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Review

Coagulation activation in diabetes mellitus: the role of hyperglycaemia and therapeutic prospects

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Summary. Numerous studies have shown that coagulation abnormalities occur in the course of diabetes mellitus, resulting in a state of thrombophilia. These observations are supported by epidemiological studies which demonstrate that thromboembolic events are more likely to occur in diabetic patients. The coagulation abnormalities observed in diabetic patients seem to be caused by the hyperglycaemia, which also constitutes the distinguishing feature of this disease. These data are also supported by in vitro studies which demonstrate how glucose can directly determine alterations in the coagulation system. The abnormalities observed involve all stages of coagulation, affecting both thrombus formation and its inhibition, fibrinolysis, platelet and endothelial function. The final result is an imbalance between thrombus formation and dissolution, favouring the former. Hyperglycaemia probably determines the onset of these abnormalities through three mechanisms which are, respectively, non-enzymatic glycation, the development of increased oxidative stress and a decrease in the levels of heparan sulphate. The first seems to affect the functionality of key molecules of coagulation in a negative sense. Oxidative stress constitutes an important pro-thrombotic stimulus, while the decrease in heparan sulphate determines a reduction in antithrombotic defenses. Good metabolic control could play a key role in controlling the coagulation irregularities in diabetes. However, considering the difficulties in achieving such an objective, it is possible that the use of drugs may represent a valid alternative. In fact, several drugs exist which are of potential interest. It is, however, necessary to perform long-term studies which demonstrate unequivocably that by controlling the coagulation abnormalities in diabetic patients, prolongation of life is possible.

Key words: Coagulation, diabetes mellitus, glycation, oxidative stress, heparan sulphate.

Patients suffering from diabetes mellitus have a high probability of developing acute cardiovascular disease, in particular myocardial infarct and cerebrovascular stroke [1]. This type of complication constitutes the cause of death in 80% of patients with Type 2 (non-insulin-dependent) diabetes [1]. The development of cardiovascular disease is clearly linked with the onset of a thrombotic event: this is supported by numerous observations which report that in patients at risk for the development of cardiovascular events, a marked thrombophilia is present [2, 3]. Therefore in this regard, diabetic patients must be considered as a high risk group. However, diabetes per se is known to constitute an additional risk of developing cardiovascular disease [4]. Therefore it is reasonable to consider which is primarily responsible for the increased risk of thrombosis. Since hyperglycaemia is the distinguishing feature of diabetes, the hypothesis that it may constitute a key factor of hypercoagulability is suggestive. The aim of this work is to demonstrate the plausibility of this hypothesis and to examine the possible mechanisms by which hyperglycaemia could lead to the development of thrombophilia, with the consequent therapeutic implications.

Evidence of thrombophilia in diabetes

Good markers of thrombin activation are currently available which make it possible to show the existence of thrombophilia. Two very reliable markers are fibrinopeptide A (FPA) and the prothrombin fragment 1 + 2 (F1 + 2). These represent respectively, the amount of fibrinogen which is transformed into fibrin [5] and that of thrombin released in the circulation [6]. An increase of both FPA [7] and F1 + 2 [8] has been reported in diabetic patients, demonstrating the existence of a true thrombophilic diathesis. In the case of FPA, the increase is, moreover, directly correlated with hyperglycaemia, both in diabetic patients [9] and normal subjects in whom a hyper-

Table 1. The observed effect of hyperglycaemia in diabetic patients on haemostatic factors

Factor	Results	References
Fibrinogen	Increased plasma levels	13, 14
VII	Increased plasma levels	16
VIII	Increased plasma levels	19
Von Willebrand	Increased plasma levels	19
X	Increased antigenic levels	18
	Reduced activation	18

glycaemic state has been artificially produced [10]. These data agree with the evidence of an increased fibrinogen turnover induced by the hyperglycaemia [11].

Coagulation factors

The thrombin activation markers mentioned above represent the dynamic expression of the coagulation activation and thus certainly offer more information than a study of the individual factors which were widely investigated in diabetology, as summarized in Table 1.

The most interesting coagulation factor is undoubtedly fibrinogen. Numerous epidemiological studies have concurred in recognizing it as having an important predictive value as a marker of cardiovascular risk [12]. Studies in diabetic patients seem to agree with these results [13, 14].

Epidemiological studies have reported that high levels of factor VII are associated with a high mortality rate for cardiovascular events [15]. Increases in circulating factor VII have also been reported in diabetes [16]. In normal subjects, a direct correlation has been reported between levels of factor VII and fasting glycaemia [17]. In agreement with this is the demonstration that glycaemia levels can directly affect the concentrations of factor VII in both diabetic patients and normal subjects [16].

The antigenic levels of factor X are also increased in diabetes [18]. The activation of this factor is however reduced by the induction of hyperglycaemia, which has been observed both in diabetic patients and in normal subjects [18]. This does not, however, result in reduced coagulation activation, which on the contrary, increases [18]. During hyperglycaemia, there is a simultaneous reduction in the functionality of antithrombin III (ATIII) which is the natural inhibitor of factor X. Thus the ratio between activated X and ATIII changes in favour of activated X, with the consequent onset of a state of hypercoagulability [18].

Coagulation inhibitors

Since the action of thrombin is the result of a balance between the pro-coagulant cascade and the action of the various inhibitors on it or on its production, it is reasonable to assume that these inhibitors may also play an important role in the genesis of thrombophilia in diabetes.

One of the most studied inhibitors is ATIII. A decrease in the biological activity of this molecule in the presence of a normal antigenic concentration has been found in diabetic patients [20]. In this case as well, hyperglycaemia seems capable of directly affecting the activity of the molecule, in both diabetic patients and in normal subjects [21, 22]. Confirming this observation, good metabolic control can increase the activity of the molecule [23, 24]. The effect of glycaemia on ATIII may have an important prothrombotic impact. In fact, its decreased biological activity results in a reduced thrombin-antithrombin complex formation with consequent hyperactivity of the thrombin [25]. Similar to ATIII, a reduction in biological activity, directly affected by metabolic control, has also been reported for heparin co-factor II, another plasma protease which inhibits thrombin [26].

High levels of alpha-2-macroglobulin have been observed in diabetes [27]. Hyperglycaemia leads to an increase in this molecule, in both diabetic patients and normal subjects [28, 29]. Protein C is another important coagulation inhibitor. A decrease in protein C has been reported in diabetes [30]. This decrease is directly caused by hyperglycaemia, and affects the entire molecule, involving both the biological activity of the protein and its antigenic concentration [31].

Protein S is an inhibitor that acts as a cofactor of activated protein C. Protein S circulates in plasma in two forms: one free and the other complexed with a fraction of the complement known as C4b-binding protein [32]. The biologically active form is the free one. A significant reduction of the latter has been reported in diabetes, due to an increase in the levels of C4b-binding protein which correlates with metabolic control [33].

Platelets

The platelet alterations that occur in diabetes have been extensively studied. An increased platelet aggregation has been reported in Type 1 (insulin-dependent) diabetic patients with poor metabolic control [34]. This phenomenon is accompanied by an increase in the circulating levels of some indices of platelet activation such as β-thromboglobulin [35] and platelet factor 4 [36]. The results on the production of thromboxane are, on the other hand, conflicting; an increase was reported in Type 1 and Type 2 diabetic patients, but seems to be affected by metabolic control only in the latter group [37, 38]. Studies in Type 1 diabetic patients have shown, however, that better metabolic control improves both the production of thromboxane [39] and platelet aggregation [40] within a very short period of time.

Platelet hyperaggregation in diabetic patients is partly determined by an exaggerated ability of the platelets to bind both thromboxane [41] and fibrinogen [42], accompanied by a reduced binding capacity for prostacyclin [43].

There are contrasting opinions as to whether the platelet alterations detected in diabetes are primitive or dependent on the presence of vascular complications. Some data indicate that hyperaggregation is also present in diabetic patients without vascular complications and in young diabetic patients [44].

Erythrocytes

Erythrocytes play a important role in coagulation processes. When the erythrocyte membrane is impaired, ADP is released, stimulating platelet aggregation. In diabetes the erythrocytes are damaged, resulting in reduced half-life [45, 46], polycythaemia [47] and increased volume [48], factors which may cause blood hyperviscosity with a consequent increase in thrombotic risk.

Fibrinolysis

Numerous studies have been published on the behaviour of fibrinolysis in diabetes, with frequently conflicting data [19].

The role of hyperinsulinaemia in causing an increase in plasminogen activator inhibitor (PAI) is unclear. An association between hyperinsulinaemia and elevated PAI level in Type 2 diabetic patients has frequently been reported [49]. Evidence to date, however, does not indicate an effect of insulin on plasma PAI as these levels are usually normal in Type 1 diabetic patients taking insulin [50] and do not increase with its acute administration [49]. Since fibrinolysis, like coagulation, is a dynamic process, it cannot be studied in diabetic patients simply by evaluating the fibrinolytic parameters alone, but should be assessed by considering the balance between thrombus formation and fibrinolysis. This point of view is consistent with the data showing that fibrinolysis in diabetes is depressed, at least relative to the coagulation activation generally found [51].

Lipoprotein (a)

Lipoprotein (a) (Lpa) at high levels has been associated with an increased risk of cardiovascular events [52]. An increase in Lpa in Type 1 diabetic subjects has been reported [53]. The most interesting fact is that its plasma levels seem to be correlated with the degree of metabolic control, varying according to the mean values of glycaemia [53].

Endothelium

It is now a well-known fact that the endothelium plays a key role in the regulation of haemostatic processes [54]. Human endothelial cells cultured under hyperglycaemic conditions demonstrate a whole series of prothrombotic alterations: a decreased production of prostacyclin [55], increased production of tissue factor [56] and an imbalance between the expression of tissue plasminogen activator and PAI favouring thrombosis [57].

Microalbuminuria

An increased urinary excretion of albumin is significantly correlated with an increased incidence of mortality for cardiovascular events, in both Type 1 [58] and Type 2 diabetic patients [59]. The pathogenesis of this association is unclear. It has recently been demonstrated that an endothelial dysfunction exists in microalbuminuria, detected by von Willebrand factor levels [60]. The presence of microalbuminuria in diabetic patients has also been associated with an increase in plasma fibrinogen [61].

Hypotheses on the pathogenetic mechanisms

From what has been described thus far, it seems clear that a series of abnormalities are present in diabetes which predispose the patient to development of a thrombotic event. This assumption is supported by epidemiological data [1]. Many of the described alterations seem to be dependent on glycaemic control. Therefore, it is feasible to consider how hyperglycaemia can cause a state of thrombophilia in diabetes.

Non-enzymatic glycation

One of the most accredited hypotheses on the pathogenesis of diabetic complications concerns the non-enzymatic glycation reactions which are capable of altering the functionality of a molecule.

This mechanism has been demonstrated in vitro for ATIII [62]. Glucose probably occupies the lysine residue which binds the ATIII to its natural cofactor, heparin, making the molecule less active [62]. This hypothesis was confirmed by the fact that both heparin and low molecular weight heparin are capable of preventing the alterations of the molecule induced by hyperglycaemia [63, 64]. Heparin co-factor II seems to have a similar fate, being actively glycated in vitro [65].

The glycation mechanism seems to intervene at other levels. Fibrin may be glycated, thus becoming less sensitive to the processes of fibrinolysis [66]. Non-enzymatic glycation may also involve the platelet membrane proteins, reducing their fluidity and making them more sensitive to the thrombin pro-aggregant stimulus [67]. Glycation of the erythrocyte membranes also leads to a decrease in fluidity, with predictable repercussions on blood viscosity [68]. Finally, the advanced end-products of glycation, which derive from this reaction, may also be implicated in diabetic thrombophilia. It has, in fact, been demonstrated in vitro that cultured endothelial cells exposed to these products express a greater procoagulant activity [69].

Oxidative stress

An increased oxidative stress has been established as present in diabetes [70]. The increase in production of free radicals is linked to processes of glucose auto-oxidation and to the possibility that the glycated proteins themselves release free radicals [71]. The production of free radicals is correlated to metabolic control and more directly to hyperglycaemia [72]. It is known that free radicals are capable of activating coagulation [73], and direct evidence already exists which indicates that oxidative

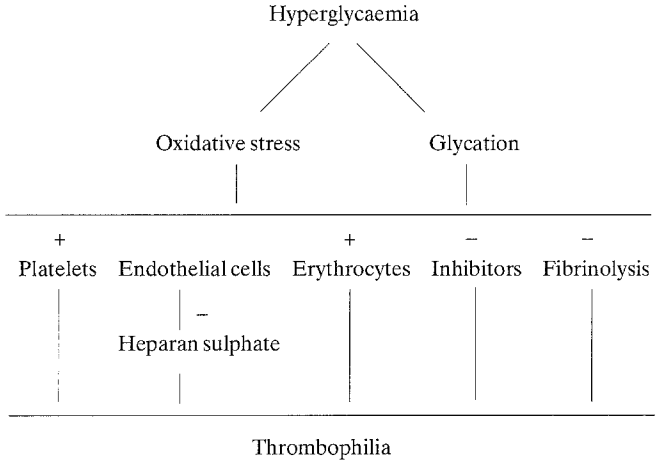

Fig. 1. Pathway linking hyperglycaemia and thrombophilia in diabetes mellitus

stress is accompanied by haemostatic alterations in diabetic patients [74]. On the basis of oxidative stress, the rapid inactivation of ATIII during induced hyperglycaemia [22] could also be included. The acute damage to the molecule could be due to a release of free radicals, since it has been demonstrated that oxidative stress is capable of reducing the activity of ATIII [75].

Hyperglycaemia-mediated oxidative stress leads to a reduction in the erythrocytes' anti-oxidant defenses, in particular of vitamin E, inducing a procoagulant state [76]. These effects can be prevented by controlling the glycaemia [76].

Reduced levels of vitamin E are reported in the platelets of diabetic subjects, accompanied by hyperaggregation [77]. Treatment with vitamin E improves this condition [77].

Recent data demonstrate that oxidative stress mediated by hyperglycaemia affects some endothelial functions [78]. The finding in vivo that vitamin E improves the production of prostacyclin by the aortic endothelium would seem to confirm this point of view [79].

Heparan sulphate

Another possible mechanism for the development of thrombophilia in diabetes is linked with the reduced synthesis of heparan sulphate [80]. Heparan sulphate is negatively charged and regulates the development of pericellular thrombotic phenomena at the level of the endothelial membrane, by interacting with ATIII [54]. It is therefore evident that a reduced synthesis of this glycosaminoglycan can lead to the appearance of thrombophilia. This hypothesis is supported by studies which reported an increased ratio between fibrinogen and ATIII, due to a decrease in ATIII [81], in the aorta of diabetic rats and a general widespread reduction of the negative charges of the blood vessels in diabetic rats [82]. These observations confirm a reduced presence of glycosaminoglycans, and of heparan sulphate, in particular, on the endothelium. This reduction is largely due to a reduced endothelial synthesis [83], and it is interesting to note that mesangial cells subjected to oxidative stress have been shown to selectively reduce the synthesis of heparan sulphate [84].

Therapeutic approach

Currently many drugs are available for which their efficacy has been demonstrated in counteracting the coagulation alterations seen in diabetes, or which are potentially of use in this disease.

Anti-oxidants can probably be used. It has recently been demonstrated that vitamin E can normalise platelet function in Type 1 diabetic patients [85]. Anti-oxidants could also be used to control Lpa levels, an effect which has been demonstrated by acetylcysteine [86]. Sulphonylureas have demonstrated a platelet antiaggregant [87] and fibrinolysis stimulating effect [88] in vivo. Promising results have been obtained with a mixture of glycosaminoglycans, sulodexide. This was able to reduce fibrinogen and FPA levels [89] and to increase the profibrinolytic response during prothrombotic stimulation induced by hyperglycaemia [51] in diabetic patients. Bezafibrate has been shown to reduce fibrinogen levels in both Type 1 [90] and Type 2 diabetic patients [91]. Omega-3 fatty acids are also of interest. The use of these compounds has been associated with a reduced incidence of arteriosclerosis and cardiovascular events [92]. There are many indications for their use in the prevention of thrombotic complications in diabetes, since they seem to be able to reduce fibrinogen levels, improve platelet aggregation [93] and reduce Lpa levels [94]. Defibrotide is a polydeoxyribonucleotide with good antithrombotic and profibrinolytic potential linked to its anti-oxidant property [95]. In view of this it may be useful in the prevention of thrombosis in diabetes.

Conclusions

Diabetes is characterized by a series of alterations that undoubtably result in a thrombophilic state in patients with this disease. This is confirmed by epidemiological studies which indicate that diabetic patients more rapidly develop a thromboembolic event [1]. The series of studies quoted herein indicate that in a large number of cases hyperglycaemia might be the pathogenetic cause of thrombophilia. Hyperglycaemia, as summarized in Figure 1, may act either by a mechanism connected with non-enzymatic glycation or by favouring an increased oxidative stress.

The presence of an alteration in glucose metabolism now seems to be a much more important cardiovascular risk factor than was previously assumed. This hypothesis is supported by the results of a number of studies. The Framingham study has shown that in non-diabetic women with an early cardiovascular event, the glycated haemoglobin levels were significantly above-normal [96]. Our preliminary studies in a cohort of normal subjects have shown that glycated haemoglobin correlates significantly with classic risk factors, such as hypertension and cholesterol [97]. On the other hand, both an increase in fi-

brinogen [12] and oxidative stress [98] are now considered to be cardiovascular risk factors. It therefore seems that thrombophilic alterations can be considered as being strictly correlated with the poor regulation of the glucose metabolism, that these alterations probably have a pathogenetic value for cardiovascular events, and that oxidative stress may be the common determining factor.

A series of potentially valid therapeutic interventions to control thrombophilia in diabetic patients have been described. Long-term studies are indicated which demonstrate unequivocally that by correcting these coagulation irregularities, the life of diabetic patients can be prolonged.

References

1. Colwell JA (1993) Vascular thrombosis in type II diabetes mellitus. Diabetes 42: 8–11
2. Theroux P, Latour JG, Leger-Gauthier C, De Lara J (1987) Fibrinopeptide A and platelet factor levels in unstable angina pectoris. Circulation 75: 156–162
3. Kruskal JB, Commerford PJ, Franks JJ, Kirsch RE (1987) Fibrin and fibrinogen-related antigens in patients with stable and unstable coronary artery disease. N Engl J Med 317: 1361–1365
4. Kannel WB, Mc Gee DL (1979) Diabetes and cardiovascular disease. The Framingham study. JAMA 241: 2035–2038
5. Nossel HL, Yudelman J, Canfield RE et al. (1974) Measurement of fibrinopeptide A in human blood. J Clin Invest 54: 43–53
6. Rabie MJ, Blashill A, Furie BC (1986) Prothrombin fragment 1.2.3, a major product of prothrombin activation in human plasma. J Biol Chem 261: 13210–13215
7. Rosove MH, Frank HSL, Harving SSL (1984) Plasma beta-thromboglobulin, platelet factor 4, fibrinopeptide A, and other hemostatic functions during improved short-term glycemic control in diabetes mellitus. Diabetes Care 7: 174–179
8. Ceriello A, Giacomello R, Colatutto A, Taboga C, Gonano F (1992) Increased prothrombin fragment 1 + 2 in type I diabetic patients. Haemostasis 22: 50–51
9. Jones RL (1985) Fibrinopeptide A in diabetes mellitus: relation to levels of blood glucose, fibrinogen disappearance, and hemodynamic changes. Diabetes 34: 836–841
10. Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Marchi E, Torella R (1989) Hyperglycemia may determine fibrinopeptide A plasma level increase in humans. Metabolism 38: 1162–1163
11. Jones RL, Peterson CM (1979) Reduced fibrinogen survival in diabetes mellitus: a reversible phenomenon. J Clin Invest 63: 485–493
12. Wilhelmsen L, Svardsudd K, Korsan-Bengtensen K, Larsson B, Welin L, Tibblin G (1984) Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 311: 501–505
13. Ganda OP, Arkin CF (1992) Hyperfibrinogenemia. An important risk factor for vascular complications in diabetes. Diabetes Care 15: 1245–1250
14. Coller BS, Frank RN, Milton RC, Gralnick HR (1978) Plasma cofactors of platelet function: correlation with diabetic retinopathy and hemoglobin A1a–c. Ann Intern Med 88: 311–316
15. Meade JW, Brozovic M, Chakrabarti RR et al. (1986) Haemostatic function and ischemic heart disease: principal results of the Northwick Park Heart Study. Lancet II: 533–537
16. Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Torella R (1988) Blood glucose may condition factor VII levels in diabetic and normal subjects. Diabetologia 31: 889–891
17. Balleisen L, Assmann G, Bailey J (1985) Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population. Baseline data on the relation to blood pressure, blood glucose, uric acid and lipid fractions. Thromb Haemostas 54: 721–723
18. Ceriello A, Quatraro A, Marchi E, Barbanti M, Dello Russo P, Lefebvre PJ (1990) The role of hyperglycaemia-induced alterations of antithrombin III and factor X activation in the thrombin hyperactivity of diabetes mellitus. Diabetic Med 7: 343–348
19. Ostermann H, van de Loo J (1986) Factors of the hemostatic system in diabetic patients. A survey of controlled studies. Haemostasis 16: 386–416
20. Ceriello A, Dello Russo P, Zuccotti C et al. (1983) Decreased antithrombin III activity in diabetes may be due to non-enzymatic glycosylation. A preliminary report. Thromb Haemostas 50: 633–634
21. Ceriello A, Giugliano D, Quatraro A et al. (1987) Daily rapid blood glucose variations may condition antithrombin III biologic activity but not its plasma concentration in insulin-dependent diabetes. Diabet Metab 13: 16–19
22. Ceriello A, Giugliano D, Quatraro A et al. (1987) Induced hyperglycemia alters antithrombin III activity but not its plasma concentration in healthy normal subjects. Diabetes 36: 320–323
23. Ceriello A, Giugliano D, Dello Russo P, Tirelli A, Passariello N, Sgambato S (1986) Metabolic control may alter antithrombin III activity but not its plasma concentration in diabetes: a possible role for nonenzymatic glycosylation. Diabetes Care 9: 32–35
24. Husted SE, Nielsen HK, Bak JF, Beck-Nielsen H (1989) Antithrombin III, von Willebrand factor antigen and platelet function in young diabetic patients treated with multiple insulin injections versus insulin pump treatment. Eur J Clin Invest 19: 90–94
25. Ceriello A, Giugliano D, Quatraro A, Marchi E, Barbanti M, Lefèbvre PJ (1990) Evidence for a hyperglycaemia-dependent decrease of antithrombin III-thrombin complex formation in humans. Diabetologia 33: 163–167
26. Ceriello A, Quatraro A, Dello Russo P, Marchi E, Milani MR, Giugliano D (1990) Evidence for a reduced heparin cofactor II biological activity in diabetes. Haemostasis 20: 357–361
27. Brownlee M (1976) Alpha-2-macroglobulin and reduced basement membrane degradation in diabetes. Lancet I: 779–780
28. Ceriello A, Giugliano D, Quatraro A, Marchi E, Barbanti M, Giugliano D (1989) Increased alpha-2-macroglobulin in diabetes: a hyperglycemia-related phenomenon associated with reduced antithrombin III activity. Acta Diabetol Lat 26: 147–154
29. Ceriello A, Quatraro A, Dello Russo P, Marchi E, Barbanti M, Giugliano D (1989) Hyperglycemia-conditioned increase in alpha-2-macroglobulin in healthy normal subjects: a phenomenon correlated with deficient antithrombin III activity. Acta Haemat 82: 61–63
30. Vukovich TC, Schernthaner G (1986) Decreased protein C levels in patients with insulin-dependent type I diabetes mellitus. Diabetes 35: 617–619
31. Ceriello A, Quatraro A, Dello Russo P et al. (1990) Protein C deficiency in insulin-dependent diabetes: a hyperglycemia-related phenomenon. Thromb Haemostas 64: 104–107
32. De Fouwuj NS, Haverkate P, Bertina RM, Koopma T, van Wijngaarden A, von Hisbergh VW (1986) The cofactor role of protein S in the acceleration of whole blood clot lysis. Blood 67: 1192–1196
33. Ceriello A, Giugliano D, Quatraro A, Marchi E, Barbanti E, Lefebvre PJ (1990) Possible role for increased C4b-binding protein level in acquired protein S deficiency in type I diabetes. Diabetes 39: 447–449
34. Davis JW, Hartman CR, Davis RF, Kyner JL, Lewis HD, Phillips PE (1982) Platelet aggregate ratio in diabetes mellitus. Acta Haemat 67: 222–224
35. Burrows AW, Chavin SI, Hockaday TDR (1978) Plasma thromboglobulin concentrations in diabetes mellitus. Lancet I: 235–237
36. Davì G, Rini GB, Averna M et al. (1982) Enhanced platelet release reaction in insulin-dependent and insulin-independent diabetic patients. Haemostasis 12: 275–281
37. Alessandrini P, Mc Rae J, Feman S, Fitzgerald GA (1988) Thromboxane biosynthesis and platelet function in type I diabetes mellitus. N Engl J Med 319: 208–212
38. Davì G, Catalano I, Averna M, Notarbartolo A, Strano A, Ciabattoni G, Patrono C (1990) Thromboxane biosynthesis and

platelet function in type II diabetes mellitus. N Engl J Med 322: 1769–1774
39. Mayfield RK, Haluskka PV, Wohltmann HS et al. (1985) Platelet function during continuous insulin infusion treatment in insulin-dependent diabetic patients. Diabetes 34: 1127–1133
40. Juhan I, Buonocore M, Jouve R, Vague P, Moulin JP, Vialettes B (1982) Abnormalities of erythrocyte deformability and platelet aggregation in insulin-dependent diabetics corrected by insulin in vivo and in vitro. Lancet I: 535–537
41. Collier A, Tymkewycz P, Armstrong R, Young RJ, Jones RL, Clarke BF (1986) Increased platelet thromboxane receptor sensitivity in diabetic patients with proliferative retinopathy. Diabetologia 29: 471–474
42. Di Minno G, Silver MJ, Cerbone AM, Riccardi G, Rivellese A, Mancini M (1986) Platelet fibrinogen binding in diabetes mellitus: differences between binding to platelets from retinopathic and retinopathic diabetic patients. Diabetes 35: 182–185
43. Betteridge DJ, El Tahir KEH, Reckless JPD, Williams KS (1982) Platelets from diabetic subjects show diminished sensitivity to prostacyclin. Eur J Clin Invest 12: 395–398
44. Winocour PD, Halushka PV, Colwell JA (1985) Platelet involvement in diabetes mellitus. In: Longenecker GL (ed) The platelets: physiology and pharmacology. Academic Press, New York, pp 341–366
45. Jones RL, Peterson CM (1981) Hematologic alterations in diabetes mellitus. Am J Med 70: 339–352
46. Ceriello A, Dello Russo P, Sgambato S, Giugliano D (1982) Glycosylated haemoglobin and reticulocyte count in diabetes. Diabetologia 22: 223 (Letter)
47. Graham JJ, Ryall RG, Wise PH (1980) Glycosylated haemoglobin and relative polycythaemia in diabetes mellitus. Diabetologia 18: 205–207
48. Ceriello A, Dello Russo P, Curcio F, Balsamo C, Pietrantuono C (1983) Red blood cell volume and glycaemic control in diabetes. Diabetologia 24: 397 (Letter)
49. Juhan-Vague I, Alessi MC, Vague P (1991) Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia 34: 457–462
50. Walmsley D, Hampton KK, Grant PJ (1991) Contrasting fibrinolytic responses in type I (insulin dependent) and type II (non-insulin dependent) diabetes. Diabetic Med 8: 954–959
51. Ceriello A, Quatraro A, Marchi E, Barbanti M, Giugliano D (1993) Impaired fibrinolytic response to increased thrombin activation in type I diabetes mellitus: the effect of the glycosaminoglycan sulodexide. Diabet Metab 19: 225–229
52. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF (1989) A potential basis for the thrombotic risk associated with lipoprotein (a). Nature 339: 301–303
53. Ramirez LC, Arauz-Pacheco C, Lackner C, Albright G, Adams BV, Raskin P (1992) Lipoprotein (a) levels in diabetes mellitus: relationship to metabolic control. Ann Intern Med 117: 42–47
54. Stern DM, Esposito C, Gerlach H et al. (1991) Endothelium and regulation of coagulation. Diabetes Care 14 [Suppl. 1]: 160–166
55. Ono H, Umeda F, Inoguchi T, Ibayashi H (1988) Glucose inhibits prostacyclin production by cultured aortic endothelial cells. Thromb Haemostas 60: 174–177
56. Boeri D, Almus FE, Maiello M, Cagliero E, Rao LVM, Lorenzi M (1989) Modification of tissue-factor mRNA and protein response to thrombin and interleukin 1 by high glucose in cultured human endothelial cells. Diabetes 38: 212–218
57. Maiello M, Boeri D, Podestà F et al. (1992) Increased expression of tissue plasminogen activator and its inhibitor and reduced fibrinolytic potential of human endothelial cells cultured in elevated glucose. Diabetes 41: 1009–1015
58. Borch-Johnsen K, Kreiner S (1987) Proteinuria: value as a predictor of cardiovascular mortality in insulin-dependent diabetes mellitus. BMJ 294: 1651–1654
59. Mogensen CE (1984) Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes mellitus. N Engl J Med 310: 356–360
60. Stehouwer CDA, Nanta JJP, Zeldenrust GC, Hackeng WHL, Donker AJA, Den Ottolander GJH (1992) Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. Lancet II: 319–323
61. Jones SL, Close CF, Mattock MB, Jarrett RJ, Keen H, Viberti GC (1989) Plasma lipid and coagulation factor concentrations in insulin dependent diabetes with microalbuminuria. BMJ 298: 487–490
62. Villanueva GB, Allen N (1988) Demonstration of altered antithrombin III activity due to non enzymatic glycosylation at glucose concentration expected to be encountered in severely diabetic patients. Diabetes 37: 1103–1107
63. Ceriello A, Giugliano D, Quatraro A et al. (1986) Heparin preserves antithrombin III biological activity from hyperglycemia-induced alterations in insulin-dependent diabetics. Haemostasis 16: 458–464
64. Ceriello A, Marchi E, Palazzini E, Quatraro A, Giugliano D (1990) Low molecular weight heparin restores antithrombin III activity from hyperglycemia induced alterations. Diabet Metab 16: 86–92
65. Ceriello A, Marchi E, Barbanti M et al. (1990) Non-enzymatic glycation reduces heparin cofactor II anti-thrombin activity. Diabetologia 33: 205–207
66. Brownlee M, Vlassara H, Cerami A (1983) Nonenzymatic glycosylation reduces the susceptibility of fibrin to degradation by plasmin. Diabetes 32: 600–604
67. Winocour PD, Watala C, Kinlough-Rathbone RL (1992) Membrane fluidity is related to the extent of glycation of proteins, but not to alterations in the cholesterol to phospholipid molar ratio in isolated platelet membranes from diabetic and control subjects. Thromb Haemostas 65: 567–571
68. Bryszewska M, Szosland K (1988) Association between the glycation of erythrocyte membrane proteins and membrane fluidity. Clin Biochem 21: 49–51
69. Esposito C, Gerlach H, Brett J, Stern D, Vlassara H (1989) Endothelial receptor-mediated binding of glucose-modified albumin is associated with increased monolayer permeability and modulation of cell surface coagulant properties. J Exp Med 170: 1387–1407
70. Baynes JW (1991) Role of oxidative stress in development of complications in diabetes. Diabetes 40: 405–412
71. Ceriello A, Quatraro A, Giugliano D (1992) New insights on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. Diabetic Med 9: 297–299
72. Ceriello A, Giugliano D, Quatraro A, Dello Russo P, Lefebvre PJ (1991) Metabolic control may influence the increased superoxide anion generation in diabetic serum. Diabetic Med 8: 540–542
73. Barrowcliffe TW, Gutteridge JM, Gray E (1987) Oxygen radicals, lipid peroxidation and the coagulation system. Agent Actions 22: 347–348
74. Collier A, Rumley AG, Paterson JR, Leach JP, Lowe GDO, Small M (1992) Free radical activity and hemostatic factor in NIDDM patients with and without microalbuminuria. Diabetes 41: 909–913
75. Gray E, Barrowcliffe TW (1985) Inhibition of antithrombin III by lipid peroxides. Thromb Res 37: 241–250
76. Jain SK, Levine SN, Duett J, Hollier B (1991) Reduced vitamin E and increased lipofuscin products in erythrocytes of diabetic rats. Diabetes 40: 1241–1244
77. Karpen CW, Pritchard KA Jr, Arnold JH, Cornwell DG, Panganamala RV (1982) Restoration of prostacyclin thromboxane A2 balance in the diabetic rat: influence of dietary vitamin E. Diabetes 31: 947–951
78. Curcio F, Ceriello A (1992) Decreased cultured endothelial cell proliferation in high glucose medium is reversed by antioxidants: new insights on the pathophysiological mechanism of diabetic vascular complications. In Vitro Cell Dev Biol 28: 787–790
79. Karpen CW, Merola AJ, Trewyn RW, Cornwell DG, Pangamala RV (1981) Modulation of platelet thromboxane A2 and arterial prostacyclin by dietary vitamin E. Prostaglandins 22: 651–661

80. Rohrbach DH, Wagner CW, Star VL, Martin GR, Brown KS, Yoon JW (1983) Reduced synthesis of basement membrane heparan-sulfate proteoglycan in streptozotocin-induced diabetic mice. J Biol Chem 258: 11672–11677
81. Witmer MR, Hadcock SJ, Peltier SL, Winocour PD, Richardson M, Hatton MW (1992) Altered levels of antithrombin III and fibrinogen in the aortic wall of the alloxan-induced diabetic rabbit: evidence of a prothrombotic state. J Lab Clin Med 119: 221–230
82. Raz I, Havivi Y, Yarom R (1988) Reduced negative surface charge on arterial endothelium of diabetic rats. Diabetologia 31: 618–620
83. Moran A, Brown DM, Kin Y, Klein DJ (1991) Effects of IGF-1 and glucose on protein and proteoglycan synthesis by human fetal mesangial cells in culture. Diabetes 40: 1346–1355
84. Kashihara N, Wanatabe Y, Makino H, Wellner EI, Kanwar YS (1992) Selective decreased de novo synthesis of glomerular proteoglycans under the influence of reactive oxygen species. Proc Natl Acad Sci USA 89: 6309–6313
85. Gisinger C, Jeremy J, Speiser P, Mikhailidis D, Daudona P, Schernthaner G (1988) Effect of vitamin E supplementation on platelet thromboxane A2 production in type I diabetic patients. Double-blind crossover trial. Diabetes 37: 1260–1264
86. Gavish D, Breslow JL (1991) Lipoprotein (a) reduction by N-acetylcysteine. Lancet I: 203–204
87. Klaff LJ, Kernoff L, Vinik AI, Jackson WPU, Jacobs P (1981) Sulfonylureas and platelet function. Am J Med 70: 627–630
88. Gram J, Jesperson J, Kold A (1988) Effects of oral antidiabetic drug on the fibrinolytic system of blood in insulin-treated diabetic patients. Metabolism 37: 937–943
89. Ceriello A, Quatraro A, Ettorre M, Marchi E, Barbanti M, Giugliano D (1993) Glucosaminoglycans administration decreases high fibrinogen plasma levels in diabetic patients. Diab Nutr Metab 6
90. Durrington PN, Winocour PH, Bhatuagar D (1990) Bezafibrate retard in patients with insulin-dependent diabetes: effects on serum lipoproteins, fibrinogen, and glycemic control. J Cardiovasc Pharmacol 16 [Suppl 9]: 30–34
91. Mathur S, Barrades MA, Mikhailidis DP, Dandona P (1990) The effect of slow release formulation of bezafibrate on lipids, glucose homeostasis, platelets and fibrinogen in type II diabetics; a pilot study. Diabetes Res 14: 133–138
92. Leaf A, Weber PC (1988) Cardiovascular effect of n-3 fatty acids. N Engl J Med 318: 549–557
93. Malasonos TH, Stacpoole PW (1991) Biological effects of omega-3 fatty acids in diabetes mellitus. Diabetes Care 14: 1160–1179
94. Simonutti M, Ceriello A, Taboga C, Faletti E, Giacomello R (1993) Effect of omega 3 fatty acids on lipid pattern in type II diabetic subjects. In: European Atherosclerosis Society "Thrombosis and the Vessel Wall Highlights '93". Ariello Bros Press, Naples, p 161 (Abstract)
95. Cirillo F, Margaglione M, Vecchione G et al. (1991) In vitro inhibition by defibrotide of monocyte superoxide anion generation: a possible mechanism for the antithrombotic effect of a polydeoxyribonucleotide-derived drug. Haemostasis 21: 98–105
96. Singer DE, Nathan DM, Anderson KM, Wilson PWF, Evans JC (1992) Association of HbA_{1c} with prevalent cardiovascular disease in the original cohort of the Framingham Heart Study. Diabetes 41: 202–208
97. Giugliano D, Quatraro A, Minei A et al. (1992) Does a positive family history of diabetes convey a more atherogenetic blood profile? Diabetologia 35 [Suppl 1]: A 93 (Abstract)
98. Riemersa RA, Wood DA, Macintyre CCA, Elton RA, Gey KF, Oliver MF (1991) Risk of angina pectoris and plasma concentrations of vitamins A, C and E and carotene. Lancet I: 1–5

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