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Oxidative Stress, Inflammation, and Diabetic Vasculopathies: The Role of Alpha Tocopherol Therapy

I. JIALAL*, S. DEVARAJ and S.K. VENUGOPAL

Laboratory for Atherosclerosis and Metabolic Research, Department of Pathology, UC Davis Medical Center, Sacramento, CA, USA

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The diabetic state confers an increased propensity to accelerated atherogenesis. In addition to the established risk factors, there is evidence for increased oxidative stress and inflammation in diabetes. Increased oxidative stress is manifested by increased lipid peroxidation (e.g. increased F_2-isoprostanes) and increased DNA damage. Evidence for increased inflammation includes increased monocyte superoxide and pro-inflammatory cytokine release (IL-1, IL-6, and TNF-α), increased monocyte adhesion to endothelium and increased levels of plasma C-reactive protein, the prototypic marker of inflammation. Most importantly, alpha tocopherol therapy, especially at high doses, clearly shows a benefit with regards to LDL oxidation, isoprostanes and a decrease in inflammatory markers such as C-reactive protein, pro-inflammatory cytokines and PAI-1 levels. Thus, it appears that, in diabetes, alpha tocopherol therapy could emerge as an additional therapeutic modality.

Keywords: Oxidative stress; Inflammation; Diabetes; Atherosclerosis; Antioxidant; Alpha tocopherol

INTRODUCTION

The current concepts with regard to atherosclerosis suggest that the earliest event in atherogenesis is endothelial cell dysfunction manifesting as deficiencies of nitric oxide and prostacyclin. This can be induced by various noxious insults including dyslipidemia, diabetes, hypertension and smoking. Following endothelial dysfunction, the next event in atherogenesis is the binding of mononuclear cells such as monocytes and T-lymphocytes to the endothelium and this is orchestrated by certain adhesion molecules present on the endothelial surface such as VCAM, ICAM, and E-selectin. Once the monocyte migrates into the sub-endothelial space it matures into a resident macrophage, takes up lipid largely through certain scavenger receptors such as SR-A and CD-36 and becomes a foam cell. In the later stages of atherogenesis, smooth muscle cells migrate to the surface and form the fibrous cap of the lesion. Plaque rupture, which is generated by lipid laden macrophages releasing matrix metalloproteinases, results in acute coronary syndromes such as myocardial infarction and unstable angina.

Diabetes mellitus is a leading cause of morbidity and mortality in the United States. It is a major public health problem and patients with diabetes have an increased propensity to accelerated atherogenesis.[1,2] The incidence of cardiovascular disease in people with diabetes mellitus is three to four times that in non-diabetic individuals. Furthermore, established risk factors such as dyslipidemia, hypertension, and smoking cannot explain this increased prevalence of macrovascular disease in diabetes. Thus, the diabetic state itself is an independent risk factor for premature atherosclerosis. Potential mechanisms that could mediate the premature atherosclerosis in diabetes are shown in Table I. Thus, there are various mediating mechanisms such as dyslipidemia, an increased pro-coagulant state, the features of the metabolic syndrome, microalbuminuria, glycation of proteins leading to advanced glycation

*Corresponding author. Address: Research One Building, 4635 Second Avenue Room 3000, Sacramento, CA 95817, USA. Tel.: +1-916-734-6590. Fax: +1-916-734-6593. E-mail: ishwarlal.jialal@ucdmc.ucdavis.edu

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TABLE I Potential atherogenic mechanisms in diabetes

- Lipid and lipoprotein aberrations
- Procoagulant state
- Hyperinsulinism and the metabolic syndrome
- Microalbuminuria
- Glycation of proteins
- Oxidative stress
- Inflammation

end-products, oxidative stress and inflammation, that could culminate in the increased propensity to vascular complications in diabetes. In this review, we will focus on evidence for increased oxidative stress and inflammation in diabetes and the effect of alpha tocopherol therapy on both of these mediating mechanisms.

Oxidative stress plays a crucial role in atherogenesis. Several lines of evidence support a pro-atherogenic role for oxidized LDL (Ox-LDL) and its *in vivo* existence.[3–5] Ox-LDL is not recognized by the LDL receptor but by the scavenger receptor pathway on macrophages, which results in unregulated cholesterol accumulation, leading to foam cell formation. Factors that may promote increased oxidative stress in diabetes include antioxidant deficiencies, increased production of reactive oxygen species (ROS), and the process of glycation and glyco-oxidation.[6,7] The most common antioxidant deficiencies reported in diabetes are lower levels of ascorbate, glutathione and superoxide dismutase.[13] Lower concentrations of reduced glutathione have been documented in diabetic neutrophils and monocytes. The most common antioxidant deficiency that has been reported in diabetes is lower concentrations of ascorbate in both diabetic plasma and mononuclear cells. The ratio of oxidation product dehydroascorbate to ascorbate is increased. Glycation and glyco-oxidation can also promote the formation of ROS, such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide, which could in turn, oxidize biomolecules such as LDL, DNA etc.[8–10] Several reports have shown that glycated LDL is more prone to oxidation.[11,12] With regards to LDL oxidizability in Type 2 Diabetes Mellitus (T2DM), studies to date show divergent results, due to the heterogeneity of the diabetic populations, glycemic control, age and the presence of vascular complications.[13] While some studies have not found increased susceptibility of LDL to oxidation in diabetic subjects, evidence for increased LDL oxidizability in diabetic subjects has been shown in at least five studies.[13] Also, T2DM has small, dense LDL, which is more prone to oxidation than large, buoyant LDL.[14] Further evidence for increased oxidative stress is the detection of circulating autoantibodies to Ox-LDL, which is considered a biological signature of *in-vivo* LDL oxidation. Increased concentrations of autoantibodies to both oxidized and glycated LDL and glyco-Ox-LDL have been documented in diabetes suggesting that in T2DM enhanced oxidative stress occurs *in-vivo* and that LDL glycation may represent a predisposing event that facilitates subsequent oxidative modification.[15,16] The presence of these circulating immune complexes has been associated with accelerated atherosclerosis, presumably as a result either of macrophage foam cell formation in response to the uptake of these complexes or stimulation of atherogenic mechanisms in cells of the arterial wall.[17]

Direct evidence of increased oxidative stress and lipid peroxidation in diabetes has been reported. F2-isoprostanes are prostaglandin-like compounds formed *in-vivo* from free radical catalyzed peroxidation of arachidonic acid and have emerged as novel and direct measures of oxidative stress. F2-isoprostane levels have been reported to be increased in both the urine and plasma of T2DM.[18–20] Another marker of increased oxidative stress in T2DM is oxidative damage to DNA as evidenced by increased concentrations of urinary oxobases[21] and DNA strand breakage on the COMET assay.[22] Also, recently it has been shown that T2DM have elevated levels of nitrotyrosine, another marker of protein oxidation.[23]

Clinical and experimental evidence support a major role for inflammation in atherogenesis as evidenced by the critical role of adhesion molecules, cytokines and chemokines.[24] The monocyte-macrophage is a crucial and the most readily accessible cell in the artery wall. The importance of studying monocyte function and atherogenesis in T2DM is further underscored by the study of Moreno *et al.*,[25] which showed that coronary tissues from diabetic subjects exhibit a larger content of lipid-rich atheroma and macrophage infiltration than tissue from non-diabetic subjects. ROS, such as $O_2^{\bullet -}$ have been shown to be increased from monocytes (Mo) and neutrophils of T2DM patients.[8–10] Studies from our laboratory show increased $O_2^{\bullet -}$ levels in lipopolysaccharide (LPS) activated Mo in T2DM patients with and without macrovascular complications.[26] The prototypic marker of inflammation is C-reactive protein. Numerous studies, especially in normal individuals, have shown that C-reactive protein levels in the highest quintile predict cardiovascular events.[27–29] Also, evidence shows that C-reactive protein levels are increased in diabetes. The first study to convincingly show this was by Pickup *et al.* who showed that plasma levels of interleukin-6 and C-reactive protein were increased in Type 2 diabetics, especially with features of the metabolic syndrome.[30] Other studies that have confirmed this observation are from Ford *et al.*,[31] who have shown that in addition to

previously diagnosed diabetics, patients with impaired fasting glucose and newly diagnosed diabetics have a greater frequency of an increased level of C-reactive protein. Also, in patients with the metabolic syndrome, there is clearly an inflammatory component and a strong correlation between C-reactive protein and measures of adiposity. However, there is scanty data on levels of CRP in T2DM patients without vascular complications. At least two groups have recently reported that T2DM have increased levels of hs-CRP.[32,33] Furthermore, in support of a pro-inflammatory state in diabetes, we have convincingly shown that monocytic release of IL1-β and IL-6 is increased in T2DM.[26,34] Also Desfaits *et al*.[35] have observed a significant increase in the levels of LPS stimulated TNF-α release from Mo in T2DM. An early event in atherogenesis is the binding of Mo to endothelial cells and their transmigration into the intima.[36] *In vitro*, hyperglycemia (HG) increases binding of Mo to human endothelial cells.[37] While increased adhesion of Mo from T2DM to endothelial cells has been reported[38,39] this has been largely in patients with hypertriglyceridemia. Recently, we showed convincingly that even patients matched with controls with regards to the lipid levels had increased adhesion of their Mo to human aortic endothelial cells.[26]

Soluble cell adhesion molecules are shed from activated cells such as endothelial cells. Increasing evidence supports the role of plasma levels of cell adhesion molecules (ICAM, VCAM, E-selectin, and P-selectin) as molecular markers of atherosclerosis.[40–42] T2DM have elevated levels of soluble adhesion molecules such as ICAM, VCAM and E-selectin[26,43–45] as shown by numerous investigators. Furthermore, increased levels of ICAM and VCAM have been reported in the atherosclerotic lesion of T2DM.[46] Both *N*-acetyl cysteine[47] and AT therapy[26] have been shown to decrease soluble CAMs.

Interestingly, recent evidence has also shown that inflammation might contribute to the pathogenesis of the diabetic syndrome. For instance, in the Women's Health study, women in the highest quartile of hs-CRP over a 4-year period have a four-fold increased risk of diabetes compared to women in the lowest quartile.[48] This has been confirmed by other studies.[32–34] Other compelling evidence for increased inflammation in diabetes is the work from Hoffman *et al*.[49] where they show diabetics with high hemoglobin A_{1C} have increased NFKB-p65 activity. Also, they show a significant correlation between NFKB-p65 and hemoglobin A_{1C}.

We recently conducted a study in which we tested the effect of high dose RRR-Alpha tocopherol (1200 IU/day) on oxidative stress and inflammation in T2DM.[16,20,26,34] In this study, we confirmed that urinary F_2-isoprostanes was increased in diabetics, and that alpha tocopherol therapy could result in a significant decrease in F_2 isoprostane levels in the urine. We also showed that alpha tocopherol therapy significantly decreased oxidative susceptibility of LDL as manifest by prolongation of the lag phase. With regards to inflammation, we showed that in the Mo of both diabetic groups with and without vascular complications that O_2^- anion release was increased and that this could be attenuated with high dose alpha tocopherol therapy (1200 IU/RRR-AT). In addition, with regards to proinflammatory cytokines we show that interleukin 1-β and interleukin-6 levels were increased in the supernates following activation of the Mo with LPS. Furthermore, we showed that alpha tocopherol therapy resulted in a reduction in IL1-β, TNF-α, and IL-6. Also, we confirmed that the prototypic marker of inflammation, C-reactive protein was elevated in T2DM patients. In addition we went on to show that high dose alpha tocopherol therapy decreased C-reactive protein measured by the highly sensitive assay. This finding was also confirmed by another group[32] that showed that alpha tocopherol at high doses reduces C-reactive protein (800 IU/day of RRR-AT).

In our study, our patients were not dyslipidemic and we went further to show that the adhesion of the Mo to aortic endothelial cells was increased. Also we showed that alpha tocopherol decreased monocyte endothelial adhesion. With regards to soluble cell adhesion molecules, we showed for the first time that alpha tocopherol therapy decreases soluble ICAM, VCAM, and E-selectin. In addition we showed that P-selectin, which in the diabetic derives largely from the platelets, was increased in diabetes confirming increased platelet activity.[50] Furthermore, we showed that alpha tocopherol therapy resulted in a significant reduction in P-selectin levels in both controls and diabetics. PAI-1 is a key regulator of fibrinolysis, and inhibits breakdown of fibrin by inhibiting tissue plasminogen activator (tPA). Decreased fibrinolysis, primarily due to increased PAI-1 activity has been demonstrated in patients with coronary artery disease. Elevated PAI-1 is considered a strong risk factor for CAD and has been shown, in some reports, to be elevated in Type 2 diabetes. Also, PAI-1 has been shown to be increased in coronary plaques in patients with type 2 diabetes. Furthermore, PAI-1 levels correlate with many variables that co-segregate with the metabolic syndrome such as BMI, waist-hip ratio, insulin, triglycerides and apo B levels. We also confirmed this in our study, however in addition, we showed that alpha tocopherol therapy reduces PAI-1 levels.[50]

With regards to molecular mechanisms, we first explored the effects of alpha tocopherol on O_2^- anion release using the monocytic cell line THP-1 cells.[51] Incubating these cells with low glucose (5.5 mmol/l)

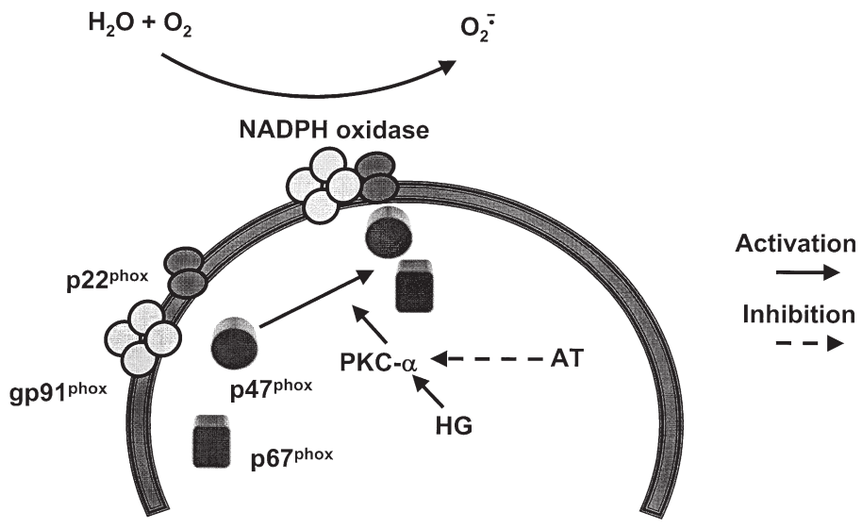

FIGURE 1

and high glucose (15 mmol/l) we showed that O_2^- anion release was increased with HG. Also we showed that PKC activity was increased in these cells. In mechanistic studies we found that O_2^- anion release was driven by PKC-α and that ROS and O_2^- are derived in the monocyte from NADPH oxidase and not from the mitochondrial respiratory chain. Using antisense oligonucleotides to PKC-α obliterated the increase in ROS and O_2^- anion release under hyperglycemic conditions in Mo, whereas antisense to PKC β-II had no effect. Alpha tocopherol also decreased O_2^- and PKC activity (both PKC α and β). Thus in this study we have shown that alpha tocopherol's inhibitory effect on O_2^- anion release in diabetic Mo is by inhibition of PKC-α resulting in a decrease in NADPH oxidase activity. Figure 1 shows our model of how under hyperglycemic conditions alpha tocopherol inhibits PKC-α resulting in impaired assembly of NADPH oxidase culminating in decreased O_2^- anion release.

With regards to alpha tocopherol therapy and vascular complications, there are limited clinical trials. The King Laboratory showed that in T1DM, high dose of RRR-AT (1800 IU/day) for 4 months normalized increased retinal blood flow and hyperfiltration.[52] Furthermore, in a recent study Gaede *et al.*[53] showed in T2DM with microalbuminuria that the combination of the antioxidants Vitamin C (1250 mg/day) and RRR-AT (680 IU/day) for 4 weeks resulted in a significant reduction in albumin excretion rates (19% reduction). Other evidence with regards to the benefit of antioxidants on biomarkers in T2DM is a study by Paolisso *et al.*[54] who have shown in 40 T2DM patients that supplementation with AT (600 mg/day of all rac AT for 8 weeks) was associated with a significant improvement in brachial artery reactivity compared to placebo; also there was an improvement in oxidative stress indices such as TBARS and total antioxidant activity. Recently, it was shown in T2DM patients that AT supplementation (1600 IU/day) improved endothelial function.[55] Thus, it appears that benefits are seen with AT at doses ≥ 600 IU/day, and that in diabetes, alpha tocopherol has effects on lipid peroxidation, platelet aggregation, inflammation and endothelial function. Recently in the SPACE (Secondary Prevention with Antioxidants of Cardiovascular Disease in End Stage Renal Disease) study, a placebo controlled randomized study, a higher dose of RRR-AT (800 IU/day) resulted in an increase in plasma levels of alpha tocopherol and a significant 54% reduction in the composite primary end point.[56] It is important to emphasize that 42% of the patients in this trial were diabetics. It is imperative to conduct a placebo controlled randomized study in T2DM to indeed see if the higher doses of alpha tocopherol that decrease inflammation and oxidative stress would culminate in reduction of cardiovascular events or atherosclerosis progression.

Acknowledgements

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References

[1] Pyorala, K., Laakso, M. and Uusitupa, M. (1987) "Diabetes and atherosclerosis: an epidemiologic view", *Diabetes* **3**, 463–524.
[2] Bierman, E. (1992) "Atherogenesis in diabetes", *Arterioscler. Thromb.* **12**, 647–656.

[3] Witztum, J. and Steinberg, D. (1991) "Role of oxidized low density lipoprotein in atherogenesis", *J. Clin. Investig.* **88**, 1785–1792.
[4] Berliner, J. and Heinecke, J. (1992) "The role of oxidized lipoproteins in atherogenesis", *Free Radic. Biol. Med.* **92**, 127–143.
[5] Devaraj, S. and Jialal, I. (1996) "Oxidized LDL and atherosclerosis", *Int. J. Clin. Lab. Res.* **26**, 178–184.
[6] Baynes, J. (1991) "Perspectives in diabetes. Role of oxidative stress in development of complications in diabetes", *Diabetes* **40**, 405–412.
[7] Ceriello, A. (2000) "Oxidative stress and glycemic regulation", *Metabolism* **49**, 27–30.
[8] Rousselot, D.B., Bastard, J.P., Jaudon, M.C. and Delattre, J. (2000) "Consequences of the diabetic status on the oxidant/antioxidant balance", *Diabetes Metab.* **26**, 163–176.
[9] Kitahara, M., Eyre, H., Lynch, R., *et al.* (1980) "Metabolic activity of diabetic monocytes", *Diabetes* **29**, 251–256.
[10] Hill, H., Hogan, N., Rallison, M., *et al.* (1980) "Functional and metabolic abnormalities of diabetic monocytes", *Adv. Exp. Med. Biol.* **69**, 621–627.
[11] Vlassara, H. (1997) "Recent progress in advanced glycation end products and diabetic complications", *Diabetes* **46**, S19–S25.
[12] Bucala, R., Makita, Z., Koschinski, T., *et al.* (1993) "Lipid advanced glycosylation: pathway for lipid oxidation *in vivo*", *Proc. Natl Acad. Sci. USA* **90**, 6434–6438.
[13] Oranje, W.A. and Wolffenbutte, B.H. (1992) "Lipid peroxidation and atherosclerosis in type II diabetes", *J. Lab. Clin. Med.* **134**(1), 19–32.
[14] Lamarche, B., Lemieux, I. and Despres, J.P. (1999) "The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects", *Diabetes Metab.* **25**, 199–211.
[15] Bellomo, G., Maggi, E., Poli, M., Agosta, F.G., Bollati, P. and Finardi, G. (1995) "Autoantibodies against Ox-LDL in NIDDM", *Diabetes* **44**, 60–66.
[16] Hsu, R.M., Devaraj, S. and Jialal, I. (2002) "Autoantibodies to oxidized low-density lipoprotein in patients with type 2 diabetes mellitus", *Clin. Chim. Acta*, Submitted for Publication.
[17] Witztum, J.L., Mahoney, E.M., Branks, M.J., *et al.* (1982) "Nonenzymatic glycation of LDL alters its biologic activity", *Diabetes* **31**, 283–291.
[18] Gopaul, N.K., Anggard, E.E., Mallet, A.I., Betteridge, D.J., Wolff, S.P. and Nourooz-Zadeh (1995) "Plasma 8-epi-PGF2alpha are elevated in individuals with NIDDM", *FEBS Lett.* **368**, 225–229.
[19] Davi, G., Ciabattoni, G., Consoli, A., Mezzetti, A., *et al.* (1999) "*In vivo* formation of 8-ep PGF2-alpha and platelet activation in diabetes mellitus-effect of improved metabolic control and vitamin E supplementation", *Circulation* **99**, 224–229.
[20] Devaraj, S., Hirany, S., Burke, R. and Jialal, I. (2002) "Divergence between LDL oxidative susceptibility and urinary F_2-isoprostanes as measures of oxidative stress in type 2 diabetes", *Clin. Chem.*, In press.
[21] Dandona, P., Thusu, K., Cook, S., Snyder, B., Makowsk, J., Armstrong, D. and Nicotera, T. (1996) "Oxidative damage to DNA in diabetes mellitus", *Lancet* **347**, 444–445.
[22] Leinonen, J., Lehtimaki, T., Toyokuni, S., Okada, K., Tanaka, T., Hiai, H., Ochi, H., Laippala, P., Rantalaiho, V., Wirta, O., Pasternack, A. and Alho, H. (1997) "New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus", *FEBS Lett.* **417**, 150–152.
[23] Ceriello, A., Mercuri, F., Quagliaro, L., Assaloni, R., Motz, E., Tonutti, L. and Taboga, C. (2001) "Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress", *Diabetologia* **44**, 834–838.
[24] Libby, P. and Hanson, G. (1991) "Biology of disease: involvement of the immune system in atherogenesis: current knowledge and unanswered questions", *Lab. Investig.* **64**, 5–13.
[25] Moreno, P.R., Murcia, A.M., Palacios, I.F., Leon, M.N., Bernardi, V.H., Fuster, V. and Fallon, J.T. (2000) "Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus", *Circulation* **102**, 2180–2184.
[26] Devaraj, S. and Jialal, I. (2000) "Post-secretory modification, monocyte function and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: the effect of alpha tocopherol supplementation", *Circulation* **102**, 191–196.
[27] Ridker, P. and Haughie, P. (1998) "Prospective studies of C-reactive protein as a risk factor for cardiovascular disease", *J. Investig. Med.* **46**, 391–395.
[28] Tracy, R. (1998) "Inflammation and cardiovascular disease, cart, horse or both", *Circulation* **97**, 2000–2002.
[29] Jialal, I. and Devaraj, S. (2001) "Inflammation and atherosclerosis: the value of the high-sensitivity C-reactive protein assay as a risk marker", *Am. J. Clin. Pathol.* **116**(Suppl.), S108–S115.
[30] Pickup, J., Mattock, M., Chusney, G., *et al.* (1997) "NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X", *Diabetologia* **40**, 1286–1292.
[31] Ford, E. (1999) "Body mass index, diabetes, and C-reactive protein among US adults", *Diabetes Care* **22**, 1971–1977.
[32] Upritchard, J.E., Sutherland, W.H. and Mann, J.I. (2000) "Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes", *Diabetes Care* **23**, 733–738.
[33] Freeman, D.J., Norrie, J., Caslake, M.J., Gaw, A., Ford, I., Lowe, G.D., O'Reilly, D.S., Packard, C.J. and Sattar, N. (2002) "C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study", *Diabetes* **51**(5), 1596–1600.
[34] Devaraj, S. and Jialal, I. (2000) "AT supplementation decreases CRP and Mo IL-6 from DM-2", *Free Radic. Biol. Med.* **29**, 790–792.
[35] Desfaits, A., Serri, O. and Renier, G. (1998) "Normalization of plasma lipid peroxides, monocyte adhesion and TNF production in NIDDM patients after gliclazide treatment", *Diabetes Care* **21**, 487–491.
[36] Ross, R. (1995) "Cell biology of atherosclerosis", *Ann. Rev. Physiol.* **57**, 791–804.
[37] Kim, J., Berliner, J., Natarajan, R., *et al.* (1994) "Evidence that glucose increases monocyte binding to human aortic endothelial cells", *Diabetes* **43**, 1103–1107.
[38] Carantoni, M., Abbasi, F., Chu, L., *et al.* (1997) "Adherence of mononuclear cells to endothelium *in vitro* is increased in patients with NIDDM", *Diabetes Care* **20**, 1462–1465.
[39] Hoogerbrugge, N., Verkerk, A., Jacobs, M., *et al.* (1997) "Hypertriglyceridemia enhances monocyte binding to endothelial cells in NIDDM", *Diabetes Care* **3**, 1122–1124.
[40] Hwang, S., Ballantyne, C.M., Sharrett, A.R., *et al.* (1997) "Circulating adhesion molecules VCAM-1-1, ICAM-1-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the atherosclerosis risk in communities (ARIC) study", *Circulation* **96**, 4219–4225.
[41] Rohde, L.E., Lee, R.T., Jamocochian, M., *et al.* (1998) "Circulating CAMs are correlated with ultrasound measurement of carotid atherosclerosis", *Arterioscler. Thromb. Vasc. Biol.* **18**, 1765–1770.
[42] Ridker, P.M., Hennekens, C.H., Roitman, J.B., *et al.* (1998) "Plasma concentration of soluble ICAM-1 and risks of future MI in apparently healthy men", *Lancet* **351**, 88–92.
[43] Albertini, J.P., Valensi, P., Lormeau, B., Aurousseau, M.H., Ferriere, F., Attali, J.R. and Gattegno, L. (1998) "Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule-1 in NIDDM. Effect of intensive insulin treatment", *Diabetes Care* **21**, 1008–1013.
[44] Fasching, P., Waldhausl, W. and Wagner, O.F. (1996) "Elevated circulating adhesion molecules in NIDDM—potential mediators in diabetic macroangiopathy", *Diabetologia* **39**, 1242–1244.
[45] Matsumoto, K., Sera, Y., Abe, Y., Ueki, Y. and Miyake, S. (2001) "Serum concentrations of soluble vascular cell adhesion molecule-1 and e-selectin are elevated in insulin-resistant patients with type 2 diabetes", *Diabetes Care* **24**, 1697–1698.
[46] Mocco, J., Choudhri, T.F., Mack, W.J., Laufer, I., Lee, J., Kiss, S., Poisik, A., Quest, D.O., Solomon, R.A. and Connolly, E.S. (2001) "Elevation of soluble intercellular adhesion molecule-1 levels in symptomatic and asymptomatic carotid atherosclerosis", *Neurosurgery* **48**, 718–721.

[47] De Mattia, G., Bravi, M.C., Laurenti, O., Cassone-Faldetta, M., Proietti, A., De Luca, O., Armiento, A. and Ferri, C. (1998) "Reduction of oxidative stress by oral *N*-acetyl-L-cysteine treatment decreases plasma soluble vascular cell adhesion molecule-1 concentrations in non-obese, non-dyslipidaemic, normotensive, patients with non-insulin-dependent diabetes", *Diabetologia* **41**(11), 1392–1396.

[48] Ridker, P.M., Buring, J.E., Shih, J., Matias, M. and Hennekens, C.H. (1998) "Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women", *Circulation* **98**(8), 731–733.

[49] Hofmann, M.A., Schiekofer, S., Isermann, B., Kanitz, M., Henkels, M., Joswig, M., Treusch, A., Morcos, M., Weiss, T., Borcea, V., Abdel Khalek, A.K., Amiral, J., Tritschler, H., Ritz, E., Wahl, P., Ziegler, R., Bierhaus, A. and Nawroth, P.P. (1999) "Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy show increased activation of the oxidative-stress sensitive transcription factor NF-kappaB", *Diabetologia* **42**(2), 222–232.

[50] Devaraj, S., Cabo Chan, A.V. and Jialal, I. (2002) "Alpha tocopherol supplementation decreases plasminogen activator inhibitor-1 and P-selectin levels in type 2 diabetic patients", *Diabetes Care*, In press.

[51] Venugopal, S.K., Devaraj, S., Yang, T.T.C. and Jialal, I. (2002) "Alpha tocopherol decreases superoxide anion release in THP-1 cells under hyperglycemic conditions through inhibition of PKC/α", *Diabetes* **2002**, In press.

[52] Bursell, S.E., Clermont, A.C., Aiello, L.P., Aiello, L., Scholossman, D.K., Feener, E.P., Laffel, L. and King, G.L. (1999) "High dose AT supplementation normalizes retinal blood flow and creatinine clearance in patients with type I diabetes", *Diabetes Care* **22**, 1245–1251.

[53] Gaede, P., Poulsen, H.E., Parving, H.H. and Pedersen, O. (2001) "Double blind randomized study of the effect of combined treatment with vitamin C and E on albuminuria in T2DM", *Diabetes Med.* **18**, 756–760.

[54] Paolisso, G., Tagliamonte, M.R., Barbieri, M., Zito, G.A., Gambardella, A., Varricchio, G., Ragno, E. and Varricchio, M. (2000) "Chronic vitamin E administration improves brachial reactivity and increases intracellular magnesium concentration in type II diabetic patients", *J. Clin. Endocrinol. Metab.* **85**, 109–115.

[55] Gazis, A., White, D.J., Page, S.R. and Cockcroft, J.R. (1999) "Effect of oral vitamin E (alpha-tocopherol) supplementation on vascular endothelial function in type 2 diabetes mellitus", *Diabetes Med.* **16**, 304–311.

[56] Boaz, M., Smetana, S., Weinstein, T., Matas, Z., Gafter, U., *et al.* (2000) "Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomized placebo controlled trial", *Lancet* **356**, 1213–1218.