Journal of Thrombosis and Haemostasis, **2**: 1282–1291

REVIEW ARTICLE

Platelet activation in type 2 diabetes mellitus

P. FERRONI,* S. BASILI,† A. FALCO‡ and G. DAVÌ‡

*Departments of *Experimental Medicine & Pathology, †Medical Therapy, University of Rome La Sapienza; and ‡G. d'Annunzio Foundation, Center of Excellence on Aging, University of Chieti, School of Medicine, Chieti, Italy*

To cite this article: Ferroni P, Basili S, Falco A, Davì G. Platelet activation in type 2 diabetes mellitus. *J Thromb Haemost* 2004; **2**: 1282–91.

Summary. The abnormal metabolic state that accompanies diabetes renders arteries susceptible to atherosclerosis, being capable of altering the functional properties of multiple cell types, including endothelium and platelets. In particular, an altered platelet metabolism and changes in intraplatelet signaling pathways may contribute to the pathogenesis of atherothrombotic complications of diabetes. A variety of mechanisms may be responsible for enhanced platelet aggregation. Among them, hyperglycemia may represent a causal factor for *in vivo* platelet activation, and may be responsible for nonenzymatic glycation of platelet glycoproteins, causing changes in their structure and conformation, as well as alterations of membrane lipid dynamics. Furthermore, hyperglycemia-induced oxidative stress is responsible for enhanced peroxidation of arachidonic acid to form biologically active isoprostanes, which represents an important biochemical link between impaired glycemic control and persistent platelet activation. Finally, increased oxidative stress is responsible for activation of transcription factors and expression of redox-sensitive genes leading to a phenotypic switch of endothelium toward an adhesive, prothrombotic condition, initial platelet activation, adhesion and subsequent platelet aggregate formation. All this evidence is strengthened by the results of clinical trials documenting the beneficial effects of metabolic control on platelet function, and by the finding that aspirin treatment may even be more beneficial in diabetic than in high-risk non-diabetic patients. Attention to appropriate medical management of diabetic patients will have great impact on long-term outcome in this high-risk population.

Keywords: atherosclerosis, diabetes mellitus, platelet activation.

Correspondence: Giovanni Davì, G. d'Annunzio Foundation, Center of Excellence on Aging, Via colle dell'Ara, 66013 Chieti, Italy.
Tel.: 0039 0871 541312; fax: 0039 0871 541313; e-mail: gdavi@unich.it

Received 8 January 2004, accepted 24 March 2004

Introduction

Diabetes mellitus (DM) is commonly associated with both microvascular and macrovascular complications [1]. Macrovascular complications manifest themselves as accelerated atherosclerosis, clinically resulting in premature coronary artery disease (CAD), increased risk of cerebrovascular disease, and severe peripheral vascular disease [1]. Patients with type 2 diabetes mellitus (T2DM) have a two- to four-fold increase in the risk of CAD and patients with DM but without previous myocardial infarction (MI) carry the same level of risk for subsequent acute coronary events as non-diabetic patients with previous MI [2,3].

During the past years, the underlying molecular mechanisms responsible for diabetic vascular complications have begun to be clarified. In this context, hyperglycemia, a well defined risk factor for accelerated atherosclerosis and vascular disease [4], may cause vessel damage through at least three apparently unrelated pathways: advanced glycation end product (AGE) formation, activation of protein kinase C (PKC), and sorbitol accumulation by way of the polyol pathway [5]. Nevertheless, these pathways could be linked by an increased production of superoxide by the mitochondrial electron transport chain [6]. Indeed, normalization of the levels of reactive oxygen species (ROS) in the mitochondria results in prevention of glucose-induced activation of PKC, AGE formation and sorbitol accumulation [6]. Furthermore, hyperglycemia via increased oxidative stress, and receptor for advanced glycation end products (RAGE) activation, increases the activation of transcription factor-κB (NF-κB) both in endothelial [7] and vascular smooth muscle cells [8]. This transcription factor regulates the expression of the genes encoding a number of mediators of atherogenesis such as leukocyte-cell adhesion molecules and/or chemoattractant proteins that recruit lymphocytes and monocytes into the vascular wall, as well as other proinflammatory mediators commonly found in atheroma. For example, transforming growth factor-β (TGF-β) plays a pivotal role in mediating extracellular matrix accumulation in the diabetic kidney [9], and vascular endothelial growth factor (VEGF) has powerful angiogenic properties that may be responsible for retinal neovascularization [10].

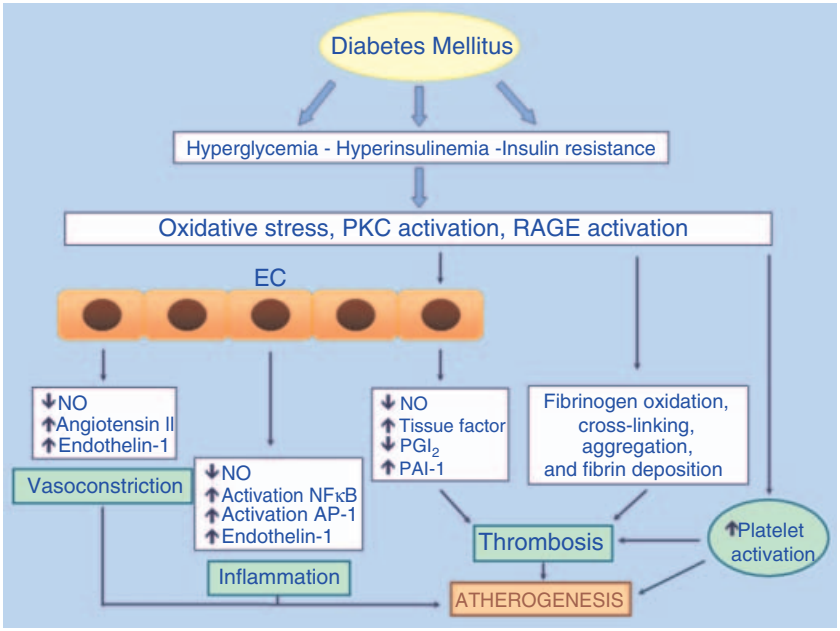

Fig. 1. Metabolic dysfunction occurring in diabetes mellitus is capable, through increased oxidative stress, protein kinase C (PKC) and receptor for advanced glycation end products (RAGE) activation, of inducing activation of endothelial cells (EC) and platelets, which causes a switch toward a pro-thrombotic, pro-inflammatory condition and contributes to the pathogenesis of atherosclerosis. NO, nitric oxide; NF-κB, nuclear transcription factor-κB; AP-1, activator protein-1; PGI_2, prostacyclin; PAI-1, plasminogen activator inhibitor-1.

Activation of the NF-κB pathway may also cause a switch of the endothelial functions toward a pro-thrombotic condition that, together with an altered platelet metabolism and changes in intraplatelet signaling pathways, contributes to the pathogenesis of atherothrombotic complications of DM (Fig. 1). It has been suggested that an enhanced activation of circulating platelets is especially apparent in diabetics with macrovascular disease [11], while other studies showed that platelet activation is rather related to the presence of DM per se, but not to the existence of vascular disease [12]. Furthermore, alterations in platelet function have been also associated with the progression of diabetic microangiopathy, but not all authors have confirmed their role in the prediction of disease progression [13]. Thus, the unresolved question is whether persistent platelet activation in DM is merely a consequence of more prevalent atherosclerotic lesions or reflects the influence of the accompanying metabolic and hemodynamic disturbances on platelet biochemistry and function.

The molecular mechanisms leading to vascular complications in DM have been the main topic of recently published articles, to which the reader may refer for a comprehensive overview of the literature [14–16]. In this review we will focus our attention on various aspects of platelet function in T2DM because these patients represent more than 90% of those with DM and atherosclerosis. Main objectives will be: to examine the major determinants of *in vivo* platelet activation in DM, to discuss the effects of glycemic control on platelet function, and to review evidences coming from clinical trials using antiplatelet drugs in this setting.

Determinants of *in vivo* platelet activation in diabetes mellitus

It is now well recognized that the abnormal metabolic state that accompanies diabetes is responsible for vascular dysfunction. Relevant abnormalities include chronic hyperglycemia, dyslipidemia, and insulin resistance. All these factors render arteries susceptible to atherosclerosis, being capable of altering the functional properties of multiple cell types, including endothelium and platelets (Fig. 1). The hypothesis that alterations in platelets and endothelial damage may occur early in the diabetic state has been suggested in animal studies showing that enhanced platelet aggregation in response to several agonists occurs well before vessel wall changes develop [17]. Indeed, enhanced platelet aggregation and thromboxane (TX) A_2 synthesis was detected within days of making rats diabetic with streptozotocin [18]. More recently, it was shown that many cytokines, released during platelet activation, stimulate migratory and proliferative responses of vascular smooth muscle cells and mediate expression of leukocyte adhesion molecules by endothelial cells, thus allowing for macrophage margination, translocation and phenotype switch, processes that are central to atherosclerotic plaque formation [19].

Increased platelet aggregation in DM was recognized already in 1965 [20]; since then many studies have demonstrated that platelet degranulation and synthesis of TX derivatives mediating further platelet activation are increased in DM [21,22], whereas platelet-mediated vasodilatation is impaired [23]. Furthermore, platelets from DM patients have been shown to have diminished sensitivity to natural antiaggregating

agents, such as prostacyclin (PGI_2) and nitric oxide (NO) [24,25]. Although there has been an expanding pool of literature on the enhanced platelet sensitivity to a variety of aggregating agents *in vitro* in T2DM [26], it is not clear at this time whether these abnormalities are intrinsic to the platelet or are a consequence of circulating factors that affect platelet function, as has been demonstrated for insulin immunocomplexes [26,27]. Recent studies indicate that the altered platelet function characteristic of DM may be related to several mechanisms, among which metabolic alterations, oxidative stress and endothelial dysfunction seems to play a pivotal role.

Metabolic alterations

Intra-platelet glucose concentration mirrors the extracellular concentration, since glucose entry into the platelet does not depend on insulin [26]. Chronic hyperglycemia has been clearly identified as a causal factor for *in vivo* platelet activation and platelet hyper-reactivity in DM patients [12,20,28] (Figs 2 and 3). In a study of 1990, Davì *et al.* demonstrated enhanced TX biosynthesis in T2DM and provided evidence for its platelet origin [22]. Tight metabolic control led to a reduction of TX levels in the same study [22]. More recently, the same authors demonstrated that the metabolic disorder rather than the attendant vascular disease appears to be responsible for persistent platelet activation in this setting [12]. Furthermore, several clues point to an important role of hyperglycemic spikes in triggering ischemic cardiovascular complications in DM [29,30]. Acute, short-term hyperglycemia induces an increased activation of platelets exposed to high shear stress conditions both *in vitro* and *in vivo* [31], and an increased sensitivity to agonists due to impaired calcium homeostasis, activation of PKC, decreased production of platelet-derived NO, and increased formation of superoxide [26,32,33]. Furthermore, hyperglycemia is responsible for nonenzymatic glycation of platelet membrane proteins that may cause changes in protein structure and conformation, as well as alterations of membrane lipid dynamics [34]. This altered dynamic of platelet membrane may, in turn, result in enhanced expression of receptors which are crucial for platelet function, such as P-selectin and GpIIb/IIIa [35], thus making these molecules much more susceptible for potential ligands [36].

Increased platelet sensitivity to aggregating agents has been also explained by an effect of low-density lipoproteins (LDL) and their glycation [37]. Hyperglycemia, in fact, induces an increase in nonenzymatically glycated LDL (glycLDL), which renders them more susceptible to oxidative stress [38, 39]. Moreover, glycLDL may cause platelet dysfunction by an increase in intracellular Ca^{2+} concentration and platelet NO production, as well as inhibition of the platelet membrane

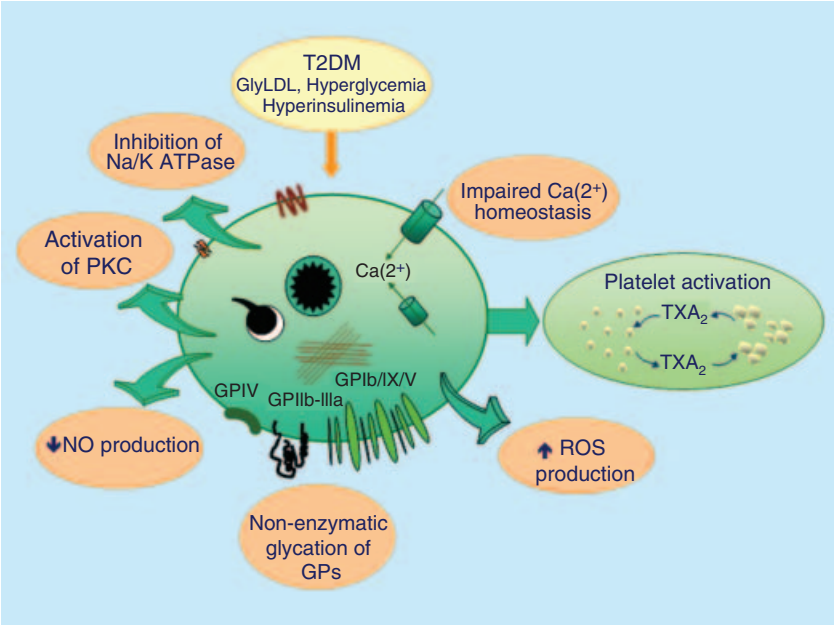

Fig. 2. Effects of altered glycemic control on platelet function. As shown, elevated glucose levels as well as glycated low-density lipoproteins (GlyLDL) and hyperinsulinemia may led to impaired calcium homeostasis, inhibition of the platelet membrane Na^+/K^+-adenosine triphosphatase (Na^+/K^+-ATPase) activity, activation of protein kinase C (PKC), decreased production of nitric oxide (NO), increased formation of reactive oxygen species (ROS) and nonenzymatic glycation of platelet membrane glycoproteins (GPs). The increased intracellular calcium concentration and activation of PKC, as well as the decreased NO bioavailability may be responsible for increased platelet sensitivity to agonists in diabetes. Similarly, generation of ROS by platelet enzymatic sources may lead to formation of lipid peroxides either from arachidonic acid or circulating low-density lipoprotein (LDL), which may cause a dose-dependent increase in calcium release from intracellular stores and platelet shape change, as well as enhanced aggregation response to subthreshold concentrations of platelet agonists. Finally, the increased extent of glycosylation of platelet membrane GPs in diabetes appears to be related to reduced membrane fluidity and altered receptor availability, contributing to platelet hyperfunction. All these events can be ultimately responsible for activation of the arachidonic pathway resulting in increased TXA_2 formation.

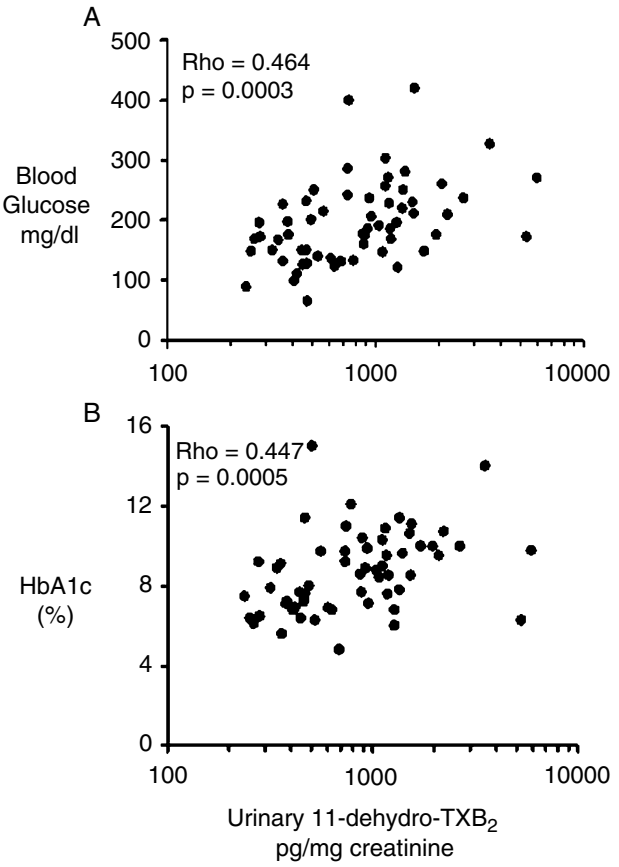

Fig. 3. Spearman rank correlation analyses of urinary 11-dehydro-TXB_2 excretion and blood glucose levels (A) or HbA_{1c} (B) in 60 patients with T2DM.

Na^+/K^+-adenosine triphosphatase (Na^+/K^+-ATPase) activity [40]. While the effects on intraplatelet calcium concentration are consistent with an enhanced sensitivity to aggregating agents, the higher NO production is expected to result in a reduction of platelet function. To explain this apparent discrepancy, Ferretti *et al*. [40] have postulated that the effects of NO might be counteracted by the contemporaneous increase in platelet calcium. Alternatively, the stimulation of NO release under conditions of unbalance between NO and ROS may lead to generation of the strong oxidant peroxynitrite [40].

Beside GlycLDL, other characteristic abnormalities can be found in the lipid profile of T2DM, including elevated triglyceride, decreased high-density lipoprotein (HDL) and increased small dense LDL levels [3]. All these may affect platelet function by interfering with membrane fluidity, or directly with intracellular systems (reviewed in [41,42]). Of interest is the finding that small dense LDL particles are more susceptible to oxidation than normal sized LDL. Oxidized LDL has been shown to decrease nitric oxide synthase (NOS) protein expression in human platelets [42]. Furthermore, the attack of LDL by ROS may cause the release of bioactive isoprostanes [43], which, in turn, are responsible for enhanced agonist-induced platelet adhesion and aggregation (see below).

Finally, it is well known that T2DM can be associated to a condition of insulin-resistance and, consequently, to increased levels of circulating insulin, especially at the beginning of diabetes natural history. Platelets have been shown to be targets of insulin action because they retain a functional insulin receptor capable of insulin binding and autophosphorylation [44]. Insulin is generally thought to reduce platelet responses to various agonists [45]. Decreased platelet insulin receptor number and affinity in subjects with T2DM have been reported [46], which suggested that reduced insulin sensitivity might account for platelet hyperactivity in T2DM, whereas other studies suggested the potential importance of insulin in maintaining normal platelet sensitivity to PGI_2 [26]. Thus, the question that arises is: are platelets the site of insulin resistance? Because insulin actions on platelets are mediated by NO, Trovati and Anfossi [47] performed a series of experiments designed to verify whether the reduced responses to insulin observed in the insulin-resistant states could be due to an impaired insulin ability to activate NOS or to an impaired NO ability to activate guanylate cyclase or to both phenomena together. The results obtained clearly demonstrated that insulin-resistant states show reduced platelet responses to both insulin and NO donors, inducing the authors to speculate that human platelets are a site of insulin resistance.

Insulin resistance, elevated triglyceride levels, decreased high-density lipoprotein (HDL) levels, and elevated fasting plasma glucose are all part of the metabolic syndrome associated to obesity and DM. Obesity, in particular android or visceral obesity, is independently associated with increased cardiovascular morbidity and mortality [48]. It is well known that adipose tissue synthesizes and secretes biologically active molecules (i.e. adiponectin, leptin, plasminogen activator inhibitor-1, tumor necrosis factor-α, and interleukin-6) that may affect cardiovascular risk factors probably through persistent low-grade inflammation and enhanced oxidant stress leading to a pro-thrombotic condition (for a recent review see [48]). Davì *et al*. recently tested this hypothesis in a population of apparently healthy women with visceral obesity. The results obtained clearly demonstrated that a low-grade inflammatory state may be the primary trigger of thromboxane-dependent platelet activation mediated, at least in part, through enhanced lipid peroxidation, a condition reverted by successful weight loss [49].

Oxidative stress

Although the underlying mechanisms are incompletely understood, it is currently recognized that oxidative stress due to chronic hyperglycemia may play an important role in the etiology of diabetic complications (Fig. 4). Hyperglycemia may induce ROS production directly via glucose metabolism and auto-oxidation and indirectly through the formation of AGE and their receptor binding. ROS, in turn, may activate other signaling molecules, such as PKC and NF-κB leading to transcription of redox-sensitive genes [50]. In addition to increased ROS production, plasma from patients whose

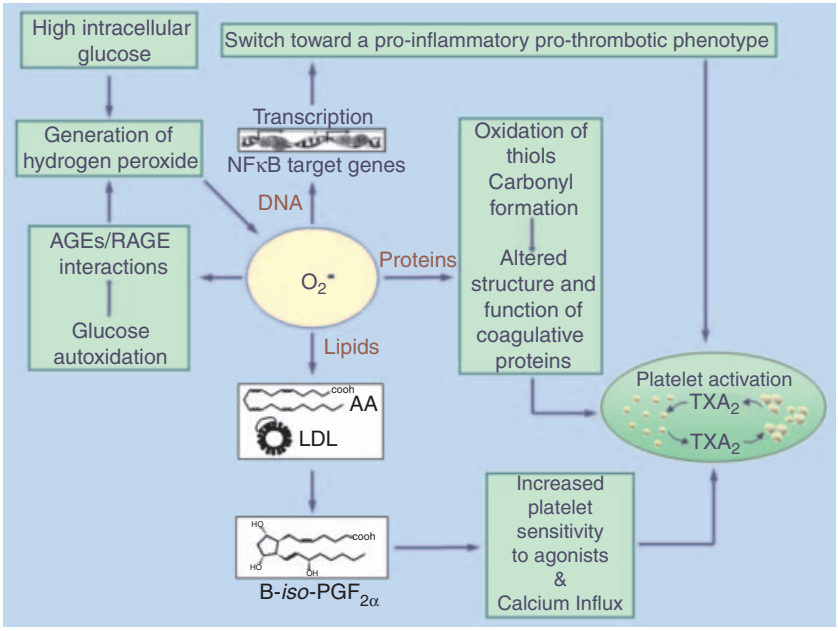

Fig. 4. Pathways of oxidative stress associated with diabetes mellitus: mechanisms of platelet activation. Hyperglycemia may induce ROS production directly via glucose metabolism and auto-oxidation and indirectly through the formation of advanced glycation end products (AGE) and their receptor (RAGE) binding. ROS, in turn, may exert their effects on: (1) lipids, leading to arachidonic acid (AA) and low-density lipoprotein (LDL) oxidation, resulting in the production of bioactive isoprostanes (i.e. 8-*iso*-prostaglandin-$F_{2\alpha}$-8-*iso*-$PGF_{2\alpha}$); (2) proteins through oxidation of thiols and carbonyl formation; (3) DNA, through activation of signaling molecules (i.e. nuclear transcription factor-κB – NF-κB) and subsequent transcription of genes encoding cytokines and adhesive proteins leading to a switch toward a prothrombotic phenotype of endothelial cells. All these phenomena may contribute to increased platelet sensitivity and activation of the AA pathway resulting in increased TXA_2 formation.

diabetes is poorly controlled has less antioxidant capacity [51] and contains increased levels of thiobarbituric acid-reactive substances (TBARS) and lipid hydroperoxides [52], and F_2-isoprostanes, such as 8-*iso*-prostaglandin (PG) $F_{2\alpha}$ [28], only partially reversible in association with improved glycemic control. 8-*iso*-$PGF_{2\alpha}$ is a nonenzymatic oxidation product of circulating LDL and arachidonic acid that is widely recognized as a reliable marker of lipid peroxidation both *in vitro* and *in vivo* [53,54]. 8-*iso*-$PGF_{2\alpha}$ induces vasoconstriction and may modify aspects of platelet function such as adhesive reactions and activation by low concentrations of other agonists (Fig. 4; [53–56]). These properties may be relevant to settings where platelet activation and enhanced free-radical formation coincide, such as in DM. The hypothesis that increased oxidant stress in T2DM could induce enhanced generation of 8-*iso*-$PGF_{2\alpha}$ and that this compound could, in turn, contribute to platelet activation is supported by the finding that 8-*iso*-$PGF_{2\alpha}$ formation correlated with the rate of TXA_2 biosynthesis in this setting [28]. Moreover, improvement of metabolic control in T2DM patients was accompanied by a significant reduction in the urinary excretion of 11-dehydro-TXB_2 (a stable metabolite of TXB_2). Thus, it was suggested that changes in the rate of arachidonate peroxidation to form biologically active iso-eicosanoids, such as 8-*iso*-$PGF_{2\alpha}$, may represent an important biochemical link between altered glycemic control, oxidant stress and platelet activation in T2DM [28].

Interestingly, enhanced lipid peroxidation and platelet activation represent early events in the development of T1DM in children and adolescents [57]. Subjects with newly diagnosed diabetes had significantly increased urinary excretion of both 8-*iso*-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 and higher plasma levels of a number of inflammatory markers. In some of these patients, oxidative stress and platelet activation were reduced after 1 year, coincident with a fall in the systemic levels of IL-6 and TNF-α. Thus, it appears that biochemical signals of oxidative stress and platelet activation can be appreciated early at the onset of diabetes mellitus and that their variable intensity is driven, at least in part, by IL-6 production and disease duration [57]. This finding is also consistent with the hypothesis that in children with T1DM the early increase in oxidative stress and platelet activation may be associated with inflammatory events that precede clinical manifestation of the disease. Once established, oxidative stress may sustain a vicious circle, because it has been shown that hydrogen peroxide induces the IL-6 promoter by activating NF-κB [58].

Furthermore, we have demonstrated that both lipid and protein oxidation are significantly elevated in patients with T2DM [59]. A potential mechanism linking lipid and protein oxidation has been recently suggested, in which oxidized polyunsaturated fatty acids are believed to form α,β-unsaturated aldehydes, that can attack lysyl side chains, contributing to the generation of stable protein carbonyl groups [60]. The strong association of F_2-isoprostane formation with both urinary 11-dehydro-TXB_2 excretion rate and plasma prothrombin fragment F1 + 2 levels suggested that lipid peroxidation can affect platelet as well as coagulative activation, thus contributing to a pro-thrombotic state in T2DM [59]. The capacity of oxidized lipids to activate platelets may constitute a

thrombotic trigger *per se*. However, it has to be remarked that oxidizing agents can down-regulate the activity and/or expression of thrombomodulin (TM) on the surface of endothelial cells [61]. Oxidation of methionine 388 in the TM molecule inactivates almost completely the anticoagulant function of the endothelial protein [62]. Moreover, oxidized lipids present on activated platelets can provide a better surface for the assembly and activation of prothrombinase complex. This may trigger a vicious cycle that, together with the oxidation-linked depression of the anticoagulant pathway, may predispose to a prothrombotic state in T2DM [59].

Endothelial dysfunction

Damage to the endothelium plays an important role in the development and progression of atherosclerosis [19]. Endothelium produces both dilating factors (e.g. NO, PGI_2) and constricting factors (endothelin, superoxide anion, angiotensin II and TX). In diabetes, the balance between dilating and constricting substances is changed and shifted towards constriction [63].

In particular, hyperglycemia inhibits production of NO by blocking eNOS activation and increasing the production of ROS [64]. These, in turn, are responsible for the activation of the transcription factors NF-κB and activator protein-1 (AP-1), which regulates the expression of the genes encoding a number of proinflammatory mediators found in atheroma, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [65]. The expression of proinflammatory cytokines and other mediators, including adhesion molecules, suggests that inflammatory processes may contribute to vascular disease in DM [19]. For example, plasma TNF-α concentration is related to insulin resistance, and it falls with dietary restriction and weight loss [66], whereas IL-6 and C-reactive protein (CRP) are known to be increased in T2DM [67]. All of them may induce a phenotypic change of endothelial cells and/or monocytes, leading to increased production of tissue factor (TF), the major procoagulant found in atherosclerotic plaques along with alterations in soluble coagulation and fibrinolytic factors [57,59,68].

Disturbances in the endothelial function and coagulation pathway may lead to initial platelet activation, adhesion and subsequent platelet aggregate formation, while the intrinsic altered platelet metabolism and changes in intraplatelet signaling pathways contribute to the overall increased platelet hyperactivity in T2DM [26]. However, as discussed above, platelet hyperactivity may not be caused solely by the onset of vascular disease. Indeed, increasing evidence is accumulating, suggesting that platelets have inflammatory actions and are a rich source of chemokines (e.g. platelet factor-4, RANTES) [69,70] and inflammatory cytokines (e.g. IL-1β, CD40L) [71,72] that are released within seconds of platelet activation, thus opening new perspectives on the contribution of platelets in inflammation and atherogenesis [73]. Accumulation of activated platelets at sites of vascular lesions, in fact, might result in high concentrations of platelet-derived substances, which in turn might support chemotaxis and recruitment of monocytes into the subendothelium at an early stage in atherogenesis [19]. In this respect, it has been suggested that adhering platelets to endothelial cells might facilitate attachment of monocytes to the endothelial layers [74] and that RANTES released by platelets and bound to the surface of inflamed, cytokine-activated endothelium supports monocyte arrest in flow [74]. Moreover, it has been demonstrated that P-selectin on the surface of activated platelets synergizes with platelet activating factor (PAF) and/or RANTES to induce the synthesis of IL-8, MCP-1 and TNF-α by monocytes, as well as tissue factor expression [73] that, together with thrombin generated during platelet activation, contributes to the activation of the coagulation cascade aforementioned.

Effects of glycemic control on platelet function

While the number of studies documenting platelet abnormalities in diabetics is large, the number documenting the response to hypoglycemic drugs is rather small [22,75–77]. Beneficial effects of insulin treatment on platelet function *in vivo* have been related to improved metabolic control, rather than to direct platelet stabilizing effects [78]. Tight metabolic control by intensive insulin treatment reduced *in vivo* platelet activation, as measured by the urinary excretion of 11-dehydro-TXB_2 [22]. Furthermore, a reduction of *in vivo* platelet activation after metabolic improvement obtained by frequent reassessment of sulfonylurea therapy was observed in a subsequent study [77]. In this respect, we should also consider that the demonstration of a benefit of metabolic control to clinical vascular disease has proved difficult to achieve in all studies of T2DM. This is due to the multifactorial nature of complications and the long duration of disease required before microvascular complications became apparent, although improvement in parameters of hyperglycemia is associated with an improvement in morbidity from large vessel disease. In T2DM, atherosclerosis coexists in the majority of patients and often predates the clinical diagnosis of diabetes. The presence of atherosclerosis, which often determines the ultimate fate of the patient, may further increase the level of oxidative stress and lipid peroxidation, amplifying the effects of hyperglycemia and potentiating vascular damage. Improved metabolic control is associated with a significant reduction in both lipid peroxidation and platelet activation *in vivo* (assessed by determination of the urinary excretion rates of 8-*iso*-$PGF_{2\alpha}$ and 11-dehydro-TXB_2), suggesting that enhanced lipid peroxidation may provide an important biochemical link between impaired glycemic control and persistent platelet activation [28]. Therefore, in diabetes, where increased glycation and oxidation are fundamental in the pathogenesis of diabetic vascular disease, antidiabetic agents with antioxidant activities may have an enhanced therapeutic role [79].

Antiplatelet drugs: results from clinical trials

Several antiplatelet strategies are currently being used or evaluated in an attempt to reduce the thrombotic complications

Table 1 Urinary excretion rates of 11-dehydro-TXB_2 in diabetic patients compared with healthy subjects

Clinical Setting	Patients	Controls	Reference
Type 1 diabetes mellitus at onset ($n = 23$ vs. 23)	1032.2 ± 460.3*	284.7 ± 56.4*	[57]
Type 2 diabetes mellitus ($n = 12$ vs. 12)	1274 ± 223*	150 – 450*†	[31]
Type 2 diabetes mellitus ($n = 72$ vs. 72)	1084 ± 1082*	208 ± 92*	[59]
Type 2 diabetes mellitus ($n = 62$ vs. 62)	1103 ± 1068*	415 ± 244*	[28]
Type 1 diabetes mellitus ($n = 23$ vs. 23)	601 ± 219*	304 ± 154*	[28]
Type 2 diabetes mellitus with peripheral artery disease ($n = 16$ vs. 16)	67.0 ± 29.0§	28.2 ± 7.9§	[12]
Type 2 diabetes mellitus ($n = 50$ vs. 32)	5.94 ± 3.68‡	1.50 ± 0.79‡	[22]

Values are expressed as mean ± SD; *pg mg^{-1} creatinine; †value expressed as range; §ng h^{-1}; ‡nmol die^{-1}.

of the atherosclerotic process. Diabetic patients present persistent thromboxane-dependent platelet activation (Table 1). Thus, low-dose aspirin represents the antiplatelet drug of choice for prevention of vascular complications in diabetes [80–82].

As discussed in the introductory section, apparently healthy T2DM patients have a risk of death from cardiovascular causes that is as high as the incidence in non-diabetic subjects who already sustained a myocardial infarction [2]. Furthermore, even subjects with impaired glucose tolerance are at a high cardiovascular risk and/or exhibit several cardiovascular risk factors. Based on the results from the US Physicians' Health Study [83] it can be reasoned that generally all subjects with T2DM are eligible for aspirin treatment, as one could deduce from a high prevalence of established cardiovascular disease in such patients. The Early Treatment Diabetic Retinopathy Study (ETDRS) is the largest single study of aspirin prophylaxis in patients with DM ($n = 3711$; 49% with a history of cardiovascular disease) [84]. Although the study was designed to examine the potential impact of antiplatelet therapy on the progression of diabetic retinopathy, it also provided the opportunity to evaluate the effects of long-term aspirin administration on cardiovascular complications. The results of a 5-year follow up indicated a statistically significant 28% reduction in MI, a non-significant 16% increase in stroke, and a significant 18% reduction in important vascular events, similar to those observed in the US Physicians' Health Study [83].

A comprehensive analysis of the effects of aspirin as an antiplatelet agent is reported in the classic papers of the Antiplatelet Trialists' (APT) Collaboration [85,86]. In these meta-analyses, the results of 145 randomized trials on antiplatelet therapy, comprising more than 100 000 patients, were analyzed. In particular, a subgroup analysis on approximately 1000 major cardiovascular events in more than 4500 patients with diabetes revealed that the benefit of antiplatelet therapy was similar to that in patients without diabetes who were enrolled in the primary and secondary-prevention trials [85,87]. Moreover, from the US Physicians' Health Study and the HOT (Hypertension Optimal Treatment) trial we learned that aspirin treatment might even be more beneficial in diabetic patients than in high-risk non-diabetic subjects [83,88]. Thus, it has been advocated to prescribe aspirin for diabetic patients who are at a high risk of cardiovascular events, even those without any manifest cardiovascular abnormalities [84,85,89]. Recommendations of the American Diabetes Association, based on the meta-analysis of all primary and secondary prevention trials with use of aspirin, support the view that aspirin therapy should be prescribed as a secondary prevention strategy in diabetic men and women with evidence of large vessel disease and as a primary prevention strategy in high-risk men and women with DM, at the age > 30 years [81,82].

A valid alternative for aspirin-intolerant patients is provided by ticlopidine [80]. The Ticlopidine Microangiopathy of Diabetes (TIMAD) Study, a randomized, double-blind, placebo-controlled trial, was designed to assess the effects of ticlopidine in reducing the progression of non-proliferative diabetic retinopathy in 435 patients followed up for 3 years [90]. The results of this study demonstrated that the progression of overall retinopathy was significantly less severe in the ticlopidine group as a result of its antithrombotic action. Despite of its beneficial effects, the hematological side-effects of ticlopidine limit wider clinical application. In an attempt to cope with the majority of the above disadvantages, the improved new generation drug, clopidogrel, was released in recent years. In the CAPRIE study, clopidogrel 75 mg daily was found to be more effective than aspirin (325 mg daily) in reducing the elevated risk for recurrent ischemic events in diabetic patients with a history of thrombosis [91].

Other antiplatelet drugs such as indobufen, dipyridamole or picotamide have been investigated over the years in clinical practice [92–95]. A randomized, placebo-controlled, short-term trial of aspirin and dipyridamole for overt T2DM nephropathy demonstrated that a combination of the two drugs significantly reduces proteinuria [95]. In patients with peripheral artery disease and diabetes picotamide reduced the relative risk of vascular events [93]. In patients with T2DM, this compound also reduced urinary albumin excretion [94].

Finally, we have to consider that attention to appropriate medical management of the diabetic patient with coronary artery disease will have great impact on long-term outcome in this high-risk subgroup. Treatment with abciximab (a chimeric monoclonal antibody against the glycoprotein complex GpIIb/IIIa) reduced the number of deaths and myocardial infarction as well as the number of emergency revascularization procedures both in diabetic and non-diabetic patients, as assessed in the EPILOG study [96]. More recently, the addition to standard antithrombotic therapy of the non-peptide platelet

GpIIb/IIIa receptor antagonists has been proposed for DM patients presenting specifically with unstable angina/non-ST-elevation MI [97]. No clear benefit of these drugs over abciximab has been demonstrated; thus, the ACC/AHA 2002 guidelines for the management of acute coronary syndromes recommend the use of abciximab in diabetic patients undergoing stent implantation [97].

Conclusions

The aberrant metabolic state that accompanies diabetes is responsible for abnormalities in endothelial and platelet function, which may contribute to the cellular events that cause atherosclerosis and subsequently increase the risk of the adverse cardiovascular events that occur in patients with T2DM and atherosclerosis. At present, it is still under debate whether enhanced platelet activation is merely a consequence of more prevalent atherosclerotic lesions (relevant to a risk of thrombosis complicating plaque rupture) or reflects the influence of the accompanying metabolic disturbances on platelet biochemistry and function. Many *in vitro* and *in vivo* studies point to a role of platelets in the early phases of the disease. Of particular relevance are the findings that platelets represent a site of insulin resistance, and that the insulin resistance syndrome of visceral obesity (a determinant for the development of T2DM) is accompanied by enhanced platelet activation mediated, at least in part, through enhanced ROS generation and lipid peroxidation. Oxidative stress-induced activation of stress-sensitive signaling pathways might therefore represent a common biochemical basis for the molecular abnormalities leading to the pro-inflammatory, pro-thrombotic state characteristic of T2DM. Better knowledge of the role of platelets in the development and progression of diabetic vascular lesions might allow the discovery of novel pharmacological targets. At present, aspirin administration is recommended as a secondary prevention strategy in diabetic men and women with evidence of large vessel disease, and as a primary prevention strategy in high-risk men and women with DM, at the age > 30 years. New therapeutic strategies, such as antioxidant agents, or statin treatment [98] in the prediabetic phase in patients with the metabolic syndrome can modify the interplay between platelets and vascular cells thus helping to prevent, and not only reverse or delay, the onset of cardiovascular disease in this setting.

References

1 Luscher TF, Creager MA, Beckman JA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences and medical therapy. *Circulation* 2003; **108**: 1655–61.

2 Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229–34.

3 Beckman JA, Creager M, Libby P. Diabetes and atherosclerosis. epidemiology, pathophysiology, and management. *J Am Med Assoc* 2002; **287**: 2570–81.

4 Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes* 1999; **48**: 937–42.

5 Cooper ME, Bonnet F, Oldfield M, Jandeleit-Dahm K. Mechanisms of diabetic vasculopathy: an overview. *Am J Hypertens* 2001; **14**: 475–86.

6 Nishikawa T, Du Edelstein DXL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; **404**: 787–90.

7 Pieper GM, Riazul H. Activation of nuclear factor-kappaB in cultured endothelial cells by increased glucose concentration: prevention by calphostin C. *J Cardiovasc Pharmacol* 1997; **30**: 528–32.

8 Yerneni KK, Bai W, Khan BV, Medford RM, Natarajan R. Hyperglycemia-induced activation of nuclear transcription factor kappaB in vascular smooth muscle cells. *Diabetes* 1999; **48**: 855–64.

9 Sharma K, Jin Y, Guo J, Ziyadeh FN. Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes* 1996; **45**: 522–30.

10 Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, Nguyen HV, Aiello LM, Ferrara N, King GL. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994; **331**: 1480–7.

11 Coppola L, Verrazzo G, La Marca C, Ziccardi P, Grassia A, Tirelli A, Giugliano D. Effect of insulin on blood rheology in non-diabetic subjects and in patients with type 2 diabetes mellitus. *Diabet Med* 1997; **14**: 959–63.

12 Davì G, Gresele P, Violi F, Basili S, Catalano M, Giammarresi C, Volpato R, Nenci GG, Ciabattoni G, Patrono C. Diabetes mellitus, hypercholesterolemia, and hypertension but not vascular disease per se are associated with persistent platelet activation *in vivo*. Evidence derived from the study of peripheral arterial disease. *Circulation* 1997; **96**: 69–75.

13 Sobol AB, Watala C. The role of platelets in diabetes-related vascular complications. *Diabetes Res Clin Pract* 2000; **50**: 1–16.

14 Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002; **23**: 599–622.

15 Natarajan R, Nadler JL. Lipoxygenases and lipid signaling in vascular cells in diabetes. *Front Biosci* 2003; **8**: s783–95.

16 Creager MA, Luscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease. pathophysiology, clinical consequences, and medical therapy: part I. *Circulation* 2003; **108**: 1527–32.

17 Colwell JA, Winocour PD, Halushka PV. Do platelets have anything to do with diabetic microvascular disease? *Diabetes* 1983; **32** (Suppl. 2): 14–9.

18 Gerrard JM, Stuart MJ, Rao GHR, Steffes MW, Mauer SM, Brown DM, White JG. Alteration in the balance of prostaglandin and thromboxane synthesis in diabetic rats. *J Lab Clin Med* 1980; **95**: 950–8.

19 Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; **340**: 115–26.

20 Bridges JM, Dalby AM, Millar JHD, Weaver JA. An effect of D-glucose on platelet stickiness. *Lancet* 1965; **1**: 75–7.

21 D'Angelo A, Micossi P, Mannucci PM, Garimberti B, Franchi F, Pozza G. Increased production of platelet thromboxane B_2 in non-insulin-dependent diabetes: relationship to vascular complications. *Eur J Clin Invest* 1984; **14**: 83–6.

22 Davì G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C. Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N Engl J Med* 1990; **322**: 1769–74.

23 Oskarsson HJ, Hofmeyer TG. Platelets from diabetic patients with diabetes mellitus have impaired ability to mediate vasodilation. *J Am Coll Cardiol* 1996; **27**: 1464–70.

24 Davì G, Rini GB, Averna M, Novo S, Di Fede G, Pinto A, Notarbartolo A, Strano A. Thromboxane B_2 formation and platelet sensitivity to prostacyclin in insulin-dependent and insulin-independent diabetics. *Thromb Res* 1982; **26**: 359–70.

25 Modesti PA, Fortini A, Gensini GF, Vanni D, Prisco D, Abbate R. Human prostacyclin platelet receptors in diabetes mellitus. *Thromb Res* 1991; **63**: 541–8.

26 Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. *Diabetes Care* 2001; **24**: 1476–85.

27 Triolo G, Davì G, Giardina E, Cardella F, Meli F, La Grutta A, Strano A, Bompiani GD. Circulating immune complexes and platelet thromboxane synthesis in patients with insulin-dependent (type I) diabetes mellitus. *Diabetes* 1984; **33**: 728–31.

28 Davì G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C. *In vivo* formation of 8-iso-prostaglandin F2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 1999; **99**: 224–9.

29 The DECODE Study Group. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. *Lancet* 1999; **354**: 617–21.

30 Temelkova-Kurktschiev TS, Koehler C, Henkel E, Leonhardt W, Fuecker K, Hanefeld M. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. *Diabetes Care* 2000; **23**: 1830–4.

31 Gresele P, Guglielmini G, De Angelis M, Ciferri S, Ciofetta M, Falcinelli E, Lalli C, Ciabattoni G, Davì G, Bolli GB. Acute, short-term hyperglycemia enhances shear stress-induced platelet activation in patients with type II diabetes mellitus. *J Am Coll Cardiol* 2003; **41**: 1013–20.

32 Assert R, Scherk G, Bumbure A, Pirags V, Schatz H, Pfeiffer AF. Regulation of protein kinase C by short term hyperglycaemia in human platelets *in vivo* and *in vitro*. *Diabetologia* 2001; **44**: 188–95.

33 Li Y, Woo W, Bose R. Platelet hyperactivity and abnormal Ca(2+) homeostasis in diabetes mellitus. *Am J Pysiol Heart Circ Pysiol* 2001; **280**: H1480–9.

34 Winocour PD. Platelet abnormalities in diabetes mellitus. *Diabetes* 1992; **41**: 26–31.

35 Tschoepe D, Driesch E, Schwippert B, Nieuwenhuis HK, Gries FA. Exposure of adhesion molecules on activated platelets in patients with newly diagnosed IDDM is not normalized by near-normoglycaemia. *Diabetes* 1995; **44**: 890–4.

36 Watala C, Gwozdzinski K, Pluskota E, Pietrucha T, Walkowiak B, Trojanowski Z, Cierniewski CS. Diabetes mellitus alters the effect of peptide and protein ligands on membrane fluidity of blood platelets. *Thromb Haemost* 1996; **75**: 147–53.

37 Watanabe J, Wohltmann HJ, Klein RL, Colwell JA, Lopes-Virella MF. Enhancement of platelet aggregation by low-density lipoproteins from IDDM patients. *Diabetes* 1988; **37**: 1652–7.

38 Kobayashi K, Watanabe J, Umeda F, Nawata H. Glycation accelerates the oxidation of low density lipoprotein by copper ions. *Endocr J* 1995; **42**: 461–5.

39 Millican SA, Schultz D, Bagga M, Coussons PJ, Muller K, Hunt JV. Glucose-modified low density lipoprotein enhances human monocyte chemotaxis. *Free Rad Res* 1998; **28**: 533–42.

40 Ferretti G, Rabini RA, Bacchetti T, Vignini A, Salvolini E, Ravaglia F, Curatola G, Mazzanti L. Glycated low density lipoproteins modify platelet properties: a compositional and functional study. *J Clin Endocrinol Metab* 2002; **87**: 2180–4.

41 Rosenson RS, Lowe GDO. Effects of lipids and lipoproteins on thrombosis and rheology. *Atherosclerosis* 1998; **140**: 271–80.

42 Byrne CD. Triglyceride-rich lipoproteins: are links with atherosclerosis mediated by a procoagulant and proinflammatory phenotype? *Atherosclerosis* 1999; **145**: 1–15.

43 Gopaul NK, Nourooz-Zadeh J, Mallet AI, Anggard EE. Formation of PGF_2-isoprostanes during the oxidative modification of low density lipoprotein. *Biochem Biophys Res Commun* 1994; **200**: 338–43.

44 Falcon C, Pfliegler G, Deckmyn H, Vermylen J. The platelet insulin receptor: detection, partial characterization, and search for a function. *Biochem Biophys Res Commun* 1988; **157**: 1190–6.

45 Abrahm DR, Hollingsworth PJ, Smith CB, Jim L, Zucker LB, Sobotka PA, Vinik AI. Decreased alpha 2-adrenergic receptors on platelet membranes from diabetic patients with autonomic neuropathy and orthostatic hypotension. *J Clin Endocrinol Metab* 1986; **63**: 906–12.

46 Udvardy M, Pfliegler G, Rak K. Platelet insulin receptor determination in non-insulin dependent diabetes mellitus. *Experientia* 1985; **41**: 422–3.

47 Trovati M, Anfossi G. Influence of insulin and of insulin resistance on platelet and vascular smooth muscle cell function. *J Diabetes Complications* 2002; **16**: 35–40.

48 Sowers JR. Obesity as a cardiovascular risk factor. *Am J Med* 2003; **115**: 37S–41S.

49 Davì G, Guagnano MT, Ciabattoni G, Basili S, Falco A, Marinopiccoli M, Nutini M, Sensi S, Patrono C. Platelet activation in obese women: role of inflammation and oxidant stress. *JAMA* 2002; **288**: 2008–14.

50 Ha H, Lee HB. Oxidative stress in diabetic nephropathy: basic and clinical information. *Curr Diab Rep* 2001; **1**: 282–7.

51 Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes* 1994; **43**: 1010–4.

52 Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM. *Diabetes* 1995; **44**: 1054–8.

53 Patrono C, FitzGerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2309–15.

54 Davì G, Falco A, Patrono C. Determinants of F_2-isoprostane biosynthesis and inhibition in man. *Chem Phys Lipids* 2004; **128**: 149–63.

55 Praticò D, Smyth EM, Violi F, FitzGerald GA. Local amplification of platelet function by 8-iso-PGF_{2a} is not mediated by thromboxane receptor isoforms. *J Biol Chem* 1996; **271**: 14916–24.

56 Minuz P, Andrioli G, Degan M, Gaino S, Ortolani R, Tommasoli R, Zuliani V, Lechi A, Lechi C. The F_{α}-isoprostane 8-epi prostaglandin $F_{2\alpha}$ increases platelet adhesion and reduces the antiadhesive and antiaggregatory effects of NO. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1248–56.

57 Davì G, Chiarelli F, Santilli F, Pomilio M, Vigneri S, Falco A, Basili S, Ciabattoni G, Patrono C. Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus: role of interleukin-6 and disease duration. *Circulation* 2003; **107**: 3199–203.

58 Zhang J, Johnston G, Stebler B, Keller ET. Hydrogen peroxide activates NFκB and the interleukin-6 promoter through NFκB-inducing kinase. *Antioxidant Redox Signal* 2001; **3**: 493–504.

59 De Cristofaro R, Rocca B, Vitacolonna E, Falco A, Marchesani P, Ciabattoni G, Landolfi R, Patrono C, Davì G. Lipid and protein oxidation contribute to a prothrombotic state in patients with type 2 diabetes mellitus. *J Thromb Haemost* 2003; **1**: 250–6.

60 Hanne HF, Refsgaard LT, Stadtman ER. Modifications of proteins by polyunsaturated fatty acid peroxidation products. *Proc Natl Acad Sci USA* 2000; **97**: 611–6.

61 Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest* 1991; **88**: 1906–14.

62 Glaser BB, Morser J, Clarke JH, Blasko E, McLean K, Kuhn I, Chang RJ, Lin JH, Vilander L, Andrews WH, Light DR. Oxidation of a specific methionine in thrombomodulin by activated neutrophil products blocks cofactor activity. *J Clin Invest* 1992; **90**: 2565–73.

63 Martina V, Bruno GA, Trucco F, Zumpano E, Tagliabue M, Di Bisceglie Pescarmona G. Platelet cNOS activity is reduced in patients with IDDM and NIDDM. *Thromb Haemost* 1998; **79**: 520–2.

64 De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. *Br J Pharmacol* 2000; **130**: 963–74.
65 Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by. UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 2001; **17**: 189–212.
66 Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-α in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* 1998; **83**: 2907–10.
67 Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-Reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *J Am Med Assoc* 2001; **286**: 327–34.
68 Kario K, Matsuo T, Kobayashi H, Matsuo M, Sakata T, Miyata T. Activation of tissue factor-induced coagulation and endothelial cell dysfunction in non-insulin-dependent diabetic patients with microalbuminuria. *Arterioscler Thromb Vasc Biol* 1995; **15**: 1114–20.
69 Brandt E, Ludwig A, Petersen F, Flad HD. Platelet-derived CXC chemokines: old players in new games. *Immunol Rev* 2000; **177**: 204–16.
70 von Hundelshausen P, Weber KS, Huo Y, Proudfoot AE, Nelson PJ, Ley K, Weber C. RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. *Circulation* 2001; **103**: 1772–7.
71 Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zimmerman GA, Weyrich AS. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J Cell Biol* 2001; **154**: 485–90.
72 Cipollone F, Mezzetti A, Porreca E, Di Febbo C, Nutini M, Fazia M, Falco A, Cuccurullo F, Davi G. Association between enhanced soluble CD40L and prothrombotic state in hypercholesterolemia: effects of statin therapy. *Circulation* 2002; **106**: 399–402.
73 Weyrich AS, Lindemann S, Zimmerman GA. The evolving role of platelets in inflammation. *J Thromb Haemost* 2003; **1**: 1897–905.
74 Lindmark E, Tenno T, Siegbahn A. Role of platelet P-selectin and CD40 ligand in the induction of monocytic tissue factor expression. *Arterioscler Thromb Vasc Biol* 2000; **20**: 2322–8.
75 Mandal S, Sarode R, Dash S, Dash RJ. Hyperaggregation of platelets detected by whole blood platelet aggregometry in newly diagnosed noninsulin-dependent diabetes mellitus. *Am J Clin Pathol* 1993; **100**: 103–7.
76 Kopp HP, Hopmeier P, Schernthaner G. Concentrations of circulating P-selectin are increased in patients with newly diagnosed insulin-dependent diabetes mellitus. *Exp Clin Endocrinol Diabetes* 1998; **106**: 41–4.
77 Davì G, Belvedere M, Vigneri S, Catalano I, Giammarresi C, Roccaforte S, Consoli A, Mezzetti A. Influence of metabolic control on thromboxane biosynthesis and plasma plasminogen activator inhibitor type-1 in non-insulin-dependent diabetes mellitus. *Thromb Haemost* 1996; **76**: 34–7.
78 Yngen M, Li N, Hjemdahl P, Wallen NH. Insulin enhances platelet activation *in vitro*. *Thromb Res* 2001; **104**: 85–91.
79 Jennings PE. The potential of gliclazide, a sulphonylurea to influence the oxidative processes within the pathogenesis of diabetic vascular disease. *Adv Exp Med Biol* 1994; **366**: 313–24.
80 Patrono C, Davì G. Antiplatelet agents in the prevention of diabetic vascular complications. *Diabet Metabol Rev* 1993; **3**: 177–88.
81 American Diabetes Association. Aspirin therapy in diabetes: position statement. *Diabetes Care* 2002; **2**: S78–9.
82 Eidelman RS, Hebert PR, Weisman SM, Hennekens CH. An update on aspirin in the primary prevention of cardiovascular disease. *Arch Intern Med* 2003; **163**: 2006–10.
83 Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. *New Engl J Med* 1989; **321**: 129–35.
84 ETDRS Investigators. Aspirin effects on mortality and morbidity in patients with diabetes mellitus. *J Am Med Assoc* 1992; **268**: 1292–300.
85 Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy-I: prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *Br Med J* 1994; **308**: 81–106.
86 Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high-risk patients. *Br Med J* 2002; **324**: 71–86.
87 Patrono C. Aspirin as an antiplatelet drug. *N Engl J Med* 1994; **330**: 1287–94.
88 Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, Menard J, Rahn KH, Wedel H, Westerling S. Effects of intensive blood pressure lowering and low-dose aspirin in patients with hypertension: principal results of the hypertension optimal treatment (HOT) randomised trial: HOT Study Group. *Lancet* 1998; **351**: 1755–62.
89 Colwell JA. Aspirin therapy in diabetes. *Diabetes Care* 1997; **20**: 1767–71.
90 TIMAD Study Group. Ticlopidine treatment reduces the progression of nonproliferative diabetic retinopathy. *Arch Ophthalmol* 1990; **108**: 1577–83.
91 Bhatt DL, Marso SP, Hirsch AT, Ringleb PA, Hacke W, Topol EJ. Amplified benefit of clopidogrel versus aspirin in patients with diabetes mellitus. *Am J Cardiol* 2002; **90**: 625–8.
92 Davì G, Patrono C, Catalano I, Custro N, Giammarresi C, Ganci A, Cosentino F, Notarbartolo A. Inhibition of thromboxane biosynthesis and platelet function by indobufen in type II diabetes mellitus. *Arterioscler Thromb* 1993; **13**: 1346–9.
93 Milani M, Longoni A, Maderna M. Effect of picotamide, an antiplatelet agent, on cardiovascular events in 438 claudicant patients with diabetes: a retrospective analysis of the ADEP study. *Br J Pharmacol* 1996; **46**: 782–5.
94 Giustina A, Perini P, Desenzani P, Bossoni S, Ianniello P, Milani M, Davì G, Romanelli G. Long-term treatment with the dual antithromboxane agent picotamide decreases microalbuminuria in normotensive type 2 diabetic patients. *Diabetes* 1998; **47**: 423–30.
95 Khajehdehi P, Roozbeh J, Mostafavi H. A comparative randomized and placebo-controlled short term trial of aspirin and dipyridamole for overt type 2 diabetes nephropathy. *Scand J Urol Nephrol* 2002; **36**: 145–8.
96 Kleiman NS, Lincoff AM, Kereiakes DJ, Miller DP, Aguirre FV, Anderson KM, Weisman HF, Califf RM, Topol EJ. Diabetes mellitus, glycoprotein IIb/IIIa blockade, and heparin: evidence for a complex interaction in a Multicenter Trial. EPILOG Investigators. *Circulation* 1998; **97**: 1912–20.
97 Meier-Ewert HK, Nesto RW. Targeting the use of glycoprotein IIb/IIIa antagonists. The diabetic patient. *Rev Cardiovasc Med* 2002; **3**: S20–7.
98 Brandle M, Davidson MB, Schriger DL, Lorber B, Herman WH. Cost effectiveness of statin therapy for the primary prevention of major coronary events in individuals with type 2 diabetes. *Diabetes Care* 2003; **26**: 1796–801.