately before the experimental feeding were 237 (SEM 2) g and 14 (SEM 0) cm, respectively. The STD rats gained weight progressively during the course of the experiment. However, as expected, the weight gain in the group fed the CAF diet was significantly higher than that of rats fed the STD diet (Fig. 3(A)). Compared with the CAF group, during the course of the experiment, there was a statistically insignificant reduction in the BW of animals administered the highest doses of LM-GSPE (Fig. 3(A)). On the 7th and the 8th weeks of the experiment, the LM-GSPE dose of 200 mg/kg per d partially counteracted the CAF effect on BW to the levels of the STD diet group (Fig. 3(A)). A tendency towards counteraction to the STD diet was also observed at the end of the experiment on BW gain in animals administered 200 mg/kg per d of LM-GSPE for 12 weeks (Fig. 3(B)).

WP of the CAF group was also found to be higher than that of the STD diet group (Fig. 3(C)). In addition, WP tended to decline with long-term administration of the highest dose of LM-GSPE without statistically significant differences (Fig. 3(C)). On the 7th week of the feeding period, the LM-GSPE doses of 100 and 200 mg/kg per d partially counteracted the CAF effect on WP to the levels of animals fed the STD diet. However, on the 8th week, only the LM-GSPE dose of 200 mg/kg per d

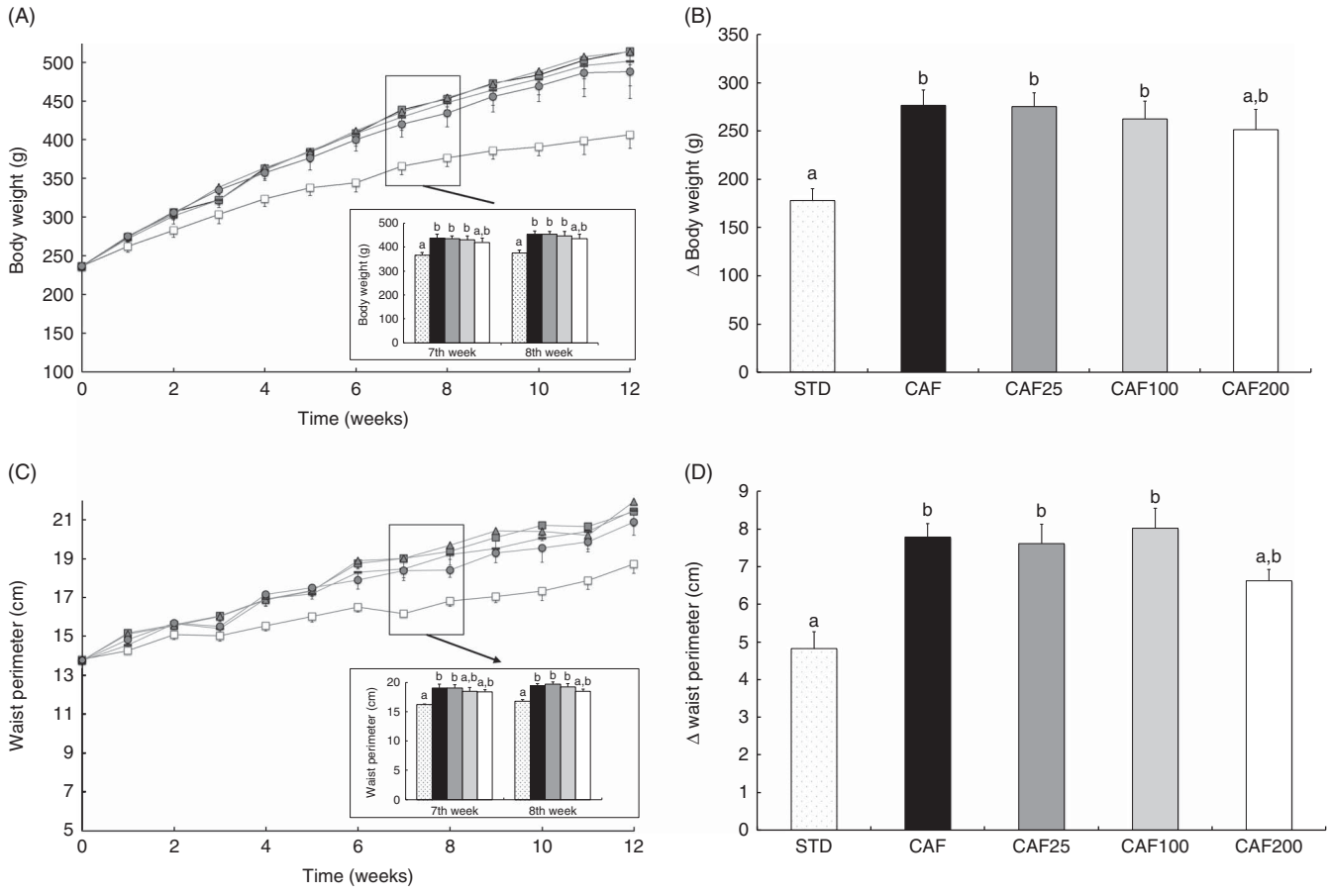

Fig. 3. (A) Changes in the body weight (BW) during the 12th week of the experiment (significant differences for overall effect: two-way ANOVA, Tukey's *post hoc* test) and on the 7th and 8th weeks of the experiment (significant differences: one-way ANOVA, Tukey's *post hoc* test); (B) changes in BW gain at the end of the experiment (12th week; significant differences: one-way ANOVA, Tukey's *post hoc* test); (C) differences in waist perimeter (WP) during the 12th week of the experiment (significant differences for overall effect: two-way ANOVA, Tukey's *post hoc* test) and on the 7th and 8th weeks of the experiment (significant differences: one-way ANOVA, Tukey's *post hoc* test); (D) changes in WP gain at the end of the experiment (12th week; significant differences: one-way ANOVA, Tukey's *post hoc* test) of the standard diet-fed rats (STD; *n* 10) and the cafeteria diet-fed rats (CAF; *n* 10) administered daily with vehicle or different doses of low-molecular-weight grape-seed polyphenol extract (25, 100 and 200 mg/kg per d: CAF25, CAF100 and CAF200, respectively; *n* 10 per group). Values are means (*n* 10 animals per group), with their standard errors. Mean values with unlike letters are significantly different ($P < 0{\cdot}05$). A, C: —□—, STD (a); —■—, CAF (b); —▲—, CAF25 (b); ——, CAF100 (b); —●—, CAF200 (b); ⊡, STD; ■, CAF; ▩, CAF25; ▩, CAF100; □, CAF200.

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partially counteracted the CAF effect on WP (Fig. 3(C)). Moreover, a tendency towards counteraction in WP gain at the end of the experimental period in animals administered 200 mg/kg per d of LM-GSPE was observed when compared with the CAF control rats (Fig. 3(D)).

Compared with the STD diet, the CAF diet produced an increase in plasma leptin concentrations at the end of the experiment (37·52 (SEM 3·54) *v.* 11·49 (SEM 0·55) ng/ml, respectively). The LM-GSPE administration (25, 100 and 200 mg/kg per d) failed to produce any significant changes in plasma leptin levels *v.* vehicle administration (40·28 (SEM 6·61), 40·07 (SEM 4·38) and 42·62 (SEM 6·69) ng/ml, respectively).

Food and liquid intakes and faecal and urine excretion

Throughout the course of the study, food intake in the CAF group was significantly higher than that in the STD group (Table 1). Such differences were not found between the CAF group and the CAF group administered the LM-GSPE. In addition, irrespective of the treatment, total protein, carbohydrate, lipid and salt intakes during the course of the experiment were significantly higher in all groups fed the CAF diet. Similarly, the total energy consumed during the course of the experiment was also found to be higher in all CAF groups *v.* the STD group, and the LM-GSPE had no effect on this parameter. In addition, the total fluid intake was significantly higher in groups fed the CAF diet, with or without LM-GSPE administration, compared with the STD diet-fed rats (Table 1).

Faecal excretion in the STD group was higher than that in the CAF group, without differences due to LM-GSPE administration. However, urine excretion was higher in all CAF groups compared with that in the STD group (Table 1).

Lipid profile

Initially, the plasma TC concentration was 98·12 (SEM 2·22) mg/dl. As expected, the CAF diet caused an increase in TC compared with the STD diet (Fig. 4(A)). Specifically, on the 7th and 10th weeks of the experiment, TC in the CAF group was found to be higher than that in the STD group, whereas at the end of experimental period (12th week) there were no significant differences between the TC values of the CAF and STD groups (Fig. 4(B)). Administration of 100- and 200-mg/kg per d doses of the LM-GSPE to the CAF group during the experimental period normalised plasma TC levels in these animals to that in the STD group (Fig. 4(A)). In addition, on the 7th week of the experimental period, the plasma TC concentration reached a maximum value in the CAF group, whereas when the LM-GSPE was administered a dose–response reduction in plasma TC levels was evident, reaching levels counteracted to STD values in the CAF200 group (Fig. 4(B)).

Plasma TAG levels were 28·85 (SEM 1·09) mg/dl at the beginning of the experiments. The CAF diet increased plasma TAG levels compared with the STD diet (Fig. 4(A)), with significant differences between these groups observed during the 7th week of the experiment (Fig. 4(B)). Although the LM-GSPE administration, at all doses assayed, produced no visible differences in plasma TAG levels during the course of the experiment (Fig. 4(A)), its administration at doses of 25 and 100 mg/kg per d reduced plasma TAG levels at the end of the experiment (12th week of the diet) (Fig. 4(D)).

Discussion

Obesity is associated with increased risk of CVD, which includes HTN, type 2 diabetes and dyslipidaemia, among others[16,17]. All these interconnected risk factors are clustered together in the term MetS[18]. The relationship between obesity and HTN has been well established. Modest reductions in BW and BP reduce the incidence of CV events[19,20]. Obese individuals present higher BP, even when within the normotensive range[21]. Studies have shown that high BMI and visceral adipose tissue are significantly associated with HTN[22].

Rats fed the CAF diet are considered a robust model of the MetS and associated co-morbidities[2]. This dietary model has been widely used to study obesity and the MetS. The development of HTN in rats fed a CAF diet for 10 weeks has been reported recently by our group[5]. In this study, we evaluated the time course of the development of HTN and other cardiometabolic parameters in this animal model. As the experiment progressed, SBP and DBP of the CAF group increased compared with the STD group. Notably, SBP in the CAF group reached a maximum of 145 mmHg starting from the 6th to the 8th week of the diet, and thereafter dropped to approximately 140 mmHg from the 9th to the 12th week, indicating that the duration of the diet can be varied to modulate the severity of HTN associated with the MetS in this experimental model. These results are in agreement with that of our previous study, which found that SBP was 140 mmHg during the 5th and 10th weeks in animals fed the CAF diet[5].

Recently, we reported that a single oral administration of 375 mg/kg of the LM-GSPE concomitantly reduced more than one risk factor of CVD by lowering BP and ameliorating hypertriacylglycerolaemia in CAF diet-fed rats[15]. The reduction in BP was similar to that caused by the short-term administration of 50 mg/kg of Captopril in these animals[5]. However, many cardiometabolic risk factors such as HTN associated with the MetS are considered chronic pathologies that require chronic treatment. In addition, because a universal drug for the treatment of the MetS and associated co-morbidities together has not been developed, a chronic treatment method for HTN that would also alleviate other components of the MetS will be highly useful[23]. Therefore, in this study, we evaluated the effects of long-term daily administration of LM-GSPE on BP and other cardiometabolic risk factors in a CAF diet-fed rat model of the MetS using a physiological dose of 25 mg/kg per d and two higher doses of LM-GSPE.

We have previously reported that after daily administration of 100 mg/kg of GSPE for 12 weeks to CAF diet- and STD diet-fed rats and 21 h after the last dosage, the flavanols and their metabolites do not accumulate in tissues but some forms target functional tissues such as the aorta[24]. The results of this study clearly showed that the long-term daily administration of LM-GSPE attenuated the development of HTN associated with the MetS in the CAF diet-fed rats. However, the antihypertensive effect of LM-GSPE has not always been dose responsive[5,7]. In particular, in the present study, the 100- and

Table 1. Physiological parameters determined during the experiment in standard (STD) diet- and cafeteria (CAF) diet-fed rats administered vehicle or different doses of low-molecular-weight grape-seed polyphenol extract (25, 100 and 200 mg/kg per d; CAF25, CAF100, CAF200, respectively) (Mean values with their standard errors)

	0th week		2nd week		4th week		6th week		8th week		10th week		12th week		Two-way ANOVA
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Total intake (g)															
STD	16·7	1·4	17·0	2·0	19·3	0·5	17·9	0·5	18·2	0·7	19·7	0·8	20·7	0·5	a
CAF	59·5	3·1	52·6	2·8	51·3	6·5	65·0	3·4	59·5	3·5	68·9	3·5	55·0	3·2	b
CAF25	57·6	2·6	56·0	2·7	54·3	3·5	65·8	4·3	57·0	4·0	63·6	3·6	53·3	4·4	b
CAF100	54·2	4·3	54·1	2·9	56·5	4·7	62·4	3·1	56·0	6·8	61·0	4·8	55·4	8·0	b
CAF200	57·9	3·0	58·5	2·6	53·0	5·0	59·7	4·0	55·9	5·8	65·0	5·3	59·0	5·3	b
Chow intake (g)															
STD	16·7	1·4	17·0	2·0	19·3	0·5	17·9	0·5	18·2	0·7	19·7	0·8	20·7	0·5	a
CAF	2·9	0·8	2·8	0·5	2·7	0·5	3·7	1·3	2·6	0·3	3·3	0·6	4·2	0·8	b
CAF25	3·6	0·7	2·6	0·5	3·9	1·5	2·6	0·6	3·6	0·7	4·1	1·0	5·7	1·0	b
CAF100	5·1	1·2	1·9	0·4	2·6	0·6	2·8	0·6	4·0	1·2	3·6	0·8	4·2	0·6	b
CAF200	3·2	0·4	2·5	0·6	3·0	0·3	3·3	0·8	2·3	0·5	4·4	1·0	4·2	1·2	b
Protein (g)															
STD	2·5	0·1	2·6	0·1	2·7	0·0	2·6	0·1	2·6	0·1	2·8	0·1	2·9	0·0	a
CAF	2·3	0·2	1·9	0·1	1·8	0·2	1·7	0·0	1·5	0·2	1·7	0·1	1·9	0·2	b
CAF25	2·5	0·1	1·7	0·1	2·0	0·3	1·7	0·1	1·3	0·1	1·8	0·1	2·1	0·2	b
CAF100	2·6	0·2	1·8	0·2	2·0	0·1	1·8	0·1	1·4	0·1	1·7	0·1	1·9	0·1	b
CAF200	2·5	0·1	2·0	0·1	2·0	0·1	2·0	0·2	1·5	0·2	1·9	0·2	1·9	0·2	b
Carbohydrates (g)															
STD	12·6	0·5	13·2	0·5	13·8	0·2	13·0	0·4	13·2	0·5	14·3	0·4	14·7	0·2	a
CAF	31·0	1·5	27·4	1·4	27·4	2·7	33·9	1·7	32·3	1·3	35·8	1·8	28·9	1·5	b
CAF25	31·3	0·8	29·2	1·4	28·5	1·8	34·1	2·1	29·9	2·1	33·3	1·8	28·4	2·2	b
CAF100	28·8	2·0	28·0	1·4	32·2	1·5	32·4	1·6	34·5	1·5	31·9	2·5	29·2	4·1	b
CAF200	29·3	1·3	30·4	1·2	27·7	2·5	31·2	2·0	29·0	2·9	34·1	2·7	31·0	2·6	b
Lipids (g)															
STD	1·4	0·1	1·5	0·1	1·6	0·0	1·5	0·0	1·5	0·1	1·6	0·0	1·7	0·0	a
CAF	19·9	1·2	16·9	0·8	17·6	2·0	21·6	1·3	21·0	0·8	23·1	1·3	18·0	1·3	b
CAF25	19·9	0·5	18·8	1·0	17·8	1·3	22·2	1·6	19·8	1·0	21·0	1·4	17·0	1·5	b
CAF100	17·5	1·7	18·3	1·0	18·9	1·7	20·9	1·1	22·5	0·5	20·2	1·7	18·1	2·7	b
CAF200	18·0	0·7	19·7	1·0	17·6	1·8	19·9	1·5	18·8	2·1	21·4	1·9	19·4	2·0	b
Salt (g)															
STD	0·046	0·002	0·047	0·002	0·048	0·001	0·045	0·001	0·045	0·002	0·048	0·001	0·051	0·001	a
CAF	0·115	0·006	0·099	0·005	0·102	0·010	0·126	0·006	0·120	0·005	0·133	0·007	0·107	0·006	b
CAF25	0·116	0·003	0·108	0·005	0·105	0·007	0·127	0·008	0·111	0·008	0·123	0·007	0·105	0·008	b
CAF100	0·106	0·008	0·104	0·005	0·109	0·009	0·120	0·006	0·132	0·004	0·118	0·009	0·108	0·015	b
CAF200	0·113	0·005	0·113	0·005	0·093	0·013	0·115	0·007	0·108	0·011	0·126	0·010	0·115	0·010	b
kJ															
STD	541·6	19·7	565·2	19·4	591·3	9·4	558·7	16·3	566·5	22·1	611·7	16·2	631·1	9·7	a
CAF	1538·8	75·8	1361·0	68·8	1358·3	135·2	1672·8	82·6	1590·6	60·6	1766·5	88·3	1423·0	75·6	b
CAF25	1552·8	38·0	1440·7	67·3	1405·5	88·8	1684·3	106·5	1462·6	101·8	1637·1	88·8	1389·6	109·7	b
CAF100	1419·2	104·4	1391·4	71·2	1526·1	97·1	1601·3	77·8	1503·5	189·3	1568·3	121·1	1433·4	200·0	b
CAF200	1413·3	48·9	1507·0	61·6	1371·3	124·1	1539·5	97·7	1431·6	141·7	1673·8	131·2	1523·1	127·7	b
Total fluid (ml)															
STD	21·4	2·6	31·5	4·5	23·5	1·7	27·7	2·1	24·6	2·7	23·9	3·0	23·7	3·4	a
CAF	51·1	3·6	51·0	4·0	49·1	4·9	64·1	4·1	61·2	5·1	65·0	4·0	55·0	5·2	b
CAF25	51·6	3·3	58·9	3·0	54·8	3·7	68·6	5·7	60·2	4·0	64·4	4·4	58·5	4·2	b
CAF100	47·8	6·4	56·5	4·1	52·7	3·7	63·3	3·8	62·6	3·9	59·0	6·0	56·3	8·3	b
CAF200	46·0	3·2	53·6	3·6	43·8	6·3	55·7	4·2	56·8	6·3	62·5	5·3	57·4	5·7	b
Faeces (g)															
STD	3·1	0·2	4·8	0·5	4·7	0·5	4·2	0·3	3·8	0·5	4·0	0·8	5·1	0·8	a
CAF	1·6	0·2	1·6	0·3	1·7	0·3	1·2	0·3	1·1	0·2	0·5	0·2	2·0	0·4	b
CAF25	1·5	0·3	1·7	0·4	1·4	0·2	1·5	0·3	1·2	0·2	1·2	0·3	1·8	0·4	b
CAF100	2·7	0·3	1·5	0·2	0·9	0·3	0·3	0·2	0·9	0·3	0·8	0·2	0·9	0·2	b
CAF200	2·0	0·3	1·3	0·4	1·0	0·3	0·8	0·2	0·5	0·2	0·9	0·4	1·3	0·3	b
Urine (ml)															
STD	9·4	1·5	14·8	2·5	12·2	1·1	15·1	2·3	15·0	2·2	15·8	3·6	17·1	3·2	a
CAF	24·5	2·7	32·5	2·5	30·6	3·8	35·4	1·4	37·1	4·0	33·1	2·3	34·6	1·7	b
CAF25	22·9	2·1	38·3	3·1	32·0	3·5	39·3	0·7	38·1	2·9	27·8	5·1	38·9	4·0	b
CAF100	27·9	2·8	30·6	1·9	31·4	2·7	33·8	2·8	42·4	1·4	32·2	3·5	35·0	3·9	b
CAF200	21·1	1·5	28·6	3·3	27·6	3·5	34·0	0·5	34·2	4·3	28·0	4·5	28·3	2·1	b

[a,b] Mean values with unlike superscript letters are statistically different, assessed by two-way ANOVA (Tukey's test) at $P < 0·05$.

200-mg/kg per d doses of LM-GSPE had similar effects on both SBP and DBP. Thus, the highest doses of LM-GSPE had no additional antihypertensive effect. Importantly, a potential hypotensive effect of LM-GSPE was ruled out because of the 100-mg/kg per d dose of LM-GSPE, which lowered both SBP and DBP during the experimental period, showed no BP-lowering effect when administered to the normotensive STD group.

Other studies on the antihypertensive effects of long-term administration of phenolic compounds such as resveratrol[25] and other flavanol-rich extracts[26,27] have also found similar chronic antihypertensive effects as described in this study. However, the experimental models used in these studies were different. In the present study, the HTN was induced by an unhealthy diet, and the results confirmed the development of hyperphagia, obesity and dyslipidaemia in these animals. This was evidenced by the observed increase in food intake, increased BW, and elevated plasma TC and TAG in addition to the HTN developed after the 12th week of the CAF diet. Therefore, these animals mimicked the classical model of the human MetS. In addition, elevated homoeostasis model assessment for insulin resistance and β levels have been found in CAF diet-fed rats, indicating the presence of peripheral insulin resistance and increased pancreatic insulin secretion[28].

It has been reported that modest changes in BP and BW are more common in populations with CVD than marked changes[19].

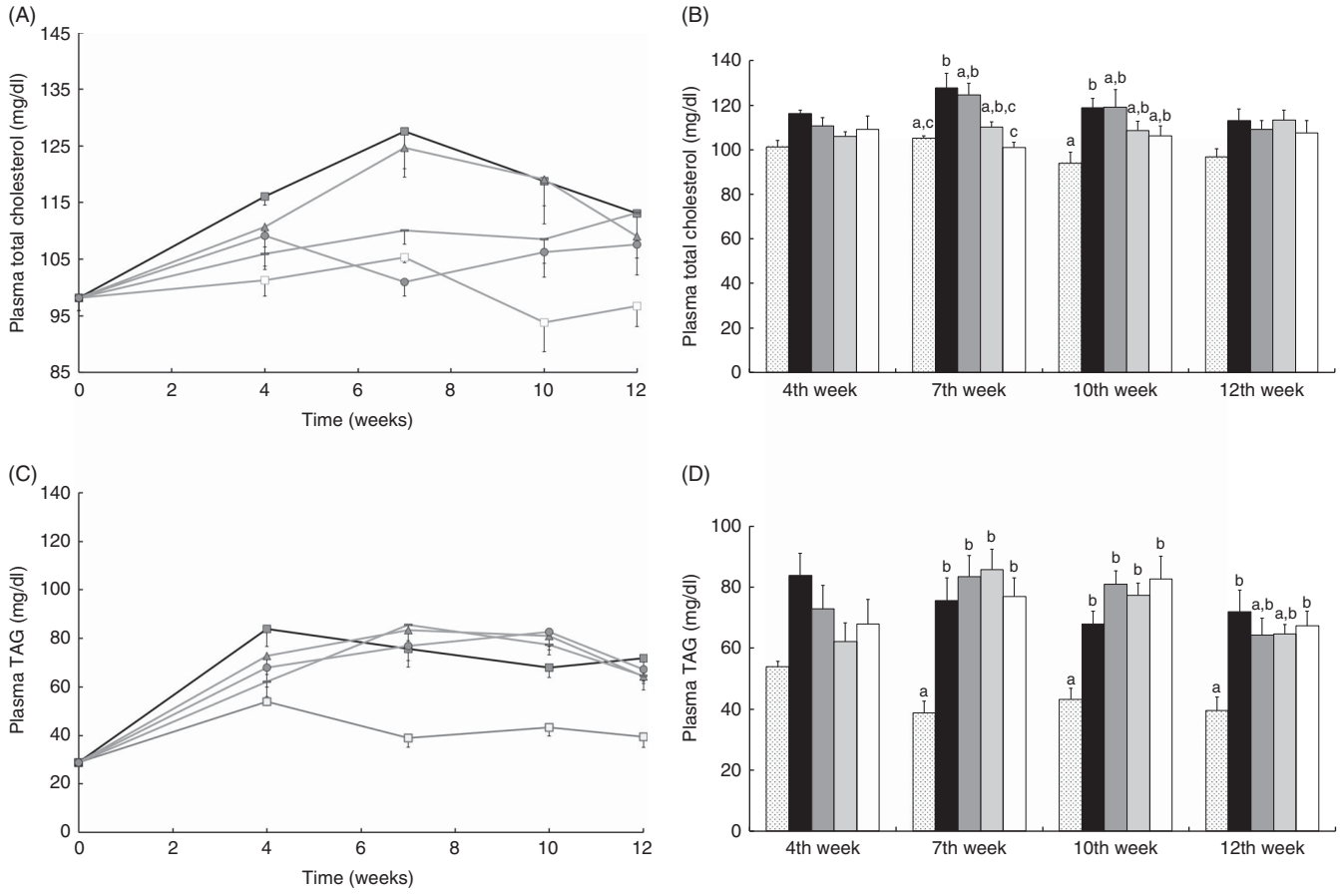

Fig. 4. Changes in plasma (A) total cholesterol during the 12th week of the experiment (significant differences for overall effect: two-way ANOVA, Tukey's *post hoc* test) and (B) at the 4th, 7th, 10th and 12th weeks of the experiment (significant differences: one-way ANOVA, Tukey's *post hoc* test); changes in plasma (C) TAG during the 12th week of the experiment (significant differences for overall effect: two-way ANOVA, Tukey's *post hoc* test) and (D) at the 4th, 7th, 10th and 12th weeks of the experiment (significant differences: one-way ANOVA, Tukey's *post hoc* test) of standard (STD; *n* 10) diet-fed rats and cafeteria (CAF; *n* 10) diet-fed rats administered daily with vehicle or different doses of low-molecular-weight grape-seed polyphenol extract (25, 100 and 200 mg/kg per d: CAF25, CAF100 and CAF200, respectively; *n* 10 per group). Values are means (*n* 10 animals per group), with their standard errors. Mean values with unlike letters were significantly different ($P<0·05$). A: —□—, STD (a); —■—, CAF (b); —▲—, CAF25 (b); —▬—, CAF100 (a, b); —●—, CAF200 (a); C: —□—, STD (a); —■—, CAF (b); —▲—, CAF25 (b); —▬—, CAF100 (b); —●—, CAF200 (b); B, D: ⊡, STD; ■, CAF; ▩, CAF25; ▤, CAF100; □, CAF200.

In the present study, we found mild decreases in BW and WP during the course of the experiment in animals that were administered LM-GSPE. Moreover, we found that the CAF200 group partially counteracted the CAF effect in total BW and WP during the 7th and 8th weeks. In addition, compared with the CAF group, a tendency towards decline in BW gain was observed in the CAF200 group at the end of the study. Previous studies have found a reduction in obesity in rats administered polyphenol extracts[29–31]. Similar beneficial effects on BW have also been observed in rats under a high-fat diet administered with cocoa, which is rich in flavanols[32,33], and in Zucker rats administered a cocoa fibre rich in cocoa flavanols[27]. However, some studies of our group found no reduction in adiposity and BW after grape-seed flavanol administration, but the unfavourable alterations in lipid and glucose metabolism or energy metabolism were ameliorated[34–36]. In contrast, a reduction in BW gain and inflammation were observed in the CAF diet-fed rats administered LM-GSPE for 19 weeks[12]. Recently, it has been described in Wistar rats that a high dose of 500 mg/kg of GSPE administered intragastrically for 8 d reduced BW and food intake, although a higher dose of 1000 mg/kg produces a rebooting effect on BW[37]. In addition, it has been reported that in high-fat diet- and STD diet-fed hamsters, the administration of LM-GSPE for 15 d resulted in a significant reduction in BW gain and a reduction in white adipose tissue weight[38]. The results of this study are in agreement with those of previous studies on grape-seed flavanols, particularly in that the effect of these polyphenolic compounds on the BW appears to be a reduction in BW gain more than a decrease in BW itself[39]. However, we also observed an effect on BW and WP during the 7th and 8th weeks in the CAF200 group.

The reduction in BW gain observed in the CAF diet-fed animals after the administration of the highest dose of LM-GSPE during the course of the study did not appear to be associated with a satiety effect. Food intake in all the LM-GSPE-administered groups was comparable and was similar to that in the group fed the CAF diet. The increased total fluid consumption observed in the groups fed the CAF diet is likely a consequence of the hyperphagia observed in these animals. Consistent with this result, we found that excretion

was also increased in the CAF groups, indicating a higher fluid intake.

The association of leptin resistance with obesity and HTN has been well documented[40–42]. Together with the expansion of the adipose tissue, leptin concentration also increases in obesity, although its circulating levels are unable to promote its central anorexigenic effects[43]. Increased levels of leptin have been found to increase appetite and obesity itself[43]. In this study, we found increased levels of leptin in the CAF groups – a consequence of diet-induced increase in BW. However, at all doses used, the long-term administration of LM-GSPE failed to modify the concentration of this hormone. Previous studies on the effect of LM-GSPE on leptin levels in obesity have reported contradicting results[36,38], likely due to the differences in animal models, different experimental conditions or the different doses administered. In this study, we found no effects on adipose tissue weight in the CAF group administered LM-GSPE (data not shown). In addition, there was no effect on energy intake. Thus, the overall results of this experiment are in concordance.

As the study progressed, the TC and TAG levels in the CAF group increased compared with the STD group. However, similar to changes in BP, these values reached a maximum before the 12th week of the CAF diet, indicating that the duration of the CAF diet may modulate the severity of some of the risk factors, such as HTN and lipid levels, associated with the MetS in this experimental model.

The effect of LM-GSPE on lipid metabolism has been extensively studied[44]. The results of this study confirm the beneficial effect of this extract in ameliorating these CV risk factors. Thus, the long-term administration of LM-GSPE (at the doses of 100 and 200 mg/kg per d) resulted in a clear reduction in the CAF diet-induced hypercholesterolaemia during the experimental feeding period. In fact, a reduced dose–response effect in TC levels after the administration of LM-GSPE was evident at the 7th week, with the levels in the CAF200 group decreasing compared with STD group values. In addition, at the 10th week of CAF diet intake, the two highest doses of LM-GSPE partially counteracted the increased plasma TC levels of the CAF diet group to STD group values. Notably, the timing of this reduction in TC levels coincided with the timing of the CAF diet-induced increase in TC levels. In addition, a reduction in TAG levels was also observed following the administration of LM-GSPE to the CAF group at the end of the 12th week of the experimental feeding period. However, no differences in TAG levels were found during the experiment. In contrast to its effect on TC, the most effective doses of LM-GSPE that produced beneficial changes in TAG levels were the 25- and 100-mg/kg per d doses, which did not include the highest dose administered. The lack of dose-dependent changes in TAG levels after the administration of LM-GSPE has been previously reported by our group[45]. Thus, the lower doses of LM-GSPE appear to be more efficient in lowering TAG levels[45].

In summary, the administration of LM-GSPE to the CAF diet-fed rats attenuated the increase in BP and improved lipid metabolism in these animals. However, although the 25- and 100-mg/kg per d doses were sufficient to produce beneficial effects on BP and lipid metabolism, the 200-mg/kg per d dose was necessary to have an effect on BW and WP. Therefore, we conclude that the LM-GSPE is a good candidate for lowering BP in the treatment of HTN associated with the MetS, particularly because it is able to simultaneously act on other cardiometabolic risk factors associated with this disorder. However, further studies are needed to establish its optimal dose and the mechanism by which LM-GSPE chronically prevent the increase in BP by the CAF diet.

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Z. P., M. M., F. I. B., A. A.-A. and B. M. designed the study; Z. P., M. M. and F. I. B. conducted the study; Z. P., A. A.-A. and B. M. analysed the data and wrote the paper. All the authors contributed to the critical revision of the manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114516004426

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