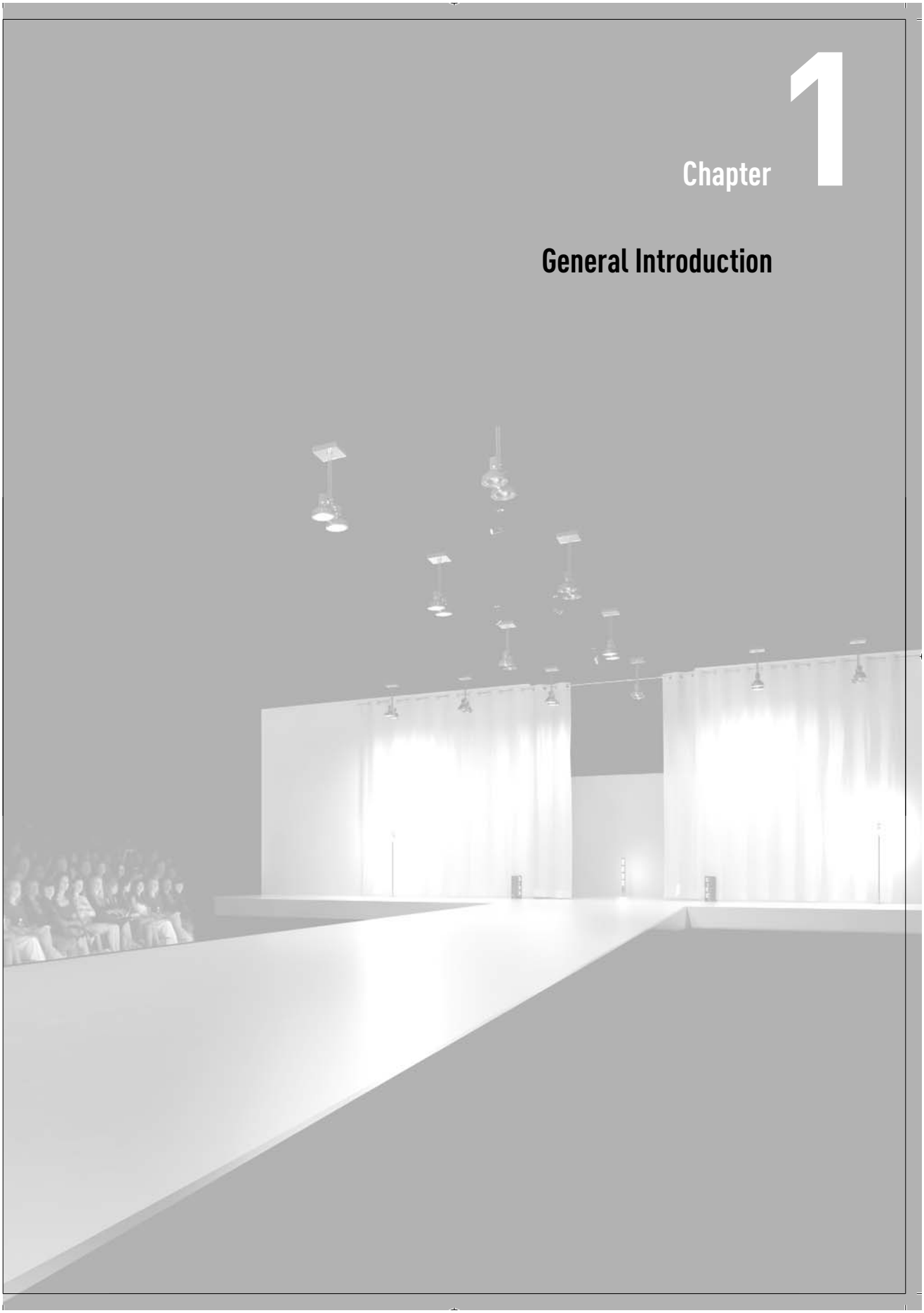
Studies on the pathophysiological aspects of the metabolic syndrome in transgenic mice

Hu, L.

Chapter 1

General Introduction



1.1 Metabolic syndrome

1.1.1 Definition

Overweight and obesity is rapidly becoming a major health problem, momentarily affecting more than 1 billion adults, and over 300 million of them clinically obese. It is predicted by the World Health Organization (WHO) that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese.[1] This overweight/obesity pandemic is not only restricted to adults, but childhood obesity is already epidemic in some regions. It is estimated that around 10% of the youths are obese worldwide. Overweight and obesity lead to adverse metabolic effects on blood pressure, lipid metabolism and insulin resistance. This clustering of pathologies is called as the metabolic syndrome (MetS) and has also started to emerge in children at young ages, a phenomenon that was inconceivable a few decades ago.

The MetS is also known syndrome X, Reaven's syndrome and insulin resistance syndrome. The latter and MetS are now commonly and interchangeably used names. MetS was first described in early 1920s by Kylin as a constellation of hypertension, hyperglycaemia and gout.[2] Over the years, different criteria were used to define the MetS. In late 1940s the MetS was redefined by Vague in which android or male-type obesity was included.[3] In 1988 Reaven stated the clinical importance of the MetS. In the landmark publication of his 1988 Banting Medal award lecture, Reaven described syndrome X as a constellation of insulin resistance, hyperglycaemia, hypertension, low high-density lipoprotein cholesterol (HDL-C) levels and increased very low-density lipoprotein (VLDL)-triglyceride levels.[4] In 1999, World Health organisation (WHO) has attempted to create an international unifying guideline.[5] However, the numbers of metabolic disorders that are associated with the MetS has increased in the last few years. Therefore, different expert groups (the European Group for the Study of Insulin Resistance (EGIR), the International Diabetes Federation (IDF) and the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (NCEP ATP III) redefined the MetS and modified the WHO definition (**Table 1**).[6-9] Nowadays, the WHO and NCEP ATP III definitions are most commonly used. The exact pathogenesis of the MetS is not clear. It is suggested that MetS is the result of increasingly sedentary lifestyles combined with ready access to energy-rich food sources in genetically susceptible individuals. Subjects with the MetS have high risk for developing insulin resistance and cardiovascular diseases.

Table 1 **The different definitions of the Metabolic Syndrome**

	Major criteria	Minor criteria				
WHO	type II diabetes mellitus or impaired fasting glucose or insulin resistance	obesity BMI > 30 kg/m^2 and/or waist/hip ratio >0.9 (♂) or >0.85 (♀)	dylipidemia HDL-C < 0.9 (♂) or < 1.0 mM ♀ TG ≥ 1.7 mM	hypertension BP > 140/90 mm Hg	microalbuminuria urinary albumin excretion rate > 20 µg/min or albumin/ creatinine ratio ≥ 30 mg/g	
EGIR	insulin resistance or fasting hyper-insulinaemia	central obesity waist circumference 94 cm (♂) 80 cm (♀)	dyslipidemia HDL-C < 1.0 mM TG > 2.0 mM	hypertension BP ≥ 140/90 mm Hg	FPG ≥ 6.1 mM	
NCEP ATP III		central obesity Waist circumference 102 cm (♂) or 88 cm (♀)	low HDL-C < 1.03 mM (♂) < 1.29 mM (♀)	triglycerides ≥ 1.7 mM	Hypertension BP ≥ 135/80 mm Hg	FPG ≥ 6.1 mM
IDF	Central obesity	low HDL-C < 1.03 mM (♂) < 1.29 mM (♀) or treatment for this abnormality	triglycerides ≥ 1.7 mM or treatment of this abnormality	hypertension systolic BP ≥130 mm Hg, diastolic BP > 85 mM Hg or treatment for previously diagnosed hypertension	FPG ≥ 5.6 mM	

HDL-C: high density lipoprotein-cholesterol
TG: triglyceride
BP: blood pressure
FPG: fasting plasma glucoses
EGIR: European Group for the Study of Insulin Resistance, IDF: International Diabetes Federation, NCEP ATP III: Third Report of the National Cholesterol Education Program's Adult Treatment Panel, HDL-C: high density lipoprotein-cholesterol, TG: triglyceride, BP: blood pressure, FPG: fasting plasma glucoses.

1.1.2 Prevalence and clinical consequences

The worldwide prevalence of MetS is not exactly known, because of different definitions are being used. Therefore comparisons in the prevalence are difficult to make. However, most studies do agree that the prevalence is rapidly increasing. Particular alarming is the increase in children. Consistent observations are that the prevalence is age-dependent and with a high ethnic variation. In the United States almost 25% of the total population has the MetS according to the NCEP ATP III definition.[10] In Europe the prevalence of the MetS varies between 24.5% in Greece and 32.6% in Spain.[11,12] In Asia, the prevalence is less than 20%.[13,14]

The most important clinical consequences of MetS are the insulin resistance and the cardiovascular diseases. MetS increases the risk for type 2 diabetes mellitus independent of insulin resistance.[15] Moreover, the MetS is associated with an increased risk for cardiovas-

cular mortality and morbidity.[16-18] Subjects with MetS have 3 times higher risk for dying of cardiovascular heart diseases when compared with subjects without MetS. MetS is also associated with chronic kidney diseases independent of the presence of type 2 diabetes mellitus or hypertension, although the underlying mechanism is not known.[19,20] Simultaneous occurrence of both metabolic syndrome and insulin resistance worsens the risk for cardiovascular diseases. Taken together, the MetS has major clinical impacts.

1.1.3 Insulin resistance

Under normal physiological conditions, ingested glucose is taken up and stored in the liver and in insulin-sensitive peripheral tissues (predominantly the skeletal muscle and the adipose tissue). Plasma glucose stimulates the production and secretion of insulin by the pancreatic insulin producing β-cells. On the one hand, insulin inhibits hepatic glucose production by inhibiting the glycogenolysis and gluconeogenesis. On the other, insulin stimulates the uptake of glucose and the formation of glycogen in the skeletal muscle. Furthermore, insulin inhibits the production of lipoprotein particles and energy substrates such as lactate and free fatty acids (FFAs) in the liver and the adipose tissue. In the adipose tissue, insulin also promotes the synthesis of triglycerides (TG) as energy storage and inhibits lipolysis. In obese state, energy intake exceeds the capacity to store energy in the adipose tissue leading to energy 'overflow' to ectopic sites. These ectopic sites are observed in the liver, skeletal muscle and pancreatic insulin-secreting β-cells.[21,22] The liver plays a pivotal role in maintaining the glucose and lipid metabolism. Several clinical studies have shown that lipid accumulation in the liver is associated with insulin resistance.[23] Patients with type 2 diabetes mellitus have a defect glucose metabolism in the liver and skeletal muscles.[24,25]

Under insulin resistance conditions, insulin fails to suppress the production of glucose and energy substrates, to stimulate the glucose uptake, and to inhibit lipolysis. Therefore, Insulin resistance is defined as a state of reduced responsiveness to normal circulating insulin levels affecting multiple organs.
Insulin resistance is a key component in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus. Numerous studies have shown that obesity and overweight are the major contributors of the metabolic syndrome. It is agreed that impaired glucose metabolism, exacerbate lipid accumulation and inflammatory processes contribute to insulin resistance. The pathogenesis of insulin resistance has been studied extensively; much is still to do since the incidence of insulin resistance has become epidemic.

1.2 PAI-1 and insulin resistance

Several epidemiological studies have shown association between increased plasma PAI-1 levels and body mass index, triglyceride levels and insulin resistance.[26-29] In the Insulin Resistance Atherosclerosis Study (IRAS) plasma PAI-1 levels predict the development of diabetes independently from other known risk factors.[26] Progression of PAI-1 plasma levels in addition to initial high plasma levels are associated with the incident diabetes.[30] Improving insulin resistance by diet, exercise or oral antidiabetic drug treatment results in decreased plasma levels of PAI-1 antigen and activity.[31-34] Although, PAI-1 is known to be synthesized by various tissues including liver and adipose tissue, the source and the mechanism of increased plasma PAI-1 levels in obesity and insulin resistance are incompletely understood. Increased plasma PAI-1 in obesity might be derived from the adipose tissue. Alternatively, increased plasma PAI-1 can be the result of local and systemic production following stimulation by adipokines.

The expression of PAI-1 in adipose tissues is positively correlated with obesity in human and rodents, suggesting a possible role in the development of obesity and insulin resistance.[35-39] This is supported by the observation made in genetically obese and insulin resistant mouse models. Disruption of the *PAI-1 gene* in ob/ob mice reduces adiposity and improves the metabolic profile determined by glucose and insulin tolerance test.[40] Two other studies showed that mice lacking PAI-1 do not develop diet-induced obesity and insulin resistance.[41,42] Downregulation of PAI-1 by angiotensin type I receptor antagonist in wild-type (WT) mice ameliorates diet-induced obesity, hyperglycemia and hyperinsulinemia. Administration of synthetic PAI-1 inhibitor induces higher insulin sensitivity in WT mice.[43]These studies suggest that PAI-1 may not merely increase in response to obesity and insulin resistance, but may have direct causal role in the development of obesity and insulin resistance. In contrast to these studies, PAI-1 deficient mice kept on a high fat diet for 3-8 weeks develop more adipose tissue.[44] In agreement with this, transgenic mice overexpressing PAI-1 have lower body weight, lower adipose tissue mass and less intraperitoneal fat.[45]
Taken together, although strong clinical evidence is present that PAI-1 plays an important role in insulin resistance and obesity, it is not clearly confirmed yet by experimental studies how enhanced PAI-1 is linked to the pathological conditions of insulin resistance and obesity. Does PAI-1 contribute to the pathogenesis of insulin resistance and obesity? Or is PAI-1 merely an epiphenomenon of insulin resistance and obesity?

1.3 Atherosclerosis

Cardiovascular disease (CVD) includes myocardial infarction, congestive heart failure, stroke and peripheral artery diseases. CVD is the leading cause of all mortality and morbidity worldwide. In North America more than 1 out the 3 persons will die of CVD and it is predicted that health costs will exceed 430 billion dollars.[46] Comparable mortality numbers hold true for The Netherlands. Atherosclerosis is the primary cause of CVD. Atherosclerosis is a progressive disease of the vessel wall that already begins in young adults. The disease primarily occurs in the large and medium-sized elastic and muscular arteries. The aetiology is very complex and it involves genetic, environmental factors and the interaction between these factors. Among the risk factors are diabetes mellitus, dyslipidemia, smoking, hypertension, gender, age and physical activity. Thus, atherosclerosis results from the combination of genetic susceptibility and unhealthy environmental influences.

1.3.1 Pathogenesis of atherosclerosis

Although the knowledge of atherosclerosis has expanded in the last decades, the exact mechanism underlying the pathogenesis is still not fully understood. The traditional view of the pathogenesis of atherosclerosis is the imbalance between cholesterol deposition and removal in the subendothelial layer after injury to the endothelium.[47] The accumulation of cholesterol can be facilitated by increased plasma LDL cholesterol levels leading to proliferation of smooth muscle cells (SMC). In the subendothelial layer, LDL cholesterol can be modified and subsequently engorged by resident macrophages to form foam cells (**Figure 1**). These foam cells form the initial fatty streak lesions which precede the formation of complex fibrous lesions.

The current concept involves inflammation and atherosclerosis is now also considered as an inflammatory disease of the large and medium-sized arteries. Inflammatory processes are present in all stages of atherosclerosis progression (**Figure 1**). Triggers of atherosclerosis, such as modified LDL can stimulate endothelial cells to produce an array of inflammatory proteins including chemotactic factors like monocyte chemoattractant protein-1 (MCP-1), growth factors such as macrophage colony-stimulating factor (M-CSF) and adhesion molecules. Among the adhesion molecules are vascular cell adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM), P-selectin and E-selectin. These adhesion molecules and chemotactic factors attract monocytes and T cells into the subendothelial layer initiating the formation of the early atherosclerotic plaque. The proliferation and differentiation of the attracted monocytes are then stimulated by M-CSF. These attracted monocytes and T cells on their turn can release inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) that further amplify the inflam-

matory activity in the vessel wall. As the atherosclerotic lesion progresses, macrophages and T cells stimulate the migration of smooth muscle cells (SMC) into the intima and the production of collagen. A fibrous cap is then formed together with extracellular lipid deposits, SMC-derived extracelluar matrix, and often with necrosis. A complex atherosclerotic lesion is then a fact. Such a complex lesion can rupture depending on the composition and vulnerability. Vulnerable plaques usually have thin fibrous caps and increased number of inflammatory cells. The fibrous cap reflects the balance between matrix production by SMC and degradation by matrix metalloproteinases. Calcification and neovascularisation can also influence the stability of the atherosclerotic plaque. In addition, thrombogenicity of a lesion depends on the presence of proteins of the coagulation cascade such as tissue factor and plasminogen activators. Usually a plaque ruptures at the edges of the lesion leading to thrombus formation and occlusion of the artery and subsequently a cardiovascular event.

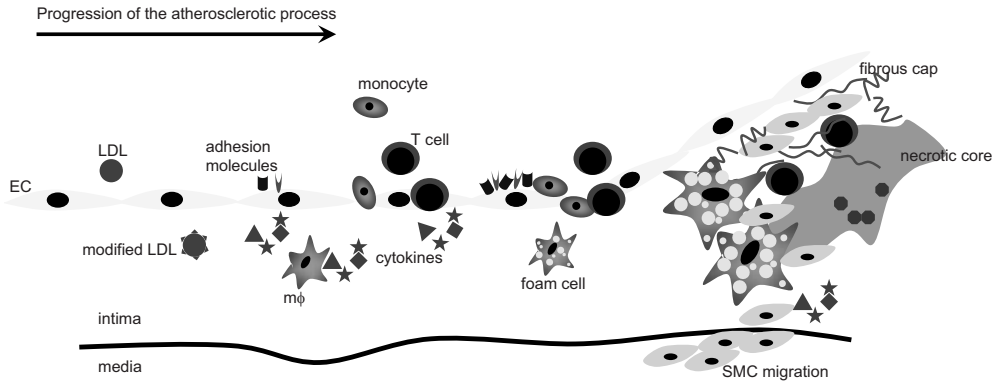


Figure 1 **Atherosclerotic process**
In the early atherosclerotic process monocytes adhere, migrate, take up modified LDL and differentiate into macrophage foam cells. The macrophage foam cells produce and release cytokines attracting even more inflammatory cells, such as T cells. In the advance process, smooth muscle cells migrate and proliferate to form fibrous cap overlying a poll of lipid-laden macrophages, T cells, necrosis, and cholesterol crystals. EC: endothelial cells, LDL: low-density lipoprotein, SMC: smooth muscle cells

1.3.2 Inflammation and atherosclerosis

As discussed above (**section pathogenesis of atherosclerosis**) inflammatory processes play a key role in the development of atherosclerosis. The nuclear factor κB (NF-κB) is a central regulatory factor of the inflammatory processes. NF-κB is considered to play a crucial role atherosclerosis locally at the vessel wall. Many inducers and target genes of NF-κB are implicated to be involved throughout the atherosclerotic process.[48] In the initial phase NF-κB can be activated in the endothelium by atherosclerogenetic stimuli such as modified LDL and

inflammatory cytokines produced at the lesion site. Additionally, NF-κB is demonstrated to be involved in the regulation of the modification of LDL, the expression chemokines and adhesion molecules.[49-56] All are important in the initial phase of the atherosclerotic process. In the advanced lesions NF-κB plays an important role in SMC migration and proliferation. The stability of an atherosclerotic plaque may also be governed by NF-κB by controlling apoptosis and necrosis. Macrophage-specific deletion of the main NF-κB activator IKK2 results in atherosclerotic lesions with increased necrosis and apoptosis.[57] However, reduced activity of NF-κB not only results in increased cell death, but also in reduced secretion of the anti-inflammatory cytokine IL-10. In the same setting reduced secretion of the pro-inflammatory cytokine TNF-α is also observed. In line with these findings, mice with p50 deficiency in the hematopoietic system show reduce less atherosclerosis, but more inflammation in the lesions.[58] Thus, this emphasizes that NF-κB as the central regulatory factor of inflammation has a complex role by influencing both pro-atherogenic and anti-atherogenic process in the vessel wall. Therefore, much is still to do to disentangle how the NF-κB activation and signalling pathways are orchestrated during the development of atherosclerosis.

In contrast, not many studies have been performed to investigate the underlying mechanisms of systemic inflammation on the development of atherosclerosis. Countless epidemiologic studies have shown that low-grade systemic inflammation is associated with metabolic syndrome. The liver is the key regulatory organ in the systemic inflammatory processes. The production of acute phase proteins, like C-reactive protein (CRP), serum amyloid A (SAA), plasminogen activator inhibitor-1 (PAI-1) are most relevant in this respect. CRP and PAI-1 are increased in subjects with the metabolic syndrome. PAI-1 has been shown to increase the risk of atherothrombotic events and may also promote the progression of atherosclerosis.[59] Experimental studies have demonstrated that CRP can activate endothelial cells to produce inflammatory markers. Furthermore, SAA can stimulate the cholesterol uptake by smooth muscle cells in an atherosclerotic plaque.[60] Therefore, hepatic inflammatory parameters are considered to be strongly associated with atherosclerosis and cardiovascular diseases. However, the exact mechanism by with systemic inflammation affects the development of atherosclerosis at the vessel wall has not been identified.

1.3.3 Endothelial progenitor cells and atherosclerosis

The first manifestation of atherosclerosis is the development of endothelial dysfunction, which is characterized by an activation of endothelial cells (EC) and decreased nitric oxide availability and deterioration of the endothelial monolayer. The initial damage is reversible. However, when no sufficient repair mechanism is present, ongoing deterioration of the endothelial monolayer can lead to the development of atherosclerotic lesions. The underlying molecular mechanism of endothelial repair is not fully understood.

A population of pluripotent cells within the peripheral blood has been described that are capable to differentiate into endothelial cells.[61] These endothelial progenitor cells (EPC) are able to home to sites of injury in the vascular endothelium and subsequently enhance neoangiogenesis after tissue ischemia. Therefore, the concept rose that EPC are recruited from the bone marrow to sites of damaged endothelium, where they can home and differentiated into mature endothelium cells (**Figure 2**). The phenotypic and functional characteristics of EPC are divergent. The widely accepted consensus defines cells positive for surface markers CD34 and vascular endothelial growth factor receptor-1 (VEGFR-2) as EPC.

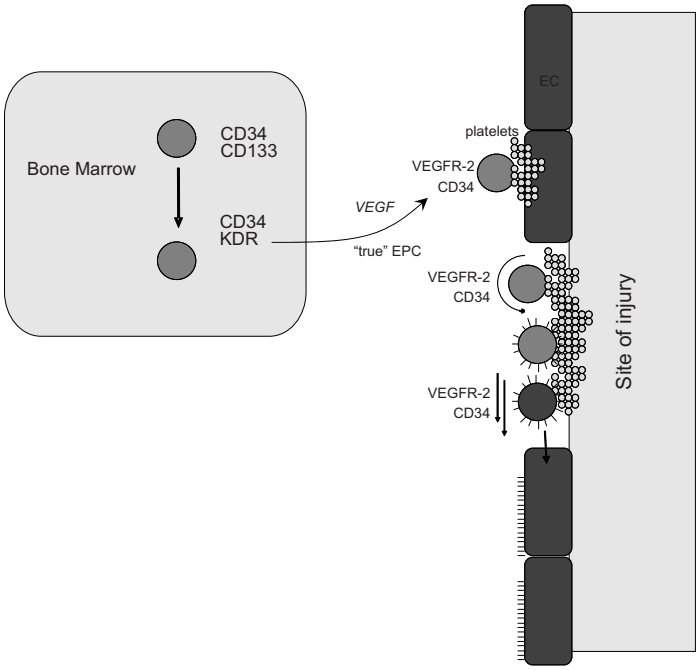


Figure 2 **Recruitment, homing and differentiation of an endothelial progenitor cell**
The endothelial progenitor cell recruited via vascular endothelial growth factor and homes to the site of injury where it differentiates into an endothelial cell. There it forms a new endothelial layer. EPC: endothelial progenitor cell, VEGF: vascular endothelial growth factor, VEGFR-2: vascular endothelial growth factor receptor-2. (courtesy of prof. dr. A.J. van Zonneveld)

The number and the functional activity of circulating EPC are correlated with cardiovascular risks. The EPC levels and the proliferation and migration activity are reduced in patients with CVD, diabetes or hypercholesterolemia.[62-65] Other cardiovascular risk factors such as smoking and CRP are also associated with impaired EPC numbers and function. In the atherosclerotic apoE-/- mouse model systemic transfusion of systemic progenitor cells inhibits the progression of atherosclerotic lesions.[66]

It is apparent that EPC can facilitate endothelial repair and is involved in the development of atherosclerosis. However, it is not clear what the exact contribution of EPC is in cardiovascular diseases.

1.4 Lipid metabolism

Cholesterol and triglycerides are of essential for many different processes in the human body and for energy storage. Since cholesterol and triglycerides are hydrophobic, they are packed into lipoproteins particles for transport in the circulation. Dietary cholesterol and triglycerides are absorbed by the intestines and packed into chylomicrons containing mainly triglyceride (**Figure 3**). Subsequently, these chylomicrons are secreted in the circulations where they acquire apolipoproteins. Once in the circulation, chylomicrons are subjected to lipolysis by endothelium-bound lipoprotein lipase (LPL) resulting in the generation in fatty acid that enters the peripheral tissues for energy storage or source. The chylomicron- remnant particles are further hydrolysed by hepatic lipase (HL) and subsequently taken up by the liver via the low-density lipoprotein receptor (LDLR) or the LDLR-related protein (LRP). The liver plays a central role in the lipid metabolism. The liver processes the cholesterol and triglycerides and secretes these again into the circulation packed into very low-density lipoprotein (VLDL) particles where they acquire apolipoproteins. Similar to chylomicrons, VLDL particles are hydrolysed by LPL and eventually resulting in low-density lipoprotein (LDL) particles. LDL in its turn can be taken up the liver via the LDLR for further processing. LPL is synthesized and secreted by parenchymal cells throughout the body. The activity of LPL is influenced by several apolipoproteins. Apolipoprotein CII serves as a co-factor, whereas apoCI and apoCIII inhibits LPL.[67-70]

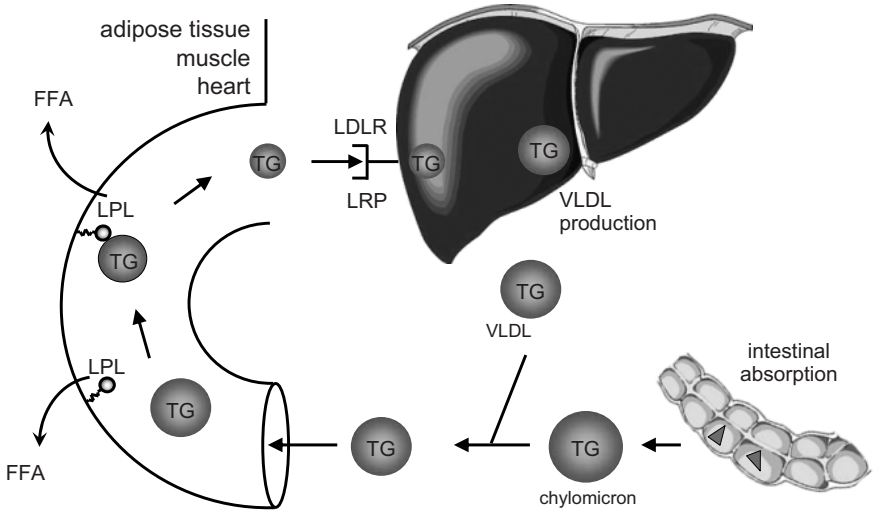


Figure 3 **Lipid metabolism**
See text for explanation. TG: triglyceride, FFA: free fatty acid, LPL: lipoprotein lipase, LDLR: low-density lipoprotein receptor, LRP: low-lipoprotein receptor-related protein, VLDL: very low-density lipoprotein receptor

Originally identified as a member of the LDLR gene family, LRP was suggested to play role in lipid metabolism. *In vitro* studies showed that LRP serves as a receptor for apoE-rich chylomicron remnants and lipoprotein lipases.[71,72]

1.4.1 Low-density lipoprotein receptor-related protein

Structure and expression

The low-density lipoprotein receptor-related protein (LRP) gene is located on chromosome 12 and was identified in 1988 by Herz J *et al*.[73] It is also known as α2-macroglobulin receptor, LRP1 and CD91.[74] LRP consists of 4544 amino acids and is synthesised as a large 600 kDa single polypeptide chain in the endoplasmatic reticulum, which is than cleaved into a 515 kDa and an 85 kDa subunit by furin in the Golgi apparatus. Both subunits remain non-covalently associated where the 515 kDa subunit binds ligands and the 85 kDa subunit is anchored in the plasma membrane. The endoplasmatic reticulum-resident chaperone protein, the 39 kDa receptor- associated protein (RAP) ensures the correct trafficking of LRP along the secretory pathway.[75] Thereby, RAP also promotes proper optimal folding of LRP and prevents premature intracellular binding to its ligands.

LRP is a member of the big low-density lipoprotein (LDL) receptor (LDLR) gene family. This family also includes the LDLR, very low-density lipoprotein (VLDL) receptor (VLDLR), apolipoprotein E receptor 2 (ApoE-R2) and megalin/LRP2/glycoprotein 330 (**Figure 4**). As other members of the LDL receptor gene family LRP contains structural domains that include: a) ligand-binding cysteine-rich complement-type repeats, b) epidermal growth factor (EGF) receptor-like cysteine-rich repeats, c) b-motifs with YWTD repeats, d) transmembrane domain and e) a cytoplasmatic domain that harbours 1-3 NPxY motifs (**Figure 4**).[73] The ligand-binding complement-type repeats are arranged in four different clusters (cluster I, II, III and IV) containing 2, 8, 10 and 11 repeats, respectively. Cluster II and IV bind most of the known ligands. A common feature of most the LDLR gene family members is their ability to bind RAP. RAP antagonizes ligand binding to all members of the LDLR gene family. Therefore, extracellular recombinant RAP is extensively exploited as a tool to study the biology of the LDLR gene family.

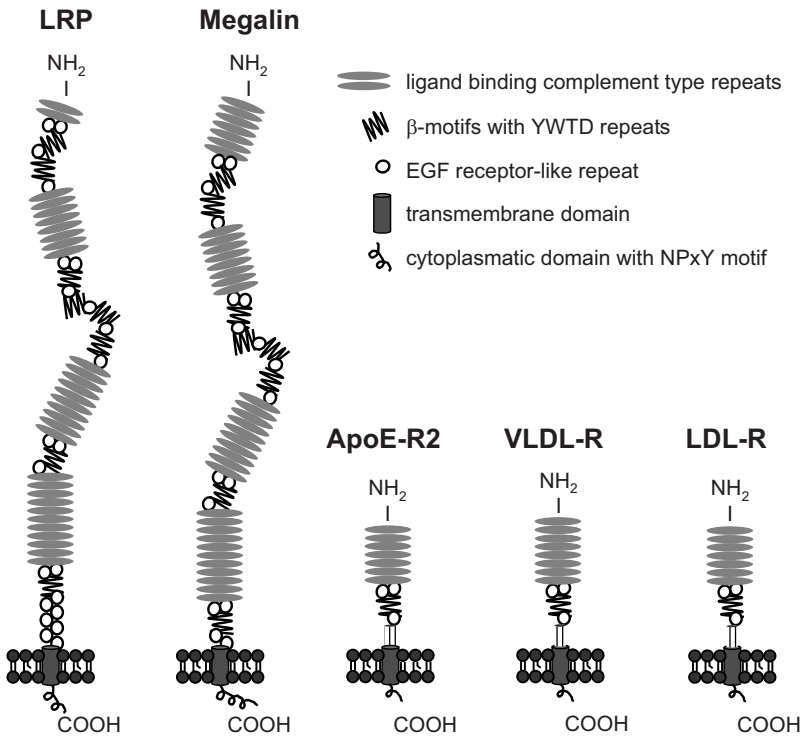


Figure 4 **The low-density lipoprotein receptor gene family**
The LDL receptor gene family consists of several homologous transmembrane receptors involved in endocytosis. All members of the LDL receptor gene family are composed of the same protein domains with similar topological organisations. LDL receptor gene family member include the low-density lipoprotein receptor-related protein (LRP), megalin (pg330 and LRP2), the apolipoprotein E receptor-2 (apoE-R2), the very low-density lipoprotein receptor (VLDLR), and the low-density lipoprotein receptor (LDLR).

LRP is widely expressed in a large variety of tissues. It is abundantly present in the liver, brain, lung, spleen, intestines, reproductive tract and fat tissue.[76] Furthermore, LRP is also expressed in a spectrum of diverse cell types, such as smooth muscle cells, macrophages and fibroblast.

Physiological functions

LRP is a multi-ligand protein. To date, LRP is known to recognise over 50 functionally and structurally numerous ligands (**Table 2**).[77,78] Originally LRP was identified as lipid metabolism receptor. Additionally, LRP is shown to serve as a regulator of the extracellular proteolytic activity by rapid internalising of the uPA/PAI-1 complex in concert with the uPAR and modulating the matrix metalloproteinase levels.[79-82] These evidences imply that LRP is a multifunctional scavenger receptor. Different mice studies confirmed that LRP is indeed

an endocytic scavenger receptor that is not only involved in the lipid metabolism, but also in haemostasis metabolism.[83-85]

Targeted deletion of the LRP gene revealed that LRP is absolutely required in the early embryonic development, suggesting that its physiological role is not restricted as a cargo transporter of extracelluar proteins.[79] The exact mechanism of embryonic lethality is unclear. However, LRP is now known also to be involved in intracellular signalling. It is thought that the cytoplasmatic tail with the NPxY motifs are involved in the interaction with numerous intracellular proteins of the signal transduction pathways.[86] Most of these proteins are adaptor proteins in the regulation of cell signalling, migration and proliferation. Depending on the phosphorylation state of LRP can regulate various intracellular signals in response to different extracellular stimuli by modifying its association with adaptor proteins.[87] LRP is shown to control cell migration and proliferation by phoshorylation in response to PDGF-BB in vascular SMC (VSMC). Failing to control the PDGF signalling in the SMC results in increased atherosclerosis (**see section LRP and atherosclerosis**).[88]

1.4.2 LRP and atherosclerosis

As abovementioned, conventional LRP knockout mice are not viable and die on day 10 of gestation. Therefore, tissue-specific disruption of LRP using the Cre/loxP recombination system has been generated to study the physiological of LRP *in vivo*. Inactivation of hepatic LRP in LDLR deficient mice (MX1Cre LRP$^{flox/flox}$) results in the accumulation of cholesterol-rich remnants lipoproteins suggesting an atherogenic lipid profile.[85] Independent of plasma cholesterol levels these mice show increased atherosclerosis on an atherogenic apoE-/- background.[89]

Next to plasma lipids levels, the proliferation and differentiation of VSCM and macrophages are important in the development of atherosclerosis (see section atherosclerosis). LRP plays a pivotal role in the vascular integrity and the prevention of atherosclerosis in the VSMC.[88] Mice lacking LRP in their VSMC have similar plasma lipid levels as mice with LRP present in the VSMC. However, VSMC LRP deficient mice show increased susceptibility to development atherosclerotic lesions. The elastic layer of the aorta is disrupted. Increase VSMC proliferation and aneurysm formation are observed as a result of abnormal control of the PDGFR expression and activation.

The role of macrophage LRP in the development of atherosclerosis is not fully known. *In vitro* studies implicate that LRP in macrophages has a pro-atherogenic potential. LRP is highly expressed in atherosclerotic lesions and upregulated in macrophages undergoing foam cell formation.[90,91] Additionally, LRP regulates ß2-integrin-mediated adhesion of monocytes to endothelial cells allowing monocytes to migrate into the intima and to differentiate into macrophages.[92] Macrophage LRP has also been demonstrated to play a role in

the translocation of 12/15-lipoxygenase, which stimulates the formation of oxidized LDL.[93,94] In concert with the LDLR, LRP can mediate the uptake of apoE-rich atherogenic lipoproteins into the macrophage.[95-97] Since all these processes promote the formation of foam cells, one would predict that LRP promotes the development of atherosclerosis at the level of macrophages.

Table 2 Extracellular LRP ligands

Lipid metabolism	Growth Factors
Apo E	PDGF
Chylomicron remnants	Midkine
Hepatic lipase	Connective tissue growth factor
Lipoprotein lipase	TGF-β
Lipoprotein (a)	
β-VLDL	*Infection and immunity*
Saposin	Aminoglycosides
Sphingolipid activator protein	Circumsporozoite protein
	Complement C3
Protease and protease/inhibitor complexes	Gentamicin
Activated α2-M*	HIV-Tat protein
Aprotinin	Lactoferin
C1s/C1q inhibitor	Minor group rhinovirus
Elastsae/α1-anti-trypsin	Polymyxcin B
FIXa	Pseudomonas exotoxin A
FVIIa/TFPI	Ricin A
FVIIIa	Saposin
FXa/TFPI	Trichosanthin
FXIa/protease-1	
Neuroserpin	*Matrix proteins*
Neuroserpin/tPA	Fibronectin
PAI-1	MMP-13
PAI-1/thrombin	MMP-9
PAI-1/tPA	TSP-1
PAI-1/uPA	TSP-2
Pregnancy zone protein/protease complexes	TSP-2/MMP-2
Pro-uPA	
TFPI	*Others*
Thrombin/anti-thrombin III	Amyloid precursor protein
Thrombin/heparin cofactor II	Amyloid β-chain
Thrombin/proteinase nexin-1	Calreticulin
tPA	Collectins
Trypsin/α1-anti-trypsin	HSP-96
TSP-2/MMP-2	RAP
uPA	
uPA/protease nexin-1	
α2-M*/protease complexes	

1.5 Outline of this thesis

In this thesis we aimed to expand our knowledge on the pathophysiological aspects of the metabolic syndrome in transgenic mice. The metabolic syndrome involves multiple aspects and has a major impact on cardiovascular diseases. In the first part of thesis the role of PAI-1 in the development of insulin resistance will addressed. This part will also focus on the mechanism of plasma PAI-1 clearance. Plasma PAI-1 is increased in patients with the metabolic syndrome. Obesity and insulin resistance are key components of the metabolic syndrome. The increased plasma PAI-1 levels are suggested to be the result of increased expression in the vascular endothelium, adipose tissue and liver. However, it is not known if the clearance also contributes to the increased plasma PAI-1 levels. **Chapter 2** describes the clearance and plasma levels of PAI-1 in a genetically and a diet-induced insulin resistant mouse models. A number of studies have shown that LRP can bind, internalise and degrade PAI-1 *in vitro*. However, it is not known whether LRP indeed plays a role in the clearance of plasma PAI-1 *in vivo*. **Chapter 3** addressed the role of hepatic LRP in the regulation of plasma PAI-1 *in vivo*. For this purpose, we studied the clearance of PAI-1 in hepatic LRP deficient mice under different conditions.

In the second part of this thesis, the roles of LRP in atherosclerosis and LPL activity in lipid metabolism are addressed. Hepatic LRP deficient mice have elevated fasted plasma cholesterol and triglyceride levels, mainly present as VLDL particles on a LDLR-/-VLDL-/- background. Since VLDL is continuously produced in the liver, VLDL remnants still need to be cleared to maintain a steady state level. **Chapter 4** addressed the whether LPL activity is important for the hepatic clearance of VLDL remnants independent of the three major apoE-recognizing receptors LRP, LDLR and VLDLR. LRP in the liver and SMC is shown to have atheroprotective role. Macrophages play a key role in the development of atherosclerosis next to SMC. Data from several *in vitro* studies suggest a pro-atherogenic of LRP in the macrophage. In **chapter 5** we investigated the role macrophage LRP in the development of atherosclerosis *in vivo*.

Finally, role of low-grade inflammation in endothelial restoration is addressed. Subjects with the metabolic syndrome have chronic low-grade inflammation and increased risk for cardiovascular diseases. **Chapter 6** describes the influence of low-grade inflammation on the number of EPC in patients with the metabolic syndrome. The association between the number of EPC and the extent of atherosclerosis in the carotid artery is also described.

The results obtained from these studies and the implications for future research are discussed in **chapter 7**.

References

1. WHO. Obesity and Overweight. *www who int* 2008.
2. Kylin E. Studien über das Hypertonie-Hyperglykämie-Hyperurikämiesyndrom. *Zentrabl f innere Med Leipz* 1923;81:105-127.
3. Vague J. Sexual differentiation. A factor affecting the forms of obesity. *Presse Med* 1947;30:339-340.
4. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595-1607.
5. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539-553.
6. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med* 1999;16:442-443.
7. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486-2497.
8. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet* 2005;366:1059-1062.
9. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433-438.
10. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002;287:356-359.
11. Athyros VG, Ganotakis ES, Elisaf M, Mikhailidis DP. The prevalence of the metabolic syndrome using the National Cholesterol Educational Program and International Diabetes Federation definitions. *Curr Med Res Opin* 2005;21:1157-1159.
12. Navarro J, Redon J, Cea-Calvo L, Lozano JV, Fernandez-Perez C, Bonet A, Gonzalez-Esteban J. Metabolic syndrome, organ damage and cardiovascular disease in treated hypertensive patients. The ERIC-HTA study. *Blood Press* 2007;16:20-27.
13. Pongchaiyakul C, Nguyen TV, Wanothayaroj E, Karusan N, Klungboonkrong V. Prevalence of metabolic syndrome and its relationship to weight in the Thai population. *J Med Assoc Thai* 2007;90:459-467.
14. Choi KC, Lee SY, Yoo HJ, Ryu OH, Lee KW, Kim SM, Baik SH, Choi KM. Effect of PPAR-delta agonist on the expression of visfatin, adiponectin, and resistin in rat adipose tissue and 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 2007;357:62-67.
15. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB, Sr., Wilson PW. Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. *Diabetes Care* 2007;30:1219-1225.
16. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001;24:683-689.
17. Hanefeld M, Koehler C, Gallo S, Benke I, Ott P. Impact of the individual components of the metabolic syndrome and their different combinations on the prevalence of atherosclerotic vascular disease in type 2 diabetes: the Diabetes in Germany (DIG) study. *Cardiovasc Diabetol* 2007;6:13.
18. de Simone G, Devereux RB, Chinali M, Best LG, Lee ET, Galloway JM, Resnick HE. Prognostic impact of metabolic syndrome by different definitions in a population with high prevalence of obesity and diabetes: the Strong Heart Study. *Diabetes Care* 2007;30:1851-1856.
19. Kurella M, Lo JC, Chertow GM. Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. *J Am Soc Nephrol* 2005;16:2134-2140.
20. Peralta CA, Kurella M, Lo JC, Chertow GM. The metabolic syndrome and chronic kidney disease. *Curr Opin Nephrol Hypertens* 2006;15:361-365.

21. Adams JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes* 2004;53:25-31.
22. Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann N Y Acad Sci* 2002;967:363-378.
23. Sakurai M, Takamura T, Ota T, Ando H, Akahori H, Kaji K, Sasaki M, Nakanuma Y, Miura K, Kaneko S. Liver steatosis, but not fibrosis, is associated with insulin resistance in nonalcoholic fatty liver disease. *J Gastroenterol* 2007;42:312-317.
24. Carey PE, Halliday J, Snaar JE, Morris PG, Taylor R. Direct assessment of muscle glycogen storage after mixed meals in normal and type 2 diabetic subjects. *Am J Physiol Endocrinol Metab* 2003;284:E688-E694.
25. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 1990;322:223-228.
26. Festa A, D'Agostino R, Jr., Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2002;51:1131-1137.
27. Kanaya AM, Wassel FC, Vittinghoff E, Harris TB, Park SW, Goodpaster BH, Tylavsky F, Cummings SR. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. *Arch Intern Med* 2006;166:350-356.
28. Meigs JB, O'Donnell CJ, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, Nathan DM, Sullivan LM, D'Agostino RB, Wilson PW. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: the Framingham Offspring Study. *Diabetes* 2006;55:530-537.
29. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. *Diabetologia* 1991;34:457-462.
30. Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. *Circulation* 2006;113:1753-1759.
31. Albertini JP, McMorn SO, Chen H, Mather RA, Valensi P. Effect of rosiglitazone on factors related to endothelial dysfunction in patients with type 2 diabetes mellitus. *Atherosclerosis* 2007.
32. Araiza P, Hewes H, Gashetewa C, Vella CA, Burge MR. Efficacy of a pedometer-based physical activity program on parameters of diabetes control in type 2 diabetes mellitus. *Metabolism* 2006;55:1382-1387.
33. Barinas-Mitchell E, Kuller LH, Sutton-Tyrrell K, Hegazi R, Harper P, Mancino J, Kelley DE. Effect of weight loss and nutritional intervention on arterial stiffness in type 2 diabetes. *Diabetes Care* 2006;29:2218-2222.
34. Hamalainen H, Ronnemaa T, Virtanen A, Lindstrom J, Eriksson JG, Valle TT, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Rastas M, Aunola S, Uusitupa M, Tuomilehto J. Improved fibrinolysis by an intensive lifestyle intervention in subjects with impaired glucose tolerance. The Finnish Diabetes Prevention Study. *Diabetologia* 2005;48:2248-2253.
35. Alessi MC, Bastelica D, Morange P, Berthet B, Leduc I, Verdier M, Geel O, Juhan-Vague I. Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. *Diabetes* 2000;49:1374-1380.
36. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med* 1996;2:800-803.
37. Landin K, Stigendal L, Eriksson E, Krotkiewski M, Risberg B, Tengborn L, Smith U. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. *Metabolism* 1990;39:1044-1048.
38. Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. *J Clin Invest* 1991;88:1346-1353.
39. Samad F, Loskutoff DJ. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. *Mol Med* 1996;2:568-582.

40. Schafer K, Fujisawa K, Konstantinides S, Loskutoff DJ. Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. *FASEB J* 2001;15:1840-1842.
41. Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, Brown NJ, Swift LL, McGuinness OP, Wasserman DH, Vaughan DE, Fogo AB. Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* 2004;53:336-346.
42. De Taeye BM, Novitskaya T, Gleaves L, Covington JW, Vaughan DE. Bone marrow plasminogen activator inhibitor-1 influences the development of obesity. *J Biol Chem* 2006;281:32796-32805.
43. Lijnen HR, Alessi MC, Van Hoef B, Collen D, Juhan-Vague I. On the role of plasminogen activator inhibitor-1 in adipose tissue development and insulin resistance in mice. *J Thromb Haemost* 2005;3:1174-1179.
44. Morange PE, Lijnen HR, Alessi MC, Kopp F, Collen D, Juhan-Vague I. Influence of PAI-1 on adipose tissue growth and metabolic parameters in a murine model of diet-induced obesity. *Arterioscler Thromb Vasc Biol* 2000;20:1150-1154.
45. Lijnen HR, Maquoi E, Morange P, Voros G, Van Hoef B, Kopp F, Collen D, Juhan-Vague I, Alessi MC. Nutritionally induced obesity is attenuated in transgenic mice overexpressing plasminogen activator inhibitor-1. *Arterioscler Thromb Vasc Biol* 2003;23:78-84.
46. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell CJ, Roger V, Rumsfeld J, Sorlie P, Steinberger J, Thom T, Wasserthiel-Smoller S, Hong Y. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2007;115:e69-171.
47. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-809.
48. de Winther MP, Kanters E, Kraal G, Hofker MH. Nuclear factor kappaB signaling in atherogenesis. *Arterioscler Thromb Vasc Biol* 2005;25:904-914.
49. Ivandic B, Castellani LW, Wang XP, Qiao JH, Mehrabian M, Navab M, Fogelman AM, Grass DS, Swanson ME, de Beer MC, de Beer F, Lusis AJ. Role of group II secretory phospholipase A2 in atherosclerosis: 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group IIa phospholipase A2. *Arterioscler Thromb Vasc Biol* 1999;19:1284-1290.
50. Zhao L, Funk CD. Lipoxygenase pathways in atherogenesis. *Trends Cardiovasc Med* 2004;14:191-195.
51. Burleigh ME, Babaev VR, Oates JA, Harris RC, Gautam S, Riendeau D, Marnett LJ, Morrow JD, Fazio S, Linton MF. Cyclooxygenase-2 promotes early atherosclerotic lesion formation in LDL receptor-deficient mice. *Circulation* 2002;105:1816-1823.
52. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell* 1998;2:275-281.
53. Aiello RJ, Bourassa PA, Lindsey S, Weng W, Natoli E, Rollins BJ, Milos PM. Monocyte chemoattractant protein-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 1999;19:1518-1525.
54. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* 2001;107:1255-1262.
55. Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, Beaudet AL. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med* 2000;191:189-194.
56. Johnson RC, Chapman SM, Dong ZM, Ordovas JM, Mayadas TN, Herz J, Hynes RO, Schaefer EJ, Wagner DD. Absence of P-selectin delays fatty streak formation in mice. *J Clin Invest* 1997;99:1037-1043.
57. Kanters E, Pasparakis M, Gijbels MJ, Vergouwe MN, Partouns-Hendriks I, Fijneman RJ, Clausen BE, Forster I, Kockx MM, Rajewsky K, Kraal G, Hofker MH, de Winther MP. Inhibition of NF-kappaB activation in macrophages increases atherosclerosis in LDL receptor-deficient mice. *J Clin Invest* 2003;112:1176-1185.

58. Kanters E, Gijbels MJ, van dM, I, Vergouwe MN, Heeringa P, Kraal G, Hofker MH, de Winther MP. Hematopoietic NF-kappaB1 deficiency results in small atherosclerotic lesions with an inflammatory phenotype. *Blood* 2004;103:934-940.
59. Vaughan DE. PAI-1 and atherothrombosis. *J Thromb Haemost* 2005;3:1879-1883.
60. Liang JS, Schreiber BM, Salmona M, Phillip G, Gonnerman WA, de Beer FC, Sipe JD. Amino terminal region of acute phase, but not constitutive, serum amyloid A (apoSAA) specifically binds and transports cholesterol into aortic smooth muscle and HepG2 cells. *J Lipid Res* 1996;37:2109-2116.
61. Asahara T, Murohara T, Sullivan A, Silver M, van der ZR, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-967.
62. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89:E1-E7.
63. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 2004;53:195-199.
64. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002;106:2781-2786.
65. Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH, Zhu JH. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. *Clin Sci (Lond)* 2004;107:273-280.
66. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, Wang T, Gregg D, Ramaswami P, Pippen AM, Annex BH, Dong C, Taylor DA. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 2003;108:457-463.
67. Havel RJ, Fielding CJ, Olivecrona T, Shore VG, Fielding PE, Egelrud T. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoproteins lipase from different sources. *Biochemistry* 1973;12:1828-1833.
68. LaRosa JC, Levy RI, Herbert P, Lux SE, Fredrickson DS. A specific apoprotein activator for lipoprotein lipase. *Biochem Biophys Res Commun* 1970;41:57-62.
69. Jong MC, Rensen PC, Dahlmans VE, van der BH, van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. *J Lipid Res* 2001;42:1578-1585.
70. Berbee JF, van der Hoogt CC, Sundararaman D, Havekes LM, Rensen PC. Severe hypertriglyceridemia in human APOC1 transgenic mice is caused by apoC-I-induced inhibition of LPL. *J Lipid Res* 2005;46:297-306.
71. Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 1989;341:162-164.
72. Beisiegel U, Weber W, Bengtsson-Olivecrona G. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc Natl Acad Sci U S A* 1991;88:8342-8346.
73. Herz J, Hamann U, Rogne S, Myklebost O, Gausepohl H, Stanley KK. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J* 1988;7:4119-4127.
74. Strickland DK, Ashcom JD, Williams S, Burgess WH, Migliorini M, Argraves WS. Sequence identity between the alpha 2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J Biol Chem* 1990;265:17401-17404.
75. Willnow TE, Armstrong SA, Hammer RE, Herz J. Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. *Proc Natl Acad Sci U S A* 1995;92:4537-4541.
76. Moestrup SK, Gliemann J, Pallesen G. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res* 1992;269:375-382.

77. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest* 2001;108:779-784.
78. Strickland DK, Gonias SL, Argraves WS. Diverse roles for the LDL receptor family. *Trends Endocrinol Metab* 2002;13:66-74.
79. Herz J, Clouthier DE, Hammer RE. LDL receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell* 1992;71:411-421.
80. Nykjaer A, Petersen CM, Moller B, Jensen PH, Moestrup SK, Holtet TL, Etzerodt M, Thogersen HC, Munch M, Andreasen PA, . Purified alpha 2-macroglobulin receptor/LDL receptor-related protein binds urokinase.plasminogen activator inhibitor type-1 complex. Evidence that the alpha 2-macroglobulin receptor mediates cellular degradation of urokinase receptor-bound complexes. *J Biol Chem* 1992;267:14543-14546.
81. Hahn-Dantona E, Ruiz JF, Bornstein P, Strickland DK. The low density lipoprotein receptor-related protein modulates levels of matrix metalloproteinase 9 (MMP-9) by mediating its cellular catabolism. *J Biol Chem* 2001;276:15498-15503.
82. Yang Z, Strickland DK, Bornstein P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. *J Biol Chem* 2001;276:8403-8408.
83. Willnow TE, Sheng Z, Ishibashi S, Herz J. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science* 1994;264:1471-1474.
84. Bovenschen N, Herz J, Grimbergen JM, Lenting PJ, Havekes LM, Mertens K, Van Vlijmen BJ. Elevated plasma factor VIII in a mouse model of low-density lipoprotein receptor-related protein deficiency. *Blood* 2003;101:3933-3939.
85. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest* 1998;101:689-695.
86. Gotthardt M, Trommsdorff M, Nevitt MF, Shelton J, Richardson JA, Stockinger W, Nimpf J, Herz J. Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *J Biol Chem* 2000;275:25616-25624.
87. Ranganathan S, Liu CX, Migliorini MM, von Arnim CA, Peltan ID, Mikhailenko I, Hyman BT, Strickland DK. Serine and threonine phosphorylation of the low density lipoprotein receptor-related protein by protein kinase Calpha regulates endocytosis and association with adaptor molecules. *J Biol Chem* 2004;279:40536-40544.
88. Boucher P, Gotthardt M, Li WP, Anderson RG, Herz J. LRP: role in vascular wall integrity and protection from atherosclerosis. *Science* 2003;300:329-332.
89. Espirito Santo SM, Pires NM, Boesten LS, Gerritsen G, Bovenschen N, Van Dijk KW, Jukema JW, Princen HM, Bensadoun A, Li WP, Herz J, Havekes LM, Van Vlijmen BJ. Hepatic low-density lipoprotein receptor-related protein deficiency in mice increases atherosclerosis independent of plasma cholesterol. *Blood* 2004;103:3777-3782.
90. Luoma J, Hiltunen T, Sarkioja T, Moestrup SK, Gliemann J, Kodama T, Nikkari T, Yla-Herttuala S. Expression of alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein and scavenger receptor in human atherosclerotic lesions. *J Clin Invest* 1994;93:2014-2021.
91. Watanabe Y, Inaba T, Shimano H, Gotoda T, Yamamoto K, Mokuno H, Sato H, Yazaki Y, Yamada N. Induction of LDL receptor-related protein during the differentiation of monocyte-macrophages. Possible involvement in the atherosclerotic process. *Arterioscler Thromb* 1994;14:1000-1006.
92. Spijkers PP, Da Costa MP, Westein E, Gahmberg CG, Zwaginga JJ, Lenting PJ. LDL-Receptor related protein regulates {beta}2-integrin mediated leukocyte adhesion. *Blood* 2004.
93. Zhu H, Takahashi Y, Xu W, Kawajiri H, Murakami T, Yamamoto M, Iseki S, Iwasaki T, Hattori H, Yoshimoto T. Low density lipoprotein receptor-related protein-mediated membrane translocation of 12/15-lipoxygenase is required for oxidation of low density lipoprotein by macrophages. *J Biol Chem* 2003;278:13350-13355.
94. Xu W, Takahashi Y, Sakashita T, Iwasaki T, Hattori H, Yoshimoto T. Low density lipoprotein receptor-related protein is required for macrophage-mediated oxidation of low density lipoprotein by 12/15-lipoxygenase. *J Biol Chem* 2001;276:36454-36459.

95. Llorente-Cortes V, Martinez-Gonzalez J, Badimon L. LDL receptor-related protein mediates uptake of aggregated LDL in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2000;20:1572-1579.
96. Kuchenhoff A, Harrach-Ruprecht B, Robenek H. Interaction of apo E-containing lipoproteins with the LDL receptor-related protein LRP. *Am J Physiol* 1997;272:C369-C382.
97. Fujioka Y, Cooper AD, Fong LG. Multiple processes are involved in the uptake of chylomicron remnants by mouse peritoneal macrophages. *J Lipid Res* 1998;39:2339-2349.