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CARNOSINE-LOADED ORAL NIOSOMES AMELIORATE HIGH-FRUCTOSE-INDUCED METABOLIC SYNDROME IN RATS VIA MODULATION OF SIRT1, A METABOLIC MASTER SWITCH

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Abstract: Metabolic syndrome is a crucial health challenge, and the available therapeutic agents are still not effective. Carnosine, a cytoplasmic dipeptide, is a potent anti-glycation, anti-oxidant, anti-inflammatory and chelating agent. However, whether carnosine would be assumed as a potential hypoglycemic agent or not, no decisive report with detailed mechanisms is found yet. As such, we suggest the carnosine-loaded in niosomes as a prospective solution to bypass its unwanted fast degradation by carnosinase which is considered as a major obstacle with the clinical application of carnosine as an oral drug therapy. Toward this, the purpose of our study is to assess the profits of oral administration of carnosine, and carnosine-loaded niosome in HFD-induced metabolic syndrome rats and to inspect some of the involved mechanisms. Initially, carnosine-loaded niosomes were prepared and characterized. Then, metabolic syndrome was provoked by 60% fructose diet in male Sprague Dawley rats where carnosine and carnosine-loaded niosomes were orally administered at doses 50 mg/kg and 25 mg/kg, respectively. In addition, biochemical and molecular studies were performed to clarify the possible mechanisms of action. Data showed that the consumption of 60% fructose diet displayed a tremendous increment in body weight, body mass index as well as a significant elevation in levels of serum glucose, insulin, TAG, TC, LDL-c, VLDL-c and FFA. Also, it showed a significant reduction in levels of serum HDL-c. Furthermore, HFD provoked up-regulation of SREBP-1c and FAS mRNA levels in adipose tissue. Also, it induced down-regulation of SIRT1, GLUT-4 mRNA levels in adipose tissue. We found that oral administration of either carnosine or carnosine-loaded niosome effectively reversed HFD-mediated alterations via SIRT1 activation. Overall, oral delivery of carnosine-loaded niosome had a better efficacy than oral carnosine, attenuating HFD-mediated alterations. Carnosine nano-formulation is a new excellent candidates for metabolic syndrome management and needs further exploration of its mechanisms.

Key words: carnosine; niosome; metabolic syndrome; fructose; SIRT1; SREBP-1c; GLUT-4

Introduction

Metabolic syndrome (MetS) is identified as a group of at least three of the five subsequent conditions: central obesity, hyperglycemia, hypertension, hypertriglyceridemia, and low serum high-density lipoprotein (1). MetS is mainly recognized by central adiposity, that is closely related to insulin resistance and facilitate further metabolic risk factors development (2). In the last years the MetS has acquired specific attention, since diabetes, obesity, and hypertension increase the risk for additional forms of COVID-19 and associated mortality (3). The co-morbidities control via efficient medications which are available for hyperglycemia, arterial hypertension, and dyslipidemia treatment is the most likely approach to hinder MetS-related outcomes (4, 5).

Carnosine (β-alanyl-L-histidine) has anti-glycation, anti-inflammatory, anti-oxidant, and chelating properties (6). carnosine has achieved an enormous attention as a possible therapy in metabolic disorders but the mechanism of the effect of carnosine on metabolic syndrome

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manifestations needs to be elucidated (7). The action of carnosine is limited in the body due to low lipophilicity and its fast hydrolysis via carnosinase. Thus, carnosine require modification to increase stability and improve bioavailability after its systemic administration (8) through incorporating it into nanostructured constructions. This is a promising strategy that may help to resist carnosinase activity and improve its target delivery (9, 10).Also, nanomaterials can reduce the amount of drug utilized and its related side effects in metabolic disorders treatment (11).

Niosome is closed vesicles which composed of non-ionic surfactants organized in concentric bilayers with cholesterol Incorporation. Niosomes are analogous to liposomes in their structure, but they provide tremendous advantages including being untoxic, having more chemical stability, and having simple and unexpensive production techniques (12). There are various studies has been displayed to improve the delivery system of some hypoglycemic agents with niosomal delivery system (13, 14). Recent studies showed that niosomes display protection from the proteolytic enzymes to the peptide and protein drugs (15). For oral delivery, niosomes of trimethyl chitosan-coated insulin are developed in order to increase insulin permeation (16). Thus this research in the field of niosomes will increase further and may lead to good market formulation in the pharma industry (15).

However, carnosine in a nano-formulation has not yet been examined for metabolic syndrome treatment. We proposed the carnosine-loaded niosomes as a potential solution to bypass its unwanted fast degradation by carnosinase which is considered as a major obstacle with the clinical application of carnosine as an oral drug therapy and to achieve high therapeutic outcome at lower dose than that previously reported with free carnosine. Toward this, the aim of our study is to evaluate the efficacy of oral free carnosine comparing to oral carnosine-loaded niosomes administration on the development of MS in a rat model provoked by high fructose diet. Also, the underlying mechanism of carnosine in the metabolic syndrome management remains mysterious. So, at the same time, there is a clear need to investigate the possible mechanism of carnosine action.

Materials and methods

Chemicals

Carnosine, Cholesterol, and Span 60 were purchased from Sigma Aldrich, USA. Fructose was purchased from uni-pharma, Egypt. All other chemicals utilized in our study were obtained from Sigma-Aldrich unless stated otherwise.

Laboratory animals

Sixty adult male Sprague Dawley rats weighing 168–170 g were procured from the animal house of the Faculty of Veterinary Medicine, Zagazig University, Egypt. They were housed for one week under standard laboratory conditions. They were kept at a temperature of 20-25 °C, relative humidity of about 60%, 12-hour light-dark cycle and free access to water and food. The study protocol got the approval of the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC/2/F/45/2022).

Preparation and characterization of Carnosine-loaded niosomes

Carnosine-loaded niosome was prepared at Nanomaterials Research and Synthesis Unit, Animal Health Research Institute, ARC, Giza, Egypt. Niosome was prepared as described by Moulahoum *et al.*, 2019 and by using the thin film method. Span 60 (100 mg) and cholesterol (20 mg) were dissolved in 10 ml chloroform. Then, by using a rotary evaporator (Buchi R-3, Switzerland), the solvent was eliminated to form a thin lipid film (120 rpm, 60 °C, 1 h). The obtained thin film was hydrated with a carnosine solution to obtain carnosine-loaded niosome. In a 10 ml solution of phosphate buffer saline (PBS), Carnosine (50 mg/ml) was dissolved at pH 7.4, 60°C to obtain the final concentration. Then, with the aqueous solution, the lipid layer was dispersed and sonicated for 5 minutes in an ultrasonic bath (Sonics & Materials Inc. USA) to obtain the niosomal formulation (9). Using the Zetasizer device, the particle size and zeta potential of carnosine-loaded niosome were measured. Using transmission electron microscopy, Its surface morphology was visualized.

Induction of metabolic syndrome

The rats were given the high fructose diet for 10 weeks to induce metabolic syndrome. The high fructose diet provides 60% of the calories from fructose and prepared by mixing standard rodent diet with 60% wt/wt fructose (17). After the high-fat diet administration for 10 weeks**,** the success of fructose-induced metabolic syndrome was verified by monitoring body weight changes and significant elevation of fasting serum glucose, insulin and triglycerides. Rats were further used for the post-treatment period (4Weeks).

Animal groups and treatments

After one week from adaptation, rats were assigned in six groups (*n* = 10 each) for 14 weeks:

Group I: served as a control group and fed standard chow diet throughout the experimental period. Instead of supplements, 0.9% saline was orally administered for equivalent handling to other experimental groups.

Group II: served as the MetS group and fed on high-fructose diet and received saline solution similar to control throughout the experimental period for 14 weeks.

Group III: rats were fed high-fructose diet and received carnosine (50 mg/kg/day; p.o) dissolved in saline throughout the experimental period as a co-treatment.

Group IV: rats were fed high-fructose diet and received carnosine-loaded niosome (25 mg/kg/day; p.o) dissolved in saline throughout the experimental period as a co-treatment.

Group V: rats were fed high-fructose diet and received carnosine (50 mg/kg/day; p.o) dissolved in saline for 4 weeks starting from week 11 of the experimental period (after confirmation of metabolic syndrome) as a post-treatment.

Group VI: rats were fed high-fructose diet and received carnosine-loaded niosome (25 mg/kg/day; p.o) dissolved in saline for 4 weeks starting from week 11 of the experimental period (after confirmation of metabolic syndrome) as a post-treatment.

The oral dose of carnosine used in the current report was selected based on the effective dose of a previous study which revealed that carnosine had a hypoglycemic effect in diabetic rats (18). Body weight and body length were recorded at baseline and at the end of the study protocol to calculate the BMI:

Body mass index (BMI) = body weight (g)/ length^2 (cm^2)

Blood and tissue samples

Following an intervention period of 14 weeks, rats were euthanized by an intraperitoneal injection of sodium thiopental (60 mg/kg), and fasting (12 hr). For biochemical estimation, the blood samples were immediately collected through the heart puncture. At room temperature for 15 min, they were allowed to clot. Then, for serum separation, they were centrifuged for 15 min at 3,000 rpm. For gene expression analyses, adipose tissues were collected, immediately frozen in liquid nitrogen and stored at - 80° C.

Biochemical assay

Biochemical estimation of serum glucose, insulin levels and HOMA-IR

Using the glucose oxidase method (Spinreact, Girona, Spain), serum glucose levels were measured. Using rat insulin ELISA kit (BioVendor Laboratory Medicine, Brno, Czech Republic), serum insulin levels were measured according to the manufacturer's protocol. The homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated as follows: HOMAIR= fasting blood glucose (mg/dl) × fasting insulin (ng/ml)/405 (19).

Biochemical estimation of lipid profile markers and free fatty acids

Serum lipid profile including serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoproteins-cholesterol (LDL-c) and triacylglycerol (TAG) were determined using commercially available kits (Spinreact, Spain). Serum levels of very low density lipoproteins-cholesterol (VLDL-C) were calculated by Friedewald equation (20) Serum concentrations of free fatty acids (FFA) were assessed by a rat ELISA kit (Cusabio Biotech Co., China) according to the manufacturer's protocol.

Quantitative real-time PCR

Using TRIzols Reagent (15596026, Life Technologies, USA), total RNA was extracted from freshly isolated adipose tissues following the manufacturer's information. Using nanodrop, RNA concentrations were measured, then using QuantiTects Reverse Transcription Kit (Qiagen,

Table 1: the primer sequences

Gene	Sequence	Accession number	References
SIRT1	Forward 5′TGG ACG AGC TGA CCC TTG A3′ Reverse 5′TCC TGC GGA TGT GGA GAT T3′	XM_017601788	(21)
SREBP-1c	Forward 5‘GCTCACAAAAGCAAATCACT3‘ Reverse 5‘GCGTTTCTACCACTTCAGG3‘	NM_001276707.1	(22)
FAS	Forward 5‘CTATTGTGGACGGAGGTATC3‘ Reverse 5‘TGCTGTAGCCCAGAAGAG3‘	NM_017332.1	(22)
GLUT-4	Forward 5‘TCATTCCTGTGAAAGTGATGACGA3‘ Reverse 5‘CTGCCACAGTGTCATATCATCCAA3‘	NM_012751.1	(23)
β-actin	Forward 5‘CCGTAA AGACCTCTATGCCAACA3‘ Reverse 5‘GCTAGGAGCCAGGGCAGTAATC3‘	NM_031144.3	(23)

USA), cDNA was synthetized based on the manufacturer's protocol. Rotor-Gene Q (Qiagen, USA) was used to determine sirtuin-1 (SIRT1), sterol regulatory element binding protein-1c (SREBP-1c), glucose transporter isoform 4 (GLUT-4) and fatty acid synthase (FAS) mRNA levels. Quantitative PCR was performed in duplicate on Maximas SYBR Green/Fluorescein qPCR Master Mix. The relative levels of SIRT1, SREBP1c, FAS and GLUT4 mRNA were calculated by the $2^{-\Delta\Delta ct}$ method, which was normalized to the β-actin housekeeping gene mRNA level. The primer sequences for SIRT1, SREBP-1c, FAS and GLUT-4 are listed in table 1.

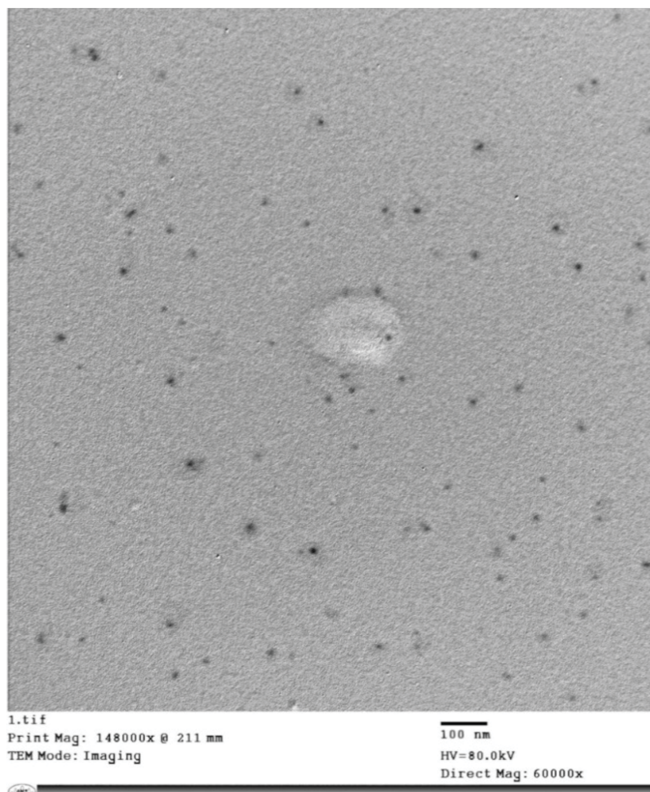

Figure 1: TEM micrograph of carnosine-loaded niosome

Statistical analysis

Data were presented as mean ± SEM and analyzed by one-way ANOVA using IBM SPSS software.

Results

Preparation and characterization of carnosine-loaded niosome

The prepared carnosine-loaded niosome showed 168 nm particle size. The zeta potential of the niosome was -2.8 mV, which indicates that the niosomes are stable against fusion due to increasing of repulsive forces between the particles (24). The morphology of carnosine-loaded niosome is shown in Figure 1.

Effect of oral carnosine, and carnosine-loaded niosome on body weight and body mass index (Anthropometric indices) in experimentally induced metabolic syndrome in rats

As shown in table 2, The baseline weight and body mass index of rats in different groups was not significantly different. It was observed that the final body weight and body mass index of all groups increased as compared to the initial measurements. The results showed that final body weight and body mass index in HFD-fed rats increased significantly when compared with the control group. The body weight and body mass index of HFD-fed rats treated with either oral carnosine or oral carnosine-loaded niosome in both co-treatment (14 weeks) and post-treatment

conditions (4 weeks) showed statistically significant decrease compared to HFD-fed rats. This decrease was significantly ameliorated especially in the co-treatment conditions. Moreover, HFD-fed rats treated with oral carnosine-loaded niosome showed the greatest effect in both co-treatment and post-treatment conditions.

Effect of oral carnosine, and carnosine-loaded niosome on serum glucose and insulin levels and the HOMA-IR index in experimentally induced metabolic syndrome in rats.

On rats fed with HFD, there was a significant increase in serum glucose and insulin levels in addition to the HOMA-IR values as compared to the normal control group, as shown in table 3. However, a significant decline in these parameters was detected in HFD-fed rats treated with either oral carnosine or oral carnosine-loaded niosome in both co-treatment (14 weeks) and post-treatment conditions (4 weeks) in comparing to HFD group. This reduction was more pronounced with the co-treatment conditions than the post-treatment conditions. Moreover, HFD-fed rats treated with oral carnosine-loaded niosome showed the greatest effect in both co-treatment and post-treatment conditions.

Effect of oral carnosine, and carnosine-loaded niosome on Lipid profile markers in experimentally induced metabolic syndrome in rats.

Serum total cholesterol (TC), triacylglycerol (TAG), low-density lipoproteins (LDL), very-low density lipoproteins (VLDL) and free fatty acids (FFA) displayed markedly raised levels, unlike HDL-cholesterol showing a dropped level, in rats fed with HFD relative to the control group, as shown in table 3. These effects were reversed by administration of either oral carnosine or oral carnosine-loaded niosome in HFD-fed rats in both co-treatment (14 weeks) and post-treatment conditions (4 weeks) and were substantially improved especially in the co-treatment conditions. Moreover, HFD-fed rats treated with oral carnosine-loaded niosome showed the greatest effect in both co-treatment and post-treatment conditions.

Effect of oral carnosine, and carnosine-loaded niosome on mRNA levels of SIRT1, SREBP-1c, FAS and GLUT-4 in adipose tissues of experimentally induced metabolic syndrome in rats.

As shown in figure 2, RT-PCR showed that HFD-fed rats exhibited a significant up-regulating

Table 2: Effect of Oral Carnosine, and Oral Carnosine-Loaded Niosome on Body Weight and Body Mass Index in HFD-fed rats

		I	II	III	IV	V	VI
B.W(g)	Day 0	168.85±2.63[a]	169.43± 2.65[a]	168.28±2.60[a]	168.71±2.91[a]	170.57±3.41[a]	168.57±2.68[a]
	14 weeks	206.43±5.98[d]	311.00±6.23[a]	281.86±5.07[b]	258.42±5.17[c]	309.43±4.95[a]	302.71±4.74[a]
BMI	Day 0	0.35±0.005[a]	0.36±0.005[a]	0.35±0.006[a]	0.35±0.006[a]	0.36±0.007[a]	0.35±0.005[a]
	14 weeks	0.44±0.01[d]	0.66±0.01[a]	0.59±0.01[b]	0.54±0.01[c]	0.65±0.01[a]	0.64±0.01[a]

Means within the same row having different superscript letters are significantly different. Significant at 0.05 probability.

Table 3: Effect of Oral Carnosine, and Oral Carnosine-Loaded Niosome on Biochemical Parameters in HFD-fed rats

	I	II	III	IV	V	VI
GLU (mg/dl)	99.42±0.75[f]	183.71±0.84[a]	134.14±0.59[d]	122.71±0.78[e]	164.85±0.74[b]	153.28±0.92[c]
INS (ng/ml)	1.85±0.11[f]	4.35±0.08[a]	2.85±0.04[d]	2.57±0.03[e]	3.72±0.04[b]	3.35±0.04[c]
HOMA-IR index	0.45±0.03[f]	1.97±0.05[a]	0.94±0.02[d]	0.78±0.01[e]	1.52±0.02[b]	1.27±0.02[c]
TC (mg/dl)	85.67±1.35[f]	107.68±0.68[a]	94.45±0.36[d]	91.49±0.34[e]	100.77±0.50[b]	96.55±0.30[c]
TAG (mg/dl)	114.01±3.85[f]	210.70±2.80[a]	156.94±0.82[d]	145.58±1.62[e]	186.26±1.40[b]	173.63±0.69[c]
HDL-C (mg/dl)	57.92±0.72[a]	44.43±0.24[f]	51.19±0.35[c]	54.68±0.40[b]	46.95±0.26[e]	48.97±0.20[d]
LDL-C (mg/dl)	4.95±0.30[f]	21.10±0.25[a]	11.87±0.19[d]	7.69±0.52[e]	16.56±0.14[b]	12.85±0.19[c]
VLDL-C (mg/dl)	22.80±0.77[f]	42.14±0.56[a]	31.39±0.16[d]	29.11±0.32[e]	37.25±0.28[b]	34.72±0.14[c]
FFA (ng/ml)	72.85±0.80[f]	102.71±0.60[a]	87.28±0.42[d]	82.71±0.78[e]	96.14±0.46[b]	91.28±0.42[c]

Means within the same row having different superscript letters are significantly different. Significant at 0.05 probability.

mRNA levels of SREBP-1c and FAS and a marked decrease in mRNA levels of SIRT1 and GLUT-4 compared to normal rats. In contrast, mRNA levels of all the aftermentioned genes were reversed in HFD-fed rats which received either oral carnosine or carnosine-loaded niosome in both co-treatment (14 weeks) and post-treatment conditions (4 weeks). These effects were significantly improved especially in the co-treatment conditions. Moreover, HFD-fed rats treated with oral carnosine-loaded niosome showed the greatest effect in both co-treatment and post-treatment conditions.

Discussion

Obesity is one of the prime components of metabolic syndrome (25). Body weight and body mass index are important anthropometric indices for assessing obesity (26). The oral administration of carnosine-loaded niosome was more effective than oral administration of carnosine in reducing obesity in HFD-fed rats as observed with decreased body weight and body mass index. The current results are in agreement with previous studies which revealed that carnosine administration reduced body weight (7, 27).

Dyslipidaemia was observed with reduced HDL-C, as well as increased VLDL-C, TAG, and LDL-C levels in HFD-fed rats. The changes in lipid metabolism are associated with free fatty acids flux secondary to insulin resistance (28). The oral administration of carnosine-loaded niosome was more effective than oral administration of carnosine in regulation of lipid metabolism. Various studies with carnosine proved altered lipid metabolism (27, 29). There are conflicting

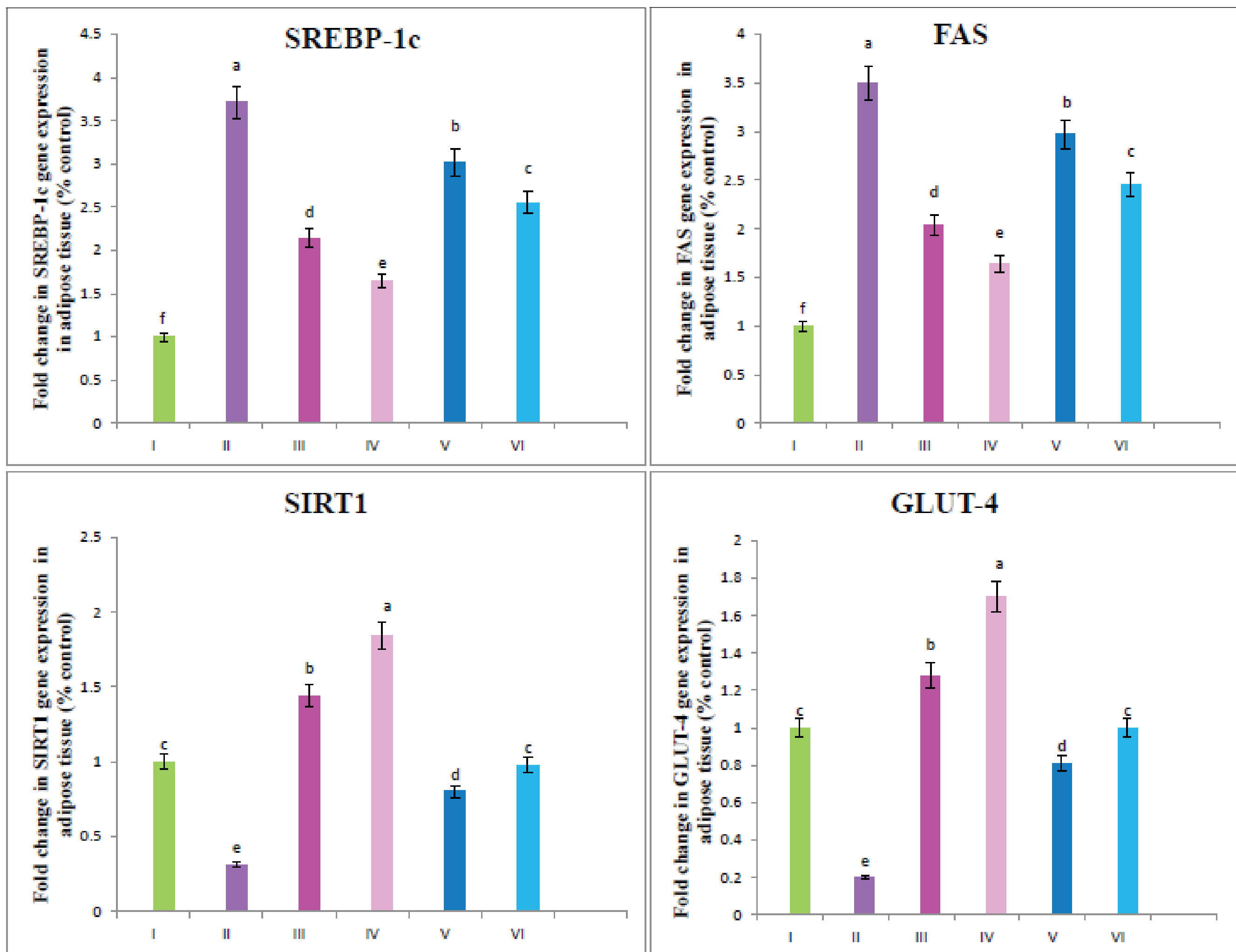

Figure 2: The effect of oral carnosine, and oral carnosine-loaded niosome on the mRNA levels of SIRT1, SREBP-1c, FAS and GLUT-4 in the adipose tissue of HFD-Induced Metabolic Syndrome in Rats (data are presented as mean ± SEM)

studies regarding the effect of carnosine on HDL (29, 30).

To further elucidate the mechanism of the anti-obesity and hypolipidemic effects of either oral carnosine or oral carnosine-loaded niosome in HFD-fed rats, we conducted a real-time PCR analysis in which we compared gene expression profiles of SREBP-1c and FAS of adipose tissue in different treatment groups. SREBP-1c, a lipogenic transcription factor, regulates lipid metabolism in cells (31, 32, 33). In the fatty acid biosynthesis pathway, FAS is the rate-limiting enzyme and its mRNA expression is under control of SREBP-1c (32). In normal tissues and cells, SREBP-1c and its regulated enzymes always have low expression and activity. But in tissues with abnormal lipid metabolism, they are stimulated (34). Our study showed that either oral carnosine or oral carnosine-loaded niosome administration, in particular carnosine-loaded niosome, downregulated the expression of SREBP-1c and FAS, indicating that they could significantly suppress lipogenesis, resulting in their anti-obesity and hypolipidemic effects in HFD-induced metabolic syndrome in rats. A previous study using carnosine also reported Similar observations (35).

Insulin resistance is a key component of MetS. The body fails to response to insulin, resulting in raising the blood glucose levels (36, 37). The oral administration of carnosine-loaded niosome was more effective than oral administration of carnosine in reducing hyperglycemia, hyperinsulinemia and insulin resistance.The hypoglycemic effect of carnosine is in agreement with previous studies (7, 30, 38, 39). In contrast, carnosine administration didn't affect the plasma glucose level (27). The reduction of insulin level and HOMA-IR by carnosine was consistent with previous studies (27, 35). There are conflicting studies regarding the effect of carnosine on insulin. A previous report did not display any significant effect of carnosine on insulin levels (7). Other studies are in contrast with our finding, it confirmed that carnosine did not alter the insulin resistance (40) and also increased plasma insulin levels (39, 41).

To determine the molecular mechanism of hypoglycemic and hypoinsulinemic effect of either oral carnosine or oral carnosine-loaded niosome in HFD-fed rats, we conducted a real-time PCR analysis for gene expression profiles of GLUT-4 of adipose tissue in different treatment groups. GLUT4, an insulin-regulated glucose transporter, is found mainly in adipose, skeletal and cardiac tissues. Insulin stimulates the GLUT4 translocation to the plasma membrane leading to a rapid elevation in the glucose uptake and therefore the decline of blood glucose level (42,43). Our results showed that either oral carnosine or oral carnosine-loaded niosome treatment, in particular carnosine-loaded niosome, significantly increased the expression of GLUT4 indicating that they are able to induce a plasma glucose-lowering effect. Therefore, they enhanced insulin sensitivity in HFD-induced metabolic syndrome in rats. A previous study is in line with our finding, it confirmed that 10 mM carnosine supplementation to glucolipotoxic-media resulted in a significant GLUT4 translocation improvement (44).

SIRT1, a NAD^+- dependent deacetylase, modulates gene expression via deacetylation of histones (45, 46). Various studies of Sirt1 agonists have showed contrasted results in the metabolic syndrome management (47, 48, 49). In high glucose-treated adipocytes, SIRT-1 enhances glucose homeostasis via regulating GLUT-4 proteins (50). SIRT1 depletion in 3T3-L1 adipocytes suppressed GLUT4 translocation and glucose uptake. But, SIRT1 activators administration to the cells led to stimulation of glucose uptake (51). In contrast, the Sirt1 overexpression does not improve IR in skeletal muscle in vivo (52). SREBP-1c is believed to be a direct target of SIRT1 (53, 54, 55). SIRT1 with AMP-activated protein kinase (AMPK) regulated SREBP-1c and FAS expression levels to alleviate NAFLD (56, 57). These data highlight the Sirt1 inhibition as a main component of the molecular pathways that produce the metabolic syndrome outcomes. Remarkably, these favorable effects of either oral carnosine or carnosine-loaded niosome are leading to relieve the metabolic disorders. Resveratrol, a direct SIRT1 activator, and carnosine appear to share many characteristics (anti-oxidants, anti-carcinogenic, anti-inflammatory and platelet anti-aggregation activity) (58). Therefore one has to consider, accepted very speculatively, the potentiality that carnosine may also stimulate SIRT1 in a method analogous to that suggested for resveratrol (59); at the minimum the two molecules are almost the same size (58). There are few studies in the literature examining whether carnosine may alter SIRT1 expression or not. The

pretreatment with carnosine in both ultraviolet -A- and 4-hydroxynonenal-treated fibroblasts restored the SIRT1 expression and prevented the acetylated proteins accumulation (60). In high glucose-induced podocytes, Carnosine upregulated the SIRT1 expression to reduce the glycative and the lipoperoxidative stress (61).

Another prime finding of our report was that encapsulation of carnosine in niosome form is predicted to enhance carnosine's bioavailability, therefore positively impact its therapeutic action. Our study gives for the first time new evidence that the developed carnosine-loaded niosome displayed superior *in-vivo* effectiveness and facilitated the way for a predicted positive effect of niosomes on enhancing carnosine therapeutic efficacy, which could benefit pharmacological studies in the future.

Finally, Carnosine has been characterized as an enigmatic and a forgotten dipeptide (58, 62) and perhaps the mystery surrounding carnosine's "true" action is just a reflection of the truth that there hasn't been a lot of experimental work in some of the areas that have been discussed; much more research is needed to discover whether any of these hypotheses are justified (58).

Conclusion

Our results emphasize the potent efficacy of oral carnosine- loaded niosome in management of metabolic syndrome. Also, this study demonstrates a novel mechanistic insights by which oral carnosine, and carnosine loaded niosome can ameliorate hyperglycaemia, hyperinsulinaemia and insulin resistance via upregulation of GLUT-4 gene expression and also can protect against obesity and dyslipidemia via downregulation of SREBP-1c and FAS gene expression in adipose tissue of HFD-induced metabolic syndrome rats. Oral carnosine and oral carnosine- loaded niosome displayed a multimodal activity through activation of SIRT1, a master metabolic switch. Conclusively, oral carnosine- loaded niosome achieved the maximum therapeutic outcome with no changing in activity. Therefore, Carnosine-loaded niosomes could be a valuable and optimistic treatment choice for metabolic syndrome. Nevertheless, this remains to be further confirmed by clinical studies.

The authors have declared no conflict of interests.

Abbreviations

HFD, high fructose diet; MetS, metabolic syndrome; TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoproteins-cholesterol; TAG, triacylglycerol; VLDL-C, very low density lipoproteins-cholesterol; FFA , free fatty acids; SIRT1, sirtuin-1; SREBP-1c, sterol regulatory element binding protein-1c; GLUT-4, glucose transporter isoform 4; FAS, fatty acid synthase; BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance; AMPK, AMP-activated protein kinase; NAFLD, Non-alcoholic fatty liver disease.

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