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Übersicht

Prevention of platelet dysfunction by vitamin E in diabetic atherosclerosis

H. Gerster

Vitamin Research Department, F. Hoffmann-La Roche, Basel, Switzerland

Schutzwirkung von Vitamin E auf Plättchenfunktion in der Atherogenese bei Diabetikern

Summary: Premature atherosclerosis and other vascular disorders are serious complications of diabetes mellitus. Contributing factors include (i) increased peroxidation of LDL leading to foam cell formation, fatty streaks and plaque formation in the arterial wall, and (ii) hyperreactivity of blood platelets leading to increased platelet adhesion and aggregation. Vitamin E may play a protective role as an antioxidant and/or membrane stabilizing agent in either mechanism. In platelets it appears to regulate arachidonic acid metabolism.

Decreased vitamin E levels in platelets are associated with increased aggregation. This is reversible by correction of the vitamin E status. In diabetics, platelet vitamin E levels tend to be reduced with concomitant increase in platelet aggregation. Several studies in patients with insulin-dependent diabetes mellitus and, to some extent, in those with non-insulin-dependent diabetes mellitus have shown that supplementation with several hundred IU vitamin E significantly reduced platelet aggregation and lipid peroxidation. In healthy volunteers high-dose supplementation had no notable effect on platelet aggregation. However, doses as low as 200 IU vitamin E significantly reduced platelet adhesion and inhibited the formation of protruding pseudopods typically occurring in activated platelets. In diabetic patients a decrease in the nonenzymatic glycation of proteins by vitamin E supplementation has been observed.

Controlled studies are needed to confirm the effect of vitamin E on platelet function in well-defined groups of diabetics, followed by large-scale trials investigating the prevention of diabetic vascular complications as clinical end point.

Zusammenfassung: Diabetes mellitus ist durch verfrühte Atherosklerose und andere vaskuläre Komplikationen gekennzeichnet. Zu den auslösenden Faktoren gehören 1. erhöhte Peroxidation von LDL, was zu Schaumzellbildung und Plaques in der Arterienwand führt, und 2. Hyperreaktivität von Blutplättchen mit erhöhter Adhäsions- und Aggregationsneigung. Vitamin E könnte in seiner Funktion als Antioxidans oder Membranstabilisator eine Schutzwirkung ausüben. In Plättchen ist es an der Regulierung des Arachidonsäuremetabolismus beteiligt.

Bei erniedrigten Vitamin-E-Konzentrationen in Plättchen ist die Aggregation erhöht. Dies wird durch Korrektur des Vitamin-E-Status normalisiert. Bei Diabetikern besteht eine Tendenz zu reduzierten Vitamin-E-Werten mit gleichzeitig gesteigerter Plättchenaggregation. Mehrere Studien in Patienten mit Insulin-abhängigem und zum Teil mit nicht-Insulin-abhängigem Diabetes mellitus haben gezeigt, daß durch die Gabe einiger hundert IU Vitamin E die Aggregation sowie die Lipidperoxidation reduziert werden können. In gesunden Kontrollen konnte keine Wirkung auf die Aggregation festgestellt werden, hingegen führten Dosen von nur 200 IU Vitamin E zu einer signifikanten Reduktion der Adhäsion, wobei die Ausstülpungen von Pseudopoden, wie sie für aktivierte Plättchen typisch sind, nicht gebildet wurden. Bei Diabetikern führte die Gabe von Vitamin E auch zu einer Verminderung der nicht-enzymatischen Glykation von Proteinen.

Diese präliminären Befunde müssen durch randomisierte und kontrollierte Studien in gut definierten

Patientengruppen ergänzt werden, um die Wirkung von Vitamin E auf die Plättchenfunktion zu erhärten.

Key words: Vitamin E – antioxidant – diabetes – platelets – atherosclerosis

Schlüsselwörter: Vitamin E – Antioxidans – Diabetes – Plättchen – Atherosklerose

Introduction

Diabetes mellitus is a major health problem in industrialized countries affecting 0.2 % of children, 1 % of young adults, 6 % of middle-aged and 8–10 % of elderly persons (1, 2, 79). Diabetes mellitus can be defined as a chronic condition of insulin deficiency or insulin resistance characterized by deranged glucose and fatty acid metabolism. This leads to hyperglycemia associated with abnormalities in fat and protein metabolism. Two major forms are usually distinguished: A) *Type I or insulin-dependent diabetes mellitus (IDDM).* This is, typically, a form of diabetes developing during childhood (previously called juvenile diabetes). The beta cells of the pancreas fail to secrete insulin, which, untreated, leads to diabetic coma and death due to acute insulin deficiency. B) *Type II or non-insulin-dependent diabetes mellitus (NIDDM).* This is usually an adult-onset form of the disease. Often, there is insulin resistance in tissues (e.g., skeletal muscle, liver and adipose tissue) associated with obesity and primary overproduction of insulin, followed by gradual exhaustion of the synthetic capacity of the pancreas. These two major forms of diabetes have numerous subforms and multiple interacting causes and risk factors such as genetic disposition and, especially regarding NIDDM, lower social class and education, female gender, black race and obesity. The distribution between the two forms is 10 % for IDDM and 90 % for NIDDM.

The disease is often not perceived as serious by the patients themselves because high blood sugar is not painful and, at least in the early stages of disease, causes no disturbing and easily recognizable symptoms. Nevertheless, the secondary complications of diabetes reduce life expectancy by about one third. Morbidity is also considerable: Half of the amputations of the lower extremities and a quarter of renal failures are caused by diabetes. It is also the leading cause of retinopathy and blindness in adults.

Quite generally, diabetes appears to accelerate the degenerative changes occurring with aging. In particular, it causes premature atherosclerosis (affecting the larger arteries) with associated complications and is also involved in microvascular disease (capillaries and arterioles) affecting retina and kidneys. Apart from hyperglycemia, diabetes-induced hypertension and hyperlipidemia contribute considerably to accelerated atherogenesis.

In diabetics, death due to atherosclerosis is two to three times more common than among the non-diabetic population. In fact, the prognosis of diabetes is determined to a major degree by the progression of atherosclerosis and its consequences such as peripheral vascular occlusion, myocardial infarction or stroke. Attention should therefore be focused on prevention and control of vascular disease.

Adequate control of blood glucose with avoidance of hyperglycemic as well as hypoglycemic episodes is the most important part of prevention. This may be achieved by dietary means, though opinions among experts about the best diabetic diet are divided. Apart from a special diet, IDDM patients require insulin substitution, and some NIDDM patients may need oral antidiabetic agents or insulin in the late stages of the disease (2, 46).

Many of the factors contributing to atherosclerosis and thrombosis cannot be easily influenced. But it may be worthwhile to single out those that lend themselves to specific modification. At a cellular level, blood platelets (thrombocytes) are intimately involved in atherogenesis and thrombogenesis and show altered features especially in diabetic patients. Vitamin E may play a role in reducing their atherogenic potential. Evidence for a protective role of vitamin E against coronary heart disease has recently been provided by a study in Scotland showing that the risk of angina pectoris was significantly lower for persons with high vitamin E plasma concentrations (54). The WHO-MONICA collaborative study suggested a strong inverse association between coronary heart disease mortality and plasma vitamin E levels in 16 different European population groups (24).

Simplified model of atherogenesis

Pathological changes in arteries proceed in multiple stages till the vessels have become rigid and occluded. The earliest lesion is a fatty streak in the intima, the space beneath the fragile, but structurally still intact endothelial lining of the arteries. The fatty streak, which may develop into a fibrous plaque, consists mainly of foam cells. These originate from circulating monocytes which differentiate into resident macrophages in the intima and are overloaded with low-density lipoproteins (LDL), the major carrier of cholesterol. The event responsible for uncontrolled lipid accumulation by the scavenger receptor of intimal macrophages is the oxidative modification of LDL. LDL are susceptible to peroxidation because they are rich in polyunsaturated fatty acids (PUFAs). Unlike the uptake of normal (native) LDL, which is not itself atherogenic, the uptake of oxidized LDL (oLDL) cannot be down-regulated. Lipid loaded monocytes/macrophages are thus converted to foam cells. Oxidation of LDL can be induced by endothelial cells, smooth muscle cells and macrophages. Reactive oxygen species (free radicals) can also initiate LDL oxidation. Structural modification renders oxidatively modified LDL cytotoxic to endothelial cells and chemotactic for circulating monocytes, potentially enlarging the foam cell streak. Peroxidized PUFAs in LDL will induce chain reactions of free radical mediated lipid peroxidation, thus contributing to the continued formation of foam cell deposits. High LDL concentrations in plasma facilitate the process of LDL oxidation. In this sense, high LDL cholesterol values constitute a risk factor for atherogenesis (64, 21). A recent study in myocardial infarct patients confirmed that the susceptibility of LDL to oxidation correlated with the severity of coronary atherosclerosis (52).

The above "lipid hypothesis" has been widely accepted although many aspects are still speculative. It elegantly explains the involvement of plasma lipids and prooxidative events in atherogenesis and offers a role for vitamin E as chain-breaking lipid-soluble antioxidant in lipoproteins and lipid cell membranes which contribute to potential protection against atherogenesis. In vitro, vitamin E has been demonstrated to prolong the lag phase of copper-induced LDL oxidation (22). Supplementation of volunteers with vitamin E was recently shown to increase LDL resistance against oxidation (17).

Previously, the "reaction-to-injury" hypothesis was considered the exclusive explanation for the induction of atherogenesis. It postulates that the initiating event is an endothelial injury leaving a denuded arterial surface rapidly covered by adhering, then by aggregating blood platelets (56). It is now realized that the two theories may complement each other (57, 65) (Fig. 1).

Endothelial injury can indeed be caused by oxidized LDL, as well as by a fatty streak releasing cytotoxic factors (3). It can also be induced by mechanical forces, e.g., by shearing stress. This is suggested by the preferential deposition of plaques close to arter-

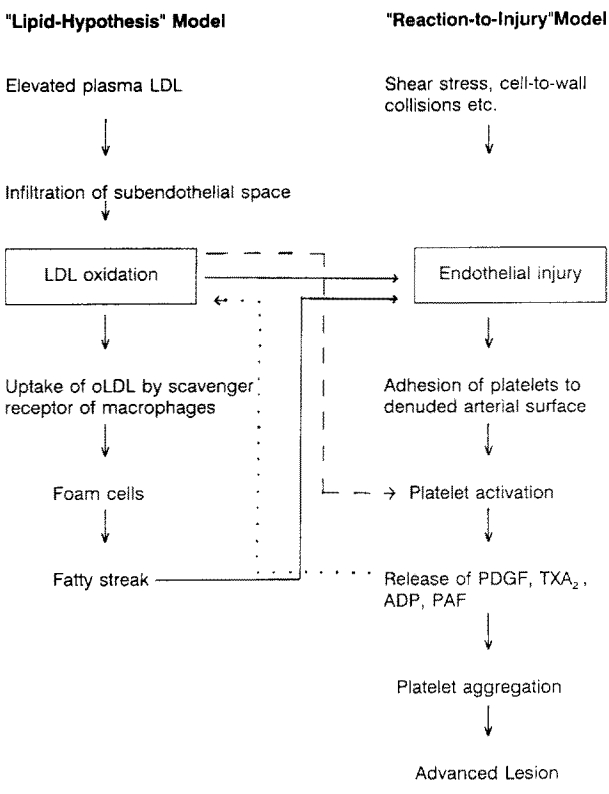

Fig. 1 Simplified complementary models of atherogenesis (adapted from refs. 65 and 57)

ial bifurcations and branching where blood flow is altered. Immune complexes and toxins may equally initiate endothelial injury (2, 66).

Apart from oxidized LDL, blood platelets, which are activated both by reactive oxygen species and by factors involved in endothelial injury, play an interesting role in atherogenesis, especially in diabetes. It appears that both LDL oxidation and platelet hyperreactivity are greatly enhanced in diabetes (5, 25).

Function of blood platelets

Platelets play a major role in hemostasis preventing fatal loss of blood from a vascular lesion. Because of this vital function, platelets have a strong tendency to stick to a suitable surface and initiate the repair process. Upon activation they cover up gaps between endothelial cells of vessel walls within seconds by adhering to the collagen of the subendothelial space. Exposure to collagen initiates the impulse for platelet activation. To enable adhesion to subendothelial connective tissue, platelets change their shape from a smooth disk to a spherical form with protruding pseudopods. Adhering platelets then release factors which, in the presence of cofactors such as fibrinogen and calcium, activate and attract other, circulating platelets, eventually leading to the formation of an occlusive clot consisting of aggregated platelets, but also red and white blood cells and

fibrin strands. Following the repair process, platelet clots normally disintegrate. However, if platelet activation is sustained, atherosclerotic lesions may be aggravated.

Platelet activation

During platelet activation arachidonic acid (AA), localized in the phospholipids of platelet membranes, is released by the enzyme phospholipase A_2. Platelets are capable of metabolizing AA to vasoactive prostanoids via two major pathways. (i) The oxidative conversion of AA by the enzyme lipoxygenase will lead to the production of 12-hydroxyeicosatetraenoic acid (12-HETE) which has strong proinflammatory properties and stimulates neutrophil chemotaxis. (ii) The other pathway involves the oxidative conversion of platelet AA by cyclooxygenase to cyclic endoperoxides and the further transformation to thromboxane A_2 (TXA_2) (Fig. 2). Thromboxane is a potent vasoconstrictor

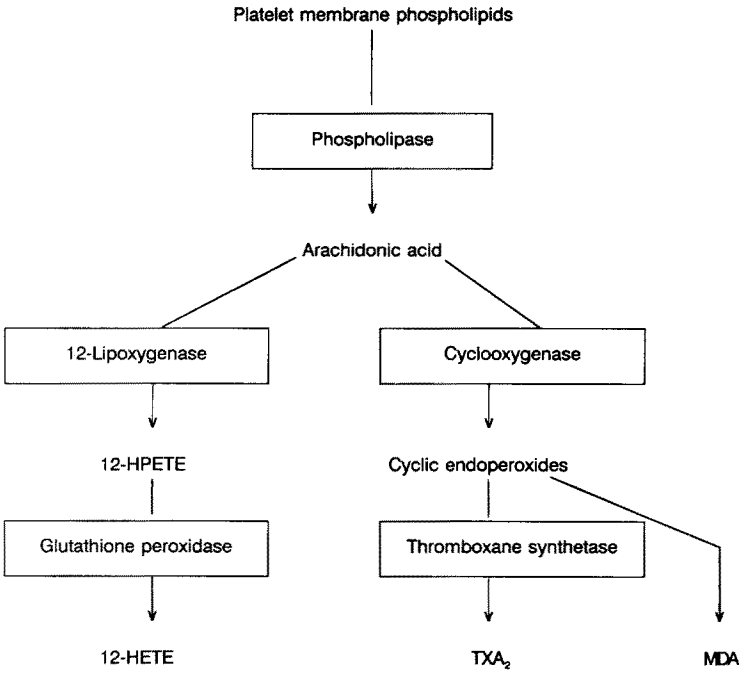

Fig. 2. Synthesis of platelet aggregating vasconstricting thromboxane A_2 (TXA_2), of proinflammatory 12-hydroxyeicosatetraenoic acid (12-HETE) and of malondialdehyde (MDA) in activated platelets.

and the most powerful promoter of platelet aggregation known. Malondialdehyde (MDA) is another end product of arachidonic acid metabolism. It is used as a crude but useful marker of lipid peroxidation. Other factors released by activated platelets which promote aggregation include adenosine diphosphate (ADP) and platelet activating factor (PAF). Among exogenous factors of platelet activation, connective tissue of the vessel wall, in particular collagenous fibrils, and thrombin, formed during the coagulation process, play an important role (10).

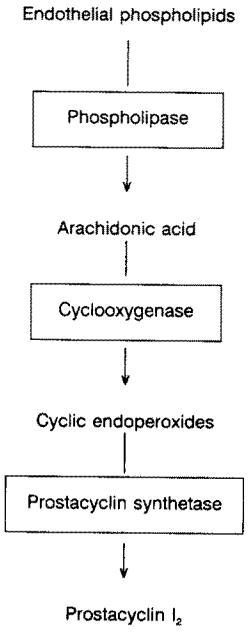

Fig. 3. Synthesis of antiaggregating vasodilating prostacyclin I_2 in endothelial cells.

The chain reaction of platelet aggregation, which is initiated and maintained by a great variety of factors, must be interrupted once the repair of the endothelial lesion has been achieved if thrombus formation and atherosclerotic deposits are to be prevented. For this purpose endothelial cells of the vessel wall synthesize another prostaglandin, which is derived from arachidonic acid in endothelial cell membranes. The enzyme prostacyclin synthetase in the vascular lining transforms cyclic endoperoxide to prostacyclin (PGI_2) (Fig. 3), which acts as a potent vasodilator and antiaggregatory agent. Thus, the balance between platelet TXA_2 and vascular PGI_2 can again be established after the repair of an injury has been accomplished by a platelet clot. The equilibrium between TXA_2 und PGI_2 is essential for the maintenance of healthy vessels with normal blood flow. Moreover, intact, functionally efficient epithelium releases enzymes such as ATPases, which transform platelet-aggregating ADP to AMP, as part of its important function of inhibiting the thrombotic tendency of blood platelets (48).

The energy requiring process of platelet activation with the generation of TXA_2 is accompanied by a sudden increase in oxygen consumption. This leads to free radical formation and destructive peroxidation of PUFAs in platelet membranes. Thus, both the lipid hypothesis and the response-to-injury hypothesis with hyperreactive platelets imply the increased generation of free radicals (65, 57).

Atherogenesis is, of course, much more complex involving other cell types and vascular responses. For instance, activated platelets release a mitogenic factor (platelet-derived growth factor, PDGF) which induces migration and proliferation of smooth muscle cells and leads to thickening of the intima, eventually causing myocardial ischemia (66, 56).

Accelerated atherogenesis in diabetes

Several pathological factors in the multistep development of atherosclerosis are aggravated by the diabetic state.

Elevated plasma concentrations of VLDL and LDL cholesterol, as they are typically found in diabetic patients, can act as substrates for increased oxidative modification of LDL. Apart from the generation of fatty streaks in the subendothelial space, oxidized LDL can proceed with the formation of free radicals which potentially damage proteins and lipid cell membranes, for instance of platelets and endothelial cells (49).

High blood sugar concentrations in diabetes can give rise to increased glycation of proteins, including serum proteins and collagen as well as crystallins in the eye lens which cross-link irreversibly. Increased glycation may in turn stimulate the oxidation of lipids and this may again stimulate glycation. This cyclic process could, hypothetically, continue and reinforce the perpetuation of oxidative stress and damage to lipids as well as to proteins in the vessel wall (6).

Increased reactive oxygen species (ROS) may be found in the plasma of diabetics. Lipid peroxide levels are particularly high in patients with poor diabetic control (60). Peroxidized fatty acids have been shown to render platelets hyperaggregable by activating phospholipase in platelet membranes (27).

Enhanced platelet reactivity. A special feature of diabetes, especially with poor glycemic control, is increased stickiness of platelets which aggregate spontaneously or with minimal stimulation. Diabetes is thus characterized by increased platelet aggregability with increased release of TXA_2. There is also decreased production of antiaggregating PGI_2. Moreover, factors released by activated platelets enhance the extent of LDL oxidation and increase the amount of lipid peroxides produced (4).

The mutually potentiating effect of these oxygen-consuming processes with free radical generation in diabetes may be expected to lead to depletion of antioxidative factors such as vitamin E. The question arises whether supplemental vitamin E interrupts the cycle of oxidative damage or has some other protective effect. It has been postulated that vitamin E inhibits TXA_2 production and stimulates PGI_2 synthesis (58). In the following, the relation of vitamin E to platelet function will be given particular consideration with special emphasis on the situation in diabetes.

Activity of vitamin E

Nomenclature

Vitamin E is the generic term for all tocols and tocotrienols having qualitatively the biological activity of RRR-α-tocopherol (previously known as d-α-tocopherol). α-Tocopherol has the highest biological activity. Other major forms include β- and γ-tocopherol and α-tocotrienol. All-rac-α-tocopherol (previously known as dl-α-tocopherol) is the synthetic form of vitamin E. Vitamin E activity in clinical trials has often been expressed in International Units (IU). One IU = 1 mg all-rac-α-tocopheryl acetate = 0.74 mg RRR-α-tocopheryl acetate = 0.67 mg RRR-α-tocopherol (44).

Antioxidative activity

Vitamin E is the major lipid-soluble chain-breaking antioxidant in tissue and plasma. In particular, it is a ubiquitous constituent of cell membranes. Vitamin E can effectively donate hydrogen to the peroxyl radical and thus interrupt the perpetuation of free radical production. In this process vitamin E turns into a weakly reactive radical itself. It

can effectively be regenerated by vitamin C, a water-soluble antioxidant. This implies that vitamin C should be abundantly present when vitamin E is involved in inactivating reactive oxygen species. Studies have demonstrated that optimal membrane protection in platelets was achieved by a vitamin E sparing action of vitamin C together with glutathione, another natural antioxidant (73). An essential vitamin E repairing function was recently confirmed for vitamin C in platelets using human cell homogenates (13).

Oxidative stress or injury occurs when the local concentration of oxidants exceeds the cellular ability to detoxify or inactivate radical species. Of major interest in recent years has been the capacity of vitamin E to delay the oxidative modification of LDL. This has permitted to formulate the concept that vitamin E may play an important role in the prevention of atherosclerosis (20, 32).

Other functions

It has variously been proposed that apart from its antioxidative effects vitamin E may have structural functions stabilizing lipid membranes. Some authors suggested that vitamin E reduces the permeability of PUFA-rich membranes and that it protects membrane phospholipids against degradation by phospholipase (43, 18). As a structural element of lipid membranes, vitamin E may contribute to decreasing membrane viscosity (68). Whether these stabilizing effects of vitamin E are physiologically relevant remains to be shown.

Effect of vitamin E on platelet function

The finding that deficiency of vitamin E in rats causes a significant increase in platelet aggregation in response to collagen was one of the first indications that vitamin E plays an important role in platelet function (45). Synthesis of platelet activating factor (PAF) was shown to be greatly enhanced in leucocytes of vitamin E deficient rats (23). Vitamin E deficiency in children was also associated with increased platelet aggregation in response to the aggregating agents collagen, ADP and epinephrine. There was no evidence of spontaneous hyperaggregation (70). A recent study in rats demonstrated that vitamin E deficiency not only enhanced platelet aggregation but, due to an increase in oxidized LDL, also abolished endothelium-dependent relaxation. The concentration of malondialdehyde (MDA) was greatly elevated as well (72). Thus, deficiency of vitamin E interfered with the cooperative interplay between platelets and endothelial cells in different ways.

Platelet vitamin E content

Platelets are comparatively rich in vitamin E with concentrations about three times higher than in erythrocytes. In a group of healthy volunteers the response of different blood compartments to supplementation with increasing doses of vitamin E (0, 30 and 100 IU/day) showed the highest uptake for platelets, followed in descending order by red blood cells, plasma lipids, plasma and finally leucocytes. Plasma lipids had no influence on platelet vitamin E content (42). The preferential uptake into platelets could be interpreted as a sign of increased requirements of these cells for vitamin E. The content of vitamin E in platelets appears to be a better measure of the nutritional status than the content in plasma since plasma vitamin E values fluctuate with plasma lipid concentrations.

A comparison of platelet vitamin E concentrations in young versus elderly persons demonstrated significantly depressed levels in older subjects. Activity of the antioxida-

tive enzyme glutathione peroxidase in platelets was reduced as well. Moreover, malondialdehyde content was increased in the elderly (75, 76). These findings could explain the age-related increase of platelet aggregability observed in an epidemiological study in Great Britain (47).

There is controversy regarding plasma and platelet vitamin E concentrations in diabetic patients. Some studies reported higher vitamin E concentrations in plasma, and normal or higher levels in platelets of diabetics than of controls. In two studies, higher plasma vitamin E concentrations were closely related to higher plasma lipid or lipoprotein values (11, 39) while in one study no such correlation was found (74).

In contrast, other investigators found significantly depressed vitamin E concentrations in platelets of diabetics compared with controls (35). This corresponds to findings in rats with streptozotocin-induced diabetes (36). Plasma vitamin E concentrations were similar in diabetics and controls (35). In one study the decrease in platelet vitamin E was particularly severe in patients with advanced diabetic complications such as proliferative retinopathy (78).

Effect of vitamin E addition to platelets in vitro

The addition of vitamin E to isolated platelets was reported to cause a dose-dependent moderate to strong inhibition of aggregation stimulated by different agonists (ADP, collagen, epinephrine). There was a concomitant inhibition of lipid peroxide formation (69). Interestingly, platelet aggregation was also reduced after the addition of tocopheryl quinone, the oxidized form of vitamin E. This finding may challenge the antioxidant hypothesis (15). Vitamin E was also shown to inhibit spontaneous platelet aggregation (33). At 1–2 mmol/L vitamin E, the concentration achieved in vitro is unphysiologically high and cannot be reached in human blood by vitamin E supplementation (66). A recent study showed that in the presence of aspirin, which inhibits TXA_2 formation, physiological concentrations of vitamin E of the order of 50–100 μmol/L inhibited cyclooxygenase independent platelet aggregation. This suggests a non-antioxidative effect of vitamin E (77). It is still uncertain whether decreased membrane viscosity by vitamin E plays a role. Another in vitro study confirmed that the addition of vitamin E reduced platelet aggregation without modifying the pathway of AA metabolism. Vitamin E could have inhibited intracellular calcium mobilization thus stabilizing membranes and rendering them less permeable to calcium ions (62). Other investigators found that vitamin E inhibited phospholipase A in platelet membranes and thus suppressed the release of arachidonic acid (19). Moreover, vitamin E could influence cyclooxygenase and lipoxygenase (51). Quite often, results from different laboratories are inconsistent. This may be due to difficulties in standardizing the methods of platelet isolation and assessment of platelet aggregation and adhesion (28).

Effect of vitamin E administration on platelets in animals and humans

Animals

In vitamin E depleted rats, supplementation with 200 mg RRR-α-tocopherol analogues/kg diet for 5 days restored platelet function, normalizing platelet aggregability as well as endothelium-dependent relaxation. Lipid peroxidation products were no longer increased (72). This study confirms earlier findings in rats (45). In rabbits, indirect platelet stimulating factors were assessed. The animals received either normal or fat-enriched diets with or without daily supplements of about 150 IU vitamin E/kg diet for 7 days. The increase in lipid peroxides due to high fat intake was completely prevented by con-

comitant vitamin E intake. Suppression of PGI_2 synthesis by the high fat diet was reduced (71).

Healthy volunteers

(Table 1). The response of platelets to vitamin E administration in humans has been variable. In six healthy volunteers given 2000 IU vitamin E for 10 days no changes were noted in collagen- or ADP-induced aggregation nor in the production of malondialdehyde (29). Supplementation of seven volunteers with 200 IU vitamin E daily for 2 weeks in another study also failed to show a modifying effect on platelet aggregation and arachidonic acid metabolism even though platelet vitamin E content had doubled (38). Doubling of platelet vitamin E concentrations with doses of 1500 IU daily for 2 weeks was also without effect on platelet response in nine healthy males (61) as was the administration of 800 IU daily to 20 volunteers for 5 weeks (63).

Investigators who had previously demonstrated moderate to potent in vitro inhibition of platelet aggregation after the addition of vitamin E (69) observed only weak antiaggregatory activity when vitamin E was given to healthy volunteers in doses of 400 up to 1000 IU daily. However, supplementation of volunteers with moderate amounts of vitamin E effectively inhibited the adhesion of platelets to various surfaces. They examined the reactivity of platelets from supplemented subjects ex vivo in a laminar flow chamber, which mimics in vivo blood flow at low shear rates as they arise in fissures of atherosclerotic plaques. At 200 IU vitamin E daily for 2 weeks there was a highly significant inhibition of platelet adhesion to surfaces such as collagen, fibronectin and fibrinogen. At 400 IU vitamin E per day the inhibition reached its maximum and could not be further increased by still higher doses. After the supplementation period there was a return to presupplementation rates of adhesion within one week. The investigators offer the following explanation for their important findings: In vitamin E-saturated platelets the elongated protruding pseudopods occurring during platelet activation were not formed, as shown by electron microscopic examination; the platelets therefore lacked an instrument for adhesion to non-platelet surfaces. The prevention of these shape changes by vitamin E occurred in a dose-dependent manner, but the underlying mechanism is unknown. Hypothetically, increased membrane stability could have prevented the extrusion of pseudopods (30, 31, 67). Since platelet adhesion may play a role in atherogenesis this possibility should be further investigated. Of particular interest will be the modulation of platelet adhesion in diabetic patients.

Table 1. Effect of vitamin E administered to healthy subjects on platelets ex vivo

Subjects	n	Vitamin E/d	Duration	Findings	Reference
Volunteers	6	2000 IU	10 d	No effect on collagen or ADP induced aggregation and on MDA	29
Volunteers	7	200 IU	2 wk	No effect on aggregation	38
Volunteers	20	800 IU or placebo	5 wk	No effect on aggregation or arachidonic acid metabolism	63
Volunteers	9	1500 IU	2 wk	No effect on aggregation	61
Volunteers	6	200 then 400 IU	2 wk 2 wk	Significant inhibition of platelet adhesion to collagen surface and prevention of pseudopod formation	30

Table 2. Effect of vitamin E administered to subject with various conditions on platelets ex vivo

Subjects	n	Vitamin E/d	Duration	Findings	Reference
Deficient children	6	1600 IU	2 wk	Normalization of aggregation and of MDA formation	70
Volunteers with reduced antioxidant status	80	100 IU (+600 mg vitamin C, 27 mg beta carotene and 75 μg Se) or placebo	5 mo	Significant reduction of platelet aggregation to ADP, of MDA and TBX_2	59
Women on oral contraceptives	30	200 IU	2 mo	Restoration of platelet vitamin E, normalization of aggregation to ADP	53
Hyperlipidemic patients	15	800 IU or placebo	3 mo	Significant reduction of aggregation to collagen and of MDA	8
Hyperlipidemic patients and controls	46 18	400 or 800 IU or placebo	2 wk	Mild suppression of aggregation to collagen at 800 IU. Normalization of MDA. No effect in controls	71

Patients with vitamin E deficiency

(Table 2). In cases of vitamin E deficiency, which is characterized by enhanced platelet aggregation and increased lipid peroxide production in both animals and humans, supplementation with vitamin E normalizes these changes as demonstrated in a study of six vitamin E deficient children. When, as in these children, vitamin E deficiency is due to fat malabsorption diseases, vitamin E doses of the order of several hundred milligrams may be needed to overcome plasma transport problems (70).

Persons with poor antioxidant status

In a double-blind randomized study in Finland 80 men with a poor antioxidant status and a comparatively high fat intake received either a combination of antioxidant nutrients (100 IU vitamin E, 600 mg vitamin C, 27 mg beta-carotene and 75 μg selenium) or placebo daily for 5 months. The treated group experienced significant improvements in platelet function. Serum lipid peroxides were reduced by 20 %, ADP-induced platelet aggregation by 24 %, ATP release during aggregation by 42 %, TXB_2 production by 57 % and plasma β-thromboglobulin by 29 % (59). These findings, which indicate that antioxidants reduce lipid peroxidation and platelet aggregability, are not in keeping with other studies in principally healthy participants (63, 61, 38). Apart from the presumably lower baseline antioxidant levels in this study population, the longer duration of supplementation may account for the observed differences.

In women using oral contraceptives (OC) containing estrogens platelet vitamin E concentrations appear to be reduced. This may explain findings showing elevated susceptibility of platelets to aggregation in OC users and may contribute to the increased risk of thrombotic complications associated with OCs. In a study including 30 women, supplementation with 200 IU vitamin E normalized platelet reactivity. The effect was particularly notable in those women who, at baseline, had shown the highest platelet reactivity (53).

Patients with elevated blood lipid levels

Hyperlipidemia often precedes or accompanies the manifestation of diabetes and exposes the patients to an increased risk of coronary heart disease. In 15 patients with hypercholesterolemia (>6.3 mmol/L) who received placebo for 3 months followed by 800 IU vitamin E for another 3 months, plasma lipid concentrations remained largely unchanged, but lipid peroxidation as well as platelet aggregability to collagen were significantly reduced (8).

The effect of 2-week supplementation with 400 or 800 IU vitamin E or placebo was investigated in a group of 46 hyperlipidemic patients in comparison with a group of 18 healthy subjects. After 2 weeks the vitamin E values in plasma lipids had doubled with either dose indicating that 400 IU were sufficient to saturate plasma lipids with vitamin E. However, the finding that only the higher dose of 800 IU vitamin E achieved mild suppression of collagen-induced platelet aggregation suggests that platelet membranes require higher concentrations. Plasma lipid peroxide levels, which had initially been higher in the patients than in the healthy controls, were normalized at the end of the 2-week supplementation period (71).

Patients with diabetes

(Table 3). In cases of advanced diabetic complications the platelet vitamin E contents were depressed and inversely correlated with TXA_2 production (measured as its metabolite TXB_2) as well as with ADP-induced platelet aggregation (78). In 16 IDDM patients challenge of vitamin E deficient platelets with ADP, collagen or thrombin also produced significantly elevated amounts of TXA_2. No increase was noted when exogenous arachidonic acid was added as substrate. This indicates that the increase in TXA_2 had been derived from platelet membrane AA and was induced by low vitamin E con-

Table 3. Effect of vitamin E administered to diabetics on platelets ex vivo

	Subjects	n	Vitamin E/d	Duration	Findings	Reference
a)	NIDDM patients CHD patients Controls	15 15 25	2000 IU	2 wk	Significant reduction of blood glucose and mild non-significant reduction of aggregation in diabetics. No effects in other subjects	9
b)	NIDDM patients (crossover)	25	2000 IU or placebo	6 wk	Same as in diabetics above	
	IDMM patients (crossover)	9	1000 mg or placebo	5 wk	Significant reduction of aggregation to ADP of TXB_2 and MDA (vitamin E form not specified)	14
	IDDM patients Controls (crossover)	22 12	400 IU or placebo	4 wk	Significant reduction of aggregation to ADP and collagen and of TXB_2	26
	IDDM patients	8	600 mg	2 wk	Significant reduction of aggregation to ADP (vitamin E in the form of α-tocopheryl nicotinate)	40
	IDDM patients Controls	14 12	600 mg	2–4 wk	Significant reduction of aggregation to ADP and of TXA_2; increase in PGI_2 (vitamin E form as above)	41

centrations (35). A similar increase was found for 12-HETE in 13 diabetic patients with low platelet vitamin E levels. This suggests that vitamin E deficiency influences AA metabolism at the level of platelet phospholipase activity permitting the increased release of platelet AA (37). Platelet aggregability was not investigated in these studies.

An intervention study including 25 healthy controls, 15 NIDDM and 15 heart disease patients investigated the effect of vitamin E supplements at daily doses of 2000 IU for 2 weeks. The variables analysed were serum lipid, glucose and vitamin E concentrations, time to aggregation and blood pressure. Vitamin E levels were greatly increased. Unexpectedly, serum glucose levels in the diabetics were significantly reduced. No significant changes were noted for the other variables. In a follow-up trial, 25 diabetic patients were given either placebo or 2000 IU vitamin E in a double-blind crossover design for two 6-week periods. Serum glucose levels were again reduced by vitamin E. Platelet aggregation time lengthened and disaggregation time shortened, but the differences were not significant. However, when the changes in aggregation time were correlated with those in serum vitamin E concentrations they became significant (9).

More impressive results were obtained in a group of nine IDDM patients who were given 1000 mg vitamin E (form not specified) or placebo in a double-blind crossover design for two 5-week periods separated by an interval of at least 3 months. The patients receiving vitamin E exhibited a small but significant reduction in ADP-induced platelet aggregation even though baseline values in these well-controlled patients had been in the normal range. Moreover, both TXB_2 and MDA production were reduced after stimulation with arachidonic acid while no effect on 12-HETE production was observed. These findings suggest that vitamin E may influence platelet arachidonic acid metabolism at the level of cyclooxygenase (see Fig. 2). In a group of healthy persons with normal platelet response at baseline, who also participated in the study, vitamin E supplements had no effect on aggregation (14).

A group of 22 IDDM patients without microvascular or macrovascular complications received supplements of 400 IU vitamin E or placebo daily for 4 weeks in comparison with a group of 12 healthy controls. The study followed a double-blind crossover protocol. TXA_2 release (measured as TXB_2) following stimulation with ADP or collagen was significantly lower in the vitamin E treated group. Platelet aggregation which, at baseline, had been in the normal range was reduced at stimulation with 0.25 μg collagen (26). Since the patients had not yet developed vascular disease, the platelet prostanoid changes observed may have been a cause rather than a consequence of vascular disease. Vitamin E supplementation would thus offer a means of preventing vascular pathology at an early stage (25).

Platelets isolated from eight NIDDM patients and from eight controls showed a dose-dependent reduction in the aggregation rate to ADP stimulation after in vitro addition of varying concentrations of vitamin E (in the form of α-tocopheryl nicotinate). Before the addition of vitamin E, the platelets of the diabetic patients showed a significantly higher rate of aggregation than those of the controls. This corresponds to the greatly reduced platelet vitamin E concentrations in the patients, especially those with proliferative retinopathy. After the addition of physiological amounts of vitamin E the decrease in aggregation was greater in patients than in controls. In a follow-up intervention study the administration of a single dose of 600 mg vitamin E (as α-tocopheryl nicotinate) to patients and controls led to a significant decrease in aggregation rate only in the control subjects. However, after the patients had been supplemented with 600 mg vitamin E daily for 2 weeks, the decrease in aggregation was significant indicating that adequate

platelet vitamin E concentrations had been achieved. The control subjects were not included in the 2-week trial (40). The investigators confirmed these findings in a study including 14 NIDDM patients suffering from proliferative retinopathy. They additionally found that TXA_2 production (measured as TXB_2) was significantly reduced and that PGI_2 (measured as the metabolite 6-keto-PGF1α) in plasma was significantly elevated by increased intake of vitamin E. The balance between TXA_2 and PGI_2, considered important for vascular health, was thus reestablished (41).

Other potential vitamin E effects in diabetics

Non-enzymatic glycation (NEG) is considered an important pathogenetic factor in secondary complications of diabetes. The reaction is comparable to the Maillard reaction known to lead to discoloration of stored and heated food and to be preventable by antioxidants (55). A study was therefore undertaken in 30 IDDM patients who received either 600 or 1200 mg vitamin E (form not specified) or placebo daily for 2 months according to a randomized double-blind design. While blood sugar levels remained unchanged, vitamin E supplementation led to a significant dose-dependent decrease in glycation of proteins (12). In another study, designed to investigate the response of platelets to vitamin E, no effect was observed on glycated hemoglobin at 1000 mg vitamin E per day (14). However, the findings confirm results from a study in streptozotocin-diabetic rats given 500 or 1000 IU vitamin E per kg diet in comparison with untreated healthy and diabetic control groups. Vitamin E supplementation caused a dose-dependent decrease in glycated hemoglobin in the diabetic rats (50). An effect on glycation has also been reported for vitamin C (16).

Conclusions

As the secondary complications of diabetes cause considerable morbidity and premature mortality involving in particular the vascular system, prevention of damage should have high priority. Vitamin E may be important in its role as the major lipid-soluble chain-breaking antioxidant in cell membranes and possibly also as a structural element stabilizing membranes. Epidemiological studies have suggested that vitamin E may reduce cardiovascular morbidity and mortality.

Two of the hypotheses attempting to explain atherogenesis incorporate a protective role for vitamin E. The *"lipid hypothesis"* postulates that oxidized LDL is at the origin of fatty streaks which then progress to plaques narrowing the arteries. Vitamin E has been shown in vitro to delay copper-induced peroxidation of LDL. Supplementation of volunteers with vitamin E has recently been shown to prolong the lag phase of copper-induced LDL oxidation though the use of copper induction of oxidation has been criticized since it may not reflect physiological conditions. Much work will be needed in animals and humans to verify the hypothesis and determine its relevance.

According to the *"reaction-to-injury-hypothesis"* activated blood platelets are involved in atherogenesis by adhering to injured vascular surfaces and attracting other platelets to aggregate, eventually obstructing blood flow. In patients with diabetes platelet aggregation is increased and the balance may be disturbed between proaggregatory, vasoconstricting thromboxane A_2 produced from arachidonic acid in platelet membranes and its antagonist, the antiaggregatory, vasodilating prostacyclin I_2 synthesized from endothelial arachidonic acid. Lipid peroxidation end products such as malondialdehyde, derived from platelet membrane phospholipids, are also increased in diabetics.

The two hypotheses, which were formerly believed to be mutually exclusive, have been reconciled since they appear to complement each other. Of course these models oversimplify the mechanism of atherogenesis which is a highly complex multifactorial and multistep process.

The non-enzymatic glycation of protein, e.g., of hemoglobin, is another mechanism involved in diabetic vascular pathology for which a protective role of vitamin E has been postulated. In the process of the formation of glycation end products, free radicals are formed leading to activation of platelets and macrophages as well as being cytotoxic to the endothelium. While one study in diabetics failed to find an effect of vitamin E on the production of glycated hemoglobin, a careful double-blind trial observed significant inhibition by vitamin E. Protection by vitamin E was confirmed in an animal experiment.

The interaction between platelets and vitamin E is particularly intriguing in diabetics because platelet vitamin E concentrations are reduced and platelet aggregation is enhanced indicating that intervention may offer potential protection. In healthy volunteers supplementation with vitamin E doses ranging from 200 to 2000 IU has not produced a notable effect on platelet aggregation. However, doses as low as 200 IU led to highly significant inhibition of platelet adhesion. The formation of protruding pseudopods, typically found in activated platelets, was equally prevented. These important findings need to be followed up in diabetic patients since platelet adhesion may be a pathogenetic factor in atherosclerosis. The inhibition of platelet adhesion by vitamin E has been proposed to function by a mechanism differing from that inhibiting platelet aggregation. It might involve the reduction of membrane fluidity with consequent increase in membrane stability. The extrusion of pseudopods might thus be inhibited.

The fact that in persons with normal platelet function aggregation was not modified by vitamin E supplementation – while in cases of pathologically increased aggregation hyperreactivity of platelets was normalized – may be beneficial since it indicates that the normal physiological function of platelets is maintained with high intakes of vitamin E. An adverse effect of high vitamin E doses in the form of increased bleeding tendency is to be expected only in cases of coagulation defects associated with vitamin K deficiency caused by malabsorption or anticoagulant therapy (7, 34).

In conditions with reduced vitamin E status supplementation with vitamin E was shown to have an inhibitory effect on platelet aggregation as well as on the production of lipid peroxides and partly of TXA_2.

Patients with diabetes have, in the majority of studies, experienced a significant reduction in platelet aggregation and in the production of lipid peroxides and TXA_2 by vitamin E supplementation. This indicates that vitamin E has a modifying effect on the hyperreactivity of platelets in diabetes. Some investigators maintain that platelet metabolism is more pronounced in diabetic patients with retinopathy, neuropathy and coronary artery disease suggesting that hyperreactivity of platelets is a consequence rather than a cause of diabetic complications. However, other investigators have identified metabolic products of platelet activation early in diabetes in the absence of vascular disease. One study in diabetics assessed the effect of vitamin E supplementation not only on platelet arachidonic acid metabolism but also on the production of vascular prostacyclin and found a significant increase in PGI_2 indicating that the balance between thromboxane and prostacyclin had been restored.

Some of the trials have been preliminary not following a rigorous study design so that no final conclusions emerge. The duration of supplementation in the different studies

has ranged from a few weeks to a few months. The doses used have also been highly variable. Before long-term large-scale trials are undertaken in patients with diabetes investigating the protective effect of vitamin E on clinical end points such as vascular complications it may be advisable to perform a few more well defined smaller studies in diabetics establishing suitable dosages to be used and verifying the mechanism of vitamin E in platelet function. Due to the limited number of studies available it is not yet possible to identify differences between IDDM and NIDDM patients in platelet response to vitamin E.

The molecular mechanism by which vitamin E reduces platelet aggregation is not known with certainty. Several steps in the arachidonic acid cascade have been postulated to lend themselves to intervention by vitamin E. Suppression of cyclooxygenase is one possibility. While drugs acting as cyclooxygenase inhibitors are likely to inhibit the production not only of TXA_2 but also of PGI_2 thus negating any benefit derived from TXA_2 reduction, it is conceivable that vitamin E, which is taken up preferentially by platelet membranes, could selectively inhibit TXA_2, sparing PGI_2 synthesis. It is also possible that vitamin E acts as an antioxidant additionally or alternatively at the level of phospholipase or thromboxane synthetase. However, the concept that the ratio of TXA_2 to PGI_2 may be the exclusive determinant of platelet aggregability in vivo may be too simple a hypothesis. Some studies have indeed shown an inhibitory effect of vitamin E on aggregation by a pathway that was independent of arachidonic acid metabolism.

Thus, much work will be required, not only at the clinical level demonstrating the relevance of platelet inhibition in diabetics, but also at the biochemical level investigating the mechanisms of platelet inhibition by vitamin E. The available evidence, while being incomplete, points to a potentially protective effect of vitamin E against platelet adhesion and aggregation in patients with diabetes and other disorders. The data are sufficiently encouraging to justify further work.

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Author's address:

Helga Gerster, Vitamin Research Department, Hoffmann-La Roche AG, Grenzacherstrasse 124, 4002 Basel, Switzerland