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GENERAL INTRODUCTION
TRIGLYCERIDE METABOLISM

Lipids and lipoproteins

Triglycerides (TG) and cholesterol are the most common lipids in our diet and essential for the human body. TG are the main source of energy, and can be stored in white adipose tissue (WAT), used for ATP production in skeletal muscle and heart, and used for generating heat in brown adipose tissue (BAT). Cholesterol is essential, as a crucial component of cellular membranes and as precursor for steroid hormone synthesis. Since lipids are hydrophobic, they are transported in the blood as constituents of soluble particles called lipoproteins. These lipoproteins are composed of a lipid-rich core containing TG and esterified cholesterol, surrounded by an amphiphilic monolayer of phospholipids (PL), unesterified free cholesterol and one or more apolipoproteins. The latter facilitate formation of the lipoproteins and modulate the activity of proteins (i.e. enzymes and transfer factors) involved in lipoprotein remodeling in the circulation. Lipoproteins are subdivided into different classes based on their density, namely (from lowest to highest density) chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Both chylomicrons and VLDL are consist mainly of TG and are therefore called TG-rich lipoproteins. The metabolism of TG-rich lipoproteins will be discussed in more detail in the following section and is schematically depicted in Figure 1.

![Figure 1. Schematic representation of TG-rich lipoprotein metabolism. See text for explanation.](image-url)
TG-rich lipoprotein metabolism

In the intestine, dietary TG are lipolyzed by pancreatic lipases into 2-monoacylglycerol and fatty acids (FA), both of which are subsequently taken up by the intestinal cells (enterocytes) where the lipolysis products are re-synthesized into TG. Enterocytes synthesize apoB that is lipidated in the endoplasmatic reticulum (ER) by the microsomal TG transfer protein (MTP), resulting in the formation of a small apoB containing particle. After fusion with larger protein-free lipid droplets, a prechylomicron is formed which is transported towards the Golgi and secreted via exocytosis. Chylomicrons are secreted into the lymph from which they are transported via the blood for storage (adipose tissue), or energy supply (skeletal muscle and heart) or heat generation (brown adipose tissue).

When no food is entering the intestine (during fasting), the liver is the organ that ensures a supply of TG by secretion of TG-rich VLDL particles. Assembly of VLDL in hepatocytes, the most predominant cell type in the liver, involves a similar pathway as chylomicron synthesis including MTP-mediated transfer of TG to apoB and fusion of the generated particle with other lipid droplets, resulting in the formation of mature VLDL that is released into the circulation. The assembly of VLDL is highly dependent on the availability of TG within the hepatocyte. The main direct sources for VLDL-TG are 1) plasma-derived FA that are released by the adipose tissue mainly in the fasted state and are esterified in the hepatocyte into TG, 2) cytosolic TG that previously accumulated in the hepatocyte and 3) de novo synthesized FA that is used for de novo synthesis of TG within hepatocytes. FA synthase (FAS) and stearyl-CoA desaturase-1 (SCD-1) represent the most important genes involved in the de novo synthesis of FA and TG, respectively.

In the circulation, TG that are derived from chylomicrons and VLDL are lipolyzed by capillary-bound lipoprotein lipase (LPL) into glycerol and FA, the latter being subsequently taken up by underlying tissues including WAT, skeletal muscle, heart, and BAT. In the postprandial state (after a meal), LPL expression is highest in WAT, thereby directing most of the chylomicron-derived TG into adipose tissue for storage. In the fasted state, LPL expression relatively increases in skeletal muscle as compared to WAT thereby ensuring VLDL-derived FA as energy source. LPL activity in heart is less regulated by the feeding status and regulation of LPL in BAT is currently unknown. Through hydrolysis of TG, and concomitant uptake of FA by the peripheral tissues, the TG-rich lipoproteins reduce in size and become residual CM remnants and VLDL remnants (i.e. IDL and LDL). These are taken up by the liver through specific receptors such as the LDL receptor and LDLr-related protein (LRP).

OBESITY AND ASSOCIATED METABOLIC DISTURBANCES

When energy intake exceeds energy expenditure (i.e. positive energy balance), TG is stored in the body as WAT. A prolonged positive energy balance will thus lead to overweight and obesity. In fact, world-wide obesity is reaching epidemic proportions.
due to a sedentary lifestyle combined with a caloric-rich diet. The adipose tissue has the largest capacity to store energy in the form of TG, by increasing both adipocyte hypertrophy (increase in cell size) as well as hyperplasia (increase in cell number). However, since expandability of adipose tissue is limited, excess TG is redirected towards non-adipose tissues such as skeletal muscle and liver, both of which are able to store TG to a more limited extent. In fact, obesity is frequently accompanied by excess fat accumulation (steatosis) in liver and muscle, which has been linked to the development of metabolic disturbances such as insulin resistance, ultimately resulting in the onset of type 2 diabetes. In addition to insulin resistance, the excess TG availability leads to an increased circulation of TG in the plasma which is called hypertriglyceridemia and an important risk factor for the development of atherosclerosis and, ultimately, cardiovascular diseases (CVD).

Obviously, obesity and associated metabolic disorders including insulin resistance and atherosclerosis could at least partly be prevented by reducing food intake and increasing regular physical activity levels. Unfortunately, these lifestyle changes have proven to be very difficult to accomplish which urges the development of therapeutic strategies combating obesity and its associated disorders.

METABOLIC INFLAMMATION

Inflammatory pathways have evolved as important mediators that may link obesity to metabolic disturbances. Obesity is accompanied by low-grade systemic inflammation, which is characterized by increased presence of cytokines and other markers of inflammation in the circulation.

Both white adipose tissue and the liver are largely involved in the onset and development of metabolic inflammation. The expansion of the adipose tissue during development of obesity coincides with the influx of macrophages and secretion of pro-inflammatory cytokines in adipose tissue of obese mice and humans. The initial trigger for macrophage infiltration in adipose tissue is suggested to be the secretion of pro-inflammatory cytokines by adipocytes upon expansion that attract and activate infiltrating macrophages, as adipocyte size is correlated to the amount of macrophage infiltration in adipose tissue. In addition to adipose tissue, HFD and obesity also induce inflammation in liver, as reflected by increased markers of hepatic inflammation (SAA and CRP) in obese subjects and increased hepatic NF-κB activity in mice fed a HFD.

The obesity-associated low-grade inflammation appears to be causally involved in the onset and development of metabolic disturbances such as insulin resistance and atherosclerosis. Pro-inflammatory cytokines such as IL-1β and TNFα that are secreted by the liver and adipose tissue can directly induce insulin resistance by interfering with insulin signaling pathways and induce formation of atherosclerotic plaques.

The transcription and activation of these pro-inflammatory cytokines is regulated by activation of other proteins, such as NF-κB or NLRP3 inflammasome-activated...
caspase-1. NF-κB is a transcription factor that regulates expression of several pro-inflammatory cytokines including IL-1β and TNFα. The NLRP3 inflammasome caspase-1 is a protein-complex that is crucial for activation of the pro-inflammatory IL-1β. The exact role of NF-κB and the NLRP3 inflammasome in metabolic inflammation in adipose tissue and liver remains to be determined, as well as its contribution to metabolic disturbances. This may reveal new potential therapeutic targets in the treatment of obesity-associated insulin resistance and atherosclerosis.

OUTLINE OF THE THESIS

Albeit that metabolic inflammation plays a key role in the development of insulin resistance and atherosclerosis, no current therapies exist that target inflammation in the treatment of these metabolic diseases. Better understanding of the interaction between inflammatory pathways and metabolic disturbances will help to develop new strategies to treat patients at risk for type 2 diabetes and CVD. The research described in this thesis was performed to gain more insight into the effect of various inflammatory pathways on the development of hyperlipidemia, insulin resistance and atherosclerosis.

The first part of this thesis focuses on the relation between inflammation and hyperlipidemia, which are both risk factors for the development of atherosclerosis. In chapter 2, current knowledge on the role of lipids and inflammatory processes in the development of atherosclerosis is described, as well as the interaction between lipid metabolism and inflammation that may aggravate the development of atherosclerosis. The effects of lipid-lowering drugs on inflammatory processes, as well as the effects of the anti-inflammatory drugs on lipid metabolism are discussed. In chapter 3, the role of the anti-inflammatory drug aspirin on lipid metabolism was evaluated. Specifically, the mechanism by which aspirin reduces plasma lipid levels was investigated in the hypertriglyceridemic APOC1 mouse model, by studying the effects of aspirin on VLDL-triglyceride metabolism.

The liver is an organ that plays an important role in triglyceride metabolism as well as inflammation. The aim of the study described in chapter 4 was to specifically determine the effect of hepatic NF-κB activity on the development of hypertriglyceridemia, by evaluating whether transgenic activation of IKK-β only in the hepatocytes of E3L mice affected VLDL-triglyceride metabolism directly. Whether the hepatic NF-κB activity affects the development of atherosclerosis was subsequently explored in chapter 5.

Toll-like receptor 4 (TLR4) is a receptor of the immune system that activates NF-κB signaling. Endotoxin, a component of Gram-negative bacteria and a well known ligand for TLR4, can induce hyperlipidemia during infection by increasing hepatic VLDL-TG production. Upon high-fat feeding, saturated FA are believed to activate TLR4/ NF-κB signaling in the liver, suggesting that TLR4 might be an important link between obesity-induced hepatic inflammation and hyperlipidemia. Therefore, the aim of chapter 6 was to investigate whether absence of TLR4 could reduce VLDL-TG production in high-fat fed mice.
The second part of this thesis describes studies exploring the role of the inflammasome-mediated caspase-1 activity in obesity and insulin resistance. The inflammasome complex is another part of the innate immune system that activates caspase-1 that is responsible for the activation of the pro-inflammatory cytokines IL-18 and IL-1β. These two pro-inflammatory cytokines have been shown to be involved in the development of obesity and insulin resistance. In chapter 7, we questioned whether absence of components of the inflammasome complex would affect HFD-induced obesity, adipose tissue inflammation and insulin resistance. Since absence of inflammasome-mediated caspase-1 markedly reduced HFD-induced obesity and insulin resistance, the mechanism by which absence of caspase-1 reduces adipose tissue mass was investigated in chapter 8, by evaluating TG-rich lipoprotein metabolism in caspase-1 deficient and wild-type mice.

Finally, the results from these studies and its implications are discussed in chapter 9.

REFERENCES