

# Thiamine reduced metabolic syndrome symptoms in rats via down-regulation of hepatic nuclear factor-k $\beta$ and induction activity of glyoxalase-I

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## ABSTRACT

**Objective(s):** Metabolic syndrome (MS) is a cause of death worldwide. The hepatic nuclear factor- NF-k $\beta$  (NF-k $\beta$ ) is the cardinal player of hepatic homeostasis, insulin sensitivity, and lipid metabolism. Thus, we investigated the effect of thiamine on hepatic gene expression of NF-k $\beta$  and its levels of activators in MS rats.

**Materials and Methods:** Male Wistar rats were randomly divided into 4 equal groups (ten rats in each group): normal, MS, and two alike groups under thiamine treatment. MS was induced in rats with a high sucrose solution (40 % in drinking water) for 4 months. Treated groups of rats received 0.18 % of thiamine daily in drinking water. Hematoxylin-Eosin stains were employed to determine the histopathological changes of the liver. Metabolic profile, glycation products, oxidative stress, inflammatory markers, the activity of glyoxalase-I, as well as NF-k $\beta$  hepatic expression of all rat groups, were determined.

**Results:** Acute hepatitis was not observed in the livers of the thiamine treated MS rats. Besides, the treatment showed an advantageous effect on glucose, lipid metabolism, and body weight via down-regulation of hepatic NF-k $\beta$  and induction of glyoxalase system activity. Furthermore, the treatment decreased diverse glycation, oxidative stress, and inflammatory markers ( $P > 0.001$ ).

**Conclusion:** Thiamine decreased body weight and improved metabolism and activity of glyoxalase-I in MS rats with anti-glycation, antioxidant, and anti-inflammatory activities. Further, the treatment had a hepatoprotective effect via reduction of NF-k $\beta$  signaling.

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## Introduction

Metabolic syndrome (MS) or insulin resistance syndrome is a cause of death worldwide (1-3). MS is marked by insulin resistance, abdominal obesity, dyslipidemia, increased blood pressure, hypercholesterolemia, and a pro-inflammatory state (2). Obesity is one of the primary risk factors for MS and type-2 diabetes. Hepatic inflammation or metabolic inflammation participates in insulin resistance, MS, type-2 diabetes, cardiovascular disease, and neurocognitive dysfunction. The hepatic nuclear factor- NF-k $\beta$  (NF-k $\beta$ ) is the cardinal player of hepatic homeostasis, stress responses (4), and insulin sensitivity. Free fatty acids (FFAs), early to end glycation products, cytokines, and oxidized LDL activate the NF-k $\beta$  pathway leading to insulin resistance, glycemia, and dyslipidemia (5, 6). Obesity and inflammation could be successive causative factors, and using anti-inflammation compounds is an effective strategy to oppose obesity and MS (7, 8).

Early, intermediate, and end glycation products as sources of oxidative stress and inflammation contribute to reduction of insulin secretion, insulin resistance, and vascular complications (9-11). Methylglyoxal (MGO) as an intermediate glycation product is a potent intracellular glycating agent that forms advanced

glycation endproducts (AGEs). The level of sera AGEs in overweight people is higher than normal and a risk factor for MS (12). The activity of the glyoxalase system is the principal defense against the accumulation of MGO ameliorating pathological conditions like insulin resistance (13, 14) and diabetes. Thus, recently reduction of glycation products and induction of activity of the glyoxalase system as an effective treatment strategy for vascular complications is considered (10, 15).

Thiamine (vitamin B1) is essential for ATP, certain neurotransmitters, nucleic acids, lipids, amino acids, steroids, and glutathione synthesis. Further, it is vital for metabolism (glucose and lipid) and normal organ function. It has antioxidant, anti-glycation, and anti-inflammatory activities (10). Thiamine deficiency is common in MS (16). It causes hyperglycemia, insulin resistance, (10) hypertension (17), oxidative stress, and inflammation, as well as elevation of glycation products (10). Based on literature review, there is evidence only about the effect of thiamine in the rat model of obesity on body weight and metabolism disorders (18). Thus, in this study, we investigated for the first time the effect of thiamine on hepatic expression of nuclear factor-k $\beta$  (NF-k $\beta$ ), and the activity of glyoxalase-I (Glo-I) in the

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MS rat model. Furthermore, the effect of treatment on glucose and lipid metabolism and glycation, oxidative stress, and inflammatory markers were studied.

## Materials and Methods

### Materials

All materials were of analytical grade and purchased from Sigma or Merck Chemical Companies.

### Rat model of metabolic syndrome

Male Wistar rats weighing  $160 \pm 10$  g, were purchased from the Faculty of Veterinary Medicine, Tehran University, Iran. Animals were kept under controlled conditions with free access to food and water. This study was approved by the Ethics Committee of Ardabil University of Medical Sciences (identification code 'IR.ARUMS.REC.1397.272'). After two weeks, forty male Wistar rats were casually allotted into four groups of normal rats (N), MS rats, and two identical ones under thiamine treatment: N (Thiamine) and MS (Thiamine). MS was induced in rats with a solution of sucrose 40 % in drinking water for 4 months (19) and the treated groups received 0.18% of thiamine hydrochloride (THC) in drinking water daily for four months. The dose of the treatment was chosen according to our recent study (10) and the literature (20). All groups were fed a standard chow diet.

At the end of the experiment, the weight of all rats was measured. Then, the percentage of alteration of body weight of rats after 4 months compared with start of the study was calculated. After 16 hr of fasting, blood samples were collected from their hearts and transferred into test tubes with and without EDTA after anesthetizing with IP injection of ketamine & xylazine (respectively, 90 and 10 mg/kg body mass). Serum samples were prepared by 10 min centrifugation of blood at 1500g, 4 °C and stored at -70 °C until measurements. Their livers were removed and weighed instantly.

### Determination of biochemical parameters

Fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), LDL, HDL, alanine transaminase (ALT), and aspartate transaminase (AST) were measured by utilizing commercial kits (Pars Azmoon, Tehran, Iran). The cardiovascular indices were determined with the calculation of LDL/HDL, TC/HDL, TG/HDL, and TG/FBG ratios. Serum FFA levels were measured with high-pressure liquid chromatography (HPLC) within one hour after rehydration (21).

The serum insulin level was determined by the enzyme-linked immunosorbent assay (ELISA) method using a rat insulin kit (ZellBio GmbH, Germany). Moreover, HOMA1 or HOMA- $\beta$  (homeostasis model assessment of insulin resistance) was determined using the equation below (22):

$$\text{HOMA1} = \frac{\text{Fasting glucose (mmol/l)} \times \text{Fasting insulin}(\mu\text{U/ml})}{22.5}$$

Also, HOMA2, beta-cell function (%B), and insulin sensitivity (%S) were determined with the HOMA2 calculator software released by the Diabetes Trials Unit, University of Oxford (23).

### Determination of glycation products in serum and the activity of Glo-I in hemolysate

Glycated albumin (g-Alb) was assayed with a colorimetric method based on the reduction of nitroblue tetrazolium chloride, and absorbance was read at a wavelength at 530 nm (24). Glycated LDL was measured based on the formation of hydroxymethyl furfuraldehyde chromogen (25). Methylglyoxal (MGO) was assayed by a reverse phase HPLC (26). AGEs measured in the serum of rats via the determination fluorescence intensity was recorded at the emission maximum (440 nm) upon excitation at 370 nm (27). The activity of glyoxalase-I (Glo-I) in hemolysate was detected by measuring the initial formation rate of S-D-lactoylglutathione and the activity of the enzymes expressed as unit/ml (U/ml) (28).

### Determination of oxidative stress and inflammatory markers in serum

The oxidative stress markers as malondialdehyde (MDA) were measured based on determination of the absorbance of thiobarbituric acid at 532 nm. Advanced oxidation protein products (AOPP) were assayed with spectrophotometric detection, according to the method of Witko-Sarsat *et al.* (29). Early products of LDL oxidation were measured spectrometrically at 234 nm (30). Fluorescence intensity of end oxidation product of LDL was detected at the emission maximum of 430 nm upon excitation at 360 nm (31). Reduced glutathione (GSH) was analyzed with UV-HPLC at 210 nm (32). The activity of paraoxonase-I (PON1) was done with a spectrophotometer by measuring the p-nitrophenol absorbance within one minute at a wavelength of 412 nm. P-nitrophenol was obtained by paraoxon hydrolysis (33). The sera activity of CAT was measured with a modified Abi method (34). Briefly, 5  $\mu$ l serum was added to a mixture (containing 10 mm of H<sub>2</sub>O<sub>2</sub> in equal volume phosphate buffer 50 mm, pH= 7 and saline) and absorbance read at 240 nm until 20 sec.

Inflammatory markers as IL-1 $\beta$  were determined using the ELISA kit (ZellBio GmbH, Germany). The sera activity of myeloperoxidase (MPO) was measured by reading the absorbance of oxidized guaiacol at wavelength 470 nm. Briefly, 10  $\mu$ l serum was added to a mixture (50 mM potassium phosphate buffer with 100 mM guaiacol and 0.0017% (w/w) hydrogen peroxide, pH 7.0 at 25 °C) and absorbance was read at 470 nm until 4 min.

### Gene expression of hepatic NF- $\kappa$ B

Total RNA was extracted from hepatic tissue using TRIzol reagent (Invitrogen, USA). The purity and concentration of extracted RNA were detected at 260 nm and purity was determined by 260/280 nm ratio with a NanoDrop spectrophotometer. cDNA was synthesized by reverse transcription following the manufacturer's protocols (MBI Fermentas, Lithuania). qRT-PCR was performed with a standard SYBR-Green PCR kit (Toyobo, Japan), and gene-specific PCR amplification was performed using an ABI 7300 (Applied Biosystems, Germany). B-actin (ACTB) was used as a housekeeping gene for normalization of gene expression data. RT-PCR primer sequences were as follows:

NF- $\kappa$ B: 5'-CCTGTCTGCACCTGTTCCAA-3' (forward)  
3'ACTCCTGGGTCTGTGTTGTT-5' (reverse)

ACTB: 5'-GGAGAA GATTTGGCACCACACT-3' (forward)  
3'-CGGTTGGCCTTAGGGTTCAGA-5' (reverse).

Relative gene expression levels were calculated using the  $2^{-\Delta\Delta CT}$  method after normalization to the mRNA level of  $\beta$ -actin. The first  $\Delta CT$  is the difference in threshold cycle between the target and reference genes:  $\Delta CT = CT(NF-k\beta) - CT(ACTB)$  (35).

#### Pathological study

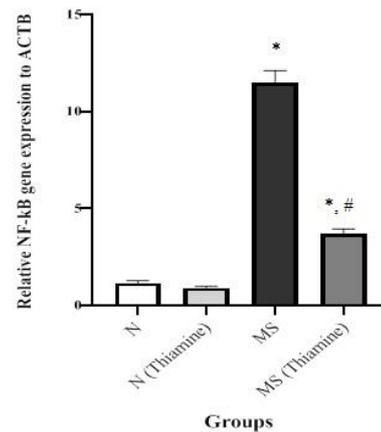
Sections of liver samples were fixed in a buffer solution containing 10% formalin and processed for paraffin embedding. Then, sections were stained with Hematoxylin-Eosin (H&E) and observed using light microscopy for histopathological parameters (36).

#### Statistical analysis

All data were expressed as mean $\pm$ SD (standard deviations). The Kolmogorov-Smirnov test represented the normal distribution of the results which was the reason for using parametric methods. Multiple analysis of variance (MANOVA-TUKEY) test was used to compare different variables used in all four groups using SPSS (ver. 16). Statistical significance was defined as  $P < 0.05$ .

#### Results

The comparison of the percentage of body weight alteration after four months and glucose and lipid metabolism in treated and untreated normal and MS rats are represented in Table 1. Induction of MS in rats via sucrose solution consumption for four months increased body weight, FBS, HOMA1, and HOMA2, as well as decreasing  $\beta$ -cell function (%B) and insulin sensitivity (%S). Furthermore, the levels of TG, TC, LDL, FFA, and different indexes composed of LDL/HDL, TC/HDL, TG/HDL, and TG/Glc elevated significantly in MS compared with normal rats. Thiamine improved glucose and lipid metabolism, insulin function and sensitivity,



**Figure 1.** Comparison of relative hepatic nuclear factor-k $\beta$  (NF-k $\beta$ ) to  $\beta$ -actin (ACTB) in untreated and thiamine treated normal (N) and metabolic syndrome (MS) groups.

\* Indicates significant difference with N group ( $P < 0.001$ )

# Indicates significant difference with MS group ( $P < 0.001$ )

decreased the alteration percentage of body weight in MS rather than the untreated one. The level of FFA in the normal treated group was lower than in the untreated one ( $P < 0.001$ ).

The levels of oxidative stress (AOPP, MDA, LDL oxidation products, GSH, the activities CAT and PON-1), inflammatory markers (IL-1 $\beta$ , MPO activity, and gene expression Nf-k $\beta$ ), and glycation (g-Alb, g-LDL, MGO, and AGEs) markers as well as the activity of GLO-I in all groups, are shown in Table 2 (except for the gene expression shown in Figure 1). Levels of all cited parameters except the activities of CAT, PON-1, GLO-I increased in the MS group. Treatment corrected

**Table 1.** Effect of thiamine on FBS, insulin, HOMA-IR, and lipid profile in normal (N) and metabolic syndrome (MS) rats

Parameter	Groups (ten rats in each group)			
	N	N (Thiamine)	MS	MS (Thiamine)
Percentage of body weight alteration (%)	58.65 $\pm$ 3.63	59.45 $\pm$ 3.80	153.60 $\pm$ 10.56*	80.79 $\pm$ 5.07*.#
Liver weight (g)	8.70 $\pm$ 0.53	8.62 $\pm$ 0.47	9.50 $\pm$ 0.69	11.84 $\pm$ 0.73*.#
Fasting blood sugar (mmol/l)	5.00 $\pm$ 0.25	4.92 $\pm$ 0.21	9.30 $\pm$ 0.53*	6.13 $\pm$ 0.45*.#
Insulin ( $\mu$ U/ml)	16.01 $\pm$ 0.83	15.93 $\pm$ 0.76	25.66 $\pm$ 1.64*	18.50 $\pm$ 1.27*.#
HOMA1	3.55 $\pm$ 0.19	3.84 $\pm$ 0.22	10.60 $\pm$ 0.73*	5.04 $\pm$ 0.41*.#
HOMA2	2.04 $\pm$ 0.06	2.03 $\pm$ 0.05	3.70 $\pm$ 0.12*	2.47 $\pm$ 0.09*.#
% $\beta$	156.23 $\pm$ 9.80	157.77 $\pm$ 10.14	70.00 $\pm$ 3.42*	116.57 $\pm$ 6.09*.#
%S	48.86 $\pm$ 2.37	49.13 $\pm$ 3.39	27.00 $\pm$ 1.16*	40.40 $\pm$ 1.35*.#
Triglyceride (mmol/l)	1.37 $\pm$ 0.09	1.39 $\pm$ 0.08	2.93 $\pm$ 0.17*	2.06 $\pm$ 0.11*.#
Total cholesterol (mmol/l)	1.83 $\pm$ 0.13	1.76 $\pm$ 0.10*.#	3.22 $\pm$ 0.19*	2.37 $\pm$ 0.15*.#
HDL (mmol/l)	0.94 $\pm$ 0.06	0.88 $\pm$ 0.04	0.72 $\pm$ 0.03*	1.00 $\pm$ 0.07*.#
LDL (mmol/l)	0.26 $\pm$ 0.11	0.24 $\pm$ 0.09	1.16 $\pm$ 0.07*	0.43 $\pm$ 0.11*.#
LDL/HDL	0.28 $\pm$ 0.01	0.27 $\pm$ 0.01*.#	1.61 $\pm$ 0.09*	0.43 $\pm$ 0.02*.#
Cho/HDL	1.94 $\pm$ 0.13	2.00 $\pm$ 0.12*.#	4.47 $\pm$ 0.25*	2.32 $\pm$ 0.16*.#
TG/HDL	1.45 $\pm$ 0.01	1.57 $\pm$ 0.01*.#	4.06 $\pm$ 0.09*	2.06 $\pm$ 0.02*.#
TG/FBG	1.45 $\pm$ 0.01	1.57 $\pm$ 0.01*.#	4.06 $\pm$ 0.09*	2.06 $\pm$ 0.02*.#
Free fatty acids ( $\mu$ mol/l)	596.45 $\pm$ 31.91	560.00 $\pm$ 27.88*.#	755.00 $\pm$ 41.64*	644.66 $\pm$ 35.71*.#

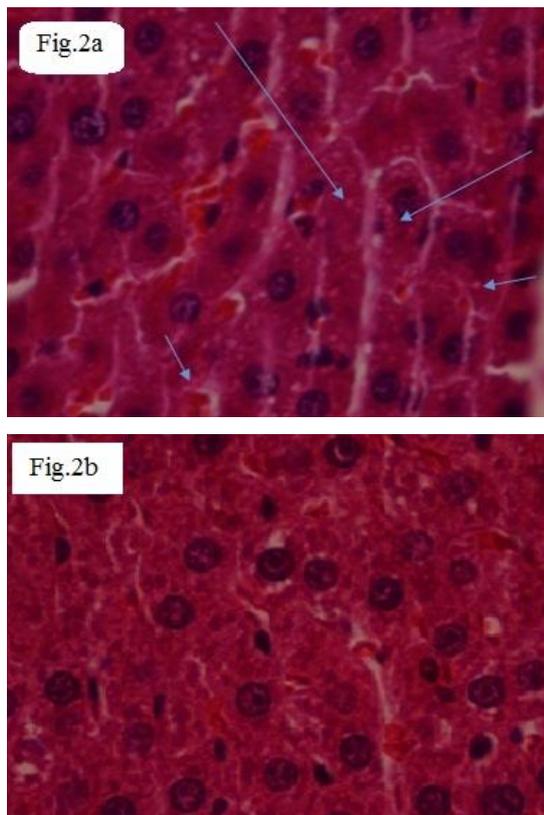
\* Indicates significant difference with N group ( $P < 0.001$ )

# Indicates significant difference with MS group ( $P < 0.001$ )

HOMA: homeostasis model assessment of insulin resistance; HDL: high density lipoprotein; LDL: low density lipoprotein; TC: total cholesterol; TG: triglyceride; FBG: fasting blood sugar

**Table 2.** Comparison between levels of glycation, oxidative stress, and inflammatory markers in the normal (N), metabolic syndrome (MS) rats, and thiamine treated ones

Parameter	Groups (Ten rats in each group)			
	N	N (Thiamine)	MS	MS (Thiamine)
Glycated albumin ( $\mu\text{mol/l}$ )	96.82 $\pm$ 5.36	85.56 $\pm$ 4.47*.#	278.38 $\pm$ 14.94*	175.03 $\pm$ 8.32*.#
Glycated LDL ( $\mu\text{mol/l}$ )	37.09 $\pm$ 1.78	23.91 $\pm$ 1.63*.#	113.55 $\pm$ 6.44*	60.66 $\pm$ 3.87*.#
Methylglyoxal ( $\mu\text{mol/l}$ )	11.62 $\pm$ 0.53	7.84 $\pm$ 0.23*.#	39.62 $\pm$ 2.22*	18.54 $\pm$ 0.82*.#
Advanced glycation end products (FI, A.U)	43.98 $\pm$ 2.35	29.91 $\pm$ 1.17*.#	307.04 $\pm$ 17.88*	80.46 $\pm$ 4.13*.#
Glyoxalase-I (U/ml)	45.23 $\pm$ 2.50	47.61 $\pm$ 2.68*.#	20.74 $\pm$ 1.38*	33.42 $\pm$ 1.83*.#
Early oxidation products of LDL ( $\mu\text{mol/l}$ )	12.54 $\pm$ 0.56	5.68 $\pm$ 0.41*.#	93.06 $\pm$ 5.45*	40.91 $\pm$ 2.68*.#
End oxidation products of LDL ( $\mu\text{mol/l}$ )	207.86 $\pm$ 12.33	185.68 $\pm$ 10.05*.#	456.83 $\pm$ 27.30*	285.37 $\pm$ 22.97*.#
Advanced oxidation protein products ( $\mu\text{mol/l}$ )	19.40 $\pm$ 1.11	13.86 $\pm$ 0.89*.#	53.51 $\pm$ 3.13*	28.42 $\pm$ 1.60*.#
Malondialdehyde ( $\mu\text{mol/l}$ )	12.68 $\pm$ 0.62	8.70 $\pm$ 0.49*.#	136.09 $\pm$ 8.47*	58.19 $\pm$ 2.86*.#
Glutathione ( $\mu\text{mol/l}$ )	181.04 $\pm$ 11.50	193.56 $\pm$ 12.07*.#	89.20 $\pm$ 5.19*	167.06 $\pm$ 9.79*.#
IL-1 $\beta$ (pg/ml)	334.30 $\pm$ 21.01	289.25 $\pm$ 17.88	729.59 $\pm$ 49.19*	410.82 $\pm$ 23.74*.#
Glyoxalase-I (U/ml)	20.74 $\pm$ 1.38	23.89 $\pm$ 1.09	20.74 $\pm$ 1.38*	33.42 $\pm$ 1.96*.#
Paraoxonase-I (U/ml)	117.03 $\pm$ 6.27	129.28 $\pm$ 7.15	45.64 $\pm$ 4.47*	97.05 $\pm$ 5.36*.#
Myeloperoxidase (U/ml)	1.40.62 $\pm$ 0.07	1.19 $\pm$ 0.05	1.58 $\pm$ 0.08*	3.21 $\pm$ 0.16*.#
Catalase (U/ml)	114.30 $\pm$ 7.69	119.26 $\pm$ 7.42*.#	38.50 $\pm$ 2.41*	79.47 $\pm$ 3.76*.#
Alanine transaminase (U/l)	23.16 $\pm$ 1.17	19.26 $\pm$ 0.93	84.39 $\pm$ 4.47*	27.86 $\pm$ 1.50*.#
Aspartate transaminase (U/l)	49.20 $\pm$ 3.26	46.43 $\pm$ 2.37	135.33 $\pm$ 6.72*	50.63 $\pm$ 2.92*.#
Lactate dehydrogenase (U/l)	597.53 $\pm$ 35.92	586.04 $\pm$ 37.51	906.52 $\pm$ 53.08*	691.74 $\pm$ 42.25*.#

\* Indicates significant difference with N group ( $P < 0.001$ )# Indicates significant difference with MS group ( $P < 0.001$ )**Figure 2.** Histopathologic views (stained by H&E and original magnification  $\times 400$ ) of the liver in the metabolic syndrome (MS) and MS (thiamine).

(a) Excessive intracellular inflammation, hydropic degeneration, and fatty changes were seen in the livers of MS rats.

(b) No signs of inflammation, hydropic degeneration, or fatty changes were observed in the livers of MS (thiamine) rats

the activities of the cited enzymes as well as reducing glycation, oxidative stress, and inflammation markers. The levels of GSH, AOPP, MDA, LDL oxidation products, IL-1 $\beta$ , MGO in the normal treated group was lower than in the untreated one ( $P < 0.001$ ).

Induction of metabolic syndrome in rats increased liver weight, motivated hepatic intracellular inflammation (Figure 2), and increased activities of ALT, AST, and LDH (Table 2) in sera. Thiamine prevented hepatitis, which was confirmed by lower levels of enzymes and gene hepatic NF- $\kappa$ B expression compared with the untreated group ( $P < 0.001$ ).

In the histopathological view (Figure 2), severe intracellular inflammation, hydropic degeneration, and fatty changes have only been observed in the livers of MS rats (Figure 2a). Thiamine prevented the cited alterations in the livers of rats (Figure 2b).

## Discussion

Thiamine improved hyperglycemia, insulin resistance, and dyslipidemia in MS rats. Also, the treatment decreased body weight and glycation, oxidative stress, and inflammatory markers along with the elevation of the activity of glyoxalase-I. Moreover, thiamine prevented histopathological changes in the liver via reduction of expression of NF- $\kappa$ B.

In this study, elevation of body weight, impaired glucose tolerance, insulin resistance, and dyslipidemia in MS rat models due to four months of high sucrose solution (HSS) consumption confirms the induction of MS. Our findings of the MS rat model are similar to those of a recent paper (19).

Pro-inflammatory cytokines such as IL-1 $\beta$  via oxidative stress induction (37), reduction of insulin signaling and glucose transport as well as elevation of FFA and up-regulation of hepatic NF- $\kappa$ B have a central

role in acute hepatitis, insulin resistance, glycemia, and dyslipidemia (5, 6, 38). Also, early to end glycation products and oxidized LDL activate the NF- $\kappa$ B pathway (5, 6). There is evidence that indicates high carbohydrate diet participates in thiamine deficiency (18). Besides, thiamine deficiency causes high expression of hepatic NF- $\kappa$ B and hepatic inflammation. The levels of glycation products have an inverse correlation with the activity of glyoxalase-I (39). Furthermore, low activity of Glo-I leads to high expression of hepatic NF- $\kappa$ B. In this study, the levels of the activators of the NF- $\kappa$ B signaling pathway including FFAs (Table 1), g-Alb, MGO, AGEs, IL-1 $\beta$ , glycated and oxidized LDL (Table 2), as well as expression of NF- $\kappa$ B (Figure 1) were higher in the metabolic syndrome group than in the normal group. Thiamine decreased hepatic expression of NF- $\kappa$ B by reducing the cited activators in the MS rats. In the pathological view (Figures 2a and 2b), hepatic intracellular inflammation confirmed the higher expression of NF- $\kappa$ B and the higher activities of ALT, AST, and LDH in the MS group rather than other groups. Further, based on liver histology, thiamine had a preventing effect against hepatic inflammation via reduction of hepatic expression of NF- $\kappa$ B. The lower activities of ALT, AST, and LDH confirmed the hepatoprotective effect of the treatment. Also, the lowered level of IL-1 $\beta$  (Table 2) and the activity of MPO (inflammatory markers) in treated MS rats verified the anti-inflammatory property of thiamine in MS rats. The effect of thiamine on hepatic expression of NF- $\kappa$ B and different activators of it in the MS group has not been reviewed yet. Lately, the ameliorative effect of thiamine on inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis via decreased relative protein expression of IL-1 $\beta$ , NF- $\kappa$ B unit p65, and phosphorylated NF- $\kappa$ B unit p65, has been presented (40).

MS induces glycemia, hyperinsulinemia, and insulin resistance following interfering with insulin secretion and insulin sensitivity (19). Thiamine has a cardinal role in glucose metabolism, insulin synthesis, and insulin secretion (41). Thiamine deficiency is common in MS (16, 42, 43); excess carbohydrate intake (18) as HSS, and diabetes (44) participate in enhancement of different glycation products following reduction of the activity of glyoxalase-I. Glycation products interfere in insulin secretion and function via induction of oxidative stress and inflammation (10, 45-47). In this study, thiamine decreased the levels of FBG and sera insulin along with reduction of insulin resistance by elevation of insulin function and sensitivity following the decrement of levels of IL-1 $\beta$ , FFA, glycation products, oxidative stress markers, and hepatic expression of NF- $\kappa$ B. Previously, the lowering effect of thiamine with 2 g/L dose in drinking water on glucose and insulin resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats has been reported (18). However, in the present study, thiamine at a much lower dose (0.18 g/L) was able to beneficially affect glucose metabolism and insulin sensitivity. Up to now, the alteration of the activity of Glo-I in MS subjects and animal models has not been presented. We reported the lower activity of Glo-I in MS rats compared with normal rats and the beneficial effect of thiamine on the activity of Glo-I.

HSS caused obesity, dyslipidemia, and increased

proneness to cardiovascular disease by elevation of different cardiovascular indices (LDL/HDL, TC/HDL, TG/HDL, and TG/FBG) in rats (Table 1). Furthermore, HSS increased lipid accumulation in the liver, resulting in liver weight gain. The liver is the main organ playing an important role in lipoprotein biosynthesis and distribution (48). Thiamine has a vital role in lipid metabolism, and its deficiency leads to dyslipidemia (49) via up-regulation of hexosamine (50) and NF- $\kappa$ B pathways (6). The treatment decreased body weight and improved lipid metabolism and decreased cardiovascular indices in MS rats with down-regulation of hexosamine and NF- $\kappa$ B pathways. Our study presented the beneficial effect of thiamine on diverse cardiovascular indices in the MS rat model for the first time. Thiamine probably has a regulatory role in lipid metabolism. The reducing effect of thiamine on body weight, TG, and TC in OLETF rats has been reported (18). However, in the present study, thiamine at a much lower dose was able to decrease the cited parameters. IL-1 $\beta$  plays a major role in fat metabolism by regulating insulin level and lipase activity (51). Therefore, reduction in its level leads to reduced insulin resistance and improved lipid metabolism (52).

Oxidative stress is one of the major risk factors for vascular disease in MS (1). HSS induced oxidative stress in MS rats with increased MDA and AOPP along with decreased GSH and activities of CAT and PON-1 (Table 2). The treatment compensated for the cited changes and showed the antioxidant property and GSH synthesis. Recently, the benefits of thiamine on PON activity in diabetic rats has been reported (53). In the present study for the first time, the advantageous effect of thiamine on the activities of PON-1 and CAT as well as GSH level in MS rats are represented.

Glycemia, hypercholesterolemia, and oxidative stress elevate LDL glycation and oxidation, which contribute to macrovascular complications (11, 54). Our results indicated the lowering effect of thiamine on the early and end oxidation products of LDL in the normal and MS rats (Table 2). The diminishing effect of the treatment on LDL glycation and oxidation are due to its antioxidant properties as well as inducing the activities of Glo-I and PON-1. The effect of thiamine on glycation and oxidation products of LDL and activities of the cited enzymes in the MS group has not been reported up to now.

Glycemia, oxidative stress, glycation, and thiamine deficiency are risk factors for hypertension (55). Moreover, thiamine deficiency contributes to vascular dysfunction and hypertension respectively via reduction of nitric oxide production and up-regulation of mRNAs implicated in the renin-angiotensin system. Failure to determine blood pressure in rats was a limitation of our study. However, thiamine by reducing the cited factors of hypertension could correct hypertension in MS rats. The advantageous effect of thiamine on hypertension in pre-diabetic subjects has been reported (17).

## Conclusion

Thiamine decreased body weight and improved glycemia, insulin function, dyslipidemia, and activity of glyoxalase-I in MS rats with anti-glycation, antioxidant, and anti-inflammatory activities. Furthermore, the treatment had a hepato-protective effect via reduction

of NF- $\kappa$ B signaling.

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### Conflicts of Interest

There are no conflicts of interest

### References

- Hess PL, Al-Khalidi HR, Friedman DJ, Mulder H, Kucharska-Newton A, Rosamond WR, *et al.* The metabolic syndrome and risk of sudden cardiac death: The atherosclerosis risk in communities study. *J Am Heart Assoc* 2017;6: 6103-6114.
- Spahis S, Borys JM, Levy E. Metabolic syndrome as a multifaceted risk factor for oxidative stress. *Antioxid Redox Signal* 2017; 26:445-461.
- Mazloomzadeh S, Karami Zarandi F, Shoghli A, Dinmohammadi H. Metabolic syndrome, its components and mortality: A population-based study. *Med J Islam Repub Iran* 2019; 33:11-16.
- Gehrke N, Schattenberg JM. Metabolic inflammation-a role for hepatic inflammatory pathways as drivers of comorbidities in nonalcoholic fatty liver disease? *Gastroenterology* 2020; 158:1929-1947.
- Petersen MC SG. Mechanisms of insulin action and insulin resistance. *Physiol Rev* 2018; 98:2133-2223.
- Piuri G, Basello K, Rossi G, Soldavini CM, Duiella S, Privitera G, *et al.* Methylglyoxal, glycated albumin, paf, and tnf- $\alpha$ : possible inflammatory and metabolic biomarkers for management of gestational diabetes. *Nutrients* 2020; 12:27.
- Wen Jin BC, Pingping Li, FangHua, Xiaoxi Lv, Jichao Zhou, Zhuowei Hu Xiaowei Zhang. 1,25-Dihydroxyvitamin D3 protects obese rats from metabolic syndrome via promoting regulatory Tcell-mediated resolution of inflammation. *Acta Pharmaceutica Sinica B* 2018; 8:178-187.
- Jayarathne S, Kobozev I, Park OH, Oldewage-Theron W, Shen CL, Moustaid-Moussa N. Anti-inflammatory and anti-obesity properties of food bioactive components: effects on adipose tissue. *Prev Nutr Food Sci* 2017; 22:251-262.
- Okura T, Ueta E, Nakamura R, Fujioka Y, Sumi K, Matsumoto K, *et al.* High serum advanced glycation end products are associated with decreased insulin secretion in patients with type 2 diabetes: a brief report. *J Diabetes Res* 2017; 2017:5139750.
- MahdaviFard S, Nakhjavani M. Thiamine pyrophosphate improved vascular complications of diabetes in rats with type 2 diabetes by reducing glycation, oxidative stress, and inflammation markers. *Med J Islam Repub Iran* 2020; 34:331-336.
- MahdaviFard S, Nakhjavani M. Effect of Pyridoxal phosphate on atherosclerosis and nephropathy progression in atherosclerotic rats. *J Adv Med Biomed Res* 2021; 29:21-27.
- Uribarri JC, Woodward W, Tripp M, Goldberg E, Pyzik L, Yee R, *et al.* Elevated serum advanced glycation endproducts in obese indicate risk for the metabolic syndrome: A link between healthy and unhealthy obesity? *J Clin Endocrinol MeTable* 2015; 100:1957-1966.
- R I. RAGE and glyoxalase in kidney disease. *Glycoconj J* 2016; 33:619-626.
- MahdaviFard S, Nakhjavani M. Effect of linalool on the activity of glyoxalase-i and diverse glycation products in rats with type 2 diabetes. *J Maz Uni Med Sci* 2020; 30:24-33.
- Nigro C, Leone A, Raciti GA, Longo M, Mirra P, Formisano P, *et al.* Methylglyoxal-glyoxalase 1 balance: the root of vascular damage. *Int J Mol Sci* 2017; 18:188-202.
- Kerns JC, Arundel C, Chawla LS. Thiamin deficiency in people with obesity. *Advances in nutrition* 2015; 6:147-153.
- Alaei-Shahmiri F, Soares MJ, Zhao Y, Sherriff J. The impact of thiamine supplementation on blood pressure, serum lipids and C-reactive protein in individuals with hyperglycemia: A randomised, double-blind cross-over trial. *Diabetes Metab Syndr* 2015; 9:213-217.
- Tanaka T, Kono T, Terasaki F, Yasui K, Soyama A, Otsuka K, *et al.* Thiamine prevents obesity and obesity-associated metabolic disorders in OLETF rats. *J Nutr Sci Vitaminol* 2010; 56:335-346.
- Souza Cruz EM, Bitencourt de Moraes JM, Dalto da Rosa CV, da Silva Simões M, Comar JF, de Almeida Chuffa LG, *et al.* Long-term sucrose solution consumption causes metabolic alterations and affects hepatic oxidative stress in Wistar rats. *Biol Open* 2020; 9:1-10
- Rabbani N, Alam SS, Riaz S, Larkin J, Akhtar MW, Shafi T, *et al.* High-dose thiamine therapy for patients with type 2 diabetes and microalbuminuria: a randomised, double-blind placebo-controlled pilot study. *Diabetologia* 2009; 52:208-212.
- Lv ZH, Ma P, Luo W, Xiong H, Han L, Li SW, *et al.* Association between serum free fatty acid levels and possible related factors in patients with type 2 diabetes mellitus and acute myocardial infarction. *BMC Cardiovasc Disord* 2014; 14:159-66.
- Matthews DR HJ, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28:412-419.
- Song YS, Hwang YC, Ahn HY, Park CY. Comparison of the usefulness of the updated homeostasis model assessment (HOMA2) with the original HOMA1 in the prediction of type 2 diabetes mellitus in koreans. *Diabetes Metab J* 2016; 40:318-325.
- Xu YJ, Wu XQ, Liu W, Lin XH, Chen JW, He R. A convenient assay of glycoserum by nitroblue tetrazolium with iodoacetamide. *Clinica Chimica Acta* 2002; 325:127-131.
- Cohen MP, Shea EA, Wu V-Y. Inhibiting LDL glycation ameliorates increased cholesteryl ester synthesis in macrophages and hypercholesterolemia and aortic lipid peroxidation in streptozotocin diabetic rats. *Metabolism* 2010; 59:658-663.
- Pappalardo M, Pappalardo L, Brooks P. Rapid and Reliable HPLC Method for the Simultaneous Determination of Dihydroxyacetone, Methylglyoxal and 5-Hydroxymethylfurfural in *Leptospermum Honeys*. *PLoS One* 2016; 11:1-9.
- Kalousova M, Skrha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. *Physiol Res* 2002; 51:597-604.
- MahdaviFard S, Bathaie S, Nakhjavani M, Heidarzadeh H. L-cysteine is a potent inhibitor of protein glycation on both albumin and LDL, and prevents the diabetic complications in diabetic-atherosclerotic rat. *Food Research International* 2014; 62:909-916.
- Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, *et al.* Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.* 1996; 49:1304-1313.
- Ahotupa M, Marniemi J, Lehtimäki T, Talvinen K, Raitakari OT, Vasankari T, *et al.* Baseline diene conjugation in LDL lipids as a direct measure of *in vivo* LDL oxidation. *Clin Biochem* 1998; 31:257-261.
- Esterbauer H, Gebicki J, Puhl H, Jürgens G. The role of lipid peroxidation and anti-oxidants in oxidative modification of

- LDL. *Free Radic Biol and Med* 1992; 13:341-390.
32. Begic A, Djuric A, Gobeljic B, Stevanovic I, Lukic V, Stanojevic I, *et al.* The simple isocratic HPLC—UV method for the simultaneous determination of reduced and oxidized glutathione in animal tissue. *Acta Chromatographica* 2017; 29:67-84.
33. Ceron JJ, Tecles F, Tvarijonaviciute A. Serum paraoxonase 1 (PON1) measurement: an update. *BMC Vet Res* 2014; 10:74-85.
34. Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984; 105:121-129.
35. Mazani M, Rezagholizadeh L, Shamsi S, Mahdavifard S, Ojarudi M, Salimnejad R, *et al.* Protection of CCl(4)-induced hepatic and renal damage by linalool. *Drug Chem Toxicol* 2020:1-9.
36. Kavita S, Neerja, R., Khushboo, G. & Saurabh, S. Probable benefits of green tea with genetic implications *J Oral Maxillofac Pathol* 2017; 21:107-114.
37. Gao D MM, Ding C, Fok M, Steele T, Ford C, *et al.* Interleukin-1beta mediates macrophage-induced impairment of insulin signaling in human primary adipocytes. *Am J Physiol Endocrinol Metab* 2014; 307:289-304.
38. Bing C. Is interleukin-1 $\beta$  a culprit in macrophage-adipocyte crosstalk in obesity? *Adipocyte* 2015; 4:149-152.
39. Mahdavifard S, Nakhjavani M. Effect of glutamine on oxidative stress, inflammatory, and glycation markers, and the activity of glyoxalase system in diabetic rats with atherosclerosis. *J Mazandaran Univ Med Sci* 2019; 28:33-42.
40. Zhang H, Peng AL, Zhao FF, Yu LH, Wang MZ, Osorio JS, *et al.* Thiamine ameliorates inflammation of the ruminal epithelium of Saanen goats suffering from subacute ruminal acidosis. *J Dairy Sci* 2020; 103:1931-1943.
41. Maguire D, Talwar D, Shiels PG, McMillan D. The role of thiamine dependent enzymes in obesity and obesity related chronic disease states: A systematic review. *Clin Nutr ESPEN* 2018; 25:8-17.
42. Fernandes LMP, Bezerra FR, Monteiro MC, Silva ML, de Oliveira FR, Lima RR, *et al.* Thiamine deficiency, oxidative metabolic pathways and ethanol-induced neurotoxicity: how poor nutrition contributes to the alcoholic syndrome, as Marchiafava-Bignami disease. *Eur J Clin Nutr* 2017; 71:580-586.
43. Page GL, Laight D, Cummings MH. Thiamine deficiency in diabetes mellitus and the impact of thiamine replacement on glucose metabolism and vascular disease. *Int J Clin Pract* 2011; 65:684-690.
44. Page G, Laight D, Cummings M. Thiamine deficiency in diabetes mellitus and the impact of thiamine replacement on glucose metabolism and vascular disease. *Int J Clin Prac* 2011; 65:684-690.
45. vinh quoc Luong K, Nguyen LTH. The impact of thiamine treatment in the diabetes mellitus. *J Clin Med Res* 2012; 4:153-160.
46. Verdile G, Keane KN, Cruzat VF, Medic S, Sabale M, Rowles J, *et al.* Inflammation and oxidative stress: the molecular connectivity between insulin resistance, obesity, and Alzheimer's disease. *Mediators Inflamm* 2015; 2015:1-17.
47. Rodrigues T, Matafome P, Sereno J, Almeida J, Castelhana J, Gamas L, *et al.* Methylglyoxal-induced glycation changes adipose tissue vascular architecture, flow and expansion, leading to insulin resistance. *Sci Rep* 2017; 7:1698-1711
48. Frizell EL, SL, Abraham V, Ozaki I, Eghbali M, Sage EH, Zern MA. Expression of SPARC in normal and fibrotic livers. *Hepatology* 1995; 21: 847-854.
49. Waheed P, Naveed AK, Ahmed T. Thiamine deficiency and its correlation with dyslipidaemia in diabetics with microalbuminuria. *J Pak Med Assoc.* 2013; 63:340-345.
50. Thornalley PJ. The potential role of thiamine (vitamin B1) in diabetic complications. *Curr Diabetes Rev* 2005; 1:287-298.
51. Cieslak M WA, Cieslak M. Role of pro-inflammatory cytokines of pancreatic islets and prospects of elaboration of new methods for the diabetes treatment. *Acta Biochim Pol* 2015; 62:15-21.
52. Rumberger JM WT, Hering MA, Marshall S. Role of hexosamine biosynthesis in glucose-mediated up-regulation of lipogenic enzyme mRNA levels: effects of glucose, glutamine, and glucosamine on glycerophosphate dehydrogenase, fatty acid synthase, and acetyl-CoA carboxylase mRNA levels. *J Biol Chem* 2003; 278:28547-28552.
53. Sarandol E, Tas S, Serdar Z, Dirican M. Effects of thiamine treatment on oxidative stress in experimental diabetes. *Bratisk Lek Listy* 2020; 121:235-241.
54. Kattoor AJ, Kanuri SH, Mehta JL. Role of Ox-LDL and LOX-1 in atherogenesis. *Curr Med Chem* 2019; 26:1693-1700.
55. Harrison DG, Gongora MC. Oxidative stress and hypertension. *Med Clin North Am* 2009;93: 621-635.