Oxidative Stress at Onset and in Early Stages of Type 1 Diabetes in Children and Adolescents

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OBJECTIVE — In diabetes, the persistence of hyperglycemia has been reported to cause increased production of oxygen free radicals through glucose autooxidation and nonenzymatic glycation. The aim of this study was to determine whether oxidative cellular damage occurs at the clinical onset of diabetes and in later stages of the disease in young patients.

RESEARCH DESIGN AND METHODS — Indicative parameters of lipoperoxidation, protein oxidation, and changes in the status of antioxidant defense systems were evaluated in single blood samples from 54 diabetic children, adolescents, and young adults and 60 healthy age- and sex-matched control subjects.

RESULTS — Malondialdehyde and protein carbonyl group levels in plasma were progressively higher in diabetic children and adolescents than in control subjects ($P < 0.0001$). The highest erythrocyte superoxide dismutase (SOD) activity was found in diabetic children at onset of clinical diabetes. In diabetic adolescents, SOD was also significantly higher ($P < 0.0001$) than in control subjects. Erythrocyte glutathione peroxidase was significantly lower in diabetic children and adolescents compared with control subjects ($P < 0.002$). A significant decline in blood glutathione content at the recent onset of diabetes was found ($P < 0.0001$). Furthermore, our results demonstrated progressive glutathione depletion during diabetes evolution. The plasma α-tocopherol/total lipids ratio and β-carotene levels during diabetes development ($P < 0.001$) were low.

CONCLUSIONS — This cross-sectional study in young diabetic patients showed that systemic oxidative stress is present upon early onset of type 1 diabetes and is increased by early adulthood. Decreased antioxidant defenses may increase the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supply in these young diabetic patients may help prevent clinical complications during the course of the disease.

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Oxygen-derived free radicals are generated in aerobic organisms during physiological or physiopathological oxidative metabolism of mitochondria. Free radicals may react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue, thereby interfering with cell function. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity (1). Impairment in the oxidant/antioxidant equilibrium in favor of the former provokes a situation of oxidative stress and generally results from hyperproduction of reactive oxygen species. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases (2).

Diabetic patients are exposed to increased oxidative stress due to several mechanisms, including glucose autooxidation and nonenzymatic protein glycation (3,4). Nonenzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Shiff bases and more stable Amadori products are formed (5). Advanced glycation end products (AGEs) are then formed through oxidative reactions and cause irreversible chemical modifications of proteins. Chronic hyperglycemia also leads to activation of NADPH-dependent aldose reductase (polyol pathway), which diminishes the NADPH available for glutathione reductase; consequently, the ratio of reduced to oxidized glutathione decreases (6).

A significant increase in serum superoxide radical production, which decreases parallel to improved glycemia and GHb levels, has been demonstrated in type 1 diabetic adults (7). Significantly higher values of thiobarbituric acid-reactive substances (TBARs) in serum, which provide an indirect measurement of lipid peroxidation and decreased erythrocyte antioxidant enzyme activities, have been observed in adult diabetic patients (8–10), although no differences have been found in these activities in pediatric diabetic patients compared with control subjects (11). A decrease in the total free radical–trapping capacity of serum from type 1 diabetic children and adolescents has been reported to be related to poor metabolic control (12,13).

The clinical onset of type 1 diabetes occurs once most pancreatic β-cells have been destroyed and therefore have stopped secreting normal insulin levels. Studies in vitro and in animal models have indicated that reactive radicals stem from macro-

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Abbreviations: AGE, advanced glycation end product; CV, coefficient of variation; DA, adolescent and young adult diabetic group; DO, diabetic onset group; GPx, glutathione peroxidase; GSH, total glutathione content; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; PCG, protein carbonyl group; SOD, superoxide dismutase; TBA, diethylthiobarbituric acid; TBAR, thiobarbituric acid-reactive substance.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

phages that infiltrate islets and contribute to their destruction (14). Furthermore, oxygen free radical–mediated lipid peroxidation and cytotoxic aldehyde production have recently been reported to be involved in cytokine-induced destruction of human islet β-cells (15).

The aims of the present study were *1*) to determine whether oxidative damage occurs, and to what degree, at the clinical onset of diabetes and after 2–22 years of disease evolution in patients without clinical manifestations of micro- and macroangiopathic diabetic complications, and *2*) to assess the oxidant/antioxidant balance in the whole diabetic group in relation to other metabolic control parameters of the disease. To this end, indicative parameters of lipid peroxidation and protein oxidation, together with some enzymatic antioxidant system activities, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and some endogenous radical scavengers (α-tocopherol, glutathione, and β-carotene) were evaluated. This study is the first to describe systemic oxidative stress at the onset of human diabetes.

Table 1—*Clinical characteristics of diabetic patients and control subjects*

Clinical data	Control subjects	Diabetic patients: At onset	Diabetic patients: During follow-up
n	60	24	30
Sex (M/F)	23/37	11/13	12/18
Age (years)	4–22	2–12	13–24
Diabetes duration (years)	0	0	2–22
HbA_{1c} (%)*†	—	9.7 ± 0.6	7.6 ± 0.3
Fructosamine (μmol/l)*†	—	422.7 ± 25	393.5 ± 15
Total cholesterol (mmol/l)†	4.3 ± 0.15	5.0 ± 0.18‡	4.7 ± 0.15
Triglycerides (mmol/l)†	0.68 ± 0.03	0.94 ± 0.1§	0.86 ± 0.06‡

Data are means ± SEM. *Reference ranges: HbA_{1c} 4–6%, fructosamine 200–300 μmol/l; †biochemical data are referred to those obtained at time of analysis; ‡$P < 0.05$, §$P < 0.001$ vs. control subjects.

RESEARCH DESIGN AND METHODS

Patients

We studied 54 type 1 diabetic patients (23 males, 31 females; ages 2–24 years). All patients were diagnosed and followed up at the Pediatric Diabetes Unit of the Vall d'Hebron Children's Hospital. Of those 54 patients, 24 prepubertal patients, ages 2–12 years, were evaluated between 7 and 10 days after the clinical onset of diabetes, when hydroelectrolytic disorders and acidosis had returned to normal with therapy (diabetic onset [DO] group). At least 5 days before and at the time of sample extraction, all DO patients had plasma bicarbonate levels within the normal range of 20–23 mEq/l; serum acid/base electrolytes were also normal, and no traces of ketones were found in urine. The remaining 30 patients, ages 13–24 years, were diabetic adolescents and young adults in whom diabetes had been diagnosed 2–22 years earlier and who were free of clinical symptoms of neuropathy, nephropathy, and retinopathy (adolescent and young adult diabetic [DA] group). The study also included 60 healthy age- and sex-matched control subjects. Total cholesterol, triglycerides, fructosamine, and GHb levels were measured enzymatically using commercial kits. The clinical and biochemical characteristics of the diabetic patients are summarized in Table 1.

Blood sample collection

Blood samples were drawn in the fasting state and processed within 1 h of collection. Samples were centrifuged for 5 min at 1,500*g* at 4°C, and erythrocytes were washed three times with NaCl 0.9% (wt/vol). Aliquots of EDTA plasma and washed erythrocytes were frozen at −70°C until analysis.

Blood samples of control subjects were obtained from blood analyses before minor surgery (e.g., hernia, fimosis). Diabetic blood samples were drawn during periodic routine control analyses. Informed consent was obtained from all individuals after the purpose and nature of the study had been explained. The present study was approved by the Ethics Committee of the Vall d'Hebron Hospitals.

Analytical methods

Plasma malondialdehyde (MDA) was determined as its diethylthiobarbituric acid adduct (TBA-MDA). Analyses were performed by reversed-phase high-performance liquid chromatography (HPLC) (16). Separation was carried out with a Symmetry C18 (10 μm) stainless steel column (4.6 × 150 mm i.d.; Waters Associates, Milford, MA). The analytical column was protected by a Waters Guard-Pak precolumn packed with the same material. The mobile phase used was acetonitrile:water (7:3 vol/vol). 1,1,3,3-tetraethoxypropane was used to generate MDA in situ under the acidic conditions required to hydrolyze plasma lipid peroxides to MDA and was used as the external standard. Injection volume was 20 μl, and the flow rate was 1.0 ml/min at room temperature. Plasma MDA was extracted with *n*-butanol, and the complex TBA-MDA was monitored by fluorescence detection (excitation, 515 nm; emission, 553 nm). Data were processed by peak automatic integration. The within- and between-run coefficients of variation (CVs) were 2.5 and 5.5%, respectively. The detection limit was 0.025 mmol/l, and analytical recovery of the standard average was 99.2%.

Plasma protein carbonyl group (PCG) levels were evaluated following the 2,4-dinitrophenylhydrazine assay (17), with slight modifications, and were expressed as nanomoles per milligram of protein. The protein concentration was determined by means of the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA), using bovine serum albumin as standard, according to the Bradford method (18).

SOD activity was measured in erythrocytes using a commercially available kit (Ransel; Randox Lab, Crumlin, U.K.). The hemoglobin concentration in milligrams per milliliters was determined by the cyanmethemoglobin method (19). Erythrocyte GPx activity was determined using a commercial kit (Ransel; Randox) and expressed as units per gram of Hb. This method is based on Paglia and Valentine (20). Reduced total glutathione content (GSH) was measured in erythrocytes by the GSH-400 kit (Bioxytech; OXIS International, Portland, OR). Erythrocyte GSH content is expressed as micromoles per gram of Hb.

Plasma α-tocopherol was also determined by reversed-phase HPLC (21) with ultraviolet detection at 280 nm (Waters Model 486 tunable absorbance detector) and peak automatic integration. Separation of α-tocopherol was carried out using a Nova-Pak C18 (5 μm) stainless steel column (3.9 × 150 mm i.d.) (Waters). The guard column was packed with the same

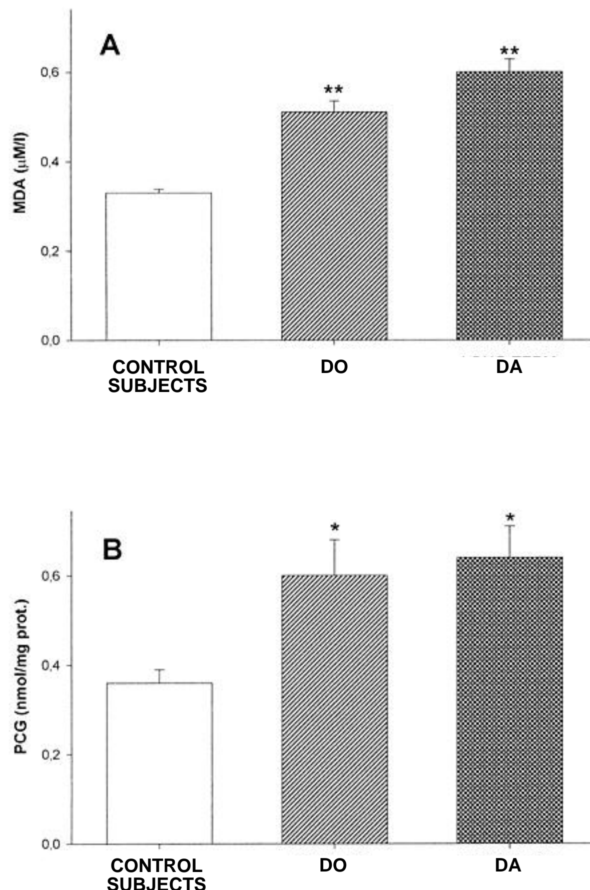

Figure 1—*Lipid peroxidation and protein oxidation parameters at onset of clinical diabetes and in later stages of diabetes in children and adolescents: plasma MDA (A) and PCG (B) levels. Data are means ± SEM. *$P < 0.001$; **$P < 0.0001$ vs. control subjects.*

material. The mobile phase used was methanol. Tocopherol acetate was used as the internal standard. The injection volume was 50 μl, and the flow rate was 2.0 ml/min at room temperature. Plasma α-tocopherol was extracted with hexane. The within- and between-run CVs were 3.7 and 6.5%, respectively; the detection limit was 1.3 μmol/l. Analytical recovery of the standard average was 99.8%. Because of the clear metabolic relationship between plasma α-tocopherol and plasma lipid parameters (22), we also expressed α-tocopherol levels as the ratio of α-tocopherol to total lipids (total cholesterol + triglycerides).

Plasma β-carotene was determined by reversed-phase HPLC (23) with visible detection at 453 nm (Waters Model 486 tunable absorbance detector) and peak automatic integration. Separation of β-carotene was carried out using a Nova-Pak C18 (5 μm) stainless steel column (3.9 × 150 mm i.d.) (Waters). The guard column was packed with the same material. The mobile phase used was acetonitrile:dichloromethane: methanol (70:20:10). Echinenone (Hoffman-La Roche, Basel, Switzerland) was used as the internal standard. The injection volume was 60 μl, and the flow rate was 1.0 ml/min at room temperature. Plasma β-carotene was extracted with hexane (0.025% butylated hydroxy toluene [BHT]). The within- and between-run CVs were 2.8 and 6.15%, respectively; the detection limit was 0.18 μmol/l. Analytical recovery of the standard average was 99.0%.

Statistical analysis

Results were statistically analyzed by analysis of variance (StatView 4.01 statistical package). Correlations between variables were studied by linear regression. Data are given as means ± SEM, and differences were considered significant when $P < 0.05$.

RESULTS — Plasma MDA levels (0.51 ± 0.025 μmol/l) had already increased significantly in DO subjects and rose even further in the DA subjects (0.6 ± 0.028 μmol/l) compared with that in control subjects (0.33 ± 0.008 μmol/l; $P < 0.0001$) (Fig. 1*A*). No correlation was found between MDA and total cholesterol ($r = 0.03$) or triglycerides ($r = 0.14$, $P > 0.3$).

Determination of PCGs in plasma showed protein oxidation levels to be significantly higher than those in control subjects (0.36 ± 0.03 nmol/mg protein; $P < 0.001$), both in DO (0.6 ± 0.08 nmol/mg protein) and DA subjects (0.64 ± 0.07 nmol/mg protein) (Fig. 1*B*).

We examined SOD activity in red blood cells, which were found to be significantly increased in diabetic children and adolescents. The highest SOD activity was found in DO subjects (4.33 ± 0.32 U/mg Hb). In DA subjects, SOD activity was also significantly higher than in control subjects (3.66 ± 0.29 vs. 2.11 ± 0.11 U/mg Hb; $P < 0.0001$) (Fig. 2*A*).

Erythrocyte GPx activity was significantly lower in the DO subjects (41.8 ± 4.6 U/g Hb) and in DA subjects (41.5 ± 3.8 U/g Hb) compared with control subjects (54.5 ± 3.2 U/g Hb; $P < 0.01$) (Fig. 2*B*).

Figure 3*A* shows that GSH content in erythrocytes was significantly lower in the DO subjects (7.11 ± 0.57 μmol/g Hb) and even lower in the DA subjects (6.11 ± 0.49 μmol/g Hb) compared with the levels of the age- and sex-matched control subjects (11.2 ± 0.42 μmol/g Hb; $P < 0.0001$).

Plasma α-tocopherol concentrations were not significantly different in DO and DA subjects than in age- and sex-matched control subjects (28.3 ± 1.1 [DO group] and 28.3 ± 0.87 [DA group] vs. 28.1 ± 0.87 μmol/l [control group]; $P < 0.8$). However, when plasma α-tocopherol levels were standardized with total plasma lipids (total cholesterol + triglycerides), the ratios in the DO (4.9 ± 0.2 μmol/mmol) and DA subjects (5.1 ± 0.2 μmol/mmol) were significantly lower than in control subjects (5.8 ± 0.17 μmol/mmol; $P < 0.01$) (Fig. 3*B*). α-Tocopherol correlated positively with total cholesterol in diabetic patients ($r = 0.34$, $P < 0.01$).

Plasma levels of β-carotene were significantly lower in the DO group (0.35 ± 0.037 μmol/l) than in control subjects (0.73 ± 0.034 μmol/l), and these values were even lower in the later stages of the disease (0.27 ± 0.028 μmol/l; $P < 0.0001$), as shown in Fig. 3*C*.

No correlations were found among metabolic control parameters (fructosamine and GHb) with regard to lipid peroxidation

(MDA) and protein oxidation (PCG) indexes, antioxidant enzymes (SOD and GPx) or intracellular (GSH) or extracellular antioxidants (β-carotene and α-tocopherol) in either of the diabetic groups.

In the present study we found no significant correlations in plasma of diabetic children 9–10 days after clinical onset of diabetes among the degree of acidosis at diagnosis (pH and bicarbonate values) and peroxidation parameters (MDA, PCG) or any of the antioxidants assessed (SOD, GPx, GSH, β-carotene, α-tocopherol).

In the control group, oxidative stress parameters and antioxidant levels in plasma were not affected by age, sex, or pubertal stage, nor were differences in GPx and SOD activities observed in red blood cells (Table 2).

CONCLUSIONS — This study demonstrated for the first time the elevated concentrations of plasma MDA, an end product of polyunsaturated fatty acid peroxidation, 8 days after clinical onset of diabetes when metabolic control had returned to normal; this suggests that oxygen free radicals may already have exerted their cytotoxic effects in this early clinical stage of the disease. To our knowledge, no studies regarding oxidative/antioxidative status at the clinical onset of type 1 diabetes in children and adolescents have been previously published. Furthermore, in children and adolescents, MDA levels continued to rise over the course of the disease, indicating overproduction of free radicals and leading to lipid peroxidation and cell oxidative injury, which is considered by some authors to be related to the development of diabetic complications (4,24).

Several studies have reported significant increases in lipid peroxides, assessed by TBAR measurement, in both type 1 and type 2 diabetic patients (8,9,24). However, spectrophotometric analysis of TBARs overestimates MDA content, since dialdehydes other than MDA and other plasma components react with TBA to form colored complexes; in fact, the limited specificity of this method is the main reason for questioning the validity of TBARs in evaluating the presence of oxidative stress. The MDA results presented herein were obtained by HPLC, which is highly specific and reproducible, since it measures only the MDA-TBA adduct without interference from other plasma components (25).

It has been proposed that unbalanced cytokine secretion and poor islet β-cell antioxidant defense status result in the initial phase of pancreatic β-cell destruction by toxic free radicals (14,15,26). Indirect or in vitro studies support the hypothesis that free radicals are present at diabetes onset, since MDA is generated and oxygen free radical scavengers reduce the incidence of diabetes (15,27). In a wider setting, we found a significant rise in plasma MDA levels, at the onset and in later stages of human diabetes, that reflect in vivo oxidative damage to lipids. This indicates that oxygen free radical–mediated cell damage has occurred, and that the extent of this injury is considerable enough to be reflected systemically.

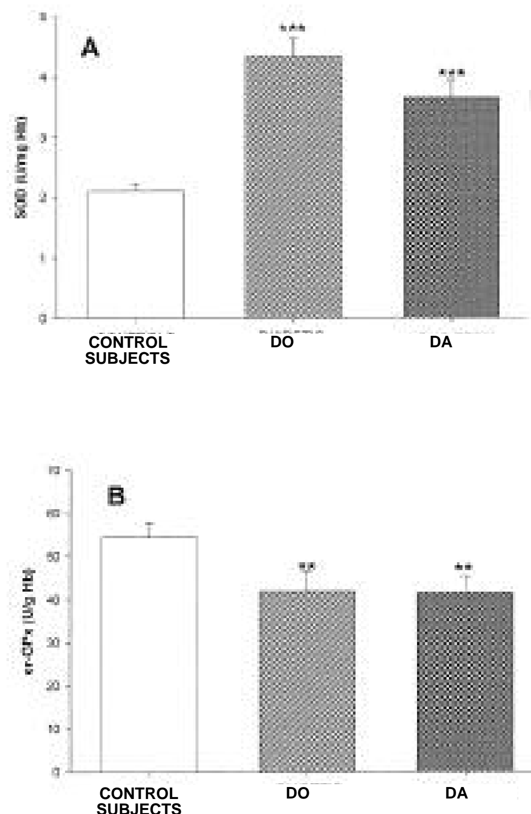

Figure 2—*Erythrocyte antioxidant enzyme activities at onset of clinical diabetes and in later stages of diabetes in children and adolescents: erythrocyte SOD (A) and erythrocyte GPx (er-GPx) (B) activity. Data are means ± SEM. **P < 0.001; ***P < 0.0001 vs. control subjects.*

The increased erythrocyte Cu/Zn-SOD activity in our young diabetic patients also supports the hypothesis of radical-mediated injury in this disease. Evidence exists that superoxide anion ($O_2^{\bullet -}$) generation measured ex vivo in serum of type 1 diabetic patients is significantly increased (7). The primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is the Cu/Zn form of the enzyme. It has also been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress (28). The highest SOD activity in red blood cells found at the onset of diabetes may be interpreted as a compensatory activation mechanism due to increased superoxide radical generation. However, the marked increase (105%) in the activity of this antioxidant enzyme at diabetes onset is not sufficient to protect cells during oxidant exposure, since increased MDA indicates that oxidative cell damage has already occurred.

The subsequent decrease in SOD activity in diabetic adolescents suggests that with longer disease duration, SOD induction and consequently its activity progressively decreases, since nonenzymatic glycation later predominates. Furthermore, hydrogen peroxide (H_2O_2) has been shown to inhibit Cu/Zn-SOD (29), and therefore the accumulation of H_2O_2 caused by the low GPx activity found in the DO group (see below) could also explain the progressive decrease in SOD in later stages of the disease.

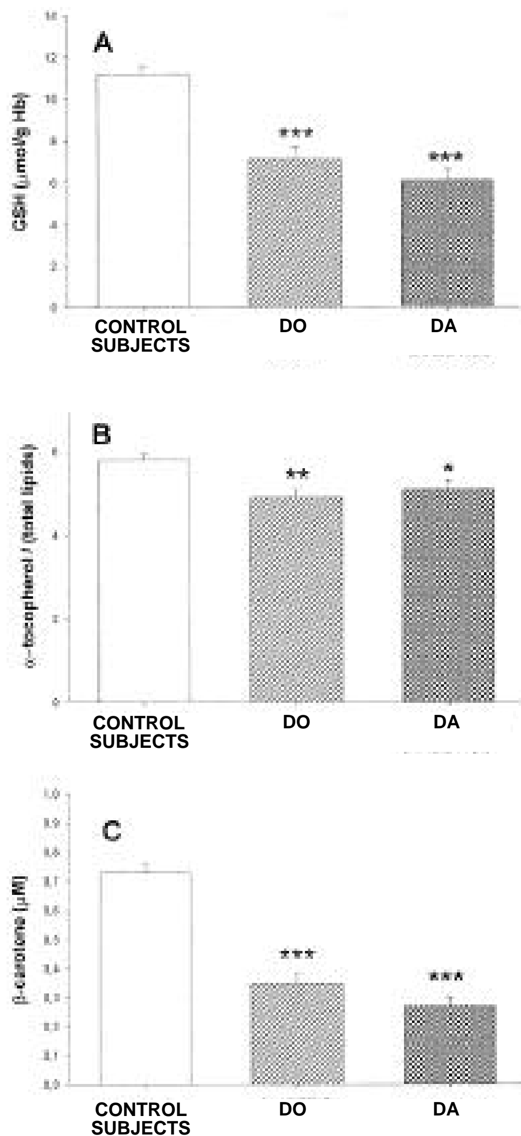

Figure 3— *Glutathione content in erythrocytes and extracellular lipid-soluble antioxidant levels at onset of clinical diabetes and in later stages of diabetes in children and adolescents: glutathione content in red blood cells (A), plasma α-tocopherol/total lipids (B), and plasma β-carotene (C). Total lipids = total cholesterol + triglycerides. Data are means ± SEM. *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$ vs. control subjects.*

Our results showed that erythrocyte GSH was depleted in the clinical course of diabetes in these young patients. This is consistent with the findings of other investigators in type 1 diabetic adolescents (30) and type 2 diabetic adults (31), suggesting that GSH metabolism is altered in both type 1 and type 2 diabetes. Several studies support the hypothesis that in diabetes, chronic hyperglycemia increases the polyol pathway as well as AGE formation and free radical generation rates, which lead to increased GSH oxidation (6,32). A relative depletion of NADPH due to aldose reductase activation and secondary to reduced production through the pentose cycle impairs GSH regeneration and leads to depletion of this free radical scavenger (32). Glutathione, the most prevalent low–molecular weight peptide antioxidant in cells, participates in many cellular functions, including detoxification processes such as protection of the sulfhydryl group of cysteine in proteins, elimination of hydrogen peroxide (GSH redox cycle), direct interaction with free radicals, and regeneration of oxidized vitamin E. Therefore, changes in GSH redox status could be considered a particularly sensitive indicator of oxidative stress. Thus the early decrease in GSH content detected in this study at the onset of diabetes may disturb antioxidant defenses that together with increased oxygen free radical activity will result in an acceleration of the oxidative damage already present in initial stages of the disease.

Our results demonstrate that erythrocyte GPx activity was significantly lower in diabetic children and adolescents at the onset and in later stages of the disease compared with control subjects, results that agree with those of Jos et al. (11), who reported decreased GPx in a group of type 1 diabetic adolescents with retinopathy. However, some authors found no differences between GPx activity of type 1 or type 2 diabetic patients and control subjects (30,31). The low GPx activity could be directly explained by the low GSH content found in diabetic patients, since GSH is a substrate and cofactor of this enzyme. Therefore, low GSH content implies low GPx activity, which may produce increased oxidative stress propensity. Moreover, pancreatic β-cells are particularly sensitive to cytotoxic damage caused by free radicals, since gene expression and activity of antioxidant enzymes such as GPx in these cells are low (26). In a recent study in pregnant diabetic experimental animals, a diabetes-induced increase was observed in Mn-SOD expression; in contrast, high glucose concentrations exerted no significant effect on GPx expression and activity (33).

Enzyme inactivation could also contribute to low GPx activity. In vitro studies have shown that although GPx is a relatively stable enzyme, it may be inactivated under conditions of severe oxidative stress (34). Arai et al. (10) showed that enzymatic inactivation might occur through glycation governed by prevailing glucose concentrations; thus increased glycation in diabetic patients and the subsequent reactions of proteins might affect amino acids close to the active sites of the molecule or disturb the stereochemical configuration, thereby provoking structural and functional changes in proteins.

On the other hand, our results showed a possible disarrangement between high enzymatic SOD activity and low GPx activity in erythrocytes of young diabetic patients. Because GPx removes H_2O_2 produced by the SOD-catalyzed reaction, an imbalance between the two enzymes may occur. In fact, activities of both antioxidant enzymes in the control group were posi-

Table 2—*Oxidative stress parameters in control group related to age, sex, and pubertal stage*

	n	MDA (μmol/l)	PCG (nmol/mg protein)	SOD (U/mg Hb)	GSH (μmol/g Hb)	Gpx (U/g Hb)	α-Tocopherol/ total lipids (μmol/mmol)	β-Carotene (μmol/l)
Age (years)								
4–12	26	0.32 ± 0.01	0.39 ± 0.04	2.31 ± 0.2	10.7 ± 0.6	55.1 ± 5.2	5.9 ± 0.3	0.80 ± 0.05
13–22	34	0.33 ± 0.01	0.33 ± 0.04	1.90 ± 0.1	11.6 ± 0.6	54.0 ± 4.1	5.6 ± 0.2	0.68 ± 0.04
Sex								
Male	23	0.33 ± 0.01	0.38 ± 0.05	1.99 ± 0.1	11.3 ± 0.6	47.0 ± 3.7	5.9 ± 0.3	0.79 ± 0.05
Female	37	0.33 ± 0.01	0.34 ± 0.04	2.21 ± 0.1	11.1 ± 0.5	59.1 ± 4.6	5.7 ± 0.2	0.70 ± 0.04
Pubertal stage								
Prepubertal	25	0.32 ± 0.01	0.40 ± 0.05	2.29 ± 0.2	11.1 ± 0.6	51.7 ± 5.2	5.9 ± 0.3	0.77 ± 0.05
Pubertal	9	0.31 ± 0.01	0.29 ± 0.05	2.00 ± 0.3	10.2 ± 0.8	47.0 ± 3.4	5.4 ± 0.2	0.75 ± 0.09
Adulthood	26	0.34 ± 0.01	0.30 ± 0.04	1.90 ± 0.1	11.6 ± 0.7	59.6 ± 5.3	5.7 ± 0.3	0.69 ± 0.05

Data are means ± SEM. Values are not significantly different.

tively correlated ($r = 0.26$, $P < 0.03$). In contrast, this correlation in the diabetic group was not maintained, which suggests that the functional relationship between the two enzymes was disrupted and therefore possibly harmful to cells.

Proteins are among the main targets of oxidation, and increased carbonyl content in proteins from aldehyde and ketone formation is an indicator of oxidative stress. Plasma PCG levels were significantly higher at the onset of diabetes and in diabetic adolescents without complications compared with control subjects, which would indicate that free radical–mediated oxidative damage of proteins is produced at diabetic onset and tends to increase in later stages of the disease. To our knowledge, there are no reports in the literature concerning plasma PCG levels in insulin-dependent diabetic patients. Carbonyl group formation is considered an early and stable marker for protein oxidation and is a method used for assessing metal-catalyzed oxidation of proteins; PCG may also be formed by other mechanisms, such as peroxidation of polyunsaturated fatty acids (PUFA) or glycation reactions (35). The average increase of 72.1% (DO group, 66.6%; DA group, 77.7%) in plasma PCG in our young diabetic patients was similar to the increase in lipid peroxidation, which enhanced MDA in plasma by 68.1% (DO group, 54.5%; DA group, 81.8%). Oxidized proteins constitute a substantial fraction of the catalytically inactive or less active forms of enzymes, which may have direct metabolic consequences.

Extracellular fluids contain nonenzymatic antioxidants that may delay or inhibit the oxidative process (1). Enhanced lipid peroxidation increases the need for lipid-soluble antioxidants, such as α-tocopherol and β-carotene. Plasma α-tocopherol levels in the DO subjects did not differ from those of control subjects. Nevertheless, when the values were normalized by total lipids, low ratios in both the DA and DO subjects were found. Changes after lipid correction were probably due to insulin-glucagon imbalances in diabetic patients, which lead to elevated plasma lipid levels with increased triglycerides and cholesterol (36). α-Tocopherol is the primary in vivo chain-breaking, lipid-soluble antioxidant in human serum and is particularly effective in lipid peroxidation inhibition (37). A relative decrease in plasma α-tocopherol may therefore be attributed to its consumption while scavenging free radicals in biomembranes or lipoproteins. A slight but nonsignificant decrease in α-tocopherol and the α-tocopherol/total lipids ratio has been reported in type 1 diabetic adults with increased TBAR levels (8) and was observed in type 1 diabetic adults with poorly controlled diabetes by Tsai et al. (38).

Plasma β-carotene was significantly lower in DO subjects than in control subjects, and a clear and progressive decrease in this antioxidant was found during diabetes evolution. To our knowledge, no studies have been reported on plasma β-carotene levels in type 1 diabetic children and adolescents. β-carotene, also a lipid-soluble and chain-breaking antioxidant, is an effective quencher of singlet oxygen and can inhibit lipid peroxidation, exhibiting effective radical-trapping antioxidant behavior only at low physiological oxygen pressures (37). The differences in plasma β-carotene concentrations between young diabetic patients and healthy control subjects may be due to rapid turnover of β-carotene, perhaps through quenching of oxygen radicals, since β-carotene is consumed faster than α-tocopherol when the radicals are generated within the lipophilic compartment of the membranes (37,39).

The findings of the present study suggest a therapeutic role for antioxidants in protecting islets from oxidative damage by free radicals in the prediabetic period of the disease. Thus, in subjects immunologically identified to be at high risk for developing IDDM, treatment with antioxidants might reduce the peroxidation rate, restore the body's antioxidant capacity, and possibly prevent or delay development of type 1 diabetes.

In summary, our results showed that an imbalance in the oxidant/antioxidant ratio is already present at the clinical onset of diabetes in children and adolescents. Diabetes-induced oxidative damage increases from childhood to early adulthood.

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