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# Diabetes-induced changes in lens antioxidant status, glucose utilization and energy metabolism: effect of DL-α-lipoic acid

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**Summary** The study was aimed at evaluating changes in lens antioxidant status, glucose utilization, redox state of free cytosolic NAD(P)-couples and adenine nucleotides in rats with 6-week streptozotocin-induced diabetes, and to assess a possibility of preventing them by DL-α-lipoic acid. Rats were divided into control and diabetic groups treated with and without DL-α-lipoic acid (100 mg · kg body weight$^{-1}$ · day$^{-1}$, i.p.). The concentrations of glucose, sorbitol, fructose, *myo*-inositol, oxidized glutathione, glycolytic intermediates, malate, α-glycerophosphate, and adenine nucleotides were assayed in individual lenses spectrofluorometrically by enzymatic methods, reduced glutathione and ascorbate – colorimetrically, and taurine by HPLC. Free cytosolic $NAD^+$:NADH and $NADP^+$:NADPH ratios were calculated from the lactate dehydrogenase and malic enzyme systems. Sorbitol pathway metabolites were found to increase, and antioxidant concentrations were reduced in diabetic rats compared with controls. The profile of glycolytic intermediates (increase in glucose 6-phosphate and fructose 6-phosphate, decrease in fructose1,6-diphosphate, increase in dihydroxyacetone phosphate, 3-phosphoglycerate, phosphoenolpyruvate, pyruvate, and no change in lactate), and 5.9-fold increase in α-glycerophosphate suggest diabetes-induced inhibition of glycolysis. Free cytosolic $NAD^+$:NADH ratios, ATP levels, ATP/ADP × inorganic phosphate ($P_i$), and adenylate charge were reduced in diabetic rats while free cytosolic $NADP^+$:NADPH ratios were elevated. Diabetes-induced changes in the concentrations of antioxidants, key glycolytic intermediates, free cytosolic $NAD^+$:NADH ratios, and energy status were partially prevented by DL-α-lipoic acid, while sorbitol pathway metabolites and free cytosolic $NADP^+$:NADPH ratios remained unaffected. In conclusion, diabetes-induced impairment of lens antioxidative defense, glucose intermediary metabolism via glycolysis, energy status and redox changes are partially prevented by DL-α-lipoic acid. The findings support the important role of oxidative stress in lens metabolic imbalances in diabetes. [Diabetologia (1998) 41: 1442–1450]

**Keywords** Lens, streptozotocin-diabetic rat, DL-α-lipoic acid, sorbitol pathway, redox and energy status

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*Abbreviations*: GSH, Reduced glutathione; GSSG, oxidized glutathione; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FDP, fructose 1,6-diphosphate; DHAP, dihydroxyacetone phosphate; 3-PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; α-GP, α-glycerophosphate; BSO, L-buthionine(S,R)-sulphoximine.

According to the data of the National Diabetes Data Group [1], the prevalence of cataracts – one of the major ocular disorders in patients with diabetes mellitus – is at least 50 % higher in both Type I (insulin-dependent) diabetes mellitus and Type II (non-insulin-dependent) diabetes than in corresponding age-matched non-diabetic subjects. Sugar cataractogenesis is initiated by osmotic stress caused by intralenticular accumulation of polyols produced by the enzyme aldose reductase [2–6]. Although growing evidence indicates the key role of an osmotic mechanism in the sequelae of biochemical and morphological chan-

ges leading to sugar cataract formation [7, 8], some recent studies also suggest that cataracts can be exacerbated by non-enzymatic glycation of lens proteins [9] and other factors. The importance of oxidative stress in various types of experimentally induced cataractogenesis is well established [10] but its role in diabetes-induced cataractogenesis remains a subject of debate. Some reports indicate an important role for oxidative damage in the onset and progression of diabetic cataracts [11], but others failed to demonstrate any substantial retardation of post-translational modification of lens crystallins [12] or cataract formation [13] with antioxidant therapy. At the same time, numerous studies in models of diabetes and galactosaemia suggest that the loss of major antioxidants such as reduced glutathione (GSH), taurine, and ascorbate is a result of osmotic compensation [14–16].

A role for changes in intermediary and energy metabolism in diabetes-induced cataractogenesis remains unclear. According to Hothersall et al. [17], energy deficiency can contribute to certain lenticular changes (i.e. impairment of membrane transport, glycoprotein and proteoglycan biosynthesis) in diabetes. Diabetes-induced impairment in lens energy status could at least in part result from the inhibition of glycolysis as it is known that 70% of ATP in the lens derives from the glycolytic pathway [18]. One of the glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase, was found to be particularly vulnerable to oxidative damage [19], which could impede the flux through the lower segment of glycolysis in the diabetic lens. It is reasonable to assume that other lenticular enzymes of intermediary and energy metabolism can also be affected by diabetes-induced oxidative stress (resulting from the loss of major antioxidants), and that these changes can be prevented by antioxidant therapy.

The purpose of the present study was to examine changes in the antioxidant status, glucose utilization in the glycolytic pathway, redox state of NAD(P)-couples, and adenine nucleotide system in lens in rats that have been diabetic for 6 weeks, and the possibility of their prevention with DL-α-lipoic acid. It is known from in vitro studies [20] that both DL-α-lipoic acid as well as its R- and S-enantiomers penetrate into the lens and are reduced to dihydrolipoate, a potent antioxidant scavenging hydroxyl, superoxide, and peroxyl radicals, as well as singlet oxygen, and regenerating other antioxidants including GSH, ascorbate, and vitamin E [21, 22]. The ability of α-lipoic acid to prevent the depletion of major antioxidants and to restore the antioxidative defense enzyme activities has been demonstrated in a number of oxidative stress models including L-buthionine(S,R)-sulphoximine (BSO)-induced cataract [22, 23].

## Materials and methods

The experiments were performed in accordance with regulations specified by the National Institutes of Health "Principles of Laboratory Animal Care, 1985 revised version" and University of Michigan Protocol for Animal Studies.

*Animals.* The experiments were performed on barrier-sustained, Caesarean-delivered male Wistar rats body weight 250–300 g, fed a standard rat chow diet (ICN Biomedicals, Cleveland, Ohio, USA) and divided into the following groups: 1) control group; 2) control group treated with DL-α-lipoic acid (Sigma Chemical Co., St. Louis, Mo., USA), at a dose of 100 mg · kg$^{-1}$ · body weight$^{-1}$ · day$^{-1}$, intraperitoneally; 3) diabetic group with 6-week duration of diabetes; 4) diabetic group with 6-week duration of diabetes, treated with DL-α-lipoic acid as described in 2) above. Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg body weight).

*Reagents.* Unless otherwise stated, all chemicals were of reagent-grade quality, and were purchased from Sigma. Methanol (HPLC grade), perchloric acid, hydrochloric acid, and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, Pa., USA) Ethyl alcohol (200 proof dehydrated alcohol, U.S.P. punctilious) was purchased from Quantum Chemical Co, (Tiscola, Ill., USA). Dihydroxyacetone phosphate dilithium salt monohydrate was purchased from Fluka BioChemika (Buchs, Switzerland). B-D-Glucose, sorbitol, N.F., *myo*-inositol, C.P., and D-fructose, U.S.P. were purchased from Pfanstiehl Laboratories (Inc., Waukegan, Ill., USA).

*Experimental procedure.* Rats from each group were anaesthetised with i.p.urethane (1–1.2 g/kg)(Sigma) and subsequently killed by cervical dislocation. Both lenses were rapidly dissected by posterior incision, carefully separated from any accompanying aqueous and vitreous humours by gentle rolling over a fine filter paper, and frozen in liquid nitrogen for subsequent biochemical analyses. In the first set of animals (5 controls, 6 controls + DL-α-lipoic acid, 7 diabetics and 7 diabetics + DL-α-lipoic acid), one lens from each rat was used for measurements of GSH, oxidized gluathione (GSSG), metabolites (except sorbitol, fructose, and *myo*-inositol) and adenine nucleotides, and the second lens was used for measurements of sorbitol, fructose, *myo*-inositol, ascorbate and taurine. In the second set of animals (5 controls, 4 diabetics and 8 diabetics + DL-α-lipoic acid), both lenses were used for measurements of metabolites and adenine nucleotides only. Blood samples for measurements of glucose were taken from the tail vein the day before killing.

*Measurements of metabolites and adenine nucleotides.* Lenses were weighed and deproteinized by homogenization with perchloric acid (1 ml of 6% $HClO_4$ per lens), followed by centrifugation (Sorvall MC 12 V). The concentrations of glucose, glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-disphosphate (FDP), dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3-PG), phosphoenolpyruvate (PEP), pyruvate, lactate, malate, α-glycerophosphate (α-GP), ATP, ADP, and AMP were assayed in neutralized extracts (neutralization was done with $K_2CO_3$ 2 mol/l) of individual lenses spectrofluorometrically (Perkin-Elmer LS-5B, Norwalk, Connecticut, USA) by enzymatic procedures as described [24]. The lower limit for all spectrofluorometric procedures in our study including glucose, sorbitol, fructose, and

*myo*-inositol was $0.1 \times 10^{-9}$ mol/l. Inorganic phosphate (Pi) concentrations were assayed by a colorimetric procedure with kits from Sigma Diagnostics.

*Measurements of sorbitol, fructose, and myo-inositol.* Lenses were weighed and homogenized in 0.8 ml 0.9 % NaCI. A 100 µl volume of 0.3 mol/l zinc sulphate, and then an equivalent of barium hydroxide, was added to 0.2 ml of homogenate for protein precipitation. The samples were centrifuged at $4000 \times g$ for 10 min and aliquots of the supernatant were taken for spectrofluorometric measurements of sorbitol, fructose, and *myo*-inositol by enzymatic procedures, using sorbitol dehydrogenase [25], fructose dehydrogenase [26], and *myo*-inositol dehydrogenase [25], respectively. In brief, the analytical mixture for sorbitol and *myo*-inositol contained 0.9 ml 0.5 mmol/l NAD in 0.1 mol/l glycine-NaOH buffer (pH 9.5) and deproteinized extract (for sorbitol, 0.1 ml for control and 0.01 ml plus 0.09 ml $H_2O$ for diabetic lenses; for *myo*-inositol, 0.1 ml for both control and diabetic lenses). The reaction was started by addition of approximately 0.8 U of sorbitol dehydrogenase or 0.5 U of *myo*-inositol dehydrogenase. The analytical mixture for fructose contained 0.9 ml of rezasurin-containing 150 mmo/l citrate buffer, pH 4.5 (1 aliquote of rezasurin (5 mg–10 ml $H_2O$) was mixed with 100 aliquotes of citrate buffer) and deproteinized extract (0.1 ml for control and 0.05 ml plus 0.05 ml $H_2O$ for diabetic lenses). The reaction was started by addition of approximately 0.5 U of fructose dehydrogenase.

*Measurement of taurine.* Lens taurine was measured by reversed phase HPLC (Waters, Milford, Mass., USA) after precolumn derivatization with o-phthalaldehyde [27]. In brief, 0.1 ml of lens homogenate (see Measurements of sorbitol, fructose and *myo*-inositol) was extracted with 1 ml of 6 % trichloroacetic acid and centrifuged at $4000 \times g$ for 10 min. The supernatants were purified on washed dual-bed, ion-exchange columns (2.5 cm AG 1-X8 100–200 mesh [Bio-Rad, Richmond, California, USA] 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 of water and lyophilized. Samples and standards were dissolved in 100 µl of water prior to HPLC analysis. Isocratic elution was carried out at a flow rate of 2 ml/min, using 43 % solvent A (0.05 mol/l $NaH_2PO_4$, pH 5.3 plus 5 mol/l NaOH) combined with 57 % solvent B (0.05 mol/l $NaH_2PO_4$ in 75 % methanol/water). Glutamine, added after ion exchange chromatography, was used as the internal standard.

*Measurements of other antioxidants.* Concentrations of GSH were assayed in perchloric acid extracts colorimetrically as described [14] and of GSSG spectrofluorometrically by enzymatic procedure. The analytical mixture contained 0.8 ml 0.1 mol/l imidazole buffer (pH 7.6), 0.2 ml of perchloric extract and 0.2–10 µmol NADPH. The reaction was started by addition of approximately 0.3 U of glutathione reductase (Type IV, Sigma). Concentration of ascorbate were measured colorimetrically with 2,6-dichlorophenolindophenol [28], after extraction of 0.4 ml of lens homogenate (see Measurements of sorbitol, fructose and *myo*-inositol) with 5 % metaphosphoric acid. All spectrophotometric measurements (GSH, $P_i$ and ascorbate) were done using the spectrophotometer Beckman DU 640, (Fullerton, Calif., USA).

*Calculations of free cytosolic $NAD^+$:NADH and $NADP^+$:NADPH ratios, mass action ratio of the ATP system, and adenylate energy charge.* According to classical publications of Krebs' laboratory [29, 30] and other studies [31], direct measurements of NAD, NADH, NADP and NADPH are not informative in terms of control of metabolism as they do not inform on compartmentalization of nicotinamide nucleotides between cytosol and mitochondria and do not separate free from protein-bound forms (only free fractions determine direction and free-energy changes of dehydrogenase reactions). The same studies proposed an alternative approach with assessment of free $NAD(P)^+$:NAD(P) 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 $NADP^+$:NADPH ratios were calculated from metabolite concentrations and the equilibrium constants of lactate dehydrogenase and malic enzyme as follows [29–31]:

$$\frac{[NAD^+]}{[NADH]} = \frac{[Pyruvate]}{[Lactate]} \times \frac{1}{k_1},$$

where $k_1$ is the equilibrium constant of lactate dehydrogenase ($1.11 \times 10^{-4}$ mol/l [29, 31].

$$\frac{[NADP^+]}{[NADH]} = \frac{[Pyruvate] \times [CO_2]}{[Malate]} \times \frac{1}{k_2},$$

where $k_2$ is the equilibrium constant of malic enzyme ($3.44 \times 10^{-2}$ mol/l) [30] (the $CO_2$ concentration was taken to be 1.16 mmol/l).

Mass action ratio of the ATP system ([ATP]:[ADP] × [$P_i$]) as a parameter of phosphorylation state of adenine nucleotides [31] and adenylate energy charge {[ATP + (ADP:2)]:ATP + ADP + AMP]} were calculated from measured concentrations of ATP, ADP, AMP and $P_i$.

*Statistical analysis.* The results are expressed as means ± standard deviation. If the standard deviation for a variable increased substantially with increase of the mean of the variable, the formal analysis was conducted on natural logarithm transformed data to reduce the heterogeneity of variances; otherwise, the analysis was conducted on the observed data. Individual pair-wise comparisons between the experimental groups (controls vs controls + DL-α-lipoic acid, controls vs diabetics, diabetics vs diabetics + DL-α-lipoic acid, and diabetics + DL-α-lipoic acid vs controls) were evaluated by the Behrens-Fisher two-sided two-sample *t*-test that does not assume equality of variances.

## Results

The body weights were lower in diabetic rats compared with those in control rats (297.5 ± 69.7 vs 439.4 ± 45.7 g, $p = 0.0004$). The initial body weights were similar in these two groups (264.7 ± 19.5 and 240.6 ± 17.8 g, respectively). No significant difference was found between body weights in control and diabetic rats treated with DL-α-lipoic acid (407.6 ± 50.4 and 282.3 ± 55.5 g, respectively) and the corresponding untreated groups.

The lens wet weights were not different among the experimental groups studied (controls: 31.5 ± 2.2 mg, diabetics: 33.2 ± 4.1, and diabetics + DL-α-lipoic acid: 31.5 ± 3.6 mg).

Plasma glucose concentrations were increased about 3.9-fold in diabetic rats compared with those in control rats (15.9 ± 2.6 vs 3.9 ± 0.3 mmol/l, $p =$

**Table 1.** Lens glucose, sorbitol, fructose, and *myo*-inositol concentrations (μmol/g wet weight) in control and diabetic rats treated with and without DL-α-lipoic acid

| | Control ($n = 5$) | Control + LA ($n = 6$) | Diabetic ($n = 7$) | Diabetic + LA ($n = 7$) |
|---|---|---|---|---|
| Glucose | 1.490 ± 0.548 | 1.160 ± 0.591 | 5.78 ± 1.26[a] | 5.45 ± 1.50[a] |
| Sorbitol | 0.683 ± 0.197 | 0.536 ± 0.067 | 13.38 ± 3.88[a] | 11.28 ± 4.22[a] |
| Fructose | 0.911 ± 0.246 | 0.986 ± 0.488 | 5.85 ± 1.62[a] | 5.66 ± 2.25[b] |
| *myo*-Inositol | 0.523 ± 0.102 | 0.479 ± 0.075 | 0.040 ± 0.029[a] | 0.048 ± 0.029[a] |

LA, DL-α-lipoic acid;
[a,b] Significantly different compared with those in controls ($p < 0.0001$ and $< 0.001$, respectively)

**Table 2.** Lens antioxidant status in control and diabetic rats treated with and without DL-α-lipoic acid (concentrations of GSH, GSSG, ascorbate, and taurine are expressed in μmol/g wet weight)

| | Control | Control + LA | Diabetic | Diabetic + LA |
|---|---|---|---|---|
| *n* | 5 | 6 | 7 | 7 |
| GSH | 5.14 ± 0.52 | 5.19 ± 0.64 | 1.75 ± 0.52[a] | 3.45 ± 1.17[b,d] |
| GSSG | 0.225 ± 0.064 | 0.226 ± 0.050 | 0.328 ± 0.130 | 0.264 ± 0.109 |
| 100GSSG : GSH | 4.41 ± 1.27 | 4.37 ± 0.92 | 19.94 ± 7.71[a] | 7.90 ± 2.75[b,d] |
| Ascorbate | 0.232 ± 0.045 | 0.259 ± 0.071 | 0.168 ± 0.035[c] | 0.200 ± 0.046 |
| *n* | 6 | 6 | 5 | 7 |
| Taurine | 7.38 ± 1.80 | 7.26 ± 0.76 | 1.21 ± 1.12[a] | 3.21 ± 2.23[b] |

LA, DL-α-lipoic acid;
[a,b,c] Significantly different compared with those in controls ($p < 0.0001$, $< 0.01$ and $< 0.05$, respectively),
[d] Significantly different compared with those in untreated diabetics ($p < 0.01$)

0.0001). DL-α-lipoic acid treatment had no effect on plasma glucose concentrations in either control (4.0 ± 0.6) or diabetic (16.5 ± 3.8) rats.

Concentrations of lens glucose, sorbitol, and fructose were increased 3.9-fold, 19.6-fold, and 6.4-fold, respectively, in diabetic rats, compared with those in controls and of *myo*-inositol levels in 6-week diabetic rats depleted to about 8% of those in controls. None of these variables was affected by DL-α-lipoic acid treatment in either control or diabetic rats (Table 1).

Lens GSH, GSSG, and ascorbate concentrations as well as 100GSSG:GSH ratios were similar in control rats treated with and without DL-α-lipoic acid (Table 2). Lens GSH, ascorbate, and taurine concentrations in diabetic rats were decreased to 34%, 72%, and 10% of those in controls. Concentrations of GSSG did not differ in the two groups but 100GSSG:GSH ratio in diabetic rats was increased 4.5-fold. Diabetes-induced depletion of GSH was partially prevented by DL-α-lipoic acid treatment. Taurine levels tended to increase although the difference from untreated diabetics did not reach statistical significance ($p = 0.068$). Concentrations of GSSG and ascorbate remained unaffected. The increase in 100GSSG:GSH ratio in diabetic rats was substantially but not completely prevented by DL-α-lipoic acid.

The concentrations of the glycolytic intermediates, G6P and F6P, were increased by 67% and 32% in the lenses of diabetic rats compared with those in controls. Those of FDP were decreased by 21% and of DHAP increased by 51%, but those of metabolites of the lower segment of glycolysis, i.e. 3-PG, PEP, and pyruvate were considerably decreased (by 29, 69 and 45%, respectively) in the diabetic rats, and lactate levels were similar in control and diabetic groups. Concentrations of all glycolytic intermediates studied were indistinguishable between control rats treated with and without DL-α-lipoic acid. The DL-α-lipoic acid treatment of diabetic rats completely prevented the decrease in FDP and 3PG levels. Accumulation of DHAP as well as decrease in pyruvate were partially prevented but PEP tended to increase although the difference with untreated diabetic group did not reach significance ($p = 0.055$). The concentrations of G6P, F6P, and lactate remained unaffected (Table 3).

Lens α-GP concentrations in 6-week diabetic rats were increased 5.9-fold (1.59 ± 0.20 μmol/g wet weight vs 0.268 ± 0.046 in controls, $p < 0.0001$). The increase was partially prevented in diabetic rats treated with DL-α-lipoic acid (0.878 ± 0.411, $p < 0.0001$ vs both untreated diabetic and controls). DL-α-lipoic acid had no effect on α-GP concentrations in control rats (0.327 ± 0.076).

Lens malate concentrations were similar in control rats treated with and without DL-α-lipoic acid (0.087 ± 0.014 and 0.087 ± 0.016 μmol/g wet weight, respectively). They were substantially depleted in diabetic rats (0.035 ± 0.012, $p = 0.0001$ vs controls). This depletion was partially prevented with DL-α-lipoic acid treatment (0.054 ± 0.011, $p = 0.001$ and $= 0.0001$ vs untreated diabetics and controls, respectively).

**Table 3.** Concentrations of glycolytic intermediates in lenses in control and diabetic rats treated with and without DL-α-lipoic acid (μmol/g wet weight)

| | Control | Control + LA | Diabetic | Diabetic + LA |
|---|---|---|---|---|
| *n* | 10 | 6 | 11 | 15 |
| G6P | 0.142 ± 0.022 | 0.135 ± 0.037 | 0.237 ± 0.037[a] | 0.233 ± 0.035[a] |
| F6P | 0.069 ± 0.012 | 0.071 ± 0.020 | 0.091 ± 0.016[c] | 0.087 ± 0.019[c] |
| FDP | 0.052 ± 0.003 | 0.054 ± 0.007 | 0.041 ± 0.005[a] | 0.056 ± 0.005[d,e] |
| DHAP | 0.045 ± 0.007 | 0.047 ± 0.015 | 0.068 ± 0.015[b] | 0.050 ± 0.015[f] |
| *n* | 11 | 6 | 9 | 14 |
| 3-PG | 0.075 ± 0.006 | 0.066 ± 0.013 | 0.053 ± 0.008[a] | 0.076 ± 0.012[e] |
| *n* | 12 | 6 | 10 | 14 |
| PEP | 0.042 ± 0.016 | 0.033 ± 0.012 | 0.013 ± 0.007[a] | 0.018 ± 0.006[a] |
| *n* | 12 | 6 | 10 | 15 |
| Pyruvate | 0.112 ± 0.024 | 0.108 ± 0.014 | 0.062 ± 0.018[a] | 0.093 ± 0.037[f] |
| Lactate | 7.93 ± 0.69 | 8.08 ± 0.60 | 8.15 ± 1.57 | 7.69 ± 1.69 |

LA, DL-α-lipoic acid;
[a,b,c,d] Significantly different compared with those in controls ($p < 0.0001$, $< 0.001$, $< 0.01$, and $< 0.05$, respectively),
[e,f] Significantly different compared with those in untreated diabetic groups ($p < 0.0001$ and $< 0.01$, respectively)

**Table 4.** Lens energy status in control and diabetic rats treated with and without DL-α-lipoic acid (concentrations of ATP, ADP, AMP, and $P_i$ are expressed in μmol/g wet weight)

| | Control ($n = 12$) | Control + LA ($n = 6$) | Diabetic ($n = 10$) | Diabetic + LA ($n = 15$) |
|---|---|---|---|---|
| ATP | 2.04 ± 0.30 | 2.17 ± 0.19 | 1.56 ± 0.34[c] | 1.95 ± 0.28[g] |
| ADP | 0.285 ± 0.043 | 0.297 ± 0.064 | 0.369 ± 0.050[b] | 0.312 ± 0.050[g] |
| AMP | 0.084 ± 0.019 | 0.075 ± 0.012 | 0.134 ± 0.020[a] | 0.091 ± 0.010[e] |
| ATP : ADP | 7.22 ± 0.87 | 7.51 ± 1.41 | 4.31 ± 1.06[a] | 6.29 ± 0.88[d,h] |
| ATP + ADP + AMP | 2.414 ± 0.347 | 2.537 ± 0.238 | 2.067 ± 0.344[d] | 2.348 ± 0.318[h] |
| Adenylate charge | 0.91 ± 0.01 | 0.91 ± 0.01 | 0.84 ± 0.03[a] | 0.89 ± 0.01[a,e] |
| $P_i$ | 2.55 ± 0.29 | 3.10 ± 0.44[b] | 2.85 ± 0.22[d] | 2.92 ± 0.26[c] |
| ATP : ADP × $P_i$ | 2.86 ± 0.41 | 2.48 ± 0.66 | 1.52 ± 0.37[a] | 2.17 ± 0.33[a,f] |

LA, DL-α-lipoic acid;
[a,b,c,d] Significantly different from those in controls ($p < 0.0001$, $< 0.001$, $< 0.01$, and $< 0.05$, respectively),
[e,f,g,h] Significantly different from those in untreated diabetic rats ($p < 0.0001$, $< 0.001$, $< 0.01$, and $< 0.05$, respectively)

Free cytosolic $NAD^+$:NADH ratios were similar in control rats treated with and without DL-α-lipoic acid (126.7 ± 23.4 and 120.1 ± 13.8) and considerably reduced in diabetic rats (69.7 ± 17.6 vs controls, $p < 0.0001$). This reduction was prevented by DL-α-lipoic acid treatment (108.7 ± 30.0, $p < 0.0005$ and = 0.088 vs untreated diabetics and controls, respectively).

Free cytosolic $NADP^+$:NADPH ratios were increased in diabetic rats vs those in controls (0.061 ± 0.010 and 0.043 ± 0.005, $p < 0.0001$). DL-α-lipoic acid treatment had no effect on free cytosolic $NADP^+$:NADPH ratios in either control (0.043 ± 0.008) or diabetic (0.054 ± 0.011) rats.

The concentrations of ATP, ADP, AMP as well as ATP:ADP ratio, total adenine nucleotide content, adenylate charge, and mass action ratio of the ATP system were similar in control rats treated with and without DL-α-lipoic acid but $P_i$ levels were slightly higher in DL-α-lipoic acid-treated group ($p < 0.02$). In diabetic rats ATP concentrations were 24% lower than in controls but ADP, AMP, and $P_i$ were increased. The total adenine nucleotide concentration as well as ATP:ADP, ATP:ADP × $P_i$ and adenylate energy charge were reduced in diabetic rats compared with controls. DL-α-lipoic acid treatment prevented the decrease in ATP and total adenine nucleotide as well as the increase in ADP and AMP. The decrease in ATP:ADP ratio, mass action ratio of the ATP system, and adenylate energy charge were partially prevented while $P_i$ concentrations remained unaffected (Table 4).

## Discussion

The absence of any difference in either body weights or blood glucose concentrations between the DL-α-lipoic acid-treated rats and those untreated is consistent with other findings [32].

The diabetes-induced increase in lens glucose, sorbitol, and fructose agrees with earlier publications [18, 33] although the accumulation of sorbitol and fructose in the diabetic group in our study was lower compared with others where measurements of the sorbitol pathway intermediates were done (probably,

due to lower blood glucose concentrations: 15.9 mmol/l in our study vs 25 mmol/l and higher in the others [33, 34]). It has been reported that α-lipoic acid stimulates glucose transport in muscle [35], as well as in peripheral nerve with resulting increases of nerve glucose, sorbitol and fructose [36]. Despite glucose transporter 1 (GLUT 1) (the translocation of which to the plasma membrane in the muscle is facilitated with DL-α-lipoic acid treatment [35]) being the most abundant glucose transporter in both lens and peripheral nerve, we found no difference in lens glucose, sorbitol and fructose concentrations in the DL-α-lipoic acid-treated control and diabetic rats and the corresponding untreated groups. These findings can probably be explained by low concentrations of glucose transporters in rat lenses as well as by their predominant localization in the nucleus and low density in the outer regions such as epithelium and cortex [37].

The depletion of *myo*-inositol in diabetic rats is consistent with the dramatic intralenticular accumulation of sorbitol and agrees with previous reports [18, 33]. The depletion of *myo*-inositol is considered to result from both osmotically-mediated decrease in ($Na^+$)-*myo*-inositol uptake and increase in *myo*-inositol efflux [38].

The diabetes-induced decrease in lens GSH concentrations is consistent with previous studies [14, 39]. The loss of GSH has been suggested to be a result of impaired ability of the lens to concentrate amino acids required for GSH biosynthesis coupled to faster GSH efflux under hyperosmotic conditions, rather than inhibition of glutathione reductase or depletion of NADPH due to increased flux through aldose reductase [14]. This conclusion is supported by 1) a further decrease in lens GSH levels in diabetic rats treated with sorbitol dehydrogenase inhibitor (that increases lens sorbitol concentrations about twofold over those in untreated diabetics) [7, 40]; 2) the absence of a reciprocal increase of GSSG concentrations in concert with the decrease in GSH ([38] and in the present study). In addition, it has been suggested that the fall in GSH in diabetic rats can be further aggravated by the decrease of ATP (which is required for de novo synthesis of GSH, both at the γ-glutamyl cysteine synthetase and glutathione synthetase steps [41]. Treatment with DL-α-lipoic acid partially prevented the diabetes-induced decrease in lens GSH concentrations which agrees with the studies on BSO-treated rats [23] and with findings in diabetic nerve [32]. The prevention of GSH depletion by DL-α-lipoic acid in diabetes and in other models of oxidative stress can not be attributed to the corresponding changes in GSSG concentrations because the decrease in GSSG with DL-α-lipoic acid treatment is either very minor (compared with the increase in GSH) [32] or absent [23], and in this study) and the decrease in GSH was substantially prevented by both racemic α-lipoic acid and its R-enantiomer [23].

Lens ascorbate concentrations in our study are comparable to those obtained by HPLC [39]. The diabetes-induced decrease in lens ascorbate agrees with other reports [39, 43]. The failure to prevent the diabetes-induced decrease in lens ascorbate by DL-α-lipoic acid in our study (vs complete prevention of BSO-induced ascorbate decrease by both racemic α-lipoic acid and its R-enantiomer [23]) indicates that the contribution of a redox cycling mechanism to the diabetes-induced decrease in ascorbate concentrations is fairly minor, and another (potentially osmotic [16]) factor could be responsible for ascorbate depletion in the lens under diabetic conditions.

Lens taurine concentrations in control rats in our study are higher compared with those of two previous reports [15, 43] but are in agreement with another [44] where taurine levels in rat lens exceeded those of GSH. Although taurine is the most abundant free amino acid in the lens [43, 44] its uptake by the lens is very low [45] which implies an endogenous taurine-synthesizing mechanism probably from cysteine and methionine. No information on the activity of cysteine sulphinate decarboxylase, the taurine-forming enzyme, in the lens is available. The observed diabetes-related depletion of taurine agrees with other findings [15], and is considered to be a result of osmotic compensation in response to intralenticular accumulation of sorbitol. A tendency towards amelioration of taurine depletion with DL-α-lipoic acid treatment concurs with the ability of the drug to restore other antioxidants in other models of oxidative stress [23]. The effect of DL-α-lipoic acid treatment on lens taurine levels seems not to be osmotically mediated as neither sorbitol nor *myo*-inositol concentrations were affected.

The pattern of glycolytic intermediates in lens in diabetic rats compared with controls (increase in G6P and F6P, decrease in FDP, increase in DHAP, decrease in 3-PG, PEP, pyruvate, no difference in lactate) suggests inhibition of glycolysis, with the sites of regulation at phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase, enolase, and pyruvate kinase. The conclusion that glycolysis is inhibited at the stage of glyceraldehyde 3-phosphate dehydrogenase is supported by the demonstration that this enzyme is a target for oxidative damage [19] and by that of a 5.9-fold increase in α-GP which can be converted both to diacylglycerol and its metabolites in the lens [46]. The observed diabetes-induced accumulation of α-GP in the lens accords with other reports [34, 40], and is consistent with the decreased free cytosolic $NAD^+$:NADH ratio which reflects the shift in equilibrium of α-GP dehydrogenase reaction towards the formation of α-GP. A complete prevention of the diabetes-induced increase in lens α-GP concentrations by an aldose reductase inhibitor (sorbinil), and only partial ( ~ 50 %) prevention by a sorbitol dehydrogenase inhibitor (CP- 166,572) [40] indi-

cates that in addition to a polyol pathway flux-linked change in $NAD^+$:NADH ratio (which is in equilibrium with the ratio of DHAP to α-GP [29]), other aldose-reductase mediated mechanism(s) contribute to a rise in lens α-GP levels under diabetic conditions. One of these putative mechanisms could be an increased reduction of GA3P to α-GP by aldose reductase [46, 47]. Others could be linked to aldose-reductase mediated oxidative stress which inhibits the flux through glyceraldehyde 3-phosphate dehydrogenase (and hence the lower segment of glycolysis, resulting in a decrease in cytosolic ATP:$ADP \times P_i$, and in $NAD^+$:NADH [31]), and may also impact on the activity of cytosolic DT diaphorase etc. The partial prevention of the diabetes-induced increase in DHAP and α-GP, complete prevention of the decrease in 3-PG and amelioration of pyruvate concentrations and $NAD^+$:NADH ratios with DL-α-lipoic acid treatment in this study supports the contribution of oxidative stress to diabetes-induced inhibition of glycolysis, accumulation of α-GP and the shift towards a more reduced state of free cytosolic NAD-couple. At the same time, a complete prevention of the decrease in FDP in DL-α-lipoic acid treated diabetic rats [48] points to an additional beneficial effect of the drug on glucose utilization at the level of phosphofructokinase. Prevention of the diabetes-induced decrease in FDP by structurally different ARIs and, on the contrary, further depletion of FDP by sorbitol dehydrogenase inhibitor [40] points to an osmotic mechanism in the down-regulation of phosphofructokinase and suggests that amelioration of osmotically-induced oxidative stress rather than "metabolic" effects of DL-α-lipoic acid by decreasing intracellular citrate [48] could prevent the diabetes associated fall in lens FDP concentrations.

Note that, in addition to a "direct" cytosolic effect of DL-α-lipoic acid on free cytosolic $NAD^+$:NADH ratio in the diabetic lens DL-α-lipoic acid can "indirectly" affect cytosolic reducing equivalent homeostasis by reduction of α-lipoate to dihydrolipoate by mitochondrial dihydrolipoamide dehydrogenase [49] (with a resulting decrease in mitochondrial NADH and stimulation of NADH transfer from cytosol to mitochondria). The ability of α-lipoic acid to decrease both lactate:pyruvate and total NADH:$NAD^+$ ratios under normoglycaemic conditions has been shown in cell culture experiments [49], however, in our in vivo study, DL-α-lipoic acid seems not to affect free cytosolic $NAD^+$/NADH ratios (calculated for *whole* lenses) of control rats. Both in vitro and in vivo studies suggest that "direct" cytosolic effects of DL-α-lipoic acid are primarily responsible for amelioration of cytosplasmic NAD-redox status in the *whole* diabetic lens, although "indirect" mitochondrial effects probably prevail in the equatorial subcapsular cortex which is rich in mitochondria and has a critical role in glucose mediated cataractogenesis [20].

In contrast to free cytosolic $NAD^+$:NADH ratios, lens free cytosolic $NADP^+$:NADPH ratios were increased in diabetic rats compared with controls. This agrees with the depletion of NADPH levels [14] as well as with the observations in a galactose model [50] and favours activation of the pentose phosphate pathway regenerating reducing equivalents (NADPH) for aldose reductase [51]. The failure of DL-α-lipoic acid to affect the redox state of free cytosolic NADP-couple is consistent with the absence of changes in sorbitol pathway metabolites, and implies a similar rate of use of NADPH by aldose reductase in DL-α-lipoic acid treated and untreated groups.

The decrease in the glycolytic flux and free cytosolic $NAD^+$:NADH ratios accords with changes in the adenine nucleotide system and imply the impairment of energy metabolism in the lens of diabetic rats. The decrease in the mass-action ratio of the ATP system and adenylate energy charge indicate a limited availability of high energy phosphates for metabolic and functional needs. That the decrease compared with controls in ATP concentrations in diabetic rats substantially exceeds the increase in ADP and AMP concentrations is in line with the previous observation of a complete prevention of diabetes-induced changes in lens adenine nucleotide system by an aldose reductase inhibitor compared with further exacerbation of energy deficiency by sorbitol dehydrogenase inhibitor [40] and indicates the role of osmotic stress in the impairment of lens energy status under diabetic conditions. Prevention of diabetes-induced changes in lens adenine nucleotide system by both DL-α-lipoic acid and by structurally different ARIs [40] suggests that polyol associated osmotic stress can impair lens energy metabolism via oxidative stress. In addition to glycolysis, oxidative stress affects the redox state of coenzyme Q [52], which leads to inhibition of mitochondrial oxidation coupled to biosynthesis of ATP. Studies in other models of oxidative stress indicate that lipoic acid counteracts those changes. Although it is known that in vitro R-lipoic acid activates the pyruvate dehydrogenase (PDH) complex [54], it is not clear if this mechanism contributes to the amelioration of lens energy status.

In conclusion, diabetes-induced changes in lens intermediary and energy metabolism are substantially prevented by antioxidant DL-α-lipoic acid which could thus help to avoid oxidative stress to the lens and thereby possibly diabetes-associated cataract formation.

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