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**Title:** Fatty acid metabolism and metabolic inflammation: two important players in the development of insulin resistance

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SUMMARY
The metabolic syndrome is a multi-component condition that includes obesity, hypertriglyceridemia and insulin resistance. The prevalence of the metabolic syndrome is rising world-wide and is associated with an increased risk for the development of cardiovascular diseases and type 2 diabetes. In the past decades it has been discovered that obese persons have slightly elevated markers of inflammation in their plasma. This low-grade chronic inflammation, also called metabolic inflammation, is hypothesized to function as the link between the various components of the metabolic syndrome. In this thesis, we have evaluated how alterations in triglyceride (TG) and fatty acid (FA) metabolism and inflammatory pathways interact in the development of obesity and insulin resistance, which are both primary risk factors for the development of type 2 diabetes.

Adipose tissue plays a central role in the development of the metabolic syndrome. Under healthy conditions, excess calories are stored in adipose tissue. When energy intake continues to exceed energy expenditure, the adipose tissue needs to expand in order to store these excess calories. When the capacity of the adipose tissue to expand is not yet reached, the excessive calories are mainly stored as fat. When the storage capacity of adipose tissue is reached, the excessive calories may be stored in non-adipose tissue (ectopic fat). This pathophysiological condition is associated with an increased risk for insulin resistance.

One of the functions of the scavenger receptor CD36 is to facilitate the entry of FA into a variety of cells, including adipocytes. In chapter 2 we have investigated the role of CD36 in preadipocyte recruitment and adipocyte functionality in gonadal, visceral and subcutaneous adipose tissue. For this purpose, we used CD36-deficient mice (CD36<sup>-/-</sup> mice) that were given a high fat diet (HFD). The CD36<sup>-/-</sup> mice showed diminished uptake of TG-rich particle derived FA in adipose tissue. As a consequence, gonadal, visceral and subcutaneous fat pads were smaller in these animals. A reduction in fat pad size can be caused by a reduction in size or a reduction in total number of adipocytes. We showed that adipocyte size was reduced in gonadal, visceral and subcutaneous fat pads of CD36<sup>-/-</sup> mice. In addition, a reduced total number of gondadal adipocytes was observed in CD36<sup>-/-</sup> mice. This reduction was caused by a decreased number of very small adipocytes (<50 µm). Our data suggest that CD36-deficiency reduces the capacity of preadipocytes to become adipocytes, since we measured a reduced number of very small adipocytes but an increased pool of preadipocytes in gonadal fat of CD36<sup>-/-</sup> mice. This deficiency in preadipocyte recruitment was not due to an intrinsic developmental defect, since in vitro differentiation experiments did not reveal a reduction in intra cellular lipid accumulation. Smaller adipocytes as observed in CD36<sup>-/-</sup> mice would be expected to have a decreased rate of lipolysis. Surprisingly, lipolysis was increased in adipocytes from CD36<sup>-/-</sup> mice. Altogether our results indicate that CD36 plays an important role in adipose tissue functionality both by regulating FA uptake and release. This in turn has an impact on the distribution of fat to adipose and non-adipose tissues. The reduced uptake of FA in adipose tissue was paralleled by an increased uptake of FA in the liver. We demonstrated that the fatty liver of CD36<sup>-/-</sup> mice was more insulin resistant.

Expansion of adipose tissue is often accompanied by infiltration of immune cells into the adipose tissue. Therefore, adipose tissue is thought to play a central role in the
onset of metabolic inflammation, although in other organs such as the liver and muscle inflammation in response to lipid accumulation can be observed as well. Immune cells are able to secrete cytokines that elicit a pro-inflammatory response that is associated with the development of insulin resistance and hypertriglyceridemia. Inflammation, insulin resistance, and deregulation of TG/FA metabolism are part of a vicious cycle that drives the progression of diabetes and cardiovascular diseases.

Systemic inflammation induces an increase in plasma TG levels. Aspirin treatment in type 2 diabetes patients resulted in a decrease in plasma TG. In chapter 3 we have aimed to investigate the mechanism by which aspirin reduced hypertriglyceridemia. Therefore we fed human apolipoprotein CI (apoCI)-expressing mice (APOCI), a mouse model with elevated plasma TG levels, as well as normolipidemic wild-type (WT) mice a HFD and treated them with aspirin. Aspirin treatment reduced hepatic NF-κB activity in HFD-fed APOCI and WT mice and in addition, aspirin decreased plasma TG levels in hypertriglyceridemic APOCI mice. This TG-lowering effect could not be explained by enhanced VLDL-TG clearance, but aspirin selectively reduced hepatic VLDL-TG production in both APOCI and WT mice without affecting VLDL-apo B production. Aspirin did not alter hepatic expression of genes involved in FA oxidation, lipogenesis and VLDL production, but decreased the incorporation of plasma-derived FA by the liver into VLDL-TG, which was independent of hepatic expression of genes involved in FA uptake and transport. We concluded that aspirin improves hypertriglyceridemia by decreasing VLDL-TG production without affecting VLDL particle production. Our results suggest that inhibition of inflammatory pathways by aspirin could be an interesting target for the treatment of hypertriglyceridemia.

In chapter 4 we have investigated the effects of a botanical component META060 on body weight development and insulin sensitivity. META060 has shown anti-inflammatory activity in vitro. Compared to WT mice fed a HFD, mice fed a HFD supplemented with META060 were protected against the development of diet-induced obesity. Moreover the mice that received META060 had improved glucose tolerance, indicating that this component has potential therapeutic value in the management of obesity and insulin resistance.

The adaptive immune system plays a clear role in metabolic inflammation. Increased amounts of specific pro-inflammatory subtypes of T-lymphocytes and activated B-lymphocytes have been found in the adipose tissue of obese subjects. Furthermore, mice fed a HFD have a restricted T Cell Receptor profile. In addition, antibodies obtained from diet-induced obese mice are able to elicit adipose tissue inflammation and insulin resistance in B-lymphocyte deficient mice. Altogether this indicates that the adaptive immune system is important in metabolic inflammation and its associated pathologies.

Antibody molecules activate cellular responses that are important for inflammation by cross-linking their Fc portion with specific membrane receptors (FcR). In chapter 5 we have investigated whether functional FcR could be important in the development of diet-induced adipose tissue inflammation and diet-induced insulin resistance by subjecting FcR γ-chain−/− mice that miss the γ-chain that is needed for proper signal transduction and surface expression of FcR, to a HFD. Compared to WT mice, FcR γ-chain−/− mice had
reduced diet-induced obesity, diminished adipose tissue inflammation and less peripheral insulin resistance, indicating that functional FcR are likely to play a role in the development of metabolic inflammation.

Taken together, the studies described in this thesis contribute to the understanding that functionality of adipose tissue and metabolic inflammation are important factors in the development of diet-induced obesity and insulin resistance. We show that CD36 is important for proper adipocyte functioning, thereby reducing the risk of ectopic fat storage in the liver. We have identified that FcR antibody effector pathways may be involved in the development of obesity and insulin resistance, which suggest new diagnostic and therapeutic possibilities for managing the metabolic syndrome. Furthermore we found that anti-inflammatory agents reduce hypertriglyceridemia and insulin resistance.