Chapter 7

General Discussion

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There’s an urgent need to increase our understanding of the metabolic syndrome

The obesity epidemic is progressively affecting larger and larger parts of the world causing increasing healthcare problems and costs in many countries. The World Health Organization (WHO) estimates that, for the first time in history, more people are dying as a result of overnutrition than of undernutrition (1). Where overweight used to be largely restricted to older people, nowadays more and more younger people, even young children, are overweight or obese. Their bodies will have to cope with the consequences of lipid overload for a larger part of their lives compared to individuals that become overweight or obese at older age. Most certainly, this will have compromising effects on their health, causing a tremendous increase in the obesity-related health burden for the society as this will cause an acceleration of the already increasing prevalence of the metabolic syndrome. The metabolic syndrome comprises a number of obesity-related health problems that are strong risk factors for the development of diabetes and atherosclerosis. A drastic lifestyle change in industrialized societies would provide the most straightforward solution for the obesity epidemic. However, attempts to improve lifestyle have had only limited success so far as the prevalence of overweight keeps rising. Hence, there is an urgent need to increase our understanding of the mechanistic bases underlying obesity-related pathophysiology to enable more effective therapeutic intervention. In recent years, a lot of research has been performed to obtain more insights into the metabolic syndrome. Although a lot has been learned from those studies, the exact etiology of the metabolic syndrome still remains incompletely understood. The general aim of this thesis therefore was to gain more insights into the pathogenesis of metabolic syndrome-related disease.

Adipose tissue, an important player affecting whole body metabolism

For a long time, adipose tissue was regarded simply a storage site for excess energy as a back-up for times of limited food availability. However, now it has become evident that major changes are taking place in adipose tissue during the development of obesity and that it is capable of affecting whole body metabolism by exerting a number of endocrine functions. Adipose tissue secretes a wide variety of soluble factors including adipokines that can have local as well as systemic effects. One of the best studied adipokines secreted by adipose tissue is leptin. Leptin secretion increases with the size of adipose tissue and functions as a satiety hormone. When a sufficient amount of energy is stored in adipose tissue, leptin signaling in the brain causes a feeling of satiety leading to reduced food intake. Thereby leptin acts as a regulator of energy homeostasis. The importance of leptin signaling in mediating energy homeostasis is exemplified in ob/ob and db/db mice, lacking leptin or the leptin receptor respectively. Both of these models become extremely obese as a result
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of overeating. However, several other adipokines are also produced by the adipose tissue, including adiponectin, resistin and visfatin for example. More recently, it was found that adipose tissue is also an important source of certain cytokines. Interestingly, obesity is associated with a disproportional production of pro-inflammatory cytokines by adipose tissue and adipose tissue infiltration of immune cells, predominantly macrophages. Because of the adipose tissue inflammation and the elevation of circulating pro-inflammatory cytokines obesity is now regarded as a state of chronic low-grade inflammation (2). Of note, it was already found more than a century ago that treatment with high dose sodium salicylate could lower glucose levels in diabetic patients (3). Although the mechanistic basis for this effect remained elusive for a long time, it was demonstrated later to depend on inhibition of IκB kinase β (IKKβ) (4). IKKβ phosphorylates IκB which then releases NF-κB, enabling its nuclear translocation and the subsequent induction of gene expression of pro-inflammatory cytokines. The first cytokine that was found to be produced by adipose tissue was TNFα in 1993 (5). It was found that TNFα expression increased with obesity and that it was associated with insulin resistance (6). Adipose tissue also is an important source of interleukin-6 (IL-6), a cytokine that plays a prominent role in obesity-related pathophysiology (7). In addition to increased expression of pro-inflammatory cytokines, histological analysis has revealed that macrophage infiltration is a predominant feature of obese adipose tissue (8-12). Most macrophages are present in so-called crown-like structures surrounding dead adipocytes (13). Monocyte chemoattractant protein-1 (MCP-1, CCL2) was identified as an important factor driving the recruitment of macrophages to the adipose tissue (14;15). Although the exact trigger for macrophage recruitment remains to be characterized, MCP-1 expression and macrophage infiltration are usually associated with larger sized adipocytes suggesting that these cells are close to the limit of their storing capacity. Macrophages might be recruited to obese adipose tissue because it needs remodeling once the limit of the storage capacity is reached and adipocytes start to die (16;17). Of interest in this respect, abolishing the need for adipose tissue remodeling by overexpression of the adipocyte differentiation stimulating factor adiponectin in leptin-deficient mice led to more, but smaller, adipocytes, reduced adipose tissue inflammation and improved insulin sensitivity (18). Also in lean adipose tissue some macrophages are present, however they are usually of the anti-inflammatory M2 phenotype. During the development of obesity, adipose tissue becomes increasingly populated with pro-inflammatory M1 macrophages causing a disbalance in the M1/M2 ratio that likely contributes to insulin resistance (19). Because of the close contact and cross-talk between adipocytes and immune cells and the systemic impact of adipose tissue inflammation, adipose tissue functions as a major site where metabolism is linked to the immune system.
Impact of 12/15-lipoxygenase on adipose tissue inflammation and insulin resistance

Chapter 2 of this thesis describes the effect of 12/15-lipoxygenase (12/15-LO) deficiency on diet-induced obesity, adipose tissue inflammation and insulin resistance. It was found that high-fat diet (HFD) fed 12/15-LO deficient mice were more obese, had more adipose tissue inflammation and were more insulin resistant compared to WT mice when fed a HFD. Similar results were obtained in chow-fed mice, although the differences between 12/15-LO/− mice and wild-type (WT) controls were somewhat less pronounced. These results differ from the observations made by two other groups that reported that 12/15-LO deficiency protects mice from HFD-induced adipose tissue inflammation and insulin resistance (20-22). Duration of the experiments and different compositions of the diets that were used in those studies may account for the opposing findings. 12/15-lipoxygenase is mainly expressed in macrophages and adipocytes (23). To explore whether the increased adipose tissue inflammation and insulin resistance were a consequence of the increased body weight of the total body 12/15-LO/− mice or whether macrophage 12/15-LO is causally involved in the development of this phenotype, bone marrow transplantation studies were performed in which bone marrow from WT or 12/15-LO/− mice was transplanted into WT mice, leading to macrophage-specific 12/15-LO deficiency. After feeding the transplanted mice a HFD, the mice that had received the 12/15-LO/− bone marrow were more insulin resistant compared to the mice that had received the WT bone marrow. Furthermore, a shift in the macrophage phenotype away from the anti-inflammatory M2 phenotype towards the pro-inflammatory M1 phenotype was consistently detected in the total body 12/15-LO/− mice as well as in the WT mice that had received the 12/15-LO/− bone marrow. Since 12/15-LO expression is strongly increased in macrophages of the M2 phenotype (24), implicating a possible role for 12/15-LO in the polarization towards this type of macrophages, in vitro experiments were performed in which macrophage polarization towards the M2 phenotype was stimulated with IL-4. In keeping with the in vivo observations, the macrophages that lacked 12/15-LO expression had an impaired ability to polarize towards the M2 phenotype. Taken together, these data indicate that macrophage 12/15-LO is important for maintaining insulin sensitivity because of its role in the polarization of macrophages towards the anti-inflammatory M2 phenotype. In a broader context these data appear also important for strengthening the emerging concept of macrophage polarization playing a role in the development of insulin resistance. Furthermore, this particular study exemplifies that the identification of therapeutic targets for obesity and insulin resistance might be a rather difficult task, in which species and particularly dietary context have a major impact. While derived from the very uniform data obtained in the different consecutive experiments presented in chapter 2 pharmacological inhibition of
12/15-LO appears to be deleterious with respect to insulin resistance/diabetes, the two other published studies using the same mouse model (21;22) would reach exactly the opposite conclusion.

**The role of the p38-MAPK target MK2 in adipose tissue inflammation and insulin resistance**

Intracellular signal transduction is governing cellular behavior in response to various environmental stimuli and is of special importance in the context of inflammation. Sequential activation of kinases facilitate rapid transduction of signals through the cell. Mitogen-activated protein kinases (MAPKs) are a group of kinases that play a central role in intracellular signal transduction. Three major MAPK pathways are present in the cell: the extracellular signal-regulated kinase (ERK), the c-Jun NH2-terminal kinase (JNK) and the p38-MAKP pathway. The latter pathway is a major regulator of inflammatory processes. The p38 pathway can be activated by a number of environmental stimuli, including cytokines. Following activation of an upstream receptor, p38 plays a pivotal role in the intracellular transduction of the signal that ultimately determines the response of the cell, e.g. the production of inflammatory cytokines. Therefore, p38 appears to be an attractive target for the action of anti-inflammatory drugs, with the intention to e.g. reduce adipose tissue inflammation. However, due to toxicity of p38 inhibitors, use of such compounds has so far not been applicable in the clinic (25). Since p38 has several downstream targets, inhibiting one or a few of those might conceivably increase the chances of therapeutic success without the undesirable side effects (26). Mitogen-activated protein kinase-activated protein kinase 2 (MK2) is a direct target of p38 that appears to be a more attractive target for future anti-inflammatory drug development (27-29). In contrast to p38α−/− mice (30), MK2−/− mice are viable without any obvious health problems (31). MK2 is involved in the maintenance of NF-κB activation and stabilization of mRNA molecules encoding pro-inflammatory cytokines like TNFα (27;31). As chronic low-grade adipose tissue inflammation has been linked to the development of insulin resistance, experiments were carried out to test whether MK2-deficiency would protect mice against HFD-induced adipose tissue inflammation and insulin resistance. As described in chapter 3 of this thesis, no differences in the degree of obesity and adipose tissue inflammation were observed between HFD fed MK2−/− and WT control mice. Surprisingly, however, MK2−/− mice showed decreased glucose tolerance following intraperitoneal administration of a glucose bolus. This coincided with decreased insulin sensitivity as became apparent during an insulin tolerance test. This unexpected phenotype suggests that MK2 not only plays a role in inflammation, but also is a more direct mediator of metabolism. Decreased expression of the insulin-responsive glucose transporter Glut4 in the adipose tissue likely contributes to the unexpected phenotype. Glut4 is a main receptor for glucose uptake in response to insulin (32). The important role of adipose tissue Glut4 in
maintaining insulin sensitivity is emphasized by the fact that adipose-specific Glut4−/− mice develop insulin resistance (33). On the other hand, mice overexpressing Glut4 specifically in adipose tissue have enhanced glucose tolerance and reduced fed insulin levels, indicative of improved insulin sensitivity (34). The pivotal role of adipose tissue Glut4 is further emphasized by the fact that overexpression of Glut4 in adipose tissue of mice that lack Glut4 expression in muscle corrects the insulin resistant phenotype in those mice (35). Interestingly, GLUT4 expression was found to be decreased in adipose tissue but not in muscle of obese individuals and in insulin resistant and type 2 diabetic patients (36;37). Taken together, the data from the studies above demonstrate that adipose tissue Glut4 expression is a central mediator of whole body glucose tolerance. Therefore, decreased adipose tissue expression of Glut4 in the MK2−/− mice in the present study conceivably offers a plausible explanation for the glucose intolerant, insulin resistant, phenotype. It remains, however, to be explored how MK2-deficiency leads to decreased adipose tissue Glut4 expression on a molecular level. In addition, the dyslipidemic phenotype observed in the HFD-fed MK2−/− mice in the experiments described in chapter 3 and in a study using Western diet-fed MK2−/−Ldlr−/− double ko mice (29) clearly indicate that MK2 is involved in mediating more processes than inflammation alone. Therefore, directly extrapolating from the studies presented in chapter 3, clinical use of MK2 inhibitors for the treatment of chronic inflammatory diseases has to be evaluated with caution, taking potential metabolic adverse effects into account. Special attention should thereby be given to the careful assessment of changes in glucose metabolism during the short-term but even more important long-term therapeutic application of such inhibitors for chronic inflammatory diseases.

HDL function in type 2 diabetes patients

Patients with type 2 diabetes have an increased risk for the development of atherosclerosis. While high plasma LDL-cholesterol (LDL-C) levels are a risk factor for atherosclerosis, HDL-cholesterol (HDL-C) levels in plasma are strongly inversely correlated with the risk of cardiovascular disease (CVD) (38-40), implying that HDL can protect against the development of atherosclerosis. Nevertheless, some patients experience CVD events despite high plasma HDL-C levels, while a certain percentage of individuals with low HDL remain free of CVD (41-43). Moreover, especially in patient populations with a high inflammatory load, the inverse relationship between plasma HDL-C levels and CVD incidence is lost (44;45). Those observations led to the emerging concept that HDL quality might be an important contributor to the protection against CVD events (42;46;47). Therefore, additional clinically relevant information could potentially be derived from assays testing the functional quality of HDL (42). Multiple mechanisms by which HDL can protect against atherosclerosis have been described, including but not limited to (i) promotion of cholesterol efflux from macrophage foam cells and reverse cholesterol transport, (ii) protection against
LDL oxidation, (iii) improving endothelial cell function and vasorelaxation and (iv) anti-inflammatory actions (48;49). As patients with type 2 diabetes have an increased CVD morbidity and mortality (50-53), altered HDL quality might conceivably contribute to disease risk in those patients. However, still little is known about HDL functionality in type 2 diabetes patients. Oxidative modification of LDL particles makes them strong ligands for uptake via scavenger receptor-mediated endocytosis by macrophages causing foam cell formation, a key event in the development of atherosclerosis (54). As ‘healthy’ HDL can protect against LDL oxidation, in chapter 4 of this thesis it was investigated whether the ability of HDL to protect against LDL oxidation is altered in type 2 diabetic patients as compared to control subjects. The potency of HDL to inhibit LDL oxidation was determined using an end-point assay with identical amounts of HDL-C. This approach was chosen because an end-point assay would be easier for application as a clinical test to screen large numbers of individuals than a dynamic assay. Normalization for HDL-C was applied to be able to directly detect functional differences of the particles independent of HDL-C levels. The data that were obtained revealed that the anti-oxidative function of HDL particles per se was not affected in type 2 diabetic patients. However, due to lower HDL levels, the total HDL-mediated protection against LDL oxidation was decreased in those patients. Calculating the total HDL-mediated protection against LDL-oxidation of an individual by multiplying the antioxidative function of the HDL particles with the individual’s plasma HDL-C level appears both justified and clinically meaningful as a reflection of an individual’s total antioxidative protection exerted by HDL.

The HDL-associated enzymes lecithin-cholesterol acyl transferase (LCAT) and paraoxonase-1 (PON-1), have been implicated to impact on HDL function (55-57). Therefore, it was also tested whether those enzymes were related to the HDL antioxidative function in the subjects of the present study detailed in chapter 4. Interestingly, the results show that higher LCAT activity was independently associated with decreased HDL antioxidative function. LCAT mediates the maturation of HDL particles by esterifying free cholesterol. Increased LCAT activity leads thereby to higher plasma HDL cholesterol levels and was therefore generally considered to be atheroprotective. However, experimental and clinical evidence for such a role of LCAT is still unclear as in experimental animals as well as in humans inconsistent data have been reported (58-72). The mechanisms responsible for the negative impact of LCAT on HDL antioxidative function are not precisely understood at present. One possible explanation is the fact that LCAT might contribute to the generation of oxygen radicals and oxidative stress in vivo. This is exemplified by an impressive reduction of oxidative stress in ApoE-deficient mice lacking LCAT (72). Although additional mechanistic studies are required to investigate the causative impact of LCAT on HDL antioxidative function, the data shown in chapter 4 suggest that interventions aimed to increase plasma HDL levels in diabetic patients without increasing LCAT activity could prove a successful strategy to restore the total antioxidative protection by HDL. Additionally, it was found that plasma glucose as well as HbA1c levels were inversely related to the antioxidative functionality of HDL in type 2 diabetes patients,
emphasizing the importance of adequate glycemic control in those patients. In the present study, PON-1 activity was lower in diabetic patients compared to controls in agreement with data published by others (73). PON-1 activity was associated with the total HDL-mediated antioxidative protection but not with the antioxidative functionality of the HDL particles. The results described in chapter 4 differ in several aspects from the conclusions of a previous study that reported the antioxidative function of HDL particles to be impaired and positively related to LCAT activity using a similar methodological approach, but including lower numbers of patients and control subjects (74). However, the diabetes-associated dyslipidaemia was more pronounced in this previous study compared with the type 2 diabetes mellitus group investigated in this thesis, as plasma triglycerides were more than twofold elevated (74), while only being approximately 30% higher in the patients with diabetes included in the present work. These differences might have translated into altered antioxidative functionality of HDL in the previous report (75) and might also provide an explanation for the contradicting results regarding the relation between LCAT activity and HDL antioxidative function. Of note, the study described in chapter 4 is a cross-sectional study so it does not address the clinically highly important question of a potential prospective role of HDL antioxidative function for the future development of cardiovascular events or diabetes progression. Prospective follow-up studies will be necessary to provide evidence for a potential causative role of impaired HDL antioxidative function in the development of CVD and diabetes progression.

Reverse cholesterol transport in experimental type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) develops following an autoimmune reaction that causes destruction of the insulin-producing β-cells in the pancreas (76). As type 2 diabetes patients, T1DM patients are at increased risk of atherosclerotic CVD. In type 1 diabetic patients, atherosclerotic CVD represents the major cause of morbidity and mortality (77;78), most likely at least in part due to suboptimal glycemic control (79). However, the underlying pathophysiology is incompletely understood. Because promoting reverse cholesterol transport is an important atheroprotective functionality of HDL, it was investigated whether experimental T1DM impacts on this process in mice. Chapter 5 of this thesis describes the data that were obtained from mice in which T1DM was induced by a single injection of alloxan, a compound which specifically induces pancreatic β-cell destruction by means of ROS generation (80). The obtained results demonstrate that experimental T1DM results in a decrease in macrophage-to-feces RCT in mice despite increased biliary sterol secretion rates. Biliary sterol secretion is thought to be a major determinant for the completion of the RCT pathway (81;82). The data presented in chapter 5 show enhanced biliary secretion of bile acids as well as cholesterol in the T1DM mice. These results are consistent with data published previously, demonstrating that alloxan-
or streptozotocin-induced diabetes increases biliary sterol secretion rates in rats (83;84). In addition, concentrations of bile acids and cholesterol in gallbladder bile were reported to be increased in T1DM mice injected with alloxan as well as in non-obese diabetic (NOD)-mice (85;86).

Aiming to delineate the mechanism underlying reduced RCT in T1DM mice, the impact on the two other key steps relevant for this process was explored, namely cholesterol efflux from macrophage foam cells and selective uptake of HDL cholesterol by the liver. It has been described that under conditions of hyperglycemia, HDL-associated proteins readily become glycated (87;88). Indeed, also the HDL proteins in the T1DM mice in the present work were glycated as indicated by an increased content of fructosamine residues. However, cholesterol efflux from macrophage foam cells towards those glycated HDL particles was not affected. Decreased efflux towards glycated Apo-AI has been described (89), however in the context of a whole HDL particle glycation does not seem to impact on cholesterol efflux (90;91). Although somewhat speculative, this apparent discrepancy may very well reflect the different impact of glycation on efflux mediated by ABCA1 and ABCG1. Since efflux of cholesterol from macrophages as well as biliary secretion did not explain the reduced RCT found in the T1DM mice, next it was tested whether the HDL isolated from T1DM mice had an impaired ability to facilitate hepatic selective uptake using an in vitro approach as well as in vivo HDL kinetics. Of note, the hepatic expression of SR-BI remained largely unchanged in T1DM mice. However, the results from the in vitro experiments as well as the in vivo HDL kinetics study revealed that HDL from T1DM mice had an impaired functionality in mediating hepatic selective uptake, presumably accounting for the reduced RCT observed in those mice. Lower rates of RCT conceivably contribute to the increased CVD risk in T1DM patients.

As indicated in figure 7 in chapter 5, the combined data of this study suggest an enhanced cycling of cholesterol between the liver and intestine that does not have a net effect on fecal neutral sterol secretion. Although direct evidence for such increased cycling of cholesterol between the liver and the intestine is not provided, data are available that would support such a phenomenon. First, type 1 diabetes leads to increased secretion of cholesterol into the bile, as found in chapter 5 and by others (83-86). This indicates an increased flux of cholesterol from the liver to the intestine. Second, insulin inhibits chylomicron production by the intestine (92) and type 1 diabetes was reported to result in higher chylomicron production in lymph cannulated rats (93), which offers an explanation for an increased flux of cholesterol from the intestine back to the liver. Combined, these data indicate that increased cycling of cholesterol between the liver and the intestine is likely in type 1 diabetes. In addition, the increased food intake in combination with the strong induction of biliary cholesterol secretion without resulting in an effect on fecal neutral sterol excretion, which was observed in the type 1 diabetic mice, suggests a reduced transintestinal cholesterol excretion (TICE). However, the data reported in chapter 5 are not all derived from the same set of mice, which complicates calculation of the TICE flux since differences in food composition between experiments might have
differentially affected the separate cholesterol fluxes. Nevertheless, the data point towards decreased TICE under type 1 diabetic conditions. Therefore, it would be worthwhile to measure cholesterol derived from food, cholesterol derived from bile, cholesterol absorption and fecal neutral sterol excretion all in the same set of animals. Such an experimental setup would allow a firmer conclusion on the effect of experimental type 1 diabetes on TICE.

Besides mediating RCT, HDL particles have additional anti-atherosclerotic properties such as inhibiting endothelial inflammation (94), promoting vascular nitric oxide generation (95) as well as protecting LDL against oxidative modification (96;97). Increasing evidence suggests that also these atheroprotective functions of HDL particles are impaired in T1DM. The anti-oxidative properties of HDL appear to be decreased in T1DM (98;99) and the ability of HDL from T1DM patients to counteract the inhibitory effects of oxidized LDL on endothelium-dependent vasorelaxation is also reduced (100). In addition, glycation of apoA-I was recently shown to decrease the potency of HDL to inhibit neutrophil infiltration and adhesion molecule expression using a carotid artery collar model in rabbits (101). In addition to decreased RCT, these alterations are expected to also contribute to an overall reduced capacity of ‘diabetic HDL’ to protect against atherosclerotic CVD. Therefore, therapies aiming to restore HDL functionality in diabetic patients could conceivably reduce the incidence of cardiovascular events in those individuals.

**Apolipoprotein O, a novel HDL-associated apolipoprotein**

As HDLs are complex particles, many factors are present that might impact on their function. The factors responsible for the atheroprotective effects of HDL are, however, as yet incompletely understood. Apolipoproteins comprise a significant part of HDL particles and impact their functionality (47;102;103). Recently, a novel apolipoprotein was identified that had been cloned from diabetic dog hearts (104). This novel protein, apolipoprotein O (apoO), was further characterized as an α-helical protein that was readily detectable in plasma where it was mainly associated with HDL (104). Very recently, it was reported that plasma levels of apoO are increased in patients with an acute coronary syndrome (105). Interestingly, apoO was not found in a comprehensive proteomics study of HDL-associated proteins (106). Although the purified protein was shown to induce cellular cholesterol efflux from J774 macrophages (104), the impact of apoO on plasma lipids and lipoprotein distribution and on HDL functionality remained unexplored. Therefore, experiments summarized in chapter 6 investigated the effects of human ApoO overexpression on plasma lipoprotein distribution and HDL functionality using human ApoA-I transgenic mice as the principal model, a humanized mouse model of HDL metabolism. Employing adenoviral overexpression of human apoO we confirmed the earlier report that apoO was mainly present on HDL, although some apoO was also detectable on LDL, but not on VLDL or in the lipid-free fractions following FPLC separation of lipoproteins.
Of interest in this respect is the fact that apoO was reported to be primarily associated with HDL in healthy subjects, while in patients with acute coronary syndrome LDL was found to contain apoO as well (105). The rather weak signal obtained from the protein blots of the FPLC fractions in the present study suggests however that the overall abundance of apoO on lipoproteins is relatively low, which may be a reason that apoO was not detected in the proteomics screen of HDL-associated proteins mentioned above (106). The fact that apoO spins off easily when HDL is isolated by ultracentrifugation, as can be deduced from the lack of apoO in lipoprotein free FPLC fractions (chapter 6 of this thesis) in combination with the abundant presence of apoO in the non-lipoprotein fraction after ultracentrifugation (104), most likely is an additional reason why apoO was not detected on HDL in the proteomics screen (106), since ultracentrifugation was used to isolate the HDL in that study. The results given in chapter 6 further demonstrate that overexpression of apoO does not impact on plasma lipids or lipoprotein distribution. In addition, using a panel of different assays to test the major established atheroprotective functions of HDL it was demonstrated that enrichment of HDL with apoO in vivo neither impacts on stimulation of cholesterol efflux from macrophage foam cells, nor on the anti-oxidative, anti-inflammatory or vasorelaxating properties of HDL. This is the first time that such a comprehensive panel of assays has been used to assess HDL functionality in a single study. Although purified apoO was reported by others to stimulate cholesterol efflux (104), the data provided in this thesis demonstrate that in the context of a whole HDL particle apoO does not impact cellular cholesterol efflux. Since no apoO was detected in the lipid free FPLC fractions, in vivo stimulation of cholesterol efflux by apoO appears unlikely. It needs to be kept in mind, however, that the here presented data do not rule out the possibility that apoO functions at very low levels. Therefore, to definitively exclude the possibility that apoO impacts on HDL metabolism or functionality, experiments using an apoO knockout model would be desirable to complement the overexpression approach. Although no impact of apoO on HDL functionality was detected, the possibility remains that in certain cells and tissues apoO carried by lipoproteins can elicit biological responses other than the ones tested in the present study as suggested by the fact that apoO was reported to be an independent predictor of an acute coronary syndrome (105). Moreover, apoO is abundantly expressed and might have, as yet unknown, intracellular functions which were not investigated in this thesis.

In conclusion, because of the vast amount of research that is being conducted at the moment, insight into the pathways causing the development of overnutrition-related health problems is growing rapidly. It is now becoming increasingly clear that adipose tissue inflammation and HDL functionality are important aspects in the development of insulin resistance and atherosclerosis, respectively. Effectively reducing adipose tissue inflammation could improve insulin sensitivity in overweight and obese individuals and thereby decrease the progression to type 2 diabetes and reduce the risk of atherosclerotic CVD. Care should be taken that inflammatory
pathways in adipose tissue are inhibited in a rather specific manner, however, in order to provide full therapeutic effectiveness without inducing undesired side effects of the treatment. As this thesis indicates, the metabolic context of the studies is an important factor. In addition, every investigation into such pathways should aim to provide a complete picture, since inflammation, obesity and insulin resistance could be selectively affected. HDL function on the other hand represents an emerging concept that certainly is worth further exploration including the design and execution of prospective studies in large patient populations. Assays quantifying HDL function in an integrative manner might conceivably predict better which individuals are at increased risk for the development of atherosclerotic CVD than the rather simple HDL-C mass measurements that focus only on one particular component of HDL. Additionally, therapies that improve the atheroprotective functions of HDL are likely to reduce the risk of cardiovascular events. Taken together, in order to successfully tackle the increasing medical challenge of the metabolic syndrome and its related complications, mechanistic research as well as multimodal therapeutic approaches will be required.
Reference List


