0031-3998/04/5605-0720
PEDIATRIC RESEARCH
Vol. 56, No. 5, 2004
Printed in U.S.A.

Effects of Vitamin E Supplementation on Intracellular Antioxidant Enzyme Production in Adolescents with Type 1 Diabetes and Early Microangiopathy

FRANCESCO CHIARELLI, FRANCESCA SANTILLI, GIUSEPPE SABATINO, ANNALISA BLASETTI, STEFANO TUMINI, FRANCESCO CIPOLLONE, ANDREA MEZZETTI, AND ALBERTO VERROTTI

Departments of Pediatrics [F.Ch., A.V., G.S., A.B., S.T.] and Medicine [F.Ci., A.M., F.S.], University of Chieti, I-66100 Chieti, Italy

ABSTRACT

Defective intracellular antioxidant enzyme production (IAP) has been demonstrated in adults with diabetic nephropathy. To evaluate the effects on IAP of vitamin E administration in adolescents with type 1 diabetes and early signs of microangiopathy, 12 adolescents (aged 11–21 y; diabetes duration 10–18) were studied. Eight had retinopathy [background (four), preproliferative (three), or proliferative (one)], four had persistent microalbuminuria, and seven had both. Skin fibroblasts were obtained by biopsies and cultured in Dulbecco's modified Eagle's medium. CuZn superoxide dismutase (SOD), MnSOD, catalase (CAT), and glutathione-peroxidase (GPX) activity and mRNA expression were measured before and after 3 mo of synthetic vitamin E supplementation (600 mg twice daily); on both occasions, IAP was evaluated at different *ex vivo* glucose concentrations (5 and 22 mM). Ten adolescents with type 1 diabetes (aged 12–20 y) without angiopathy and eight healthy volunteers (aged 15–22 y) participated as control subjects. Vitamin E serum levels were measured throughout the study. In normal glucose concentrations, CuZnSOD, MnSOD, CAT, and GPX activity and mRNA expression were not different among the groups. In high glucose, CuZnSOD activity and mRNA increased similarly in all groups [angiopathics: 0.96 ± 0.30 U/mg protein; 9.9 ± 3.2 mRNA/glyceraldehyde-3-phosphate dehydrogenase). CAT and GPX activity and mRNA did not increase in high glucose only in adolescents with angiopathy (0.35 ± 0.09; 4.2 ± 0.1 and 0.52 ± 0.14; 2.4 ± 0.9, respectively). MnSOD did not change in any group. Vitamin E supplementation had no effect on any enzymatic activity and mRNA in both normal and hyperglycemic conditions. Adolescents with early signs of diabetic angiopathy have defective IAP and activity, which are not modified by vitamin E. (***Pediatr Res*** **56: 720–725, 2004)**

Abbreviations

CAT, catalase
DMEM, Dulbecco's modified Eagle's medium
GAPDH, glyceraldehyde-3-phosphate dehydrogenase
GPX, glutathione-peroxidase
SOD, superoxide dismutase
SSC, standard sodium citrate

It is widely known that oxidative stress may play a relevant role in the pathogenesis of diabetic vascular complications (1–3). Increased production of reactive oxygen metabolites and species is a direct consequence of high glucose concentrations (3,4). Hyperglycemia is able to increase the levels of oxygen radical scavenging enzymes in cultured endothelial cells (5) and in the kidney of rats with diabetes induced by streptozotocin (6,7). Finally, hyperglycemia can induce formation of free radicals and activation of oxidative stress through nonenzymatic glycation of proteins (8,9), auto-oxidative glycation (10), activation of protein kinase C (11), and increased polyol pathway (12). In normal individuals, exposure to high glucose concentrations induces an antioxidant defensive mechanism in skin fibroblasts; in adults with type 1 diabetes with macroalbuminuria and overt nephropathy, this defensive mechanism is absent (13).

Recently, we demonstrated that fluorescent products of lipid peroxidation and malondialdehyde both are increased in adolescents and young adults with early nephropathy (14). Concurrently, vitamin E levels were markedly reduced in these individuals. In the present study we evaluated intracellular

Received August 19, 2003; accepted March 19, 2004.
Correspondence: Francesco Chiarelli, M.D., Department of Pediatrics, University of Chieti, Ospedale Policlinico, Via dei Vestini, 5, I-66100 Chieti, Italy; e-mail: chiarelli@unich.it
This study was supported by a grant of Italian Ministry of Research (grant COFIN) and a generous donation by Fondazione Carlo Erba, Italy.

DOI: 10.1203/01.PDR.0000141990.12375.13

antioxidant enzyme production in skin fibroblasts of young patients with persistent microalbuminuria and early diabetic nephropathy; we also investigated whether administration of vitamin E (600 mg twice daily for 3 mo) is able to modify this cellular antioxidant mechanism.

METHODS

Participants

All patients gave their informed consent to the study, which was approved by the Ethics Committee of the School of Medicine, University of Chieti, Italy. Twelve adolescents with type 1 diabetes agreed to participate; their age ranged from 11 to 21 y, and duration of diabetes ranged from 10 to 18 y. Eight of these patients had retinopathy [background (four), preproliferative (three), or proliferative (one)], four had persistent microalbuminuria [defined as an albumin excretion rate >50 μg/min in two of three overnight urinary collections), and seven had both. Skin fibroblasts obtained by skin biopsies were taken by excision under local anesthetic from the anterior surface of the forearm and cultured in Dulbecco's modified Eagle's medium (DMEM). CuZn superoxide dismutase (SOD), MnSOD, catalase (CAT), and glutathione-peroxidase (GPX) activity and mRNA expression were measured before and after 3 mo of vitamin E supplementation (600 mg twice daily); on both occasions, antioxidant enzyme activity was evaluated *ex vivo* at different glucose concentrations (5 and 22 mM). Ten adolescents (aged 12–20 y) without diabetic angiopathy and eight healthy volunteers (aged 15–22 y) participated in the study as control groups. Clinical characteristics of participants enrolled in the study are summarized in Table 1.

Arterial blood pressure was measured in all patients and control subjects following the recommendations of the American Heart Association and the American Academy of Pediatrics (15,16). Glomerular filtration rate (GFR) was measured as previously described (17). Vitamin E serum levels were evaluated every 2 wk and measured as previously described (as α-tocopherol by HPLC) (18).

Cell Culture

Fibroblasts were cultured in DMEM (ICN Biochemicals, Thame, UK) supplemented with 20% FCS (Life Technologies, Paisley, Scotland, UK), 2 mM glutamine (Sigma Chemical Co., Dorset, UK), 50 U/mL of penicillin (Life Technologies), and 50 μg/mL of streptomycin (Life Technologies). At the fourth passage, cells were cooled gradually and then frozen at −180°C in 10% DMSO in DMEM until used for the experiments. It is well recognized that even long-term cryopreservation does not affect fibroblasts' functional activities (19).

Experiments

All experiments were conducted between the sixth and eighth passages, using the same batches of medium and FCS. The purchased medium contained 5 mM of glucose, to which mannitol or glucose was added to obtain iso-osmolal experimental media; in other words, mannitol was added to the medium to ensure that the high glucose culture media had the same osmolarity. Cells were cultured in iso-osmolal normal (5 mM) *ex vivo* glucose and in high *ex vivo* glucose concentrations (22 mM).

Each sample of cells was grown for 12 wk, with renewal of the medium every second day. For each culture condition (normal or high glucose), 12 80-cm^2 plastic tissue culture flasks were used. Three flasks were used for RNA extraction, three flasks for enzyme activity measurement, three flasks for the evaluation of cell membrane lipid peroxidation, and three flasks to determine cell number.

Cell Counting

The medium was aspirated, and the monolayers were washed twice with PBS and detached by treatment with 2.5 mL trypsin-EDTA (Life Technologies) for 4–6 min at 37°C. Trypsin activity was stopped by the addition of 7 mL of medium that contained serum, after verification under the microscope of the complete detachment of the cells. The cell suspension was passed several times through a fine Pasteur pipette to disaggregate cell clumps, and 1 mL was counted in an electronic Coulter counter (ZBI model; Coulter Electronics, Beds, UK) equipped with a 100-μm aperture.

Antioxidant Enzyme Activity

CAT and GPX activities. The monolayers were rinsed twice with ice-cold PBS, and the cells were harvested with a sterile rubber cell scraper. The cells were sedimented for 4 min at 1600 × *g* and processed either for enzyme/protein or for mRNA analyses. For enzyme/protein lysates, cells were resuspended in 50 mM of potassium-phosphate buffer that contained

Table 1. *Characteristics of adolescents with diabetic angiopathy (DA), adolescents without diabetic angiopathy (NDA), and nondiabetic control subjects (CS)*

	DA	NDA	CS
n (F/M)	12 (6/8)	12 (6/8)	10 (5/5)
Age (y)	17 (14–21)	16 (12–22)	18 (16–22)
Diabetes duration (y)	14 (12–19)	14 (11–20)	—
HbA1c (%)	10.1 (8.2–11.3)	8.2 (7.0–9.2)	4.5 (4.0–4.9)
IR (IU · day^{-1} · kg^{-1})	1.2 (0.9–1.5)	1.1 (0.8–1.2)	—
MBP (mm Hg)	96 (91–102)	95 (90–98)	92 (89–94)
AER (μg/min)	71 (28–135)	11 (6–20)	5 (3–9)
GFR (ml · min^{-1} · 1.73 m^{-2})	153 (142–160)	130 (124–137)	—

Data are median (range).

IR, insulin requirement; MBP, mean blood pressure; AER, albumin excretion rate.

0.5% Triton X-100 and sonicated (in an ice-water bath) for two 30-s bursts on a Branson sonicator B15 (position 2, continuous setting; Branson Ultrasonic, Danbury, CT) with a 30-s cooling interval. Total protein concentration was determined according to the procedure of Bradford (20). For CAT and GPX activities, sonicates were first spun 5 min at 800 × *g* (4°C). The supernatants were assayed according to the procedure of Clairborne (21) for CAT activity and Gunzler and Flohè (22) for GPX activity.

SOD measurements. Cells were suspended in 100 mM of triethanolamine-diethanolamine buffer and homogenized with a Teflon glass Dounce homogenizer. The homogenate was centrifuged at 105,000 × *g* for 1 h (4°C), and the supernatant was passed through a small Sephadex G25 (coarse) column to remove low-molecular-weight substances that interfere with the enzyme assay (23). An aliquot of the eluate was applied onto a 5.5% polyacrylamide gel to localize SOD activity (24), with the exception that no tetramethyl-ethylenediamine was used for staining.

MnSOD activity. MnSOD activity was determined in mitochondrial fractions that were prepared by differential centrifugation, as previously described (25). Mitochondria were disrupted by freezing-thawing in a high ionic strength buffer [0.25 mM of sucrose, 0.12 M of KCl, and 10 mM of Tris-HCL (pH 7.4)]. Mitochondrial membranes were removed by sedimentation at 105,000 × *g* for 1 h (4°C), and enzyme activity was measured in the supernatant.

Northern blot analysis. Total RNA was prepared according to the procedure of Chirgwin *et al.* (26). Briefly, 10 μg of total RNA was electrophoresed on a 1.4% agarose-formaldehyde gel and then transferred to gene screen membranes. The filters were prehybridized in 50 mM of Tris-HCl (pH 7.5), 0.1% sodium pyrophosphate, 0.2% Ficoll, 5 mM of EDTA, 1% SDS, 2.2% poly(vinylpyrrolidone), 50% formamide, 0.2% BSA, 1× standard sodium citrate (SSC), and 150 μg/mL of denatured salmon sperm DNA at 65°C for 6 h. Blots were hybridized with ^{32}P-labeled probes for human CuZnSOD (27), human CAT (27), human MnSOD (28), and bovine GPX (29), to a specific activity of 1×10^6 cpm/mL in hybridization fluid at 65°C overnight. The filters were washed at 65°C twice for 15 min with 2× SSC-0.1% and twice for 15 min with 0.1× SSC-0.1% SDS and then subjected to autoradiography using an intensifying screen at −85°C. Densitometry was performed on an LKB laser scanning densitometer. Hybridization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as internal control to correct for loading inequalities.

The filters were probed for the four antioxidant enzymes separately, and GAPDH was also used separately. The results were normalized against an ideal reference value obtained from healthy individuals at 5 mmol glucose/L *ex vivo*.

Lipid peroxidation. Cells were trypsinized and centrifuged at 250 × *g* for 10 min at 4°C. Cell pellets were resuspended in 1 mL of cold PBS for assay of thiobarbituric acid–reactive substances and conjugated dienes, as previously described (30).

Statistical Analysis

ANOVA was used to test differences among the three groups. Paired *t* test was used to compare, for each group of fibroblasts, the results under conditions of normal *versus* high *ex vivo* glucose concentration, whereas Fisher least significant differences test was used to evaluate the difference among the three different groups in either normal or high glucose condition. A $p < 0.05$ was considered significant. Data are expressed as means ± SD or as median and range.

RESULTS

CuZnSOD. In normal *ex vivo* glucose concentration, CuZnSOD activity and mRNA expression were not different among the four groups. In high *ex vivo* glucose conditions, CuZnSOD mRNA and activity increased similarly in all groups (p = NS by ANOVA).

MnSOD. In normal *ex vivo* glucose concentration, MnSOD activity and mRNA expression were not different among the four groups. In high *ex vivo* glucose conditions, MnSOD did not change in any group.

CAT and GPX activity. In normal *ex vivo* glucose concentration, CAT and GPX activity and mRNA expression were not different among the four groups. In high *ex vivo* glucose conditions, CAT and GPX mRNA ($p < 0.001$) and activity ($p < 0.001$) were significantly different between the groups by ANOVA (Figs. 1 and 2). Comparing the groups in high glucose

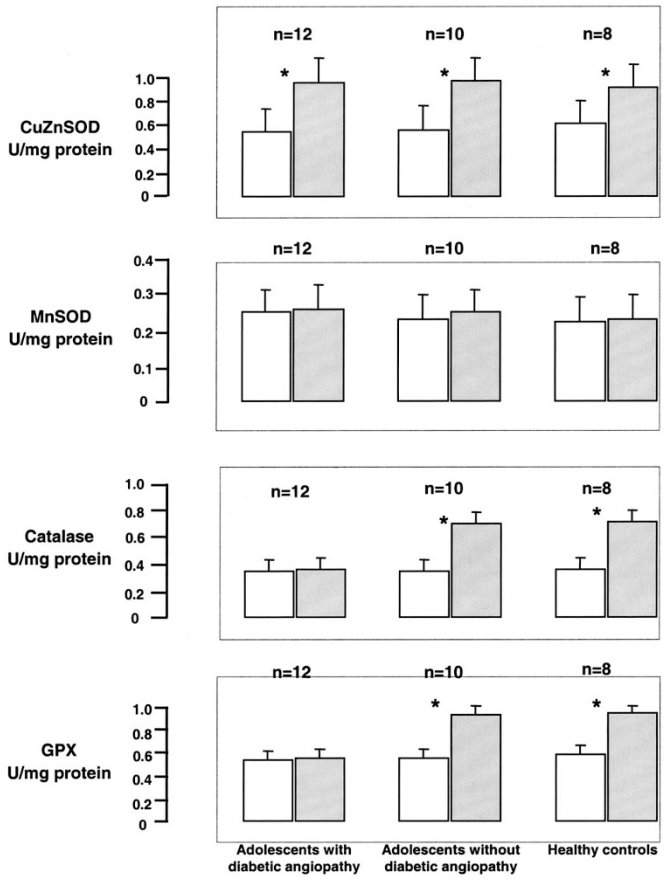

Figure 1. Antioxidant enzyme activity in skin fibroblasts from adolescents with diabetic angiopathy (n = 12), adolescents without diabetic angiopathy (n = 10), and healthy control subjects (n = 8). Enzyme activity was measured in normal glucose concentration (5 mmol/L; □) and in high glucose condition (22 mmol/L; ■); *$p < 0.001$.

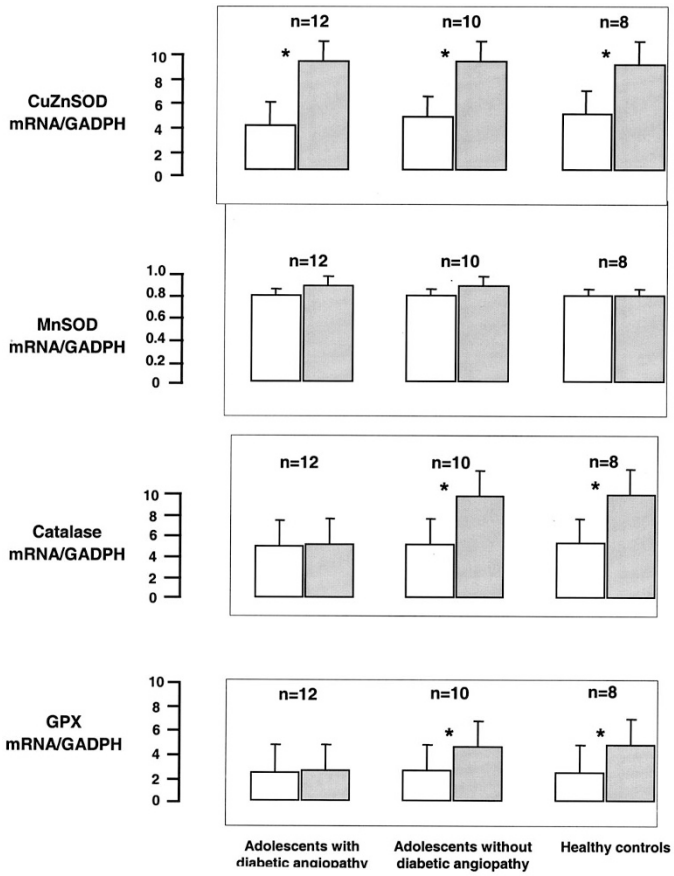

Figure 2. mRNA expression of antioxidant enzymes in skin fibroblasts from adolescents with diabetic angiopathy (n = 12), adolescents without diabetic angiopathy ($n = 10$), and healthy control subjects ($n = 8$). mRNA expression was measured in normal glucose concentration (5 mmol/L; □) and in high glucose condition (22 mmol/L; ■); *$p < 0.001$.

conditions, CAT and GPX mRNA expression and CAT and GPX protein activity were significantly higher in control subjects and diabetic subjects without angiopathy *versus* angiopathic diabetic subjects, with no difference between adolescents without diabetic angiopathy and control subjects (Figs. 1 and 2).

Lipid peroxidation. High *ex vivo* glucose concentrations significantly increased lipid peroxidation in every group of cells. Higher levels were found in cells of adolescents and young adults with diabetic angiopathy ($p < 0.001$).

Vitamin E supplementation. Vitamin E serum levels increased 2–3 wk after the administration and remained high throughout the study. No adverse event was evident in any patient. Vitamin E supplementation (600 mg twice daily for 3 mo) did not change significantly any of the enzymatic activity in both normal and hyperglycemic conditions (Table 2). With regard to mRNA expression, vitamin E was not able to modify the mRNA/GAPDH ratio for CuZnSOD at 5 and 22 mM *ex vivo* glucose concentrations [diabetics with angiopathy (DA): 4.6 ± 1.6, 10.1 ± 2.9; diabetics without angiopathy (NDA): 4.9 ± 1.6, 10.3 ± 3.1], for MnSOD (DA: 0.9 ± 0.4, 1.1 ± 0.2; NDA: 0.9 ± 0.5, 1.2 ± 0.4), for catalase (DA: 4.3 ± 1.3, 4.5 ± 1.4; NDA: 4.4 ± 1.5, 8.4 ± 2.7), and for GPX (DA: 2.3 ± 1.0, 2.5 ± 0.9; NDA: 2.4 ± 1.1, 4.3 ± 1.1).

DISCUSSION

The present study indicates that exposure to high *ex vivo* glucose concentrations induces an increase in mRNA levels and biologic activity of CuZnSOD, CAT, and GPX in fibroblasts from control subjects and adolescents without diabetic angiopathy; by contrast, in fibroblasts from diabetic adolescents with angiopathy, only CuZnSOD is increased. This finding may have important consequences concerning glucose-induced oxidative stress damage to the cell; in fact, glucose-induced oxidative stress has been demonstrated to damage several cells, including endothelial cells (2,5).

Both CuZnSOD, which is located primarily in the cytoplasm, and MnSOD, a structurally distinct protein located in the mitochondria, catalyze the reaction $O_2^- + O_2^- + 2H^+ = O_2 + H_2O_2$ (28). H_2O_2 is converted to H_2O in peroxisomes by the antioxidant enzyme CAT and in the cytoplasm by GPX (31). These antioxidant enzymes protect the cell from oxidative stress, but the threshold of protection can vary dramatically as a function of their activity and balance (32). CAT and GPX are far more efficient than CuZnSOD in protecting fibroblasts against oxidative stress (32,33). However, in several instances, cells with increased levels of CuZnSOD are hypersensitive to oxidative stress rather than protected from it (32). This happens because CuZnSOD increases the formation of H_2O_2, which, if not efficiently converted to H_2O by an adequate level of CAT and GPX, may be detrimental to the cell (32). It is therefore not surprising that generally an increase in CuZnSOD is accompanied by a concomitant increase in CAT and GPX (32). In the presence of high *ex vivo* glucose concentrations, we confirmed this phenomenon in the fibroblasts derived from control subjects and diabetic young patients without microvascular complications. In the fibroblasts of young patients with childhood-onset diabetes and angiopathy, however, high glucose induced a significant increase only in CuZnSOD but no change in the activity of CAT and GPX. These results are largely confirmatory of previous results obtained in adult diabetic patients with

Table 2. *Antioxidant enzyme activity after 3 months of vitamin E administration in skin fibroblasts from adolescents with (DA) and without (NDA) diabetic angiopathy*

	DA		NDA	
	5 mmol/L glucose	22 mmol/L glucose	5 mmol/L glucose	22 mmol/L glucose
CuZnSOD (U/mg protein)	0.58 ± 0.24	0.99 ± 0.32*	0.59 ± 0.27	0.98 ± 0.31*
MnSOD (U/mg protein)	0.31 ± 0.09	0.34 ± 0.11	0.32 ± 0.10	0.34 ± 0.12
Catalase (U/mg protein)	0.37 ± 0.11	0.38 ± 0.12	0.36 ± 0.10	0.71 ± 0.20*
GPX (U/mg protein)	0.58 ± 0.11	0.59 ± 0.10	0.59 ± 0.14	0.99 ± 0.34*

Enzyme activity was measured in normal (5 mmol/L) and high (22 mmol/L) glucose concentrations (* $p < 0.001$). Data are mean ± SD.

macroproteinuria and overt nephropathy (13) and suggest that cells of youths with type 1 diabetes and incipient angiopathy are not able to adjust their antioxidant defenses when high *ex vivo* glucose concentration–induced oxidative stress is produced, so they are more susceptible to oxidative stress. Alternatively, one could argue that in the absence of the ability to increase CAT and GPX, the cells may "decide" not to enhance CuZnSOD and MnSOD, in that the mechanism could simply be switched-off. However, this event should be operative also in individuals with diabetes without angiopathy and in control subjects.

High glucose concentrations *in vitro* and hyperglycemia *in vivo* are well-known stimuli for the production of free radicals and the generation of oxidative stress, with a consequent increase in the expression and activity of antioxidant enzymes (1–3), which act as a defense system against cell damage (33). Hyperglycemia is also a necessary factor for the development of the glomerular lesions of diabetes. The observation that, despite hyperglycemia, only a portion of the population of patients with type 1 diabetes will progress to diabetic microangiopathy indicates that there is individual diversity in cell response to high *ex vivo* glucose concentrations. It is therefore of great relevance that a disturbance in the mechanisms of protection from oxidative stress was found only in the cells of adolescents with angiopathy. By contrast, in adolescents and young adults with long-term type 1 diabetes without angiopathy, a group that seems to be protected from vascular complications, the defense mechanisms against high glucose–induced oxidative stress were intact or similar to those of nondiabetic individuals.

The novel finding of this study is that vitamin E supplementation (600 mg twice daily for 3 mo) was unable to substantially modify the antioxidant enzyme production and activity in young patients with early signs of angiopathy. Contrasting results have been obtained on the effects of vitamin E on markers of oxidative stress: recently, we were able to demonstrate that administration of vitamin E (at the same dosage used in the present study) was able to reduce plasma concentrations of MCP-1 (an inflammatory chemokine possibly involved in the pathogenesis of diabetic angiopathy) (14); fluorescent products of lipid peroxidation and malondialdehyde were also reduced after treatment and vitamin E levels increased.

In a double-blinded, placebo-controlled study, high-dose vitamin E supplementation (~1230 mg/d) was able to normalize retinal blood flow and creatinine clearance in patients with type 1 diabetes (34). In patients with type 2 diabetes, supplementation with vitamin E (~550 mg/d) induced a significant reduction of risk factors for macrovascular complications (35). Even low doses of vitamin E (~70 mg/d) were able to reduce glutathione and lower lipid peroxidation and HbA1c concentrations in the erythrocytes of patients with type 1 diabetes (36). At variance, in a recent study, a lower dose of vitamin E (~270 mg/d) taken orally for 8 wk had no significant effect on oxidatively induced LDL or DNA damage in patients with type 1 diabetes, but the same dosage regimen did reduce susceptibility to LDL oxidative change in control subjects (37). These studies are difficult to compare, because they examine patients with type 1 and type 2 diabetes; in some, natural vitamin E is used; in others, synthetic vitamin is used, different doses are given, and the duration of treatment is also different. These factors must be taken into consideration when comparing studies. Furthermore, clinical trials with vitamin E failed to demonstrate any beneficial effect on the development of diabetic complications (38). On this matter, it was suggested recently that antioxidant therapy with vitamin E or other antioxidants is limited to scavenging already-formed antioxidants and therefore may be considered a more "symptomatic" rather than a "causal" treatment for vascular oxidative stress (39,40). Some studies have documented that vitamin E is able to inhibit protein kinase C activation and consequently to induce a beneficial effect on endothelial cell dysfunction and diabetic angiopathy (41,42); however, the lack of effect of vitamin E in the present study was evident in both youths with diabetes and angiopathy and in those with no signs of diabetic vascular disease. Therefore, at least at the doses and for the time used in the present study, vitamin E is not effective in modifying the defective intracellular (in skin fibroblasts) antioxidant enzyme production in young adults with childhood-onset diabetes and signs of incipient retinopathy and nephropathy.

CONCLUSION

In conclusion, this study confirms that exposure to high *ex vivo* glucose concentrations induces an antioxidant defense mechanism in skin fibroblasts of normal young subjects and that a failure of this defensive mechanism is present in fibroblasts obtained from young patients with childhood-onset diabetes and early signs of diabetic retinopathy and nephropathy. Vitamin E supplementation (at least at the dose of 600 mg twice daily for 3 mo) is unable to significantly modify these cellular antioxidant mechanisms. Consequently, treatment with these doses of vitamin E should not be used routinely as an adjunct treatment for secondary prevention of angiopathy in patients with childhood-onset type 1 diabetes. Similar to the Diabetes Control and Complications Trial, researchers need to do a long-term clinical trial with a large patient population to assess whether vitamin E supplementation (in different doses and for longer periods) may help to lower the incidence of development and progression of microvascular complications in patients with diabetes (43).

Acknowledgments. We acknowledge the editorial assistance provided by Antonella Bascelli. We also thank the Regional Juvenile Diabetes Association (AGDA) and in particular Mrs. Doriana D'Alimonte D'Attilio for support in childhood diabetes care and research.

REFERENCES

1. Baynes JW 1991 Role of oxidative stress in development of complications in diabetes. Diabetes 40:405–412
2. Ceriello A, Giugliano D 1997 Oxidative stress and diabetic complications. In: Alberti KGMM, Zimmet P, De Fronzo RA (eds) International Textbook of Diabetes Mellitus. John Wiley & Sons, Chichester, pp 1453–1461
3. Baynes JW, Thorpe SR 1996 The role of oxidative stress in diabetic complications. Current Opin Endocrinol 3:277–284
4. Giugliano D, Ceriello A, Paolisso G 1996 Oxidative stress and diabetic vascular complications. Diabetes Care 19:257–267

5. Ceriello A, dello Russo P, Amstad P, Cerutti P 1996 High glucose induces antioxidant enzymes in human endothelial cell in culture. Evidence linking hyperglycemia and oxidative stress. Diabetes 45:471–477
6. Sechi LA, Ceriello A, Griffin CA, Catena C, Amstad P, Schambelan M, Bartoli E 1997 Renal antioxidant enzyme mRNA levels are increased in rats with experimental diabetes mellitus. Diabetologia 40:23–29
7. Reddi AS, Bollineni JS 1997 Renal cortical expression of mRNAs for antioxidant enzymes in normal and diabetic rats. Biochem Biophys Res Commun 235:598–601
8. Mullarkey CJ, Edelstein D, Brownlee M 1990 Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 173:932–939
9. Ceriello A, Quatraro A, Giugliano D 1992 New insight on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. Diabet Med 9:297–299
10. Wolff SP, Dean RT 1987 Glucose autooxidation and protein modification. The potential role of "autoxidative glycosylation" in diabetes. Biochem J 245:243–250
11. DeRubertis FR, Craven PA 1994 Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. Diabetes 43:1–8
12. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, Tilton RG 1993 Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42:801–813
13. Ceriello A, Morocutti A, Mercuri F, Quagliaro L, Moro M, Damante G, Viberti GC 2000 Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. Diabetes 49:2170–2177
14. Chiarelli F, Cipollone F, Mohn A, Marini M, Iezzi A, Tumini S, Fazia M, De Cesare D, Pomilio M, Pierdomenico SD, Cuccurullo F, Mezzetti A 2002 Circulating monocyte chemoattractant protein-1 and early development of nephropathy in type 1 diabetes. Diabetes Care 25:1829–1834
15. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee 2003 The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 289:2560–2572
16. Task Force on Blood Pressure Control in Children; National Heart, Lung, and Blood Institute, Bethesda, Maryland 1987 Report of the Second Task Force on Blood Pressure Control in Children—1987. Pediatrics 79:1–26
17. Chiarelli F, Cipollone F, Romano F, Tumini S, Costantini F, di Ricco L, Pomilio M, Pierdomenico SD, Marini M, Cuccurullo F, Mezzetti A 2000 Increased circulating nitric oxide in young patients with type 1 diabetes and persistent microalbuminuria: relation to glomerular hyperfiltration. Diabetes 49:1258–1263
18. Mezzetti A, Guglielmi MD, Pierdomenico SD, Costantini F, Cipollone F, De Cesare D, Bucciarelli T, Ucchino S, Chiarelli F, Cuccurullo F, Romano F 1999 Increased systemic oxidative stress after elective endarterectomy: relation to vascular healing and remodeling. Arterioscler Thromb Vasc Biol 19:2659–2665
19. Kearney JN 1991 Cryopreservation of cultured skin cells. Burns 17:380–383
20. Bradford M 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
21. Clairborne A 1985 Catalase activity. In: Greenwald R (ed) Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton, pp 283–284
22. Gunzler W, Flohé L 1985 Glutathione peroxidase. In: Greenwald R (ed) Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, pp 285–290
23. Paoletti F, Aldinucci D, Mocali A, Caparrini A 1986 A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Anal Biochem 154:536–541
24. Beauchamp B, Fridovich I 1971 Superoxide dismutase improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287
25. Lemasters JJ, Grunwald R, Emaus RK 1984 Thermodynamic limits to the ATP/site stoichiometries of oxidative phosphorylation by rat liver mitochondria. J Biol Chem 259:3058–3063
26. Chirgwin J, Przbyla A, MacDonald RJ, Rutter WJ 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299
27. Amstad P, Peskin A, Shah G, Mirault ME, Moret R, Zbinden I, Cerutti P 1991 The balance between Cu, Zn-superoxide dismutase and catalase affects the sensitivity of mouse epidermal cells to oxidative stress. Biochemistry 30:9305–9313
28. Ho YS, Crapo JD 1998 Isolation and characterization of complementary DNAs encoding human manganese-containing superoxide dismutase. FEBS Lett 229:256–260
29. Mullenbach GT, Tabrizi A, Irvine BD, Bell GI, Tainer JA, Halliwell RA 1988 cDNAs of three glutathione peroxidases: selenocystine incorporation. In: Cerutti P, Fridovich I, McCord JM (eds) Oxy-radicals in Molecular Biology and Pathology. Alan R. Liss, New York, pp 313–326
30. Freeman BA, Young SL, Crapo JD 1983 Liposome-mediated augmentation of superoxide dismutase in endothelial cells presents oxygen injury. J Biol Chem 258:12534–12542
31. Michiels C, Raes M, Houbion A, Remacle J 1991 Association of antioxidant systems in the protection of human fibroblasts against oxygen derived free radicals. Free Radic Res Commun 14:323–334
32. Michiels C, Raes M, Toussaint O, Remacle J 1994 Importance of Se-glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med 17:235–248
33. Beckam JS, Freeman BA 1986 Antioxidant enzymes as mechanic probes of oxygen-dependent toxicity. In: Taylor AE, Matalon S, Ward P (eds) Physiology of Oxygen Radicals. American Physiological Society, Bethesda, pp 39–53
34. Bursell SE, Clermont AC, Aiello LP, Aiello LM, Schlossman DK, Feener EP, Laffel L, King GL 1999 High-dose vitamin E supplementation normalizes retinal blood flow and creatinine clearance in patients with type 1 diabetes. Diabetes Care 22:1245–1251
35. Upritchard JE, Sutherland WH, Mann JI 2000 Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammation activity in type 2 diabetes. Diabetes Care 23:733–738
36. Jain SK, McVie R, Smith T 2000 Vitamin E supplementation restores glutathione and malonildialdehyde to normal concentrations in erythrocytes of type 1 diabetic children. Diabetes Care 23:1389–1394
37. Astley S, Langrish-Smith A, Southon S, Sampson M 1999 Vitamin E supplementation and oxidative damage to DNA and plasma LDL in type 1 diabetes. Diabetes Care 22:1626–1631
38. Marchioli R, Schweiger C, Levantesi G, Gavazzi L, Valagussa F 2001 Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids 36:S53–S63
39. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D 2001 Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 53:135–159
40. Ceriello A 2003 New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 26:1589–1596
41. Venugopal SK, Devaraj S, Yang T, Jialal I 2002 Alpha-tocopherol decreases superoxide anion release in human monocytes under hyperglycaemic conditions via inhibition of protein kinase C-alpha. Diabetes 51:3049–3054
42. Abiko T, Abiko A, Clermont AC, Shoelson B, Horio N, Takahashi J, Adamis AP, King GL, Bursell SE 2003 Characterization of retinal leukostasis and hemodynamics in insulin resistance and diabetes: role of oxidants and protein kinase-C activation. Diabetes 52:829–837
43. Jain SK 1999 Should high-dose vitamin E supplementation be recommended to diabetic patients? Diabetes Care 22:1242–1244