The obesity epidemic

The cause for the susceptibility of the human species to develop obesity originates from the past. For the longest time in human evolution food was frequently scarce and our ancestors had to commit a lot of effort to collect sufficient food to maintain their caloric needs. However, during periods of starvation, our species would most likely have become extinct if there would not be a means to store excess energy in times of prosperity for use in times of scarcity. The farmost storage of redundant energy is conducted by a specialized tissue, the adipose tissue. Consisting of multiple depots, adipose tissue can expand tremendously when energy is stored in the form of triglycerides (fat) in periods where energy intake exceeds utilization. In times where limited food is consumed, stored triglycerides are hydrolyzed and released from the adipose tissue into the blood circulation, thereby becoming available as a source of energy for other cells and tissues in the body. As a consequence, energy buffers decline and adipose tissue volume decreases again. For thousands of years the ability to store energy in adipose tissue for usage in times of necessity has helped the human species to survive. However, in the modern era, food is available at all times and in nearly unlimited quantities in large parts of the world. In addition, due to industrialization, energy expenditure has decreased and a growing number of people now has a sedentary lifestyle. As a result, many people have a positive energy balance, meaning that they consume more energy than they spend, which is stored as fat and results in weight gain. When energy consumption exceeds energy expenditure for long periods of time, the expansion capacity of the adipose tissue is pushed to the limit. At a certain moment, the adipose tissue is not able to store anymore energy and fat will be stored ectopically in organs and tissues like liver and muscle (1). However, this interferes with the normal function of those tissues and contributes to the development of diseases such as insulin resistance and type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease (NAFLD). During the last decades, there has been a strong increase of the number of overweight (body mass index (BMI) >25) and obese individuals (BMI >30). One could say that, in parts of the world like the United States and Europe, obesity incidence is reaching epidemic proportions. The World Health Organization (WHO) estimated that, in 2008, worldwide 1.5 billion adults were overweight of which 500 million were classified as obese. These numbers are predicted to rise further in the forthcoming years. As might be expected, the prevalence of obesity-associated diseases like type 2 diabetes (2) and cardiovascular disease (CVD) (3) is also increasing at a substantial rate. Consequently, obesity-associated mortality is a major cause of death in developed countries these days and will likely increase further in the upcoming years (4). Obesity-related complications have been grouped together in the so-called metabolic syndrome, also known as syndrome X. The term metabolic syndrome comprises a cluster of conditions including obesity, dyslipidemia, insulin resistance and diabetes mellitus. According to WHO guidelines, a person has metabolic syndrome if the following criteria are met: (i) presence of at least one of the following conditions: diabetes mellitus, impaired
glucose tolerance, impaired fasting glucose or insulin resistance in combination with (ii) two of the following: high blood pressure, dyslipidemia, central obesity or microalbuminuria. Metabolic syndrome usually coincides with a chronic low-grade inflammation, e.g. in adipose tissue. This aspect of the metabolic syndrome has gained a lot of interest the last couple of years.

The immune system in a nutshell

Most organisms, even certain bacteria and plants, have developed some sort of immune system that protects them from being infected by pathogens. In mammals, the immune system consists of a complex network of circulating factors, cells, tissues and organs that defend the host against foreign invaders, often in a collaborative manner. In some cases, the immune system becomes autoreactive and starts to attack the bodies’ own cells and tissues, causing autoimmune disease. Well-known examples of such autoimmune diseases are rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and type 1 diabetes mellitus (T1DM), which is described in more detail below. On the other hand, when the immune system is suppressed, e.g. in people treated with immunosuppressive drugs or in patients with the acquired immunodeficiency syndrome (AIDS), resulting from infection with the human immunodeficiency virus (HIV), pathogens are not adequately eliminated and the individual becomes more susceptible to infections. A properly functioning immune system efficiently attacks foreign invaders but leaves the organisms’ own cells and tissues unharmed.

The immune system consists of two branches, the innate and the adaptive immune system with the latter branch only being present in higher animals (5). The innate immune system serves to attack foreign invaders in a quick, however rather unspecific, manner and no immunological memory is attained. The innate immune system comprises humoral (e.g. the complement system) and cellular components. Effector cells of the innate immune system include, amongst others, macrophages, dendritic cells, neutrophils and eosinophils. The innate immune system plays a major role in adipose tissue inflammation that occurs in obese individuals (see below for more details). The adaptive or acquired immune system on the other hand serves to attack invading organisms in a highly specific manner by the recognition of pathogen-specific antigens. Compared to innate immune responses, however, adaptive immune responses need more time to develop. The main effector cells of the adaptive immune system are the T and B lymphocytes. These cells carry receptors that recognize specific antigens that, when presented in combination with appropriate signals from co-stimulatory molecules, causes these lymphocytes to become activated. Once activated, antigen-specific T and B cells will start to proliferate in a process known as clonal expansion. These expanded, antigen-specific, clones then act to eliminate the invading pathogen. T helper cells will start to produce a variety of cytokines that stimulate the other cells of the immune system to attack the pathogen. Cytotoxic
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T cells will eliminate cells that are infected with a pathogen and express pathogen-derived peptides on their cell surface in the context of MHC class I molecules, whereas regulatory T cells produce anti-inflammatory factors to prevent an uncontrolled immune response. B cells produce antibodies that 'label' infected pathogens in a process called opsonisation which makes the pathogens easier recognizable for other cells of the immune system. A clear hallmark of the adaptive immune system is the feature that memory can be acquired which optimizes elimination of a pathogen during subsequent infections. Although a clear distinction between the innate and acquired branches of the immune system is usually made in text books, they are closely connected and there is ample cross-talk between the cells of these different branches within an organism (5).

During inflammation, a cell receives many signals via receptors located at the plasma membrane. Signaling cascades facilitate communication between the plasma membrane and intracellular compartments where regulatory targets are located. Sequential activation of kinases provide means to quickly respond to signals from the environment. Mitogen-activated protein kinases (MAPK's) are a group of kinases that play a pivotal role in transducing extracellular signals to the nucleus. Three major MAPK pathways have been identified: The extracellular signal-regulated kinase (ERK), the c-Jun NH2-terminal kinase (JNK) and the p38-MAKP pathway. The latter two are prominently involved in the transduction of inflammatory signals (6). MAPK's contribute to numerous cellular responses, including cell proliferation and apoptosis but also affect cell morphology. However, these features are beyond the scope of this thesis and are reviewed in detail elsewhere (7).

Bioactive lipids have been shown to be crucially involved in inflammatory processes (8). Many bio-active lipid species have been characterized. Well known bio-active lipids are (phospho)sphingolipids and arachidonic acid-derived bio-active lipids. The role of sphingolipids, including sphingosine-1-phosphate, as bio-active lipids has been extensively studied (9-11), but is not a subject of this thesis and will therefore not be further discussed here. Arachidonic acid can be liberated from membrane phospholipids by the actions of phospholipase A2 enzymes that catalyze phospholipid hydrolysis at the sn-2 position (12). The released arachidonic acid serves as a substrate for enzymes like cyclooxygenases and lipoxygenases that covert it into other lipid mediators that can either potentiate or inhibit inflammatory processes. Some enzymes have a wide-ranging substrate specificity and therefore are able to produce distinct products, depending on the substrate they encounter. For example, 12/15-lipoxygenase (12/15-LO) is involved in the biosynthesis of lipoxins and D-series resolvins (from arachidonic acid and docosahexaenoic acid (DHA) respectively), bio-active lipids that aid in the resolution of inflammation, (13-15). On the other hand, 12/15-LO is also involved in the biosynthesis of bio-active lipids that potentiate inflammation (16). In chapter 2, data are presented regarding the role of 12/15-LO in diet-induced adipose tissue inflammation and insulin resistance.
Adipose tissue, more than just an energy storage depot

For decades, adipose tissue was regarded as a not too meaningful, simple, tissue that functioned solely for the storage of excess energy. However, observations made from the end of the 20th century until now have drastically changed this view on adipose tissue. Nowadays, adipose tissue is regarded as a complex endocrine tissue capable of influencing whole body metabolism (17-19). The increased appreciation for adipose tissue started in 1993 when Hotamisligil and coworkers published that TNFα expression in adipose tissue is linked to insulin resistance in animal models (20). Two years later, the same group reported that TNFα expression is increased in human obesity and insulin resistance as well (21). Soon after these initial publications, adipose tissue was identified as a source of multiple other cytokines too, including plasminogen activator inhibitor-1, transforming growth factor-β, interleukin-6 and monocyte chemoattractant protein 1 (MCP1/CCL2) (22-25). Several years later, it was reported that adipose tissue of obese individuals as well as mice is inflamed and infiltrated with macrophages (26-30). By means of adipocyte-specific overexpression, Mcp1 was shown by two groups to be a major factor responsible for recruitment of macrophages into adipose tissue and the subsequent development of insulin resistance (31;32). However, the important role of Mcp1 in the recruitment of macrophages into the adipose tissue could not be confirmed by another group using mice that were Mcp1-deficient (33). How nutrient excess exactly triggers the production of chemoattractive factors and immune cell infiltration of adipose tissue is incompletely understood (34). However, adipocyte hypertrophy has been linked to increased infiltration of immune cells into the adipose tissue (35;36). It is presumed that, when adipocytes are overloaded with lipids and reach a certain size, their condition deteriorates causing an increased production of chemoattractive factors (37). This hypothesis is supported by the typical infiltration pattern that is observed upon immunohistological examination of ‘obese’ adipose tissue. Macrophages that have infiltrated adipose tissue are usually present in so-called crown-like structures, surrounding dead adipocytes (38). It is believed that they gather around these dead adipocytes to clean the remainders (39;40). A considerable number of macrophages are also present in adipose tissue of lean individuals and mice. However, in contrast to obesity, adipose tissue macrophages in the lean condition mainly exert an anti-inflammatory phenotype known as M2-polarization (41). When someone becomes obese, not only macrophage numbers in the adipose tissue increase but the phenotype of the adipose tissue macrophages also changes. With increasing obesity, macrophage polarization shifts from an anti-inflammatory M2 phenotype to a pro-inflammatory M1 phenotype (41). M1 macrophages produce a more inflammatory cytokine profile compared to M2 macrophages (42-44) and M1-skewed macrophage polarization in the adipose tissue has been linked to insulin resistance (45-48) while M2-skewed macrophage polarization was demonstrated to have beneficial effects on insulin sensitivity (49;50). Whereas most research on adipose tissue inflammation so far has focused on macrophages, recently other types of immune cells have been
observed in adipose tissue as well. Several groups reported the presence of T cells in adipose tissue, and specific subpopulations have been linked to protection (51;52) or exacerbation (53-55) of adipose tissue inflammation and insulin resistance. B cells have also been reported to be present in adipose tissue and impact on insulin sensitivity (56). Although macrophages are the most abundant type of immune cells present in inflamed adipose tissue, the reports mentioned above indicate that obesity-induced adipose tissue inflammation involves a comprehensive and complex immunological reaction in which many cell types take part.

Adipose tissue also is a source of adipokines like leptin, adiponectin, resistin and visfatin. The best studied adipokines are leptin and adiponectin. Serum leptin levels correlate with the amount of adipose tissue. Leptin serves as a sensor for the amount of adipose tissue present in an individual and acts as a regulator of food intake by functioning as a satiety hormone (57). Normally, when a person has a sufficient amount of adipose tissue, leptin signaling in the brain will result in satiety causing a reduction in food intake. This working mechanism is exemplified in mice that lack either leptin (ob/ob mice) or the leptin receptor (db/db mice). In those mice lack of leptin signaling leads to increased food intake, resulting in severe obesity and insulin resistance. It has therefore been proposed that leptin resistance in the hypothalamus might be causally involved in the development of obesity and insulin resistance (58).

Interestingly, leptin can also act as a stimulator of inflammation. Although the various effects of leptin on the immune system are far from elucidated, leptin is known to stimulate macrophage activation and to act as an inducer of T helper-1 cells (59). In contrast to leptin, serum adiponectin levels are often inversely related with the amount of adipose tissue. Adiponectin stimulates the differentiation of adipocytes, leading to a smaller average adipocyte size (60;61). These beneficial effects of adiponectin are demonstrated in ob/ob mice that overexpress adiponectin specifically in adipocytes. These transgenic mice display reduced adipose tissue inflammation and increased insulin sensitivity despite an enormous increase in body weight compared to normal ob/ob mice (62). This was most likely achieved by an improved differentiation of subcutaneous adipocytes, facilitating increased storage of lipids while maintaining a relatively small adipocyte size. A number of anti-inflammatory properties have also been attributed to adiponectin, including a role in decreasing the production of the inflammatory cytokines TNFα and IFNγ while on the other hand stimulating the production of the anti-inflammatory cytokine IL10 (63).

**Insulin, an indispensable mediator of metabolism**

Insulin, one of the most important hormones, is highly conserved between species and functions as a major regulator of metabolism. The main function of insulin is to maintain the blood glucose concentration below harmful levels. In response to elevation of blood glucose levels after a meal, insulin is released from the β-cells. These are located in the islets of Langerhans in the pancreas, where insulin is
produced and stored in vesicles enabling a quick release in response to increasing blood glucose levels. Once in the circulation, insulin stimulates glucose uptake in target tissues like muscle and adipose tissue, that are responsible for most insulin-induced glucose uptake from the circulation. Insulin signals via the insulin receptor, a receptor that belongs to the subfamily of receptor tyrosine kinases. Binding of insulin to the insulin receptor induces a cascade of phosphorylation reactions including the phosphorylation of the insulin receptor itself, but also leading to phosphorylation of insulin receptor substrate (IRS), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (PKB, Akt), ultimately leading to translocation of the insulin-responsive glucose transporter GLUT4 to the cell membrane resulting in increased glucose uptake (64;65). Furthermore, insulin increases storage of glucose in the form of glycogen in muscle and liver and suppresses hepatic gluconeogenesis. Combined, these actions lead to a reduction of the blood glucose level, preventing potential detrimental consequences of hyperglycemia. However, insulin not only affects glucose metabolism. For instance, insulin also stimulates lipogenesis in the liver (66). In adipose tissue, insulin inhibits lipolysis by inhibiting hormone sensitive lipase (HSL) (67). In addition, insulin stimulates glucose conversion into glycerol-3-phosphate in adipocytes which, combined with fatty acid uptake, enables triglyceride formation (68). Insulin is also known to influence other processes such as vascular tone (69) and even memory function (70).

**Insulin resistance & Type 2 diabetes mellitus**

Type 2 diabetes mellitus (T2DM) usually is preceded by a period in which an individual gradually becomes more insulin resistant. During this phase, fasting blood glucose levels are not markedly elevated because the reduced response of the body to insulin is compensated by an increased insulin production in the pancreas. Insulin resistance, as well as T2DM, is a strong risk factor for the development of cardiovascular disease (CVD). Decreased glucose tolerance, increased insulin tolerance and elevated fasting insulin levels are indicative of insulin resistance. Insulin resistance can most sensitively be detected by performing a hyperinsulinemic euglycemic clamp. In short, this method comprises a continuous infusion of insulin combined with a variable infusion of glucose which is adjusted to maintain stable, usually post-prandial (± 7 mmol l\(^{-1}\)) blood glucose levels. The more glucose that needs to be infused to maintain euglycemia, the more insulin sensitive a subject is. A low glucose infusion rate therefore indicates insulin resistance. The hyperinsulinemic euglycemic clamp method can be further sophisticated by the addition of (stably) labeled glucose to the infusate, enabling calculation of the glucose disposal rate and hepatic glucose production, reflecting peripheral and hepatic insulin sensitivity, respectively. Symptoms directly associated with insulin resistance are not so obvious and many people do not know they are insulin resistant. At the moment where insulin production is not sufficient anymore to maintain adequate glycemic control,
type 2 diabetes arises, resulting in hyperglycemia and associated clinical symptoms. These include diabetic retinopathy leading to impaired eye sight, neuropathy and peripheral artery disease resulting in poor blood circulation in the limbs, gangrene and sometimes amputation. Although genetic susceptibility plays a role in the development of insulin resistance and type 2 diabetes, obesity represents by far the most prevalent risk factor (71).

**Type 1 diabetes mellitus**

T1DM is one of the most common severe autoimmune diseases, affecting 1 in 300 individuals (72). Like T2DM, the prevalence of T1DM is rising fast. The reasons underlying the increasing prevalence of T1DM are, however, poorly understood (73). It has been estimated that the number of newly diagnosed cases in Europe will have increased 70% by the year 2020 as compared to 2005 (74). Susceptibility to T1DM has a strong genetic component, mainly linked to HLA haplotype, however the factors that trigger the onset of disease remain largely unknown (75). T1DM is caused by a loss of self-tolerance to the insulin-producing pancreatic β-cells in the islets of Langerhans, leading to β-cell destruction, deficient insulin production and, consequently, hyperglycemia (72;75). Autoimmune reactivity starts several years before the actual type 1 diabetes-associated hyperglycemia emerges (72). In genetically susceptible individuals loss of tolerance to β-cell autoantigens probably occurs very early in life, often at the age of only a couple of months (76). T cells are the most abundant immune cells found in islets that have developed insulitis, but also macrophages are prominently present (77). The autoimmune reaction appears to be specifically targeted to β-cells as the immune infiltrate resolves after destruction of β-cells is complete (77). Although a number of immunotherapeutic strategies have shown some efficacy in mice and humans, these treatment strategies need further optimization and are, as yet, inadequate to be used for the prevention or treatment of type 1 diabetes mellitus (72). Replacement therapy with exogenous insulin is applied to regulate blood glucose levels in T1DM patients. Nevertheless, patients with T1DM are at increased risk for the development of atherosclerotic CVD, which represents the major cause of morbidity and mortality in this population (78;79). Suboptimal glycemic control during treatment with exogenous insulin most likely is accountable for the increased CVD incidence, at least to a certain extent (80). However, the mechanisms underlying the relation between T1DM and incidence of atherosclerotic CVD are, as yet, not fully elucidated and need further clarification.

**Atherosclerosis**

Atherosclerosis is characterized by thickening of the arterial wall, leading to narrowing of the vessel lumen and reduced blood flow to the distal organ. Ultimate plaque rupture
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is the major threat in atherosclerosis and the resulting blood clots are the pathological substrate for myocardial infarction or stroke in atherosclerotic patients. Although the mechanisms driving formation and progression of atherosclerotic plaques are, as yet, incompletely understood, a number of processes that contribute to plaque development have been identified. Current knowledge concerning the development and progression of atherosclerotic plaques has been extensively reviewed elsewhere (81-83) and will therefore be summarized only briefly below. Apolipoprotein (apo) B-containing lipoproteins penetrate the endothelial barrier and migrate to the sub-endothelial space where ‘fatty-streaks’ are formed and the particles become modified (84). In response to the retention of these atherosclerotic lipoproteins, endothelial cells upregulate the expression of adhesion molecules and chemokines leading to attraction and retention of immune cells. During diapedesis - migration through the endothelial cell layer – attracted monocytes differentiate into macrophages. Once in the sub-endothelial space, macrophages start ingesting modified LDL particles which leads to foam cell formation. Foam cells are cholesterol-loaded macrophages that are the hallmark cells of atherosclerotic plaques. Accordingly, an inflammatory cascade is initiated leading to the attraction of more immune cells and progression of the plaque. Thus, in addition to LDL-cholesterol, inflammation plays a pivotal role in the development of atherosclerotic plaques. Therapies aiming to reduce plasma LDL-cholesterol levels are currently the most applied treatment to prevent myocardial infarction or stroke. A class of drugs that inhibit the cholesterol synthesizing enzyme Hmg-CoA reductase, named statins, are the mainstay of primary as well as secondary prevention strategies. However, the widespread use of statins has only resulted in a 15-40% reduction of CVD events although efficient LDL-cholesterol lowering was achieved (85). This rather disappointing number indicates the need for alternative and/or additive strategies to achieve better atheroprotection. Currently, several other LDL lowering strategies are being tested in clinical trials (82). Increasing HDL, which increases ‘offloading’ of cholesterol from macrophage foam cells, as well as therapies aiming at reducing local inflammation in plaques are likely to reduce progression of atherosclerosis (82;86). Although, simply increasing HDL-C levels might not be effective as can be deduced from the failure of CETP-inhibition to improve cardiovascular outcome (87;88). Increasing HDL particle number and function instead of HDL-C levels might be more effective (89). The anti-atherogenic properties of HDL are discussed in more detail below.

Cholesterol metabolism and lipoproteins

Cholesterol is an essential component of cell membranes that modulates membrane fluidity and permeability and is involved in signaling via its role in the formation of lipid rafts (90-92). Furthermore, cholesterol is used as a substrate for the production of bile acids, steroid hormones and vitamin D. Cholesterol is, however, very hydrophobic and unable to circulate in the aqueous environment of blood.
Therefore, it is transported through the blood compartment by lipoproteins. Except for pre-β HDL, which has a discoidal shape, lipoproteins can be envisioned as spherical particles, with a hydrophilic surface and a hydrophobic core, that circulate in the blood and facilitate transport of hydrophobic molecules, i.e. cholesterol and triglycerides. The surface of lipoproteins consists of phospholipids, the hydroxyl group of free cholesterol and (apolipo)proteins, whereas the more hydrophobic cholesterol esters and triglycerides are the predominant constituents of the lipoprotein core. Categorization of lipoprotein subclasses is primarily based on their relative densities, which are in decreasing order: High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Intermediate Density Lipoproteins (IDL), Very Low Density Lipoproteins (VLDL) and chylomicrons. The latter class is responsible for the transport of dietary lipids that have been absorbed in the intestine, via the lymphatic system, into the body. VLDL, IDL and LDL are characterized by the presence of apolipoprotein B and facilitate the transport of mainly triglycerides and cholesterol from the liver throughout the body in the so-called forward pathway. VLDL particles are synthesized by the liver (Figure 1). Once in the circulation, the triglycerides transported by these particles are hydrolyzed by lipoprotein lipase (LPL), resulting in smaller particles with an increased cholesterol:triglyceride ratio and a higher density, LDL particles. HDL, the lipoprotein subclass identified by the presence of apolipoprotein A-I, is considered to be responsible for the transport of cholesterol from the periphery back to the liver in a process called reverse cholesterol transport (RCT) which is described in more detail below.

Atheroprotective functionalities of HDL

Large population studies have conclusively demonstrated a robust inverse correlation between plasma HDL cholesterol levels and CVD risk (93-95), indicating that HDL protects against CVD development by some means. Indeed, multiple atheroprotective functions have been attributed to HDL (96;97). In addition to its key role in cholesterol efflux from macrophage foam cells and RCT, atheroprotective functions of HDL include enhancement of endothelial function (98), protection against LDL oxidation (99;100) and suppression of inflammation (101). HDL functionality represents an emerging concept and conceivably valuable information can be derived from characterizing HDL function. Recently, the capacity of HDL to induce efflux from macrophages was shown to be a predictor of coronary artery disease (CAD), independent of HDL cholesterol levels (102). Interestingly, a number of disease conditions has recently been described to coincide with impaired HDL functionality (103-105), which might contribute to the increased incidence of cardiovascular disease risk in those patients. Although the factors determining HDL functionality are as yet ill defined, oxidation of phospholipids and ApoA-I as well as a change in protein composition or activity of enzymes associated with HDL particles might impact on HDL function (105;106). Enzymes that might affect
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Figure 1. Simplified schematic of cholesterol metabolism

Dietary cholesterol is absorbed in the intestine and transported to the liver by chylomicrons. In addition the liver can import cholesterol from lipoproteins, via e.g. scavenger receptor type B-I (SR-BI) and LDL receptor (LDLR), mediating uptake of HDL- and LDL-cholesterol, respectively. Furthermore, the liver can produce cholesterol through de novo synthesis (DNS). The liver secretes cholesterol and triglycerides in VLDL particles that have a low cholesterol:triglyceride ratio. The triglycerides contained within the VLDL particles are hydrolyzed through lipolysis mainly mediated by lipoprotein lipase (LPL), resulting in the formation of smaller particles with an increased cholesterol:triglyceride ratio and a higher density, LDL particles. Especially after modification, e.g. by oxidation, LDL particles are high affinity ligands for uptake by scavenger receptors into e.g. macrophages resulting in the formation of foam cells, the hallmark of atherosclerotic plaques.

Cholesterol can be effluxed by macrophages towards HDL particles, where it is esterified by lecithin-cholesterol acyltransferase (LCAT). This atheroprotective cholesterol efflux process is mediated via ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1). HDL transports the cholesteryl ester (CE) to the liver, where it is taken up via SR-BI-mediated selective uptake. Once in the liver, cholesteryl ester is hydrolyzed by cholesteryl ester hydrolase (CEH). Subsequently, cholesterol can be re-secreted within VLDL or secreted into the bile, either as free cholesterol or after conversion into bile acids (BA). Transport of cholesterol from the liver into the bile is mediated to the major part (approximately 75%) by ABCG5/G8, but also, with relevance for RCT, by apical SR-BI. BA on the other hand are secreted by the bile salt export pump (BSEP, ABCB11). Biliary secreted cholesterol and BA enter the intestine and can either be excreted with the feces or be reabsorbed and cycle back to the liver. In contrast to mice, humans express cholesteryl ester transfer protein (CEPT), an enzyme that generates a net transfer of CE from HDL towards apolipoprotein B-containing lipoproteins in exchange for triglycerides. Transintestinal cholesterol excretion provides an additional pathway for the body to eliminate cholesterol, however the details of this process remain, as yet, to be characterized. To keep the figure concise, the transporters involved in the trafficking of cholesterol and bile acids and most of the enzymes involved in sterol metabolism are not depicted.
HDL function include lecithin-cholesterol acyl transferase (LCAT), the enzyme that converts free cholesterol into more hydrophobic cholesteryl esters and is critical for HDL maturation, and paraoxonase-1 (PON-1) which has been associated with antioxidative properties of HDL (106-108).

**Reverse cholesterol transport**

Elimination of sterols from the body can be accomplished by the liver via biliary secretion of cholesterol and bile acids, and by the intestine via modulation of absorption rates and transintestinal cholesterol excretion (TICE). In the case of CVD, biliary sterol secretion is a critical step in RCT (109;110). Current knowledge about TICE is reviewed elsewhere (111) and is not further discussed here because it is beyond the scope of this thesis. The role of HDL in macrophage cholesterol efflux and RCT represents the best established atheroprotective feature of this lipoprotein. RCT is defined as the transport of cholesterol from peripheral cells, of which macrophages are of major interest from a clinical perspective, out of the body via the feces. The macrophage-to-feces RCT pathway comprises three crucial steps: (i) The efflux of cholesterol from macrophage foam cells towards HDL and the subsequent transport through the blood circulation, (ii) the uptake of HDL cholesterol by the liver and (iii) the secretion of sterols (cholesterol and bile acids) from the liver into the bile and the intestine followed by the subsequent excretion from the body with the feces. As mentioned before, cholesterol-loaded macrophage foam cells are a predominant cell type in atherosclerotic plaques and HDL can mediate cholesterol efflux from these cells, thereby protecting against atherosclerosis (102). Cholesterol efflux towards HDL acceptor particles is mediated by two different ATP transporters, ATP-binding cassette sub-family A member 1 (ABCA1) and ABCG1. ABCA1 mediates transport of phospholipids and cholesterol mainly towards ApoA-I and small discoidal pre-β HDL particles, whereas ABCG1 mediates efflux of cholesterol towards larger, more mature, HDL particles. The importance of ABCA1 as mediator of a key anti-atherogenic process in underscored by the fact that patients with Tangier disease, who completely lack ABCA1 activity due to mutations in the respective gene, have a 4 to 6-fold increase in CVD risk (112). Moreover, numerous animal studies have demonstrated that ABCA1 expression protects against the formation of atherosclerotic lesions while lack of function promotes atherosclerosis (113;114). Data regarding the impact of ABCG1 on atherosclerosis development is, however, less conclusive (115-122). A recent study suggests that the effect of ABCG1 on atherosclerosis might be dependent on the stage of atherogenesis (123).

Effluxed cholesterol that has been taken up by HDL is transported via the blood stream to the liver. In the liver, HDL-cholesteryl ester (CE) can be taken up by hepatocytes through SR-BI in a process called selective uptake. As the name already suggests, this process comprises the selective uptake of cholesteryl ester from HDL particles without importing the protein component of the lipoprotein particle.
HDL-holoparticle uptake, a P2Y13-mediated process which involves the uptake of the whole HDL particle (124), has also been described to contribute to RCT (125). After being taken up in the liver, cholesteryl ester is hydrolyzed by cholesteryl ester hydrolase. Part of the cholesterol is used as a substrate for the synthesis of bile acids. HDL-derived cholesterol can therefore be excreted from the liver into the bile either in the form of free cholesterol or after conversion into bile acids. Biliary phospholipid and bile acid secretion facilitate secretion of cholesterol into the bile (109;126;127). Biliary phospholipids and bile acids form mixed micelles, which are essential as acceptors for biliary cholesterol secretion (109). Whereas phospholipids are mainly secreted into the bile by multidrug resistance protein 2/3 (ABCB4), biliary bile acid secretion is mainly carried out by the bile salt export pump (BSEP or ABCB11). Mutations in the genes encoding BSEP and MDR2/3 are associated with progressive familial intrahepatic cholestasis (PFIC) (128). The majority of cholesterol is transported into the bile by the heterodimeric transporter ABCG5/ABCG8. Mice that are homozygous for the deletion of either gene, Abcg5 or Abcg8, or both genes secrete significantly reduced amounts of cholesterol into the bile (129-131). However, also SR-BI contributes to biliary cholesterol secretion and notably the activity of SR-BI is independent of functional expression of the ABCG5/ABCG8 transporters (132;133). Secreted biliary sterols usually are temporarily stored in the gallbladder, transported to the intestine via the bile duct in response to a meal and either leave the body with the feces or are absorbed in the intestine and cycle back to the liver.
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Outline of this thesis

Patients with the metabolic syndrome, but also patients with T1DM, are at increased risk of cardiovascular disease (CVD), a major cause of death in developed countries. The number of patients with the metabolic syndrome as well as the number of patients with T1DM is increasing, which will lead to a further increase in CVD-related morbidity and mortality in the near future. The pathophysiology of the metabolic syndrome and T1DM, and their association with increased CVD risk, has been subject of intensive study. Nevertheless, current knowledge about those diseases is far from complete. This thesis aimed to provide better understanding of the pathophysiology leading to insulin resistance and to explore a potential role for altered HDL functionality in diabetes contributing to increased CVD risk. Chapter 2 and 3 concern the effects of modulation of inflammatory pathways on adipose tissue inflammation and insulin sensitivity, whereas chapter 4 and 5 focus on atheroprotective functionalities of HDL in diabetes. Chapter 6 presents data on HDL functionality after enrichment with a recently discovered apolipoprotein that was found to be upregulated in diabetic dog hearts.

In chapter 2, data are presented describing that 12/15-lipoxygenase-deficient mice are more insulin resistant compared to wild-type mice. This phenotype became more pronounced when the mice were fed a high-fat diet (HFD). Using bone marrow transplantation, we identified macrophage 12/15-lipoxygenase expression as an important factor in maintaining insulin sensitivity due to its role in the polarization of macrophages towards the anti-inflammatory M2 phenotype. Chapter 3 describes the phenotype of HFD-fed mice deficient in the expression of the inflammatory mediator mitogen-activated protein kinase-activated protein kinase 2 (MK2). We found that these mice had a surprising glucose intolerant, insulin resistant, phenotype. Mechanistically, we identified decreased GLUT4 expression in the adipose tissue as a factor conceivably contributing to the observed phenotype. In chapter 4, data are presented regarding the ability of HDL to protect against LDL oxidation in type 2 diabetic patients. These data show that the HDL anti-oxidative function per se was not affected in type 2 diabetic patients. However, the total HDL-mediated protection against LDL oxidation was reduced in those patients due to lower HDL levels. Furthermore, fasting plasma glucose levels and lecithin-cholesterol acyl transferase (LCAT) activity were inversely correlated with HDL anti-oxidative functionality. In chapter 5, data concerning reverse cholesterol transport in type 1 diabetic mice are described. We found that macrophage-to-feces reverse cholesterol transport was reduced in type 1 diabetic mice despite strongly increased biliary sterol secretion. Impaired hepatic uptake of HDL cholesterol, likely due to glycation of HDL particles, was identified as a conceivable mechanistic explanation for the decreased macrophage-to-feces reverse cholesterol transport in those mice. Data presented in chapter 6 describe a spectrum of HDL atheroprotective functions in mice overexpressing a novel apolipoprotein that was only discovered recently, apolipoprotein O. Overexpression of this apolipoprotein resulted in increased
presence on HDL particles, but did not affect HDL-mediated efflux from macrophage foam cells, protection against LDL oxidation, protection against endothelial cell inflammation or endothelial cell-mediated vasorelaxation.
Reference List


Introduction


