

# Total Antioxidant Status and Lipid Peroxidation in Diabetic Patients

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## Introduction

Lipid peroxidation is thought to be one of the major factors involved in atherogenesis and carcinogenesis. Occurring at low levels in all cells, the peroxidation process involves the oxidative conversion of unsaturated fatty acids to lipid hydroperoxides as the primary product, together with the formation of a variety of secondary metabolites. In atherosclerosis, current research suggests that lipid peroxidation results in macrophage stimulation of foam cell formation, leading to endothelial damage, which is thought to initiate the formation of atherosclerotic plaques<sup>(1,2)</sup>. Diabetics are known to be at increased risk of cardiovascular disease, a phenomenon which has previously been linked to the lipid peroxidation process<sup>(3)</sup>. Increased lipid peroxidation in diabetic subjects has been reported<sup>(1)</sup>, with malondialdehyde recognised as the major product of this process<sup>(4)</sup>.

The aim of this work was to present a comparative study of some factors involved in antioxidant protection. Those chosen were Total Antioxidant Status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), which were measured with malondialdehyde (MDA) as an indicator of lipid peroxidation, in 100 diabetic samples and 22 controls.

## Materials and Methods

Five ml of blood was taken from each of 100 diabetic subjects and 22 normal controls and placed into EDTA-treated tubes. Samples were assayed within 24h of being taken. Two ml of whole blood was removed and centrifuged at 2,000 x g for 10 min. The plasma was removed and retained for assay of TAS, GR and MDA. The remainder was retained for assay of SOD and GPx.

Assays for TAS, SOD, GPx and GR were performed at 37°C using a Cobas Fara II centrifugal analyser (Roche, Switzerland).

### Total Antioxidant Status

Total Antioxidant Status (TAS) was measured using a kit supplied by Radox Laboratories Ltd. (Cat. No. NX2332). The plasma sample volume was 5 µl, in a total assay volume of 305 µl. Colour production is measured at 600 nm with a read time of 5 min.

### Superoxide dismutase

Superoxide dismutase (SOD) was measured using a kit supplied by Radox Laboratories Ltd. (Cat. No. SD125) using an appropriate whole blood SOD control (Cat. No. SD126).

Aliquots of whole blood (0.5 ml) were centrifuged at 2,000 x g, washed x 4 with 0.9% NaCl, and lysed in a total volume of 2 ml of ice-cold double deionised H<sub>2</sub>O. The lysate was then diluted 1 in 25 with RANSOD diluting buffer (Cat. No. 366MS). This preparation was used for measurement of SOD. The reaction was measured at 500 nm, using 5 µl of sample in a total reaction volume of 230 µl.

### Glutathione peroxidase

Glutathione peroxidase (GPx) was measured using a kit supplied by Radox Laboratories Ltd. (Cat. No. RS505), using the appropriate whole blood control (SC692). Whole blood 50 µl was diluted with 1 ml RANSEL diluting agent and incubated for 5 min. One ml of double-strength Drabkin's solution was added, and assays were performed within 20 min. GPx activity was measured at 340 nm, using a sample volume of 5 µl in a total reaction volume of 285 µl.

### Glutathione reductase

Glutathione reductase (GR) was measured using a kit supplied by Radox Laboratories Ltd. (Cat. No. GR2368). The decrease in absorbance is measured at 340 nm. The assay requires a sample volume of 10 µl in a total reaction volume of 310 µl.

### Malondialdehyde

Malondialdehyde (MDA) was assayed as a marker of lipid peroxidation using a colorimetric reaction which uses 1-methyl-2-phenylindole as chromogen. Condensation of one molecule of MDA with 2 molecules of 1-methyl-2-phenylindole under acidic conditions results in the formation of a chromophore with an absorbance maximum at 586 nm.

A 7.6 mM solution of 1-methyl-2-phenylindole (MPI) was prepared immediately prior to use, in 33% methanol in acetonitrile. A 650 µl aliquot of MPI was placed in each test tube, to which was added 200 µl of plasma. The tubes were mixed well, and 150 µl of 10 M HCl was added. After mixing once more, the tubes were sealed, and incubated for 60 min. at 45°C. After incubation, the tubes were chilled on an ice bath, and spun at 10,000 x g for 5 min. to remove debris. The absorbance at 586 nm was measured and subtracted from the blank value, obtained by replacing plasma with water. A calibration graph was prepared using 4 µmol/L, 8 µmol/L, 16 µmol/L and 20 µmol/L of 1,1,3,3-tetramethoxypropane in 20 mM Tris-HCl, buffer, pH 7.4.

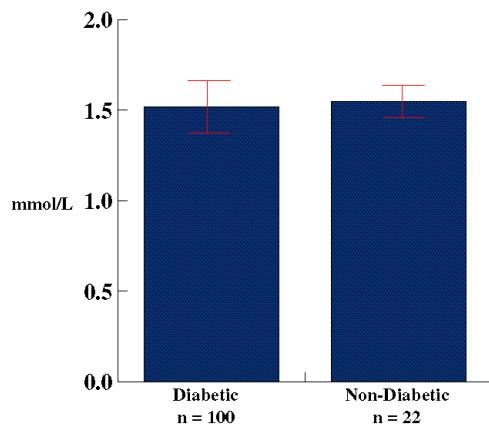
## Statistics

Statistical significance was assessed using Student's t-test. Results were deemed statistically significant where p<0.05. Correlation coefficients were calculated using least squares linear regression, and were deemed significant when r>0.9.

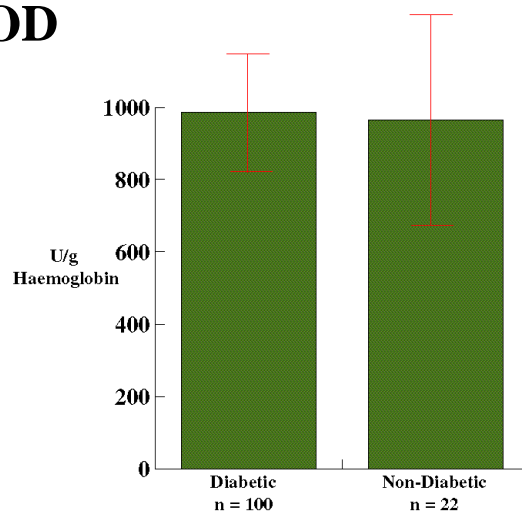
## Results

Levels of MDA were found to be significantly higher in diabetics compared to normal subjects, with a mean value of 3.103 µmol/L for diabetics, compared to 1.266 µmol/L in normal controls. In addition, GPx levels were significantly lower in diabetics, with a mean value for diabetics of 44.2 U/g Hb compared to 56.1 U/g Hb in normal subjects. No significant difference in TAS, SOD and GR levels was found between the two groups. (Table 1) Antioxidant markers were correlated with TAS levels by least squares linear regression. A slight association between TAS and MDA levels was noted, although this was not deemed to be statistically significant (r = -0.617). No association was noted between TAS and SOD, GPx or GR (Table 2).

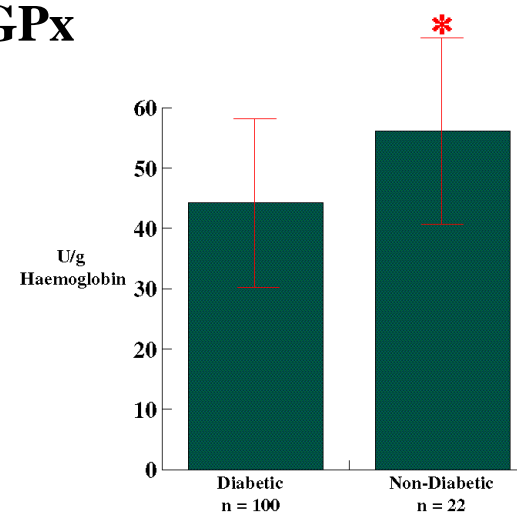
## TAS



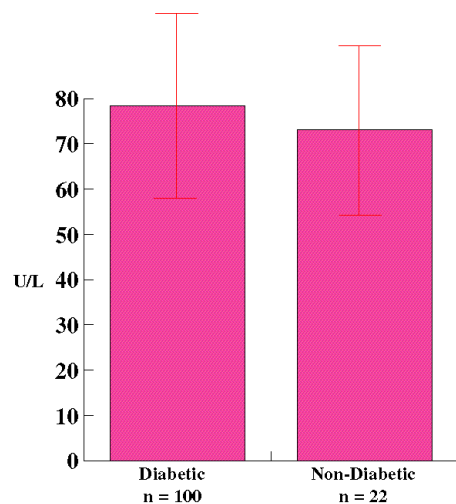
## SOD



## GPx



## GR



## MDA

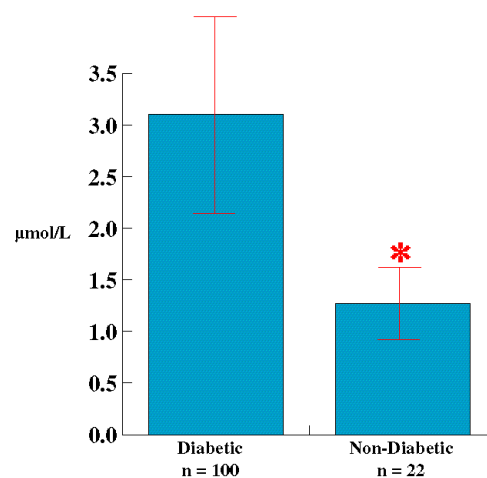


Table 1: Comparison of Antioxidant Markers between Diabetic (n=100) and Non-Diabetic (n=22) subjects

Marker	Diabetic Mean ± S.D.	Non-Diabetic Mean ± S.D.	p (T-Test)
MDA	3.103 ± 0.938	1.266 ± 0.331	1.7 x 10 <sup>-15</sup> *
TAS	1.515 ± 0.148	1.545 ± 0.088	0.176 NS
SOD	985.8 ± 163.18	965.9 ± 292.0	0.330 NS
GPx	44.187 ± 14.04	56.091 ± 15.43	0.0003*
GR	78.329 ± 20.52	73.061 ± 18.77	0.131 NS

\* - Significantly different  
NS - Not significantly different

Table 2: Correlation between Antioxidant Markers and Total Antioxidant Status in Diabetic Subjects

Marker	Correlation Coefficient*
MDA	-0.617 NS
SOD	0.109 NS
GPx	0.197 NS
GR	-0.251 NS

Table 3: Correlation between Antioxidant Markers and Total Antioxidant Status in Non-Diabetic Subjects

Marker	Correlation Coefficient*
MDA	0.256 NS
SOD	0.342 NS
GPx	0.349 NS
GR	-0.097 NS

\*Correlation by least squares linear regression

## Discussion

The increased susceptibility of diabetics to cardiovascular disease may, in part, be related to the increased rates of lipid peroxidation, as observed in this and other studies<sup>(1,5)</sup>. Interestingly, GPx was significantly decreased. GPx is one of the enzymes responsible for the removal of H<sub>2</sub>O<sub>2</sub> produced as part of cellular metabolism, and there is possible significance in the occurrence of increased MDA (an indicator of lipid peroxidation) together with reduced levels of GPx in this diabetic group. It is possible that the observed reduction in GPx in these diabetic samples may indirectly lead to increased lipid peroxidation, since lipid hydroperoxides are destroyed by GPx<sup>(7)</sup>. Another possibility is that erythrocyte GPx activity may in some way be inhibited by the presence of higher levels of MDA. TAS, SOD and GR levels showed no significant difference between the two groups. An earlier study of six diabetic patients<sup>(6)</sup> also showed low GPx and normal levels of SOD. The monitoring of antioxidant parameters in diabetic patients could prove of vital importance in the study of the disease process.

TAS : Total Antioxidant Status  
SOD : Superoxide Dismutase  
GPx : Glutathione Peroxidase  
GR : Glutathione Reductase  
MDA : Malondialdehyde

## References

1. Esterbauer, H., Striegl, G., Puhl, H. and Rotheneder, G. (1989) Free Radical Res. Commun. **6**, 67-75.
2. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C. and Witztum, J.L., (1989) N. Engl. J. Med. **320**, 915-924.
3. Uzel, N., Sivas, A. and Uysal, M. (1987) Horm. Metab. Res. **19**, 89-90.
4. Schauenstein, E., Esterbauer, H. and Zollner, H. (1977) in Aldehydes in Biological Systems: Their natural occurrence and Biological Activities Pion Press, London.
5. Arshad, M.A.Q., Bhadra, S., Cohen, R.M. and Subbiah, M.T.R. (1991) Clin. Chem. **37**, 1756-1758.
6. Faure, P., Corticelli, P., Richard, M.J., Arnaud, J., Coudray, C., Halimi, S., Favier, A. and Roussel, A.M.
7. Ames, R.N., Shigenaga, M.K. and Hagen, T.M. (1993) Proc. Natl. Acad. Sci. USA **90**, 7915-7922