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

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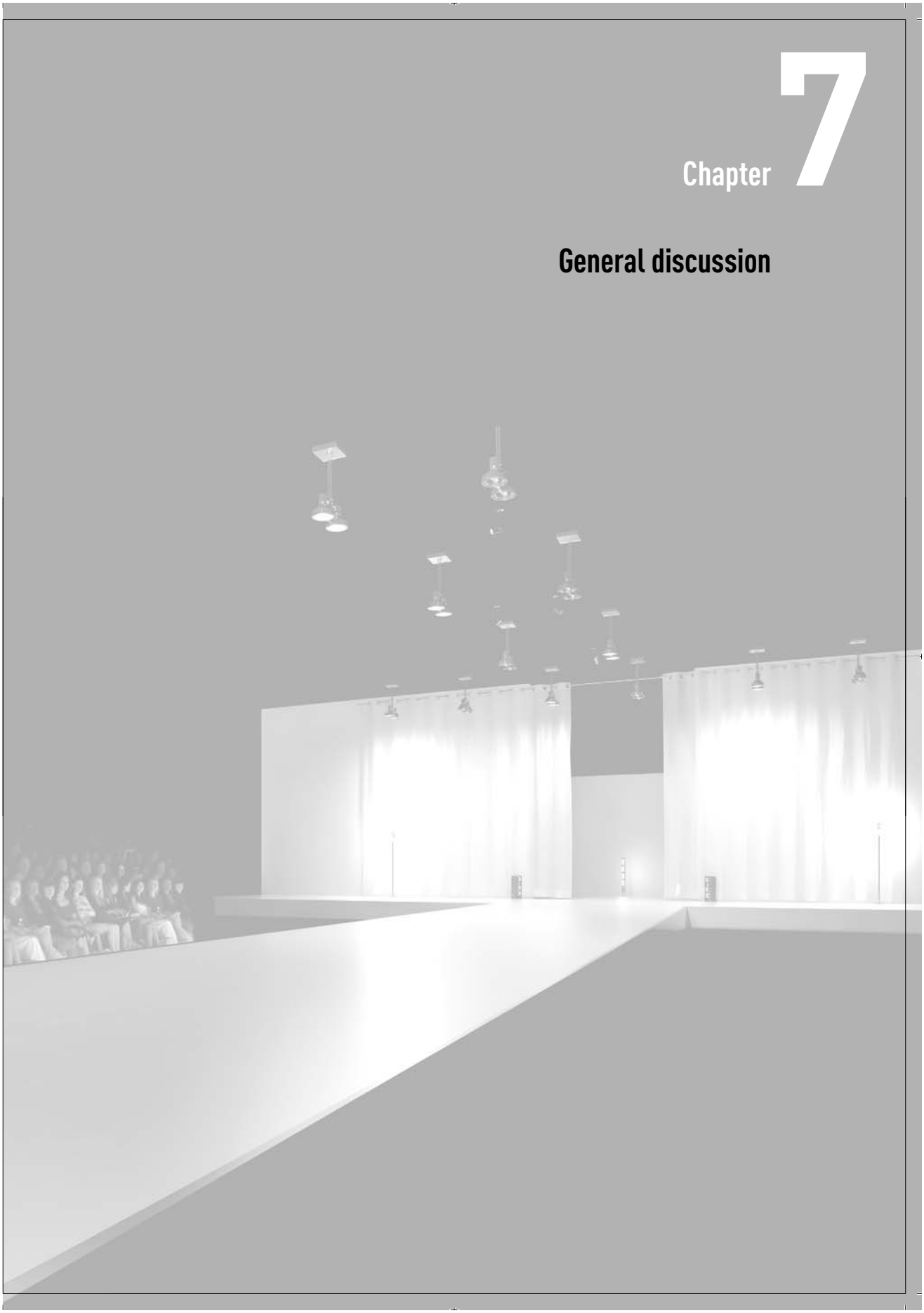
Chapter 7

General discussion



Overweight and obesity is reaching pandemic proportions and rapidly becoming a major health problem in Western societies, momentarily affecting more than 1 billion adults.[1]The pandemic of overweight and obesity is associated with an increased incidence of the metabolic syndrome (MetS). The MetS is now known to be associated with diabetes and increased cardiovascular morbidity and mortality.

The research described in this thesis was designed to gain further inside in the different aspects of the MetS. The studies were in particular directed to the physiological impact of plasminogen activator inhibitor-1 (PAI-1) and the low-density lipoprotein receptor-related protein (LRP) with respect to the MetS and atherosclerosis in mouse models. In addition, the effect of low-grade inflammation on the endothelial function in human subjects with MetS was addressed. The major conclusions and implications of our findings as well as the future perspectives will be discussed in this chapter.

PAI-1 catabolism and its role in insulin resistance and obesity

The low-density lipoprotein receptor (LDLR)-related protein (LRP) is a large cell-surface multi-ligand endocytic clearance and signalling receptor of the LDLR gene family.[2,3] LRP was originally identified as an endocytic receptor for apolipoprotein (apoE)-rich lipoproteins. LRP is known to recognise >50 structurally and functionally different ligands, including plasminogen activator inhibitor-1 (PAI-1).[4,5] PAI-1 is the primary inhibitor of the plasminogen activation *in vivo*. It is not known wether this PAI-1/LRP interaction is of physiological importance. Furthermore, it is unknown by what molecular mechanism PAI-1 is cleared from the circulation. By using the hepatic LRP deficient mouse model, we studied the role of LRP in the clearance of plasma PAI-1 (**Chapter 3**). We showed that plasma PAI-1 level is not regulated by LRP. However, inhibition of the LDLR family by receptor-associated protein (RAP) does significantly increase plasma PAI-1 levels.

The LDLR gene family consists of several members. Among them are the LDLR, very low-density lipoprotein receptor (VLDLR), apolipoprotein E receptor (apoE-R) and megalin. We demonstrated that next to LRP both LDLR and VLDLR are also not involved in the regulation of plasma PAI-1 levels. Which of the other RAP-sensitive mechanisms regulates plasma PAI-1 levels remains to be elucidated. The RAP-sensitive megalin might be good candidate for the regulation of plasma PAI-1. Megalin is shown to interact with PAI-1 and mediates the endocytosis of PAI-1 for degradation *in vitro*.[6,7] Although PAI-1/LRP interaction does not influence plasma levels, the interaction between PAI-1 and LRP might be of importance locally at the cellular level. This PAI-1/LRP interaction might be of importance in the clearance of proteins of the haemostasis system, such as thrombin. PAI-1 can promote the clearance of thrombin via LRP.[7] Additionally, PAI-1 is a potent chemo-attractant molecule, which activity depends on the interaction with LRP for cell signalling.[8] Altogether, we propose that the interaction between PAI-1 and LRP is of importance on a local level rather than on the plasma level.

In 1986, PAI-1 was firstly described to be linked to insulin resistance.[9] Increased plasma PAI-1 levels and further progression of the increased plasma PAI-1 levels correlate strongly with insulin resistance.[10,11] Improvement of insulin resistance by diet or pharmacologic intervention is correlated with decreased plasma PAI-1 levels.[12,13] Diet-induced obesity and insulin resistance were prevented in PAI-1 deficient mice on a wild-type background.[14] Increased plasma PAI-1 levels are suggested to be the result of increased expression of PAI-1 by adipose tissue and ectopic fat depots.[10] These studies together generate the hypothesis that PAI-1 can cause the development of obesity and insulin resistance. It is not known whether attenuated plasma PAI-1 clearance contributes to the increased plasma PAI-1 levels observed in insulin resistance. In **chapter 2** we demonstrated that increased plasma PAI-1 levels in diet-induced insulin resistant obese mice is not due the decreased plasma PAI-1 clearance. Moreover, we showed that plasma PAI-1 levels followed obesity and insulin resistance in time with delay of weeks suggesting that PAI-1 is not causally related to insulin resistance. Several other studies support our data. The study by Morange *et al* showed that PAI-1 deficient mice develop more adipose tissue.[15] Transgenic mice overexpressing PAI-1 have lower body weight, lower adipose tissue mass and less intraperitoneal fat.[16] A new hypothesis might be proposed that the increased PAI-1 levels observed in the epidemiological studies and animal studies are an epiphenomenon of inflammation in the setting of insulin resistance and obesity.

PAI-1 is an acute phase protein and can be induced by inflammation such as induced by lipopolysaccharide injection.[17] Obesity and insulin resistance are positively correlated with low-grade inflammation. Inhibition of inflammation is demonstrated to decrease plasma PAI-1 levels in subjects with insulin resistance.[13] The pro-inflammatory marker tumor necrosis factor-α (TNF-α) is elevated in obese human subjects and rodents.[18] Obese mice deficient of TNF-α remain insulin sensitive.[19] TNFα is known to induce the expression of PAI-1.[20] Taken altogether, these findings support our hypothesis that increased plasma PAI-1 levels are an epiphenomenon in the setting of insulin resistance and obesity rather than a causal factor. The data from the different PAI-1 deficient mouse models could be explained by the different environment/housing or genetically different inflammatory background. This epiphenomenon of increased plasma PAI-1 levels in the setting of insulin resistance could be of significant clinical importance in regard to cardiovascular events. Subjects with insulin resistance have increased cardiovascular risk at the same amount of atherosclerotic burden compared to subjects without insulin resistance. It is apparent that many studies are still needed to investigate the exact mechanisms underlying the involvement of PAI-1 in the development of insulin resistance and obesity.

Role of LRP in atherosclerosis

The traditional view of the development of atherosclerosis is the accumulation of cholesterol in the vessel wall facilitated by elevated plasma cholesterol levels packed in lipoproteins. These lipoproteins are processed and hydrolysed during the transport before taken up by the liver by the LDLR and LRP or the peripheral tissue by the VLDLR. Alternative pathways for the clearance of the triglyceride (TG)-rich lipoproteins are heparin sulphate proteoglycans (HSPGs) and the scavenger receptor class B type I (SR-BI).[21-23] Lipoprotein lipase (LPL) is the key enzyme responsible for the hydrolysis of TG-rich lipoproteins and is suggested to play a role in the uptake of the TG-rich lipoproteins by the LDLR and LRP *in vitro*.[24] By using the LRP-LDLR-/-VLDLR-/- mouse model (**Chapter 4**), we demonstrated that LPL activity also regulates the hepatic uptake of TG-rich lipoproteins via a pathway different from the lipoprotein receptors pathway. We confirmed that both the HSPG and SR-BI pathways are also involved in the clearance of the TG-rich lipoproteins. Therefore plasma lipoprotein levels not only depend on the activity of the lipoprotein receptors, but also on the activity of LPL and the non specific receptors HSPGs and SR-BI. As a consequence, impaired activity of these pathways can result in the development of hyperlipidemia, and eventually atherosclerosis.[25,26]

Atherosclerosis is also considered as an inflammatory disease of the vascular wall.[27] Macrophages play a central role in the pathogenesis of atherosclerosis. Several lines of evidence suggest that LRP in the macrophages promotes the development of atherosclerosis, since it stimulates foam cell formation.[28-35] However, these are all indirect *in vitro* studies. Therefore, we took the advantage of the unique mouse model of macrophage specific LRP deficiency to investigate the role of LRP in macrophages in the development of atherosclerosis **(Chapter 5)**. We demonstrated that LRP in the macrophages protects against the development of atherosclerosis. The mechanism by which macrophage LRP modulates atherosclerosis is not clear yet. A careful control of the balance between the pro-atherogenic and anti-atherogenic LRP ligands is obviously necessary. As important is a strict regulation of cell migration and remodelling of the extracellular matrix. Therefore, it can be postulated that the LRP in the macrophages plays a central role in both controlling the balance between the pro-atherogenic and anti-atherogenic ligands, and the regulation of the extracellular matrix, since it is a multi-ligand multifunctional receptor.[36-40] This hypothesis is supported by tissue specific LRP deficient mouse models. Hepatic LRP deficiency results in increased plasma apoE-rich lipoproteins. Additionally, independent of the plasma lipoproteins LRP deficiency in the liver and smooth muscle cell (SMC) results in increased atherosclerosis and impaired vascular structure.[40,41] In the absence of LRP, the accumulation of pro-atherogenic ligands was observed and the tight regulation of cell migration and the remodelling of the extracellular matrix obliterated. We demonstrated that LRP deficiency in the macrophage results in increased extracellular collagen matrix. Since the extracellular collagen accumulates

in the atherosclerotic plaque in the absence of LRP in the macrophage, one can propose that LRP deficiency results in a stable plaque. However, the definition of a stable plaque is disputable. One could also propose that LRP in the macrophage controls the regulation of cell migration and remodelling of the extracellular matrix as LRP in the SMC. The extracellular collagen is tightly controlled amongst others by the matrix metalloproteinase (MMP)/tissue inhibitor metalloproteinase (TIMP) system and interferon-γ (IFN- γ).[42,43] MMP-9 is an important representative of the MMP/TIMP system and is a known ligand of LRP.[44] The role of MMP-9 is not unambiguous. We did not observe increase of MMP-9. On the other hand, extracellular collagen is modulated by T cells via IFN- γ. T cells inhibit the production of the extracellular matrix stimulated by INF- γ. We demonstrated that the number of T cells was decreased in the atherosclerotic plaques when LRP is absence in the macrophages. One could also propose that LRP in the macrophages controls the inflammatory process in the development of atherosclerosis. This hypothesis is supported by the observed increased inflammatory markers, such as the monocyte chemo-attractant protein type-1 (MCP-1) and TNF-α, present in the atherosclerotic plaques in macrophage LRP deficient mice.[45] It is also suggested that LRP in the macrophage suppresses the inflammation via the NF-κB pathway.[46] All these evidences are in line with the current conception that atherosclerosis is an inflammatory disease of the vascular wall.

Role of inflammation in atherosclerosis in subjects with MetS

MetS is associated with chronic low-grade systemic inflammation. More and more evidence is accumulating that inflammatory markers are predictive to cardiovascular risks. One of the inflammatory markers associated with MetS is the C-reactive protein (CRP).[47] CRP is shown to be associated with endothelial damage and atherosclerosis.[48] Under physiological conditions endothelial damage is restored by the circulating endothelial progenitor cells (EPC). After incorporation into the endothelial monolayer, the EPC stimulate the proliferation of the neighbouring endothelial cells restoring the damaged endothelium.[49] Circulating EPC are thought to be derived from hemangioblastic cells that reside in the bone marrow. Lower numbers of circulating EPC are observed in subjects with cardiovascular diseases.[50] We demonstrated that elevated inflammation as measured by CRP levels is associated with decreased numbers of circulating EPC in subjects with the MetS (**Chapter 6**). The presence of atherosclerotic lesions as assessed by magnetic resonance imaging (MRI) also correlates with lower number of EPC. Very interesting is the similar observation in other chronic inflammatory diseases such as rheumatoid arthritis.[51] Therefore, one can propose that lower circulating EPC levels are associated with a chronic inflammatory state. The mechanism underlying these observations is not completely clear. Is the increased utilisation of EPC by the damaged endothelium an explanation for the low number of circulating plasma EPC? Or is it the inflammatory state that suppresses the production of EPC? Or is it both? We demonstrated that the number hematopoietic stem cells (HSC) is decreased when

low-grade inflammation is present. We also showed that the lower numbers of EPC and HSC are accompanied by higher levels of inflammatory markers like TNF-α and p-selectin. Therefore, suppressed production might be an explanatory factor. However, the questions remain unsolved since our study was an observational study. To elucidate the underlying mechanism, *in vitro* studies need to be performed on how inflammatory markers can influence the number or the function of the EPC and HSC.

Concluding remarks

It should be realised that multiple factors are involved in the pathophysiology of the metabolic syndrome and its increased risk for cardiovascular diseases. Although intensive research is being performed, much research is still required before therapeutic interventions targeting the metabolic syndrome can be developed.

In this thesis we showed the PAI-1 catabolism is facilitated by a RAP-sensitive mechanism other than LRP, LDLR and VLDLR. The increased plasma PAI-1 levels observed in insulin resistance and obesity is not explained by impaired clearance of PAI-1. The increased plasma PAI-1 levels might be an epiphenomenon of the chronic inflammatory state of insulin resistance or obesity. Furthermore, alternative pathways other than the traditional lipoprotein receptors are involved in the regulation of plasma cholesterol and triglyceride levels. The development of atherosclerosis is multi-factorial in which the balance between the anti- and pro-inflammatory processes plays a central role. Macrophage LRP might be one of the features that control this balance. Inflammation not only promotes to the development of atherosclerosis, but might also be involved in the processes that restore the damaged vascular wall. Insulin resistance and cardiovascular diseases are becoming epidemic in the western world concerning both children and adults. Since increased chronic inflammation is particularly fundamental to this, demanding research on this aspect of metabolic syndrome is very much needed.

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