

Impact of L-Carnitine and Cinnamon on Insulin-Like Growth Factor-1 and Inducible Nitric Oxide Synthase Gene Expression in Heart and Brain of Insulin Resistant Rats

Mona A. Mohamed

Biochemistry Division, Department of Chemistry, Faculty of Science,
Al-Azhar University, Cairo, Egypt

Abstract: Problem statement: Evaluate the effects of daily administration of L-carnitine and cinnamon extract for two weeks on the expression of Insulin-like Growth Factor-1 (IGF-1) and inducible Nitric Oxide Synthase (iNOS) genes in cardiac and brain tissues of rats with Insulin Resistance (IR). **Approach:** Rats were divided into 4 groups (8 animals each): Group (1) rats fed control diet (60% starch) as control while groups (2, 3 and 4) fed high fructose diet (60% fructose). At the beginning of the 3rd week of feeding, rats of group (3) were treated with L-carnitine (300 mg kg⁻¹ body weight/day, i.p.) and animals of group (4) received a daily oral dose of cinnamon aqueous extract (0.5 mL rat⁻¹). The animals were maintained in their respective groups for 4 weeks. **Results:** Feeding high fructose diet causes significant reduction in Insulin Receptor Substrate-1 (IRS-1) (amounted 30.65%) and elevation in iNOS expression (reached 51%) in the cardiac tissues as compared to control. In brain tissues, the IGF-1 mRNA was reduced in fructose loaded groups (28.81%). Administration of either L-carnitine or cinnamon extract significantly improves the expression of the cardiac studied genes but with no effects on the brain tissues. **Conclusion:** The present study illustrated that CE was more potent than L-carnitine in improving the IR.

Key word: Insulin resistance, insulin-like growth factor-1, inducible nitric oxide synthase, insulin receptor substrate-1

INTRODUCTION

Fructose consumption has largely increased most likely as a result of this increased use of high fructose corn syrups and pre-packaged foods that contains between 55-90% fructose. Diets specifically high in fructose have been shown to contribute to a metabolic disturbance in animal models resulting in weight gain, hyperlipidemia and insulin resistance (Hwang *et al.*, 1987). Insulin Resistance (IR) is accompanied by deleterious metabolic consequences including hyperinsulinemia, hyperglycemia, glucose intolerance and hypertriglyceridemia in rodents. Therefore fructose-fed rat is used as an animal model of IR (Kannappan *et al.*, 2006).

Insulin is known to promote the actions of various growth factors and increase in cell growth in multiple cell types (Wang *et al.*, 2006). Insulin-like Growth Factor-1 (IGF-1) is synthesized mainly by the liver and kidneys but also, in a paracrine and autocrine way, by endothelial and vascular smooth muscle cells and cardiac myocytes (Conti *et al.*, 2001).

Several lines of evidence indicate that IR or hyperinsulinemia may be causal factors in the

development of thick left ventricular walls and left ventricular hypertrophy. Insulin at high concentrations is capable of inducing hypertrophic effects via IGF-1 receptor (which are abundant in the heart), but also at low concentrations via the insulin receptor (Hill and Milner, 1985; Frystyk and Orskov, 2004).

Some investigators revealed a correlation between IR and defects of the Nitric Oxide (NO) system (Shinozaki *et al.*, 1999; Kuboki *et al.*, 2000). Inducible Nitric Oxide Synthase (iNOS; EC 1.14.13.39) is assumed to be one of the candidates that mediate inflammation involved in insulin resistance (Fujimoto *et al.*, 2005). Among the multiple alterations associated with heart failure, the induction of myocardial expression of iNOS has gained particular attention because this high output isoform of NO synthases releases high levels of NO product, which in cardiac myocytes is known to induce apoptosis (Taimor *et al.*, 2001). In view of this detrimental potential, the concept was developed that cardiac iNOS induction might be an important factor in the pathogenesis of heart failure.

Although the effects of the high fructose diet on the different biochemical parameters in serum and tissues

have been discussed, little is known about its effect on brain tissue. There is a substantial literature indicates that the chemistry and the function of both the developing and mature brain can be influenced by the diet (Kurban *et al.*, 2007).

Cheng *et al.* (2000) had shown that IGF-1 is a key regulator of glucose transport and utilization in the developing murine brain. They also demonstrated that the caloric content and macronutrient composition of the diet exert independent effects on brain IGF-1 gene expression.

Pharmacological therapy is a critical step in the management of patients with metabolic syndrome when life style modifications fail to achieve the therapeutic goals (Deedwania and Gupta, 2006). Much scientific research has focused on the health benefits of herbs and spices. Some of these benefits are broad-based, but others are specific to one or a few physiological functions in the body. Naturally-occurring compounds that have been shown to improve insulin sensitivity include L-carnitine and cinnamon. L-carnitine (β -hydroxy-6-trimethyl aminobuturate) is a ubiquitous constituent of mammalian plasma and tissues, mainly distributed among skeletal and cardiac muscles. Carnitine is supplied to the body through dietary sources (e.g., meat and dairy products) and by biosynthesis from lysine and methionine. Carnitine transports long chain fatty acids across the inner mitochondrial membrane into the matrix for β -oxidation and has effects on oxidative metabolism of glucose in tissues (Rajasekar and Anuradha, 2007).

Cinnamon (*Cinnamom zeylanicum*) is a spicy, which is added to food preparations to impart taste and aroma. Cinnamon has insulin-potentiating properties and may be involved in the alleviation of the signs and symptoms of diabetes and cardiovascular disease related to IR (Anderson, 2008).

Considering all these, the present study was initiated to evaluate the effects of L-carnitine and cinnamon extract supplementation on the expression of IGF-1 and iNOS genes in heart and brain tissues of rats fed high fructose diet as a well-characterized model of IR.

MATERIALS AND METHODS

Chemicals: L-carnitine, other chemicals and solvents were of high analytical grade and were purchased from Sigma Chemical Company.

Cinnamon extract: Cinnamon bark (*Cinnamom zeylanicum*) was purchased from the local market and extracted according to the method of Kannappan *et al.*

(2006). This extract was diluted with water (1:10) and was administered orally to rats.

Animals and treatment: Male Wister rats at the age of 22 weeks (n = 32) and body weight ranging from 120-170 g were obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). Rats were housed 2/cage under controlled condition 12 h light/12 h dark cycle. All animals received standard pellet diet for one week and water *ad libitum*.

After acclimatization, animals were divided into the following groups consisting of 8 rats each: Group 1: (Con)/control animals received the control diet which contained corn starch (60 g 100 g⁻¹) as a sole source of carbohydrate. Group 2: (HFD)/Fructose-fed rats received a high fructose diet (60 gm 100 g⁻¹). Group 3: (HFD + CAR)/fructose-fed animals received the fructose-diet and treated with L-carnitine (300 mg kg⁻¹ body wt/day, i.p.) at the beginning 3rd week of experimental period. Group 4: (HFD + CE)/Fructose-fed rats received a daily oral dose of CE extract (0.5 mL rat⁻¹) from the 3rd week of experimental period.

The animals were maintained in their respective groups for 4 weeks and body weight changes were recorded weekly. At the end of the experimental period, animals in all groups were weighed and blood samples were taken from the retro-orbital venous plexus under light ether anesthesia after a fast of 12 h. Serum was separated by centrifuging blood samples at 3000 rpm for 10 min. Serum samples were aliquoted and stored at -20°C until analysis, except for glucose which was determined on the same day. After blood collection, all animals were sacrificed by decapitation and for each animal heart and brain were excised, rinsed with shield saline, weighed and rapidly frozen in liquid nitrogen then stored at -70°C until assayed.

Serum analysis: Serum was used for determination of glucose and Triacylglycerol (TG) according to the methods described by Mahfouz *et al.* (2010). The remaining part of serum was used for determination of insulin which was assayed by monoclonal immunoradiometric assay using kit supplied by Diagnostic Products Corporation (DPC) according to Marschner *et al.* (1974). Homeostasis Model Assessment (HOMA) correlates positively with insulin-resistance and was calculated according to Matthews *et al.* (1985).

Extraction of tissue lipids: Extraction was carried out according to the procedure of Refaie *et al.* (2005). Briefly, total lipids from tissues were extracted by homogenization with chloroform and methanol (2:1).

The extraction solvent was evaporated and lipids re-suspended in methanol. Cholesterol and triacylglycerol concentrations were assayed according to the method of Yousef *et al.* (2006) and Foster and Dunn (1973).

Determination of tissue MDA and NO: The concentrations of Malondialdehyde (MDA) and nitric oxide (NO as nitrite and nitrate) were estimated in the whole tissue homogenate (5%) according to the methods of Esterbauer and Cheeseman (1990) and Moshage *et al.* (1995), respectively.

Gene expression of IGF-1 and iNOS by RT-PCR: Total RNA was extracted from the tissues by the acid guanidinium thiocyanate-phenol chloroform method of Shaker *et al.* (2009). The sequence of the primers used to amplify IGF-1 and iNOS genes was illustrated in Table 1.

Reverse transcription-polymerase chain reaction was done using the RT-PCR kit (Promega, Madison, WI). Briefly, 1 μ g extracted RNA, 6 μ l dNTP, 6 μ l 5x buffer and 30pM specific downstream primer were brought up to a total volume of 28 μ l. The mixture was heated at 65°C for 5min. and chilled on ice for 5min. Exactly, 0.5 μ L RNasin and 1 μ l AMV were added and the mixture was incubated at 42°C for 2 h. Then, 5 μ L of cDNA was added to 200 μ M dNTP, 2.5U Taq polymerase and 3.3 nM MgCl₂ in a final volume of 30 μ L. The reaction mixture was placed in a Hybaid Express thermal cycler for 3 min at 95°C and then subjected for 32 cycles of 95°C for 1.5 min, 55°C for 1 min, 72°C for 1.5 min and a final step at 72°C for 10 min. All the PCR products were applied to specified wells into an ethidium bromide-stained 2% agarose gel in known order, then semi-quantities using the gel documentation system (Bio Doc Analyzer) supplied by Biometra. The content of IGF-1 and iNOS mRNA were calculated and expressed as cDNA relative densitometric units (ratio of IGF-1 or iNOS cDNA/ β -actin).

Cardiac Insulin Receptor Substrate-1 (IRS-1): Western blot analysis was used to determine the cardiac (IRS-1) (ug mL⁻¹) according to the method of Bezerra *et al.* (2000). For brain tissue, IRS-1 was not performed.

Table 1: Sequence of the primers used to amplify IGF-1, iNOS and β -actin genes

Gene	Primer sequence
IGF-1: Forward	GTGTGGACCGAGGGGCTTTACTTC
Reverse	GCTTCAGTGGGGCACAGTACATCTC
iNOS: Forward	GGCTTGCCCTGGAAGTTTCTCTTCAAAGTC
Reverse	AAGGAGCCATAATACTGGTTGATG
B-actin: Forward	TGTTGTCCCTGTATGCCTCT
Reverse	TAATGTCACGCACGATTTC

Statistical analysis: All results were expressed as the mean \pm SD. Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 11.0, Chicago, IL, USA). The data were analyzed by one-way Analysis of Variance (ANOVA). To compare the difference among the groups, post hoc testing was performed by the Bonferroni test. The p-value<0.05 was considered statistically significant (Dawson, 2000).

RESULTS

At the end of the experiment, the percentage change in the body weight gain and the relative heart and brain weights in rats of the studied groups were not statistically significant, compared to control rats (Table 2).

Data in Table 3 illustrate that the serum levels of glucose, insulin and HOMA index as well as triacylglycerol were significantly elevated (p<0.0001) in HFD group after 4 weeks of fructose-feeding, compared to control group. Administration of L-carnitine for 2 weeks caused significant reductions in glucose, HOMA and TG (p<0.0001), while insulin level did not change significantly, compared to HFD group. Cinnamon treated rats reduced these studied parameters to the normal control values, except serum cholesterol which did not altered in all groups.

Concentrations of cholesterol and triacylglycerol in addition to MDA and NO products in cardiac and brain tissues were illustrated in Table 4. Feeding rats with HFD did not affect significantly the concentrations of both cardiac and brain cholesterol and NO product, compared to control rats. The levels of cardiac TG and MDA were elevated significantly (p<0.0001), while no changes were observed in the brain tissues, compared to control.

With regard to carnitine treated group, all the studied cardiac parameters were non significantly changed, except the cardiac concentration of MDA which showed significant reduction, compared to HFD groups (p<0.001) and was toward the control value. In addition, carnitine reduced brain cholesterol level (p<0.001), compared to HFD group.

Cinnamon treatment did not affect cardiac cholesterol and NO levels, but it significantly reduced the cardiac levels of TG and MDA (p<0.001), compared to HFD group. On the other hand, cinnamon reduced significantly the levels of cholesterol (p<0.0001 and 0.001) and NO (p<0.0001 and 0.001), compared to control and HFD groups, respectively.

Table 2: Body weight gain% and organs weights (mean ± SD) in different studied groups

Weights (%)	Con	HFD	HFD +CAR	HFD +CE
Body wt. gain	20±3.47	19±6.13	27±2.92	20±6.03
Relative heart wt.	0.34±0.04	0.32±0.02	0.31±0.03	0.35±0.03
Relative brain wt.	0.84±0.12	0.79±0.07	0.80±0.11	0.85±0.14

Relative organ wt. % = (organ wt / body wt) X 100

Table 3: Metabolic serum parameters (mean ± SD) in the different studied groups

Parameters	Con	HFD	HFD +CAR	HFD +CE
Glucose (mg dL ⁻¹)	92.20±9.78	155.06±16.80 ^a	97.43±8.30 ^b	89.19±13.50 ^b
Insulin (µIU m L ⁻¹)	19.46±3.60	36.29±6.70 ^a	28.47±6.90 ^a	20.04±4.20 ^b
HOMA	4.44±0.60	13.88±2.20 ^a	6.86±1.60 ^{a,b}	4.43±1.20 ^b
Cholesterol (mg d L ⁻¹)	75.27±10.30	84.28±12.40	76.58±10.20	77.43±10.70
TG (mg d L ⁻¹)	78.30±12.20	165.78±13.50 ^a	95.92±9.80 ^{a,b}	79.92±10.50 ^b

^a: p<0.0001 Vs CON; ^b: p< 0.0001 Vs. HFD

Table 4: Concentration of cholesterol, TG (mg g⁻¹) and MDA (µmol g⁻¹) as well as NO product (µM g⁻¹) (mean ± SD) in cardiac and brain tissues

Parameters	Con	HFD	HFD +CAR	HFD +CE
Cardiac tissues				
Cholesterol	2.34±0.61	2.98±0.56	2.64±0.51	2.99±0.20
TG	19.53±1.9	23.10±1.65 ^a	22.65±3.75	17.09±1.34 ^b
MDA	22.10±3.47	41.19±4.54 ^a	28.19±1.66 ^{a,b}	28.73±2.89 ^{a,b}
NO	0.97±0.12	1.17±0.27	1.21±0.21	1.34±0.46
Brain tissues				
Cholesterol	13.10±1.50	14.50±1	12.50±0.46 ^b	10.16±1.30 ^{a,b}
TG	16.60±1.40	14.8±1.19	15.80±0.54	15.50±1.37
MDA	35.30±4.90	40.23±3.50	41.50±4.30	41.54±4.63
NO	3.30±0.68	2.85±0.22	2.38±1.20	0.99±0.26 ^{a,b}

^a: p<0.0001 Vs CON; ^b: p<0.001 Vs HFD

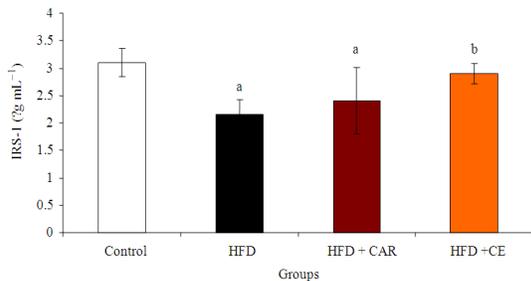


Fig. 1: Effects of high fructose diet, L-carnitine and cinnamon extract on cardiac IRS-1 level

Significant reduction was observed in the levels of cardiac IRS-1 in rats fed high fructose diet (p< 0.0001), compared to control rats. Carnitine administration slightly elevated the level of IRS-1 toward control but this elevation was not significant as compared to HFD group. Administration of cinnamon improved the receptor level (p<0.001), compared to HFD group (Fig. 1).

Figure 2 illustrate that there was significant elevation in the expression of cardiac IGF-1 in groups treated with carnitine and cinnamon (p<0.001), compared to HFD group. The expression of iNOS was significantly elevated (p<0.0001) in HFD group, compared to control. Oral administration of cinnamon reduced significantly (p<0.001) the iNOS expression, compared to HFD group.

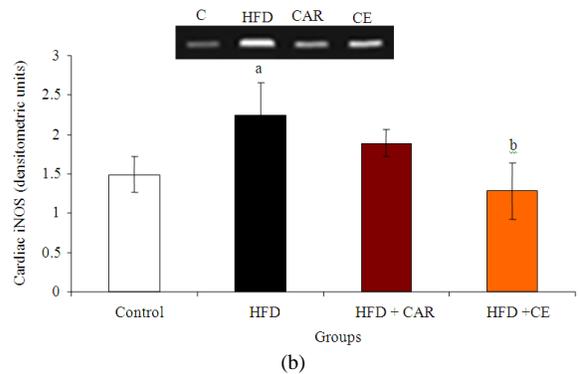
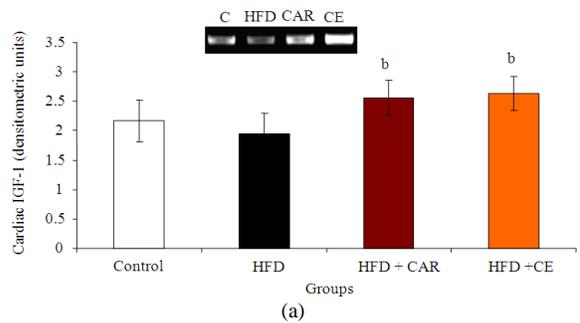


Fig. 2: Effects of high fructose diet, L-carnitine and cinnamon extract on cardiac IGF-1 (a) and iNOS (b) expression

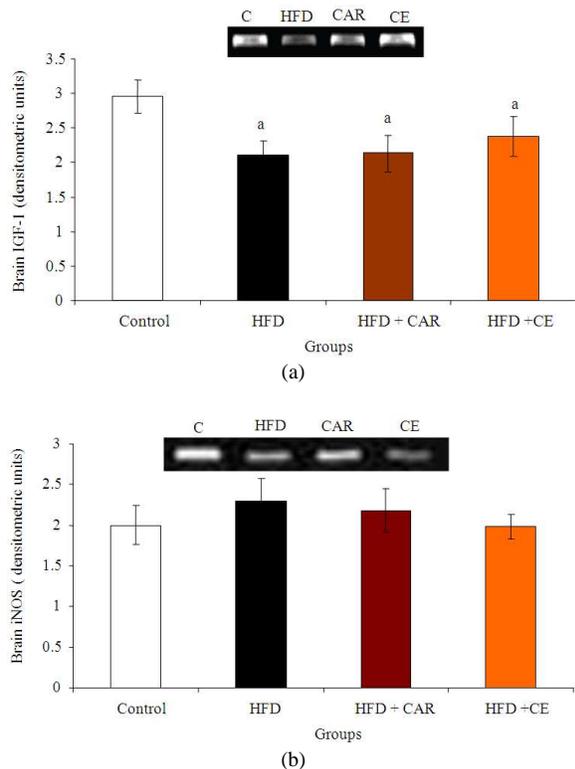


Fig. 3: Effects of high fructose diet, L-carnitine and cinnamon extract on brain IGF-1 (a) and iNOS (b) expression.

In regard to brain tissue, the expression of IGF-1 was significantly reduced in groups fed HFD ($p < 0.0001$) with non significant changes in the expression of iNOS, compared to control group (Fig. 3).

DISCUSSION

Metabolism of dietary fructose which occurs mainly in liver differs from that of glucose. Fructose enters the glycolytic pathway at the triose level. This difference in initial metabolism of fructose not only acutely affects carbohydrate metabolism by changing the supply of intermediates, but also induces metabolic adaptation including changes in gene expression (Koo *et al.*, 2008).

The present study illustrates that feeding high fructose diet for 4 weeks caused IR, which characterized by hyperglycemia, hyperinsulinemia and hypertriglyceridemia. These results are in line with those of Nandhini *et al.* (2002) and Mahfouz *et al.* (2009).

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) has emerged as a practical and

simple method for estimating insulin resistance. While this method is currently not routinely used as a cardiovascular risk marker, Bertoluci *et al.* (2010) hypothesized that it could be potentially useful since hyperglycemia and hyperinsulinemia are both related to cardiovascular diseases.

HOMA index was significantly elevated in rats fed the fructose diet. Administration of L-carnitine and CE reduced significantly the HOMA index. The insulin level did not change significantly in the serum of carnitine treated rats, while cinnamon treated rats reduced the level to the control value.

The present work showed significant elevation in the cardiac TG and MDA concentrations as compared to control group. The rate of uptake of lipids, unlike that of glucose, is not regulated by a hormone. Therefore, increased circulating lipids will increase uptake and thereby fatty acid oxidation (Clerk *et al.*, 2002). When the rate of uptake is greater than the rate of oxidation, fatty acids are shuttled to the TG synthesis pathway. Fructose loaded rats, which showed the characteristic features of IR, also displayed an imbalance between the peroxidation process and the antioxidant system. These results agree with (Busserolles *et al.*, 2002), who studied the effects of two weeks of high sucrose on both oxidative stress parameters and stress-related gene expression in rat heart. Both increased TBARS and lower Cu-Zn-SOD activity were found in heart, despite the elevation in Cu-Zn-SOD mRNA level from high sucrose fed rats compared to rats on a starch diet.

Accumulation of palmitate in cardiomyoblasts produces both increased reactive oxygen and ceramide production, resulting in apoptosis (Wang *et al.*, 2006) which cause cardiac hypertrophy.

The administration of L-carnitine or cinnamon extract to insulin resistant rats mitigated the adverse effect of fructose load on the glycemic status. In addition, they reduced the TG accumulation and the lipid peroxidation in the cardiac tissue.

Rodrigues *et al.* (1988) and Solfrizzi *et al.* (2006) stated that L-carnitine supplementation is beneficial for the treatment and prevention of diabetes associated metabolic and cardiac defects, since L-carnitine facilitates the transport of fatty acids across the mitochondrial membrane for eventual oxidation and energy production.

The present results for cinnamon effects are in line with those of Qin *et al.* (2004) who stated that cinnamon extract fed to animals consuming a high-fructose diet prevents the development of the metabolic syndrome. Cinnamon bark was reported to have antioxidant effects by increasing the activities of

antioxidant enzymes including glutathione S-transferase, superoxide dismutase and catalase in rat livers and hearts (Bell *et al.*, 2008).

The present study attributed the marked reduction in the cardiac level of IRS-1 in rats fed high fructose to the oxidative stress. This in agreement with the findings reported by Bezerra *et al.* (2000) and Catena *et al.* (2003) who found significant reductions in hepatic and skeletal muscle IRS-1 tyrosine phosphorylation and receptor numbers in fructose fed rats. In addition, it was observed that exposure of hepatoma cells to an oxidant stress (H₂O₂) induced increases in phosphorylation at serine 307 and serine 632 of IRS-1 (Bloch-Damti *et al.*, 2003) and this oxidant exposure was associated with degradation of the IRS-1 protein (Potashnik *et al.*, 2003).

L- carnitine and CE improved the IR in the fructose loaded rats. The effect of aqueous extract of cinnamon was accompanied by remarkable increase in the level of IRS-1, while the improvement produced by carnitine was toward the normal control level, but still less than that of cinnamon.

Results of Qin *et al.* (2004) suggested that CE administration to HFD-fed rats prevents the development of insulin resistance at least in part by enhancing insulin signaling. Water-soluble cinnamon compounds stimulate the autophosphorylation of the insulin receptor and inhibit phosphotyrosine phosphatase activity that inactivates the insulin receptor leading to increased insulin sensitivity (Imparl-Radosevich *et al.*, 1998). Also, these polyphenols increase the amount of insulin receptor β protein (Cao *et al.*, 2007).

Cardiac cells of diabetic patients do not develop IR to the same extent as seen in skeletal muscle. Some studies have not observed IR in hearts of type II diabetic patients, even when other diabetic tissues of the same patients exhibit significant IR (Utriainen *et al.*, 1998; Jagasia *et al.*, 2001). Thus under conditions of diabetic hyperinsulinemia, the less-resistant heart is being stimulated by relatively high levels of insulin. Consequently, this can explain the non significant reduction observed in the cardiac IGF-1 expression in the studied rats fed HFD, since insulin promotes the action of IGF-1. In the present work supplementation with either L-carnitine or CE increased significantly the IGF-1 expression as compared to HFD group. This elevation may be due to the improved glycemic status. Consequently, IGF-1 enhances insulin sensitivity and increases plasma glucose disappearance and tissue glucose utilization (Conti *et al.*, 2001).

Inflammation induces the expression of iNOS which produces inflammatory mediator NO (Kumar *et al.*, 2004). The expression of iNOS is upregulated by most,

if not all, inducers of insulin resistance, including proinflammatory cytokines, free fatty acids (Shimabukuro *et al.*, 1998), hyperglycemia and oxidative stress (Ceriello *et al.*, 2002). In fact, elevated expression of iNOS was observed in skeletal muscle of high fat diet-fed mice (Perreault and Marette, 2001), in heart of Zucker diabetic fatty rats (Zhou *et al.*, 2000) and in skeletal muscle of patients with type 2 diabetes (Torres *et al.*, 2004).

In this study, non significant change in the cardiac level of NO product was observed, despite the elevation of the cardiac iNOS expression associated with IR which induced by feeding rats with high fructose diet. This may be due to either post-transcriptional or translational regulations of iNOS expression or due to deficiency of one or more of cofactors required for NOS activity. Alonso *et al.* (1997) reported that Tumor Necrosis Factor- α (TNF α) involved in numerous processes in the cardiovascular system, reducing nitric-oxide synthase gene expression through post-transcriptional regulation of mRNA stability. The non significant level of cardiac NO may also be partly mediated by scavenging of NO by superoxide anions (generated due to the oxidative stress state) to produce peroxynitrite (NO₃⁻). Tetrahydrobiopterin (essential NOS cofactor, BH₄) is efficiently oxidized to 7,8-dihydrobiopterin (BH₂) by NO₃⁻ in vitro and in vivo. With deficiency of BH₄, NOS uncoupling can predominate (Upmacis *et al.*, 2007).

Although L-carnitine and CE administration improved the expression of iNOS gene, the impact of CE was more pronounced. Kanuri *et al.* (2009) stated that cinnamon extract was associated with an inhibition of the iNOS mRNA expression found in livers of alcohol-treated animals. These results confirm that the oxidative stress was the reason of that alteration in iNOS expression. Inhibition of iNOS expression has been recently proposed to be a new mechanism of actions of insulin sensitizers (Kohnen *et al.*, 2001). Perreault and Marette (2001) reported that iNOS deficiency protected against high fat diet induced insulin resistance.

A number of studies have been reported concerning the effects of insulin on circulating lipid or lipid contents in the peripheral tissues. However, lipid metabolism in the central nervous system CNS with insulin resistance/diabetes is not well characterized in spite of its potential importance.

This study demonstrates that feeding rats with high fructose diet for 4 weeks has no effect on the brain lipid content.

Brain is the most cholesterol-rich organ, containing approximately 25% of the cholesterol present in the body and most of its cholesterol is a product of local

synthesis (Kurban *et al.*, 2007). Kalman *et al.* (2001) found that, after 10 weeks of hypercholesterolemic diet the rabbits developed significant increase in plasma and liver cholesterol levels. In contrast, the cholesterol content of the brain cortex did not change. In addition, Haris *et al.* (1993) showed that cholesterol content of brain is not affected by either high fat or high cholesterol diet, which confirms the present results.

This study concerning the expression of brain IGF-1 and iNOS illustrated the presence of reduction in the expression of IGF-1 with no change in iNOS gene in both untreated and treated HFD groups.

These results agree with Cheng *et al.* (2003) who stated that systemic glucose levels possibly regulate IGF-1 expression in brain, given that blood glucose levels were reduced in rats fed caloric restriction diets either carbohydrate-enriched or isocaloric fat-based diet. However brain IGF-1 mRNA levels were equally reduced in both groups, whereas glucose levels were more profoundly reduced in the fat-based diet. He postulated that another unidentified factor related to nutritional status influences brain IGF-1 expression.

Brain inflammation occurs behind the blood-brain barrier and thus differs from inflammation in the periphery by the relative absence of macrophages. Microglia are brain macrophages that produce oxidants and induce expression of iNOS if they detect pathogens or damage (Brown, 2007). This study showed non significant alteration in brain lipid peroxidation, consequently no brain damage.

Concerning all these, we postulate that brain is not affected with the IR state present in that model.

CONCLUSION

This study illustrates that cinnamon aqueous extract was more potent than L-carnitine in improving the IR.

REFERENCES

- Alonso, J., L. Sanchez de Miguel, M. Monton, S. Casado and A. Lopez-Farre, 1997. Endothelial cytosolic proteins bind to the 3 untranslated region of endothelial nitric oxide synthase mRNA: Regulation by tumor necrosis factor alpha. *Mol. Cell. Biol.*, 17: 5719-5726. PMID: PMC232420
- Anderson, R.A., 2008. Chromium and polyphenols from cinnamon improve insulin sensitivity. *Proc. Nutr. Soc.*, 67: 48-53. DOI: 10.1017/S0029665108006010
- Bell, D.S., J.H. O'Keefe and P. Jellinger, 2008. Postprandial dysmetabolism: The missing link between diabetes and cardiovascular events. *Endocr. Pract.*, 14: 112-124. PMID: 18238751
- Bertolucci, M.C., A.S. Quadros, R. Sarmiento-Leite and B.D. Schaan, 2010. Insulin resistance and triglyceride/HDLc index are associated with coronary artery disease. *Diabetol. Metab. Syndr.*, 2: 11. PMID: PMC2830967
- Bezerra, R.M., M. Ueno, M.S. Silva, D.Q. Tavares and C.R. Carvalho *et al.*, 2000. A high fructose diet affects the early steps of insulin action in muscle and liver of rats. *J. Nutr.*, 130: 1531-1535. PMID: 10827205
- Bloch-Damti, A., R. Potashink, J.F. Tanyi, Y. le Marchand-Brustel and A. Rudish *et al.*, 2003. IRS-1 protein degradation induced by oxidative stress is associated with distinct time-course of phosphorylation on serine 307 and serine 632. *Diabetologia*, 46: A209-A209.
- Brown, G.C., 2007. Mechanisms of inflammatory neurodegeneration: INOS and NADPH oxidase. *Biochem. Soc. Trans.*, 35: 1119-1121. DOI: 10.1042/BST0351119
- Busserolles, J., W. Zimowska, E. Rock, Y. Rayssiguier and A. Mazur, 2002. Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. *Life Sci.*, 71: 1303-1312. DOI: 10.1016/S0024-3205(02)01846-5
- Cao, H., M.M. Polansky and R.A. Anderson, 2007. Cinnamon extract and polyphenols affect the expression of tris-tetraprolin, insulin receptor and glucose transporter 4 in mouse 3T3-L1 adipocytes. *Arch. Biochem. Biophys.*, 459: 214-222. DOI: 10.1016/j.abb.2006.12.034
- Catena, C., G. Giacchetti, M. Novello, G. Colussi and A. Cavarape *et al.*, 2003. Cellular mechanisms of insulin resistance in rats with fructose-induced hypertension. *Am. J. Hypertens*, 16: 973-978. DOI: 10.1016/S0895-7061(03)01002-1
- Ceriello, A., L. Quagliaro, M.D. Amico, C. di Filippo and R. Marfella *et al.*, 2002. Acute hyperglycemia induces nitrotyrosine formation and apoptosis in perfused heart from rat. *Diabetes*, 51: 1076-1082. DOI: 10.2337/diabetes.51.4.1076
- Cheng, C.M., R.R. Reinhardt, W.H. Lee, G. Joncas and S.C. Patel *et al.*, 2000. Insulin-like growth factor-1 regulates developing brain glucose metabolism. *Proc. Natl. Acad. Sci. USA*, 97: 10236-10241. PMID: 10954733
- Cheng, C.M., B. Kelley, J. Wang, D. Strauss and D.A. Eagles *et al.*, 2003. A ketogenic diet increases brain insulin-like growth factor receptor and glucose transporter gene expression. *Endocrinology*, 144: 2676-2682. DOI: 10.1210/en.2002-0057
- Clerk, L.H., S. Rattigan and M.G. Clark, 2002. Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake *in vivo*. *Diabetes*, 51: 1138-1145. DOI: 10.2337/diabetes.51.4.1138

- Conti, E., F. Andreotti, A. Sciahbasi, P. Riccardi and G. Marra *et al.*, 2001. Markedly reduced insulin-like growth factor-1 in the acute phase of myocardial infarction. *J. Am. Coll. Cardiol.*, 38: 26-32. PMID: 11451284
- Dawson, B.K., 2000. *Basic and Clinical Biostatistics*. 3rd Edn., Appleton and Lange, USA., ISBN: 0838505104, pp: 399.
- Deedwania, P.C. and R. Gupta, 2006. Management issues in the metabolic syndrome. *J. Assoc. Physicians India*, 54: 797-810. PMID: 17214277
- Esterbauer, H. and K.H. Cheesman, 1990. Determination of aldehyde lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.*, 186: 407-410. PMID: 2233308
- Foster, L.B. and R.T. Dunn, 1973. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clin. Chem.*, 19: 338-340. <http://www.clinchem.org/cgi/reprint/19/3/338.pdf>
- Frystyk, J. and H. Orskov, 2004. IGF-I, IGF-II, IGF-Binding Proteins and Diabetes. In: *International Textbook of Diabetes Mellitus*, De Fronzo, R.A., Ferrannini, E., H. Keen and P. Zimmet (Eds.). John Wiley, Chichester, pp: 1685-1694.
- Fujimoto, M., N. Shimizu, K. Kunii, J.A. Martyn and K. Ueki *et al.*, 2005. A role for iNOS in fasting hyperglycemia and impaired insulin signaling in the liver of obese diabetic mice. *Diabetes*, 54: 1340-1348. DOI: 10.2337/diabetes.54.5.1340
- Haris, K.B., H.R. Cross, W.G. Pond and H.J. Mersmann, 1993. Effect of dietary fat and cholesterol level on tissue cholesterol concentrations of growing pigs selected for high or low serum cholesterol. *J. Anim. Sci.*, 71: 807-810. <http://jas.fass.org/cgi/content/abstract/71/4/807>
- Hill, D.J. and R.D. Milner, 1985. Insulin as a growth factor. *Pediatr. Res.*, 19: 879-886. PMID: 2413420
- Hwang, I.S., B.B. Hoffman and G.M. Reaven, 1987. Fructose-induced insulin resistance and hypertension in rats. *Hypertension*, 10: 512-516. PMID: 3311990
- Imparl-Radosevich, J., S. Deas, M.M. Polansky, D.A. Baedke and T.S. Ingebritsen *et al.*, 1998. Regulation of PTP-1 and insulin receptor kinase by fractions from cinnamon: Implications for cinnamon regulation of insulin signaling. *Horm. Res.*, 50: 177-182. DOI: 10.1159/000023270
- Jagasia, D., J.M. Whiting, J. Concato, S. Pfau and P.H. McNulty, 2001. Effect of non-insulin-dependent diabetes mellitus on myocardial insulin responsiveness in patients with ischemic heart disease. *Circulation*, 103: 1734-1739. PMID: 11282903
- Kalman, J., B.J. Kudchodkar, R. Krishnamoorthy, L. Dory and A.G. Lacko *et al.*, 2001. High cholesterol diet down regulates the activity of activator protein-1 but not nuclear factor-kappa B in rabbit brain. *Life Sci.*, 68: 1495-1503. DOI: 10.1016/S0024-3205(01)00955-9.
- Kannappan, S., T. Jayaraman, P. Rajasekar, M.K. Ravichandran and C.V. Anuradha, 2006. Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. *Singapore Med. J.*, 47: 858-863. PMID: 16990960
- Kanuri, G., S. Weber, V. Volynets, A. Spruss and S.C. Bischoff *et al.*, 2009. Cinnamon extract protects against acute alcohol-induced liver steatosis in mice. *J. Nutr.*, 139: 482-487. DOI: 10.3945/jn.108.100495.
- Kohnen, S.L., A.A. Mouithys-Mickalad, G.P. Deby-Dupont, C.M. Deby and M.L. Lamy *et al.*, 2001. Oxidation of tetrahydrobiopterin by peroxynitrite or oxoferryl species occurs by a radical pathway. *Free Radic. Res.*, 35: 709-721. DOI: 10.1080/10715760100301221
- Koo, H., M.A. Wallig, B.H. Chung, T.Y. Nara and B.H. Simon *et al.*, 2008. Dietary fructose induces a wide range of genes with distinct shift in carbohydrate and lipid metabolism in fed and fasted rat liver. *Biochim. Biophys. Acta*, 1782: 341-348. DOI: 10.1016/j.bbadis.2008.02.007
- Kuboki, K., Z.Y. Jiang, N. Takahara, S.W. Ha and M. Igarashi *et al.*, 2000. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and *in vivo*: A specific vascular action of insulin. *Circulation*, 101: 676-681. PMID: 10673261
- Kumar, V., N. Fausto and A. Abbas, 2004. *Acute and Chronic Inflammation*. In: *Robbins and Cotran Pathologic Basis of Disease*. 7th Edn., Saunders, Philadelphia, ISBN: 0721601871, pp: 1552.
- Kurban, S., I. Mehmetoglu and G. Yilmaz, 2007. Effect of diet oils on lipid levels of the brain of rats. *Indian J. Clin. Biochem.*, 22: 44-47.
- Mahfouz, M.H., H.M. Ghanem and M.A. Mohamed, 2009. Therapeutic effect of L-carnitine on sialic acid, soluble Fas (sFas) and other biochemical variables in hyperinsulinemic rats. *Life Sci. J.*, 6: 76-82.
- Mahfouz, M.H., H.M. Ghanem and M.A. Mohamed, 2010. Modulation of insulin receptor substrate-1 and some inflammatory variables in hyperinsulinemic rats treated with cinnamon extract. *Am. J. Biochem. Biotech.*, 6: 11-18. DOI: 10.3844/ajbbsp.2010.11.18
- Marschner, I., P. Bottermann, F. Erhardt, R. Linke and G. Löffler *et al.*, 1974. Group experiments on the radioimmunological insulin determination. *Horm. Metab. Res.*, 6: 293-296. PMID: 4413601

- Matthews, D.R., J.P. Hosker, A.S. Rudenski, B.A. Naylor and D.F. Treacher *et al.*, 1985. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28: 412-419. DOI: 10.1007/BF00280883
- Moshage, H., B. Kok, J. Huizenga and P. Junsen, 1995. Nitrite and nitrate determinations in plasma: A critical evaluation. *Clin. Chem.*, 41: 892-896. PMID: 7768008
- Nandhini, A.T., S.D. Balakrishnan and C.V. Anuradha, 2002. Taurine improves lipid profile in rats fed a high fructose-diet. *Nutr. Res.*, 22: 343-354.
- Perreault, M. and A. Marette, 2001. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat. Med.*, 7: 1138-1143. PMID: 11590438
- Potashnik, R., A. Bloch-Damti, N. Bashan and A. Rudish, 2003. IRS-1 degradation and increased serine phosphorylation can not predict the degree of metabolic insulin resistance induced by oxidative stress. *Diabetologia*, 46: 639-648. DOI: 10.1007/s00125-003-1097-5
- Qin, B., M. Nagasaki, M. Ren, G. Bajotto and Y. Oshida *et al.*, 2004. Cinnamon extract prevents the insulin resistance induced by a high-fructose diet. *Horm. Metab. Res.*, 36: 119-125. DOI: 10.1055/s-2004-814223.
- Rajasekar, P. and C.V. Anuradha, 2007. Effect of L-carnitine on skeletal muscle lipids and oxidative stress in rats fed high-fructose diet. *Exp. Diabetes Res.*, 2007: 727-741. PMID: 17641743
- Refaie, F.M., A.Y. Esmat, S.M. Abdel Gawad, A.M. Ibrahim and M.A. Mohamed, 2005. The antihyperlipidemic activities of 4(3H) quinazolinone and two halogenated derivatives in rats. *Lipids Health Dis.*, 4: 22-32. DOI: 10.1186/1476-511X-4-22
- Rodrigues, B., H. Xiang and J.H. McNeill, 1988. Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. *Diabetes*, 37: 1358-1364. DOI: 10.2337/diabetes.37.10.1358
- Shaker, O.G., D.A. Sourour and M. Taha, 2009. Effects of statins on insulin-like growth factor-1 gene expression in fructose induced metabolic syndrome in rats. *Open Endocrinol. J.*, 3: 28-33. <http://www.bentham.org/open/toej/articles/V003/28TOEJ.pdf>
- Shimabukuro, M., M. Higa, Y.T. Zhou, M.Y. Wang and C.B. Newgard *et al.*, 1998. Lipopoptosis in beta-cells of obese prediabetic fa/fa rats: Role of serine palmitoyl transferase overexpression. *J. Biol. Chem.*, 273: 32487-32490. DOI: 10.1074/jbc.273.49.32487
- Shinozaki, K., A. Kashiwagi, Y. Nishio, T. Okamura and Y. Yoshida *et al.*, 1999. Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent relaxation through nitric oxide/O₂ imbalance in insulin-resistant rat aorta. *Diabetes*, 48: 2437-2445. DOI: 10.2337/diabetes.48.12.2437
- Solfrizzi, V., C. Capurso, A.M. Colacicco, A.D. Intronio and C. Fontana *et al.*, 2006. Efficacy and tolerability of combined treatment with L-carnitine and simvastatin in lowering lipoprotein (a) serum levels in patients with type 2 diabetes mellitus. *Atherosclerosis*, 188: 455-461. DOI: 10.1016/j.atherosclerosis.2005.11.024
- Taimor, G., A. Rakow and H.M. Piper, 2001. Transcription Activator Protein 1 (AP-1) mediates NO-induced apoptosis of adult cardiomyocytes. *FASEB J.*, 15: 2518-2520. DOI: 10.1096/fj.01-0353fje
- Torres, S.H., J.B. de Sanctis, M. de L. Briceno, N. Hernandez and H.J. Finol, 2004. Inflammation and nitric oxide production in skeletal muscle of type 2 diabetic patients. *J. Endocrinol.*, 181: 419-427. PMID: 15171690
- Upmancis, R.K., M.J. Crabtree, R.S. Deeb, H. Shen and P.B. Lane *et al.*, 2007. Profound biopterin oxidation and protein tyrosine nitration in tissues of ApoE-null mice on an atherogenic diet: Contribution of inducible nitric oxide synthase. *Am. J. Physiol. Heart Circ. Physiol.*, 293: 2878-2887. DOI: 10.1152/ajpheart.01144
- Utriainen, T., T. Takala, M. Luotolahti, T. Ronnema and H. Laine *et al.*, 1998. Insulin resistance characterizes glucose uptake in skeletal muscle but not in the heart in NIDDM. *Diabetologia*, 41: 555-559. PMID: 9628273
- Wang, J., Y. Song, Q. Wang, P.M. Kralik and P.N. Epstein, 2006. Causes and characteristics of diabetic cardiomyopathy. *Rev. Diabet. Study*, 3: 108-117. DOI: 10.1900/RDS.2006.3.108
- Yousef, M., M. Haroun, M. El-Masry and R. Ateia, 2006. Biochemical and immunological study on the effects of barley and its components as hypoglycemic agents in diabetic rats. *Am. J. Biochem. Biotech.*, 2: 1-8. DOI: 10.3844/ajbbsp.2006.1.8
- Zhou, Y.T., P. Grayburn, A. Karim, M. Shimabukuro and M. Higa *et al.*, 2000. Lipotoxic heart disease in obese rats: Implications for human obesity. *Proc. Natl. Acad. Sci. USA.*, 97: 1784-89. PMID: 10677535