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The effects of L-Carnosine on development of metabolic syndrome in rats

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ABSTRACT

Aims: The prevalence of metabolic syndrome (MetS) is increasing in several countries. The MetS is characterized by the occurrence of at least three of the following risk factors: decreased high-density lipoprotein cholesterol, increased blood pressure, raised fasting blood glucose, elevated triglycerides, and abdominal obesity. There is a growing evidence of the role of L-carnosine in improving lipid profile and enhancement of the antioxidant activity. However, the effects of L-carnosine on development of MetS are unknown.

Main methods: Male Wistar rats were randomly assigned to receive either; conventional diet (control), high-fat high-carbohydrate diet (HFHCD), L-carnosine and conventional diet (L-Car), or L-carnosine and high-fat high-carbohydrate diet (HFHCD and L-Car) for 16 weeks. Central obesity, systolic blood pressure, lipid profile, glucose hemostasis, levels of leptin and adiponectin were evaluated on week 16.

Key findings: Rats that received HFHCD for 16 weeks showed MetS phenotype such as central obesity, increased blood pressure and glucose, as well as an altered lipid profile ($P < 0.05$). L-Carnosine supplementation to MetS rats significantly reduced abdominal obesity, blood pressure and glucose, and normalized total cholesterol and low density lipoprotein cholesterol levels ($P < 0.05$). Insulin, leptin and adiponectin concentrations were not affected by L-Carnosine ($P > 0.05$).

Significance: L-carnosine has beneficial effects on ameliorating the manifestations of MetS in rats.

1. Introduction

The term metabolic syndrome (MetS) expresses a constellation of biochemical and clinical abnormalities that are featured primarily as central obesity and insulin resistance, and is considered as a serious public health problem [1]. The prevalence of MetS among US adults was estimated as ~34% utilizing data of the National Health and Nutrition Examination Survey 2007–2014 [2], and it could be as high as 84% among other populations [3]. While there are some little differences in the syndrome definition, the presence of three or more of the following components can characterize the disease: decreased high-density lipoprotein (HDL) cholesterol, increased blood pressure, raised fasting blood glucose, elevated triglycerides, and abdominal obesity [4]. Physical inactivity and high-fat and carbohydrate diet represent the major risk factors for the development of MetS [5]. The complex trait of MetS is unequivocally linked to elevated risk of life-threatening complications such as type 2 diabetes mellitus [6] and cardiovascular disease [7] among others.

The pathophysiology of MetS is diverse with contribution of hereditary, epigenetic and environmental factors that are related to insulin resistance, fatty acid excess, and inflammation [8]. Various experimental models of MetS have been developed using pharmacological agents, dietary manipulations, or genetic modifications [9]. Fat and carbohydrate enriched diet is commonly used to induce MetS because of its multifactorial effect on glucose, lipids and hormone metabolism [10]. For example, Rubio-Ruiz and colleagues showed that rats with MetS induced by sucrose-enriched diet had elevated blood pressure, insulin resistance, increased lipid peroxidation, and decreased antioxidant capacity [11]. Another recent animal model of MetS using high-fat high-glucose diet showed increased levels of glucose, insulin, total cholesterol, and triglycerides in MetS rats [12]. Other investigations also revealed that adipokines such as leptin and adiponectin that are expressed in adipose tissue appear to play critical roles in the development of MetS [13]. The two proteins have opposite effects on insulin resistance and inflammation. The pathophysiological abnormalities of MetS can be modulated by the use of natural products such as resveratrol and quercetin [11].

Carnosine is a natural imidazole dipeptide (β-alanyl-L-histidine) that is primarily located in skeletal muscle and presents as an over the counter food additive [14]. It enhances the cellular buffering capacity and stabilizes cell membrane [15]. L-carnosine has been implicated in multiple biological effects such as antioxidant, anti-inflammatory,

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antiaging, and antiproliferative functions [16]. It has been proven to be effective in attenuation of several disease conditions such as liver cirrhosis [17], atherosclerosis [18] and diabetes and its complications [19]. Supplementation of rats with L-carnosine increased the activity of antioxidants, decreased lipid peroxidation and improved lipid profile [20]. In a mouse model of diabetes and hyperlipidemia, treatment with carnosine decreased triglycerides and modulated the atherosclerotic lesions [21]. Clearly, carnosine has received high attention as a potential therapeutic agent in metabolic disorders. Thus, the goal of this study was to examine the effects of L-carnosine on the development of MetS in rats.

2. Materials and methods

2.1. Animals

Young adult male Wistar rats (200–250 g) were housed in the animal care unit at Jordan University of Science and Technology (JUST, Irbid, Jordan). Rats were kept at a 12:12 light/dark cycle at standard room temperature and had free access to water with *ad libitum* food consumption. Animals were adapted for 10 days and then were randomly assigned into four groups (n = 9–10 each) to receive either: conventional diet (control), high-fat high-carbohydrate diet (HFHCD), L-carnosine and conventional diet (L-Car), or L-carnosine and high-fat high-carbohydrate diet (HFHCD and L-Car). Sucrose was added to drinking water for the HFHC groups at 20% to increase the carbohydrate load and to ensure induction of MetS. The duration of the study was for 16 weeks. The Animal Care and Use Committee (ACUC) of JUST approved all experimental procedures and protocols, which comply with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

2.2. Conventional and HFHC diets

Ingredient and chemical compositions of the conventional diet and HFHCD are presented in Table 1. The control diet was commercially prepared diet (pellets) that are routinely used in our animal care unit at JUST. The HFHCD was prepared in our laboratory by manually mixing the ingredients (sucrose, margarine, and milk powder) with 15% of the control diet. The final texture of the HFHCD was solid dough which was then manually cut into small pieces and offered to rats.

Table 1
Ingredient and chemical composition of the conventional and HFHC diets.

	Treatment Diets[a]	
	Conventional	HCHF
Ingredients, %		
Standard rat diet	100	15
Sucrose	0	40
Margarine	0	20
Full-fat milk Powder	0	25
Chemical composition, %		
Carbohydrate (starch & sugars)[b]	49.5	58.6
Protein	21.7	9.5
Lipid (ether extract)	2.4	19.6
Fiber	4.9	0.7
Ash	3.5	2.6
Moisture	9.8	6.4
Gross energy, KCal/g	4.4	4.9
Energy from fat, %	5	36
Energy from CHO, %	45	48

[a] The standard rat diet contained (%) barley (50%), corn (20%), protein concentrate (26%), wheat bran (2%), and minerals/vitamins premix (2%). HFHC = high-fat high-carbohydrate.
[b] Estimated by difference.

2.3. L-carnosine administration

L-carnosine (Sigma–Aldrich Corp., MI, USA) was dissolved in 0.9% normal saline and 250 mg/kg/day was administered as intraperitoneal injection [22] for 16 weeks. Groups without L-carnosine treatment received intraperitoneal injection of the vehicle only.

2.4. Body weight and central obesity measurements

Weight of rats and their abdominal circumference were measured at the end of the exposure duration. The central obesity was evaluated by weighing the excised retroperitoneal white adipose tissues in euthanized rats as described previously [23].

2.5. Systolic blood pressure measurement

Systolic blood pressure was measured in rats at the end of 16 weeks exposure by computerized tail-cuff plethysmography method (Tail cuff plethysmography blood pressure system, IITC Life Science, Woodland Hills, CA), and the systolic blood pressure was recorded by a special software (IITC software version 1.1, IITC Life Science, Woodland Hills, CA).

2.6. Hormonal, lipid profile and glucose hemostasis

Rats were euthanized by rapid decapitation without anesthesia since anesthsia may affect the level of glucose, insulin and lipid profile [24]. Blood was collected and immediately centrifuged for 20 min at 4000 g. Serum was separated and stored at -80 °C till further analysis. Serum levels of leptin and adiponectin (R&D Systems, USA) were measured by ELISA technique following the manufacturers' instructions.

Serum levels of triglyceride, total cholesterol and HDL cholesterol (Biosystems, Barcelona, Spain) were measured according to the manufacturer's instructions. Friedewald's low-density lipoprotein (LDL)-cholesterol estimation formula was used to calculate the level of LDL cholesterol [25].

The level of fasting blood glucose was determined by blood glucose meter (ACCU-CHEK, Roche diagnostic, USA) from rats' tails. Serum level of insulin (MyBioSource, SanDiego, CA, USA) was measured using ELISA technique according to the manufacturer's instructions. The absorbance was read by Epoch Biotek microplate reader (BioTek, Winooski, VT, USA) at the appropriate wavelengths.

2.7. Statistics

Data were presented as mean ± SEM. D'Agostino & Pearson omnibus and Shapiro-Wilk normality tests were used to check for the normality of data. One-way ANOVA followed by Tukey's multicomparison test or Kruskal-Wallis test were used to analyze and compare the difference between multiple groups with respect to biochemical parameters. Statistical analysis was performed utilizing GraphPad Prism 5® software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Conventional and HFHCD

The HFHCD contained relatively more fat (by about 716%) and carbohydrate (by about 18%) compared to the control diet. Subsequently, the HFHCD provided more energy coming from fat compared to the control diet (36% vs. 5%). Both diets were relatively isocaloric. Sucrose (20%) was also added to drinking water for the HFHCD to increase the carbohydrate supply for rats in those groups.

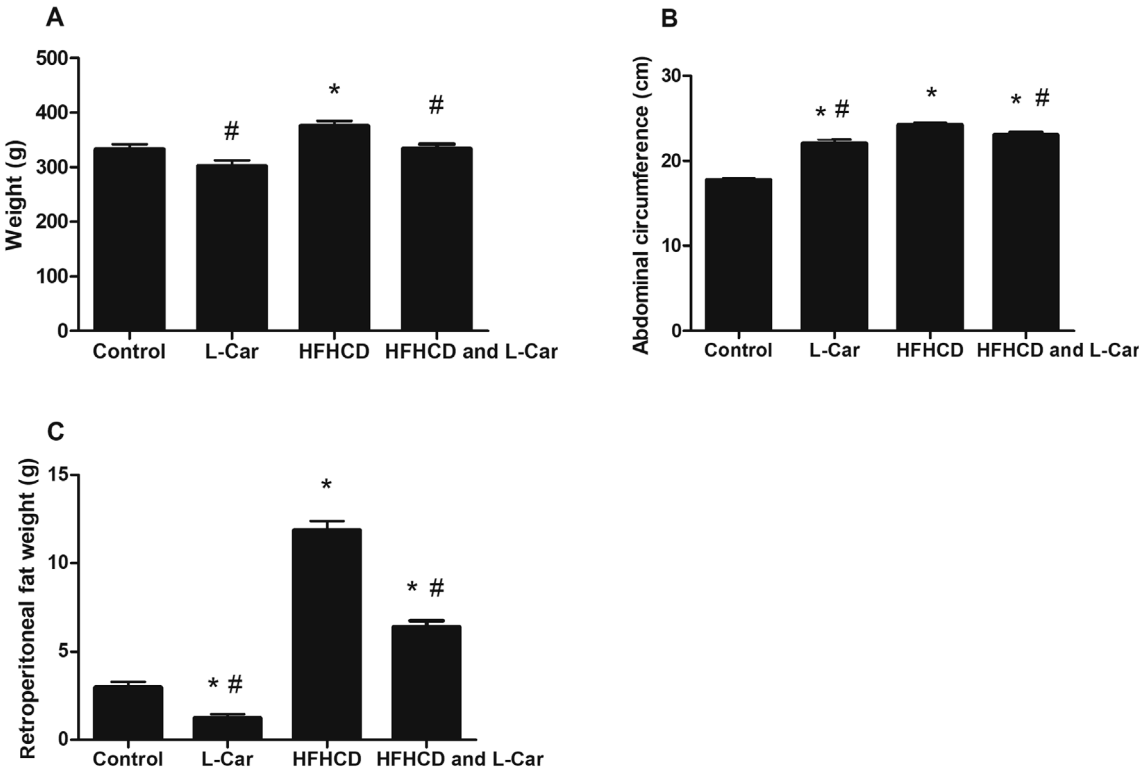

Fig. 1. Effect of L-carnosine on HFHCD induced central obesity. Animals were exposed to HFHCD and L-carnosine for 16 weeks then, (A) animals weight, (B) abdominal circumference and (C) retroperitoneal fat weight were measured. * and # indicate significant difference from control and HFHCD respectively. Values are expressed as mean ± SEM of 9–10 animals per group. $P < 0.05$ was considered statistically significant.

3.2. Effect of L-carnosine on central obesity

The administration of HFHCD significantly increased body weight (333.0 ± 9.3 g in control group versus 376.2 ± 8.8 g in HFHCD group, $P < 0.05$), abdominal circumference (17.7 ± 0.2 cm in control group versus 24.3 ± 0.2 cm in HFHCD group, $P < 0.05$), and retroroperitoneal fat weight (3.0 ± 0.3 g in control group versus 11.9 ± 0.5 g in HFHCD group, $P < 0.05$) compared to control on week 16 (Fig. 1A, B and 1C).

The co-administration of L-carnosine with HFHCD significantly reduced body weight (376.2 ± 8.78 g in HFHCD group versus 333.9 ± 8.6 g in HFHCD and L-carnosine group, $P < 0.05$), abdominal circumference (24.3 ± 0.2 cm in HFHCD group versus 23.1 ± 0.3 cm in HFHCD and L-carnosine group, $P < 0.05$), retroroperitoneal fat weight (11.9 ± 0.5 g in HFHCD group versus 6.4 ± 0.4 g in HFHCD and L-carnosine group, $P < 0.05$) compared to HFHCD administration (Fig. 1A, B and 1C).

3.3. Effect of L-carnosine on systolic blood pressure

The administration of HFHCD significantly increased systolic blood pressure (98.9 ± 2.1mmHg in control group versus 141.5 ± 1.2mmHg in HFHCD group, $P < 0.05$) compared to control on week 16 (Fig. 2). However, the co-administration of L-carnosine with HFHCD significantly reduced systolic blood pressure (141.5 ± 1.2mmHg in HFHCD group versus 120.8 ± 1.1mmHg in HFHCD and L-carnosine group, $P < 0.05$) compared to HFHCD group, but is still significantly higher than the control group (Fig. 2).

3.4. Effect of L-carnosine on glucose hemostasis

The administration of HFHCD significantly increased fasting blood glucose (78.8 ± 1.6 mg/dl in control group versus 135.2 ± 1.9 mg/dl in HFHCD group, $P < 0.05$) compared to control (Fig. 3A). In contrast,

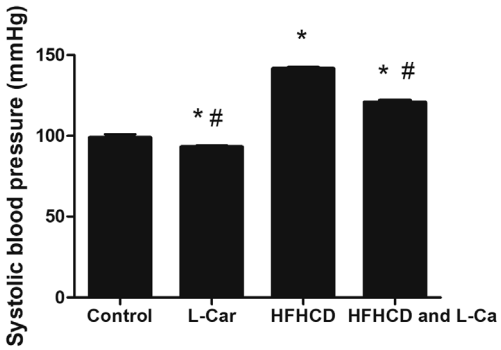

Fig. 2. Effect of L-carnosine on HFHCD induced increased systolic blood pressure. Animals were exposed to HFHCD and L-carnosine for 16 weeks then systolic blood pressure was measured. * and # indicate significant difference from control and HFHCD respectively. Values are expressed as mean ± SEM of 9–10 animals per group. $P < 0.05$ was considered statistically significant.

the co-administration of L-carnosine with HFHCD significantly reduced fasting blood glucose (135.2 ± 1.9 mg/dl in HFHCD group versus 120.8 ± 1.5 mg/dl in HFHCD and L-carnosine group, $P < 0.05$) compared to HFHCD group, but is still significantly higher than the control group (Fig. 3A). Further, L-carnosine reduced the level of glucose compared to control (78.8 ± 1.6 mg/dl in control group versus 69.3 ± 1.1 mg/dl in HFHCD and L-carnosine group, $P < 0.05$) (Fig. 3A).

The level of fasting insulin was not affected by HFHCD or L-carnosine co-administration (146.5 ± 23.4 pg/ml in control group, 88.9 ± 10.8 pg/ml in L-carnosine group, 109.2 ± 20 pg/ml in HFHCD group and 126.1 ± 17.7 pg/ml in HFHCD and L-carnosine group, $P > 0.05$) (Fig. 3B).

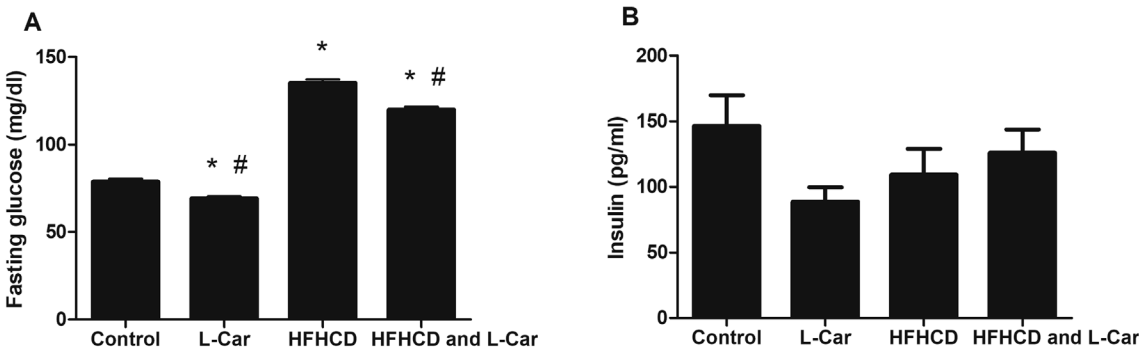

Fig. 3. Effect of L-carnosine on HFHCD induced glucose hemostasis disturbances. Animals were exposed to HFHCD and L-carnosine for 16 weeks then the concentration of (A) fasting glucose and (B) fasting insulin were measured. * and # indicate significant difference from control and HFHCD respectively. Values are expressed as mean ± SEM of 9–10 animals per group. $P < 0.05$ was considered statistically significant.

Table 2
Effect of L-carnosine on HFHCD induced lipid profile disturbances.

Lipid Profile	Control	L-Car	HFHCD HFHCD and L-Car
Total Cholesterol (mg/dl)	64.9 ± 2.9	40.7 ± 0.9*	98.1 ± 4.2* 72.8 ± 4.6#
LDL (mg/dl)	5.8 ± 1.4	3.3 ± 0.9	21.6 ± 4.1* 9.1 ± 4.0#
HDL (mg/dl)	52.1 ± 3.1	31.1 ± 1.1*	54.1 ± 5.1 43.4 ± 1.7
Triglycerides (mg/dl)	34.6 ± 1.5	31.1 ± 1.9	112.0 ± 13.4* 101.1 ± 8.0

*and # indicate significant difference from control and HFHCD respectively. Values are expressed as mean ± SEM of 9–10 animals per group. $P < 0.05$ was considered statistically significant.

3.5. Effect of L-carnosine on lipid profile

HFHCD increased level of total cholesterol, triglycerides and LDL compared to control. However, HFHCD administration did not affect the level of HDL compared to control (Table 2).

L-carnosine reduced the increased level of total cholesterol and LDL that were induced by HFHCD administration, while the levels of HDL and triglycerides were not affected by L-carnosine compared to HFHCD alone (Table 2).

3.6. Effect of L-carnosine on leptin and adiponectin levels

The administration of HFHCD significantly increased leptin concentration (900.8 ± 92.9 pg/ml in control group versus 4832 ± 808.3 pg/ml in HFHCD group, $P < 0.05$) compared to control (Fig. 4A). On the other hand, the co-administration of L-carnosine with HFHCD did not affect the leptin concentration (4832 ± 808.3 pg/ml in HFHCD group versus 4109 ± 481.9 pg/ml in HFHCD and L-carnosine group, $P > 0.05$) compared to HFHCD group, but is still significantly higher than the control group (Fig. 4A).

The adiponectin concentration was significantly increased by HFHCD (5355 ± 504.9 pg/ml in control group versus 9860 ± 1014 pg/ml in HFHCD group, $P < 0.05$) compared with control (Fig. 4B). However, L-carnosine co-administration did not affect the increased concentration of adiponectin by HFHCD (9860 ± 1014 pg/ml in HFHCD group versus 7774 ± 635.2 pg/ml in HFHCD and L-carnosine group, $P > 0.05$) (Fig. 4B).

4. Discussion

The MetS is a prevalent worldwide health problem that is associated with dyslipidemia, hypertension, insulin resistance, and abdominal obesity. It is linked to various genetic and environmental factors that encompass oxidative stress and inflammation. This study evaluated the role of L-carnosine in modulating MetS induced by HFHCD in rats. The current study revealed that co-treatment of animals with L-carnosine significantly reduced abdominal obesity, blood pressure, blood glucose, LDL and total cholesterol that were initially increased by the HFHCD. Overall, the study's findings suggest that L-carnosine could be a potential agent for ameliorating the phenotype of MetS.

One of the primary features of MetS is the abdominal obesity that was demonstrated in the current model after feeding the rats with HFHCD. The mean abdominal circumference reported in this study among HFHCD group was comparable to that reported in an experimental model of MetS where rats were treated with a high-fat/high-glucose diet for 20 weeks (24.3 ± 0.2 cm vs. 25.5 + 0.44 cm, respectively) [12]. The administration of L-carnosine was effective in reducing central obesity as observed with decreased retroroperitoneal fat weight, abdominal circumference, and total body weight. As these measures reflect viseral adipose tissue [26], the effect seen with L-carnosine may suggest its role in modulating the secretion of

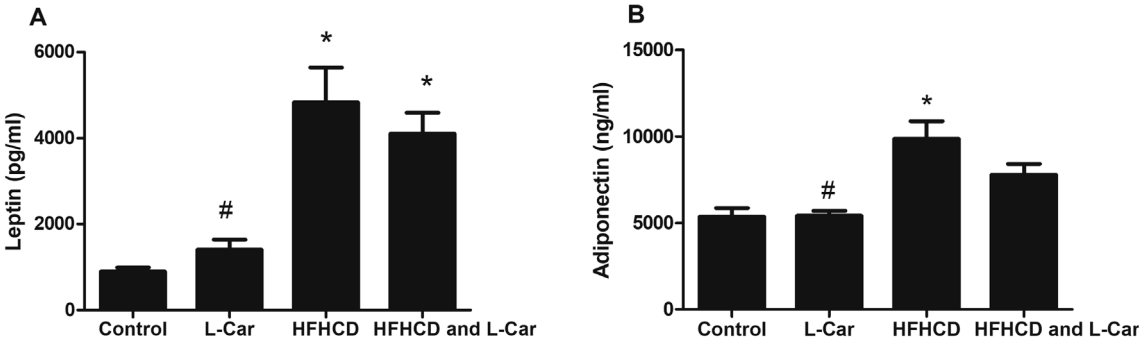

Fig. 4. Effect of L-carnosine on HFHCD induced hormonal disturbances. Animals were exposed to HFHCD and L-carnosine for 16 weeks then the concentration of (A) leptin and (B) adiponectin were measured. * and # indicate significant difference from control and HFHCD respectively. Values are expressed as mean ± SEM of 9–10 animals per group. $P < 0.05$ was considered statistically significant.

proinflammatory adipokines from adipose tissue [27].

Increased blood pressure (by 34%) and glucose (by 71.6%) were also hallmarks of MetS that were observed in the current study among HFHCD treated group. Interestingly, co-treatment of HFHCD group with L-carnosine decreased systolic blood pressure and glucose levels observed following HFHCD feeding. However, this effect was not completely normalized to the levels of controls. Carnosine was shown to affect the activity of sympathetic and parasympathetic nervous system at various organs such as liver, kidney, and adrenal glands resulting in reducing blood pressure and sugar [28]. A subject with congenital deficiency of carnosine degrading enzyme (carnosinase) had a remarkable decrease in blood pressure [29]. Moreover, administration of L/D-carnosine (30 mg/kg/day) to obese rats for 24 weeks decreased blood pressure [30]. The antihypertensive effect of L-carnosine could be explained through its effect on attenuating the renal sympathetic nerve activity or its action on histamine (H3) receptor [28]. Decreased blood sugar with L-carnosine treatment could be attributed to the suppression of sympathetic nervous system activity at the adrenal gland and liver [31]. Furthermore, treatment of animals with H3 receptor antagonist reversed the effect of L-carnosine on blood sugar, indicating the contribution of a histaminergic mechanism [31]. In humans, overweight or obese subjects with impaired glucose intolerance who were treated with L-carnosine (2 g) daily for 12 weeks showed decreased 2-h glucose levels [32]. The finding that insulin was not affected by HFHCD was inconsistent with a previous observation of increased insulin level in rats administered with high-fat/high-glucose diet [12]. However, a small yet significant drop in blood pressure and fasting blood glucose was also observed among the control group treated with L-carnosine in the current study. This might imply that the effect of L-carnosine in reducing blood pressure and sugar is not specific to MetS group, but it is more profound when the syndrome is developed. Future experiments should focus on elucidating the mechanism of the effect of L-carnosine on blood pressure, glucose and insulin.

In the present study, hyperlipidemia induced by HFHCD was also mitigated with co-treatment with L-carnosine. The initial increase in cholesterol and LDL with HFHCD was lowered when rats were treated concurrently with L-carnosine. A trend was also observed toward decreased the elevated levels of triglycerides. Various studies showed altered lipid metabolism with carnosine [20,30]. For example, decreased triglycerides and cholesterol levels in heart and liver was reported when diabetic mice were treated with carnosine [33]. Moreover, supplementation with L-carnosine to patients with diabetes resulted in decreased blood sugar and triglycerides as compared to placebo [34]. It was suggested that the positive effect of carnosine on lipid profile might be mediated through its action on insulin [33]. As the current study did not show any significant effect of carnosine on insulin levels, additional investigations are warranted to further validate this observation. Other reports proposed that the lipid-lowering effect of carnosine could be via its effect on lipoprotein lipase activity [20,35]. Assessing the activity of lipoprotein lipase should be considered in future studies. There are conflicting findings in the literature regarding the effect of L-carnosine on HDL [20,34]. It has been shown that higher levels of HDL does not always mean that it has a protective role [36,37]. Therefore, the function of HDL is poorly reflected by its concentration and assessing the activity of HDL, such as the cholesterol acceptor activity, is recommended in future studies to withdraw conclusion about the effect of L-carnosine, either alone or in combination with HFHCD, on HDL.

As adipokines play a critical role in the development of MetS components such as insulin resistance, diabetes and obesity [38]; the present study evaluated the impact of L-carnosine supplementation on serum adipokine levels. Treatment with HFHCD increased serum levels of adiponectin. In a recent model of MetS, increased adiponectin and a trend toward increased leptin were observed in rats fed with high-fat/high-glucose diet [12]. Similar observations were also reported in rats fed with high sucrose diet where development of MetS was not linked to decreased adiponectin level [39]. Additionally, low or high concentrations of adiponectin were observed among various models encompassing MetS manifestations [40,41]. It is possible that adiponectin release from adipose tissue during the development of MetS is time dependent [39]. Thus, it is necessary to monitor adipokine levels at different time intervals during model development. The finding of increased leptin level with HFHCD was consistent with previous models of MetS in rodents [9].

The present study also showed that L-carnosine supplementation did not have an effect on the elevated levels of adiponectin and leptin. This result is in agreement with a previous finding that carnosine supplementation to nondiabetic overweight or obese patients did not affect adiponectin levels [32]. Conversely, a follow-up study revealed reduced serum resistin concentration and a trend toward decreased leptin level with L-carnosine [27]. Supplementation with a combination of cinnamon, chromium and carnosine to prediabetic overweight or obese subjects had a negligible effect on leptin level [42]. The variations in results could be attributed in part to differences in MetS features between humans and rodents which may have an effect on baseline levels of adipokines. Another possibility is that the development of MetS in this model is not primarily represented by changes in inflammation.

The current study has some limitations. First, the effects of L-carnosine and HFHCD on oxidative stress biomarkers as glutathione reductase activity and peroxynitrite concentration in different tissues were not examined. Second, the mechanism of the effect of L-carnosine and HFHCD on body weight were not fully examined as the level of other hormones as ghrelin, resistin and glucagon like-peptide 1 were not measured.

5. Conclusions

In conclusion, this study has demonstrated that treatment of rats with HFHCD for 16 weeks was associated with the development of MetS manifestations such as central obesity, increased blood pressure and sugar, as well as an altered lipid profile. L-carnosine supplementation to rats with MetS reduced abdominal obesity, blood pressure and glucose, and normalized total cholesterol and LDL levels. L-carnosine did not affect leptin and adiponectin concentrations. Overall, the findings suggest that L-carnosine could have a potential effect in ameliorating MetS manifestations. Future investigations are warranted to confirm these results in patients with MetS.

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