Taurine prevents collagen abnormalities in high fructose-fed rats

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Background & objectives: Accumulation of collagen and changes in its physiochemical properties contribute to the development of secondary complications of diabetes. We undertook this study to see the effects of taurine on the content and characteristics of collagen from tail tendon of rats fed with high fructose diet.

Methods: The rats were divided into four groups of six each: control group (CON), taurine-supplemented control group (CON+TAU), taurine supplemented (FRU+TAU) and not supplemented fructose-fed group (FRU). The physico-chemical properties of collagen isolated from the tail tendon were studied.

Results: Fructose administration caused accumulation of collagen in tail tendon. Enhanced glycation and advanced glycation end products (AGE)-linked fluorescence together with alterations in aldehyde content, solubility pattern, susceptibility to denaturing agents and shrinkage temperature were observed in fructose-fed rats. Elevated β component of type I collagen was evidenced from the SDS gel pattern of collagen from the fructose-fed rats. Simultaneous administration of taurine alleviated these changes.

Interpretation & conclusion: Taurine administration to fructose-rats had a positive influence on both quantitative and qualitative properties of collagen. The results of the present study suggested a role for the action of taurine in delaying diabetic complications and the possible use of taurine as an adjuvant therapeutic measure in the management of diabetes and its complications.

Key words Collagen - crosslinking - fructose diet - glycation - tail tendon - taurine

Changes in the structure and functions of proteins caused by the process of non-enzymatic glycation and advanced glycation end products (AGEs) are implicated in the development of secondary complications in diabetes. Collagen, the most abundant connective tissue protein, contains several dibasic amino acids and has slow turnover rate. This makes collagen, highly susceptible to modification by glycation.

High fructose-fed rat is frequently used as an animal model for insulin resistance. Fructose-rich diet induces insulin resistance, hyperinsulinaemia, glucose intolerance, hypertriglyceridaemia and hypertension in rats. Fructose feeding has been shown to cause glycation and crosslinking of skin collagen and to promote aging process in rats.

Taurine (2-amino ethane sulphonic acid) is an intracellular amino acid that is present in millimolar
concentrations in the plasma, tissues and interstitial media. Taurine has various biological and physiological functions and has been extensively reviewed with focus on its involvement in diabetes and its complications. We have earlier shown that taurine can improve insulin action and prevent fructose-induced metabolic alterations in insulin resistant rats. No experimental data are available on the effect of taurine on collagen structure and properties. This prompted us to study whether taurine administration in high fructose-fed rats would prevent collagen accumulation, glycation and AGE-linked fluorescence in tail tendon, and improve variables such as solubility, shrinkage temperature, glycation and fluorescence.

Material & Methods

Animals and treatment: Male Wistar rats (body weights 170-190 g) obtained from Central Animal House, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar, were housed two per cage under 12 h light and dark cycle. The rats were fed for one week with a standard pellet diet obtained from Karnataka State Agrofeeds Corporation Private Ltd., Agro Feeds Division, Bangalore, India. The animals were cared in accordance with principles and guidelines of the Institutional Animal Ethics Committee (IAEC), Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. The experimental protocol was approved by the IAEC.

Experimental groups: The animals were divided into four groups of six rats each. Group 1, control animals (CON), received the control diet containing 61 per cent starch and tap water ad libitum [61% starch, 20% casein, 0.7% methionine, 5% groundnut oil, 9.7% wheat bran and 3.5% salt mixture (The mineral mix in a kg contained MgSO_4·7H_2O-30.5 g; NaCl-65.2 g; KCl-105.7 g; KH_2PO_4-200.2 g; MgCO_3·5H_2O-40.0 g; CaCO_3-512.4 g; KI-0.8 g; NaF-0.9 g; CuSO_4·5H_2O-1.4 g; MnSO_4·0.4 and CONH_3·0.05 g)] and water ad libitum. Group 2, fructose-fed animals (FRU), received the fructose enriched diet, which was similar in composition to the control diet (except for starch which was replaced by 61% fructose). Group 3, fructose-fed animals (FRU-TAU), received the fructose diet and were allowed to drink 2 per cent taurine solution ad libitum. Group 4, control animals (CON-TAU), received the control diet and were given 2 per cent taurine solution ad libitum.

The diets were prepared fresh everyday based on the method of Cohen et al (1977). The animals were maintained in their respective groups for 45 days. Body weight changes were measured weekly. At the end of the experimental period the rats were sacrificed by cervical decapitation. Tail tendons were dissected out.

Analytical procedures: A weighed amount of the tail tendon was defatted with chloroform:methanol (v/v, 2:1) mixture and lyophilized. The lyophilized sample was hydrolysed with 6N HCl for 18 h at 110°C. After hydrolysis the digested sample was evaporated to dryness, dissolved in water and hydroxyproline content was measured. Total collagen content was determined by multiplying the hydroxyproline content by a factor of 7.46. The extent of glycation was determined by the method of Rao and Pattabiraman using concentrated sulphuric acid and 80 per cent phenol.

Collagen-linked fluorescence was measured by the method of Monnier et al. The tail tendons were minced in phosphate buffered saline (PBS, 10 mM pH 7.4) and washed with chloroform:methanol and N-2-hydroxyethylpiperazine N-2-ethane sulphonic acid (HEPES) - buffer (0.02 M, pH 7.5). The pellet was suspended in HEPES buffer containing 120 units of type VII collagenase and digested at 37°C for 48 h and centrifuged. The supernatant was used for the fluorescence assay.

The solubility pattern was determined by the method of Miller and Rhodes. Tail tendons were extracted with neutral salt solvent containing 20 mM ethylene diamine tetra acetic acid (EDTA) and 2 mM N-ethyl maleimide. The supernatants were pooled and used for the assay of hydroxy-proline. The residue obtained from neutral salt extraction was again extracted with 0.5 M acetic acid. The supernatants obtained were used for hydroxyproline assay. The
residue obtained from acid extraction was extracted with pepsin. The pooled supernatants were used for the hydroxyproline assay.

Aliquots of insoluble collagen samples were analysed for aldehyde content according to the method of Paz et al.\textsuperscript{11}, and expressed as \( \mu \text{mol acetaldehyde/100 mg collagen} \). The levels of thiobarbituric acid reactive substances in the insoluble collagen was assessed by the method of Iqbal et al.\textsuperscript{12}. The procedure of Adam et al.\textsuperscript{13} was followed to assess the susceptibility of insoluble collagen to denaturing agents such as urea and potassium thiocyanate (KCNS). Insoluble collagen was suspended in 6\( M \) urea and 2\( M \) KCNS separately for 24 h and then centrifuged. The supernatant obtained were analysed for hydroxyproline content. The shrinkage temperature of collagen was determined as described by Nutting and Borasky.\textsuperscript{14}

For performing gel electrophoresis, collagen was extracted from tail tendons by acid extraction\textsuperscript{15}. The samples were treated with eight parts (v/v) of 0.5\( M \) acetic acid overnight. The suspension was centrifuged at 25,000 g for 30 min. The supernatant was processed further to obtain acid-soluble collagen while pellet obtained represented acid-insoluble collagen. The supernatant was precipitated with addition of solid NaCl to reach a concentration of 5 per cent (w/v) and kept overnight. The pellet obtained was suspended in 0.5\( M \) acetic acid containing 100 mg pepsin/g wet tissue, centrifuged and the supernatant was precipitated with solid NaCl. The fractions were processed separately as follows. The solutions were centrifuged and the precipitate obtained was dissolved in 0.5\( M \) acetic acid and dialyzed overnight against 0.02\( M \) disodium hydrogen orthophosphate for atleast 5-6 changes. The purified collagen was lyophilized and used for further analysis.

To determine \( \alpha \) and \( \beta \) components of acid- and pepsin-soluble collagen, the collagen samples were subjected to sodium dodecyl sulphate (SDS) gel electrophoresis using a 3 per cent stacking gel with a 5 per cent running gel and Coomassie brilliant blue staining. The gels were scanned with a densitometer. The ratio of \( \alpha \) to \( \beta \) subunits of acid- and pepsin-soluble collagen was determined.

Statistical analysis: Values are expressed as means \( \pm \) SD. Data within the groups were analysed using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). \( P<0.05 \) was considered statistically significant.

Results

The rats fed fructose diet showed increased glycation, hydroxyproline content and fluorescence as compared to control rats. These rats also showed elevated aldehyde content and peroxidation and shrinkage temperature, depicting collagen accumulation and modification. Taurine administered fructose-fed rats registered a near-normal levels of all the collagen parameters. No significant changes were observed in control rats treated with taurine (Table I).

Collagen from fructose-fed rats showed altered solubility pattern. They showed decreased solubility in neutral salt and acid but an increase in solubility with pepsin. Taurine-treated fructose-fed rats showed improved solubility with neutral salt and acid as compared to fructose-fed rats. The susceptibility to denaturing agents was lower in fructose-fed rats to an extent of 44 and 33 per cent as compared to that from control rats (Table II).

Figs 1 and 2 showed the representative SDS-gel patterns of acid-soluble and pepsin- soluble collagen samples respectively of control and experimental rats. Fructose-treated rats showed increased bandwidth of different subunits of type I collagen as compared to other groups. The lowest \( \alpha \)-to-\( \beta \) chain ratio was observed in the fructose-fed rat, whereas the control group showed the highest ratio for both acid- and pepsin- soluble collagen. The \( \alpha /\beta \) ratio was significantly decreased in fructose-fed rats. Taurine treated control (CON+TAU) and fructose-fed (FRU- TAU) groups showed intermediate values (Table III).

Discussion

The changes in the content and properties of collagen in fructose-fed rats indicated increased crosslinking and maturation of collagen.

Changes in collagen crosslinking, and an increase in aldehyde content and shrinkage temperature have been reported in various pathological conditions
Covalent crosslinks in collagen may be attributed to either non-enzymatic reaction between reducing sugars (fructose/glucose) and the protein or to the action of lysyloxidase, the only enzyme known to initiate crosslinking an collagen\textsuperscript{17}.

\textit{In vitro} studies with collagen showed that fructose is a more potent glycating agent than glucose\textsuperscript{18}. McPherson et al\textsuperscript{19} postulated that some of the protein crosslinking that occurs in diabetes \textit{in vivo} could be fructose induced. Sakai et al\textsuperscript{20} suggested that fructose enhanced reactive oxygen generation, the fluorescence of glycated collagen and reduced the digestibility of collagen by collagenase, showing the closer involvement of fructose rather than glucose in crosslinking. Malondialdehyde, an end product of lipid peroxidation can react with the free amino groups of collagen and stimulate cross-linking\textsuperscript{21}. Fructose

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\hline
\textbf{Parameters} & \textbf{CON} & \textbf{FRU} & \textbf{FRU+TAU} & \textbf{CON+TAU} \\
\hline
Total collagen (mg/100 mg tissue) & 76.22 ± 0.78 & 161.8 ± 7.14* & 79.67 ± 2.17** & 75.04 ± 0.48 \\
Glycation (µg glucose/mg collagen) & 11.55 ± 0.55 & 25.75 ± 1.31* & 12.63 ± 1.01** & 12.04 ± 0.72 \\
AGE-linked fluorescence (AU/µmol hydroxyproline) & 38.38 ± 1.04 & 62.60 ± 2.88* & 37.77 ± 0.91** & 39.80 ± 2.54 \\
Aldehyde content (µmol acetaldehyde/100 mg collagen) & 13.56 ± 1.76 & 16.25 ± 1.46* & 14.45 ± 1.20** & 14.05 ± 1.35 \\
Lipid peroxidation (nmol/mg protein) & 9.14 ± 0.65 & 15.5 ± 0.26* & 9.43 ± 0.23** & 8.98 ± 0.30 \\
Shrinkage temperature (°C) & 70.93 ± 1.08 & 82.50 ± 2.23* & 69.53 ± 0.50** & 70.00 ± 1.46 \\
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\end{tabular}
\caption{Total collagen, extent of glycation, advanced glycation end product (AGE) linked fluorescence, aldehyde content, lipid peroxidation levels and shrinkage temperature in tail tendon of control and experimental animals}
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\hline
\textbf{Parameters} & \textbf{CON} & \textbf{FRU} & \textbf{FRU+TAU} & \textbf{CON+TAU} \\
\hline
Neutral salt (µg/100 mg collagen) & 131.12 ± 9.98 & 92.62 ± 7.05* & 129.52 ± 10.01** & 131.52 ± 9.59 \\
Acid (mg/100 mg collagen) & 3.46 ± 0.26 & 2.39 ± 0.18* & 3.39 ± 0.26** & 3.44 ± 0.27 \\
Pepsin (mg/100 mg collagen) & 40.98 ± 3.12 & 61.28 ± 4.67* & 42.37 ± 3.23** & 41.10 ± 3.13 \\
\textit{Denaturing agents:} & & & & \\
6 M Urea (mg/100 mg collagen) & 14.90 ± 2.32 & 9.36 ± 1.98* & 13.89 ± 1.88** & 14.29 ± 1.48 \\
2M KCNS (mg/100 mg collagen) & 10.54 ± 1.65 & 8.26 ± 1.37* & 11.12 ± 1.04** & 11.09 ± 0.96 \\
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\caption{Solubility of collagen in neutral salt, acid and pepsin and the levels of susceptibility to denaturating agents}
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\hline
\textbf{Groups} & \textbf{Acid soluble collagen} & \textbf{Pepsin soluble collagen} \\
& (% of total collagen) & (% of total collagen) \\
\hline
CON & 2.03 ± 0.15 & 2.61 ± 0.20 \\
FRU & 1.67 ± 0.13* & 1.98 ± 0.15* \\
FRU+TAU & 1.96 ± 0.15** & 2.21 ± 0.17** \\
CON+TAU & 1.98 ± 0.15 & 2.26 ± 0.17 \\
\hline
\end{tabular}
\caption{Effect of taurine on the α/β ratio of acid- and pepsin-soluble collagen in skin of control and experimental animals}
\end{table}

including diabetes\textsuperscript{16}. Covalent crosslinks in collagen may be attributed to either non-enzymatic reaction between reducing sugars (fructose/glucose) and the
Fig. 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of acid-soluble collagen from tail tendon in control and experimental rats. The rats were administered with control diet (CON), fructose diet (FRU), fructose diet and 2 per cent taurine solution (FRU+TAU) and control diet and 2 per cent taurine (CON+TAU), $\alpha$, $\beta$ and $\gamma$ represent the fractions of type I collagen.

Fig. 2. SDS-PAGE pattern of pepsin-soluble collagen from tail tendon in control and experimental rats. The rats were administered with control diet (CON), fructose diet (FRU), fructose diet and 2 per cent taurine solution (FRU+TAU) and control diet and 2 per cent taurine (CON+TAU), $\alpha$, $\beta$ and $\gamma$ represent the fractions of type I collagen.
consumption was shown to facilitate oxidative damage in rat tissues and cause increased formation of peroxides and aldehydes.\(^{22}\)

The SDS gel pattern confirmed accumulation and increased crosslinking of collagen in high fructose-fed rats. A decreased ratio of \(\alpha\) to \(\beta\) components of collagen in tail tendon of fructose-fed rats was also observed. The relative abundance of high molecular weight collagen chains was demonstrated by increased \(\beta\) component.

Taurine abolished fructose-induced accumulation of collagen and alterations in its properties. Collagen from taurine-administered fructose-fed rats displayed decreased glycation, AGE linked fluorescence, aldehyde and peroxide content in tail tendon collagen, together with a decline in total collagen content as compared to fructose-fed rats. The solubility pattern was improved with a relative increase in neutral salt and acid soluble collagen. These changes indicated a reduction in the crosslinking of collagen proteins in taurine-treated fructose-fed rats.

High dietary fructose can increase the formation of glyceraldehydes and methylglyoxal, malondialdehyde and 4-hydroxynonenal, the products of lipid peroxidation.\(^{23}\) The carbonyl carbon of these aldehydes reacts readily with the free amino and sulphydryl groups of functional enzymes and membrane proteins to form Schiff base that could alter their functions.\(^{24}\)

Taurine might act as an antiglycative compound. Taurine was shown to provide free amino groups that could compete for the reducing sugars and aldehydes.\(^{25}\) As a free amino group donor, taurine competitively inhibited the formation of pentosidine and other AGEs \textit{in vivo}.\(^{26}\)

The results of the present study suggested that taurine might play an important role in delaying diabetic complications through its antiglycation and antioxidant properties and might be useful as an adjuvant therapeutic measure in the management of diabetic disease and its complications. One limitation of this study is that taurine-induced effects were not compared with known antiglycating and antioxidant agents (positive controls). In a recent study\(^ {27}\) taurine alone failed to decrease skin and kidney damage in long-term experimental diabetes. Further studies focusing on other indices of AGE formation and a comparison with other known antiglycation agents are needed for a better understanding of the mechanism of taurine action.

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References


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