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Insulin resistance syndrome: interaction with coagulation and fibrinolysis

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Summary

Insulin resistance represents a common metabolic abnormality leading to cardiovascular disease, the major cause of morbidity and mortality in most parts of the world. Insulin resistance is also associated with an increased risk of type 2 diabetes which is strongly associated with obesity. The insulin resistance of obese people and subjects with type 2 diabetes is characterised by defects at many levels, affecting insulin receptor concentration, glucose transport mechanisms and the activities of intracellular enzymes. Around 25% of western populations show some features of the insulin resistance syndrome (often referred to as syndrome X or the metabolic syndrome) ie, a clustering of metabolic, atheromatous risk factors, including hypertriglyceridaemia, hyperinsulinaemia, hypertension, hypercholesterinaemia and obesity. However, the known metabolic cardiovascular risk factors associated with the insulin resistance syndrome do not sufficiently explain the excess vascular risk attributed to this syndrome. The observation, that increased plasma plasminogen activator inhibitor 1 (PAI-1) levels were associated with insulin resistance and atherothrombosis added for the first time a pathological basis for an association of the insulin resistance syndrome not only with metabolic, atheromatous (atherosclerotic) risk but also with atherothrombotic risk. It is very likely that not only PAI-1, but also other abnormalities in haemostatic variables contribute to this excess vascular risk. Knowledge of how haemostatic variables cluster with classical metabolic risk factors associated with the insulin resistance syndrome could help to better understand the pathogenesis of cardiovascular diseases. Indeed, many coagulation and fibrinolytic proteins have been shown to be associated with features of the insulin resistance syndrome and these associations suggest that some coagulation and fibrinolytic proteins have a role in atherothrombotic disorders, principally through an association with other established metabolic (atheromatous) risk factors in the presence of underlying insulin resistance. Interestingly, new therapeutic approaches in the prevention and treatment of insulin resistance do show some influence on coagulation and fibrinolysis. The newest drugs are the thiazolidinediones, a totally novel class of insulin sensitisers. They have the potential to offer improvements both in glycaemic control and in cardiovascular events.

Key words: insulin resistance syndrome; coagulation; fibrinolysis; thiazolidinediones

Insulin resistance syndrome: clustering of atheromatous risk factors

Introduction

Insulin resistance can be seen as a genetic and molecular condition involving defective insulin signalling and glucose transport into cells. Insulin resistance also represents a common metabolic abnormality leading to cardiovascular disease, the major cause of morbidity and mortality in most parts of the world. In addition, insulin resistance is associated with an increased risk of type 2 diabetes, which is strongly associated with a sedentary lifestyle and obesity [1]. Diabetes mellitus is one of the main threats to human health in the 21st century [2, 3]. Around 25% of the western population show some features of the insulin resistance syndrome [4]. Over twenty years ago, Albrink et al. showed probably for the first time that a cluster of metabolic, atheromatous risk factors, including hypertriglyceridaemia, and obesity, are associated with increased risk for coronary artery disease [5]. In the 1960's, the observation that many diabetics were actually hyperinsulinaemic lead to the evolution of the definition of the insulin resistance syndrome (often referred to as syndrome X or the metabolic syndrome) and its link to both hyper-

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triglyceridaemia and coronary artery disease [6]. The observation also made in the sixties, that hypertension was often associated with hyperinsulinaemia, added more information to the subsequent definition of the insulin resistance syndrome and its components [7]. Recently, the Insulin Resistance Atherosclerosis Study (IRAS) showed a link between a direct measure of insulin resistance itself and atherosclerosis [8].

In summary, insulin resistance is not simply a problem of deficient glucose uptake in response to insulin but a multifaceted syndrome (insulin resistance syndrome) which is associated with dyslipidaemia, hyperinsulinaemia, obesity, hypertension, and atherosclerosis.

Lipids and adipose tissue

The inability of insulin-resistant fat cells to store triglyceride (TG) is possibly the first step in the development of the dyslipidaemia characteristic of insulin resistance. The phenotype of insulin resistance includes a dyslipidaemia characterised by an elevation of very low-density lipoprotein (VLDL) triglyceride, a reduction in high-density lipoprotein (HDL) cholesterol, and the presence of low-density lipoprotein (LDL) particles, which are smaller, cholesterol depleted and triglyceride-enriched [9]. Adipose tissue plays an important role in insulin resistance. Circulating free fatty-acids (FFA's) derived from adipocytes are elevated in many insulin-resistant states and it has been suggested that they contribute to the insulin resistance of diabetes and obesity by inhibiting glucose uptake, glycogen synthesis and glucose oxidation, and by increasing hepatic glucose output [10]. The association between increased circulating FFA's and insulin resistance might involve accumulation of triglycerides in muscle and liver. This could be confirmed by nuclear magnetic resonance spectroscopy, which has shown a close correlation between intramyocellular triglyceride content and whole-body insulin resistance in subjects with obesity and type 2 diabetes [11].

Hypertension

Hypertension is not as commonly associated with insulin resistance as dyslipidaemia [12]. Only about 50% of hypertensive subjects are insulin-resistant. The underlying mechanisms between the link of hypertension and insulin resistance are not fully understood. Abnormalities in blood flow and vasodilatation have been suggested [13]. Laakso et al showed that insulin given intravenously causes vasodilatation only in normal subjects but not in obese, insulin-resistant subjects and in patients with type 2 diabetes [13]. In addition, a direct relationship between plasma insulin concentration and blood pressure has been noted [4]. Based on these results, it seems reasonable to conclude that resistance to insulin-stimulated glucose uptake, glucose intolerance, and hyperinsulinaemia are characteristic of a certain proportion of patients with hypertension. These abnormalities however do not necessarily improve when hypertension is treated with common pharmacological anti-hypertensive agents.

Hyperglycaemia induced microvascular damage

Early in the course of diabetes, intracellular hyperglycaemia causes changes in blood flow and increased vascular permeability. This reflects decreased activity of nitric oxide, increased activity of angiotensin II and endothelin-1 [14]. Angiotensin II produces acute vasoconstriction, leading to an increase in blood pressure. Endothelin-1 is also an important regulator of vascular tone and has been implicated in the pathogenesis of atherosclerosis. In addition, abnormalities of extracellular matrix contribute to an irreversible increase in vascular permeability. Hyperglycaemia itself leads not only to an decreased endothelial production of nitric oxide, which represents an anti-atherogenic molecule, but also to an increased production of a potent inhibitor of fibrinolysis, namely plasminogen activator inhibitor 1 (PAI-1) [14].

What causes insulin resistance?

The insulin resistance of obese people and subjects with type 2 diabetes is characterised by defects at many levels affecting insulin receptor concentration, glucose transport mechanisms and the activities of intracellular enzymes [15].

In addition, acquired and genetic factors can also influence insulin sensitivity. Type 2 diabetes must be considered as a polygenetic disorder and may involve polymorphisms in several genes encoding the proteins involved in insulin secretion and insulin signalling [16].

Early-onset coronary artery disease. Association with insulin resistance.

Early-onset coronary heart disease (CHD) could be due to genetic or environmental factors independent of adverse changes in known conventional metabolic risk factors. However, adverse changes in features of the insulin resistance syndrome in families with early-onset CHD caused by genetic and environmental factors could explain the increased vascular risk in those subjects. Kareinen et al. determined the levels of cardiovascular risk factors among siblings with and without severe early-onset CHD drawn from 101 Finnish families [17]. Siblings with premature familial CHD had higher levels of insulin, higher levels of total and VLDL triglycerides, and lower levels of HDL cholesterol. They also had higher levels of fibrinogen, a marker for increased atherothrombotic risk. These data suggest that the clustering of features of the insulin resistance syndrome is likely to contribute to early-onset CHD in these families.

Insulin resistance syndrome: clustering of thrombotic risk factors

Introduction

Prospective and case controlled studies have indicated that many of the coagulation and fibrinolytic proteins that could theoretically contribute to a thrombotic tendency are in practice related to the presence or development of coronary artery disease. Suppression of fibrinolysis due to high plasma concentrations of PAI-1, and increased plasma concentrations of factor VII (FVII), fibrinogen and von Willebrand factor (vWF) have all been related to the development of myocardial infarction [18–21]. In addition, high concentrations of tissue plasminogen activator (t-PA) and D-Dimer, the latter a measure of fibrinolysis, are related to risk of myocardial infarction [21,22].

Already in the 1970's, haemostatic variables and their possible association with diabetes were investigated in 154 diabetic subjects [23]. Mean values for FVII, factor X (FX) and fibrinogen were higher in the diabetics compared to controls. These findings suggested a potentially important association between a thrombogenic tendency and vascular disease in diabetes. Whether some of these haemostatic abnormalities precede the onset of clinically manifest vascular complications or are a consequence of them was not known.

The observation, that increased plasma PAI-1 levels were associated with insulin resistance and atherothrombosis added for the first time a pathological basis for an association of the insulin resistance syndrome not only with metabolic, atheromatous (atherosclerotic) risk but also atherothrombotic risk [24].

The known metabolic cardiovascular risk factors associated with the insulin resistance syndrome do not sufficiently explain the excess vascular risk attributed to this syndrome, and it is therefore very likely that apart from PAI-1, other abnormalities in haemostatic variables contribute to this excess risk. Knowledge of how haemostatic variables cluster with classical metabolic risk factors associated with the insulin resistance syndrome could help to better understand the pathogenesis of cardiovascular diseases.

If markers of coagulation correlate with individual metabolic variables, this may reflect separate underlying processes or common pathological pathways. If the latter is true, haemostatic and fibrinolytic parameters should be included in the features and characterisation of the insulin resistance syndrome. Such an association would suggest that some coagulation and fibrinolytic proteins have a role in atherothrombotic disorders, principally through an association with other established metabolic (atherothrombotic) risk factors in the presence of underlying insulin resistance (figure 1).

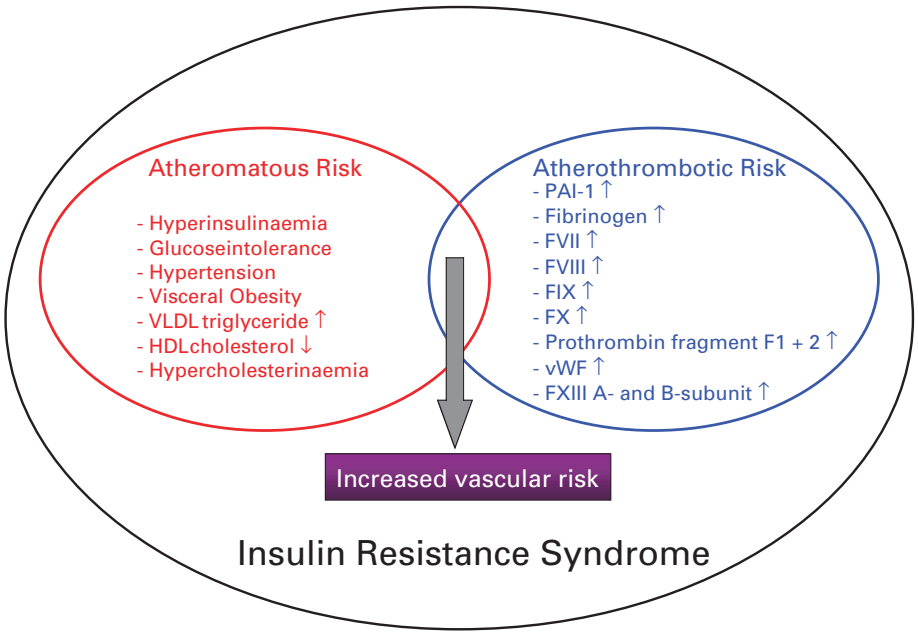


Figure 1

Insulin Resistance Syndrome. The insulin resistance syndrome is not only characterised by its clustering of metabolic cardiovascular risk factors (shown in red) but also by its association with several disorders of coagulation and fibrinolytic proteins (shown in blue).

Insulin resistance syndrome: Interaction with coagulation proteins

Vitamin K-dependent coagulation factors (FII, FVII, FIX and FX)

Sakkinen A et al. performed factor analysis on 10 metabolic risk factors associated with the insulin resistance syndrome and vitamin K-dependent coagulation proteins (FII, FVII, FIX, FX) in elderly non-diabetic men and women to examine the clustering of the metabolic and haemostatic risk markers [25]. The following haemostatic variables were determined: FVII:C (clotting activity), FIX:C, FX:C, and prothrombin fragment F1+2. Markers of thrombin activity (prothrombin fragment F1+2) were not related to metabolic factors. Factor VII:C,IX:C and X:C were strongly associated with triglycerides. The association between triglycerides and factor VII:C may be due to a role of triglyceride-bearing lipoproteins as procoagulant surfaces [26]. It has been shown that FVII concentrations are elevated in subjects with non-insulin-dependent diabetes mellitus (NIDDM) and the concentrations are in relation to features of insulin resistance [27]. In this population, cholesterol, insulin and gender remained as independent predictors of FVII levels [27]. Increased levels of FVII could also be shown in first degree relatives of patients with NIDDM [28]. There was also a strong association of FVII levels and features of insulin resistance [28]. An association of insulin resistance with prothrombin fragment F1+2 concentrations could also be shown in first-degree relatives of type 2 diabetic patients [29]. In addition, FVII activity has been shown to increase during postprandial hyperlipidaemia, suggesting a risk for acute CAD events after consumption of a high-fat meal [30]. Little data is available about the anticoagulatory proteins, protein C and protein S. However, one study was able to show that insulin sensitivity was negatively associated with protein S and protein C concentrations [31]. Both proteins were also associated with PAI-1 activity. This may represent a mechanism, which counteracts the concomitant hypofibrinolysis, which occurs in insulin-resistant subjects.

Fibrinogen activation products, fibrin degradation products, fibrinogen and fibrin

Sakkinen A et al. also analysed a marker of fibrinogen activation (fibrinopeptide A), and D-Dimer as a marker for fibrin degradation through activation of endogenous fibrinolysis ie, plasminogen activation and subsequent fibrin degradation. However, fibrinopeptide A and D-Dimer showed no relation to metabolic risk factors [25].

The formation of stable cross-linked fibrin is the ultimate step in coagulation processes. Fibrin therefore plays an important role in the pathogenesis of atherothrombotic disorders. In advanced arteriosclerotic coronary lesions, platelet-fibrin thrombi form on ruptured plaques, leading to unstable angina and myocardial infarction [32]. Fibrin gels formed in plasma of patients with myocardial infarction have tighter and more rigid network structures than do those formed in plasma from control subjects [33]. Fibrinogen levels showed a weak correlation with fasting glucose levels and high density lipoprotein cholesterol levels [25]. Fibrinogen levels are also increased in first degree relatives of patients with NIDDM and are associated with features of insulin resistance [28].

Folsom et al. analysed which characteristics might influence fibrinogen levels with data from the Atherosclerosis Risk in Community Study (ARIC), a prospective study designed to assess risk factors for the development of atherosclerotic diseases, obtained from over 12'000 men and women, aged 45–64 years [34]. In general, fibrinogen levels increased with age, smoking, body size, diabetes, fasting serum insulin, LDL cholesterol, lipoprotein (a), leukocyte count, and menopause, and decreased with ethanol intake, physical activity, HDL cholesterol, and female hormone use [34]. These data again indicate, that some features of the insulin resistance syndrome such as diabetes, fasting serum insulin concentrations and dyslipidaemia increase fibrinogen levels with increased vascular risk. The influence of fibrinogen on the risk of cardiovascular diseases and the development of diabetes was also investigated prospectively [35]. Diabetics had higher levels of fibrinogen, hypertension, hypertriglyceridaemia, and obesity, but lower HDL cholesterol values [35]. Again, the increased fibrinogen levels represented a thrombogenic component to the metabolic risk factor clustering.

FVIII and von Willebrand factor

Levels of vWF cluster weakly with risk factors associated with insulin resistance in first degree relatives of patients with NIDDM [28] but are not elevated compared to healthy control subjects. However, vWF concentrations are elevated in patients with NIDDM compared to controls but are also only weakly related to features of the insulin resistance syndrome. Age and insulin levels remained as independent predictors of vWF levels [36]. As part of the ARIC study, baseline measurements of FVIII and von Willebrand factor (vWF) were performed to determine their relationship to the development of atherosclerosis [37]. Both factors were positively associated with diabetes, body mass index (BMI), waist-to-hip ratio, serum insulin, and plasma triglycerides, all markers of the insulin resistance syndrome. The Framingham Offspring Study evaluated associations between fasting insulin levels and haemostatic factors in subjects with normal and impaired glucose homeostasis. Mean levels of vWF as well as FVII, PAI-1 and t-PA increased across fasting insulin quintiles among subjects with normal glucose tolerance but among subjects with glucose intolerance, only vWF, PAI-1 and t-PA increased, but not FVII [38].

Factor XIII (FXIII)

South Asians have increased morbidity and mortality from cardiovascular disease and an increased prevalence of insulin resistance. Kain et al suggested, that elevated PAI-1 and fibrinogen in Asians of both genders may contribute to the increased vascular risk experienced in this population [39]. Warner et al. showed in the same population, that FXIIIB-Subunit levels correlated with waist:hip ratio, HbA1c, fasting triglycerides, total cholesterol and PAI-1 antigen. These results suggest an underlying association of FXIII B-subunit antigen levels with insulin resistance [40]. It has been recently shown that possession of a common polymorphism in the FXIII gene (FXIIIVal34Leu) is protective against myocardial infarction suggesting for the first time a role of FXIII in thrombotic disorders [41–44]. Subjects with the protective Leu allele, who nonetheless had a history of MI were further investigated [41,45]. These subjects had higher concentrations of PAI-1, insulin, proinsulin, and an increased BMI, changes which were not observed in subjects possessing the Val/Val genotype [46]. These findings indicated that inhibition of fibrinolysis through increased PAI-1 levels negates the protective effect of the Leu allele and suggest an interaction with insulin resistance. Levels of FXIII A- and B-subunit antigen are elevated in subjects with type 2 diabetes, and levels of FXIII A-subunit antigen are also elevated in relatives of subjects with type 2 diabetes [47]. In addition, levels of the FXIII B-subunit antigen (carrier protein) show a consistent pattern of correlation with other vascular risk markers, which supports the possibility of an underlying association with the insulin resistance syndrome [47]. These new findings regarding FXIII also shed light on the interaction between the haemostatic system and metabolic features of the insulin resistance syndrome. In addition, the relation between FXIII and classical metabolic risk factors helps further explain the association between insulin resistance and the risk of thrombosis.

Insulin resistance syndrome: interaction with fibrinolytic proteins

Plasmin-α_2-antiplasmin

In elderly non-diabetic men and women, plasmin-α_2-antiplasmin complexes showed significant associations with weight, fasting insulin and fasting glucose levels [25]. Data on the role of this complex in insulin-resistant states are limited. However the study suggests that markers of the insulin resistance syndrome correlate with these complexes which lead to inhibition of fibrinolysis.

Plasminogen activator inhibitor-1 (PAI-1). Regulation of PAI-1 transcription by peroxisomal proliferator-activated receptor-gamma (PPAR-γ)

The importance of the fibrinolytic system as a major regulator of fibrin deposition in the vessel wall raises the role of perturbations in this system in the development of vascular disease. A decrease in fibrinolysis for example due to elevated PAI-1 might be expected to result in an increase in fibrin deposition and subsequent thrombus formation. In practice high plasma PAI-1 levels are indeed associated with various thrombotic disorders [48–51]. There is an association between the presence of coronary artery disease and low plasma fibrinolytic activity due to increased

PAI-1 [52]. In the prospective Northwick Park Heart study there was a strong, long-term relation between low fibrinolytic activity and the incidence of coronary artery disease, suggesting that low fibrinolytic activity preceded heart disease [53].

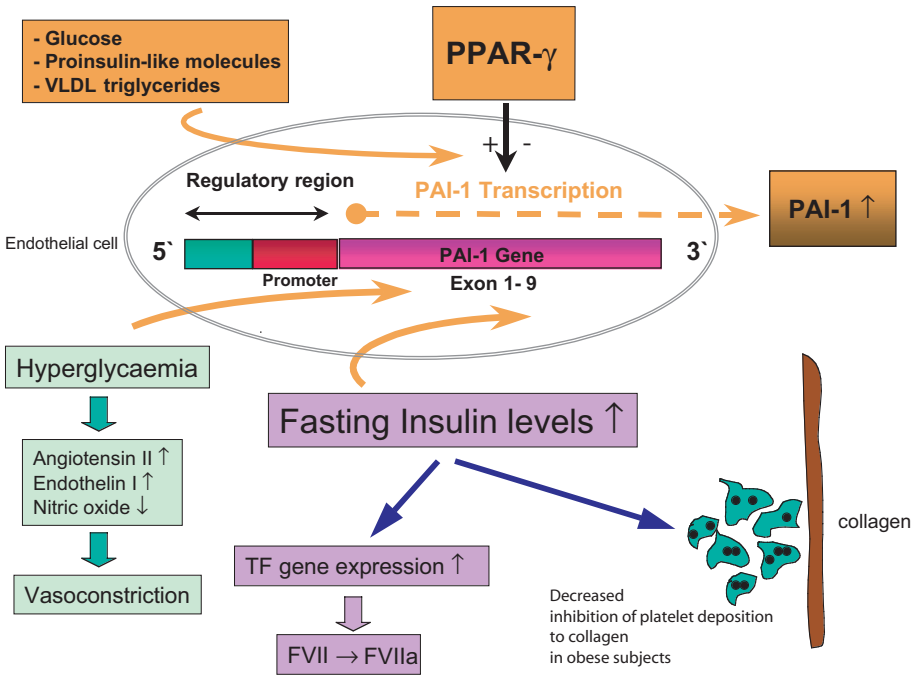
Patients with insulin resistance, whether they have normal glucose tolerance or diabetes, have increased plasma PAI-1 concentrations and global suppression of fibrinolysis [54]. Interventions that lower insulin resistance such as weight loss are invariably accompanied by a reduction in plasma PAI-1 concentrations [55]. Clinical studies indicate that patients with coronary artery disease are both insulin-resistant [56] and have increased PAI-1 plasma concentrations [19, 51, 57]. These findings imply a role for PAI-1 in atherothrombotic disorders, principally through clustering with other established risk markers in the presence of underlying insulin resistance.

Both glucose and insulin increase the synthesis and secretion of PAI-1 in human vascular endothelial and vascular smooth muscle cells in vitro [58, 59]. Clinically, improved control of hyperglycaemia has been shown to decrease plasma PAI-1 activity in patients with type 2 diabetes [60].

Incubation of endothelial cells with VLDL increases PAI-1 synthesis and secretion. A putative VLDL response element, recently identified in the gene for PAI-1, may be responsible for this induction [61]. High levels of VLDL stimulate increased PAI-1 synthesis and secretion by cultured human endothelial cells. This transcriptional regulation of PAI-1 expression by VLDL has been shown to be genotype-specific (PAI-1 4G/5G promoter polymorphism) [62]. The effect of diabetes on PAI-1 levels in the arterial wall has been investigated recently. Blood samples and small tissue specimens from the mammary artery were obtained from 11 diabetic and 10 non-diabetic subjects who underwent coronary artery bypass graft surgery [63]. PAI-1-related immunofluorescence was increased in the arterial wall of diabetic subjects and plasma fibrinolytic activity was reduced. This study provided evidence that diabetes is as-

Figure 2

Features of insulin resistance: Interaction with PAI-1 and TF gene expression, platelet activation and vascular tone. Glucose, proinsulin-like molecules and VLDL triglycerides increase PAI-1 transcription in endothelial cells and therefore lead to increased PAI-1 plasma concentrations. Peroxisomal proliferator-activated receptor-gamma (PPAR-γ) may also regulate PAI-1 expression in human endothelial cells. Hyperglycaemia not only induces PAI-1 transcription but also leads to an increase in angiotensin II and endothelin I and a decrease in nitric oxide, therefore leading to vasoconstriction. Increased fasting insulin levels induce tissue factor (TF) gene expression and a decrease in platelet inhibition, both leading to a procoagulant state. Tissue factor activates FVII to FVIIa. This initiates the coagulation process of the extrinsic pathway.



sociated with increased PAI-1 expression in the arterial wall. Taken together, PAI-1 is today considered as a very strong marker of insulin resistance [64].

For a long time, the regulation of PAI-1 transcription by endothelial cells, a major source of PAI-1, was not fully understood. Peroxisomal proliferator-activated receptor-gamma (PPAR-γ) is a ligand-activated transcription factor that regulates gene expression in response to various mediators [65]. It could be shown that

PPAR-γ may regulate PAI-1 expression in human endothelial cells (figure 2). Activators of PPAR-γ such as thiazolidinediones can decrease basal and tumour necrosis factor (TNF)-alpha-stimulated PAI-1 secretion and mRNA expression in human umbilical cord vein endothelial cells (HUVEC) [65].

Insulin resistance syndrome: interaction with platelets

Human platelets have insulin receptors that participate in the regulation of platelet function [66]. Studies in vivo as well as in vitro have demonstrated that insulin inhibits platelet aggregation in healthy non-obese subjects [67, 68]. How insulin regulates platelet activation in non-obese and obese subjects has recently been investigated [69]. In vivo insulin infusion inhibited platelet deposition on collagen in non-obese subjects but failed to do so in the obese subjects. Furthermore, only in non-obese subjects did insulin significantly increase platelet cGMP concentrations, contributing to the inactivation of platelets after adhesion. These data demonstrate that normal in vivo insulin action inhibits platelet interactions with collagen. These platelet-inhibiting actions of insulin are absent in obese subjects and could therefore provide a further mechanism linking insulin resistance to atherothrombotic disease (figure 2).

Relationship of insulin and its precursors to markers of coagulation and fibrinolysis across different states of glucose tolerance

It has been suggested that proinsulin rather than insulin might determine PAI-1 expression in diabetic and non-diabetic subjects [70] (figure 2). Proinsulin has been related to cardiovascular risk factors, such as hypertension and dyslipidaemia, in diabetic and non-diabetic subjects [71, 72]. However, there were few data available on the association of proinsulin and/or its breakdown products with the haemostatic system, especially in subjects with impaired glucose tolerance or type 2 diabetes. Festa et al. investigated the relationship of insulin and its precursors (intact proinsulin, proinsulin breakdown products) to markers of coagulation and fibrinolysis in a large population across a range

of different states of glucose tolerance [73]. PAI-1 antigen levels were significantly different with varying glucose tolerance status (normal, impaired and type 2 diabetes) irrespective of age, sex and ethnic group. The highest levels were found in subjects with type 2 diabetes. Insulin and proinsulin (breakdown products) independently and significantly contribute to PAI-1 levels. Fibrinogen levels also increased with impairment of glucose tolerance in subjects with normal glucose tolerance (NGT), impaired glucose tolerance and type 2 diabetes. Fibrinogen levels were positively related to insulin and its precursors and the relationship was strongest in subjects with NGT and weaker or absent in subjects with impaired glucose tolerance and type 2 diabetes [73]. This data may have important clinical implications in the risk assessment and prevention of macrovascular disease, not only in patients with overt diabetes but also in non-diabetic subjects who are "only" hyperinsulinaemic.

Tissue factor gene expression in obesity

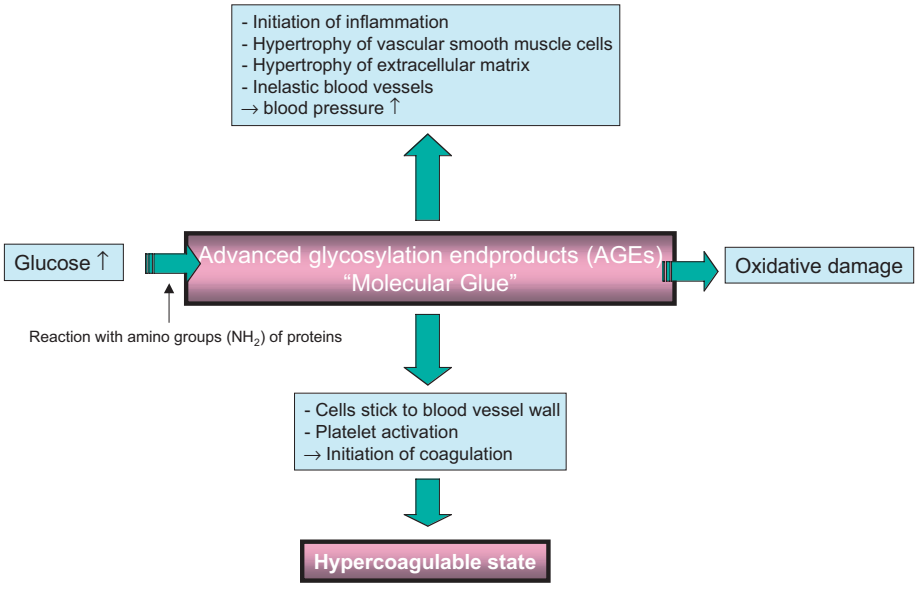
Tissue factor (TF) is a cellular initiator of the coagulation cascade and represents an initiator of the extrinsic coagulation pathway through activation of FVII. A recent study examined changes in TF mRNA in various tissues from lean and obese mice [74]. Administration of insulin to lean mice induced TF mRNA in the kidney, brain, lung, and adipose tissue suggesting that hyperinsulinaemia associated with insulin resistant states, such as obesity and non-insulin-dependent diabetes mellitus, may induce local TF gene expression in multiple tissue (figure 2). The elevated TF possibly also contributes to the increased risk of atherothrombotic disease that accompanies insulin-resistant states.

Advanced glycosylation end-products (AGEs): Influence on atherogenesis and thrombosis

The molecule glucose (D-glucopyranose) is notably unreactive and therefore an excellent reason for evolution to have chosen it to be an important cellular fuel. However, it can become harmful through its transformation into advanced glycosylation end-products (AGEs) [75–77]. This reaction requires no enzymatic catalysis. The six-carbon ring spontaneously opens, converting into a linear molecule, which reacts with an amino (NH_2) group of a protein. One good example is the formation of haemoglobin A_{1C} in which glucose has hooked itself onto the β-chain of haemoglobin. Other macromolecules such as collagen and lipids are subject to analogous modifications, especially in insulin-resistant states such as diabetes. As AGEs, glucose becomes a molecular glue that initiates inflammation leading to hypertrophy of vascular smooth muscle cells and extracellular matrix and makes blood vessels inelastic and stenotic. It appears, that glycosylation of proteins enhances their potential for oxidative damage [78]. Collagen molecules in vessels offer numerous amino groups

Figure 3

AGEs: Initiation of vascular remodelling and coagulation. Advanced glycosylation endproducts (AGEs) act as a molecular glue. This initiates many adverse reactions leading to inflammation, cellular remodelling, oxidative damage and hypercoagulability.



for formation of AGE's. Normally, collagen molecules slide freely over one another, and vessels dilatation as well as constriction can take place. After formation of AGEs, vessels become stiffened and inelastic leading to high blood pressure. AGE accumulation causes circulating molecules and cells to stick to the blood vessel wall [79]. This reaction can also include platelets leading to platelet activation and initiation of blood coagulation. Through these processes, AGEs can lead to hypercoagulable states and therefore increased thrombotic risk (figure 3). It is interesting to note, that biguanides were the first antihyperglycaemic drugs designed to inhibit glycosylation reactions. Dimethylbiguanide (also known as metformin) reduces the risk of cardiovascular complications in type 2 diabetes. The process of glycosylation-related protein cross-linking is similar to fibrin cross-linking catalysed by activated FXIII. Standeven et al. therefore investigated whether the cardio-protective effect of dimethylbiguanide could be related to effects on clot stabilisation [80]. Thrombin-induced cleavage of the activation peptide from FXIII was inhibited in a dose-dependent manner. Fibre thickness and pore size of fibrin clots decreased significantly with dimethylbiguanide. These results suggest that dimethylbiguanide interferes with blood coagulation FXIII activation and fibrin polymerisation and provides further evidence for an interaction between features of insulin resistance and blood coagulation. Newer drugs are also under investigation. They are called "AGE-breakers" and do not prevent AGE formation but disrupt AGEs that have already formed [77]. It has been shown that concentration of AGE proteins are increased in the arterial wall of diabetics compared with matched non-diabetics [81].

Therapeutic approaches in the prevention and treatment of insulin resistance with influence on coagulation and fibrinolysis

Some oral anti-diabetic agents go far beyond glucose-level lowering. Such effects include lipid modifying actions, antithrombotic and profibrinolytic properties, and direct action at the level of the vessel wall to improve endothelial function or prevent smooth muscle cell hyperplasia (figure 4). Particularly newer oral anti-diabetic agents promise through such mechanisms a reduction of cardiovascular complications in insulin-resistant states. The newest drugs are the thiazolidinediones, a totally novel class of insulin sensitisers. They have the potential to offer improvements both in glycaemic control and in cardiovascular events.

Biguanides (metformin)

As an insulin sensitiser, metformin acts predominantly on the liver, where it suppresses glucose release. The effects of weight change and metformin on fibrinolysis and the vWF in obese non-diabetic subjects have been investigated [82]. The subjects were randomly allocated to a 1-year treatment with metformin or placebo, in addition to diet and exercise recommendations. Metformin did not have any significant additional effect on PAI-1 decrease, which occurred mainly in subjects who lost weight. But there was a significantly greater decrease in t-PA and vWF antigen levels in the metformin group than in the placebo group,

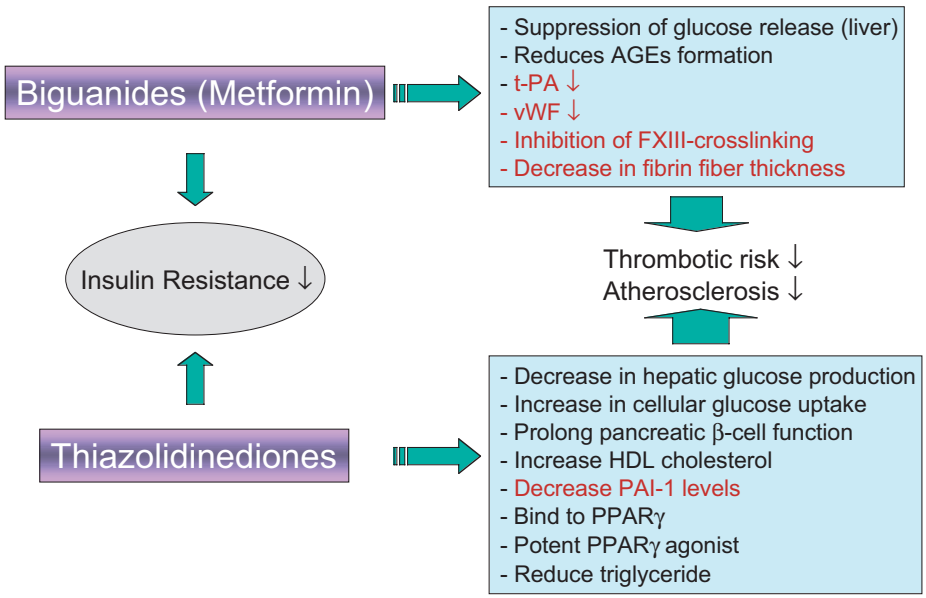


Figure 4

Therapeutic approaches in the prevention and treatment of insulin-resistant states: Influence on coagulation and fibrinolysis. Biguanides and Thiazolidinediones go far beyond glucose-level lowering. Such effects include lipid modifying actions, reduction in advanced glycosylation endproducts (AGEs) formation and profibrinolytic and antithrombotic properties (shown in red).

factors which are mainly secreted by endothelial cells. As mentioned above, dimethylbiguanide interferes with blood coagulation FXIII activation and fibrin polymerisation leading to decreased fibre thickness and pore size of fibrin clots [80].

In a very recent study, 3234 non-diabetic subjects with elevated fasting and post-load plasma glucose concentrations were randomly assigned to placebo, metformin, or a lifestyle-modification programme [83]. The average follow-up was 2.8 years. The lifestyle intervention reduced the incidence of diabetes by 58 percent and metformin by 31 percent as compared with placebo. This study concluded, that lifestyle changes and treatment with metformin both reduce the incidence of diabetes in subjects with increased risk and that lifestyle intervention was more effective than therapy with metformin.

Thiazolidinediones: ligands for the nuclear receptor, PPARγ

Thiazolidinediones bind to peroxisome proliferator-activated receptor-γ (PPARγ), a member of a family of intranuclear DNA-binding proteins [84]. In fact, thiazolidinediones are very potent PPARγ agonists. A given thiazolidinedione's affinity for PPARγ correlates with its glucose-lowering potency by an increase in cellular uptake of glucose into skeletal muscle. They are also useful for patients with type 2 diabetes because they decrease hepatic glucose production and prolong pancreatic β-cell function by preventing apoptosis of β-cells [84]. Thiazolidinediones such as rosiglitazone and pioglitazone have been shown to increase HDL cholesterol and reduce triglycerides [85]. In addition, rosiglitazone has been shown to decrease blood pressure in humans with diabetes [86] and to decrease circulating PAI-1 and C-reactive protein (CRP) levels in patients with diabetes [87, 88]. Their potential protective effect on β-cell function and on the development of macrovascular complications is of particular interest. However, thiazolidinediones share as major undesirable effects a risk of peripheral oedema, of anaemia due to plasma volume expansion and of weight gain due to the development of subcutaneous adipose tissue [89, 90]. Although the increase adiposity is paradoxical to an improvement in insulin sensitivity, this change should be viewed in context of the qualitative changes in adipose tissue, including the remodelling of adipocytes to a smaller size with higher lipid storage potential [91]. This shift in energy balance is likely to result in lower circulating free fatty acid levels, improving insulin sensitivity. Synthetic non-thiazolidinediones which also serve as PPARγ ligands are currently under investigation. These newer ligands should be able to separate the problem of weight gain and improvement in insulin sensitivity [92]. In summary, thiazolidinediones improve the metabolic, vasoactive, inflammatory and thrombogenic milieu to potentially retard the atherosclerotic process and reduce insulin resistance and components of the insulin resistance syndrome. PPAR receptors are exciting targets for therapeutic compounds likely to impact on insulin sensitivity, lipid and glucose homeostasis and vascular disease. The future certainly looks promising in this area.

Conclusion

It is important to realise that insulin resistance is not only associated with classic metabolic cardiovascular risk factors such as glucose intolerance, hyperinsulinaemia, dyslipidaemia, hypertension, and visceral obesity, but also with several disorders of coagulation and fibrinolysis. The understanding of these complex interactions allows treatment to be focussed not only on hyperglycaemia but also on a more aggressive treatment of other cardiovascular risk factors. Newer oral anti-diabetic agents show therefore lipid modifying actions, antithrombotic and profibrinolytic properties, and direct action at the level of the vessel wall. All the clinical studies mentioned above point at an increasing awareness of the role of insulin-resistant states in disturbances of coagulation and the fibrinolytic pathway. Excess risk for cardiovascular disease associated with the insulin resistance syndrome may be mediated in part by enhanced potential for acute thrombosis through hypercoagulability. This link between atheromatous, atherosclerotic (metabolic) risk factors and atherothrombotic risk factors explains the complex pathophysiological processes leading to plaque formation, plaque rupture and subsequent formation of blood clots leading to thrombotic diseases such as myocardial infarction and ischaemic stroke. The original description of the insulin resistance syndrome by Reaven in 1988 [6] proposed a clustering of atheromatous risk factors in insulin-resistant states. Elevated concentrations of many coagulation factors and inhibitors of fibrinolysis in insulin-resistant subjects and the association of these coagulation factors with features of insulin resistance now also indicate the presence of a thrombotic component to this syndrome.

In summary, a large segment of the adult population of industrialised countries develops insulin resistance, produced by genetic, hormonal and lifestyle factors such as physical inactivity, and obesity because of certain nutrient excesses. This disease is not only characterised by the clustering of hyperinsulinaemia, dyslipidaemia (atherogenic lipid profile), hypertension, glucose intolerance, visceral obesity, type 2 diabetes but also by abnormalities of blood coagulation and fibrinolysis. Be-

cause patients with the insulin resistance syndrome accumulate all these cardiac risk factors, special attention should be given in terms of diagnosis and treatment.

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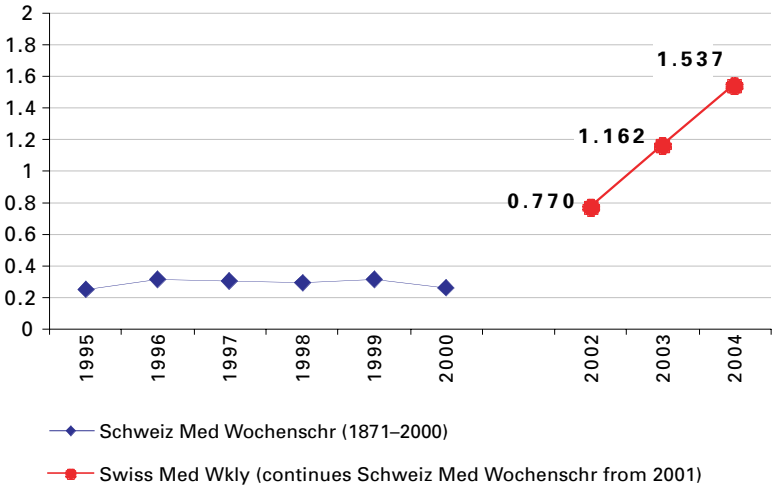

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