Taurine modifies insulin signaling enzymes in the fructose-fed insulin resistant rats

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SUMMARY

Objectives: High fructose feeding induces insulin resistance and hyperinsulinemia in rats. The present study was proposed to elucidate the derangements in the insulin signaling pathway in high fructose-fed rats and whether taurine, a sulphur-containing amino acid could improve insulin action by modulating the signal transduction pathway.

Methods: Male Wistar rats of body weight 170-190 g were divided into 4 groups of 6 rats each. Control rats received control diet and water ad libitum. Fructose fed animals received high fructose diet (> 60% of total calories) and water ad libitum. Fructose + taurine rats received fructose diet and 2% taurine solution ad libitum. Control + taurine rats received control diet and 2% taurine solution ad libitum. After the experimental period of 30 days, the effects of taurine on certain parameters on glucose metabolism were determined. The activities of protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTP) were assayed in liver.

Results: The activities of the glycolytic enzymes were significantly lower while the activities of the glucoegenic enzymes were higher in untreated fructose-fed rats as compared to control animals. Depletion of liver glycogen was observed in fructose-fed rats. Fructose-fed rats showed alterations in the activities of insulin signaling enzymes PTK and PTP. Taurine administration improved insulin sensitivity and controlled hyperglycemia and hyperinsulinemia in fructose-fed rats. Taurine treatment also restored the glucose metabolizing enzyme activities in fructose-fed rats.

Conclusions: Taurine supplementation might have a beneficial effect in overcoming insulin resistance and its associated abnormalities by modifying the post-receptor events of insulin action.

Key-words: Fructose · Taurine · Insulin signaling · Glucose metabolism · Glycogen.

RÉSUMÉ

La taurine modifie les enzymes de la voie de signalisation de l’insuline dans un modèle de rats rendus insulino-résistants par un régime enrichi en fructose

Objectifs : Un régime enrichi en sucrose entraîne chez le rat une insulinorésistance et une hyperinsulinémie. Cette étude avait pour but de préciser dans ce modèle les anomalies de la voie de signalisation de l’insuline induites par un régime enrichi en sucrose, et de chercher si la taurine, aminoacide contenant un sulfure, était susceptible d’améliorer l’action de l’insuline en modulant la voie de transmission du signal.

Méthodes : Des rats Wistar males pesant de 170 g à 190 g ont été répartis en 4 groupes de six rats. Les rats témoins recevaient un régime standard et de l’eau à volonté. Les rats du groupe fructose ont été soumis à un régime enrichi en fructose (> 60 % des calories totales) de l’eau à volonté. Les rats du groupe fructose + taurine ont été soumis à un régime enrichi en fructose et ont reçu une solution de taurine 2 % à volonté. Les rats du groupe témoin + taurine ont été soumis à un régime standard et ont reçu une solution de taurine 2 % à volonté. Après une période expérimentale de 30 jours, les effets de la taurine sur certains paramètres métaboliques du glucose ont été déterminés. L’activité hépatique de la protéine tyrosine kinase (PTK) et celle de la protéine tyrosine phosphatase (PTP) ont été mesurées.

Résultats : L’activité des enzymes de la glycolyse était significativement plus basse et celle des enzymes de la glucogénèse plus haute chez les rats recevant un régime en fructose comparés aux rats témoins. Une déplication en glycogène hépatique a été observée chez les rats recevant un régime enrichi en fructose. Les rats recevant un régime enrichi en fructose présentaient des altérations des enzymes de la voie de signalisation de l’insuline PTK et PTP. L’administration de taurine a amélioré l’insulinosensibilité et contrôlé l’hyperglycémie et l’hyperinsulinémie des rats recevant un régime enrichi en fructose. Le traitement par la taurine resta à la même activité des enzymes impliquées dans le métabolisme du glucose chez les rats recevant un régime enrichi en fructose.

Conclusions : Une supplémentation en taurine pourrait avoir un effet bénéfique vis à vis l’insulinorésistance et des anomalies associées, en modifiant les événements post-récepteur de l’action de l’insuline.

Mots-clés : Fructose · Taurine · Signalisation de l’insuline · Métabolisme du glucose · Glycogène.
The insulin resistance described in an animal model involving a high fructose diet is associated with glucose intolerance, increased insulin concentration and decreased insulin sensitivity [1]. A high fructose diet alters the activity of several enzymes regulating hepatic carbohydrate metabolism and lowers insulin-stimulated glucose oxidation leading to hepatic insulin resistance [2, 3].

Recent insights into the mechanism of insulin action have demonstrated that reversible tyrosine phosphorylation of the insulin receptor and its cellular substrate proteins play a crucial role in the mechanism of insulin action [4]. Insulin signaling is initiated by binding of insulin to the extracellular α-subunit of insulin receptor, resulting in the stimulation of β-subunit, which contains intrinsic receptor tyrosine kinase activity, autophosphorylation of the receptor at multiple tyrosine residues [5, 6]. Autophosphorylation of the receptor enhances the intrinsic tyrosine kinase activity and evokes a series of phosphorylation events. These include tyrosyl phosphorylation of intracellular substrates named insulin receptor substrates (IRS 1 to 4) and other molecules. The phosphorylated proteins mediate the cellular actions of insulin.

Protein tyrosine phosphatases (PTP) are the enzymes responsible for the selective dephosphorylation of tyrosine residues. The extent of tyrosyl phosphorylation on a given protein is controlled by the reciprocal actions of protein-tyrosine kinase (PTK) and PTP activities. Increased activities of PTP have been reported in liver of fructose-fed rats [7] and in several insulin-resistant states including obesity and in some models of diabetes [8]. Specific inhibition of particular PTPs that are negative modulators of insulin action may have potential to improve insulin sensitivity [9].

Taurine, 2 amino ethane sulphonic acid, is one of the most abundant amino acids in the plasma and cytosol. Besides its role in bile acid conjugation, taurine has been shown to be involved in various important physiological functions such as maintenance of the structural integrity of the membrane, regulation of calcium homeostasis, modulation of protein phosphorylation, osmoregulation, neurotransmission and neuromodulation [10].

Exogenous taurine has been implicated to modulate carbohydrate metabolism. For e.g. the antidiabetic action of taurine has been demonstrated earlier in experimental animals [11]. Some observations suggest that taurine affects glucose utilization and interacts with insulin receptor [12]. Previously we have reported that taurine improves glucose metabolism in fructose-fed rats presumably via improved insulin action and glucose tolerance [2].

The importance of taurine in diabetes is well-documented [13] and accumulated evidence show that diabetic condition is associated with taurine depletion [14]. However, the mechanisms by which taurine could act have not been identified. Thus in the present study, we have attempted to investigate the specific locations of impairment of insulin signaling pathway in the liver of insulin resistant rats and the modulatory effects of taurine.

Materials and methods

Materials

Taurine was purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals were of analytical grade procured from local commercial sources.

Animal model

Male adult Wistar rats of body weight ranging from (170-190 g) were obtained from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Annamalai University. They were housed two per cage under controlled conditions (22-25°C) on a 12 h light/12 h dark cycle. They all received a standard pellet diet (Karnataka State Agro Corporation Ltd, Agro feeds division, Bangalore, India) and water ad libitum. The animals used in the present study were cared for as per the principals and guidelines of the Institutional Animal Ethical Committee, Annamalainagar in accordance with the Indian National Law on animal care and use.

Experimental groups

After acclimatisation the animals were divided into the following groups consisting of 12 rats each.

Group 1 — Control animals (CON) received the control diet containing starch and tap water ad libitum.

Group 2 — Fructose-fed animals (FRU) received the fructose-enriched diet and water ad libitum.

Group 3 — Fructose-fed animals (FRU-TAU) received the fructose diet and were allowed to drink 2% taurine solution ad libitum.

Group 4 — Control animals (CON-TAU) received the control diet and were given 2% taurine solution ad libitum.

The composition of control and fructose diet are given in Table I. The diets were prepared fresh daily.

The animals were maintained in their respective groups for 30 days. Food intake, fluid intake and body weight changes were measured regularly.

Insulin sensitivity tests

An oral glucose tolerance test was carried out in awaked rats two days before the sacrifice. For this the rats were fasted overnight and glucose (2 g/kg b.w) was given from 30% solution by oral gavage. Tail blood samples were collected before glucose load and sequentially for every half an hour after glucose load up to 90 minutes and were immediately analysed for glucose.
Assay for PTP activity

PTP activity in the liver homogenate was determined by the method of Chen et al. [22]. Liver tissue was stimulated with insulin by opening the abdomen and exposing the portal vein by injecting $10^3 \text{ mol/L}$ insulin. Within 30s, the liver sample was removed, minced coarsely with the buffer. The tissue homogenate was prepared with Tris-EDTA-sucrose (TES) buffer and then centrifuged at 400 g, 4°C to sediment nuclei. An aliquot of the tissue homogenate (0.05 ml) was added to 450 µl of reaction mixture which contained 50 mM morpholino ethane sulphonylic acid, 150 mM NaCl, 2.5 mM EDTA, 0.1% bovine serum albumin, 2 mM dithiothreitol and 50 mM p-nitro phenyl phosphate and incubated for 10 min at 30°C. The reaction was stopped by the addition of 500 ml of 2M KOH and the amount of product (p-nitrophenol) produced was measured by determining the absorbance at 405 nm in a spectrophotometer. Nonspecific absorbance was corrected for by subtracting the absorbance at 405 nm determined in the absence of tissue homogenate. p-nitrophenol (5-25 mmol) was used as the standard. Values were expressed as mmoles of p-nitrophenol liberated/min/mg protein.

Statistical analysis

All the values were expressed as means ± SD of 6 rats from each group and statistically evaluated by two-way analysis of variance considering diet and treatment as two factors. When significance was found, the means were tested for significance by Tukey’s test for multiple comparisons. A value of $P < 0.05$ was considered significant.

Results

Body weight gain was similar for all groups and the final body weights were not significantly different from
each other (CON-178.33 g ± 1.97; FRU-182.83 g ± 4.81; FRU+TAU-181.33 g ± 3.24; CON+TAU-178.50 g ± 2.63).

The food intake was 84.9 ± 10.1 g/kg b.w/day for rats fed the high fructose diet and 83.8 ± 11.21 g/kg b.w/day for those fed the control diet. Food intake was not significantly different from each other throughout the experiment.

Taurine administered fructose-fed and control rats showed significantly higher fluid intake (P < 0.05) than that of untreated control or fructose-fed animals. The daily intake of taurine was about 1-1.5 g/kg b.w, which amounts to an average intake of 35-40 g/kg b.w over the treatment period of 30 days.

The content of taurine was significantly decreased in plasma (µmol/L) (CON-102.1 ± 4.17; FRU-92.0 ± 8.2; FRU+TAU-143.0 ± 5.8; CON+TAU-158.0 ± 10.6) and liver (µmol/mg protein) (CON-441.8 ± 22.4; FRU-402.0 ± 27.6; FRU+TAU-494.0 ± 23.4; CON+TAU-517.7 ± 26.0) of the fructose-fed rats as compared to control rats. Significance was found at p < 0.05. Supplementation restored taurine levels in plasma and liver.

Table II gives the levels of plasma glucose, fructose and insulin values of control and experimental animals respectively. The levels were significantly elevated in fructose-fed rats as compared to control rats. Treatment with taurine caused significant reduction in the levels in fructose-fed rats as compared to untreated fructose-fed rats.

Table III summarizes the activities of glucose metabolising enzymes and glycogen content in liver of control and experimental animals. The activities of enzymes hexokinase, glucose-6-phosphate dehydrogenase and pyruvate kinase were significantly decreased whereas the activities were significantly increased for glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase in fructose-fed rats as compared to control rats. Taurine treatment prevented these alterations and the enzyme activities were normal in taurine treated fructose rats. The activities remained unaltered in control rats treated with taurine. Significant reduction in liver glycogen content was observed in fructose-fed rats as compared with those of normal rats. The glycogen content in the liver was near normal in taurine-treated fructose rats and control rats.

Figure 1 depicts the results of the oral glucose tolerance test in experimental animals. The fasting glucose level was higher in fructose-fed rats as compared to control rats and the level was significantly lower in fructose-fed rats treated with taurine than the untreated fructose-fed rats. Significant elevations were observed in the glucose levels after the oral glucose load at all the time points in fructose-fed rats. The response was normal in taurine-treated rats.

The mean SSPG levels during the continuous infusion of epinephrine, propranolol, exogenous insulin and glucose are shown in Figure 2. Untreated fructose-fed rats showed increased levels of glucose in response to infusion of exogenous insulin and glucose in the proposed concentrations. The supplementation of taurine to fructose-fed animals attenuated the effect of fructose. Thus taurine treated rats had a significantly lower SSPG value than the fructose-fed rats.

Figure 3 represents the PTP and PTK activity in liver of control and experimental animals. PTP activity was significantly increased by 40% whereas PTK activity was significantly decreased in liver of fructose-fed rats as compared to that of control rats. Taurine supplemented fructose rats showed significant reduction in the activity of PTP and a significant increase in the activity of PTK as compared to untreated fructose-fed rats. No significant alterations were observed in control rats supplemented with taurine.

Discussion

Feeding high dosage of fructose (60 g/100 g diet) can have deleterious metabolic effects including insulin resistance, hyperinsulinemia, hyperglycemia and glucose intolerance in rats. These phenomena have been attributed to the loss of in vivo insulin sensitivity and to a low level of insulin — stimulated glucose oxidation in liver, skeletal muscle and adipose tissue.

**Table II**

Levels of plasma glucose, insulin and fructose values of control and experimental animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>FRU</th>
<th>FRU+TAU</th>
<th>CON+TAU</th>
<th>Anova</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.68 ± 0.43</td>
<td>5.36 ± 0.13*</td>
<td>4.82 ± 0.34*</td>
<td>4.67 ± 0.36</td>
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</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>54.21 ± 4.03</td>
<td>90.70 ± 4.00*</td>
<td>59.63 ± 5.49*</td>
<td>57.40 ± 8.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Fructose (mmol/L)</td>
<td>0.42 ± 0.02</td>
<td>0.64 ± 0.07*</td>
<td>0.43 ± 0.09*</td>
<td>0.42 ± 0.02</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD from 6 animals in each group.
* compared with CON, P < 0.05; * compared with FRU, P < 0.05
(Two-way ANOVA followed by Tukey’s test). NS- not significant.
Taurine influences glucose metabolism in fructose rats

Increase in plasma glucose levels associated with hyper-insulinemia in fructose-fed rats suggests impaired insulin action. Under conditions of severe hyperglycemia, high dietary fructose leads to increased plasma fructose concentrations. Dietary consumption of fructose might affect the concentrations of fructose and fructose metabolites in blood and tissues.

The changes in the enzyme activities and glycogen content in fructose-fed rats are indicative of the liver being in the gluconeogenic state. The activities of the regulatory enzymes like glucokinase, glucose-6-phosphatase, glycogen synthase and hexokinase were altered during chronic fructose feeding leading to hepatic insulin resistance. Furthermore, fructose feeding has been shown to lead to a decrease in the ability of insulin to suppress activation of hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activity.

Reduction in the hepatic glycogen concentration has been reported in this model [24]. Fasting liver and soleus muscle glycogen were markedly reduced in fructose-fed rats compared with glucose-fed rats. Hyperglycemia per se can increase hepatic glycogen synthesis and contribute to the direct pathway to total glycogen synthesis in rats [25].

Decreased PTK activity and increased PTP activity in liver of fructose-fed rats has been observed in the present study. The PTP functions in concert with PTK to balance the cellular level of phosphotyrosine. Many studies have reported the aberrant regulation of insulin signaling pathways in this diet-induced rat model of insulin resistance.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>CON</th>
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<th>FRU+TAU</th>
<th>CON+TAU</th>
<th>Anova</th>
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<tbody>
<tr>
<td></td>
<td>Diet</td>
<td>Treatment</td>
<td>Interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (µmol/L)</td>
<td>102.1±4.17</td>
<td>92.0±8.2*</td>
<td>143.0±5.8**</td>
<td>158.0±10.6*</td>
<td>NS 0.05 0.05</td>
</tr>
<tr>
<td>Liver (µmol/mg protein)</td>
<td>441.8±22.4</td>
<td>402.0±27.6*</td>
<td>494.0±23.4**</td>
<td>517.0±27.0*</td>
<td>NS 0.05 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD from 6 animals in each group.
* compared with CON, P < 0.05; ** compared with FRU, P < 0.05.
(Two-way ANOVA followed by Tukey’s test). NS- not significant.

<p>| Table IV | Effect of taurine on certain enzymes of glucose metabolism and the glycogen content in liver of control and experimental animals. |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>FRU</th>
<th>FRU+TAU</th>
<th>CON+TAU</th>
<th>Anova²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 6 phosphatase (µg of Pi liberated /min/mg protein) x 10⁻²</td>
<td>4.05±0.63</td>
<td>6.15±0.53*</td>
<td>4.33±0.54#</td>
<td>4.06±0.40</td>
<td>0.05 0.05 0.05</td>
</tr>
<tr>
<td>Fructose 1,6 bis phosphatase (µg of Pi liberated /min/mg protein)</td>
<td>3.94±0.25</td>
<td>11.60±1.87*</td>
<td>5.09±0.39#</td>
<td>4.10±0.08</td>
<td>0.05 0.05 0.05</td>
</tr>
<tr>
<td>Hexokinase (µmoles of glucose phosphorylated/hr/mg protein)</td>
<td>0.22±0.006</td>
<td>0.18±0.007*</td>
<td>0.21±0.01#</td>
<td>0.22±0.01</td>
<td>0.05 0.05 0.05</td>
</tr>
<tr>
<td>Pyruvate kinase (µmoles of pyruvate formed/min/g protein)</td>
<td>106.0±6.26</td>
<td>68.0±5.36*</td>
<td>99.0±5.87#</td>
<td>103.66±4.7</td>
<td>0.05 0.05 0.05</td>
</tr>
<tr>
<td>Glucose 6 phosphate dehydrogenase (mIU/mg protein x 10⁻⁴)</td>
<td>4.61±0.34</td>
<td>3.80±0.27*</td>
<td>4.38±2.16#</td>
<td>4.45±0.26</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Glycogen phosphorylase (mg of Pi liberated /min/mg protein)</td>
<td>4.39±0.34</td>
<td>4.85±0.33*</td>
<td>4.24±0.23#</td>
<td>4.32±0.29</td>
<td>NS 0.05 NS</td>
</tr>
<tr>
<td>Glycogen (mg of glucose/g tissue)</td>
<td>42.93±2.99</td>
<td>35.10±2.50*</td>
<td>42.70±2.52#</td>
<td>40.30±2.55</td>
<td>0.05 0.05 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD from 6 animals in each group.
* compared with CON, P < 0.05; # compared with FRU, P < 0.05.
(ANOVA followed by Tukey’s test). NS- not significant.
Boyd et al. [26] have noticed the reduction of insulin receptor kinase activity in vivo in sucrose-fed rats. Reduction in the phosphorylation of pp185 (IRS-1/IRS-2) in the liver and muscle of fructose-fed rats contribute to insulin resistance [27]. Recently, Taghibiglou et al. [7] have suggested that hepatic insulin resistance induced by high fructose feeding in part could be attributed to increase in expression of a non-transmembrane PTP called PTP-1B. Bhanot et al. [28] observed elevation of basal and insulin-stimulated activities of protein-serine kinase B (PKB) and phosphatidylinositol 3-kinase (PI3K) in fructose-fed hypertensive rats.

Figure 1
Plasma glucose concentrations in response to oral glucose load in control and experimental animals (means ± S.D, n = 6).
* - compared with CON, P < 0.05
# - compared with FRU, P < 0.05.

Figure 2
SSPG concentrations during the insulin suppression test in control and experimental animals. (means ± S.D, n = 6). Significant differences among mean are analyzed by two-way ANOVA followed by Tukey’s test.
* - compared with CON, P < 0.05
# - compared with FRU, P < 0.05.

Figure 3
Protein tyrosine phosphatase and protein tyrosine kinase activity of control and experimental animals (means ± S.D, n = 6). Significant differences among mean are analyzed by two-way ANOVA followed by Tukey’s test.
* - compared with CON, P < 0.05
# - compared with FRU, P < 0.05.
Taurine administration was effective in mitigating the adverse effects of fructose loading on glucose and insulin levels. Glucose tolerance and insulin sensitivity were improved upon taurine treatment in fructose-fed rats. Restoration of enzyme activities in taurine-treated fructose-fed rats could be the consequence of potentiation of insulin action and glucose utilization by taurine. Taurine could reduce basal hepatic glucose output through an effect on hepatic glycogen synthesis or breakdown. In the present study taurine lowered the breakdown of glycogen by causing a reduction in glycogen phosphorylase. We have earlier shown that taurine can increase glucose utilization at both normal glucose and high glucose concentrations and reduce glycogen phosphorylase. We have earlier shown that taurine can increase glucose utilization at both normal glucose and high glucose concentrations and reduce glycogen phosphorylation of proteins and lipid peroxidation in RBCs exposed to high glucose [29].

Taurine is reported to control hyperglycemia without increasing serum insulin levels [11] indicating that the hypoglycemic action of taurine is unrelated to a direct stimulation of pancreatic insulin release. Trachtman et al. [30] have reported that taurine supplementation prevented hyperglycemia and diabetic nephropathy in streptozotocin diabetic mice. Taurine also had a beneficial effect on insulin sensitivity and prevented diabetic complications in a rat model of type 2 diabetes [31].

Taurine modulates the insulin signal transduction pathways by inhibiting the cellular PTP activity that negatively regulates insulin signaling and also activates tyrosine phosphatase. Thus taurine has the potential ability to prolong as well as increase insulin signaling. Taurine has been identified as an inhibitory modulator of serine phosphorylation of certain proteins in subcellular and membrane fractions of retina, heart and brain [32] and hence a regulator of signal transduction. We speculate that in this diet-induced model, activation of serine kinases coupled with inhibition of tyrosine phosphorylation could result in insulin resistance. Taurine may block phosphorylation of selective serine residues to increase the phosphorylation of tyrosine residues.

Changes in redox balance can activate certain stress-induced serine kinases which in turn can cause inactivation of PTP [33]. Thus it is also possible that taurine being an antioxidant, would make the cells less susceptible to the consequence of stress-induced activation of serine kinases. The present data suggest that taurine supplementation could be helpful in overcoming insulin resistance and the associated metabolic changes. Increase in the tyrosine phosphorylation and inhibition of dephosphorylation by taurine in liver of fructose-fed rats may contribute to explain some aspects of taurine action in this model. The regulatory effects of taurine on these systems indicate a need for further studies.

Acknowledgement – The financial support in the form of Senior Research Fellowship to Ms. A.T. Anitha Nandhini from Indian Council of Medical Research (ICMR), New Delhi is gratefully acknowledged.

References