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Improved islet function is associated with anti-inflammatory, antioxidant and hypoglycemic potential of cinnamaldehyde on metabolic syndrome induced by high tail fat in rats

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ABSTRACT

Cinnamon is used in traditional medicine and foods. In this study the protective effects of cinnamaldehyde, one of the most abundant compound of cinnamon against metabolic syndrome induced by high fat diet, were investigated. To induce metabolic syndrome, male Wistar rats were given high fat diet for 16 weeks. Cinnamaldehyde was administrated orally (143.8 μmol/kg body weight) concomitant with high fat feed. Changes in islet morphology, lipid profile, TNF-α, TBARS, insulin resistance were analyzed. Metabolic syndrome was induced by high fat diet. Cinnamaldehyde reversed this process and significantly reduced insulin secretion and content in isolated islets of high fat diet. Beta cell enlargement, TNF-α and TBARS significantly increased with high fat diet, cinnamaldehyde restored both to the control level. Cinnamaldehyde prevented all symptoms of metabolic syndrome by improving oxidative and inflammatory conditions in pancreatic islets with no effect on insulin secretion but by enhancing insulin reserve and preventing beta cell damage.

1. Introduction

Metabolic syndrome (MetS) is a multifactorial disease, including a complex of metabolic risk factors for cardiovascular disease and diabetes. The criteria consist of obesity, dyslipidaemia [elevated triacylglycerol (TAG), low high density lipoprotein-cholesterol (HDL)], and hypertension and insulin resistance (hyperglycaemia/hyperinsulinaemia). Studies show that in most countries in the world, 30% of adult populations are suffering from MetS and its prevalence considerably increases with obesity and age (Ervin, 2009). The prevalence of

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obesity is increasing, such that about 500 million adults worldwide are obese. Epidemiological studies have shown that high fat containing diets lead to weight gain and the similar calorie intake from fat compared with protein or carbohydrates is associated with the higher increase in the body weight (Buettner, Scholmerich, & Bollheimer, 2007). Similar to humans, fat rich diets induce obesity and metabolic disorders in experimental animal models. To induce obesity in animal models, numerous high fat diets with different types of fat (plant or animal; with different degrees of unsaturation) with various percent of fat have been used (Lia et al., 2014; Mashmoul et al., 2014). It is estimated that each year about 50,000 tonnes of tail tissue (Alipanah & Emamjomeh Kashan, 2011) are used as a source of fat production. It is therefore important to investigate the suitability of this fat for human consumption. The underlying mechanisms of the condition are various and complicated; MetS and obesity are associated with low grade inflammatory condition (Cano et al., 2009) and oxidative stress also is involved in the pathogenesis of metabolic syndrome (Roberts & Sindhu, 2009). There are various anti-obesity drugs in the market, but most of them affect physiological function by mechanisms regulating body weight and display several side effects. This highlights the importance of functional food for management of metabolic syndrome and associated disorders (Ritesh et al., 2013). Cinnamon in most societies, especially those in Asia is widely used as a spice. Cinnamon essential oil, the main constituent of the plant which makes up 1% of the bark, is widely used in medicine, cooking and cosmetics. Cinnamaldehyde (CNMA) is an oily and yellowish liquid with a warm, sweet odour and pungent taste which is composed of 90% of the oil (Wijesekera, 1978). In traditional medicine, cinnamon is also used for therapeutic purposes. It especially lowers blood sugar without any side effects for years, although some clinical studies have reported conflicting results (Plaisier et al., 2011; Rafehi, Ververis, & Karagiannis, 2012). Prospective randomized controlled trials have shown that consumption of cinnamon did not significantly alter haemoglobin A_{1C} level, fasting plasma glucose, or plasma lipid profiles in type 1 and type 2 diabetes (Baker, Gutierrez-Williams, White, Kluger, & Coleman, 2008). It does not improve whole-body insulin sensitivity or oral glucose tolerance and does not modulate blood lipid profile in postmenopausal patients with type 2 diabetes (Vanschoonbeek, Thomassen, Senden, Wodzig, & van Loon, 2006). To our knowledge so far the effects of CNMA on the islets of pancreas histology and their insulin release and depot have not been reported. In this study we induced metabolic syndrome using high fat diet in rat and have looked for possible protective effects of CNMA against induced MetS.

2. Methods

2.1. Animals and diets

A total of 40 male Wistar rats (12 weeks old, 220–250 g,) were supplied from Pasteur Institute, Tehran, Iran. All experiments were conducted in accordance with standard ethical guidelines and the study was approved by the local ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences (No: 91/08/168). The animals were housed individually per cage (dimensions 45, 25, 15 cm) under standard conditions (12 h light–12 h dark cycle starting at 0700, at 24 °C in a controlled humidity) throughout the experimental period with free access to food and water. After 1 week period of adaptation to laboratory situation, rats were randomly assigned to four different experimental groups (n = 10 per group): Group A; as a control was fed control chow diet, Group B; was fed a semi-purified high fat diet (HFD) consisting of the 25% (w/w) of fat (1% soya bean oil and 24% fat), Group C; was fed a regular chow diet with CNMA and Group D; was fed a HFD with CNMA supplement. Animals were orally given CNMA (W228613, Sigma-Aldrich, Shanghai, China, purity ≥ 95%) 143.8 μmol/kg body weight in corn oil daily, through gavages for the duration of experiment (16 weeks) as reported previously (Gowder & Devaraj, 2006). The control chow diet and HFD were supplied from Javaneh, Khorasan animal food company, Mashhad, Iran. High fat food was prepared freshly and stored at 4 °C during the duration of the experiment (16 weeks). The composition of the control regular chow diet was 20% protein, 1% soybean oil and 14% fibre by weight, while HFD group received high fat food consisting 18% protein, 1% soybean oil, 24% tail fat and 10% fibre by weight. The amount of energy (per 100 g diet), in regular chow and HFD was 1172.3 kJ (280 kcal) and 1716.58 kJ (410 kcal), respectively. The main fatty acid composition of soybean oil was saturated fatty acids (SFA; C16:0, 11.65%; C18:0, 4.45%), 16·1%, monounsaturated fatty acid (MUFA; C18:1, 20.02%; C20:1, 98%) 22.0% and polyunsaturated fatty acid (PUFA; C18:2 n-6, 47.57%; C18:3 n-3, 12.11%) 61.5%. The fatty acid composition of tail fat was analyzed by gas chromatography and included SFA (C14:0, 3.64; C16:0, 22.55; C17:0, 4.10; C18:0, 12.54%) 44.9%; MUFA (C14:1, 1.44; C16:1, 4.18; C17:1, 2.18; C18:1, 41.15%) 51.64%; PUFA (C18:2 n-6, 3.11%; C18:3 n-3, 0.34%) 3.45% and the cis form of fatty acids was 93.82%. A curved stainless steel gavage needle with ball tip was used for gavaged rats and was washed thoroughly and wiped between animals. Oral gavages were performed by skilled personnel so daily CNMA gavages were not stressful in rats that have been acclimated to handling.

2.2. Experimental procedures

2.2.1. Anthropometrical parameters

Body weight and food intake were recorded between 0900 and 1200 during 16 weeks of experiments. Corrections were made for the small quantities (g) of food spilled per cage. Naso-anal length was measured in anaesthetized rats at the end of the study in a ventral position; one observer stretched the animals until snout, spinal column, and middle of the pelvis were in a straight line, while the other observer measured nose-to-anal length by use of a caliper (mm) and then the Lee index was calculated: $\text{Lee Index} = \frac{\sqrt[3]{\text{Weight (g)}}}{\text{Length (cm)}} \times 1000$. The values of Lee Index greater than 310 were considered as obesity.

2.2.2. Measurement of blood pressure (BP)

Blood pressure was determined using a non-invasive tail-cuff method (Narco Bio-Systems, Inc., Houston, TX, USA) in conscious animals at the beginning and at the end of the study. To acclimate, the animals were placed in a constant-temperature (29 ± 2 °C) chamber for at least 15 min on 2–3

separate days. Subsequently, several recordings of BP were made while the animals were relaxed and the mean of the three stable consecutive measures (~1 min apart) was calculated (Bunag, 1973).

2.2.3. *Intraperitoneal glucose tolerance test (IPGTT)*

At the end of the study an IPGTT was performed in conscious animals after overnight fasting (12–14 h). After obtaining the first blood sample from the tail vein at time zero, glucose solution (50% w/v, 0.4 ml/100 g body weight) was administered as an ip injection at a dose of 2 g/kg, and blood samples (0.3 ml each) were taken from the tail vein at 15, 30, 60, 90 and 120 min into heparinized tubes for glucose and insulin measurements (Matthews et al., 1985). Blood samples were centrifuged immediately (3000 g; 10 min, 4 °C) and plasma were then separated and stored at –20 °C until analysis (2 weeks).

2.2.4. *Insulin tolerance test*

Following 3–4 weeks recovery from IPGTT, an ip insulin tolerance test was performed in non fasting state on six animals in all groups. Tail blood samples (100 μl) were obtained before and after 15 and 30 min of an ip bolus injection of regular human insulin (1 unit/kg in 1 ml saline) for glucose measurement. Finally, animals (fasted for 12 h) were anaesthetized by ip injection of sodium pentobarbital (60 mg/kg body weight). Blood samples were collected, and serum was obtained by centrifugation for leptin and lipid analyses. Pancreas from four animals in each group after a medium laparotomy were removed and digested (details are explained later) and then retroperitoneal, epididymal and mesenteric fat tissues were dissected and weighed. Adiposity was calculated from the ratio of the three depot fats to the body weight, and then homeostasis model assessment of insulin resistance (HOMA-IR) $\text{HOMA-IR} = \frac{(\textbf{Glucose}\,(\textbf{mmol/l}) \times \textbf{Insulin}\,(\mu\textbf{U/ml})}{22.5}$ was calculated.

2.2.5. *Islet isolation*

For islet isolation 10 ml ice-cold Hank's balanced salt solution (HBSS) [pH, 7.4; containing NaCl, 136; KCl, 5.36; $CaCl_2$, 1.26; $MgSO_4$ $7H_2O$, 0.8; Na_2HPO_4 $2H_2O$, 0.33; KH_2PO_4, 0.44; $NaHCO_3$, 4.16 all in mM (Merck, Darmstadt, Germany)] containing 0.5 mg/ml of collagenase P (Roche, Cat. # 1213, Penzberg, Germany) was injected into the pancreatic duct, and then the islets were removed, transmitted to a 50 ml falcon tube and digested in a 37 °C water bath for 15 min (Farrokhfall, Seyed Hashtroudi, Ghasemi, & Mehrani, 2014). Digestion was terminated by adding ice-cold HBSS and the tube was shaken for 1 min. After two washes with cold HBSS, the suspension was filtered through a 500 μm plastic mesh to discard any undigested tissue particles. Following one additional washing, the islets were hand-picked under a stereomicroscope.

2.2.6. *Glucose stimulated insulin secretion (GSIS) and islet insulin content*

For evaluation of insulin secretion in vitro, batches of five islets (three replicas for each condition from four animals) were transferred into 1.5 ml plastic tubes, containing 1 ml of Krebs–Ringer solution [(pH, 7.4); NaCl 115; KCl 5; $MgCl_2$ $6H_2O$ 1; $CaCl_2$ 2.5; $NaHCO_3$ 24 (Merck); 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 16 (Sigma, St. Louis, MO, USA) all in mM] and 5 g/dl BSA (Fluka, St. Louis, MO, USA). Then different amounts of glucose were added into four separate tubes, in which the final glucose concentrations were 2.8, 5.6, 8.3, 16.7 mmol/l. Then, all tubes were incubated for 60 min in 37 °C water bath, and gassed with 95% O_2:5% CO_2 for 5 min at the beginning. All samples were centrifuged at 500 g (14 g), 2 min and then aliquots of supernatant were collected and stored at –20 °C for insulin determination.

Groups of 10 islets were extracted overnight at 4 °C in 1 ml of acid/ethanol for measurement of their insulin content (Welsh & Sjoholm, 1988).

2.3. *Biochemical analyses of serum parameters*

Serum concentrations of total cholesterol (TC), HDL and TAG were determined using standard laboratory techniques (Zistchem Co., Tehran, Iran). Plasma concentrations of insulin and leptin and TNF-α were measured using the ELISA procedures (insulin, Mercodia, Sweden; leptin, Causobio, Japan; and TNF-α, Diaclone, Besançon, France) and glucose concentration was measured using glucose oxides procedure (Pars Azmoon Co., Tehran, Iran) respectively. Low density lipoprotein-cholesterol (LDL) was calculated using Friedewald equation.

2.4. *Thiobarbituric reactive substances (TBARS) assay*

The levels of thiobarbituric acid-reactive substances, as by-product of lipid peroxidation, were determined in samples. The analysis was performed according to the previously described method (Mihara & Uchiyama, 1978), based on the reaction with thiobarbituric acid in acidic pH at 90–100 °C. The absorbance of the resulting pink product was measured spectrophotometrically at 535 nm. Briefly, 0.5 ml serum was added to a reacting solution containing trichloroacetic acid 10% (v/v) and TBA reagent 0.67% (w/v) in separate capped plastic tubes and incubated in boiling water bath for 45 min. After cooling to room temperature, 1 ml of *n*-butanol was added to each tube and vortexed. Following centrifugation the upper butanol layer was separated and absorbance was measured using a spectrophotometer at 532 nm. 1, 1, 2, 3-tetraethoxypropane, as MDA precursor, (1–20 μmol/l) was used as a standard. The levels of TBARS were calculated from the calibration curve with malonaldehyde bis(dimethyl acetal) used as standard and expressed in MDA equivalents. For islet TBARS measurement, 200 islets in 200 μl buffer were sonicated and then supernatant was separated and assayed as explained above.

The intra-assay coefficients of variation for leptin, insulin, TNF-α, MDA, glucose, serum TAG, TC and HDL were 9.23, 8.42, 6.23, 5.67, 2.5, 3.79, 3.64 and 3.41%, respectively. The inter-assay coefficients of variation for insulin and glucose were 11.85% and 4.35%, respectively. Detection limits for plasma insulin, leptin and TNF-α were 0.15 μg/l, 0.078 ng/ml and 15 pg/ml, respectively. All of the measurements were performed in triplicate.

2.5. *Histology*

Whole pancreas (n = 3 rats in each group) were carefully dissected and fixed in 10% buffered formalin. Paraffin blocks that

consist of two 1 cm pieces; one from the tail (mesenteric end) and the other from the head (splenetic end) of pancreas, were sectioned (3 μ thick) and mounted on glass slides. Sections were deparafinized and boiled for 6 min in 10 mM citric acid (pH 6.0) for antigen retrieval. Then, subsequently incubated with primary antibody (Catno 1542, DAKO Diagnostics, Carpinteria, CA, USA) and with reagent alone (as negative control) for 60 min at room temperature followed by biotinylated secondary antibody (DAKO Diagnostics) for 30 min. Colour development was performed with DAB (DAKO Diagnostics) according to the manufacturer's protocol. For morphometric analysis, three sections with 100 μm distance were taken from each paraffin block. Images of all insulin immunoreactive stained islets were captured by a colour video camera attached to a light microscope and the islet areas were measured. Islet area was calculated by Image J software. β-Cell number per islet was achieved by counting the number of cell nuclei within the insulin immunoreactive islet area. β-Cell size was calculated by dividing the insulin immunoreactive area on β-cell number per islet (Harndahl et al., 2004).

2.6. Statistical analysis

All analysis were validated by D'Agostino and Pearson (omnibus K2 test performed with Prism version 5) normality test. All data are expressed as mean ± SEM of triplicate experiments and data on IPGTT and ITT were analyzed by two-way ANOVA and the rest by one way ANOVA using GraphPad Prism software (Version 5). The glucose and insulin concentration was assessed by calculating the area under curve (AUC) of IPGTT. Two-way and one way ANOVAs were followed by Bonferroni post-hoc tests. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Biometrical data

Throughout the study all animals were alive without any fatality. Caloric consumption in the HFD and HFD + CNMA groups was significantly higher than those of the control and control with CNMA groups (Table 1, $P < 0.001$).

Initial mean body weight was similar in all animals (243.15 ± 0.775). During the experiment the body weight in all groups were significantly increased, especially in the HFD. At the end of the 16th week, the BW increment in groups containing high fat was significantly higher than those in control and control with CNMA groups (Table 1, $P < 0.001$). Cinnamaldehyde consumption significantly reduced body weight in HFD animals (Table 1, $P < 0.05$). The adiposity was increased in the HFD compared to the other experimental groups. The high fat diet significantly increased body lipid index compared with the control and the CNMA groups ($P < 0.01$, Table 1). Cinnamaldehyde consumption reversed above parameters and caused a significant decline in adiposity in HFD groups ($P < 0.05$, Table 1).

3.2. Plasma levels of leptin, TNF-α, TBARS, lipid profile and islet TBARS

As presented in Table 1, no significant differences were observed in serum levels of leptin, TC, TAG, HDL-C and LDL-C among the experimental groups. Fat feeding significantly increased serum TNF-α ($P < 0.05$, Table 1) in HFD group, compared to other experimental groups. Conversely, the concurrent use of CNMA with tail fat diet decreased TNF-α level in this

Table 1 – Anthropometrical parameters, plasma leptin, TNF-α, MDA and lipid profiles of rats in different experimental groups.

Variable	Groups			
	Control Mean ± (SEM)	Control + CNMA Mean ± (SEM)	HFD Mean ± (SEM)	HFD + CNMA Mean ± (SEM)
Energy consumption (kcal/day)	61.349 (1.36)	61.25 (0.77)	68.6 (1.84)***	67.94 (1.67)†††
Body weight gain (g/16 week)	119.2 (7.45)	110.2 (6.10)	165.4 (7.20)***	137.8 (7.39)†††,‡
Adiposity (% BW)	2.75 (0.24)	2.74 (0.19)	4.22 (0.34)***	3.2 (0.24)‡
Lee index	309.5 (1.14)	304.1 (1.74)	321.14 (1.54)***	311.5 (2.46)‡‡‡
Plasma leptin (ηg/ml)	1.03 (0.05)	0.97 (0.05)	1.25 (0.10)	1.04 (0.12)
Plasma TNF-α (pg/ml)	9.06 (2.40)	7.86 (2.13)	20.09 (1.60)***	8.22 (3.31)‡
Plasma TBARS (μmol/l)	5.20 (1.06)	4.50 (1.09)	11.55 (0.41)***	8.20 (0.22)‡
Islet TBARS (μmol/l)	ND	ND	0.105 (0.076)	ND
Lipid profile				
TC (mg/dl)	75.65 (2.05)	74.4 (1.38)	80.59 (2.0)	80.83 (1.34)
TAG (mg/dl)	118.0 (3.23)	119.64 (3.44)	118.90 (2.38)	121.4 (2.66)
HDL (mg/dl)	41.45 (0.35)	42.99 (0.76)	43.67 (0.62)	43.42 (0.44)
LDL (mg/dl)	11.19 (2.45)	7.88 (2.03)	12.23 (2.80)	11.17 (2.1)

Statistical comparison between groups was made using a one way ANOVA followed by a Bonferroni post-hoc test, values are mean ± SEM, n = 10 each group.
*** $P < 0.001$, statistically significant differences between different diets (Control and HFD).
††† $P < 0.0001$, statistically significant differences between different diet and treatment (Control and HFD + CNMA).
‡ $P < 0.05$, ‡‡‡ $P < 0.0001$, statistically significant differences between different treatments (HFD + CNMA and HFD) in the outlined sites. Sampling was conducted on fasting.
ND, not detected.

Table 2 – Haemodynamic factors in the experimental groups.

Groups				
Variable	Control	Control + CNMA	HFD	HFD + CNMA
BP[16] (mmHg) Mean (SEM)	106.65 (2.22)	104.86 (2.38)	125.45 (3.95)	117.17 (2.45)
PR[16] (pulse/min) Mean (SEM)	367.6 (8.13)	365 (9.96)	374.3 (4.62)	366.5 (6.47)
BP raise (mmHg) Mean (SEM)	2.66 (0.95)	0.26 (0.12)†††	21.86 (2.39)***	7.66 (2.57)‡

Statistical comparison between groups was made using a one way ANOVA and followed by a Bonferroni post-hoc test, n = 10 each group, values are means ± SEM.

††† $P < 0.001$, statistically significant differences between different diet and treatment (HFD and Control + CNMA).

*** $P < 0.001$, statistically significant differences between different diets (Control and HFD).

‡ $P < 0.05$ statistically significant differences between different treatments (HFD + CNMA and HFD) in the outlined sites. [16] At the end of study (week 16).

animal's group ($P < 0.05$). TBARS level significantly increased in the HFD group ($P < 0.001$, Table 1) and CNMA restored it to the control levels ($P < 0.05$). Islet TBARS level in HFD was 0.105 µmol/l but was not detectable in other groups (Table 1). The amount of increase in TBARS is proportional for lipid peroxidation. This means that decrease in TBARS in the presence of cinnamaldehyde is related to its antioxidant activity.

3.3. *Blood pressure*

The average of initial BP in all animals was 104.33 (2.38). High-fat feeding for 16 weeks increased BP by approximately 22 mmHg. The increase of BP in the HFD group compared with the control groups was significant ($P < 0.001$, Table 2). Cinnamaldehyde consumption significantly lowered BP, especially in HFD animals ($P < 0.05$, Table 2).

3.4. *Plasma glucose and insulin concentrations in response to intraperitoneal injection of glucose*

Glucose tolerance test results for all groups are shown in Fig. 1. Fasting plasma glucose (FPG) levels in the HFD group were significantly higher than the control and CNMA fed groups (min 0 FPG: 185.38 ± 19.38 in HFD vs. 108.58 ± 3.2, 107.71 ± 2.97 and 114.08 ± 4.18 mg/dl in the control chow diet, the control chow diet with CNMA and HFD with CNMA respectively, $P < 0.001$). After administration of glucose, the HFD rats exhibited impaired glucose tolerance during the test. In HFD rats, mean plasma glucose concentrations were significantly higher throughout the IPGTT, compared to the control and CNMA groups (min 15 364.06 ± 25.74 in HFD vs. 220.92 ± 15.91, 226.94 ± 8.53 and 270.78 ± 16.07 mg/dl in control, control with CNMA and HFD with CNMA respectively, $P < 0.0001$). Cinnamaldehyde consumption in HFD animals for 16 weeks significantly decreased mean plasma glucose concentration (Fig. 1a) after glucose administration in all time intervals (min 30: 285.45 ± 8.19 vs. 364.07 ± 21.51 mg/dl, $P < 0.01$; min 60: 255.95 ± 6.72 vs. 323.10 ± 18.72 mg/dl, $P < 0.01$; min 90: 229.8 ± 7.62 vs. 306.14 ± 18.39 mg/dl, $P < 0.01$; min 120: 215.46 ± 8.38 vs. 300.01 ± 25.23 mg/dl, $P < 0.0001$ vs, HFD). However, CNMA consumption in high fat diet group was unable to bring the glucose concentration to the control level ($P < 0.05$, Fig. 1a). In HFD rats the AUC for plasma glucose concentration during IPGTT was

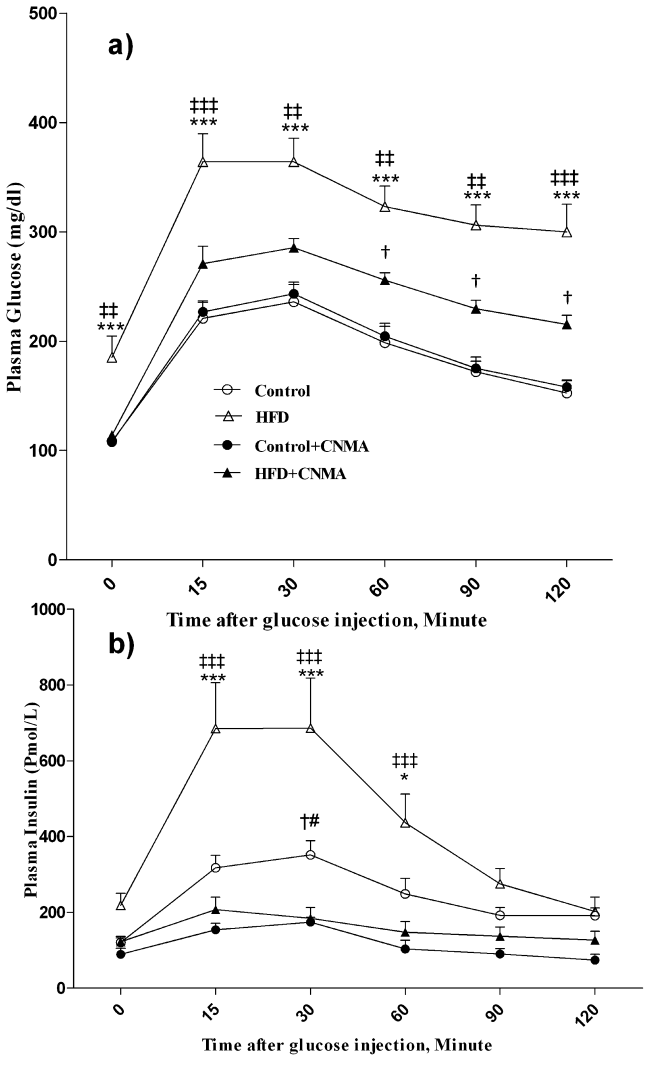

Fig. 1 – Intraperitoneal glucose tolerance test in animals: (a) Plasma glucose concentration and (b) insulin level. Values are mean ± SEM. Statistical comparison between groups was made using two way ANOVA and followed by a Bonferroni post-hoc test, n = 10 each group; HFD vs control (*$P < 0.05$, ***$P < 0.001$), HFD + CNMA vs control (†$P < 0.05$), HFD vs HFD + CNMA (‡‡$P < 0.01$, ‡‡‡$P < 0.001$) and Control + CNMA vs Control (#$P < 0.05$). The experiment was conducted on fasting.

Table 3 – Variations of plasma glucose and insulin concentrations during IPGTT in different experimental groups.

Groups				
Variable	Control (Mean ± SEM)	Control + CNMA (Mean ± SEM)	HFD (Mean ± SEM)	HFD + CNMA (Mean ± SEM)
Glucose AUC (mmol/l/120 min)	1265 (85.76)	1302 (43.02)	2132 (128.6)***‡‡‡	1618 (46.33)†
Insulin AUC (Pmol/l/120 min)	30.59 (2.84)	13.84 (1.64)#	51.75 (7.64)**‡‡‡	18.63 (2.51)
HOMA IR index	3.92 (0.44)	3.91 (0.75)	16.9 (1.8)***‡‡‡	4.65 (0.87)

Statistical comparison between groups was made using a one way ANOVA and followed by a Bonferroni post-hoc test, n = 10 each group, values are means ± SEM.

† $P < 0.05$, statistically significant differences between different diet and treatment (Control and HFD + CNMA).

** $P < 0.01$, *** $P < 0.001$, statistically significant differences between different diets (Control and HFD).

‡‡‡ $P < 0.001$, statistically significant differences between different treatments (HFD + CNMA and HFD).

# $P < 0.05$, statistically significant differences between different treatments (Control + CNMA and Control) in the outlined sites.

significantly higher as compared to the other groups ($P < 0.0001$, Table 3).

As shown in Fig. 1b, basal insulin level in the HFD animals was higher than those of other experimental groups but not significant (min 0 insulin: 120.82 ± 12.15, 218.7 ± 31.57, 122.6 ± 13.51 and 97.21 ± 13.2 pmol/l in control chow diet, HFD, HFD with CNMA and control chow diet with CNMA, respectively). Although in high fat consumed animals, the means plasma insulin concentrations were higher throughout the IPGTT compared to the other groups, the differences were significant only at 15, 30 and 60 min. Cinnamaldehyde consumption with high fat feeding lowered plasma insulin level [min 15 insulin: 207.9 ± 32.42 vs. 685.4 ± 120.5 pmol/L, $P < 0.0001$; min 30 insulin: 184.4 ± 27.83 vs. 686.32 ± 131.96 pmol/L, $P < 0.0001$; min 60 insulin: 147.4 ± 28.58 vs. 436.67 ± 75.16 pmol/L, ($P < 0.05$; Fig. 1b)]. Cinnamaldehyde consumption decreased plasma insulin level, and a significant difference was prominent in min 30 compared to the control group (Fig. 2). The AUC of the plasma insulin concentration of HFD group was significantly higher compared to the other groups. Cinnamaldehyde significantly decreased plasma insulin level in control + CNMA and HFD + CNMA groups compared to control and HFD groups (Table 3).

3.5. *Plasma glucose concentration in response to intraperitoneal injection of insulin*

Non-fasting plasma glucose in HFD feeding group was significantly increased compared to the control and CNMA groups ($P < 0.05$ and $P < 0.01$ respectively; Fig. 3). Insulin administration caused decline in plasma glucose levels in all groups, but hypoglycaemia in the other groups was significantly higher than the HFD group ($P < 0.01$).

3.6. *Homeostasis model assessment of insulin resistance index*

The HOMA-IR index of the HFD group was significantly higher than those of other experimental groups ($P < 0.001$, Table 3). These results show that HFD induced insulin resistance but CNMA improved insulin sensitivity ($P < 0.001$, Table 3).

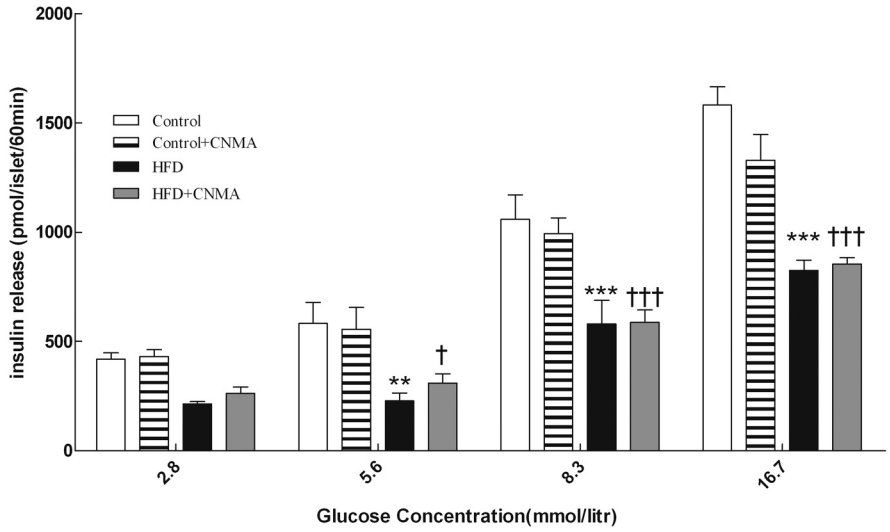

Fig. 2 – GSIS assay on isolated islets from experimental groups. Insulin release was measured during 1 hour from groups of five islets at increasing glucose concentrations (2.8–16.8 mmol/l) after overnight fasting. Values are mean ± SEM for eight cups (two cups each containing five islets for each condition from each animal; four animals in each group); HFD vs Controls ($P < 0.01$, ***$P < 0.001$), HFD + CNMA vs control (†$P < 0.05$, †††$P < 0.001$).**

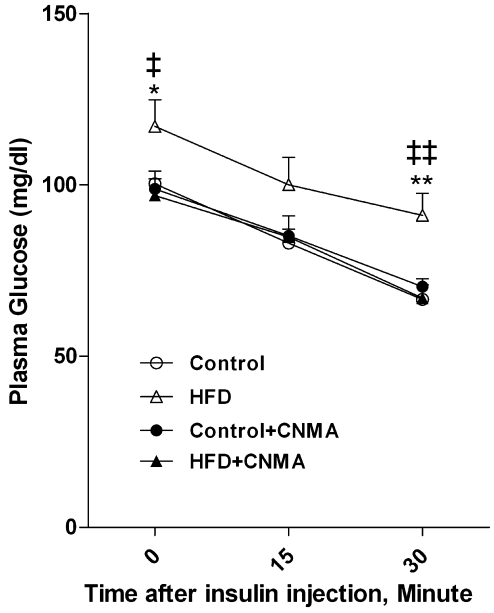

Fig. 3 – Insulin tolerance test in experimental groups. Plasma glucose concentration during insulin tolerance test in experimental groups in feeding status. Statistical comparison between groups was made using a two way ANOVA and followed by a Bonferroni post-hoc test, n = 6 each group; HFD versus control (*P < 0.05, **P < 0.01), HFD vs HFD + CNMA (‡P < 0.05, ‡‡P < 0.01).

3.7. *Insulin content and secretion from the isolated islets*

As evident from Fig. 2, in the HFD rats, the GSIS of isolated islets in all concentration of glucose (2.8, 5.6, 8.3 and 16.7 mmol/l) was significantly reduced, compared with the control and control + CNMA group. However, in the HFD + CNMA group, insulin secretion when stimulated with 8.3 mM of glucose was significantly lower compared to the control groups. Conversely, the insulin secretion from islets of this group was severely decreased when stimulated with supra physiological concentration of glucose (16.7 mmol/l). The results show that in both control groups (control and control + CNMA) insulin secretion from islets in response to all concentrations of glucose were similar (Fig. 2).The results in Fig. 4 shows that insulin content of islets from fat feeding animals compared to the controls group was significantly reduced ($P < 0.001$) and CNMA consumption significantly increased the content of insulin in the HFD group ($P < 0.0$).

3.8. *Immunohistochemical staining of pancreas*

Immunohistochemical staining of pancreas by insulin antibody was used to observe the density of islet and possible intensity of insulin content in the islets. The immunostaining section of pancreas did show any significant differences in insulin contents in islets among the different experimental groups, but mean of β-cell area was significantly increased with high dietary fat (Fig.5a , $P < 0.05$) compared to other groups. Cinnamaldehyde consumption with high tail fat prevented the enlargement of the β-cell area and maintained it to control level. Intake of CNMA had no effect on mean islet area in the control chow diet animals. β-cell size markedly increased in high fat feed animals compared to the control groups (Fig. 5b, $P < 0.001$) and CNMA ingestion significantly decreased β-cell volume (Fig. 5b, $P < 0.001$).

4. Discussion

The results of this study showed that high tail fat feeding can induce certain signs of metabolic syndrome, such as obesity and insulin resistance, as well as high blood pressure. Basal insulin and glucose level also increased in high tail fat fed animals. Conversely, islet insulin secretion and islet insulin content both were significantly decreased with high tail fat feeding. Cinnamaldehyde consumption compensated the abnormal responses to glucose challenge test and decreased plasma glucose and insulin to nearly normal values. To our belief, these are the first results showing that the CNMA consumption had no incremental effect on islets insulin secretion while causing increase in islet insulin content. To study the possible protective effect of CNMA on islets of Langerhans in experimental metabolic syndrome, a preliminary investigation has been conducted using tail fat which revealed the significant elevation in insulin content along with marked reduction of TNF-α and MDA levels in serum and islets.

Based on recent studies, to create a model for metabolic disorders of human obesity particularly insulin resistance in rats, the use of HFD is recommended (Buettner et al., 2007). In this study, tail fat – the main constituent of Iranian fat diet – is used. Wistar rats fed with animal fats are more appropriate for this

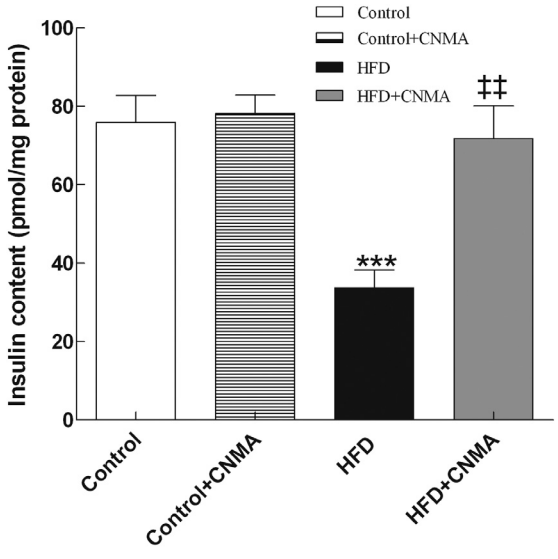

Fig. 4 – Insulin content of islets in experimental groups. Insulin content was measured in groups of 10 islets. Statistical comparison between groups was made using a one way ANOVA and followed by a Bonferroni post-hoc test, n = 7 each group HFD vs Controls (*P < 0.001), HFD + CNMA vs HFD (††P < 0.01).**

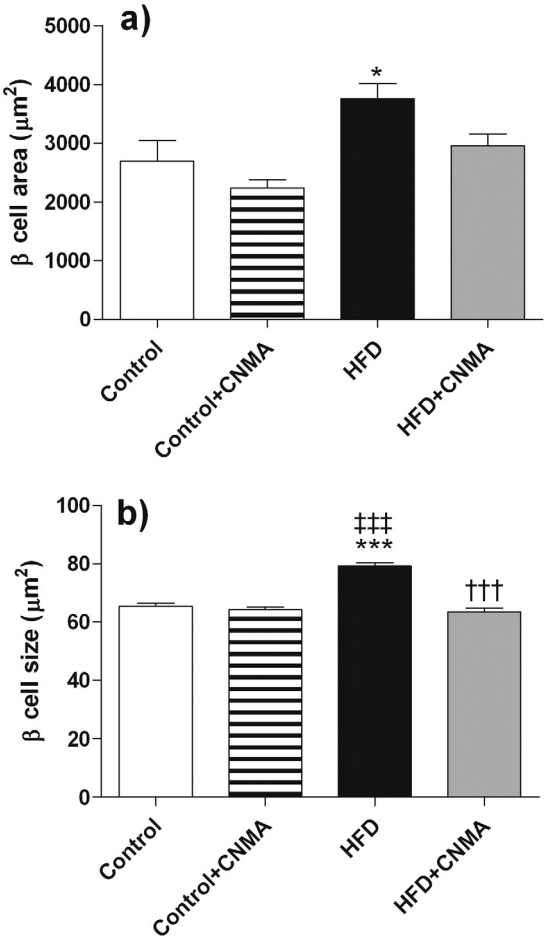

Fig. 5 – Effect of high tail fat feeding and Cinnamaldehyde consumption on islet morphology. Beta cell area (a) and beta cell size (b) in islets from rats fed either a control or a high-fat diet for 16 weeks; starting dieting at 12 weeks of age. All islets in three sections of two different parts of pancreas were examined in three rats from each group. Results are reported as means ± SEM. HFD vs control (*$P < 0.05$, ***$P < 0.001$), HFD + CNMA vs control (†††$P < 0.001$), HFD vs HFD + CNMA (‡‡‡$P < 0.001$).

propose (Buettner et al., 2007). In this animal model, both duration of HFD consumption and the age of the animal are important. Body weight and food intake progressively increase in young animals and it has been shown that the adult rats fed with high-fructose diet produce signs of metabolic syndrome, but young rats do not (de Moura, Ribeiro, de Oliveira, Stevanato, & de Mello, 2009). In this study, adult rats (12 weeks) were used. Rats fed with high fat consumed significantly less food than the other groups although they had higher energy intake compared to the control groups (Table 1). Palatability, energy density and a weak satiety signal from fatty diets have been suggested to play a role in higher energy intake (Hariri, Gougeon, & Thibault, 2010; Hariri & Thibault, 2011). Comparing the Lee index, the animals fed with HFD had a high weight gain than those on the control diet and this was further confirmed by adiposity results (Table 1). Study has shown that SFA is the most obesogenic (Kien, Bunn, & Ugrasbul, 2005) and the tail fat has high saturated fat content (49% and ≈25% food calorie). Concomitant CNMA ingestion with tail fat feeding could significantly lower body weight and adiposity index, indicating that CNMA inhibits adipogenesis. In this study, there was no significant increase in serum leptin after high-fat feeding. It is proposed that serum leptin level is not affected by foods rich in SFA such as tail fat as previously confirmed (Okere et al., 2006).

In relation to lipid abnormalities we could not find any significant dyslipidaemia in our experimental groups. Similar results have also been reported in other studies using tallow and lard (Ryu & Cha, 2003; Zaman et al., 2011). Conversely, Touati et al. (2011) and Ryu and Cha (2003) reported an increase in LDL-cholesterol and a decrease in HDL-cholesterol at the end of a HFD consumption period; this may be due to low cholesterol levels in our high fat diet food (0.1%). There were no changes in basal concentration of plasma TAG in our study. This is not consistent with the decreased insulin sensitivity possibility because of species difference. The mechanisms underlying this species difference are complicated. It has been confirmed that dissociation between the peripheral and hepatic resistance to the metabolic insulin effects and sustained sensitivity to the transcriptional insulin effects/liposynthetic effect in liver (de novo lipogenesis in liver), is the major determinant of this discrepancy.

In our study, HFD feeding also increased blood pressure which is in agreement with that reported by Yoshioka et al. (2000) that chronic feeding with lard increased BP in rats. Abnormality of carbohydrate metabolism is associated with high blood pressure in this experimental study; the study in the absence of dyslipidaemia, insulin resistance in vascular tissues is the major factor for hypertension. Cinnamaldehyde digestion significantly reduced BP. Considering that pulse rate was similar in all groups and that insulin resistance was improved in HFD + CNMA group, it seems likely that CNMA may improve peripheral action of insulin by its antioxidant activity and inhibit the development of vascular resistance.

In this experiment, the HFD animals had impaired glucose tolerance test. There are two possibilities for this observation. First, the high level plasma glucose may be due to insulin resistance in peripheral tissues and second, high fat diet has created lipotoxicity and decreased insulin secretion. To further explore the former possibility, HOMA-IR index (in fasting state) and plasma insulin during IPGTT and insulin tolerance test (in postprandial period) were performed. HOMA-IR index and insulin tolerance test showed higher insulin resistance in both situations. To assess the insulin status, the islet insulin content and islet insulin secretion histological study were investigated. Islet insulin content was significantly decreased in high fat animals and CNMA consumption markedly increased insulin contents and restored it to control level (Fig. 4). Islet insulin secretion in different concentration of glucose was significantly reduced in high fat feed group. Immunohistochemistry obviously showed increase in β-cell volume that improved with CNMA consumption (Fig. 5), this is in accordance with insulin resistance. Ahren, Ahren, and Wierup (2010) have previously shown similar results with 60% diet from fat for 3 months in mice. It is known that beta cells adapt to an

increased insulin demand such as insulin resistance condition by high insulin secretion via improvement of beta cell secretary function and/or increased beta cell volume/number (Ahren & Pacini, 2005). According to decompensation against insulin resistance and decrease in islet insulin secretion in HFD rats, the complete compensation was not achieved in beta cell secretary function, but only the structural compensation was applied. Cinnamaldehyde reversed the histological changes in islets and prevented beta cell damag.

According to these results, it is proposed that, in this model of metabolic syndrome, insulin sensitivity and insulin secretion were reduced. Variable effects of a high-fat diet on insulin secretion have been reported in the literature (Ahren et al., 1999; Lu et al., 2012); the discrepancies may be explained by variations in the diet composition and or in the balance between the effects of lipids on insulin sensitivity or on insulin secretion. Other documented studies have observed an increase in plasma insulin levels, which is attributed to insulin resistance in rats fed a high-fat diet (Jobgen et al., 2009; Zaman et al., 2011) and is in agreement with our results. This study and the others have shown that CNMA has hypoglycaemic effect in insulin resistance and streptozotocin-induced diabetic rats (Couturier et al., 2010; Jia et al., 2009). In this study, compared to the study of Jia et al. (2009), the plasma insulin was not increased with CNMA consumption. This controversial result may be due to different kinds of experiments where they used type 1 diabetes model. Based on the absence of an increase in islet insulin secretion it is suggested that CNMA may have an insulin like effect and hence increase insulin sensitivity; therefore, the requirement of insulin secretion may be diminished and the islet capacity to insulin secretion is preserved (protective effect on islet). Plasma/islet TBARS and plasma TNF-α levels were reduced in CNMA treated HFD rats, indicating decreased lipid peroxidation and general inflammation. Previously it was reported that CNMA decreased plasma TBARS and increased glutathione in plasma of streptozotocin diabetic rats (Kumar, Vasudeva, & Sharma, 2012). Wang et al. (2014) showed that high level of TBARS (5–10 μM, it is the same as plasma TBARS of HFD animals in the study) promoted insulin secretion. Therefore, peroxidation of lipids is thought to play a crucial role in hyperinsulinaemia associated with insulin resistance and its normalization by CNMA consumption. It is known that macrophages, adipocytes and even purified pancreatic islets are a potent source of TNF-α and this cytokine induces insulin resistance and decreases islet insulin secretion (Zhang & Kim, 1995). It has been shown that CNMA suppresses the production of TNF-α from LPS stimulated human blood monocytes/macrophages in culture medium (Chao et al., 2008). Our results show that CNMA decreases TNF-α level and this might improve insulin resistance and carbohydrate metabolism in HFD animals. On the other hand TBARS and TNF-α represent cause and effect on islet toxicity (Rabinovitch, Suarez, Thomas, Strynadka, & Simpson, 1992) and CNMA can reduce both compounds. Therefore it is suggested that the protective effect of CNMA on islet might be due to inhibition of cytotoxic action of cytokine, free radical production and lipid peroxidation. However, the decreased secretary responsiveness of the islets in HFD + CNMA animals may be linked to the adaptation of the islets to low insulin secretion in vivo.

5. Conclusion

These results for the first time revealed that high tail fat feeding (25%, 16 weeks) effectively induced metabolic syndrome (especially its hallmark "insulin resistance") in rats and facilitating glucose metabolism of CNMA is not associated with increase of islet insulin secretion and enlargement of beta cell area/volume. In addition, protective effect of CNMA on islets is achieved by inhibition of islet oxidant and inflammatory toxicity and by reducing the workload of the pancreas. Therefore due to a wide traditional use of cinnamon, it can be recommended for pancreas health and prevention of metabolic syndrome in susceptible individuals.

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