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The Association between the Total Antioxidant Potential of Plasma and the Presence of Coronary Heart Disease and Renal Dysfunction in Patients with NIDDM

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Oxidative stress may be an important pathogenetic factor in the development of diabetic vascular complications. The total antioxidative potential of plasma reflects the ability of an individual to resist oxidative stress. We measured the plasma total peroxyl radical-trapping potential (TRAP) and the concentrations of four plasma chain-breaking antioxidants in 81 patients with non-insulin-dependent diabetes mellitus (NIDDM) nine years after diagnosis and in 102 well-matched non-diabetic control subjects. The association between the total antioxidative potential and the presence of coronary heart disease (CHD) and diabetic kidney disease were also studied. There were no significant differences in plasma TRAP between NIDDM patients and control subjects (1250 ± 199 vs. 1224 ± 198 μM). Nor were there any significant differences in the concentrations of plasma uric acid, ascorbic acid, α-tocopherol, and protein thiols between NIDDM patients and control subjects. Patients with a low glomerular filtration rate and/or high urinary albumin excretion had elevated plasma uric acid. Plasma TRAP was not, however, associated with renal dysfunction. The plasma of NIDDM patients with CHD had a significantly higher value of unidentified antioxidative potential than that of patients without CHD. This relation was strongly dependent upon smoking. In conclusion, these data demonstrate that there are no major defects in the antioxidative potential of plasma caused by NIDDM *per se*. CHD and diabetic renal dysfunction were not associated with changes in plasma TRAP.

Keywords: Diabetes mellitus, non-insulin-dependent, oxidative stress, coronary disease, antioxidants, total peroxyl radical-trapping potential

INTRODUCTION

Oxidative stress is defined as a harmful imbalance between oxidizing reactions and the

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antioxidative defense of the organism.[1] Patients with non-insulin-dependent diabetes mellitus (NIDDM) may be especially prone to oxidative stress which enhances the development of diabetic complications.[2] Hyperglycemia induces non-enzymatic glycation of proteins and DNA and promotes the formation of reactive oxygen species, which leads to tissue damage.[3]

Due to the extremely short half-life of free radicals, their presence in biological systems evades direct detection in clinical situations.[1,4] The amount of oxidative stress may to a some extent be estimated by measuring the total antioxidative potential of plasma.[4] This consists of the preventive actions of metal binding proteins, like transferrin and ceruloplasmin, and the radical scavenging actions of chain-breaking antioxidants, i.e. uric acid, ascorbic acid, α-tocopherol, and protein thiols (sulphydryl groups, -SH).[5,6] The total peroxyl radical-trapping potential (TRAP) originally introduced by Wayner *et al.* in 1985,[7] and the total antioxidant status (TAS), developed by Miller *et al.* in 1993,[8] are currently the most widely used methods to assess the total antioxidative potential of biological fluids. Both methods are based on the inhibition of reactions due to peroxyl radicals and this inhibition is dependent upon the activity of chain-breaking antioxidants.

Total antioxidant potential of plasma has been previously assessed in NIDDM patients, but both decreased and unchanged values have been observed.[9–13] Thus, the association between the total antioxidant activity of plasma and the occurrence of major diabetic complications has not been established. Therefore, we studied plasma TRAP and the concentrations of four chain-breaking antioxidants and explored the possible association of these variables with the presence of a coronary heart disease (CHD) and diabetic kidney dysfunction in NIDDM patients and well-matched non-diabetic control subjects.

PATIENTS AND METHODS

Patients

One-hundred and fifty NIDDM outpatients attended at the Health Care Center of the City of Tampere during the years 1985–1988.[14] NIDDM patients fulfilled the WHO diagnostic criteria of NIDDM.[15] One-hundred and fifty non-diabetic patients attending the same facilities, matched individually for age and gender, were recruited as control subjects. Subjects with a serious disease or a shortened life expectancy (e.g. cancer, cirrhosis, etc.) were excluded from both study groups. Of these study groups, 81 NIDDM patients and 102 non-diabetic control subjects were re-evaluated affer a nine year follow-up period.

At the time of this study NIDDM patients were treated with diet alone ($n = 13$), with a combination of a diet and oral antidiabetic drugs (sulphonylurea or metformin) ($n = 44$), or with insulin ($n = 24$). CHD was diagnosed (CHD+) if the study participant had a myocardial infarction as evaluated by hospital records, if there were recorded symptoms of angina pectoris, or if the ECG fulfilled one or more of the Minnesota codes[16] 1.1–1.3, 4.1–4.3, 5.1–5.3 or 7.1. Diabetic patients with urinary albumin excretion (UAE) between 30 mg/24-h and 300 mg/24-h (microalbuminuria, $n = 25$) or with UAE over 300 mg/24-h (clinical nephropathy, $n = 6$) were recorded.

All subjects gave written informed consent. The study was approved by the ethics committees of the Tampere University Hospital and the Health Care Center of the City of Tampere.

Methods

Subjects attended after an overnight fast in the morning between 7 am and 10 am. The body mass index (BMI) was calculated as body weight (kg)/height^2 (m). A physician asked about whether the study participant smoked (yes/no currently). Ten milliliters of blood was drawn from an antecubital vein into EDTA-containing Vacutainer tubes.

Plasma was separated within 30 min by centrifugation at 2000*g* for 10 min at +4°C. For measurement of ascorbic acid, 100 µl of plasma was mixed with 100 µl of 5% meta-phosphoric acid. The storage time of samples at −70°C did not exceed six months.

Total Peroxyl Radical-trapping Potential (TRAP)

Plasma TRAP was measured by a chemiluminescence-enhanced method, as has been described.[17–19] Briefly, 2,2′-azobis [2-amidipropanone] hydrocloride (ABAP, Polysciences, Warrington, PA, USA) generates peroxyl radicals at a constant rate at 37°C by thermal decomposition, and they can be detected by chemiluminescence reaction enhanced with luminol (Sigma Chemical Co., St. Louis, MO, USA). When a plasma sample is added, this reaction is inhibited for a time that is directly proportional to the total peroxyl radical-trapping antioxidative potential of the sample. The extinction time of the plasma sample is compared to that of 1 mol of the water soluble tocopherol analog Trolox C (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, a gift from F. Hoffman–La Roche Ltd, Basel, Switzerland) – a substance capable of scavenging 2 mol of peroxyl radicals. The plasma TRAP is expressed as micromoles of peroxyl-radicals trapped by one liter of the sample (µM). The calculated TRAP ($TRAP_{Calc}$) is derived from the concentrations of the individual antioxidants based on their stoichiometric peroxyl radical-trapping factors as follows: $TRAP_{Calc} = [\text{uric acid}] * 2.0 + [\alpha\text{-tocopherol}] * 2.0 + [\text{ascorbic acid}] * 0.7 + [\text{–SH}] * 0.4$. Provision is left for an unidentified fraction of measured TRAP, ($TRAP_{Unid}$), which is the difference between the measured TRAP and $TRAP_{Calc}$ ($TRAP_{Unid} = TRAP - TRAP_{Calc}$).

Plasma Antioxidants

The concentrations of uric acid and ascorbic acid were measured by high-performance liquid chromatography (HPLC) with an electrochemical detector.[20] Plasma α-tocopherol was measured by a modified HPLC method,[21] where ultraviolet detection was replaced by LC-amperometric detector (Bioanalytical systems Inc., West Lafayette, IN, USA). A potential of +1.0 V was used. The protein thiol groups were determined as described.[22]

Other Laboratory Measurements

Serum cholesterol and triglycerides were determined by the dry slide technique on an Ektachem 700 analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). High density lipoprotein (HDL) cholesterol was measured with the same technique after precipitation of low-density lipoprotein (LDL) and of very low-density lipoprotein with dextran sulphate/magnesium chloride.[23] Serum LDL was estimated using the Friedewald's formula[24] if triglycerides did not exceed 4 mM. Fasting blood glucose was determined enzymatically, and glycosylated hemoglobin (HbA_1c) by liquid chromatography. Renal glomerular filtration rate (GFR) was measured using ^{51}Cr-EDTA and the single injection method.[25]

Statistics

Analysis of covariance (ANCOVA) or Student's *t*-test were used to compare continuous variables between groups. The χ^2-test was used to compare discontinuous variables. Pearson's correlation matrix was used for correlation analysis. Stepwise multiple regression analysis was used to search for individual determinants of plasma TRAP. Data are given as mean ± standard deviation. Statistical analyses were performed using a microcomputer and the Statistica/Win 5.0 (Statsoft Inc., Tulsa, OK, USA) software. A p value < 0.05 was considered statistically significant.

RESULTS

The demographic and biochemical characteristics of the study groups are given in Table I. NIDDM patients had higher BMI, higher triglycerides, lower total and LDL cholesterol, higher systolic blood pressure, and higher 24-h UAE than the non-diabetic control subjects (Table I). These changes are in accordance with the basic characteristics of NIDDM and thus demonstrate the metabolic differences between the study groups that are clearly distinguishable after a nine-year follow-up period. NIDDM patients also had a significantly higher prevalence of overt CHD and microalbuminuria (Table I).

There were no significant differences in plasma TRAP or in the levels of antioxidants between NIDDM patients and non-diabetic control subjects (Table II), even when analyzed separately for non-smokers and smokers, or for gender. Plasma α-tocopherol concentration corrected for total cholesterol also did not significantly differ between NIDDM patients and control subjects (5.9 vs. 5.6 μM/mM cholesterol, $p = 0.27$). Smoking NIDDM patients had lower plasma ascorbic acid concentration than non-smokers ($p = 0.02$). In a three-way ANCOVA, where the dependent variables were the study group, gender, and smoking, and GFR was used as a covariate, significant effects were observed only in the level of ascorbic acid: NIDDM patients and smokers independently had lower plasma ascorbic acid than non-diabetic subjects or non-smokers ($p = 0.03$ and $p = 0.007$, respectively).

NIDDM patients with overt CHD had a significantly higher prevalence of microalbuminuria than NIDDM patients who had no CHD, and they also had a significantly lowered GFR (Table III), but there were no significant differences in the indices of blood glucose control or in the lipid values. There were no significant differences in plasma TRAP or in the levels of antioxidants between NIDDM patients with and without CHD. NIDDM patients with CHD had a significantly higher $TRAP_{Unid}$ than their counterparts without CHD ($p = 0.006$, (Table III). However, this effect of CHD on $TRAP_{Unid}$ lost its statistical significance if the smokers were excluded ($p = 0.08$) because the smokers with CHD ($n = 3$) had markedly higher $TRAP_{Unid}$ than the

TABLE I Demographic and biochemical characteristics of the study groups

	NIDDM patients ($n = 81$)	Controls ($n = 102$)
Gender (male/female *n*, %)	46/35, 57/43	52/50, 51/49
Age (years, range)	64.4 ± 7.8, 48.5–74.9	65.1 ± 6.9, 49.6–74.8
Smoking (*n*, %)	8, 10	14, 14
Duration of disease (years)	9.3 ± 0.8	—
CHD (*n*,%)	36, 44*	28, 27
Subjects with UAE 30–300 mg/24-h (*n*, %)	22, 27*	4, 4
Subjects with UAE > 300 mg/24-h (*n*, %)	5, 6	2, 2
BMI (kg/m^2)	30.1 ± 5.3*	27.6 ± 4.5
Fasting blood glucose (mM)	9.3 ± 3.0*	4.7 ± 0.6
HbA_1c (mM)	8.4 ± 1.6*	5.6 ± 0.4
Serum fasting insulin (mU/l)	21.3 ± 13.8*	11.7 ± 6.5
Triglycerides (mM)	2.1 ± 1.4*	1.5 ± 0.8
Total Cholesterol (mM)	5.3 ± 1.0*	5.8 ± 1.1
HDL Cholesterol (mM)	1.1 ± 0.4	1.2 ± 0.4
LDL Cholesterol (mM)	3.3 ± 0.9*	3.9 ± 1.1
Blood pressure (syst/diast, mmHg)	162 ± 22*/88 ± 9	153 ± 20/87 ± 9
24-h UAE (mg)	85.0 ± 175.7*	20.8 ± 55.1
GFR ($ml/min/1.73\,m^2$)	96.6 ± 23.7	90.0 ± 20.1

*$p < 0.05$ between groups by Student's *t*-test or by χ^2-test. CHD, coronary heart disease; UAE, urinary albumin excretion; GFR, glomerular filtration rate.

smokers without CHD ($n=5$) (454 ± 61.0 vs. 171 ± 122.9, $p=0.01$). Control subjects with and without CHD did not differ significantly in any of the measured biochemical variables.

The plasma concentration of uric acid correlated inversely with GFR in both study groups ($r=-0.51$, $p<0.001$ in NIDDM patients, $r=-0.26$, $p=0.04$ in control subjects). Accordingly, plasma TRAP also had a significant inverse correlation with GFR (Figure 1). The concentration of plasma ascorbic acid had a positive correlation with GFR in control subjects ($r=0.32$, $p=0.01$). However there were no differences in plasma TRAP or in the measured concentrations of plasma antioxidants between NIDDM patients with normal 24-h UAE, microalbuminuria, or clinical nephropathy. This is partly explained by the lack of correlation between GFR and 24-h UAE in NIDDM patients ($r=0.01$, $p=$ N.S.). Due to the low number of non-diabetic subjects with increased 24-h UAE, this analysis was performed only in NIDDM patients.

There were no significant differences in plasma TRAP or in the concentrations of antioxidants between patients on diet, oral drug, or insulin treatment. Plasma TRAP did not correlate with the indices of blood glucose control, and patients with high HbA_1c ($HbA_1c > 7.5\%$) did not have significantly different plasma TRAP or antioxidant values than patients with $HbA_1c <$ 7.5% NIDDM patients with high serum LDL (≥ 4.1 mM, $n=13$) had significantly higher plasma TRAP (1344 ± 198 vs. 1180 ± 195,

TABLE II Plasma TRAP and antioxidants in NIDDM patients and control subjects. There were no significant differences between groups

	NIDDM patients	Control subjects	p-value
TRAP (μM)	1250 ± 199	1224 ± 198	0.38
$TRAP_{Calc}$ (μM)	993 ± 149	966 ± 152	0.23
$TRAP_{Unid}$ (μM)	257 ± 105	258 ± 130	0.95
Uric acid (μM)	338 ± 73.6	320 ± 73.5	0.10
–SH (μM)	552 ± 78.9	566 ± 81.5	0.25
α-tocopherol (μM)	30.9 ± 10.8	31.5 ± 7.9	0.68
Ascorbic acid (μM)	48.6 ± 19.8	52.8 ± 23.9	0.21

p values by ANCOVA (glomerular filtration rate as a covariant).

TABLE III Plasma TRAP, antioxidants and other biochemical and clinical parameters in NIDDM patients according to the presence of CHD at the time of this study

	CHD+ ($n=36$)	CHD− ($n=45$)	p-value
TRAP (μM)	1296 ± 205	1213 ± 188	0.06
$TRAP_{Calc}$ (μM)	1004 ± 167	984 ± 135	0.56
$TRAP_{Unid}$ (μM)	292 ± 101	228 ± 101	0.006
Uric acid (μM)	346 ± 77.0	332 ± 71.1	0.38
–SH (μM)	537 ± 96.5	564 ± 60.0	0.13
α-tocopherol (μM)	31.9 ± 9.5	30.1 ± 11.7	0.46
Ascorbic acid (μM)	46.9 ± 21.2	50.0 ± 18.7	0.50
Fasting blood glucose (mM)	9.5 ± 2.7	9.1 ± 3.2	0.45
Fasting serum insulin (mU)	22.3 ± 13.5	20.5 ± 14.1	0.56
HbA_1c (%)	8.2 ± 1.7	8.4 ± 1.5	0.64
Triglycerides (mM)	2.2 ± 1.2	1.9 ± 1.5	0.29
Total Cholesterol (mM)	5.4 ± 1.1	5.2 ± 1.0	0.42
HDL Cholesterol (mM)	1.1 ± 0.4	1.1 ± 0.5	0.35
LDL Cholesterol (mM)	3.3 ± 0.9	3.3 ± 0.9	0.77
24-h UAE (mg)	84.2 ± 135.4	85.6 ± 208.1	0.97
GFR (ml/min/1.73 m^2)	88.3 ± 23.4	104.3 ± 21.7	0.007
Patients with microalbuminuria (n, %)	20, 56	5, 11	<0.001
Patients with nephropathy (n, %)	2, 6	4, 9	0.57
Smoking subjects (n, %)	3, 8	5, 11	0.68

*p values by ANCOVA (for TRAP and antioxidants), by Student's t-test or by χ^2-test. CHD+/−, coronary heart disease yes/no; GFR, glomerular filtration rate; UAE, urinary albumin excretion.

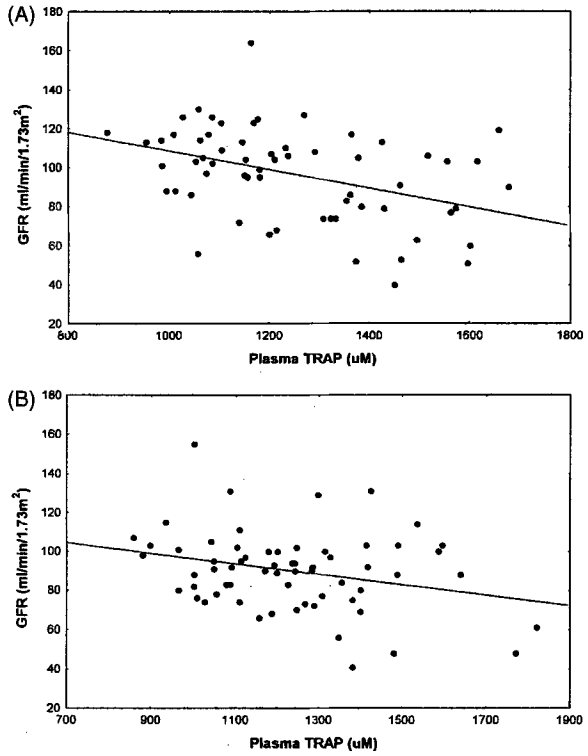

FIGURE 1 The correlation between plasma TRAP and GFR in NIDDM patients (panel A, $r=-0.41$, $p=0.001$) and in non-diabetic control subjects (panel B, $r=-0.28$, $p=0.03$).

$p=0.02$), $TRAP_{Calc}$ (1081 ± 167 vs. 926 ± 149, $p=0.006$) and plasma level of uric acid (370 ± 77.8 vs. 309 ± 77.6, $p=0.03$) than patients with low serum LDL (≤ 2.8 mM, $n=24$). There were no such differences in the non-diabetic control subjects, analyzed also separately for smokers and non-smokers.

There was a strong positive correlation between measured plasma TRAP and $TRAP_{Calc}$ in control subjects ($r=0.76$, $p<0.001$) and in NIDDM patients ($r=0.86$, $p<0.001$). Plasma TRAP correlated also strongly with the level of uric acid in NIDDM patients ($r=0.84$, $p<0.0001$) and in control subjects ($r=0.72$, $p<0.0001$). In NIDDM patients plasma TRAP also correlated with the plasma α-tocopherol concentration ($r=0.25$, $p=0.03$), and there was a correlation between plasma TRAP and protein thiols ($r=0.27$, $p=0.005$) in control subjects. In a multiple regression analysis in all study subjects, where the independent variables were age, fasting blood glucose, and insulin HbA_1c, serum triglycerides, LDL and HDL, GFR, and the plasma antioxidants measured, the only significant individual determinants of plasma TRAP were uric acid ($\beta=0.749$, $p<0.0001$) and serum triglycerides ($\beta=0.174$, $p=0.03$). No significant determinants for $TRAP_{Unid}$ could be specified by a similar analysis.

DISCUSSION

Our data indicate that the plasma total antioxidant potential of NIDDM patients is not different from that of non-diabetic control subjects, as assessed by plasma TRAP and by the concentrations of four main chain-breaking antioxidants. Penabad *et al.*[11] observed no difference in serum TRAP between NIDDM patients in good glycemic control and non-diabetic subjects. There was no significant difference in total antioxidant activity between 24 NIDDM patients and non-diabetic controls in the study of Maxwell *et al.*[9] In the studies of Ceriello *et al.*,[10,13] NIDDM patients had a lower plasma TRAP and lower uric acid level than control subjects, but this was not the case in our study. Since uric acid is the most powerful antioxidant in the TRAP assay, this alone is likely to explain the differences in TRAP between these studies. NIDDM is, in fact, often associated with hyperuricemia,[26] and hyperuricemia has, in itself, been proposed as being an individual risk factor for CHD.[27,28] Reduced TRAP has been associated with poor blood glucose control,[10,11] but in the present study there was no association between blood glucose control and plasma TRAP or antioxidants. The recent results of Wells-Knecht *et al.*[29] also suggest that oxidative stress is not increased by diabetes alone. However, we have previously reported that NIDDM patients had a markedly increased level of 24-h urinary excretion of 8-OHdG,[30] a marker of systemic oxidative

DNA damage and oxidative stress.[31] Thus it is interesting that neither plasma TRAP nor the concentrations of plasma chain-breaking antioxidants were altered despite the increased systemic oxidative damage that is assumed to prevail in diabetes. Therefore, TRAP and corresponding assays may thus not be the tests of choice for quantifying the amount of oxidative stress.

There were no differences in the plasma antioxidant potential or in the levels of antioxidants between non-diabetic subjects with and without CHD as well as between non-smoking NIDDM patients with and without CHD. It was interesting, however, that the three smoking NIDDM patients with CHD had a markedly higher $TRAP_{Unid}$ than the five smoking NIDDM patients who did not have overt CHD. The numbers of those subjects are, of course, too low for reliable analysis. Thus it seems that subjects in this study who had an overt CHD, either diabetic or not, did not have significant defects in their extracellular antioxidant defense. Increased oxidative stress, supposed to be associated with the development of diabetic vascular complications, did not alter the total antioxidant potential of NIDDM patients. NIDDM patients with CHD did not have higher plasma uric acid than patients without CHD, although they had markedly lower GFR. Thus, hyperuricemia due to the renal dysfunction does not seem to explain the CHD in these patients. A change in $TRAP_{Unid}$ may indicate that some extracellular, unmeasured or even uncharacterized, antioxidative components become activated. At least bilirubin, flavonoids, β-carotene, and melatonin have been put forward as candidates acting as $TRAP_{Unid}$.[32,33]

Only uric acid of the measured antioxidants had a clear positive correlation with the plasma TRAP. A strong association between serum TRAP and protein thiols ($r = 0.639$) has been reported,[34] and this correlation was also significant in the non-diabetic subjects in the present study. However, the only significant determinants of plasma TRAP shown by multiple regression analysis were uric acid and to a lesser extent serum triglycerides. A high plasma level of uric acid was related to low GFR in both NIDDM patients and in non-diabetic subjects. However, concentrations of ascorbic acid, α-tocopherol or protein thiols were not associated with glomerular output, and NIDDM patients with increased UAE did not differ from patients with normal UAE with regard to plasma TRAP or any antioxidants measured. Thus it seems that there are no changes in the plasma levels of antioxidants, other than the elevated uric acid, in NIDDM patients with renal dysfunction.

NIDDM patients with low serum LDL had substantially lower plasma TRAP than the patients with high serum LDL, which is in agreement with the results of Muldoon *et al.*[34] This association was not due to high protein thiols, but to high uric acid, and is not likely to indicate that subjects with low LDL are prone to increased oxidative stress. The dependency of plasma TRAP on the level of uric acid obviously sets limitations to the interpretation of TRAP values. The use of antioxidant supplements was not determined in this study, and we cannot completely exclude the possibility that they may have affected the results. However, the plasma concentrations of ascorbic acid and α-tocopherol were not different between the study groups. It has also been demonstrated that dietary antioxidant supplementation does not have significant effects on plasma TRAP value.[35–37]

As a conclusion, there were no differences in plasma TRAP or in the plasma levels of four chain-breaking antioxidants between NIDDM patients and non-diabetic control subjects. No significant changes in the total antioxidant potential were observed in NIDDM patients with CHD or increased UAE, but high uric acid and plasma TRAP were associated with lowered GFR.

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References

[1] H. Sies (1997) Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, **82**, 291–295.

[2] J.W. Baynes (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes*, **40**, 405–412.

[3] S.P. Wolff (1993) Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *British Medical Bulletin*, **49**, 642–652.

[4] B. Halliwell (1996) Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research*, **25**, 57–74.

[5] B. Halliwell and J.M.C. Gutteridge (Eds.) (1990) Antioxidants of extracellular fluids. In: *Free Radicals in Biology and Medicine*, 2nd edn., Oxford: Clarendon Press, pp. 253–260.

[6] D.D. Wayner, G.W. Burton, K.U. Ingold, L.R.Barclay and S.J. Locke (1987) The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochimica et Biophysica Acta*, **924**, 408–419.

[7] D.D. Wayner, G.W. Burton, K.U. Ingold and S. Locke (1985) Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins. *FEBS Letters*, **187**, 33–37.

[8] J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan and A.D. Milner (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*, **84**, 407–412.

[9] S.R. Maxwell, H. Thomason, D. Sandler, C. Leguen, M.A. Baxter, G.H. Thorpe, A.F. Jones and A.H. Barnett (1997) Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *European Journal of Clinical Investigation*, **27**, 484–490.

[10] A. Ceriello, N. Bortolotti, E. Falleti, C. Taboga, L. Tonutti, A. Crescentini, E. Motz, S. Lizzio, A. Russo and E. Bartoli (1997) Total radical-trapping antioxidant parameter in NIDDM patients. *Diabetes Care*, **20**, 194–197.

[11] C.R. Penabad, I.B. Conde and C.P. Marqui (1994) Total free radical-trapping capacity of serum from diabetics. *Clinical Chemistry*, **40**, 2116–2117.

[12] S.A. Santini, P. Cotroneo, A. Manto, G. Marra, P. Magnani, B. Giardina, A. Mordente, A.V. Greco and G. Ghirlanda (1996) Serum antioxidant status in diabetes mellitus. *Diabetes*, **45**(S2), 64A.

[13] A. Ceriello, N. Bortolotti, M. Pirisi, A. Crescentini, L. Tonutti, E. Motz, A. Russo, R. Giacomello, G. Stel and C. Taboga (1997) Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. *Diabetes Care*, **20**, 1589–1593.

[14] O.R. Wirta and A.I. Pasternack (1995) Glomerular filtration rate and kidney size in type 2 (non-insulin-dependent) diabetes mellitus. *Clinical Nephrology*, **44**, 1–7.

[15] WHO expert committee on diabetes mellitus (1980) The second report: 646. Technical Report Series. WHO. Geneva.

[16] G.A. Rose and H. Blackburn (1968) Cardiovascular survey methods. WHO Monograph Series 56.

[17] T. Metsä-Ketelä (1991) Luminescent assay for total peroxyl radical capability of plasma. In: *Bioluminescence and chemiluminescence current status* (Eds.P. Stanley and L. Kricka), John Wiley and Sons, Chichester, pp. 389–392.

[18] J.T. Uotila, A.L. Kirkkola, M. Rorarius, R.J. Tuimala and T. Metsä-Ketelä (1994) The total peroxyl radical-trapping ability of plasma and cerebrospinal fluid in normal and preeclamptic parturients. *Free Radical Biology and Medicine*, **16**, 581–590.

[19] R. Aejmelaeus, T. Metsä-Ketelä, P. Laippala and H. Alho (1996) Is there an unidentified defence mechanism against infection in human plasma? *FEBS Letters*, **384**, 128–130.

[20] B. Frei, R. Stocker and B.N. Ames (1988) Antioxidant defences and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 9748–9752.

[21] G. Catignani and J. Bieri (1983) Simultaneous determination of retinol and alpha-tocopherol in serum or plasma by liquid chromatography. *Clinical Chemistry*, **29**, 708–712.

[22] G. Ellman (1959) Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics*, **82**, 70–77.

[23] G.K. Warnick, J. Benderson and J. Albers (1983) In: G.R. Cooper (Ed.) *Selected Methods of Clinical Chemistry*, American Association for Clinical Chemistry, pp. 91–99.

[24] W.T. Friedewald, R.I. Levy and D.S. Fredrickson (1972) Estimation of plasma low density lipoprotein cholesterol concentration without use of preparative ultrasentrifuge. *Clinical Chemistry*, **18**, 499–502.

[25] E.S. Garnett, V. Parsons and N. Veall (1967) Measurement of glomerular filtration-rate in man using a ^{51}Cr-edetic-acid complex. *Lancet*, **15**, 818.

[26] G.M. Reaven (1995) Pathophysiology of insulin resistance in human disease. *Physiological Reviews*, **75**, 473–486.

[27] D.S. Freedman, D.F. Williamson, E.W. Gunter and T. Byers (1995) Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I epidemiologic follow-up study. *American Journal of Epidemiology*, **141**, 637–644.

[28] F.N. Brand, D.L. McGee, W.B. Kannel, J.D. Stokes and W.P. Castello (1985) Hyperuricemia as a risk factor of coronary heart disease: The Framingham study. *American Journal of Epidemiology*, **121**, 11–18.

[29] M.C. Wells-Knecht, T.J. Lyons, D.R. McCance, S.R. Thorpe and J.W. Baynes (1997) Age-dependent increase in *ortho*-tyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. *Journal of Clinical Investigation*, **100**, 836–846.

[30] J. Leinonen, T. Lehtimäki, S. Toyokuni, K. Okada, T. Tanaka, H. Hiai, H. Ochi, P. Laippala, V. Rantalaiho, O. Wirta, A. Pasternack and H. Alho (1997) New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Letters*, **417**, 150–152.

[31] M. Shigenaga, C. Gimeno and B.N. Ames (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proceedings of the National*

Academy of Sciences of the United States of America, **86**, 9697–9701.

[32] J. Lindeman, D. van Zoeren-Grobben, J. Schrijver, A. Speek, B. Poorthuis and H. Berger (1989) The total free radical-trapping ability of cord plasma in preterm and term babies. *Pediatric Research*, **26**, 20-24.

[33] R. Aejmelaeus, T. Metsä-Ketelä, T. Pirttilä, A. Hervonen and H. Alho (1997) Unidentified antioxidant defences of human plasma in immobilized patients: a possible relation to basic metabolic rate. *Free Radical Research*, **26**, 335–341.

[34] M.F. Muldoon, S.B. Kritchevsky, R.W. Evans and V.E. Kagan (1996) Serum total antioxidant activity in relative hypo- and hypercholesterolemia. *Free Radical Research*, **25**, 239–245.

[35] C.W. Mullholland and J.J. Strain (1993) Total radical-trapping potential (TRAP) of plasma: Effects of supplementation of young healthy volunteers with large doses of α-tocopherol and ascorbic acid. *International Journal of Vitamin and Nutrition Research*, **63**, 27–30.

[36] C. Calzada, M. Bizzotto, G. Pganga, J.J. Miller, K.R. Bruckdorfer, A.T. Diplock and C.A. Rice-Evans (1995) Levels of antioxidant nutrients in plasma of and low density lipoproteins: A human volunteer supplementation study. *Free Radical Research*, **23**, 489–503.

[37] K. Lönnrot, T. Metsä-Ketelä, G. Molnár, J.-P. Ahonen, M. Latvala, J. Peltola, T. Pietilä and H. Alho (1996) The effect of ascorbate and ubiquinone supplementation on plasma and CSF total antioxidant capacity. *Free Radical Biology and Medicine*, **21**, 211–217.