Cardiovascular Disease in Type 2 Diabetes From Population to Man to Mechanisms

The Kelly West Award Lecture 2008

MARKKU LAAKSO, MD

Epidemic of diabetes, affecting about 3–5% of Western populations, is one of the main threats to human health in the 21st century (1). Changes in the human environment, behavior, and lifestyle have resulted in a dramatic increase in the incidence and prevalence of diabetes in people with genetic susceptibility to diabetes. The global number of people with diabetes was 151 million in 2000, and it is projected to increase to 221 million in 2010 (an increase of 46%) both in developed and developing countries (2).

Chronic hyperglycemia leads to many long-term complications in the eyes, kidneys, nerves, heart, and blood vessels. Individuals with pre-diabetes, undiagnosed type 2 diabetes, and long-lasting type 2 diabetes are at high risk of all complications of macrovascular disease, coronary heart disease (CHD), stroke, and peripheral vascular disease. More than 70% of patients with type 2 diabetes die of cardiovascular causes (3). Therefore, the epidemic of type 2 diabetes will be followed by an epidemic of diabetes-related cardiovascular disease (CVD).

Over the years, epidemiological studies have produced important information on the prevalence and incidence of diabetes complications in different populations. They have also given important information on different risk factors determining susceptibility to diabetes complications (Fig. 1). This information is crucial for mechanistic studies in physiology at the tissue level and for molecular biology studies at the cellular level. A good example is glycated hemoglobin. Several studies have indicated that glycated hemoglobin is associated with diabetes complications in prospective epidemiological studies. That informa-

tion has been crucial for the planning of clinical trials to test the hypothesis that the treatment of chronic hyperglycemia leads to reduction in long-term diabetes complications. Moreover, information from epidemiology has led to several mechanistic studies and the elucidation of molecular level insights how insulin resistance and hyperglycemia lead to diabetes complications.

Defining the problem: type 2 diabetes and pre-diabetes increase the risk of CVD

The risk of CVD mortality in type 2 diabetic patients is more than double compared with that in age-matched subjects. Stroke events and all manifestations of CHD, myocardial infarction (MI), sudden death, and angina pectoris are at least twofold more common in patients with type 2 diabetes than in nondiabetic individuals (4). A high proportion of patients with type 2 diabetes die after an acute MI within 1 year, and a considerable number of patients die outside the hospital (5). Relative risk for CHD events is higher in female patients with type 2 diabetes than in male patients with type 2 diabetes. The reason for the sex difference is largely unknown but could be at least in part explained by a heavier risk-factor burden and a greater effect of blood pressure and atherogenic dyslipidemia on the risk of CVD in diabetic women than in diabetic men (6).

The prognosis of patients with type 2 diabetes is highly dependent on the presence of CVD. We compared the 7-year incidence of fatal and nonfatal MI among 1,373 nondiabetic subjects with the incidence among 1,059 subjects with type 2 diabetes (7). Our study suggested that pa-

tients with type 2 diabetes without previous MI have as high a risk of MI as nondiabetic patients with previous MI. Thus, our results indicated that type 2 diabetes is a "coronary heart disease equivalent." These results were recently replicated by a 18-year follow-up study of our original cohort (8) (Fig. 2) and by a Danish study including 3.3 million subjects (9).

One of the paradoxes in the studies of cardiovascular complications in type 2 diabetes is that at diagnosis individuals with type 2 diabetes already have substantially increased prevalence of CHD and stroke (4). Although a part of this risk could be attributed to asymptomatic hyperglycemia, fulfilling the criteria for diabetes years before diagnosis, it is unlikely that this could explain the increased risk of CVD because duration of diabetes is not a very strong risk factor for CVD in subjects with type 2 diabetes. In agreement with these results are the findings from the UK Prospective Diabetes Study (UKPDS) (10). At low glycated hemoglobin levels, even in the normal range, the risk of CHD events was substantially increased compared with the risk of retinopathy, indicating that risk factors other than hyperglycemia must explain increased CHD events. Studies in pre-diabetic individuals give further evidence. Several studies show that subjects with impaired glucose tolerance (IGT, 2-h plasma glucose levels 7.8–11.0 mmol/l) or impaired fasting glucose (IFG, plasma glucose 5.6–6.9 mmol/l) have about twofold higher risk for CVD events than normoglycemic subjects (11).

The Whitehall study was the first to show an increased risk of CVD when the 2-h level exceeded 5.5 mmol/l (12). A meta-analysis of 20 studies including 95,783 nondiabetic individuals followed for 12.4 years showed that high fasting, 1-h, and 2-h glucose levels increased the risk for CVD events (11). The DECODE (Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe) study analyzed 10 prospective European cohort studies including 15,388 men and 7,126 women (13). The relationship be-
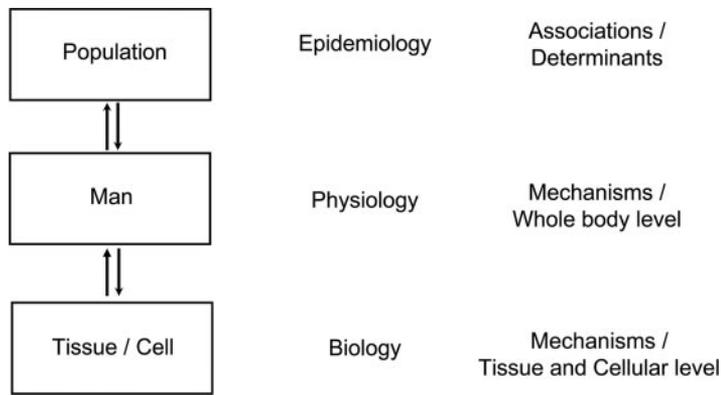

Figure 1—*Different approaches in studies of cardiovascular complications in type 2 diabetes.*

tween glycemia and CVD mortality already was observed within the normal glucose range and exhibited a linear relationship without any indication of a threshold effect.

Pre-diabetes—key for the understanding of cardiovascular complications in type 2 diabetes

Impaired insulin action (insulin resistance) in combination with impaired insulin secretion is a major pathophysiological mechanism leading to elevated fasting or postprandial glucose in pre-diabetic individuals. Impaired insulin action is observed in several tissues, particularly in skeletal muscle, adipose tissue, endothelium, and the liver. Compensatory hyperinsulinemia is often seen in pre-diabetic individuals because the pancreas compensates for insulin resistance in peripheral insulin-sensitive tissues.

Pre-diabetes is a heterogeneous entity. Both IFG and IGT are characterized by insulin resistance. Previous small studies demonstrated that individuals with IGT have a more pronounced degree of insulin resistance, whereas individuals with IFG are characterized by a more pronounced β-cell defect when related to the ambient glucose levels and the degree of insulin sensitivity (14,15). We recently carried out a study including almost 1,000 offspring of type 2 diabetic patients and showed that participants with isolated IFG had impaired basal insulin secretion, reduced first-phase insulin response, and reduced insulin sensitivity compared with normoglycemic individuals (16). Subjects with IFG also have reduced hepatic sensitivity to insulin (17). In contrast, a characteristic finding in subjects with isolated IGT is increased insulin resistance (16).

Several studies have provided evidence that high insulin level is associated with risk of CHD in nondiabetic subjects in prospective population studies (summarized in 18). We were the first to show that insulin resistance per se is directly associated with atherosclerosis, even in normoglycemic subjects. We measured insulin sensitivity by the euglycemic-hyperinsulinemic clamp and asymptomatic atherosclerosis with ultrasound method in the femoral or carotid arteries (19). Healthy nonobese subjects without any medication but who had atherosclerotic plaques and corresponding control subjects without signs of atherosclerosis were included in the study. Subjects with asymptomatic atherosclerosis exhibited an approximate 20% decrease in insulin-mediated glucose. Our study provides evidence that the primary events responsible for atherothrombosis could be related to insulin resistance per se.

Changes in cardiovascular risk factors in pre-diabetes and type 2 diabetes

Mechanisms linking pre-diabetes and type 2 diabetes with CVD remain poorly understood. Both of these conditions share insulin resistance in several tissues, and when frank hyperglycemia develops there are several potential mechanisms for high glucose to increase the risk of atherothombosis (20). Pre-diabetic subjects often have a clustering of different CVD risk factors, insulin resistance, obesity, central obesity, elevated blood pressure, elevated total triglycerides, and low HDL cholesterol. Therefore, it is unclear whether hyperglycemia per se in the nondiabetic range is causally associated with the risk of CVD. Type 2 diabetic patients are at least as insulin resistant as pre-diabetic subjects. Therefore, insulin resistance–related risk factors in the pre-diabetic state and insulin resistance–related and hyperglycemia–related risk factors in type 2 diabetes are likely to explain a major part of enhanced atherothrombosis in these conditions (Fig. 3).

Metabolic changes in pre-diabetes include impaired endothelial function, sub-clinical inflammation (21), changes in adipokines, development of atherogenic dyslipidemia, increased levels of free fatty acids (FFAs), and changes in thrombosis and fibrinolysis (22).

Impaired endothelial function

The earliest finding in the pathogenesis of atherosclerotic lesions is impaired endothelial function, which is tightly linked to insulin resistance. We demonstrated that insulin-stimulated increase in leg glucose disposal and blood flow were coupled in a dose-dependent manner (23,24). The va-
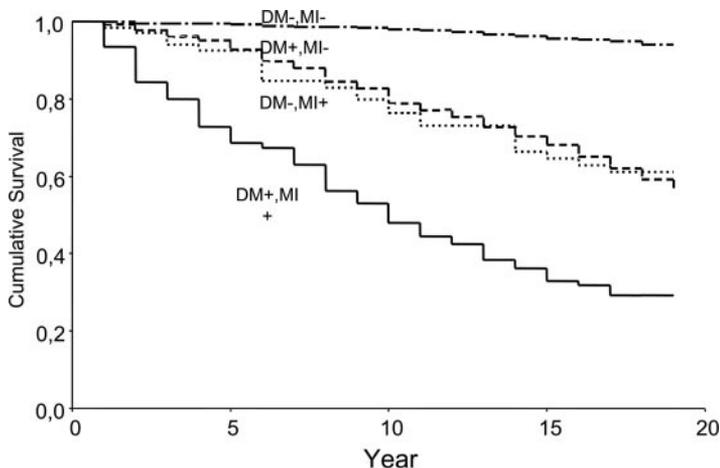

Figure 2—*Incidence per 1,000 person-years and Cox model hazard ratio for CHD death (adjusted for age, area of residence, and sex) during the 18-year follow-up according to the presence of diabetes and prior evidence of MI in 1,373 nondiabetic and 1,059 diabetic subjects. Adapted from ref. 8.*
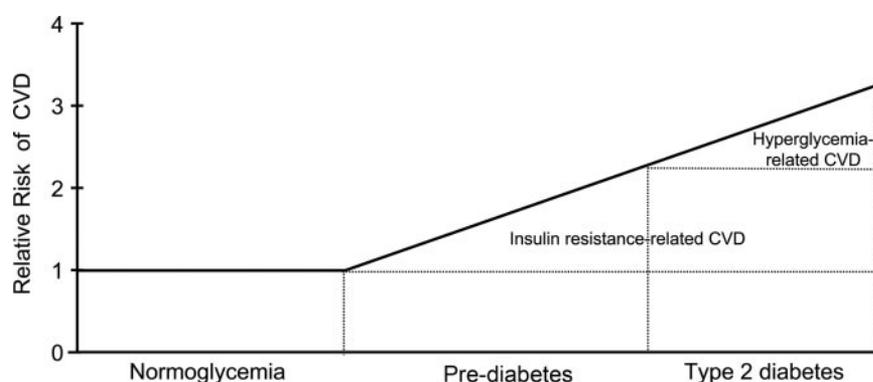
Figure 3—*Relative risk of CVD in normoglycemia, pre-diabetes, and type 2 diabetes.*

sodilatory action of insulin is dependent on nitric oxide (NO) generation, since blocking insulin-induced increases in blood flow with NO synthase inhibitor N^G-monomethyl-L-arginine diminished both blood flow and glucose uptake (25). Thus, vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action (26). Indeed, NO-dependent increases in blood flow to skeletal muscle could account for 25 to 40% of the increase in glucose uptake in response to insulin stimulation (27).

As shown in Fig. 4 phosphatidylinositol 3-kinase (PI 3-kinase)-dependent insulin signaling pathways in the endothelium related to the production of NO share striking similarities with metabolic pathways in skeletal muscle that promote glucose uptake. Insulin-stimulated glucose uptake requires PI 3-kinase–dependent signaling pathways that involve insulin re-

ceptor substrate 1 (IRS-1), PI 3-kinase, phosphoinositide-dependent kinase 1 (PDK-1), Akt, and downstream effectors to contribute to insulin-stimulated translocation of insulin-responsive GLUT4. PI 3-kinase activation is necessary but not sufficient for insulin-stimulated production of NO, resulting in vasodilatation. Shc/Ras/mitogen-activated protein (MAP) kinase pathway is a distinct nonmetabolic branch of the insulin-signaling pathway regulating secretion of the vasoconstrictor endothelin-1, one of the most potent vasoconstrictors, and vascular cell adhesion molecule 1 (VCAM-1) in endothelium as well as growth and mitogenesis. Metabolic insulin resistance is characterized by pathway-specific impairment in PI 3-kinase–dependent signaling induced, e.g., by proinflammatory cytokines (tumor necrosis-α [TNF-α], interleukin [IL]-1β, IL-6, C-reactive protein [CRP]), which in the endothelium may cause imbalance between

production of NO and glucose uptake, resulting in insulin resistance and endothelial dysfunction.

Hyperglycemia inhibits production of NO, leads to elevated FFA levels due to impairment in insulin's antilipolytic effect, and increases the production of reactive oxygen species, contributing to the reduction of NO synthesis (28). Hyperglycemia also increases the production of endothelin-1 (27). In addition, diabetes leads to abnormal vascular smooth muscle cell function due to impaired NO-mediated vasodilation, increased levels of endothelin-1, angiotensin II, and plasminogen activator inhibitor 1 (PAI-1) (29). Therefore, in hyperglycemia the PI 3-kinase pathway is even more down-regulated and the Shc/Ras/MAP-kinase pathway even more upregulated than in the pre-diabetic state.

Subclinical inflammation

Low-grade inflammation is linked to insulin resistance and is involved in the pathogenesis of type 2 diabetes (30). Inflammatory and insulin signaling pathways are tightly linked, both of which lead to insulin resistance and endothelial dysfunction, contributing to cardiovascular complications. Adipose tissue is an active endocrine and paracrine organ that releases a large number of cytokines and bioactive mediators, such as leptin, adiponectin, IL-6, and TNF-α, that influence insulin resistance, inflammation, and atherosclerosis (31). Obesity is also associated with more generalized, systemic inflammation involving circulating inflammatory proteins such as CRP, IL-6, PAI-1, P-selectin, VCAM-1, and fibrinogen. Adhesion molecule expression is induced by proinflammatory cytokines such as IL-1β, TNF-α, and CRP produced by the liver in response to IL-6 (32).

Our study in offspring of subjects of patients with type 2 diabetes who are at high risk of developing diabetes and CVD demonstrated the presence of insulin resistance, an excess of intra-abdominal fat mass, hypoadiponectinemia, and multiple defects in glucose and energy metabolism in these individuals (33). We also found high levels of high-sensitivity CRP (hs-CRP), IL-6, IL-1β, IL-1 receptor antagonist, and adhesion molecules (P-selectin, intracellular adhesion molecule 1, ICAM-1) among these pre-diabetic subjects, indicating that low-grade inflammation and markers of endothelial dysfunction are characteristic findings in
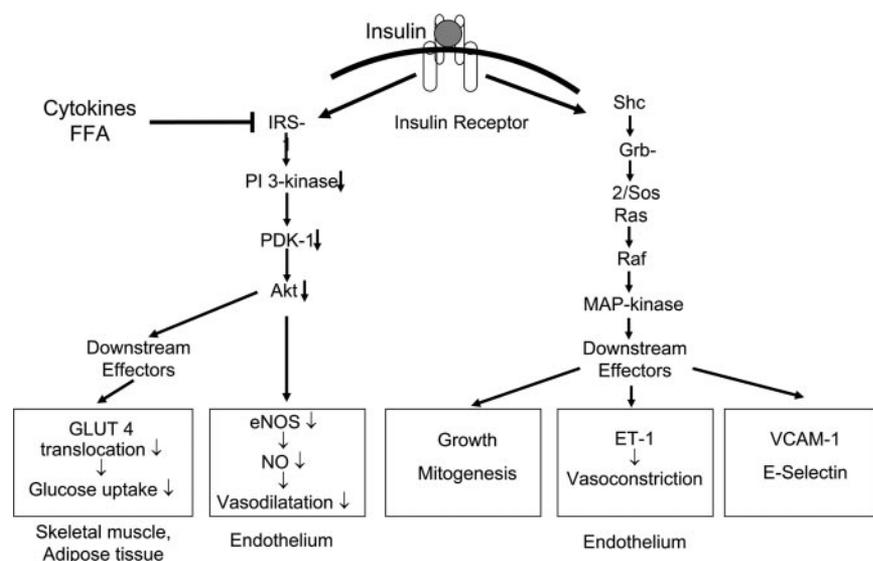

Figure 4—*Parallel insulin-signaling pathways mediating metabolic and vascular effects in peripheral tissues. eNOS, endothelial nitric oxide synthase; ET-1, endothelin 1. Modified from ref. 27.*

subjects at high risk of type 2 diabetes and CVD (33,34).

Adipokines

Several adipokines, such as leptin, adiponectin, TNF-α, IL-6, resistin, visfatin, and retinol-binding protein 4, have been suggested to be associated with insulin resistance (31). Adiponectin has important anti-atherogenic, antidiabetic, and anti-inflammatory properties and is expressed abundantly in adipocytes. In subjects with an excess of intra-abdominal fast mass, adiponectin levels are low, which might be explained by an increase in TNF-α secretion from visceral fat. High adiponectin level correlates with high insulin sensitivity (35). Adiponectin inhibits the expression of ICAM-1, VCAM-1, and E-selectin through the inhibition of nuclear factor-κB (NF-κB) activation and has several antiatherogenic and anti-inflammatory properties (35).

Atherogenic dyslipidemia

Insulin resistance and type 2 diabetes are associated with several changes in lipids and lipoproteins. We measured insulin resistance by the euglycemic-hyperinsulinemic clamp technique in subjects with varying degrees of glucose tolerance (36). Insulin-resistant subjects had higher total and VLDL triglycerides and lower HDL cholesterol than subjects with high insulin sensitivity. Elevated levels of triglyceride-rich lipoproteins, either in the fasting or postprandial state (37), are characteristic findings in patients with type 2 diabetes (VLDL, metabolites of VLDL, chylomicron remnants). Low HDL cholesterol, often associated with high levels of total and VLDL triglycerides, is another characteristic lipid abnormality in patients with type 2 diabetes (38).

The fundamental defect in lipid metabolism in patients with type 2 diabetes is the hepatic overproduction of large VLDL particles, particularly VLDL$_1$ (38). Overproduction of VLDL particles initiates a series of other changes in lipoproteins, resulting in high levels of remnant particles, small dense LDL, and low HDL cholesterol levels. In addition to reduced levels of HDL cholesterol and apolipoprotein A-I (3), there are abnormalities in the size and composition of the HDL particles (38) (decreased particle numbers, changes in particle composition) in patients with type 2 diabetes. Reduced concentrations of HDL and apolipoprotein A-I promote the accumulation of choles-terol in the vessel wall and lead to atherosclerosis.

Total and LDL cholesterol levels are usually normal in type 2 diabetic individuals, but compositional changes in LDL particles occur (small dense LDL, high triglyceride content, and oxidative modification of LDL particles), and the number of LDL particles is increased (37). Because each LDL particle contains one apolipoprotein B molecule, patients with type 2 diabetes also have increased levels of apolipoprotein B. An increased number of LDL particles might contribute to atherogenesis (39). Small dense LDL particles rapidly enter the arterial wall and can be toxic to endothelial cells, cause greater production of procoagulant factors, and can be oxidized more readily than the large buoyant particles. VLDL$_1$-triglyceride level is the major predictor of LDL size in individuals with or without type 2 diabetes.

Thrombosis and fibrinolysis

Insulin resistance and diabetes are associated with prothrombotic risk (coagulation factors VII, XII, and fibrinogen) and with suppression of fibrinolysis due to elevated concentrations of the fibrinolytic inhibitor PAI-1 (40). In insulin-resistant states, impaired endothelial function suppresses NO production and prostacyclin synthesis and platelets tend to aggregate. Hyperglycemia and glycation contribute to the generation of clot that is resistant to fibrinolysis (40). Elevated levels of PAI-1 have been shown to be associated with insulin resistance and type 2 diabetes independent of obesity and poor glycemic control (41).

Risk factors predicting CVD in subjects with type 2 diabetes—classic risk factors

All major risk factors, elevated high total and LDL cholesterol, low HDL cholesterol, elevated blood pressure, and smoking are similar risk factors in patients with type 2 diabetes and nondiabetic individuals (42). However, at every level of risk factor, diabetic subjects have at least two-fold higher risk than nondiabetic individuals. This indicates that although classic risk factors are very important determinants of increased risk of CVD in type 2 diabetic subjects, they do not explain the higher risk of CVD events in these individuals. Therefore, to understand excess risk of CVD in type 2 diabetes, other risk factors have to be considered.

Markers of impaired endothelial function

In the Framingham Offspring Study, high levels of von Willibrand factor (vWF), a biomarker of endothelial damage and dysfunction, were associated with increased risk of new-onset CVD over 11 years of follow-up of a community-based sample (43). In the Hoorn study, markers of endothelial dysfunction and low-grade inflammation explained ~43% of the increase in CVD mortality conferred by type 2 diabetes (44).

Markers of inflammation

Previous studies have shown that high levels of CRP, IL-6, and TNF-α predict type 2 diabetes (45). We measured hs-CRP in 1,045 subjects with type 2 diabetes. Subjects with hs-CRP >3 mg/l had a higher risk for CHD death than patients with hs-CRP $\leq$3 mg/l, even after the adjustment for confounding factors (46). Therefore, in our large cohort of type 2 diabetic patients, hs-CRP was an independent risk factor for CHD deaths. In another recent study, hs-CRP was independently associated with short-term mortality risk in normoalbuminuric type 2 diabetic individuals and in those without a previous diagnosis of CVD (47).

Insulin resistance

Insulin resistance is a characteristic abnormality in glucose metabolism in patients with pre-diabetes and type 2 diabetes. It often clusters with elevated blood pressure, obesity, central obesity, elevated levels of total triglycerides, low levels of HDL cholesterol, and hemostatic abnormalities. This clustering of CVD risk factors exists in nondiabetic individuals and patients with type 2 diabetes and predicts CHD (48,49). Whether hyperinsulinemia itself is a predictor of CVD has been debated (18). Ruige et al. (50) performed a meta-analysis of published studies and showed that a weak positive association was found between high insulin levels and CVD events.

Since insulin resistance is clustering with several other risk factors, conventional statistical methods underestimate the true significance of insulin resistance in increasing the risk of CVD events. Therefore, factor analysis that can include several intercorrelated variables in the same model has been applied. By applying factor analysis and principal component analysis, we showed that "hyperinsulinemia cluster" (a factor having high positive loadings for BMI, trig-

lycerides, and insulin and a high negative loading for HDL cholesterol) was predictive of death from CHD in middle-aged and elderly patients with type 2 diabetes (49,51).

A recent study applied the Archimedes model to estimate the proportion of MIs that would be prevented by maintaining insulin resistance and other risk factors at healthy levels (52). Person-specific data from the National Health and Nutrition Examination Survey 1998–2004 were used to create a simulated population representative of young adults in the U.S. In young adults, preventing insulin resistance would prevent 42% of MIs. Insulin resistance was more important than systolic blood pressure (36%), HDL cholesterol (31%), LDL cholesterol (16%), and fasting plasma glucose and smoking (both 9%) in the prevention of MI.

Hyperglycemia
Several prospective population-based studies including a large number of patients with type 2 diabetes have shown that glycemic control is important for the risk of CVD (53–55). However, this risk is not particularly strong for CHD. The UKPDS (10) and our 7-year follow-up study on 1,059 Finnish patients with type 2 diabetes (53) showed that the most important risk factors for CHD were classic risk factors, particularly dyslipidemia (high total and LDL cholesterol, high total triglycerides, and low HDL cholesterol). However, in both of these studies poor glycemic control also predicted CHD events, but the association was much weaker than for classic risk factors.

We compared the impact of hyperglycemia on the risk of CVD mortality between patients with type 1 and type 2 diabetes (56). An increment of 1 unit (%) of glycated hemoglobin increased CVD mortality by 52.5% in type 1 diabetic subjects and by 7.5% in type 2 diabetic subjects. These results are consistent with previous studies indicating that hyperglycemia is probably the most important risk factor for CVD in type 1 diabetes (57), whereas in type 2 diabetes classic risk factors and insulin resistance are more important risk factors for CVD than hyperglycemia.

Advanced glycation end products (AGEs), modification products formed by glycation or glycoxidation of proteins and lipids, have been linked to premature atherosclerosis in patients with diabetes. However, evidence from prospective studies has been missing. We investigated whether increased serum levels of AGEs predict total and CVD mortality in a random sample of 874 Finnish diabetic study participants who were followed for 18 years. AGEs were significantly associated with total and CVD mortality in women but not in men (58).

Pathophysiology of insulin resistance– and diabetes-related CVD
Insulin resistance and diabetes cause accelerated atherosclerosis via several mechanisms affecting endothelium, vascular wall, smooth muscle cells, and platelets. Insulin resistance is associated with impaired vasodilatation, increased oxidative stress, and high concentration of FFAs, vasoconstrictors, cellular adhesion molecules, PAI-1, cytokines, and other mediators of low-grade inflammation and thrombosis formation. Type 2 diabetes further enhances these abnormalities and induces multiple adverse changes in the function and structure of vessel wall including and excess formation of AGEs (3).

Initiation of atherosclerosis process
The primary event in the process of atherosclerosis is the accumulation of LDL cholesterol in the subendothelial matrix in diabetic and nondiabetic individuals (37). Small and dense LDL particles are likely to enhance atherogenesis and CVD risk in type 2 diabetes. In vitro studies show that small LDL particles rapidly enter the arterial wall, cause greater production of procoagulant factors, and are more readily oxidized (59). Activation of NF-κB signaling cascade leads to the production of E-selectin, ICAM-1 and VCAM-1, as well as chemoattractant cytokines (37). Increased concentrations of FFAs may also induce inflammation, worsen insulin resistance, and impair endothelium-dependent vasodilation (25). Low HDL cholesterol and apolipoprotein A1 levels are likely to contribute to impaired removal of excess cholesterol from atherosclerotic plaques (60).

Hyperglycemia-induced changes
In experimental models, hyperglycemia plays a central role in early development of atherosclerosis by enhancing monocyte adhesion to endothelial cells (61), by activation of NF-κB, and by production of AGEs formed by sustained exposure of proteins and lipids to hyperglycemia. Glucose- and AGE-mediated inhibition of NO production by endothelial cells is associated with impaired endothelial function (62). High glucose concentrations can also stimulate the proliferation of vascular smooth muscle cells and smooth muscle cell migration to the intima, where they participate in the formation of a fibrous cap.

Acute coronary syndrome is usually a consequence of a coronary plaque rupture. Thin fibrous cap, caused by decreased collagen production or degradation of collagen and matrix by proteinases, and inflammation, both often observed in type 2 diabetes, increase susceptibility to plaque rupture (3). Platelet activity and blood coagulability are increased in type 2 diabetes, resulting in enhanced thrombus formation. Advanced atherosclerotic lesions in diabetic patients have reduced number of vascular muscle cells and therefore these lesions are more vulnerable for rupture.

Animal models
Animal models are needed to understand the pathophysiology of atherothrombosis in type 2 diabetic patients. However, no mouse model has been available where the effect of type 2 diabetes on atherosclerosis had been investigated without significant concomitant changes in plasma lipid levels. Therefore, we crossbred two genetically modified mouse strains to achieve a model expressing both atherosclerosis and characteristics of type 2 diabetes (63). For atherosclerotic background we used LDL receptor–deficient mice synthesizing only apolipoprotein B100 (LDLR$^{-/-}$ ApoB$^{100/100}$). Diabetic background was obtained from transgenic mice overexpressing insulin-like growth factor-II (IGF-II) in pancreatic β-cells. IGF-II transgenic LDLR$^{-/-}$ ApoB$^{100/100}$ mice exhibited insulin resistance and hyperglycemia compared with hypercholesterolemic LDLR$^{-/-}$ ApoB$^{100/100}$ controls. IGF-II/LDLR$^{-/-}$ ApoB$^{100/100}$ mice displayed significantly increased lesion calcification, which was more related to insulin resistance than glucose levels. Lipid levels of IGF-II/LDLR$^{-/-}$ ApoB$^{100/100}$ mice did not differ from LDLR$^{-/-}$ ApoB$^{100/100}$ controls at any time. Therefore, in this animal model a combination of insulin resistance and hyperglycemia induced increased calcification and lesion progression.

Vascular endothelial growth factors (VEGFs) are potent angiogenic factors that can affect plaque neovascularization. Therefore, we determined the effect of diabetes on atherosclerosis and expression

of angiogenesis-related genes in athero-sclerotic lesions (64). Alloxan was used to induce diabetes in male Watanabe heritable hyperlipidemic rabbits. Accelerated atherogenesis was observed in the diabetic rabbits, and atherosclerotic lesions had an increased content of macrophages and showed significant increases in immunostainings for VEGF-A, VEGF-D, VEGF receptor-1, VEGF receptor-2, receptor for AGE (RAGE), and NF-κB. These results suggest that diabetes up-regulates VEGF-A, VEGF-D, and VEGF receptor-2 expression and increases NF-κB, RAGE, and inflammatory responses in atherosclerotic lesions.

"Common soil" hypothesis of diabetes complications

Elegant studies of Brownlee et al. (65) have shown that a single unifying mechanism of diabetes complications might be hyperglycemia-induced overproduction of superoxide by the mitochondrial electron transport chain, which activates four damaging pathways: polyol pathway, hexosamine pathway, protein kinase C pathway, and AGE formation. These authors also showed that insulin resistance induced by high FFA levels caused increased production of superoxide in arterial endothelial cells (66). The FFA-induced overproduction of superoxide activates proinflammatory signals and leads to impaired endothelial function.

Our study showed that proliferative retinopathy predicted CVD and CHD death in type 2 diabetic subjects who were free of CVD at baseline (67). The association of retinopathy with mortality was independent not only of conventional CVD risk factors but also of glycemic control and duration of diabetes. Similar results have been published previously (68). Our results are in agreement with the concept that similar underlying processes are responsible for micro- and macrovascular complications in diabetes.

Diabetic retinopathy and atherothrombosis share pathophysiological similarities. Both processes include impaired endothelial function, inflammation, neovascularization, apoptosis, and the hypercoagulable state. The neovascularization of the vessel wall has been found to be a consistent feature of the development of atherosclerotic plaque, and vasa vasorum neovascularization precedes endothelial dysfunction (69). Because proliferative retinopathy has been a more important predictor than background retinopathy in several studies, in-
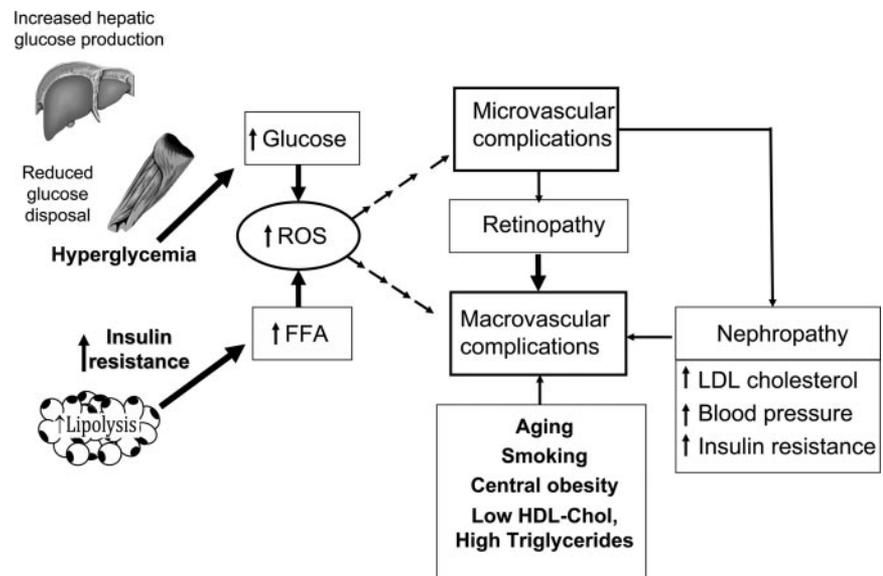

Figure 5—*The "common soil" hypothesis of diabetes complications.*

cluding our study (67), this may imply that neovascularization is an especially important common pathway leading to micro- and macrovascular complications.

Micro- and macrovascular disease in type 2 diabetes are likely to share common pathways (Fig. 5). Both insulin resistance and hyperglycemia lead to oxidative stress and mitochondrial overproduction of superoxide and activate damaging pathways leading to diabetes complications. The Diabetes Control and Complications Trial showed that insulin-resistant type 1 diabetic patients at their baseline visit were at the highest subsequent risk of developing micro- and macrovascular complications (70). Insulin-resistant diabetic patients often have nephropathy, and a clustering of CVD risk factors that further accelerate atherothrombosis.

Concluding remarks

Several mechanisms described in this review can contribute to accelerated atherothrombosis in patients with type 2 diabetes, but only limited data from prospective population studies are available to evaluate the significance of these potential mechanisms on CVD risk. Direct proof of these new mechanisms identified through in vitro experiments is difficult to obtain due to a lack of simple markers applicable for large epidemiological and clinical studies.

Recent trials on the prevention of CVD events by the treatment of hyperglycemia in patients with type 2 diabetes have been disappointing. This is likely to reflect the dominant role of insulin resis-

tance in CDV events in patients with type 2 diabetes. In fact, insulin resistance is regulating almost all mechanisms known to be associated with CVD in pre-diabetic and diabetic subjects. Studies published so far underestimate the true role of insulin resistance because of the lack of statistical models for the analysis of cross-talk between different insulin-sensitive tissues and networks of insulin resistance–related mechanisms. A recent article including a mathematical analysis of CVD risk factors and their interrelated pathways has suggested a major role of insulin resistance and obesity as potential causes for high risk of CVD among diabetic patients (52). Therefore, more research is needed to elucidate the mechanisms of insulin resistance– and hyperglycemia-related CVD both in pre-diabetic and diabetic individuals.

References
1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414:782–787
2. Amos A, McCarty D, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Med 1997;14:S1–S85
3. Laakso M. Cardiovascular disease in type 2 diabetes: challenge for treatment and prevention. J Intern Med 2001;249:225–235
4. Laakso M, Lehto S. Epidemiology of ma-

crovascular disease in diabetes. Diabetes Rev 1997;5:294–315

5. Miettinen H, Lehto S, Salomaa V, Mähönen M, Niemelä M, Haffner SM, Pyörälä K, Tuomilehto J. Impact of diabetes on mortality after the first myocardial infarction: the FINMONICA Myocardial Infarction Register Study Group. Diabetes Care 1998;21:69–75

6. Juutilainen A, Kortelainen S, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Gender difference in the impact of type 2 diabetes on coronary heart disease risk. Diabetes Care 2004;27:2898–2904

7. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229–234

8. Juutilainen A, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Type 2 diabetes as a "coronary heart disease equivalent": an 18-year prospective population-based study in Finnish subjects. Diabetes Care 2005;28:2901–2907

9. Schramm TK, Gislason GH, Køber L, Rasmussen S, Rasmussen JN, Abildstrøm SZ, Hansen ML, Folke F, Buch P, Madsen M, Vaag A, Torp-Pedersen C. Diabetes patients requiring glucose-lowering therapy and nondiabetics with a prior myocardial infarction carry the same cardiovascular risk: a population study of 3.3 million people. Circulation 2008;117:1945–1954

10. Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR, for the United Kingdom Prospective Diabetes Study Group: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). Br Med J 1998;316:823–828

11. Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events: a metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999;22:233–240

12. Fuller JH, Shipley MJ, Rose G, Jarrett RJ, Keen H. Mortality from coronary heart disease and stroke in relation to degree of glycaemia: the Whitehall Study. Br Med J 1983;287:867–870

13. DECODE Study Group, the European Diabetes Epidemiology Group. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. Arch Intern Med 2001; 161:397–405

14. Weyer C, Bogardus C, Pratley RE. Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 1999;48:2197–2203

15. Festa A, D'Agostino R Jr, Hanley AJ, Karter AJ, Saad MF, Haffner SM. Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. Diabetes 2004;53:1549–1555

16. Laakso M, Zilinskaite J, Hansen T, Boesgaard TW, Vänttinen M, Stancáková A, Jansson PA, Pellmé F, Holst JJ, Kuulasmaa T, Hribal ML, Sesti G, Stefan N, Fritsche A, Häring H, Pedersen O, Smith U, EUGENE2 Consortium: Insulin sensitivity, insulin release and glucagon-like peptide-1 levels in persons with impaired fasting glucose and/or impaired glucose tolerance in the EUGENE2 study. Diabetologia 2008;51:502–511

17. Bock G, Dalla Man C, Campioni M, Chittilapilly E, Basu R, Toffolo G, Cobelli C, Rizza R. Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 2006;55:3536–3549

18. Laakso M. Insulin resistance and coronary heart disease. Curr Opin Lipidol 1996;7:217–226

19. Laakso M, Sarlund H, Salonen R, Suhonen M, Pyörälä K, Salonen JT, Karhapää P. Asymptomatic atherosclerosis and insulin resistance. Arteriosl Thromb 1991;11:1068–1076

20. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes 1999;48:937–942

21. Festa A, Hanley AJ, Tracy RP, D'Agostino R Jr, Haffner SM. Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulin secretion. Circulation 2003;108:1822–1830

22. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB Sr, Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. Diabetes 2005;54:3252–3257

23. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: a novel mechanism for insulin resistance. J Clin Invest 1990;85:1844–1852

24. Laakso M, Edelman SV, Brechtel G, Baron AD. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. Diabetes 1992;41:1076–1083

25. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest 1997;100:1230–1239

26. Barrett EJ, Eggleston EM, Inyard AC, Wang H, Li G, Chai W, Liu Z. The vascular actions of insulin control its delivery to musle and regulate the rate-limiting step in skeletal muscle insulin action. Diabetologia 2009;52:752–764

27. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006; 113:1888–1904

28. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000;49:1939–1945

29. Bian K, Doursout MF, Murad F. Vascular system: role of nitric oxide in cardiovascular diseases. J Clin Hypertens 2008;10:304–310

30. Dandona P, Aljada A, Chaudhuri A, Bandyopadhyay A. The potential influence of inflammation and insulin resistance on the pathogenesis and treatment of atherosclerosis-related complications in type 2 diabetes. J Clin Endocrinol Metab 2003; 88:2422–2429

31. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875–880

32. Szmitko PE, Wang CH, Weisel RD, de Almeida JR, Anderson TJ, Verma S. New markers of inflammation and endothelial cell activation: part I. Circulation 2003; 108:1917–1923

33. Salmenniemi U, Ruotsalainen E, Pihlajamäki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E, Laakso M. Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. Circulation 2004;110:3842–3848

34. Ruotsalainen E, Vauhkonen I, Salmenniemi U, Pihlajamäki J, Punnonen K, Kainulainen S, Jalkanen S, Salmi M, Laakso M. Markers of endothelial dysfunction and low-grade inflammation are associated in the offspring of type 2 diabetic subjects. Atherosclerosis 2008;197:271–277

35. Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett 2008;582:74–80

36. Laakso M, Sarlund H, Mykkänen L. Insulin resistance is associated with lipid and lipoprotein abnormalities in subjects with varying degrees of glucose tolerance. Arteriosclerosis 1990;10:223–231

37. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. Lancet 2008;371:1800–1809

38. Adiels M, Olofsson SO, Taskinen MR, Borén J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome.

Arterioscler Thromb Vasc Biol 2008;28:1225–1236

39. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype: a proposed genetic marker for coronary heart disease risk. Circulation 1990;82:495–506

40. Grant PJ. Diabetes mellitus as a prothrombotic condition. J Int Med 2007;262:157–172

41. Natali A, Toschi E, Baldeweg S, Ciociaro D, Favilla S, Saccà L, Ferrannini E. Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. Diabetes 2006;55:1133–1140

42. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993;16:434–444

43. Frankel DS, Meigs JB, Massaro JM, Wilson PW, O'Donnell CJ, D'Agostino RB, Tofler GH. Von Willebrand factor, type 2 diabetes mellitus, and risk of cardiovascular disease: the Framingham Offspring Study. Circulation 2008;118:2533–2539

44. de Jager J, Dekker JM, Kooy A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Endothelial dysfunction and low-grade inflammation explain much of the excess of cardiovascular mortality in individuals with type 2 diabetes: the Hoorn Study. Arterioscl Thromb Vasc Biol 2006;26:1086–1093

45. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes in adults (Atherosclerosis Risk in Communities Study): a cohort study. Lancet 1999;353:1649–1652

46. Soinio M, Marniemi J, Laakso M, Lehto S, Rönnemaa T. High-sensitivity C-reactive protein and coronary heart disease mortality in patients with type 2 diabetes: a 7-year follow-up study. Diabetes Care 2006;29:329–333

47. Bruno G, Fornengo P, Novelli G, Panero F, Perotto M, Segre O, Zucco C, Deambrogio P, Bargero G, Perin PC. C-reactive protein and 5-year survival in type 2 diabetes: the Casale Monferrato Study. Diabetes 2009;58:926–933

48. Lempiäinen P, Mykkänen L, Pyörälä K, Laakso M, Kuusisto J. Insulin resistance syndrome predicts coronary heart disease events in elderly nondiabetic men. Circulation 1999;100:123–128

49. Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Cardiovascular risk factors clustering with endogenous hyperinsulinaemia predict death from coronary heart disease in patients with type II diabetes. Diabetologia 2000;43:148–155

50. Ruige JB, Assendelft WJ, Dekker JM, Kostense PJ, Heine RJ, Bouter LM. Insulin and risk of cardiovascular disease: a meta-analysis. Circulation 1998;97:996–1001

51. Kuusisto J, Lempiäinen P, Mykkänen L, Laakso M. Insulin resistance syndrome predicts coronary heart disease events in elderly type 2 diabetic men. Diabetes Care 2001;24:1629–1633

52. Eddy D, Schlessinger L, Kahn R, Peskin B, Schiebinger R. Relationship of insulin resistance and related metabolic variables to coronary artery disease: a mathematical analysis. Diabetes Care 2009;32:361–366

53. Lehto S, Rönnemaa T, Haffner SM, Pyörälä K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. Diabetes 1997;48:1354–1359

54. Kuusisto J, Mykkänen L, Pyörälä K, Laakso M. NIDDM and its metabolic control predict coronary heart disease in elderly subjects. Diabetes 1994;43:960–967

55. Kuusisto J, Mykkänen L, Pyörälä K, Laakso M. Non-insulin-dependent diabetes and its metabolic control are important predictors of stroke in elderly subjects. Stroke 1994;25:1157–1164

56. Juutilainen A, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Similarity of the impact of type 1 and type 2 diabetes on cardiovascular mortality in middle-aged subjects. Diabetes Care 2008;31:714–719

57. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643–2653

58. Kilhovd BK, Juutilainen A, Lehto S, Rönnemaa T, Torjesen PA, Hanssen KF, Laakso M. Increased serum levels of advanced glycation endproducts predict total, cardiovascular and coronary mortality in women with type 2 diabetes: a population-based 18 year follow-up study. Diabetologia 2007;50:1409–1417

59. Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. Ann Intern Med 2001;135:447–459

60. Moore RE, Navab M, Millar JS, Zimetti F, Hama S, Rothblat GH, Rader DJ. Increased atherosclerosis in mice lacking apolipoprotein A-I attributable to both impaired reverse cholesterol transport and increased inflammation. Circ Res 2005;97:763–771

61. Otsuka A, Azuma K, Iesaki T, Sato F, Hirose T, Shimizu T, Tanaka Y, Daida H, Kawamori R, Watada H. Temporary hyperglycaemia provokes monocyte adhesion to endothelial cells in rat thoracic aorta. Diabetologia 2005;48:2667–2674

62. Bucala R, Tracey KJ, Cerami A. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. J Clin Invest 1991;87:432–438

63. Heinonen SE, Leppänen P, Kholová I, Lumivuori H, Häkkinen SK, Bosch F, Laakso M, Ylä-Herttuala S. Increased atherosclerotic lesion calcification in a novel mouse model combining insulin resistance, hyperglycemia and hypercholesterolemia. Circ Res 2007;101:1058–1067

64. Roy H, Bhardwaj S, Babu M, Kokina I, Uotila S, Laitinen T, Hakumäki J, Laakso M, Herzig K-H, Ylä-Herttuala S. VEGF-A, VEGF-D, VEGF receptor-1, VEGF receptor-2, NFkB and RAGE in atherosclerotic lesions of diabetic Watanabe Heritable Hyperlipidemic rabbits. Faseb J 2006;20:E1550–E1559

65. Brownlee M. The pathobiology of diabetes complications: a unifying mechanism. Diabetes 2005;54:1615–1625

66. Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. J Clin Invest 2006;116:1071–1080

67. Juutilainen A, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Retinopathy predicts cardiovascular mortality in type 2 diabetic men and women. Diabetes Care 2007;30:292–299

68. Stratton IM, Kohner EM, Aldington SJ, Turner RC, Holman RR, Manley SE, Matthews DR. UKPDS 50: risk factors for incidence and progression of retinopathy in type II diabetes over 6 years from diagnosis. Diabetologia 2001;44:156–163

69. Herrmann J, Lerman LO, Rodriguez-Porcel M, Holmes DR Jr, Richardson DM, Ritman EL, Lerman A. Coronary vasa vasorum neovascularization precedes epicardial endothelial dysfunction in experimental hypercholesterolemia. Cardiovasc Res 2001;51:762–766

70. Kilpatrick ES, Rigby AS, Atkin SL. Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes: "double diabetes" in the Diabetes Control and Complications Trial. Diabetes Care 2007;30:707–712