INTRODUCTION

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General introduction

Cardiovascular disease is the most common cause of morbidity and mortality in the Western world. In developing countries, a transition is taking place towards urbanization, industrialization, and more Western life styles which will inevitably lead to a further increase in cardiovascular disease. The term cardiovascular disease includes clinical manifestations of arterial atherosclerosis, such as peripheral artery disease, cerebrovascular disease, and coronary artery disease. Well-established risk factors are dyslipidemia, smoking, diabetes mellitus, hypertension, and abdominal obesity. The pathogenesis of atherosclerosis is considered to be multifactorial, i.e. caused by a combination of genetic predisposition, lifestyle, and environmental influences.

Pathogenesis of atherosclerosis

For decades, atherosclerosis has been considered a disease of the arteries caused by excessive cholesterol storage. In recent years, advances have been made in our understanding of the pathophysiological mechanisms that lead to atherosclerosis. Accumulating evidence suggests that the atherosclerotic disease process in the arterial wall is the consequence of an intricate interplay between numerous related and interacting processes and pathways. The endothelial cells that line the luminal vascular surface play a pivotal role in maintaining the hemostatic balance. Ross proposed a model for the pathogenesis of atherosclerosis that was based on the response to injury. Injury to the endothelium is an initiating event in atherogenesis. It triggers the immune system and the coagulation cascade. Endothelial cells (EC) produce adhesion molecules (VCAM-1 and ICAM-1) and monocyte-chemotactic-protein-1 (MCP-1), but also prostaglandin-2, nitric oxide (NO), endothelium-derived relaxing factor (EDRF) and thromboxane A2, all influencing vascular tone, vascular structure and hemostatic properties of the vessel wall. The main determinant of the anti-atherosclerotic properties of the endothelium, endothelium-derived NO, is intimately involved in the regulation of vascular tone, vascular growth and coagulation.

The immunological and inflammatory response is generated by the adhesion of leukocytes to the endothelium. Adhered monocytes synthesize and express tissue
factor, factor VII, high-affinity binding sites for fibrinogen and also produce many cytokines including IL-6 and TNF-α. The adhesion of platelets to leukocytes is mediated by P-selectin expressed on the platelet plasma membrane. Adhesion is followed by migration to the sub-endothelium and differentiation, in appearance of oxidized low-density lipoprotein (ox-LDL), into macrophages. Monocyte derived macrophages and T-lymphocytes are observed in all stages of atherosclerosis. Early lesions, the so-called fatty streaks, consist of isolated groups of macrophages containing lipid droplets (macrophage foam cells). The genesis of fatty streaks consists of transport of cholesterol into the arterial wall, reverse cholesterol transport from peripheral tissues back to the liver, oxidation of constituents of these lipoproteins which may trigger an inflammatory response in the vessel wall, pro-inflammatory mediators which may enhance lipoprotein uptake by macrophages and enhance their pro-inflammatory response, protection against oxidation and inflammation by enzymes on some lipoproteins, and pro-oxidative enzymes which may interfere with the reverse cholesterol transport. The arterial inflammatory reaction stimulates proliferation and migration of vascular smooth muscle cells (VSMC)
that become intermixed with the area of inflammation. A continuous inflammation and the formation of fibrous tissue lead to further progression and enlargement of the lesion. In advanced atherosclerotic lesions a fibrous cap overlies an atheroma-tous core of lipid, necrotic and sometimes calcified material. It is now generally accepted that rupture of a vulnerable plaque due to infiltration of inflammatory cells rather than gradual occlusion of an artery, often leads to an acute clinical manifestation of atherosclerosis. In the presence of one or more risk factors for athero-genesis (e.g. dyslipidemia, diabetes mellitus, hypertension), early atherosclerotic lesions may progress and become mature atherosclerotic plaques. The dangerous and potentially lethal consequences of atherosclerosis are the destabilization of the advanced plaque, which may culminate in sudden disruption of the plaque surface, triggering luminal thrombosis. Plaque disruption with superimposed thrombosis is the most frequent pathoanatomic substrate underlying the acute coronary syndromes (unstable angina, acute myocardial infarction, and sudden coronary death) and ischemic stroke.
References

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Endothelium

Cardiovascular risk factors such as hypercholesterolemia, hypertension, diabetes and smoking may impair the normal protective function of the vascular endothelium. The endothelium is a selective barrier located between the circulating blood and the VSMC. It is a large organ, which covers an area of approximately 1 to 7 m² and weighs about 1 kg in an adult human. The endothelium is metabolically active and functional integrity of the endothelium is important for the maintenance of blood-flow and the prevention of atherothrombosis. The endothelium releases factors that are involved in both pro-atherogenic actions such as vasoconstriction, platelet aggregation, leukocyte adhesion, thrombogenesis, and VSMC proliferation and anti-atherogenic actions such as vasorelaxation, fibrinolysis, inhibition of platelet aggregation, and inhibition of leukocyte adhesion, in a balanced way. In endothelial dysfunction, a disturbance of this balance between vasoconstricting, prothrombotic, and proliferative factors (endothelin-1 (ET-1), reactive oxygen species (ROS), angiotensin-II (Ang-II), thromboxane A₂) and vasodilating, anti-thrombotic, and anti-proliferative factors (NO, endothelium-derived hyperpolarizing factor, natriuretic peptides, and prostacyclin) occurs, which results in a pro-atherogenic state. Endothelial function in humans can be measured in several ways and serves as a tool for early assessment of cardiovascular risk.

Old fashioned measurements of endothelial function

Several endothelial factors have been put forward as tools for assessing endothelial function. Endothelial function can be estimated by measuring endothelium-derived proteins such as von Willebrand factor (vWF), plasma ET-1, modulators of coagulation such as plasma thrombomodulin, plasma tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1), as well as adhesion molecules such as VCAM-1 and ICAM-1. It should be recognized that these endothelium-derived proteins can be affected by a variety of stimuli. Non-specific confounders limit the use of vWF and P-selectin as specific markers of endothelial function in subjects with acute-phase reactions and platelet activation. Although some of these markers are elevated in cardiovascular diseases and correlations with cardiovascular diseases or other risk factors have been shown, they lack sensitivity and/or specificity for assessment of endothelial function in individual patients.
Nitric oxide
NO is an important endothelium-derived vasodilating factor.\textsuperscript{14,15} Besides vasodilation, NO also inhibits adhesion of leukocytes to the endothelium,\textsuperscript{16} inhibits platelet-vessel wall interaction,\textsuperscript{17,18} decreases endothelial permeability,\textsuperscript{19} inhibits VSMC proliferation and migration,\textsuperscript{20,21} and reduces vessel tone.\textsuperscript{22} NO was originally described as endothelium-derived relaxing factor (EDRF).\textsuperscript{14} NO is synthesized by NO synthase from L-arginine.\textsuperscript{23} The conversion from L-arginine to NO can be inhibited by false substrates for the NO synthase, e.g. by NG-monomethyl-L-arginine (L-NMMA) or NG-nitro-L-arginine methyl ester (L-NAME).\textsuperscript{24} Two subtypes of NO-synthases may be distinguished: a constitutive NO is produced by endothelial cells in response to shear stress by the enzyme endothelial nitric oxide synthase (eNOS), and an inducible isoform (iNOS) as an important inflammatory mediator released by macrophages in response to immunological stimuli.\textsuperscript{25} Different co-factors are necessary, such as calcium, calmodulin, heme, tetrahydrobiopterin (BH\textsubscript{4}), and reduced nicotamine adenine dinucleotide phosphate (NADPH).\textsuperscript{26,27} NO rapidly diffuses to either the blood or abluminally to the underlying VSMC, where it activates the enzyme guanylate cyclase, inducing an accumulation of cyclic guanosine monophosphate (cGMP) in the VSMC.\textsuperscript{28} NO not only stimulates cGMP but is also rapidly inactivated by binding to heme and by reacting with oxygen radicals, yielding methemoglobin, nitrate and peroxynitrite.\textsuperscript{29} Therefore, NO-bioavailability is the result of the balance between NO-synthesis and breakdown. Under normal, healthy conditions, low concentrations of NO and nanomolar concentrations of peroxynitrite are produced, both resulting in vasodilative and/or anti-atherosclerotic actions. However, in pathophysiological situations like diabetes,\textsuperscript{29-33} hypercholesterolemia,\textsuperscript{34-38} and smoking,\textsuperscript{39-42} NO-availability \textit{in vivo} is impaired as a result of reduced NO-formation, enhanced NO-breakdown or both. Reduced NO-formation can be the result of decreased availability of L-arginine,\textsuperscript{34} or co-factors for eNOS.\textsuperscript{34,40,42} Nevertheless, a major factor of impaired NO-availability may be enhanced degradation of NO by reactions with oxygen radicals produced by enzymes such as xanthine oxidase,\textsuperscript{44} NADH- and NADPH oxidase.\textsuperscript{45,46} Moreover, under certain pathophysiological conditions, eNOS itself can synthesize mainly superoxide instead of NO.\textsuperscript{47-49} This NO-uncoupling phenomenon is characterized by reduced availability of NO and increased production of highly cytotoxic oxidant peroxynitrite. Because of its reactivity, direct measurement of NO itself \textit{in vivo} is virtually impossible. Alternatively, NO-dependent vasodilation is probably one of the most reliable and practically useful estimations of endothelial function. There are different ways of stimulating or inhibiting NO-release and different ways to measure this effect in order to assess NO-dependent vasodilation.
Coronary endothelial assessment

Coronary angiography is routinely performed to detect stenotic coronary artery lesions in symptomatic patients. In addition to morphological details of the coronary arteries, endothelial function of resistance and epicardial conduit vessels can be simultaneously investigated. The resistance vessels regulate coronary blood flow. Endothelial function of these resistance vessels can be demonstrated using an intracoronary Doppler flow velocity transducer. Endothelial function of coronary conduit vessels can be demonstrated by measuring the changes in diameter. Intracoronary infusion of acetylcholine at a dose between $10^{-8}$ to $10^{-4}$ mmol/l is generally used as a distinctive agonistic provocation. In healthy arteries this will result in increased coronary dilation and flow due to specific stimulation of the muscarinic 1 receptor on the luminal side of the endothelial cell. Coronary flow decreases and epicardial vessels constrict if endothelial function is impaired due to lack of NO availability, thus allowing a direct effect of acetylcholine on the subendothelial muscarinic 2 receptor on the VSMC, establishing a paradoxal vasoconstriction.

Determination of forearm blood flow (FBF) using venous occlusion plethysmography

Plethysmography has been used to measure blood flow in resistance vasculature beds for more than a century. For endothelial function measurements venous occlusion plethysmography of the forearm is most widely used. The mean blood flow of FBF: volume changes measured using mercury in elastic strain gauges and the intra-arterial canula for local infusion of vasoactive compounds.

Figure. Determination of FBF using occlusion plethysmography
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the forearm is measured by recording an increase in forearm volume that occurs during temporary interruption of venous outflow by inflating a pneumatic cuff to 40 mmHg at the upper arm leaving arterial inflow unimpeded. A wrist cuff, inflated 40 mmHg above systolic blood pressure, excludes flow from the hand, since the latter reflects thermoregulation rather than a resistance vessel bed. The initial increase in volume is due to arterial inflow. The volume change can be measured using mercury in elastic strain gauges wrapped around the forearm at 1/3 of the forearm length distal from the olecranon. At this part the forearm contains the largest proportion of muscle. An intra-arterial canula is placed to allow for local infusion of vasoactive compounds. The basal blood flow is measured during infusion of 0.9% saline.

Endothelium-dependent vasodilation can be induced using compounds such as serotonin, acetylcholine and bradykinin, which all more or less stimulate NO-formation. Serotonin, physiologically released by platelets, acts via the luminal serotonin receptor and specifically stimulates eNOS. Acetylcholine binds to the muscarinic 1 receptor on the endothelial cell and activates eNOS as well as endothelium-dependent VSMC-hyperpolarization. In physiologic situations acetylcholine is not a natural NO-stimulator. Acetylcholine is rapidly degraded by cholinesterase. In many human studies acetylcholine was used as agonist of NO-release. However, firstly acetylcholine has been shown to induce multiple vascular effects such as NO-release, release of vasodilative and vasoconstrictive prostaglandins and release of EDHF. Secondly, since muscarinic receptors on VSMC directly mediate vasoconstriction, blunting of the acetylcholine-induced vasodilation may be unrelated to a change in the NO-pathway. Serotonin induces a large initial rise in bloodflow, followed by a vasodilatation that sustains less. In contrast to the partial inhibition of acetylcholine-induced vasodilation by the NOS-inhibitor L-NMMA, the sustained vasodilation induced by serotonin is completely abolished by co-infusion of L-NMMA. Bradykinin acts via the luminal bradykinin receptor on endothelial cells and stimulates besides NO also EDHF. Bradykinin causes vasodilation independent of NO, predominantly through hyperpolarization. Therefore, we prefer serotonin to investigate specific NO-mediated vasodilation. Nitroprusside, serving as exogenous NO-donor, induces endothelium-independent vasodilation by directly acting on the VSMC. Inhibition of basal NO-activity is possible with specific NO-blockers such as L-NMMA and L-NAME. Changes in FBF in the measured arm are the result of vaso-active compounds. Besides this local reaction, systemic factors such as sympathetic arousal and blood pressure also can influence FBF. Comparison of flow between the measurement arm and the contralateral arm (M/C ratio) excludes these systemic factors.
Non-invasive measurement of flow-mediated dilation (FMD) of the conduit brachial artery

A non-invasive technique to measure endothelial function is the assessment of FMD using an ultrasound device.\textsuperscript{60,61} Assessment of endothelial function is based on changes in vessel diameter. Endothelium-dependent vasodilation is non-invasively induced by blood flow increase or shear stress. To induce an increase in blood-flow in the forearm, a cuff is inflated to 50 mmHg above systolic pressure distal to the transducer. Deflating this cuff after 4-5 minutes results in hyperemia lasting for approximately 2 minutes while causing vasodilation for up to 20 minutes.\textsuperscript{62} In healthy subjects, a brachial artery normally dilates approximately 5-10\%.\textsuperscript{60,61} L-NMMA abolishes the vasodilative effect of hyperemia in the brachial artery, demonstrating that NO is the main mediator of FMD.\textsuperscript{63} The non-endothelium-dependent vasodilative response can be measured using sublingually administrated nitroglycerine. It is important to be aware of influencing factors like baseline diameter. The vasodilator responses are inversely related to the vessel size. The accuracy and reproducibility are good. An interobserver variability of 2.9\% and a response variability of 1.4\% are reported.\textsuperscript{64} The number of patients required in an intervention trial depends on the hypothesized improvement in FMD and the type of study; thus,

\textbf{Figure.} FMD of the conduit brachial artery

\includegraphics[width=\textwidth]{fmd.png}

FMD: measuring endothelial function using an ultrasound device; the ultrasonic view of the brachial artery.
larger groups are needed for a parallel compared to a cross-over design.\textsuperscript{65,66} There is a close relationship of vascular function between the coronary and forearm conduit vessels. Patients with coronary artery endothelial dysfunction, manifested as vasoconstriction in response to intracoronary acetylcholine, had significantly impaired FMD in the brachial artery compared to patients with normal coronary endothelial function (FMD 4.8\% vs. 10.8\%, p<0.01). Also patients with coronary artery disease had an attenuated FMD response compared to patients with angiographically smooth coronary arteries (FMD 4.5\% vs. 9.7\%, p<0.02). Both coronary endothelial dysfunction and presence of coronary artery disease were strong predictors of reduced FMD responses. The positive predictive value of reduced FMD response (FMD < 3\%) in predicting coronary endothelial dysfunction was 95\%.\textsuperscript{67}

**Conditions for proper assessment of endothelial function**

Noteworthy, different factors affect measurements of endothelial function, which demand rigid standardization to avoid modulating effects. The sympathetic nervous system is an important regulator of coronary and peripheral vascular tone.\textsuperscript{68-75} In this respect, FMD was practically abolished during sympathetic stimulation by baroreceptor unloading which was prevented during local alpha adrenergic blockade with phentolamine.\textsuperscript{76} Thus symptomatic treatment modalities which affect sympathetic outflow (e.g. diuretics, β-blockers) may also improve FMD secondary to a decreased sympathetic activity. Circadian rhythm is important in coronary conduit vessels. In coronary segments with endothelial dysfunction, the constrictor response to acetylcholine or the dilator response to nitroglycerine was significantly greater in the morning than in the afternoon. This suggests a potentially protective role for the endothelium in modulating variations in coronary tone that may contribute to increased incidence of cardiovascular events in the early morning.\textsuperscript{77} According to this phenomenon, FBF and FMD have found to be markedly reduced in the morning compared to the afternoon.\textsuperscript{78,79} Hypercholesterolemia is associated with endothelial dysfunction and can be improved by treatment with cholesterol lowering drugs.\textsuperscript{35-38,80-82} Also high-fat meals impair endothelial function while several compounds such as vitamin C and E, folic acid, ACE-inhibition and AT-I receptor blockade, prevent this impairment.\textsuperscript{83-88} In addition, elevated glucose levels can impair endothelial function as well.\textsuperscript{89-92} FMD response of the brachial artery is impaired in postmenopausal women and related to the estradiol level in premenopausal women. This suggests that estrogens have a protective effect on the endothelium.\textsuperscript{93-98} Endothelium-mediated vasodilation of coronary and peripheral arteries is inversely correlated to age.\textsuperscript{99-103}
Several studies revealed impaired endothelial function of the coronary and brachial conduit vessels and forearm resistance vessels in smokers compared to non-smokers. This is probably due to decreased NO-availability by radical stress or decreased eNOS-activity since the L-NMMA-induced vasoconstrictive effect was diminished. BH4, an important co-factor for eNOS, improves the endothelial function in both forearm resistance- and conduit vessels.

As discussed above, some factors improve endothelium-mediated vasodilation, whereas others deteriorate endothelial relaxation. Thus, for greatest accuracy, it is important that the patient is investigated in a quiet room at constant room temperature (20-24°C) in a comfortable position. The patient should have been fasting for 6-12 hours.

**Relation between endothelial dysfunction and cardiovascular morbidity**

Endothelial dysfunction plays an important role in the pathogenesis of coronary artery disease and is considered to be an early manifestation of atherosclerosis. Multiple investigators have clearly demonstrated impaired endothelial function of coronary conduit and resistance arteries in atherosclerotic patients. In addition, impairment of peripheral endothelium-dependent vasodilation has been demonstrated in patients with coronary artery disease. Moreover, the progressive impairment of the endothelium correlates with the progression of coronary atherosclerosis. Even more important, endothelial dysfunction is not only associated with atherosclerosis and its risk factors but also with cardiovascular outcome. To identify cardiac events using FMD in the brachial artery in patients with non-specific chest pain a specificity of 51%, a sensitivity of 86% and a negative predicting value of 93% was found.
References


**INSULIN RESISTANCE**

**General introduction**

The prevalence of type 2 diabetes (DM2) has soared in the past decades because of changing lifestyles and eating habits. Obesity associated with insulin resistance is one of the main determinants of the increased incidence of DM2. The major long-term complications of DM2 are an increased risk of myocardial infarction, stroke and peripheral vascular disease. Although microvascular complications cause considerable morbidity in patients with DM2, up to 80% of patients die from macrovascular pathology. Treatment of individual risk factors has been shown to reduce cardiovascular events in DM2, a concept supported by observations in the UK Prospective Diabetes Study (UKPDS). Patients with impaired glucose tolerance (IGT) and/or impaired fasting glucose are so called pre-diabetic and are at risk for developing DM2. Although in DM2 elevated plasma glucose concentrations may induce vascular damage, in pre-diabetic patients the increased cardiovascular risk is most likely the result of the pathophysiological phenomenon of insulin resistance which leads to the occurrence of risk factors (e.g. elevated bloodpressure, low HDL-cholesterol, elevated triglycerides) and causes impaired vasoreactivity.

**Pathophysiology of insulin resistance**

Insulin has several physiological functions. This hormone is the most important regulator for the plasma glucose level by stimulating the disposal of glucose in skeletal muscle- and adipose tissue and decreasing the hepatic glucose production. Furthermore insulin stimulates the lipogenesis and glycogen- and protein-synthase activity in adipocytes, liver and skeletal muscle, and inhibits glycogenolysis, lipolysis and protein breakdown. In addition, insulin plays a role in the regulation of cell growth and differentiation. Insulin resistance means that when insulin binds to the insulin receptor on the cell surface, this stimulation is not strong enough to induce the normal intracellular signal transduction with the consequence of insufficient reaction of target organs on plasma levels of insulin. In case of a sufficient pancreatic β-cell-function, insulin resistance will lead to a compensatory hyperinsulinemia to maintain normoglycemia. Hyperglycemia, follows by further increase in insulin resistance and after failing of the pancreatic β-cell-function. Adipose tissue, especially intra-abdominal obesity, plays an important role in insulin resistance. Adipose tissue is no longer only a depot, but can be seen as an endocrine organ. Several products of adipose tissue may influence
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development of insulin resistance. Important adipocyte-derived factors are FFA, but the adipokine TNF-α, adiponectin, and leptin. High levels of free fatty acids (FFA) have been linked to the induction of insulin resistance because increased FFA production in the liver leads to increased gluconeogenesis and decreased glucose metabolism in skeletal muscle. Furthermore high levels of FFA will lead to an increase of very low-density lipoprotein (VLDL) together with an increase of plasma triglycerides. In an insulin resistant state there is an attenuated lipoprotein lipase (LPL) activity. Lipoprotein lipase is involved in the lipolysis of VLDL. Decreased breakdown of VLDL particles leads to reduced availability of small VLDL fragments and an increase in triglyceride-rich high-density lipoprotein (HDL) particles via cholesterolesentertransferprotein (CETP). Triglyceride-rich HDL particles are faster cleared by the liver resulting in a decreased HDL plasma concentration and an increased triglyceride concentration.

The expression of TNF-α by adipose tissue is upregulated in obesity and TNF-α levels are increased in patients with features of the insulin resistance syndrome (such as endothelial dysfunction) inducing a higher risk of recurrent coronary events. Adiponectin is an adipocyte-derived hormone that decreases insulin resistance. A low adiponectin plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. The role of leptin in insulin resistance is controversial, but leptin might interfere with insulin signalling in certain cell types. Hyperinsulinemia also causes hypertension. Normally insulin has a vasodilating effect, however with insulin resistance, insulin can cause sympathetic activation, production of the vasoconstrictor ET-1, and increase of renal salt retention. Together with, or leading to, endothelial dysfunction these mechanisms can result in hypertension.

Figure. Pathophysiology of insulin resistance

![Pathophysiology of insulin resistance](image)
Insulin resistance syndrome
Taken together, insulin resistance is generally regarded as an important feature of a cluster of risk factors of cardiovascular disease. These risk factors (dyslipidemia, hypertension, glucose intolerance, hyperinsulinemia, obesity, low-grade inflammation, endothelial dysfunction and hypercoagulability) often precede clinically manifest DM2. However, the interaction between different cardiovascular risk factors and defects in insulin signalling is very complex. Most persons with multiple metabolic risk factors are insulin resistant which leads to the concept that insulin resistance is the major cause of the cluster metabolic syndrome.¹⁵

Metabolic syndrome
The metabolic syndrome, which involves a cluster of cardiovascular risk factors including hypertension, obesity, glucose intolerance, endothelial dysfunction, dyslipidemia and a proinflammatory state, is generally considered to be of major importance in the pathophysiology of DM2 and is associated with an increased risk for cardiovascular complications.¹⁶-¹⁸ According to the Adult Treatment Panel III (ATP III) the metabolic syndrome is diagnosed when 3 or more criteria are present. The ATP III definition of the metabolic syndrome is the most commonly used definition and most practical for clinical use:¹⁹

1) Abdominal obesity (waist circumference > 102cm in men and >88cm in women).
2) High blood pressure (≥ 130mmHg systolic or ≥ 85mmHg diastolic).
3) Hypertriglyceridemia (serum triglycerides ≥ 1.70mmol/l).
4) Low serum HDL cholesterol (<1.04mmol/l in men and < 1.29mmol/l in women).
5) High fasting serum glucose (≥ 6.1mmol/l).

The increase in prevalence of the metabolic syndrome is of great concern worldwide. The for age adjusted prevalence of the MS among assumable healthy persons is around 24% worldwide.²⁰,²¹ In a French study, using a modified WHO-definition for the metabolic syndrome, the prevalence for women was 12 % and for men 23%.²² For patients with manifest arterial disease the prevalence is 45%²³ and for patients with DM2 80%.²⁴ In a population based cohort study the odds ratio (adjusted for age, sex, and follow-up duration) for the development of diabetes in patients with impaired fasting glucose was 10.0 (95% CI 6.1-16.5).²⁴ The presence of the metabolic syndrome at baseline increased the risk for the development of diabetes mellitus almost
2-fold in American Indians\textsuperscript{25} and in Finnish men a 4-fold increase was shown.\textsuperscript{26} Presence of the metabolic syndrome gives a nearly 3-fold increase in cardiovascular related mortality compared to subjects without the metabolic syndrome.\textsuperscript{27} In addition, the metabolic syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm\textsuperscript{28} and untreated essential hypertension.\textsuperscript{29} It is assumable that the cardiovascular risk for patients with the metabolic syndrome can just be explained by the sum of the separate cardiovascular risk factors. However, Scuteri et al. showed that the age-associated increases in vascular thickness and stiffness was more than expected from the separate cardiovascular risk factors in relation to the metabolic syndrome.\textsuperscript{30} Moreover, the increased cardiovascular risk for patients belonging to the metabolic syndrome can not be completely explained by the traditional models for risk-scoring like the Framingham-score.\textsuperscript{31} This increased cardiovascular risk may be due to the combination of nontraditional markers, which all have a relation with insulin resistance, together with the separate traditional components of the metabolic syndrome (hyperglycemia, hypertension, low plasma HDL cholesterol, high plasma triglyceride levels, and obesity). The nontraditional markers are endothelial dysfunction, inflammation, hyperinsulinemia, oxidative stress hypercoagulability together with decreased fibrinolysis, and increased small-dense-LDL.\textsuperscript{32-34} Several studies showed a relation between hyperinsulinemia, increased plasma FFA levels, and more oxidized small-dense LDL with endothelial dysfunction.\textsuperscript{35-37} Also enhanced low-grade inflammation induces endothelial dysfunction by increased production of cytokines like C-reactive protein (CRP), TNF-$\alpha$, and IL-6.\textsuperscript{38-40} In addition, increased hs-CRP is associated with obesity, insulin resistance, and endothelial dysfunction.\textsuperscript{41} The more components of the metabolic syndrome are present in the same person, the higher the plasma CRP concentration.\textsuperscript{42} Noteworthy is that CRP not only seems to function as an indicator for cardiovascular disease, but CRP may also play a role as a risk factor in atherogenesis.\textsuperscript{43} Endothelial dysfunction, an early step in atherogenesis, is also present in the metabolic syndrome and may be a cause or a consequence of insulin resistance. The Framingham study already showed that abdominal obesity is the main hypertensinogenic factor.\textsuperscript{44} Like already mentioned earlier, obesity is also the cause of insulin resistance, diabetes mellitus type 2, left ventricular hypertrophy, hyperlipidemia, and thus related to an increased risk for atherosclerotic diseases. A direct association between hypertension and BMI has been observed in cross-sectional and longitudinal population studies from early childhood to old age.\textsuperscript{45} The mechanism
by which obesity raises blood pressure is not fully understood, but increased BMI is associated with an increase in plasma volume and cardiac output. These alterations and blood pressure can be decreased by weight loss in both normotensive and hypertensive subjects. Furthermore, blood pressure in obese adolescents is sodium-sensitive, and fasting insulin is the best predictor of this sensitivity; after weight loss the blood pressure decreases and the salt sensitivity is reduced. The variables that best predict sodium sensitivity are fasting plasma insulin, plasma aldosterone, and plasma norepinephrine, supporting the hypothesis that blood pressure is sensitive to dietary sodium and that this sensitivity may be due to the combined effect of hyperinsulinemia, hyperaldosteronism, and increased activity of the sympathetic nervous system. Hyperinsulinemia increases both sympathetic nerve activity and sodium and water retention and the expected vasodilation, upon binding of insulin to the endothelial insulin receptor and causing activation of eNOS, is impaired in insulin resistant states. It is also the heterogeneous effect of endogenous NO on proliferation along the vascular tree that relates to the two different phenomenons of insulin resistant vascular dysfunction. Endothelial dysfunction exists in conduit arteries, while elevated vascular resistance of resistance arteries is observed in essential hypertension.
Although a pathophysiological construct seems plausible, future research must unravel pathophysiology and clinical use before the metabolic syndrome can be designated as a ‘syndrome’.\textsuperscript{51} The individual components that make up the syndrome should be treated coherently. These are the visible mountains of the floating iceberg above the water. However there are other risk factors underneath the water surface. Awareness of the underlying disorders is important for understanding the pathophysiology and thus coherent treatment: be aware for insulin resistance and its associated (non-) traditional risk factors.
References


Our understanding of the so-called cardiovascular dysmetabolic syndrome has been improved by the discovery of nuclear peroxisome proliferator-activated receptors (PPARs). PPARs are ligand-activated transcription factors belonging to the nuclear receptor superfamily. As transcription factors, PPARs regulate the expression of numerous genes and affect glycaemic control, lipid metabolism, vascular tone and inflammation. Activation of the subtype PPAR-\(\gamma\) improves insulin sensitivity. Expression of PPAR-\(\gamma\) is present in several cell types involved in the process of atherosclerosis. Thus, modulation of PPAR-\(\gamma\) activity is an interesting therapeutic approach to reduce cardiovascular events. Thiazolidinediones are PPAR-\(\gamma\) agonists and constitute a new class of pharmacological agents for the treatment of type 2 (non-insulin–dependent) diabetes mellitus. Two such compounds are currently available for clinical use: rosiglitazone and pioglitazone. Thiazolidinediones improve insulin sensitivity and glycaemic control in patients with type 2 diabetes. In addition, improvement in endothelial function, a decrease in inflammatory conditions, a decrease in plasma levels of free fatty acids and lower blood pressure have been observed, which may have important beneficial effects on the vasculature.

Several questions remain to be answered about PPAR-\(\gamma\) agonists, particularly with respect to the role of PPAR-\(\gamma\) in vascular pathophysiology. More needs to be known about the adverse effects of Thiazolidinediones, such as hepatotoxicity, increased low-density lipoprotein cholesterol levels and increased oedema. The paradox of adipocyte differentiation with weight gain concurring with the insulin-sensitising effect of Thiazolidinediones is not completely understood. The decrease in blood pressure induced by Thiazolidinedione treatment seems incompatible with an increase in the plasma volume, and the discrepancy between the stimulation of the expression of CD36 and the antiatherogenic effects of the Thiazolidinediones also needs further explanation. Long-term clinical trials of Thiazolidinediones with cardiovascular endpoints are currently in progress.

In conclusion, studying the effects of Thiazolidinediones may shed more light on the mechanisms involved in the insulin resistance syndrome. Furthermore, Thiazolidinediones could have specific, direct effects on processes involved in the development of vascular abnormalities.
**Outline of the Thesis**

**Cardiovascular disease** is the leading cause of morbidity and mortality in Western countries. The term cardiovascular disease comprises clinical manifestations of arterial atherosclerosis, such as peripheral artery disease, cerebrovascular disease, and coronary artery disease. Well-established risk factors are dyslipidemia, smoking, diabetes mellitus, hypertension, and clustered in association with abdominal obesity. In the pathophysiology of atherosclerosis, based on the response to injury mechanism, the pathophysiological phenomena **endothelial dysfunction** and **inflammation** are playing a pivotal role.

The endothelium has been identified as the central transducer through which risk factors can cause atherosclerosis and its clinical complications. The central pathway in this event is the activation of the endothelium. Basically this involves the switch from a healthy condition with low concentrations of NO resulting in vasodilative and/or anti-atherosclerotic actions. However, in pathophysiological situations NO-availability **in vivo** is impaired because of redox signalling in the activated endothelium, as a result of reduced NO-formation, enhanced NO-breakdown or both. There are two major consequences of this endothelial activation:

First, it will lead to the production of chemokines and expression of adhesion molecules. This will support the recruitment of inflammatory cells into the vessel wall. Although this system is physiological in the context of host-defense it may become inappropriate with prolonged periods of endothelial cell activation secondary to cardiovascular risk factors. As a result, an inflammatory phenotype evolves in the vessel wall which leads to atherogenesis and plaque rupture.

Second, the loss of NO-bioavailability by the endothelium, due to risk factors, affects the vessel wall structure. There are some preliminary indications that NO inhibits VSMC-proliferation in the vascular wall. As a result one could hypothesize that loss of NO-activity would result in increased VSMC-proliferation. This may accelerate the development of the atherosclerotic plaque, but may also lead to thicker, remodelled vessels. As a result these vessels become stiffer and start to produce pressure load on the heart.

In Chapter 1 we first would like to explore the hypothesis that endothelial function (in the sense of NO-bioavailability) is an important determinant of vessel wall structure. In conduit arteries, endothelial dysfunction is initiating atherogenesis. The other phenomenon of endothelial dysfunction is the elevated vascular resistance of resistance arteries (microcirculation) as observed in essential hypertension.
We addressed this heterogeneity of vascular remodeling along the vascular tree and postulated that this regional difference may be related to a heterogeneous effect of eNOS on proliferation in conduit arteries vs. resistance vessels.

In the current thesis we would like to explore several aspects of the described model of vascular injury in the setting of type 2 diabetes. Type 2 diabetes is emerging as a worldwide epidemic and currently about 200 million people are affected worldwide. An important driver for this increased incidence is the associated increase in patients with insulin resistance (approximately 400 million worldwide right now). This insulin resistance is driven by obesity and secondary to obesity, free fatty acid fluxes towards other tissues than adipocytes (like the muscle and liver). However, genetic factors, particularly in Asian people, seem to play a role as well.

Clinically it would be useful to correlate the severity of insulin resistance to the severity of alterations in vessel wall structure due to loss of NO-activity. In chapter 2 we investigated in a cross-sectional survey (2105 patients) to determine whether carotid artery stiffness was increased in (‘pre-diabetic’) patients with the metabolic syndrome and in patients who developed overt type 2 diabetes.

Because our group extensively studied all kind of other markers of endothelial dysfunction in diabetes and obesity (and thus insulin resistance), we did not further explore these concepts and took the experience already gained, to address possible treatment. Thinking about a treatment of the underlying disorders, the discovery of nuclear peroxisome proliferator-activated receptors (PPARs) and subsequent insight into their role in several metabolic pathways was a major breakthrough in our understanding of pathophysiological mechanisms underlying the insulin resistance syndrome.

Thiazolidinediones (like pioglitazone) are clinically available agonists of the PPAR-γ subtype and constitute a new class of antihyperglycaemic agents. Activation of PPAR-γ not only improves insulin sensitivity but may also have additional beneficial vascular effects.

The aim of a main part of this thesis is to focus on the potential role of Thiazolidinediones in the pathophysiological mechanisms involved in vascular disease.

Chapter 3 is an overview of the metabolic and additional vascular effects of Thiazolidinediones.

In chapter 4 we investigated the direct vascular effects of pioglitazone on the capacity of the vasculature to maintain its NO-release, in type 2 diabetic patients, in a double blind and crossover design. As measurement of NO-activity in the vessel wall, flow-mediated dilation in the conduit brachial artery was used. We focused on relatively short-term application of pioglitazone to tease out direct vascular effects from its indirect metabolic effects.
We subsequently investigated whether a beneficial effect of TZDs on NO-bioavailability in type 2 diabetes also translates into better protection of the vessel wall from inflammatory stimuli.

To this end we investigated in chapter 5, whether diabetic subjects could maintain NO dominated endothelial function in the presence of increased concentrations of TNF-α. Therefore we first investigated the effect of TNF-α on endothelium-dependent vasodilation and secondly the effects of short-term pioglitazone treatment on TNF-α induced endothelial dysfunction in patients with type 2 diabetes mellitus.

In addition, we investigated ex vivo whether TZDs changed the properties of monocytes to adhere to the endothelium, or to produce cytokines.

Therefore, we examined in chapter 6, in a parallel controlled, ex vivo study, the effects of incubation with TZDs, on monocyte-endothelium-adherence under flow conditions.

In chapter 7 we studied the cytokine production in whole blood from type 2 diabetes patients after short-term pioglitazone treatment, in a double blind and crossover design.