Effect of Dietary Taurine Supplementation on GSH and NAD(P)-Redox Status, Lipid Peroxidation, and Energy Metabolism in Diabetic Precataractous Lens

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PURPOSE. To evaluate changes in glutathione and NAD(P)-redox status, taurine and malondialdehyde (MDA) levels, glucose utilization, and energy metabolism in diabetic precataractous lenses and to assess whether these changes can be prevented with dietary taurine supplementation.

METHODS. The experimental groups included control and streptozotocin-diabetic rats with a 3-week duration of diabetes fed unsupplemented or taurine (1% or 5%)-supplemented diets. The levels of glucose, sorbitol, fructose, myo-inositol, oxidized glutathione (GSSG), glycolytic intermediates, malate, α-glycerophosphate, and adenine nucleotides were assayed in individual lenses spectrofluorometrically by enzymatic methods, reduced glutathione (GSH) spectrofluorometrically with O-phthaldialdehyde, MDA colorimetrically with N-methyl-2-phenylindole, and taurine by high-performance liquid chromatography. Free cytosolic NAD+/NADH and NADP+/NADPH ratios were calculated from the lactate dehydrogenase and malic enzyme systems.

RESULTS. Sorbitol pathway metabolites and MDA were increased, and GSH and taurine levels were reduced in diabetic rats versus controls. The profile of glycolytic intermediates (an increase in glucose 6-phosphate, no change in fructose 6-phosphate and fructose 1,6-diphosphate, an increase in dihydroxyacetone phosphate, a decrease in 3-phosphoglycerate, phosphoenolpyruvate, and pyruvate, and no change in lactate), and a 9.2-fold increase in α-glycerophosphate suggest diabetes-induced inhibition of glycolysis. Free cytosolic NAD+/NADH ratios, ATP levels, ATP/ADP, and adenylate charge were reduced, whereas free cytosolic NADP+/NADPH ratios were elevated. Lens taurine levels in diabetic rats were not affected by supplementation with 1% taurine. With 5% taurine supplementation, they were increased approximately 2.2-fold higher than those in untreated diabetics but remained 3.4-fold lower than in controls. Lens GSH levels were similar in diabetic rats fed unsupplemented and 5% taurine-supplemented diets, whereas GSSG and MDA levels and GSSG/GSH ratios were reduced by 5% taurine supplementation. The decrease in free cytosolic NAD+/NADH, ATP/ADP, and adenylate energy charge were ameliorated by 5% taurine supplementation, whereas accumulation of sorbitol pathway intermediates, depletion of myo-inositol, inhibition of glycolysis, a decrease in ATP and total adenine nucleotide, and an increase in free cytosolic NADP+/NADPH were not prevented.

CONCLUSIONS. Dietary taurine supplementation ameliorates MDA levels, GSSG/GSH, and NAD+/NADH and fails to prevent the osmotically mediated depletion of GSH and taurine and the decrease in glucose utilization and ATP levels in diabetic precataractous lens. Dietary taurine supplementation cannot be regarded as an alternative to aldose reductase inhibition in eliminating antioxidant and metabolic deficits contributing to diabetes-associated cataractogenesis. (Invest Ophthalmol Vis Sci. 1999;40:680–688)
Effect of Taurine on Lens Metabolism in Diabetic Rats

Materials and Methods

The experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the University of Michigan Protocol for Animal Studies.

Animals

The experiments were performed on male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), body weight 200 to 250 g, divided into the following groups: (1) control group (10 rats); (2) diabetic group with 3-week duration of diabetes fed a standard rat chow diet (ICN Biomedical, Cleveland, OH; 10 rats); (3) diabetic group with 3-week duration of diabetes fed the above-mentioned diet containing 1% taurine (10 rats); and (4) diabetic group with 3-week duration of diabetes fed the diet containing 5% taurine (10 rats). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg body weight). Because of the potential problems with taurine entry into the diabetic (hyperosmotic) lens outlined above, we applied high-dose (1% and 5%) taurine diets that were well tolerated and were not associated with any adverse side effects in other studies.

Reagents

Unless otherwise stated, all chemicals were of reagent grade quality and were purchased from Sigma Chemical (St. Louis, MO). Methanol (high-performance liquid chromatography [HPLC] grade), perchloric acid, hydrochloric acid, and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA). Ethanol (200 proof dehydrated alcohol, U.S. Pharmacopeia puncilicus) was purchased from Quantum Chemical (Tischola, IL). Dihydroxyacetone phosphate dilithium salt monohydrate was purchased from Fluka BioChemika (Buchs, Switzerland). β-glucose, sorbitol, myo-inositol, and α-fructose, (U.S. Pharmacopoeia) were purchased from Pfänstiehl Laboratories (Waukegan, IL). Kits for MDA assay were purchased from Oxis International (Portland, OR).

Experimental Procedure

Rats from each group were sedated with carbon dioxide and gently rolled over a fine filter paper, and frozen in liquid nitrogen for subsequent biochemical analyses. One lens from each rat (except the group fed the 1% taurine diet in which only lens taurine levels were assessed) was used for measurements of GSH, oxidized glutathione (GSSG), glucose, glycolytic intermediates, malate, and adenine nucleotides, and the second lens was used for measurement of sorbitol, fructose, myo-inositol, MDA, and taurine. Blood samples for measurements of glucose were taken from the tail vein the day before the rats were killed.

Measurement of Metabolites and Adenine Nucleotides

Lenses were weighed and deproteinized by homogenization with perchloric acid (1 ml of 6% HClO₄ per lens), and then centrifuged (Sorvall MC 12V; NEN-Dupont, Wilmington, DE). The levels of glucose, GSH, GSSG, glycolytic intermediates (except 1,3-diphosphoglycerate and 2-phosphoglycerate), α-glycerophosphate (α-GP), malate, ATP, ADP, and AMP were assayed in neutralized extracts (neutralization was performed with concentrated K₂CO₃) of individual lenses spectrophotometrically (LS-5B; Perkin-Elmer, Norwalk, CT) by enzymatic procedures as described by Lowry and Passonneau.

Measurements of Sorbitol, Fructose, and myo-Inositol

Lenses were weighed and homogenized in 0.8 ml 0.9% NaCl. A 100-μl volume of 0.3 M zinc sulfate was then added to 0.2 ml of homogenate for protein precipitation, followed by an equivalent of barium hydroxide. The samples were centrifuged at 4000g for 10 minutes, and aliquots of the supernatant were taken for spectrofluorometric measurement of sorbitol, fructose, and myo-inositol by enzymatic procedures, using sorbitol dehydrogenase, 30 fructose dehydrogenase, 31 and myo-inositol dehydrogenase, 32 respectively. In brief, the analytical mixture for sorbitol and myo-inositol contained 0.9 ml 0.5 mM NAD in 0.1 M glycine-NaOH buffer (pH 9.5) and deproteinized extract (for sorbitol, 0.1 ml for control and 0.01 ml plus 0.09 ml H₂O for diabetic lenses; for myo-inositol, 0.1 ml for control and diabetic lenses). The reaction was started by the addition of ca. 0.8 U sorbitol dehydrogenase or 0.5 U myo-inositol dehydrogenase. The analytical mixture for fructose contained 0.9 ml of reagents-containing 150 mM citrate buffer, pH 4.5 (1 aliquot of reasurin, 5 mg/10 ml H₂O, was mixed with 100 aliquots of citrate buffer) and deproteinized extract (0.1 ml for control and 0.05 ml plus 0.05 ml H₂O for diabetic lenses). The reaction was started by the addition of ca. 0.5 U fructose dehydrogenase.

Measurement of Taurine

Lens taurine was measured by reverse-phase HPLC (apparatus: Waters 501, pumps: Waters 717; autosampler: Waters 470; scanning fluorescence detector: A excitation: 360 nm; λ emission: 455 nm; and Waters Millenium Software; Waters Corp., Milford, MA) after precolumn derivatization with o-phthalaldehyde reagent. 33 (10 mg o-phthalaldehyde dissolved in 0.2 ml absolute ethanol were mixed with 10 μl mercaptoethanol and 5 ml 0.5 M borate buffer, pH 10.3). In brief, 0.1 ml of lens homogenate (see Measurement of Sorbitol, Fructose, and myo-
Inositol section) and taurine standards in the range of 1-10 × 10⁻⁹ M were extracted with 1 ml of 6% trichloroacetic acid and-centrifuged at 4000g for 10 minutes. The supernatants were purified on washed dual-bed ion-exchange columns (2.5 cm AG 1-X8 100-200 mesh; Bio-Rad, Richmond, CA; in the chloride form over 2.5 cm AG 50W-X8 200/400 mesh [Bio-Rad] in the hydrogen form) by elution with 2 ml H₂O. The eluents were washed twice with H₂O-Saturated ether and lyophilized. Samples and standards were dissolved in 100 μl of H₂O before HPLC analysis. Isocratic elution was carried out at a flow rate of 2 ml/min using 43% solvent A (0.05 M NaH₂PO₄, pH 5.3, plus 5 M NaOH) combined with 57% solvent B (0.05 M NaH₂PO₄ in 75% methanol/H₂O) filtered with type FH 0.5 μm Millipore filter and degassed. Glutamine, added after ion exchange chromatography, was used as the internal standard.

Measurements of GSH and GSSG

GSH levels were assayed in perchloric extracts spectrofluorometrically. In brief, 0.1 ml of extract was mixed with 0.89 ml of 20 mM EDTA in 1.0 M Tris–HCl buffer (pH, 8.1), and the reaction was initiated by the addition of 0.01 ml o-phthaldialdehyde (10 mg/ml methanol). GSSG levels were assayed spectrofluorometrically by enzymatic procedure. The analytical mixture contained 0.8 ml 0.1 M buffer (pH 7.6), 0.2 ml perchloric extract, and 0.2 μM to 10 μM NADPH. The reaction was started by the addition of approximately 0.3 U of glutathione reductase (Type IV; Sigma).

Measurements of MDA

For measurements of MDA, individual lenses were weighed and homogenized in 20 mM Tris buffer (pH 7.4) containing 5 mM butylated hydroxytoluene. The homogenates were centrifuged at 4000g for 10 minutes. All further steps followed exactly the protocol described in the kit (Biotech LPO-586; Oxis International).

Calculations of Free Cytosolic NAD⁺/NADH, NAD⁺/NADPH, and Adenylate Energy Charge

According to classic reports from the Krebs' laboratory and other studies, direct measurement of NAD, NADH, NADP, and NADPH is not informative in terms of control of metabolism because they do not provide information on compartmentalization of nicotinamide nucleotides between cytosol and mitochondria and because they do not separate free from protein-bound forms (only free fractions determine direction of dehydrogenase reactions). The same studies proposed an alternative approach, implying asessment of free NAD(P)⁺/NAD(P)H ratios in the cytoplasm and mitochondria by measuring the ratios of the concentrations of the oxidized and reduced metabolites of suitable NAD(P)-linked dehydrogenase systems. Using this approach, free cytosolic NAD⁺/NADH and NAD⁺/NADPH ratios were calculated from metabolite concentrations and the equilibrium constants of lactate dehydrogenase and malic enzyme as follows:

\[
\frac{[\text{NAD}^+]_{\text{free}}}{[\text{NADH}]} = \frac{[\text{pyruvate}]}{[\text{lactate}]} \times \frac{1}{k_1},
\]

where \( k_2 \) is the equilibrium constant of malic enzyme (3.44 × 10⁻² M) (the CO₂ concentration was taken to be 1.16 mM).

Adenylate energy charge ([ATP + 1/2ADP])/[ATP + ADP + AMP]) is a parameter of phosphorylation of adenine nucleotide system, was calculated from measured concentrations of ATP, ADP, and AMP.

Statistical Analysis

The results are expressed as mean ± SD. Differences among experimental groups were determined by ANOVA, and the significance of differences between these groups was assessed with the Student-Newman-Keuls multiple range test. Significance was defined at \( P = 0.05 \).

RESULTS

The body weights were lower in diabetic rats compared with those in controls (227.6 ± 48.2 versus 357.3 ± 45.7 g, \( P < 0.01 \)). The initial body weights were similar in these two groups. No statistically significant difference was found between body weights in diabetic rats treated with 1% and 5% taurine (218.8 ± 46.4 and 223.5 ± 35.8 g, respectively) and the corresponding untreated groups.

The lens wet weights were not different among the experimental groups studied (controls: 28.5 ± 1.8 mg, diabetics: 27.8 ± 3.0 mg, diabetics + 1% taurine: 30.4 ± 2.4 mg, diabetics + 5% taurine: 29.2 ± 2.2 mg).

Plasma glucose levels were increased approximately 6.1-fold in diabetic rats compared with those in control rats (408 ± 34 versus 59 ± 8 mg/dl, \( P < 0.01 \)). Neither 1% nor 5% taurine supplementation had an effect on plasma glucose levels in diabetic rats (414 ± 45 and 598 ± 7 mg/dl, respectively) and the corresponding untreated groups.

Lens taurine levels in control and diabetic rats fed either unsupplemented or taurine-supplemented diets are presented in Figure 1. Lens taurine levels in 3-week diabetic rats were decreased 7.9-fold compared with those in control rats (\( P < 0.01 \)). One percent dietary taurine supplementation failed to prevent diabetes-induced lens taurine depletion. Lens taurine levels in diabetic rats fed 5% taurine diet were 2.2-fold higher.

\[
\text{FIGURE 1.} \quad \text{Lens taurine levels in controls and diabetic rats fed unsupplemented, 1% taurine-supplemented, or 5% taurine-supplemented diets (mean ± SD, n = 8–10). C, control; D, diabetic; T, taurine.}
\]

\[
\frac{[\text{NAD}^+]_{\text{free}}}{[\text{NADH}]} = \frac{[\text{pyruvate}]}{[\text{lactate}]} \times \frac{1}{k_2},
\]
TABLE 1. Lens Glucose, Sorbitol, Fructose, and myo-Inositol Levels in Control and Diabetic Rats Fed Taurine Unsupplemented or 5% Taurine-Supplemented Diets

<table>
<thead>
<tr>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + 5% Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.52 ± 0.09</td>
<td>13.58 ± 2.29*</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.439 ± 0.145</td>
<td>27.50 ± 3.39*</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.948 ± 0.237</td>
<td>9.88 ± 2.92*</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>1.213 ± 0.157</td>
<td>0.196 ± 0.058*</td>
</tr>
</tbody>
</table>

Values are micromoles per gram wet weight; n = 8 to 9 for all groups. * Significantly different compared with those in controls (P < 0.01).

The levels of dihydroxyacetone phosphate (DHAP) in diabetic rats were increased 1.4-fold compared with those in control rats, whereas the levels of metabolites of the lower segment of glycolysis (i.e., 3-phosphoglycerate [3-PG] and pyruvate) were decreased (1.6-, 1.5-, and 1.6-fold, respectively), and lactate levels were similar in control and diabetic groups. Five percent taurine supplementation decreased G6P compared with the levels in untreated diabetics. Accumulation of DHAP and decrease in pyruvate were partially prevented. The levels of F6P, FDP, 3-PG, and PEP remained unaffected while lactate levels were decreased below those in untreated diabetics and controls.

Lens α-GP levels were increased 9.1-fold in diabetic rats versus controls (2.24 ± 0.28 μmol/g wet weight versus 0.245 ± 0.059 μmol/g wet weight, P < 0.01). The increase was partially prevented in diabetic rats fed the 5% taurine-supplemented diet (1.52 ± 0.24 μmol/g wet weight, P < 0.01 versus untreated diabetic and control rats).

Lens malate levels were substantially depleted in diabetic rats (0.036 ± 0.007 μmol/g wet weight versus 0.091 ± 0.020 μmol/g wet weight in control rats, P < 0.01). This depletion was not prevented in diabetic rats fed the 5% taurine-supplemented diet (0.040 ± 0.001 μmol/g wet weight, P < 0.01 versus untreated diabetic control rats).

Free cytosolic NAD+/NADH ratio (Fig. 3A) was markedly reduced in diabetic rats versus control rats (P < 0.01). This reduction was prevented in diabetic rats fed the 5% taurine-supplemented diet (P < 0.01 versus untreated diabetic rats).

TABLE 2. Glutathione Redox Status in Control and Diabetic Rats Fed Unsupplemented or 5% Taurine-Supplemented Diets

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + 5% Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>5.75 ± 1.00</td>
<td>1.09 ± 0.33*</td>
<td>1.19 ± 0.18*</td>
</tr>
<tr>
<td>GSSG</td>
<td>0.123 ± 0.025</td>
<td>0.136 ± 0.034</td>
<td>0.046 ± 0.011††</td>
</tr>
<tr>
<td>GSSG/GSH</td>
<td>0.022 ± 0.005</td>
<td>0.12 ± 0.047*</td>
<td>0.040 ± 0.0013††</td>
</tr>
</tbody>
</table>

Levels of GSH and GSSG are expressed in micromoles per gram wet weight. † Significantly different compared with those in controls (P < 0.01 and <0.05, respectively). †† Significantly different compared with those in untreated diabetics (P < 0.01).
TABLE 3. Levels of Glycolytic Intermediates in Lens in Control and Diabetic Rats Fed Unsupplemented or 5% Taurine-Supplemented Diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + 5% T</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6P</td>
<td>0.131 ± 0.025</td>
<td>0.195 ± 0.050*</td>
<td>0.107 ± 0.047§</td>
</tr>
<tr>
<td>F6P</td>
<td>0.038 ± 0.015</td>
<td>0.052 ± 0.019</td>
<td>0.042 ± 0.004</td>
</tr>
<tr>
<td>FDP</td>
<td>0.050 ± 0.010</td>
<td>0.037 ± 0.015</td>
<td>0.029 ± 0.013</td>
</tr>
<tr>
<td>DHAP</td>
<td>0.035 ± 0.009</td>
<td>0.050 ± 0.008*</td>
<td>0.038 ± 0.013‡</td>
</tr>
<tr>
<td>3-PG</td>
<td>0.067 ± 0.005</td>
<td>0.042 ± 0.009*</td>
<td>0.044 ± 0.010*</td>
</tr>
<tr>
<td>PEP</td>
<td>0.051 ± 0.007</td>
<td>0.035 ± 0.009‡</td>
<td>0.041 ± 0.010</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.109 ± 0.027</td>
<td>0.068 ± 0.013*</td>
<td>0.079 ± 0.016‡</td>
</tr>
<tr>
<td>Lactate</td>
<td>8.13 ± 1.12</td>
<td>7.56 ± 1.72</td>
<td>5.74 ± 0.68**</td>
</tr>
</tbody>
</table>

Values are in micromoles per gram wet weight; n = 6 to 8 for all groups. T, taurine.

*† Significantly different compared with those in controls (P < 0.01 and < 0.05, respectively).
‡§ Significantly different compared with those in untreated diabetic groups (P < 0.01 and < 0.05, respectively).

Discussion

The increase in lens glucose, sorbitol, and fructose levels in diabetic rats is in agreement with previously published reports and is indicative of a severe intralenticular osmotic stress.

Lens taurine levels in control rats in the present study were higher compared with those reported by Malone et al. and Heinamaki et al. but are in agreement with the study of Reddy in which taurine levels in rat lens exceeded those of GSH. The diabetes-related depletion of taurine is in agreement with other observations for lens and other targets for diabetic complications and is consistent with osmotic regulation of lens taurine levels. The failure to achieve amelioration of taurine levels in diabetic precataractous lenses with 1% taurine supplementation and the only slightly beneficial effect of 5% taurine supplementation (which corresponds to the extremely high dosage of 1 to 2 g/kg/d) is consistent with reports of very low lens taurine uptake, and osmotically induced downregulation of Na+/taurine cotransporter manifested by decreased levels of Na+/taurine cotransporter mRNA. It is also consistent with the absence of any effects of dietary 5% taurine supplementation on the diabetes-induced increase in lens sorbitol and fructose levels, which is indicative of a similar degree of intralenticular osmotic stress, the major factor underlying diabetes-induced lens taurine depletion, in diabetic rats fed either unsupplemented or taurine-supplemented diets.

The depletion of myo-inositol in diabetic rats is consistent with the dramatic intralenticular accumulation of sorbitol and is in agreement with previous reports. The depletion of myo-inositol in diabetic rats is consistent with the dramatic intralenticular accumulation of sorbitol and is in agreement with previous reports. The depletion of myo-inositol in diabetic rats is consistent with the dramatic intralenticular accumulation of sorbitol and is in agreement with previous reports.
myo-inositol is considered to result from both an osmotically mediated decrease in (Na⁺)-myo-inositol uptake and an increase in myo-inositol efflux.23,44 In contrast to findings in the sciatic nerve,26 5% dietary taurine supplementation did not result in a further depletion of lens myo-inositol levels. This is consistent with the compatible osmolyte hypothesis26 and is explained by the fact that a 2.4-μmol increase in lens taurine levels with 5% dietary taurine supplementation does not make a significant contribution to intralenticular hyperosmolarity in diabetic precataractous lens, whereas a corresponding increase in nerve taurine levels26 (comparable with sorbitol levels) markedly shifts osmotic equilibrium and potentiates osmotic stress.

The diabetes-induced decrease in lens GSH levels is consistent with previous studies.21,22 According to Lou et al.,21 the loss of GSH is a result of an impaired ability of the lens to concentrate amino acids required for GSH biosynthesis coupled to faster GSH efflux under hyperosmotic conditions rather than an inhibition of glutathione reductase or depletion of NADPH due to increased flux through aldose reductase. This conclusion is supported by a further decrease in lens GSH levels in diabetic rats treated with sorbitol dehydrogenase inhibitor (which increases lens sorbitol levels approximately twofold over those in untreated diabetics)45,46 and the absence of a reciprocal increase of GSSG levels in concert with the decrease in GSH levels (see Ref. 22 and in the present study). In addition, it has been suggested that the decrease in GSH levels in diabetic rats may be further affected by the decreased level of ATP (which is required for de novo synthesis of GSH, both at the γ-glutamyl cysteine synthetase and glutathione synthetase steps45). The absence of any effect of dietary taurine supplementation on lens GSH and ATP levels in the diabetic model and normalization by taurine of GSH and ATP in another model of oxidative stress (menadione-induced cataractogenesis)11 is indicative of different mechanisms underlying depletion of GSH and high energy phosphates in the two models. The findings of the present study and other reports demonstrating beneficial effects of aldose reductase inhibitors on lens GSH levels in experimental models of diabetes and galactosemia21,48 imply that elimination of osmotic stress (i.e., prevention of lens polyol accumulation) is essentially required for normalization of lens GSH levels.

Lens GSSG levels in the present study were in agreement with other reports.22,49 Interestingly, GSSG levels (which were not significantly different between control and 3-week untreated diabetic animals, consistent with the study by Mitton and Trevithick25) were markedly lower in diabetic rats fed the 5% taurine-supplemented diet. This fact is difficult to explain on the basis of contemporary contradictory information on antioxidant properties of taurine. Although some studies report the ability of taurine to decrease luminal-dependent chemiluminescence elicited by chemically generated hydroxyl radicals and t-butyl hydroperoxide,50,51 and lipid peroxidation,13,15,28 others indicate that hypotaurine, a taurine precursor, rather than taurine itself, has antioxidative properties against the aforementioned reactive oxygen species.25,52 It is also known that both taurine54 and glutathione54 scavenge hypochlorite, which reacts with SH- and -S-S- groups of glutathione.55 and that lens contains hypochlorite-producing enzyme, myeloperoxidase.56 Further studies of antioxidative properties of taurine, and, in particular, its relationships with glutathione and other antioxidants, are necessary to better understand the biochemical basis of GSSG decrease with high-dose taurine supplementation.

Levels of MDA, an indicator of lipid peroxidation, in the lens of nondiabetic rats in the present study were consistent with those obtained by HPLC in another study.13 The dramatic 3.8-fold increase in lens MDA levels found in diabetic rats is consistent with findings in lenses cultured in 30 mM galactose medium.15 The ameliorating effect of taurine on MDA levels in diabetic precataractous lens found in the present study is consistent with an inverse correlation between taurine and MDA levels in the aforementioned report13 and with findings for other tissues in diabetic15 and nondiabetic58,57 models of oxidative injury.

The pattern of glycolytic intermediates in the lens of diabetic rats compared with control animals (increase in G6P, no change in F6P and FDP, increase in DHAP, decrease in 3-PG, PEP, pyruvate, no difference in lactate) suggests inhibition of glycolysis, with the sites of downregulation at its glyceraldehyde-3-phosphate dehydrogenase reaction and the lower segment (i.e., enolase and pyruvate kinase). The conclusion that glycolysis is inhibited at the stage of glyceraldehyde-3-phosphate dehydrogenase is supported by the demonstration of vulnerability of this enzyme to oxidative damage58,59 and by the finding of a 9.1-fold increase in the levels of α-GP (a by-product of glycolysis), which can be further converted to diacylglycerol and products of its metabolism in the lens.66 The diabetes-induced accumulation of α-GP in the present study agrees with other reports61,62 and contributes to a decrease in

### Table 4. Lens Energy Status in Control and Diabetic Rats Fed Unsupplemented or 5% Taurine-Supplemented Diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Diabetic + 5% Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>2.75 ± 0.32</td>
<td>2.04 ± 0.27*</td>
<td>1.96 ± 0.23*</td>
</tr>
<tr>
<td>ADP</td>
<td>0.433 ± 0.054</td>
<td>0.562 ± 0.132†</td>
<td>0.334 ± 0.072§</td>
</tr>
<tr>
<td>AMP</td>
<td>0.126 ± 0.015</td>
<td>0.186 ± 0.046*</td>
<td>0.087 ± 0.022§</td>
</tr>
<tr>
<td>ATP/ADP</td>
<td>6.36 ± 0.72</td>
<td>3.88 ± 1.31*</td>
<td>6.07 ± 1.15§</td>
</tr>
<tr>
<td>ATP + ADP + AMP</td>
<td>3.31 ± 0.34</td>
<td>2.79 ± 0.26*</td>
<td>2.39 ± 0.318§</td>
</tr>
<tr>
<td>Adenylate charge</td>
<td>0.90 ± 0.08</td>
<td>0.83 ± 0.04*</td>
<td>0.89 ± 0.027</td>
</tr>
</tbody>
</table>

Levels of ATP, ADP, and AMP are expressed in micromoles per gram wet weight; n = 6 to 8 for all groups. T, taurine.

*† Significantly different from those in controls (P < 0.01 and < 0.05, respectively).

§§ Significantly different from those in untreated diabetic rats (P < 0.01 and < 0.05, respectively).
free cytosolic NAD\(^+\)/NADH ratio, which is known to be in equilibrium with DHAP/a-GP under steady-state conditions of the system.\(^{55,57}\) The decrease in free cytosolic NAD\(^+\)/NADH ratio (consistent with a reduction in total NAD\(^+\)/NADPH\(^\\alpha\)) is a very early metabolic marker of poorly controlled diabetes.\(^{64}\) Although studies in rats with a 4- to 10-day duration of diabetes\(^{65,66}\) indicate that NAD-redox imbalances in the lens (which has exceptionally high sorbitol pathway activity compared with other targets for diabetic complications) at least partially result from increased NAD-dependent oxidation of sorbitol to fructose, the present findings and our studies with antioxidant \(\alpha\)-lipoic acid\(^{65}\) imply that with prolongation of diabetes other mechanisms of NAD-redox changes (i.e., those associated with oxidative stress) prevail. The prevention of the diabetes-induced increase in DHAP, amelioration in a-GP, and pyruvate levels and NAD\(^+\)/NADH ratios with taurine and \(\alpha\)-lipoic acid\(^{65}\) together with the lack of any effect of both compounds on sorbitol pathway intermediates, indicate that amelioration of diabetes-induced lens NAD-redox imbalances can be successfully achieved with improvement of antioxidant status, in the absence of sorbitol dehydrogenase inhibition.

The elevation in free cytosolic NAD\(^+\)/NADPH ratio in diabetic rats versus control rats is in accordance with activation of the pentose phosphate pathway under conditions of increased flux through the sorbitol pathway.\(^{66}\) The lack of any effect of taurine on this parameter is consistent with the absence of any changes in the sorbitol pathway intermediates.

The decrease in steady-state concentrations of key glycolytic intermediates (PEP, pyruvate) in the lower segment of glycolysis and free cytosolic NAD\(^+\)/NADH ratios are in concert with changes in the adenine nucleotide system (a decrease in ATP and total adenine nucleotide level and the ATP/ADP ratio and adenylate energy charge), and imply the impairment of energy metabolism in the lens in diabetic rats. Although taurine supplementation ameliorated both ATP/ADP and adenylate energy charge (which are normally indicative of availability of high-energy phosphates for metabolic and functional needs\(^{67}\) and parallel NAD-redox changes\(^{57}\)), the absolute levels of ATP remained unaffected, and ADP, AMP, and total adenine nucleotide were depleted below those in nondiabetic rats. The lack of amelioration in ATP levels (consistent with the lack of amelioration of glycolysis manifested by unaffected PEP and decreased lactate levels) and the depletion of other adenine nucleotides indicate that the normal relationship between NAD-redox and energy state (i.e., ↑ NAD\(^+\)/NADH → ↑ glycolysis → ↑ ATP and total adenine nucleotide) is impaired under conditions of high-dose taurine supplementation. Similar changes in high-energy phosphates were observed in studies with the sorbitol dehydrogenase inhibitor.\(^{65}\)

A number of studies in experimental models of diabetes and galactosemia demonstrate that GSH, ATP, and taurine depletion; other antioxidant deficits; lipid peroxidation; redox imbalances; and decrease in the glycolytic flux are prevented by structurally different aldose reductase inhibitors that eliminate the origin of these imbalances (i.e., sorbitol-accumulation–linked osmotic stress).\(^{19-21,46-48,61,68-70}\) The present study indicates that dietary taurine supplementation, which does not affect the sorbitol pathway flux and fails to prevent lens taurine depletion, ameliorates only part of these defects, being, even at extremely high dosages, totally ineffective against loss of GSH and ATP and inhibition of glycolysis. Taking into consideration the importance of GSH for preventing cross-linking of lens crystallins and maintaining lens transparency and the vitaly essential role of ATP in all major functional and metabolic processes, one can draw the conclusion that taurine supplementation cannot be regarded as an alternative to aldose-reductase inhibition in eliminating antioxidant and metabolic deficits contributing to diabetes-associated cataractogenesis. From the present study it is unclear whether a more effective repletion of lens taurine levels (e.g., by dietary taurine supplementation combined with overexpression of Na\(^+\)/tauine cotransporter gene or selective inhibition of taurine efflux) would result in a better prevention of diabetes-induced changes in lens biochemistry.

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References

Effect of Taurine on Lens Metabolism in Diabetic Rats


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