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GENERAL DISCUSSION
The metabolic syndrome, a multi-component condition including obesity, insulin resistance, dyslipidemia and hypertension, is associated with an increased risk for cardiovascular diseases and diabetes. The prevalence of the metabolic syndrome, currently around 30%, is rising worldwide and this rise parallels the increase in the prevalence of overweight and obesity. In 2009, 47% of all Dutch adults had overweight of which 12% were obese.

Chronic low-grade inflammation is thought to function as the link between the various components of the metabolic syndrome and the development of the associated pathologies. Disturbances in triglyceride (TG) and fatty acid (FA) metabolism can drive the inflammatory response and vice versa, the inflammatory response can drive disturbances in TG/FA metabolism. However, the mechanisms underlying these interactions between TG/FA metabolism and inflammation remain to be fully understood. In this thesis, we have investigated how modulation of TG/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.

6.1 ROLE OF FA TRANSPORT IN ADIPOSE TISSUE DEVELOPMENT AND INFLAMMATION

Adipose tissue is the first visually affected organ in the development of obesity and the metabolic syndrome. Excessive calories are stored in adipose tissue as TG. Energy storage in adipocytes is partly regulated by FA transporters in adipocytes that facilitate FA entry.

In chapter 2 we found that the FA transporter CD36 is important for adipocyte recruitment and adipocyte functionality. CD36⁻/⁻ mice become less obese on high fat diet (HFD), although their body weight is still increased compared to mice fed a normal chow diet. Adipose tissue of CD36⁻/⁻ mice has some healthy aspects compared to adipose tissue of wild-type (WT) mice, since we found that the adipocytes of CD36⁻/⁻ mice are more insulin sensitive and the adipose tissue of CD36⁻/⁻ mice is less inflamed. Nevertheless, the adipose tissue of these animals should be considered as dysfunctional. The adipocytes of CD36⁻/⁻ mice cannot store surplus FA and also the recruitment of new adipocytes is impaired. As a result the FA are translocated to the liver and stored ectopically, which results in hepatic insulin resistance.

Differences in storage capacity of adipose tissue are important in the development of the metabolic syndrome. Human cohort studies indicate that there are individuals who are obese but seem to remain metabolically healthy. These individuals have a healthy lipid profile, e.g. lower TG, higher high density lipoprotein (HDL) cholesterol, lower fasting glucose and insulin concentrations and better glucose tolerance compared to unhealthy obese individuals, despite a similar level of total body fat. Several studies have shown that the expandability of subcutaneous fat is critical for maintaining healthy obesity. If this capacity to expand is large, an individual is more likely to remain healthy, even when obese. If the expandability of subcutaneous fat is low, excess fat is likely to be stored intra-abdominally (in visceral fat) or in non-adipose tissues (ectopic fat deposition), which is associated with an adverse metabolic profile.
Different factors could play a role in the capacity of the subcutaneous fat to expand. Fat mass, fat distribution and adipocyte numbers are for a large part genetically determined. Twin and population studies have indicated that 25-70% of the variability in these traits can be explained by genetics. For example, variation in the LPL gene and mutations in the \( \beta3 \)-adrenergic receptor gene have been associated with visceral obesity.

Additional important factors that could play a role in maintaining healthy adipose tissue are nutrition and exercise. A controlled-feeding trial demonstrated that consumption of a diet enriched in saturated FA (SFA) led to an increased expression of genes involved in inflammatory processes, while a diet enriched in monounsaturated FA (MUFA) led to decreased expression of inflammatory genes in adipose tissue of overweight subjects. Thus, the type of dietary FA seems to affect inflammation in adipose tissue.

Physical activity also likely contributes to healthy obesity. It has been found that active obese men (fat fit men) have increased HDL cholesterol levels, decreased TG levels, decreased alanine aminotransferase (ALT) levels (which is a marker for liver damage), and improved insulin sensitivity, as measured by HOMA-index, compared to unfit obese men (fat unfit men). In addition, visceral fat and liver fat were decreased in fat fit men compared to fat unfit men. It has been postulated that men who are fit and fat have a greater capacity to store fat in subcutaneous adipose tissue.

If the expandability capacity of subcutaneous adipose tissue is decreased, excessive FA are more likely to be stored as visceral fat or in non-adipose tissues as ectopic fat. In its most extreme form this is illustrated by patients with lipodystrophy, who despite a normal BMI display features of the metabolic syndrome such as insulin resistance and hypertriglyceridemia. These patients have genetic defects in the development of adipose tissue and are characterized by extremely fatty livers. Insight in the genetics of lipodystrophy provides direct insight in adipose tissue development and function, and contributes to a better understanding of the onset of the pathology associated with the metabolic syndrome.

A less extreme form of lipodystrophy may affect individuals who despite a normal weight and BMI, display signs of insulin resistance and dyslipidemia. These individuals have been identified as metabolically obese normal weight (MONW) individuals. MONW individuals are characterized by an increased visceral fat mass, which is associated with decreased insulin sensitivity. Seppala-Linderoos et al. have found increased hepatic TG content in nonobese men who have high levels of plasma insulin and TG.

Physical inactivity may be an important characteristic of MONW individuals. An inverse relationship between maximal oxygen uptake \( (V_{\text{O2max}}) \) and insulin insensitivity has been shown. Several studies have demonstrated that regular exercise, either in combination with a healthy diet ameliorates metabolic abnormalities and reverses adverse fat distribution. These studies illustrate the necessity of some basal level of physical activity even for those that are not obviously overweight.
Adipose tissue expansion and obesity are associated with the development of low-grade inflammation. Expansion of adipose tissue is beyond a certain point physically limited by nutrient and oxygen supply. The subsequent hypoxia, endoplasmatic reticulum stress and apoptosis may lead to infiltration of immune cells into adipose tissue such as macrophages, activated T-lymphocytes and activated B-lymphocytes. Under normal physiological conditions, this response is self-limited and geared towards a new metabolic and inflammatory equilibrium. However, under conditions of chronic overnutrition, the low-grade inflammation can also become chronic. This chronic low-grade inflammation has been termed metabolic inflammation, since it is hypothesized that this inflammation is triggered by a surplus of nutrients. Metabolic inflammation is characterized by increased plasma levels of pro-inflammatory cytokines, insulin resistance and a deregulation of TG/FA metabolism. Inflammation, insulin resistance, and deregulation of TG/FA metabolism are part of a vicious cycle that drives the progression of diabetes and cardiovascular diseases.

Inflammation itself can directly affect lipoprotein metabolism as illustrated by the observation that patients with acute infections have elevated VLDL TG levels. In addition, administration of lipopolysaccharide (LPS), a strongly pro-inflammatory component of the cell wall of Gram-negative bacteria, and cytokines produce hypertriglyceridemia. Conversely, disturbances of lipid metabolism can also produce a pro-inflammatory state. SFA can stimulate inflammatory pathways via Toll Like Receptor (TLR) 4 or via increased reactive oxygen species (ROS) production. Recently it was found that SFA-induced ROS can activate the inflammasome complex, which results in increased release of the pro-inflammatory cytokine IL-1β.

In chapter 3 and 4 of this thesis, we have investigated the effect of anti-inflammatory compounds on TG metabolism (chapter 3) and glucose tolerance (chapter 4). In chapter 3 we show that high-dose aspirin, which decreases total hepatic NF-κB activity, also reduces hepatic VLDL-TG production in HFD-fed mice, thereby reducing the plasma TG levels of hypertriglyceridemic mice. In chapter 4 we showed that a botanical agent that has shown anti-inflammatory activity in vitro, was able to reduce body weight gain and diminish glucose intolerance in mice fed a HFD. Anti-inflammatory agents might therefore be interesting to treat symptoms of the metabolic syndrome.

High-dose aspirin has been shown to improve insulin sensitivity in obese type 2 diabetics. In this study also a reduction of plasma TG was observed. Since prolonged use of high-dose aspirin can lead to gastrointestinal bleedings, clinical trials are being performed with salsalate, another anti-inflammatory agent based on the active compound salicylic acid, with fewer side effects than aspirin. Recently, high-dose salsalate treatment has shown to improve glycemic control, to decrease CRP levels and to decrease TG levels in type 2 diabetic patients. Botanical compounds like META060 that we have tested in chapter 4 are anticipated to have even fewer side effects.
Even when unwanted potential side effects of anti-inflammatory compounds can be avoided, one could argue that reducing the inflammatory response in itself may be dangerous, since the capacity to destroy potential pathogens is also diminished. In this respect, adverse effects of anti-TNF-α have been reported. Treatment with TNF-α blockers has long been established for patients with rheumatoid arthritis (RA) and Crohn’s disease. Because of the tight association between inflammation and insulin resistance and the positive effects on glucose and insulin levels in patients with RA, anti-TNF-α agents have been tested in individuals with the metabolic syndrome. Etanercept, an anti-TNF-α agent, was found to improve fasting glucose levels in individuals with the metabolic syndrome. Extensive use of anti-TNF-α agents in treatment of the metabolic syndrome was however not recommended, since increased risk of infection has been reported in patients with RA and Crohn’s disease. A more promising anti-inflammatory agent is Anakinra. Anakinra is an IL-1 receptor antagonist (IL-1ra) that counterbalances the pro-inflammatory cytokine IL-1β. Administration of Anakinra resulted in improved glycemic control due to improved β-cell function in type 2 diabetic patients, although insulin sensitivity was unchanged. In line with these results it was shown that in non-diabetic subjects treatment with IL-1ra improved β-cell function, but insulin sensitivity remained similar. In contrast to treatment with anti-TNF-α, no significant increase in incidence of infection in RA patients treated with Anakinra have been reported. Also for salsalate, there are no known effects for increased risk of infection.

Although anti-inflammatory agents are thus promising in treating insulin resistance and hypertriglyceridemia, a large randomized clinical trial has shown that intensive lifestyle intervention, i.e. weight loss due to caloric restriction and physical exercise, is also able to reduce inflammation as demonstrated by lower CRP levels. In comparison with metformin, intensive lifestyle intervention was able to prevent diabetes to a greater extent in patients with impaired glucose tolerance. Thus, anti-inflammatory agents may be useful to reduce the inflammatory burden on the short term, whereas intensive lifestyle intervention and maintenance of this novel life style could do so on the long term.

6.3 ROLE OF THE ADAPTIVE IMMUNE SYSTEM IN METABOLIC INFLAMMATION

Recent studies have shown that the adaptive immune system plays a clear role in metabolic inflammation. Increased amounts of T_h1 and CD8+ T-lymphocytes in adipose tissues of obese individuals have been reported. Yang et al. found that T-lymphocytes that reside in adipose tissue of diet-induced obese mice have a restricted T Cell Receptor (TCR) profile, which suggest that there is ongoing antigen exposure eliciting an active adaptive immune response. Recently, it was found that transfer of IgG antibodies of diet-induced obese mice to B-lymphocyte deficient mice induces adipose tissue inflammation and insulin resistance in these mice.

In chapter 5, we revealed that functional activating Fc receptors (FcR) likely play a role in diet-induced obesity and associated diet-induced adipose tissue inflammation and insulin resistance. Membrane receptors for the Fc portion of antibody molecules can be found on...
many hematopoietic cells, including macrophages. Cross-linking of these receptors activates cellular responses that are important for inflammation and immunity. The observation that functional FcR are likely involved in diet-induced obesity and inflammation suggest that antibodies are able to elicit a pro inflammatory response via cross-linking with FcR, which subsequently contributes to metabolic abnormalities seen in obese subjects.

Currently it is unclear against which antigens these antibodies are directed. Also it is unclear how B-lymphocytes acquire antigens. It could be that antigens arise from stressed apoptotic adipocytes, since it was observed that IgG antibodies localize with crown-like structures. Invariant natural killer T (iNKT) cells may also play a role in B-lymphocyte activation. iNKT cells react to lipid stimuli and are able to activate antibody production by B-lymphocytes. Recently it was found that mice fed a HFD have an increased amount of activated iNKT cells. It was also discovered that iNTK cell-deficiency protects against diet-induced insulin resistance and adipose tissue inflammation. Since similar results were obtained with db/db mice fed a chow diet, it is unlikely that exogenous lipids activate iNKT cells. Instead, the authors suggest that dyslipidemia in obesity results in iNKT cells loaded with self-lipids. These lipids could be presented to iNKT cells by antigen presenting cells such as macrophages. The increased amount of activated iNKT cells in obese mice may contribute to an increased B-lymphocyte activation, which subsequently leads to adverse metabolic effects.

The observation that the adaptive immune system is involved in the onset of insulin resistance offers new therapeutic possibilities. HFD-fed mice treated with CD3 specific antibody, which inactivates pro inflammatory T, showed improved glucose tolerance in addition, deletion of B-lymphocytes with CD20 specific monoclonal antibody in HFD-fed mice also resulted in improved glucose tolerance. More B-lymphocyte or antibody modulating agents such as inhibitors against CD19 and CD22 are available for usage in humans. Specific deletion of iNKT cells by an iNKT cell-specific antibody in individuals prone to develop the metabolic syndrome could also be protective in these individuals. However, just as mentioned in the previous section on anti-inflammatory compounds, a possible side effect of interference in the adaptive immune system may be enhanced susceptibility to invading pathogens. Therefore a target-tissue approach might be important. For instance, agents that specifically target the pro inflammatory response in adipose tissue would attenuate adipose tissue inflammation and associated insulin resistance, without disrupting other immune functions in the body of an individual.

6.4 ROLE OF THE GUT IN METABOLIC INFLAMMATION

Recently, attention has been drawn to the role of microbiota and epithelial barrier function of the gut in metabolic inflammation. It was found that innate immune suppression, by deletion of components of the inflammasome, can lead to altered gut microbiota, which results in transfer of bacterial products to the portal circulation where TLR 4 and 9 are activated, which
ultimately leads to enhanced pro-inflammatory signaling. Another study showed that gut microbiota per se can induce obesity and insulin resistance in mice that are genetically protected against obesity and insulin resistance (the mice used in this study were TLR 2 deficient) by increasing the gut permeability, which leads to increased LPS absorption.

We observed that both CD36-deficient mice as well as FcR γ-chain deficient mice have increased intestinal length (data not shown), indicating that alterations in lipid metabolism and inflammation are associated with alterations in the intestine. Human and mice studies have indicated that there is an association between immune suppression and lipid malabsorption.

Since microbiota and the epithelial barrier function can be changed by diet, the role of altered microbiota/epithelial barrier function is another factor that should be taken into account when studying the onset of the metabolic syndrome. Already, a few years ago it was shown that weight loss by following a fat or carbohydrate restricted low calorie diet, could induce a shift in bacterial phyla of obese people. Recently, it was found by Everard et al. that the botanical compound META060 that we have used in chapter 4 increased expression of two key intestinal tight junction proteins, thereby reducing the gut permeability.

Prebiotics, non-digestible nutrients such as dietary fibre and frutan, are also able to change gut microbiota. In obese individuals, fructan administration has shown a positive effect on BMI. Additional studies have demonstrated favorable effects of fructans on gastro-intestinal peptides such as PYY and GLP-1, which may contribute to the positive effects of fructan.

Which gut microbial species are responsible for these positive effects is currently unknown, since many are changed upon treatment with prebiotics.

6.5 CONCLUDING REMARKS

The increasing prevalence of the metabolic syndrome world-wide urges the development of new drugs and therapies that reduce the progress of metabolic disturbances such as hypertriglyceridemia and insulin resistance. In the last decades it has become clear that FA metabolites and chronic inflammation are involved in the pathology of the metabolic syndrome. The epithelial barrier function of the gut also has to be taken into account when studying the metabolic syndrome. For the development of new drugs and therapies it is essential to evaluate how FA metabolites and inflammatory pathways contribute to the development of hypertriglyceridemia and insulin resistance. In this thesis we showed that CD36 plays a role in proper storage of FA in mature adipocytes as well as in recruitment of new adipocytes, thereby reducing ectopic fat deposition. Also, we 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 inhibition of inflammatory pathways reduces hypertriglyceridemia and insulin resistance. Although anti-inflammatory agents might be helpful in treatment of the metabolic syndrome, caution is needed, since an inflammatory
response is necessary for destroying pathogens. Therefore, lifestyle-changing programs that help patients with improving (and maintaining) their dietary habits and adapting to a more physical active lifestyle deserve attention as well, since these interventions have proven to be successful in managing the metabolic syndrome.

REFERENCES


