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# Oxidative Stress in Normal and Diabetic Rats

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## Summary

Parameters related to oxidative stress were studied in a group of 10 Wistar diabetic rats and 10 control rats. The levels of total erythrocyte catalase activity in the diabetic animals were significantly ($p<0.001$) greater than the control levels. The diabetic animals presented an amount of vitamin E far greater ($p<0.0001$) than the controls, as was also the case for the vitaminE/polyunsaturated fatty acid (PUFA) and vitaminE/linoleic acid (C18:2) ratios. Greater vitaminE/triglyceride (TG) ratio, however, appeared in the control group. The corresponding vitamin A ratios (vitaminA/TG, vitaminA/PUFA, vitaminA/C 18:2) were higher in the control group. Our work corroborates the findings that fatty acid metabolism presents alterations in the diabetes syndrome and that the antioxidant status is affected.

**Key words**

Oxidative stress • Diabetes • Catalase activity • Vitamin A • Vitamin E

## Introduction

Free radicals are considered to be of great importance as the causes of many disorders in general, and of diabetes in particular. In recent years, the diabetes syndrome has been associated with enhanced lipid peroxidation, which can contribute to tissue damage under chronic conditions (Nourooz-Zadeh *et al.* 1997). The mechanisms generated by free radicals seem to include alterations of LDL by oxidation- or glycosylation-provoking cytotoxic reactions in the endothelial cells, and the selective accumulation of modified LDL.

All living organisms generate or make use of a great variety of hydro- and liposoluble antioxidant compounds (vitamins A and E, glutathion, etc.), and also synthesize a series of antioxidant enzymes (catalase, superoxide dismutase, glutathion peroxidase) which are responsible for deactivating the reactive intermediaries of oxygen. Despite their extreme importance, antioxidant substances and enzymes are not completely effective in preventing oxidative damage (Davies 1995). A review of the literature on this subject reveals contradictory results. Numerous studies have reported an increase of lipoperoxidation products in diabetic patients (Katoh 1992), whereas others have either not found such differences (Velazquez *et al.*1991), or an increase only in diabetic patients with complications (Mooradian 1991) or those who are poorly controlled (Altomare *et al.* 1992).

The aim of the present work was to study the influence of developing insulin-dependent diabetes on a series of parameters that indicate the animal's anti-oxidative status and its defenses.

## Method

*Animals*

We used 20 female Wistar rats, of 230 g body weight, which were divided into 2 groups: control (n=10)



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and diabetic (n=10). Water and food were supplied *ad libitum*. The food was commercial feed from B.K. Universal, S.A. Diabetes was induced by an intraperitoneal injection of streptozotocin in a dose of 65 mg/kg body weight to non-fasted animals.

Fifteen days after the streptozotocin administration, both groups of animals were weighed and arterial blood samples were withdrawn.

*Lipoperoxidation parameters (LPO)*

*Fatty acid determination.* Plasma fatty acid extraction was performed by the method of Folch *et al.* (1957), as modified by Castela *et al.* (1985). The composition was determined by gas chromatography using a Hewlett-Packard model 6890 analytical chromatograph with an autoinjector and BPX70 50m capillary column. The results for each fatty acid were expressed as a percentage of total fatty acids.

*Catalase determination.* Erythrocyte catalase activity was assessed following Aebi (1984). This method is based on the decomposition of hydrogen peroxide by catalase. The decrease in absorbance at 240 nm was measured at room temperature. The catalase activity was expressed in micrograms of enzyme per milligram of hemoglobin.

*Malondialdehyde determination.* MDA assays were performed in plasma and erythrocytes by high-performance liquid chromatography according to the technique of Esterbauer *et al.* (1984).

*Vitamin analysis.* Plasma vitamins A and E were assayed by high-performance liquid chromatography as described by Shearer *et al.* (1986).

*Biochemical parameters*

Plasma glucose (hexokinase-peroxidase glucose) and triglycerides (oxidase-peroxidase lipase-glycerate) were measured using a Coulter C.P.A. autoanalyzer. Hemoglobin determinations were performed by the technique of Zijlstra and Van Karpen (1960), using spectrophotometry.

*Statistics*

All values are given as mean ± standard deviation. Statistical analysis was performed using the Mann-Whitney test. The differences were taken as statistically significant when $p<0.05$.

**Table 1.** Biochemical parameters in control and diabetic rats

| | Control (n=10) | Diabetic (n=10) |
|---|---|---|
| Glucose (mg/dl) | 142±12 | 814±108*** |
| Triglycerides (mg/dl) | 111±43 | 1272±798*** |
| TFA (mg/ml) | 0.644±0.102 | 1.565±0.699***** |
| SFA (mg/ml) | 0.401±0.055 | 0.779±0.262** |
| MUFA (mg/ml) | 0.067±0.014 | 0.219±0.118*** |
| PUFA (mg/ml) | 0.175±0.040 | 0.567±0.293*** |
| C18:2 (mg/ml) | 0.075±0.018 | 0.311±0.160**** |

*TFA: total fatty acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acid, C18:2: linoleic acid. Diabetic group vs control group: *p<0.05, **p<0.005, ***p<0.001, **** p<0.0005, *****p<0.0001 .*

**Table 2.** Oxidative parameters in control and diabetic rats

| | Control (n=10) | Diabetic (n=10) |
|---|---|---|
| Catalase/hemoglobin (μg/mg) | 0.96±0.18 | 1.15±0.15 * |
| Plasma malondialdehyde (μM) | 2.9±.9 | 2.3±1.4 |
| Erythrocyte malondialdehyde (μM) | 14.1±3.5 | 15.3±9.6 |
| Vitamin E (mg/l) | 12.0±1.6 | 73.4±43.9***** |
| Vitamin A (mg/l) | 18.9±7.6 | 21.2±11.8 |

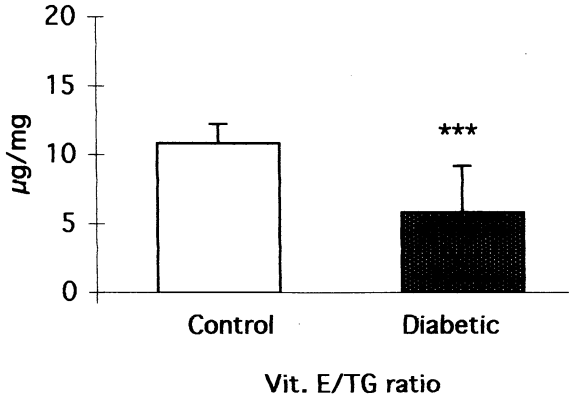
*Diabetic group vs control group: *p<0.05, ***** p<0.0001.*

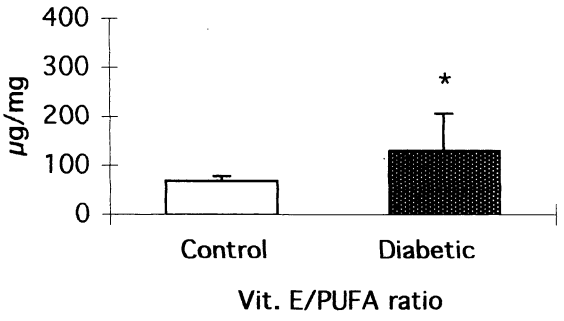
## Results

The body weight of the diabetic animals was by 8 % lower than the basal weight ($p<0.05$) and than the weight of control group ($p<0.05$) (data not shown).

Table 1 lists the concentrations of plasma triglycerides and glucose, as well as of monounsaturated, polyunsaturated and saturated fatty acids, total fatty acids, and linoleic acid which were always elevated in diabetic rats as compared with control rats.

The values of erythrocyte catalase in the two groups (controls: 561.9±45.0 ng/ml; diabetic animals: 727.8±54.5 ng/ml, $p<0.001$). There were no significant variations in the levels of erythrocyte hemoglobin found in the two groups of animals (control group: 59.8±9.9 mg/dl; diabetic group: 64.3±9.6 mg/dl). The catalase levels per milligram of hemoglobin were significantly lower in the controls than in the diabetic group ($p<0.05$) (Table 2).



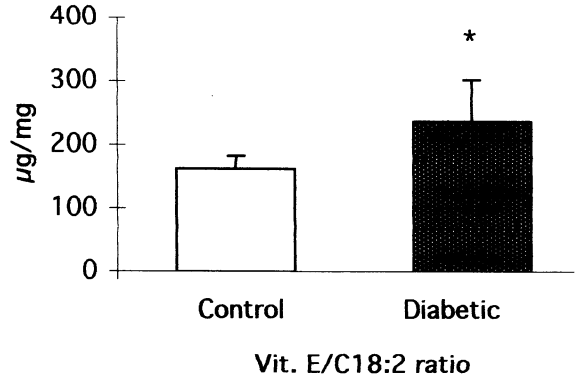
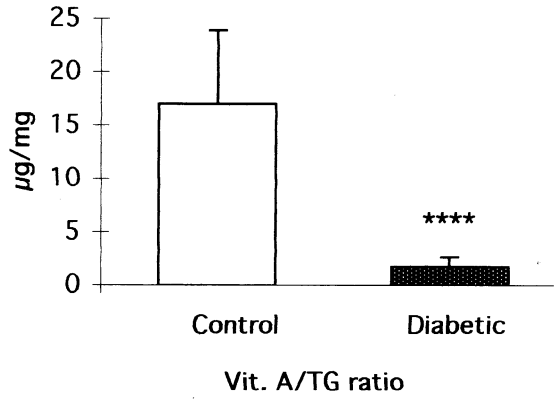
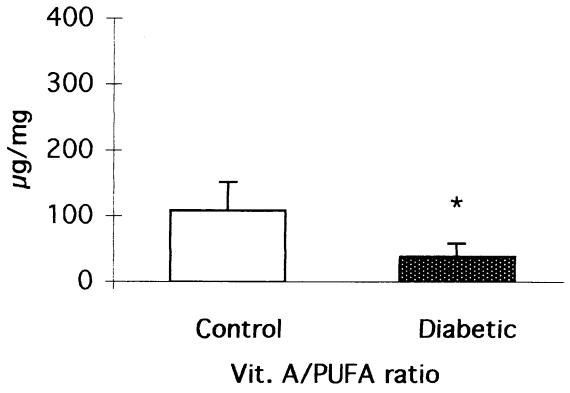




**Fig. 1.** *Vitamin E /triglycerides, vitamin E/poly-unsaturated fatty acids and vitamin E/linoleic acid ratios in control and diabetic rats. * $p<0.05$, *** $p<0.005$.*



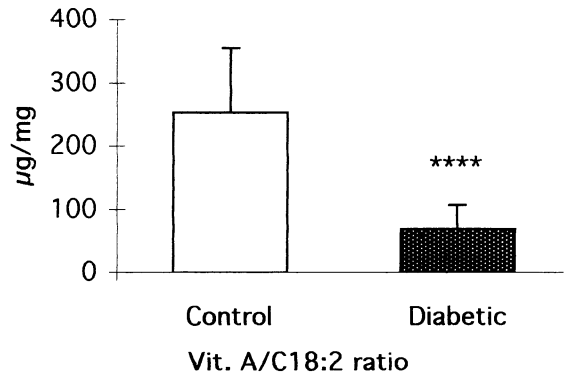




**Fig. 2.** *Vitamin A/triglycerides, vitamin A/poly-unsaturated fatty acids and vitamin A/linoleic acid ratios in control and diabetic rats. * $p<0.05$, *** $p<0.005$, **** $p<0.0001$.*

The plasma levels of vitamin E were significantly elevated in the diabetic animals ($p<0.0001$) (Table 2). The ratio between the plasma levels of vitamin E and plasma triglycerides (Fig. 1) was 10.8±1.4 μg/mg in the control group and 5.8±3.4 μg/mg in the diabetic group ($p<0.005$). The vitamin E levels divided by the levels of polyunsaturated fatty acids were 68.8±9.1 μg/mg in the control group, and 129.4±77.5 μg/mg in the diabetic group ($p<0.05$). The ratios between plasma levels of vitamin E and linoleic acid (C18:2) were 161.0±21.3 μg/mg in the controls, and 235.9±65.8 μg/mg in the diabetic rats ($p<0.05$).

The plasma levels of vitamin A found in the two groups of animals did not differ significantly (Table 2). When the vitamin A values were divided by the plasma triglyceride levels (Fig. 2), significant differences appeared between the two groups of animals (17.0±6.9 μg/mg in the control group and 1.7±0.9 μg/mg in the diabetic group, $p<0.0001$). Dividing the plasma levels of vitamin A by the plasma levels of polyunsaturated fatty acids yielded 108.0±43.6 μg/mg in the control group and 37.5±20.8 μg/mg in the diabetic group ($p<0.05$). The plasma vitamin A levels divided by those of linoleic acid were 252.7±102.1 μg/mg in the control group and 68.3±37.9 μg/mg in the diabetic group ($p<0.0001$).

## Discussion

Fatty acid metabolism undergoes alterations in diabetes. Streptozotocin diabetic rats have higher levels of linoleic acid than non-diabetic controls, these levels becoming normalized by the administration of insulin, as reported by Faas and William (1980). Our results convincingly corroborate these data. When we studied the proportion of linoleic acid presented with respect to the spectrum of other fatty acids in our animals, we observed a major rise of this fatty acid in the diabetic animals compared to control animals (Table 1). Curiously, opposite changes have been reported in non-insulin-dependent diabetic patients, i.e. a decline in linoleic acid and a rise in PUFA (Pelikánová *et al.* 1991) which was related to a greater incidence of cardiovascular disorders. In a study by Vessby *et al.* (1994), alterations of palmitoleic, linoleic and dihomogammalinoleic fatty acids were found in healthy subjects who developed diabetes years afterwards. One mechanism, that might explain the lipid alterations found in diabetics, is the destruction of PUFA by free radicals. The double bonds in this type of fatty acid make them highly susceptible to oxidation and their destruction would lead to lesions that are characteristic for diabetic complications.

Evidence has accumulated that the generation of reactive oxygen species, i.e. oxidative stress, may play a major role in the etiology of diabetic complications (Ceriello *et al.* 1991). The exposure of endothelial cells to a high concentration of glucose leads to an overproduction of superoxide anions. This may interfere with the metabolism of nitric oxide, a powerful vasodilator produced by the endothelium which participates in the general homeostasis of vascular muscles contractions. Furthermore, many of the adverse effects of hyperglycemia on endothelial functions are reversed with antioxidants (Giugliano *et.al.* 1996).

Many pathological states alter the activity of antioxidant enzymes. Balashova *et al.* (1994) reported that catalase activity rises by approximately 50 % in insulin-dependent patients with angiopathies. The antioxidant status is fairly poor in both glucose intolerance and non-insulin-dependent diabetes, so that it is possible that antioxidant therapy might mitigate or retard the progress of glucose intolerance (Sundaram *et al.* 1996).

In our present work, the total erythrocyte catalase activity in diabetic animals were significantly greater than in the controls. This would indicate that the activation of oxidative defenses is enhanced, as has been argued by Ohrvall *et al.* (1994). One way of comparing individuals is to consider the ratio between erythrocyte catalase activity and hemoglobin content. Our values for this ratio still indicate a greater enzymatic activity in the diabetic animals with respect to control animals.

There are many free radical generating mechanisms, but their presence is translated into a series of lipid peroxidation products which include malondialdehyde, a protein modifying agent (Hartley *et al.* 1997). It is this compound which is most commonly used as a marker of the degree of lipoperoxidation. Despite the importance of oxidative stress, its *in vivo* evaluation has not led to conclusive results. The interpretation of the relationship between the products of lipoperoxidation and diabetes is still contradictory (Nourooz-Zadeh *et al.* 1997). A possible explanation of the different conclusions reached in various studies may be that not only there is a rise in free radicals in diabetes, but there is also enhanced vitamin and enzyme defenses of the organism. We found no differences in the plasma or erythrocyte levels of malondialdehyde between the two

groups of rats (control and diabetic). In a day-by-day study of a large number of subjects, it was found that the great variations in MDA may question the usefulness of this parameter as an indicator of lipoperoxidation (Nielsen *et al.* 1997).

Vitamins act as inhibitors of free radical action. Vitamin E or alpha tocopherol is highly soluble in lipids, so that it is the main antioxidant of lipoproteins and cell membranes. Vitamin A, derived from beta-carotenes, is also liposoluble. Some studies have shown that the control of diabetes improves with the administration of high doses of vitamin E to patients, since it protects the fatty acids of cell membranes and thereby preserves their reactions with respect to insulin (Caballero 1993). It has been found that the daily administration of 600 mg of vitamin E for 3 months to a group of non-insulin-dependent diabetic patients produced a decline in basal insulin levels (Škrha *et al.* 1997). Insulin-dependent patients have similar levels of total plasma vitamin E as the controls. But when the ratio of vitamin E to the amount of lipids is taken into account, it lower than in normal subjects (Tsai *et al.* 1994). The absolute levels of vitamin A are significantly lower in insulin-dependent patients than in normal subjects (Tsai *et al.* 1994). It has been shown in studies with well-controlled non-insulin-dependent patients that both vitamin A and vitamin E levels are significantly higher than in healthy subjects taken as controls. This is also the case in diabetic patients with hyperlipidemia.

Our results show that the levels of vitamin E in diabetic animals are far greater than those in control animals. However, when we considered the ratio of vitamin E to plasma triglycerides as a measure of the concentration of the liposoluble vitamin in its natural medium, we observed that the highest values of this ratio appeared in control animals (see Fig. 1). The rario of vitamin E levels to those of the PUFA, and especially of linoleic acid, which the vitamin protects from peroxidation, indicate that the diabetic animals exhibit significantly higher values than the control animals. However, te data obtained with respect to vitamin A (Fig. 2) show the opposite difference between the control and diabetic animals, the higher levels being observed in the control group.

It has been concluded that diabetes induces disturbances in the determined oxidative parameters that could be responsible for several diabetic complications.

## References

AEBI RH: Catalase in vitro. *Meth Enzymol* **105**: 121-126, 1984.

ALTOMARE E, VENDEMIALE G, CHICCO D, PROCACCI V, CIRELLI F: Increased lipid peroxidation in type 2 poorly controlled diabetic patients. *Diabete Metab* **18**: 264-271, 1992.

BALASHOVA TS, GOLEGA EN, RUD'KO IA, BALABOLKIN MI, KUBATIEV AA: Effect of biosynthetic insulin on lipid peroxidation in erythrocyte membranes in patients with type I diabetes mellitus. *Probl Endokrinol (Mosk)* **40**: 12-15, 1994.

CABALLERO B: Vitamin E improves the action of insulin. *Nutr Rev* **51**:339-340, 1993.

CASTELA PM, MESIAS JL, MAYNAR JL: Evolution of total, neutral and polar lipids in Macabeo grapes throughout their vegetative cycle. *Sci Aliment* **5**: 587-597, 1985.

CERIELLO A. GIUGLIANO D, QUATRARO A, DELLO RUSSO P, LEFEBVRE PJ: Metabolic control may influence the increased superoxide generation in diabetic serum. *Diabetes Med* **8**: 540-542, 1991.

DAVIES KJ: Oxidative stress: the paradox of aerobic life. *Biochem Soc Symp* **61**: 1-31, 1995.

ESTERBAUER H, LANG J, ZADRAVEC S, SLATER TF: Detection of malondialdehyde by high-performance chromatography. *Meth Enzymol* **105**: 319-28, 1984.

FAAS FRED H, WILLIAM JC: Altered fatty acid desaturation and microsomal fatty acid composition in the streptozotocin diabetic rat. *Lipids* **15**: 953-961, 1980.

FOLCH J, LEES M, STANLEY SLOANE GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**: 497-509, 1957.

GIUGLIANO D, CERIELLO A, PAOLISSO G: Oxidative stress and diabetic vascular complications. *Diabetes Care* **193:** 257-267, 1996.

HARTLEY DP, KROLL DJ, PETERSEN DR: Prooxidant-initiated lipid peroxidation in isolated rat hepatocytes: detection of 4-hydroxynonenal- and malondialdehyde-protein adducts. *Chem Res Toxicol* **10**: 895-905, 1997.

KATOH K: Possible relevance of lipid peroxidation and thromboxane production to the initiation and/or evolution of microangiopathy in non-hyperlipidemic type 2 diabetes mellitus. *Diabetes Res Clin Pract* **18**: 89-98, 1992.

MOORADIAN AD: Increased serum conjugated dienes in elderly diabetic patients. *J Am Geriatr Soc* **39**: 571-574, 1991.

NIELSEN F, MIKKELSEN BB, NIELSEN JB, ANDERSEN HR, GRANDJEAN P: Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem* **43**: 1209-1214, 1997.

NOUROOZ-ZADEH J, RAHIMI A, TAJADDINI-SARMADI J, TRISCHLER H, ROSEN P, HALLIWELL B, BETTERIDGE DJ: Relationship between plasma measures of oxidative stress and metabolic control in NIDDM. *Diabetologia* **40**: 647-653, 1997.

OHRVALL M, TENGBLAD S, EKSTRAND B, SIEGBAHN A, VESSBY B: Malondialdehyde concentration in plasma is inversely correlated to the proportion of linoleic acid in serum lipoprotein lipids. *Atherosclerosis.* **108**: 103-110, 1994.

PELIKÁNOVÁ T, KOHOUT M, VÁLEK J, BAŠE J, ŠTEFKA Z: Fatty acid composition of serum lipids and erythrocyte membranes in type 2 (non-insulin-dependent) diabetic men. *Metabolism* **40**: 175-80, 1991.

SHEARER MJ: Vitamins. In: *HPLC of Small Molecules.* CK LIM (ed.), IRL Press Ltd., Oxford, 1986, pp 157-220.

ŠKRHA J, ŠINDELKA G, HILGERTOVÁ J: The effect of fasting and vitamin E on insulin action in obese type 2 diabetes mellitus. *Ann NY Acad Sci* **827**:556-560, 1997.

SUNDARAM RK, BHASKAR A, VIJAYALINGAM S, VISWANATHAN M, MOHAN R, SHANMUGASUNDARAM KR: Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clin Sci* **90**: 255-260, 1996.

TSAI EC, HIRSCH IB, BRUNZELL JD, CHAIT A: Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes* **43**: 1010-1014, 1994.

VELAZQUEZ E, WINOCOUR PH, KESTEVEN P, ALBERTI KG, LAKER MF: Relation of lipid peroxides to macrovascular disease in type 2 diabetes. *Diabet Med* **8**: 752-758, 1991.

VESSBY B, ARO A, SKARFORS E, BERGLUND L, SALMINEN I, LITHELL H: The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. *Diabetes* **42**: 1353-1357, 1994.

ZIJLSTRA W, VAN KARPEN EJ: Standardization of hemoglobinometry. *Clin Chem* 7: 96-100, 1960.

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