Beneficial effects of metformin on haemostasis and vascular function in man

PJ Grant

Summary
Type 2 diabetes is characterised by insulin resistance in association with clustering of atherothrombotic risk factors (dysglycaemia, hyperinsulinaemia, hypertension, raised triglyceride, low HDL cholesterol and increased levels of plasminogen activator inhibitor-1 (PAI-1) and clotting factor VII). There is a 3-5 fold increase in risk of myocardial infarction rising to 10-20 fold in the presence of microalbuminuria and overall around 70-75% of subjects with type 2 diabetes die of cardiovascular disease. However, classical risk factors which associate with insulin resistance do not account for all the increased burden of vascular disease in diabetic subjects. Metformin is a biguanide compound which is antihyperglycaemic, reduces insulin resistance and has cardioprotective effects on lipids, thrombosis and blood flow. Metformin has a weight neutral/weight lowering effect and reduces hypertriglyceridaemia, elevated levels of PAI-1, factor VII and C-reactive protein. In addition recent studies indicate that metformin has direct effects on fibrin structure/function and stabilises platelets, two important components of arterial thrombus. The United Kingdom Prospective Diabetes Study (UKPDS) reported that metformin was associated with a 32% reduction in any diabetes related endpoint (p < 0.002), a 39% reduction in myocardial infarction (p < 0.01) and a non-significant 29% fall in microvascular complications. The figures for macrovascular complications compare favourably for those described for other cardioprotective agents such as ACE inhibitors and statins. These findings confirm metformin as first line therapy in the management of obese insulin resistant type 2 diabetes and in the prevention of the vascular complications of this common condition.

Key-words: Metformin · Type 2 Diabetes · Haemostasis · Fibrinolysis · Macrovascular complications · Thrombosis.

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Type 2 diabetes is a condition characterised by fasting hyperglycaemia, usually in the presence of insulin resistance, and a marked increase in cardiovascular complications. Several studies have indicated that there is a 3-5 fold increase in risk of cardiovascular disease in diabetic subjects with loss of cardioprotection in females. The development of microalbuminuria is accompanied by a 20 fold increase in risk. Diabetic subjects generally do less well after acute events or surgical interventions and overall around 77% of deaths in diabetes are due to vascular disease. Studies indicate that conventional risk factors do not account for all the increased risk associated with type 2 diabetes and attention has turned to novel risk markers that cluster with insulin resistance such as prothrombotic factors and abnormalities in platelet function and blood flow. Metformin reduced vascular risk in the UKPDS and evidence indicates that as well as having beneficial effects on some classical risk markers, it also affects systems involved in the regulation of less traditional vascular markers mentioned above. This review will summarise current knowledge of the effects of metformin in these areas.

Mechanisms of thrombus formation

Coagulation

The intrinsic pathway was described in detail by the “waterfall” and “cascade” theories of coagulation presented in 1964 by Davie & Ratnoff [1] and by Macfarlane [2] (Fig 1). Although recognised since the 19th Century that brain and other tissues contained a factor that induced clotting of blood, further characterisation of the extrinsic pathway was not achieved until 1966 when it was discovered that this was tissue factor (TF) acting as a cofactor for FVII [3].

Intrinsic pathway activation

The view that negatively charged surfaces triggered coagulation has been replaced by the concept that the main trigger for coagulation is cellular injury with tissue factor (TF) release [4]. There are circumstances, such as extracorporeal circulation, where blood is exposed to negatively charged surfaces, although contact activation does not always occur under these circumstances [5]. Contact activation does play an important role in the reciprocal activation of the coagulation, fibrinolysis, complement and kinin pathways during thrombosis and sepsis.

Extrinsic pathway activation

TF is abundantly expressed on cells that surround blood vessels to ensure that clotting is readily triggered upon vascular injury. Intravascular cells such as monocytes and endothelial cells also express TF after stimulation with cytokines [6]. Functionally active TF has been localised in the lipid-rich core of the atherosclerotic plaque, particularly in association with macrophage-derived foam cells [7]. Studies show there is a pool of circulating TF which propagates thrombus formation under flow conditions [8].

Cascade of zymogen-enzyme conversions

FVII(a) is a serine protease that in complex with TF proteolytically cleaves FX, which, with FVa, cleaves prothrombin to yield fragment 1 + 2 and thrombin. In addition to activation of FX, the FVII(a)/TF complex also cleaves FIX. This leads to further thrombin generation as FIXa with FVIIIa cleaves more FX. Further reactions include activation of FV, FVIII and FXI by thrombin. FVa and FVIIIa act as cofactors for FXa and FIXa respectively. von Willebrand factor (vWF) serves as a carrier for FVIII and is involved in platelet aggregation.

Figure 1
Simplified scheme of the coagulation and fibrinolytic pathways with main components related to the insulin resistance syndrome shown in black boxes. Under circumstances of tissue damage, release of tissue factor activates factor VII which can directly activate factor X or indirectly activate factor X through activation of factor IX. A scanning electron micrograph of cross-linked fibrin, the final product of the coagulation cascade is shown. In coronary artery thrombosis, the fibrin clot is platelet rich, whilst in venous thrombosis the clot is platelet poor. The fibrinolytic pathway consists of enzymes which promote the formation of plasmin from plasminogen. Plasmin lyses cross-linked fibrin to produce fibrin degradation products. Plasminogen activator-1 (PAI-1) is the principal inhibitor of plasminogen activators and is strongly related to the insulin resistance syndrome.
Formation of cross-linked fibrin

The formation of a platelet rich fibrin clot is characteristic of an occluded arterial lesion following plaque rupture. Thrombin, generated by activation of the coagulation pathway (described above) is central to these processes, and catalyses three reactions that lead to clot formation: (a) activation and aggregation of platelets, (b) conversion of fibrinogen into fibrin, and (c) activation of FXIII. Thrombin induced cleavage of fibrinogen leads to the formation of half-staggered overlapping protofibrils that laterally aggregate into thicker fibre bundles [9, 10]. The fibres form a three-dimensional network to which platelets and other proteins adhere, forming the structural basis of the blood clot. Fibrin is covalently cross-linked by FXIIIa [11] which enhances the chemico/physical stability of the clot. FXIII cross-links α2-antiplasmin to fibrin, increasing resistance to fibrinolysis [12].

Fibrinolysis

A natural defence system against deposition of cross-linked fibrin exists in vivo to maintain vascular patency which, like the coagulation cascade, is based on a series of serine proteinase reactions (Fig 1). Central to fibrinolysis is plasmin, a serine proteinase that degrades fibrin [13]. Plasminogen is converted to plasmin by cleavage by two distinct plasminogen activators, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Activation of plasminogen by tPA is regulated by the presence of fibrin; in the absence of fibrin, tPA is a poor enzyme. Activation of plasminogen by uPA is regulated by the binding of uPA to a cellular receptor (u-PAR). α2-antiplasmin is the main physiological inhibitor of plasmin [14] and inhibition of both tPA and uPA occurs by plasminogen activator inhibitor-1 (PAI-1), a glycoprotein of the serpin family [15].

Cellular interactions

Blood cells provide many of the proteins involved in haemostasis, as well as the surface at which crucial enzyme and ligand-receptor reactions occur. Whilst thrombosis in the venous system occurs under low flow conditions, with the formation of an erythrocyte-rich fibrin clot, thrombi in the arterial vascular bed are platelet-rich and occur under high shear stress.

Platelets

Blood platelets are small discoid anucleoid cells that adhere to collagen exposed after vessel injury to form the primary haemostatic plug. This adhesion occurs via an interaction between collagen and vWF, which binds to the platelet receptor glycoprotein Ib-IX. Platelets are activated by thrombin cleavage of two members of the protease-activated receptor (PAR) family, PAR-1 and PAR-4 [16]. Platelets lose their typical discoid shape on activation, become irregular in appearance and release the contents of α-granules which contain a rich mixture of prothrombotic factors (calcium, fibrinogen, FV, vWF, FXI, kininogen and PAI-1), and adhesive proteins (thrombospondin, fibronectin and vitronectin). Fibrin is generated around platelet aggregates, which interact with the fibrin network through the glycoprotein Ib/IIIa receptor [17].

Endothelium

Under normal conditions, the vascular endothelium maintains an anticoagulant and vasodilatory phenotype to prevent intravascular clotting. There are two major anticoagulant mechanisms at work on the endothelium. One consists of the proteoglycan layer that covers the luminal surface of the endothelium which enhance the anticoagulant activity endothelium. The second anticoagulant mechanism is maintained by the expression of thrombomodulin which binds thrombin and alters its specificity from procoagulant to anticoagulant, by activation of protein C [18]. Lesions of the endothelium expose the procoagulant subendothelium rich in tissue factor and the endothelium expresses a thrombogenic phenotype.

Insulin resistance: an atherothrombotic syndrome

Type 2 diabetes is associated with a two to four-fold greater annual risk of cardiovascular disease (CVD) in males with a five-fold increase observed in female type 2 diabetics. The prevalence of diabetes in the UK has increased by around 30% since 1991, with approximately 6% of the population now diagnosed with the disease [19, 20]. Increased resistance to insulin-mediated glucose disposal underpins the development of the majority of cases of type 2 diabetes. In 1988 Gerald Reaven proposed the existence of a syndrome in which atherogenic risk factors cluster in the presence of underlying insulin resistance more commonly than would be expected to occur by chance alone [21]. In the original description, insulin resistance was associated with relative hyperinsulinaemia, abnormal glucose metabolism, dyslipidaemia (hyper-triglyceridaemia and low HDL cholesterol), and hypertension. More recently, a pro-coagulant aspect to the syndrome has been identified which includes elevated levels of the fibrinolytic inhibitor plasminogen activator inhibitor-1 antigen (PAI-1), tissue-type plasminogen activator antigen (t-PA), factor VII, factor XII and fibrinogen (Fig 2). It is thought that atherothrombotic clustering accounts for the high incidence of atherothrombotic disorders (myocardial infarction, stroke and peripheral vascular disease) associated with both type 2 diabetes and impaired glucose tolerance. However, studies have indicated that insulin resistance occurs in up to 25% of the population [22] and a similar clustering of risk factors is seen in subjects with apparently normal glucose metabolism but with evidence of abnormal insulin responses [23].
Insulin resistance and coagulation/fibrinolysis

Since the original description of the insulin resistance syndrome, evidence has accumulated that clustering of thrombotic risk also occurs in relation to underlying insulin resistance. Disturbances in the fibrinolytic system due to elevated concentrations of PAI-1 antigen and accompanied by elevated t-PA antigen concentrations occur in relation to normoglycaemic insulin resistance and type 2 diabetes. Additionally, pro-coagulant proteins, in particular, factor VII, factor XII and fibrinogen have been linked with the insulin resistance syndrome.

Plasminogen activator inhibitor-1 and tissue plasminogen activator

Clinical studies have identified strong relationships between features of the insulin resistance syndrome and levels of PAI-1 and t-PA [24-28]. In the recently reported Framingham Offspring Study, Meigs et al. examined the relationship between insulin levels and haemostatic factors in glucose tolerant and intolerant individuals. Levels of PAI-1 and t-PA were higher in subjects with glucose intolerance and increased significantly across insulin quintiles in both glucose tolerant and intolerant subjects groups [29]. Elevated PAI-1 levels have been found in the non-diabetic first-degree relatives of type 2 diabetic probands [30] and in patients with established cardiovascular disease [31, 32]. In these studies, the increases in PAI-1 have invariably been related to insulin concentration, dyslipidaemia and obesity, further strengthening the association between PAI-1, insulin resistance and cardiovascular disease.

The mechanism(s) by which changes in fibrinolytic factors occur in relation to insulin resistance remain unclear. Cell culture studies using human hepatocytes and HepG2 cells demonstrate an increase in PAI-1 mRNA after addition of insulin or insulin-like growth factor-1. However, the lack of corroborative in vivo data has led to alternative theories, including the indirect action of insulin on hepatic PAI-1 secretion via increased triglyceride [33] and the stimulation of PAI-1 production by adipocytes [34]. Pro-inflammatory cytokines, such as IL-6 and TNF-α, are over-expressed in adipose tissue of obese subjects. Increased TNF-α expression affects insulin signalling [35] and stimulates PAI-1 production by adipose tissue [36]. A 4G/5G polymorphism in the promoter region of the PAI-1 gene, 675 base pairs upstream of the transcription start site influences PAI-1 levels. Subjects homozygous for the 4G allele have higher plasma PAI-1 concentrations than subjects of 5G/5G genotype, a difference which is more pronounced in the presence of hypertriglyceridaemia [37, 38]. This is thought to be due to a VLDL-triglyceride sensitive site in the promoter region adjacent to the 4G/5G position and provides evidence of a specific gene-environment interaction within the insulin resistance syndrome [39] which acts to recruit thrombotic risk into the cluster. Elevated t-PA concentrations in association with insulin resistance superficially appear to be counterintuitive in relation to vascular risk but are likely to reflect underlying PAI-1 levels given that the majority of t-PA circulates as a t-PA/PAI-1 complex. Increased t-PA concentrations also occur as a consequence of endothelial cell dysfunction and damage, and chronic infection or inflammatory processes.

Factor VII

Elevated levels of factor VII coagulant activity (FVII) predicted fatal but not non fatal cardiovascular events in the Northwick Park Heart Study (NPHS), however, subsequent reports have largely failed to support an association between FVII and CAD [40-44]. After 16 years of follow-up, reanalysis of NPHS data demonstrated a specific relationship...
between FVIIc levels and death due to CAD [45] and in the PROCAM study [40] were of borderline significance when analysed for fatal events only. Increased levels of FVII are associated with the insulin resistance syndrome in patients with type 2 diabetes [46] and have been reported in hyperinsulinaemic subjects with normal glucose tolerance. The healthy, first-degree relatives of diabetic probands show evidence of increased expression of insulin resistance [47, 48] and FVII was elevated in the non-diabetic relatives of type 2 diabetic patients [49]. This difference was attenuated after adjustment for other features of the insulin resistance syndrome and in separate regression models FVII was independently related to insulin levels. There is evidence of increased FVII expression in the presence of hypertriglyceridaemia [50] and genotype-specific interactions between triglyceride and FVII gene polymorphisms.

**Fibrinogen**

Fibrinogen is a strong and independent predictor of myocardial infarction and stroke in non-diabetic subjects and elevated levels relate to macrovascular complications in diabetic patients [51]. A relationship between fibrinogen and insulin has been demonstrated in glucose tolerant women [45] and subjects with abdominal obesity [52] although, overall, fibrinogen is less strongly associated with features of the insulin resistance syndrome than PAI-1 and factor VII. Fibrinogen may be of particular importance in the context of microalbuminuria which predicts excess cardiovascular mortality in healthy subjects [53] and is associated with insulin resistance in non-diabetic individuals [54]. Several studies have demonstrated a consistent increase in plasma fibrinogen in both type 1 and type 2 subjects with microalbuminuria [55-57], and a recent study identified an independent association between fibrinogen and microalbuminuria in non-diabetic men [58].

**Metformin and vascular homeostasis**

The biguanides are a class of drugs, originally derived from the Goat’s rue or French Lilac, used for the management of diabetes in medieval Europe [59]. It was not until the 1950s and 60s that an awareness developed as to the specific clinical indications for the use of the biguanides and at around the same time studies appeared that suggested potential benefit in vascular disorders. The latter views were predicated on developments in vascular biology that indicated that imbalances in clot formation and lysis led to vessel occlusion and that enhancement of fibrinolytic processes could have the potential to prevent or ameliorate this process. At around this time a wide variety of pharmaceutical agents were tested for their effects on fibrinolysis and in 1965 the first report on the effects of metformin appeared in *The Lancet* [60]. In this small study in subjects with coronary artery disease, the use of metformin was associated with modest falls in cholesterol and fibrinogen and an increase in fibrinolytic activity as reflected by a global test of the fibrinolytic system, the euglobulin clot lysis time. It was concluded that the changes in fibrinolysis were due to increases in a plasminogen activator, although it was another 20 years until it was demonstrated that these changes were, in fact, due to a relative increase in activator activity due to a fall in the fibrinolytic inhibitor, PAI-1. Further work from this group confirmed the increase in fibrinolytic activity with metformin in patients with peripheral vascular disease [61] although not all studies in non-diabetic subjects were in agreement and conflicting results were obtained [62]. In 1987, Vague et al. reported that 15 days’ treatment with metformin in obese non-diabetic women led to an increase in fibrinolytic activity mediated by a fall in PAI-1 activity [63]. With some perspicacity they suggested that this effect was likely to be mediated either directly or indirectly through the effects of metformin on insulin, an hypothesis that was subsequently supported by their own in vitro work using cultured hepatic cells [64].

**Metformin and fibrinolysis in type 2 diabetes**

Several studies in type 2 diabetic subjects have reported that the use of metformin is associated with an increase in fibrinolysis [65] due to a fall in PAI-1 concentrations in plasma [66-69] in both white [67, 69] and Asian subjects from the Indian subcontinent [68] and Korea [66]. Two studies have reported an associated fall in tPA [67, 69] and two no effect [66, 68]. There is evidence that whilst the conventional risk factor response is dose dependent, this is not true for the PAI-1 response which appears to be independent of dosage [69].

**Metformin and thrombosis in type 2 diabetes**

The effects of metformin on coagulation have mostly centred on fibrinogen, presumably because of its central role in cardiovascular disease. Generally these studies have suggested either a small fall [60] or no change [61, 62, 66] in fibrinogen levels associated with metformin use. There is one study that has indicated that metformin use is associated with a reduction in coagulation FVII levels [70]. Interestingly, metformin also reduced levels of FXIII activity and FXIII A and B subunits in plasma after 12 weeks compared to placebo. Additionally, metformin altered fibrin structure/function by interfering with the processes involved in fibrin polymerisation and lateral aggregation [71]. It seems plausible that effects on clotting factor levels are mediated indirectly through altering insulin resistance. The effects on fibrin crosslinking are most likely explained by the effects of metformin on the formation of advanced glycation end products [72] as both processes share common amino acid interactions which metformin appears to interrupt.

**Metformin and platelet function**

There have been no studies that have attempted to systematically investigate the effects of metformin on platelet function. Studies of metformin and platelet function have
generally been reported as a component of other studies and have reported conflicting results. In a study of elderly type 2 diabetic patients treated with sulphonylurea or metformin, there was a reduction in levels of platelet factor 4 and beta thromboglobulin with metformin [73]. These proteins are viewed as markers of platelet activation and the results imply that metformin stabilises the platelet in some way. These findings are supported by evidence that metformin exerts an antioxidant effect on the platelet [74]. Other work, however, has not shown any effect of metformin on platelet function [68]. It remains unclear at present as to whether metformin has direct effects on platelet abnormalities related to the presence of insulin resistance [75] or whether potential beneficial effects are secondary to other changes induced by metformin.

Metformin and blood flow

Effects of metformin on blood flow are potentially of importance as the combination of increased blood flow and reduced atherothrombotic risk may preserve tissues at risk. In a small group of 15 type 2 patients treated with metformin 850 mg tds there was a significant improvement in post-ischaemic blood flow after up to 6 months treatment [76]. Metformin increases haemodynamic responses to L-arginine, the precursor of vasodilatory nitric oxide [77] an effect which may be explained by the observation that metformin lowers levels of asymmetric dimethylarginine, the endogenous inhibitor of nitric oxide synthase, in patients with type 2 diabetes [78]. The effects of metformin on blood flow seem to extend to both skeletal muscle and adipose tissue [79]. Although good evidence exists for an effect of metformin on blood flow, probably mediated through nitric oxide metabolism, there is little to support the view that this is translated into improvements in blood pressure [80].

Implications for cardiovascular risk

Metformin is an interesting pharmaceutical agent, in part because of its diversity of metabolic actions (Table I) and in part because we are still limited in our understanding of precisely how it works. Recent epidemiological studies have breathed new life into our evidence base for the management and prevention of diabetes and cardiovascular disease and remarkably metformin has been shown to both reduce micro- and macrovascular disease [81] and the conversion to type 2 diabetes of subjects with impaired glucose tolerance [82]. The results of the UKPDS study in overweight type 2 diabetic patients [81] indicated that the use of metformin was associated with a reduction in cardiovascular mortality compared to insulin or sulphonylureas. As all three therapies were associated with equivalent improvements in glycaemic control, these findings suggest that metformin is exerting effects on cardiovascular risk through additional mechanisms. The insulin resistance associated with pre-diabetic conditions and with type 2 diabetes itself is characterised by atherothrombotic risk factor clustering that includes both classical (hypertension, dyslipidaemia, dysglycaemia) and non-classical (hyperinsulinaemia, prothrombotic risk) vascular risk factors. This concept has moved us away from a glucocentric view of cardiovascular complications to one in which management of multiple risk factors concurrently is of paramount importance, a view supported by the results of the recent Steno 2 study [83]. The effects of metformin on the insulin resistance syndrome are diverse and potentially explain the cardioprotective actions reported in UKPDS. Metformin has a similar effect on glycosylated haemoglobin to the sulphonylureas, but in contrast is relatively insulin sparing whilst being at worst weight neutral. Evidence points to metformin having a

Table I

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<thead>
<tr>
<th>Risk marker</th>
<th>Effect</th>
<th>Reference</th>
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<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>Marked reduction</td>
<td>65-69</td>
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<tr>
<td>Factor VII</td>
<td>Reduction</td>
<td>70</td>
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<tr>
<td>Fibrinogen</td>
<td>Equivocal, some studies report reduction, others no effect</td>
<td>60, 61, 62, 66</td>
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<td>Factor XIII</td>
<td>Reduces A and B subunit</td>
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<tr>
<td>Fibrin</td>
<td>Alters structure/function</td>
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<tr>
<td>C-reactive protein</td>
<td>Reduction</td>
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<td>Platelets</td>
<td>Reduction in platelet factor 4 and β thromboglobulin</td>
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<td>Stabilises platelet</td>
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<td>Blood flow</td>
<td>Metformin increases haemodynamic responses to L-arginine,</td>
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<td>Lowers levels of asymmetric dimethylarginine</td>
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<td>Improves post ischaemic blood flow</td>
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<td>Improves blood flow in both skeletal muscle and adipose tissue</td>
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triglyceride-lowering effect in subjects who are hypertriglyceridaemic and recent unpublished data from our unit indicates that metformin lowers C reactive protein in type 2 subjects. These effects are compatible with amelioration of plaque progression and possibly plaque stabilisation. In addition metformin enhances fibrinolysis by reducing elevated PAI-1, reduces levels of some thrombotic risk factors and appears to have direct effects on fibrin structure function whilst stabilising the circulating platelet. These latter effects are consistent with the formation of a fibrin platelet mesh that is less prothrombotic and which is more easily lysed, due to both changes in its intrinsic structure and because fibrinolysis itself is enhanced. Taken together it would appear that metformin has beneficial effects on the atherothrombotic processes that enhance vascular risk in insulin resistant subjects. The diverse effects of metformin on atherothrombotic processes probably explain the cardio-protective results from UKPDS and emphasise the importance of metformin in the management of type 2 diabetes and the prevention of vascular complications.

References


