INFLAMED FAT: immune modulation of adipose tissue and lipid metabolism

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Addendum

Summary

Samenvatting

Dankwoord

List of publications

Curriculum vitae

SUMMARY

The worldwide prevalence of obesity is steadily increasing. Obesity leads to insulin resistance and atherosclerosis, which are the pathologies underlying type 2 diabetes and cardiovascular disease, respectively. Inflammation is an important factor connecting obesity to these disorders, but the exact mechanisms connecting obesity, the immune system, type 2 diabetes and cardiovascular disease are still under investigation. The research described in this thesis was performed 1) to gain more insight into the role of the immune system in obesity, dyslipidemia, insulin resistance and atherosclerosis, 2) to study whether inflammation contributes to the disadvantageous metabolic phenotype of a human population with a particularly high risk to develop type 2 diabetes and cardiovascular disease, and 3) to study the therapeutic potential of decreasing inflammation by pharmacological strategies to reduce obesity and improve glucose and lipid metabolism in pre-clinical models. **Chapter 1** serves as a general introduction to the different processes and players that are important in obesity and associated disorders, including regulation of lipid metabolism, the physiology of adipose tissue, and the interaction between the immune system and adipose tissue function.

We first studied the effect of a potent inflammatory trigger, *i.e.* Bacille-Calmette-Guérin (BCG), on lipid metabolism and atherosclerosis. BCG is prepared from attenuated live *Mycobacterium bovis* and modulates atherosclerosis development as currently explained by immunomodulatory mechanisms. However, whether BCG is pro- or anti-atherogenic remains inconclusive as the effect of BCG on cholesterol metabolism, the main driver of atherosclerosis development, has remained underexposed in previous studies. In **chapter 2**, we aimed to elucidate the effect of BCG on inflammation in relation to cholesterol metabolism and atherosclerosis development in a mouse model of human-like lipoprotein metabolism. To this end, we fed hyperlipidemic APOE*3-Leiden.CETP mice a Western-type diet and treated them with a single intravenous injection of BCG. We found that BCG-treated mice had hepatic mycobacterial infection and hepatomegaly. Enlargement of the liver coincided with severe immune cell infiltration and a higher cholesterol content. Moreover, BCG reduced plasma total cholesterol levels. This was due to accelerated hepatic clearance of cholesterol, as evident from clearance studies with intravenously injected [^{14}C]cholesteryl oleate-labeled lipoprotein-like particles, and to reduced intestinal plant sterol and cholestanol absorption. Ultimately, BCG decreased the ability of peritoneal macrophages to become foam cells and tended to decrease atherosclerotic lesion area and reduced lesion severity in the aortic root of the heart: BCG delayed atherosclerotic lesion progression. Consequently, we concluded that BCG reduces the plasma levels of atherogenic lipoproteins and delays atherosclerotic lesion formation in hyperlipidemic mice.

In **chapter 3**, we switched our focus from immune modulation of lipid metabolism and atherosclerosis to glucose metabolism and type 2 diabetes. During the development of obesity, B cells accumulate in white adipose tissue (WAT) and produce IgG, which has previously been suggested to contribute to the development of glucose intolerance.

IgG signals by binding to Fcγ receptors (FcγR) and by activating the complement system (including C3). We aimed to study whether activation of FcγR and/or complement C3 mediates the development of high fat diet-induced glucose intolerance. To this end, we studied high-fat diet-fed mice lacking all four FcγRs, only the inhibitory FcγRIIb, only the central component of the complement system C3, and mice lacking all FcγRs as well as C3. We found that absence of the inhibitory FcγRIIb increases adipose tissue IgG, but that absence of FcγRs and/or C3 does not protect against high-fat diet induced glucose intolerance. We therefore concluded that if obesity-induced IgG contributes to the development of glucose intolerance, this is not mediated by FcγR or complement activation.

In **chapter 4**, we studied the inflammatory state of South Asians, a human population with a particularly high risk to develop glucose intolerance, compared to white Caucasians. More specifically, we compared mRNA expression of 144 markers of immune function in skeletal muscle and WAT of middle-aged overweight pre-diabetic Dutch South Asian and matched white Caucasian men. We found that expression of especially interferon signaling genes was lower in South Asians, both in muscle (IFIT3, IFI44) and WAT (IFI35, IFI44, IFIT2, IFIT3, IFIT5, OAS1, STAT1). Ingenuity pathway analysis highlighted the anti-inflammatory interferon α/β signaling pathway to be lower in South Asians. From this, we concluded that South Asians have impaired interferon signaling in metabolic tissues. Since recent evidence from rodent studies shows that impaired interferon α/β signaling in adipose tissue induces insulin resistance and glucose intolerance, impaired interferon signaling in South Asians may contribute to their high risk of type 2 diabetes.

In the next part of this thesis, we focused on the treatment of metabolic inflammation. The anti-inflammatory compound salsalate lowers glucose intolerance and dyslipidemia in type 2 diabetes patients, but the mechanism was still unknown. The aim of **chapter 5** was to unravel the molecular mechanisms involved in these beneficial metabolic effects of salsalate by treating mice with salsalate during and after development of high-fat diet-induced obesity. We found that salsalate attenuates and reverses high-fat diet-induced weight gain, in particular fat mass accumulation, improves glucose tolerance, and lowers plasma triglyceride levels. Mechanistically, salsalate selectively promotes the uptake of triglyceride-derived fatty acids by BAT, as evident from clearance studies with glycerol tri[^{3}H]oleate-labeled lipoprotein-like emulsion particles, decreased the intracellular lipid content in BAT, and increased rectal temperature, all pointing to more active BAT. Moreover, treatment of T37i brown adipocytes *in vitro* with salsalate increased uncoupled respiration, expression of the uncoupling protein-1 (*Ucp1*) and lipolysis. These latter two effects were abolished by the inhibition of cAMP-dependent protein kinase A (PKA). We concluded that salsalate activates BAT, presumably by directly activating brown adipocytes via the PKA pathway. This suggests a novel mechanism that may explain its beneficial metabolic effects in type 2 diabetes patients.

Finally, we studied the therapeutic potential of targeting GPR120, a free fatty acid receptor that is highly expressed in BAT and WAT, is regulated by cold exposure, and is a mediator of anti-inflammatory and insulin-sensitizing effects. Since the effects of GPR120 on lipid metabolism and substrate utilization have not been studied to date, the aims

of **chapter 6** were to assess the role of GPR120 in lipid metabolism and to evaluate the therapeutic potential of a selective GPR120 agonist in energy consumption. When fed a high-fat diet, GPR120 deficient mice had higher fat mass, lower physical activity, and lower energy expenditure during the dark phase, while relative substrate utilization (*i.e.* glucose *vs.* fatty acids) did not differ compared to wild-type mice. GPR120 deficiency reduced expression of *Ucp1*, *Prdm16* and *Pparα*, which are markers of BAT activity, thus suggesting less active BAT. Treatment with the GPR120 agonist TUG891 during high-fat diet-feeding reduced fat mass after 2.5 weeks. Furthermore, short-term treatment (5 days) with TUG891 on a chow diet acutely increased fat oxidation and reduced glucose oxidation, and already tended to reduce fat mass. From these data we concluded that GPR120 deficiency increases fat mass while GPR120 agonism reduces fat mass and increases fat oxidation. Therefore, stimulation of GPR120 holds therapeutic potential to combat obesity.

To conclude, we evaluated the results of this thesis in **chapter 7** and discussed the potential of targeting adipose tissue (and) inflammation to treat obesity-associated disorders such as type 2 diabetes and cardiovascular disease. Taken together, the studies described in this thesis have increased our understanding of the role of inflammation in adipose tissue function and lipid metabolism during the development of type 2 diabetes and cardiovascular disease. Moreover, novel potential therapeutic strategies were identified to combat obesity, metabolic inflammation and associated metabolic disorders, such as treatment with interferons, salsalate and GPR120 agonists.