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Systemic and white adipose tissue inflammation in obesity and insulin resistance

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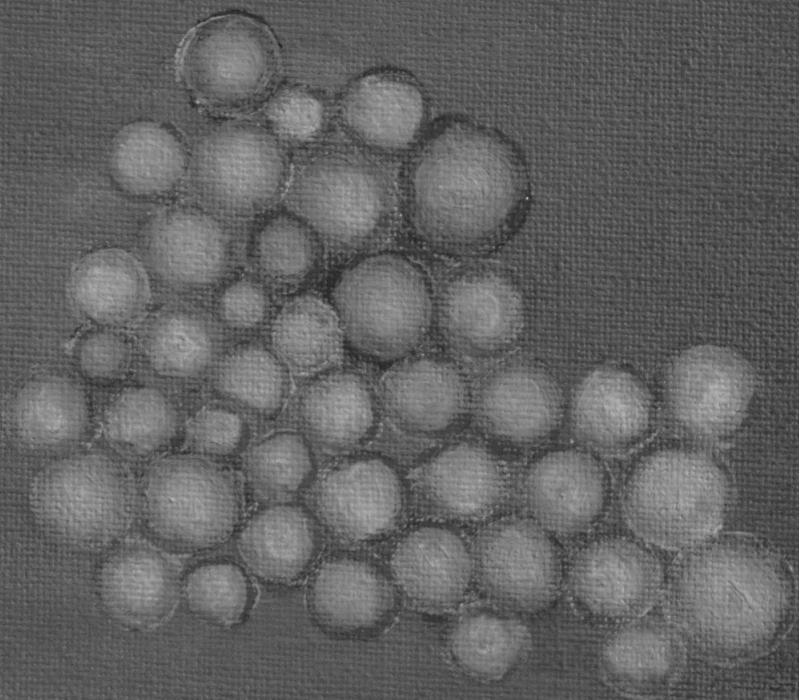
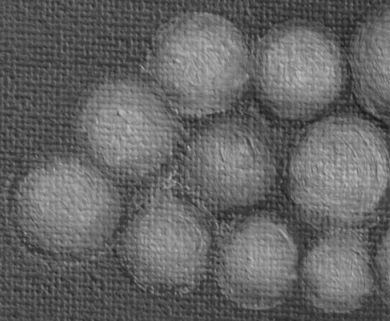
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Summarizing discussion



Discussion

As the obesity epidemic is still increasing, strategies to prevent and treat obesity and related pathologies are urgently needed. Obesity-induced inflammation is thought to contribute to the development of metabolic disorders. Therefore, inflammatory pathways that play a role in obesity-induced inflammation are potential promising targets in the treatment of metabolic disorders. Extensive knowledge on obesity-induced inflammation and the role of inflammatory pathways in the development of metabolic disorders can benefit the development of these therapeutic strategies. Mouse models are widely used to study obesity and related disorders. However, to what extent mouse-derived results translate to humans has not been studied extensively yet. Obesity-induced inflammation and its role in the development of insulin resistance, as well as the similarities of these processes between humans and mice have been addressed in this thesis. The new findings described in this thesis will be summarized and discussed in this final chapter. Additionally, clinical implications of obesity-induced inflammation as target to treat metabolic disorders and future perspectives will be addressed.

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Summary of the new findings

In this thesis, we determined to what extent metabolic health of obese humans is associated with obesity-related inflammation. WAT depot expandability and composition of the immune cells were determined during the development of obesity in mice, and the immune cell composition of the circulation and WAT were compared between obese humans and mice. Additionally, the role of Fc-receptors and the complement pathway in obesity-induced insulin resistance were studied. The main findings of this thesis are listed below:

- Metabolic health is associated with inflammatory status; obese women with T2DM have increased numbers of circulating leukocytes and higher levels of IL6 in the circulation as compared with metabolically healthy obese women (**Chapter 2**)
- DIO male C57Bl/6J mice exhibit metabolic dysfunction from a body weight of 40 grams onwards, which seems to be directed by the limited storage capacity of gWAT (**Chapter 3**)
- The different WAT depots of male C57Bl/6J mice differ with respect to expandability and immune cell composition (**Chapter 3**)
- The composition of immune cells in the circulation and WAT differs remarkably between obese humans and mice; mice have relatively higher numbers of B cells in both the circulation and WAT (**Chapter 4**)
- The effect of obesity on circulating immune cells shows similarities between humans and mice, including increased activation of lymphocytes and higher numbers of monocytes (**Chapter 4**)
- FcR γ -chain deficiency reduces the development of diet-induced obesity and associated WAT inflammation. This can, at least partly, be explained by increased lean mass and altered gut microbiota of the FcR γ -chain deficient mice (**Chapter 5**)

- IgG in WAT increases with increasing body weight in mice (**Chapter 6**)
- If obesity-induced IgG antibodies contribute to the development of insulin resistance, this is not dependent on FcγR or complement mediated pathways (**Chapter 6**)

Adipocytes in the development of metabolic disorders

Although obesity is known as a primary risk factor for the development of metabolic disorders, 20-25% of obese subjects are seemingly metabolically healthy, and it is not yet known why specifically some people remain healthy whereas others develop T2DM. Metabolically healthy obese subjects are considered as individuals with excessive weight, who are relatively insulin sensitive with a healthy lipid profile including low TG and fasting glucose levels, and who lack any signs of metabolic dysfunction (1). Adipocytes are thought to play an important role in the development of obesity related metabolic disorders. As long as obesity coincides with healthy normal functioning adipocytes, triglycerides (TG) can be properly stored in the adipose tissue and will not accumulate in peripheral tissues. Additionally, healthy non-stressed adipocytes produce less pro-inflammatory mediators compared to enlarged stressed adipocytes. Thus, normal functioning adipocytes that are still able to expand and thus can cope with the excessive lipid load during obesity, may be crucial for metabolic health. However, what are the determinants for adipocytes to expand without getting stressed, and how does this translate to metabolically healthy obesity? Metabolically healthy obese persons might have a genetic advantage that leads to better processing and storage of the increased lipid load. Whether metabolically healthy obese individuals always remain healthy seems questionable. Especially when obesity progresses, at some point the adipocytes will reach their limit for healthy expansion, and this will eventually and unavoidably lead to metabolic dysfunction.

Adipose tissue distribution is known as a major factor in the development of metabolic disorders and varies widely between individuals. Especially vWAT has been associated with the development of metabolic disorders (2), and is thought to exert more negative effects on health as compared with sWAT. Adipose tissue distribution might also contribute to the difference in metabolic health between equally obese individuals, however, the factors determining body fat mass and fat distribution are still largely unknown (3-5). A role for developmental genes in regulating body fat distribution has been suggested by several GWAS studies (6-9). Genetic variation in adipose tissue depot specific expression patterns of developmental transcription factors involved in embryological development, may contribute to differences in proliferation and differentiation capacity of the adipocytes in different depots and regulate fat distribution (10, 11). Inflammatory signals may also regulate adipose tissue depot specific growth and fat distribution. Recently, a role for the inflammatory component NLRP3-inflammasome has been described in the limited expansion capacity of sWAT and the development of insulin resistance in humans. The inflammasome down regulates adipogenesis (12). In **chapter 2** we found no differences in waist circumference, total body fat percentage, or adipocyte size of the different depots between morbidly obese women with and without T2DM. Thus, in our study population, fat distribution did not seem to be associated with metabolic health, or to explain the

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difference in inflammation. However, as our study population is already morbidly obese for at least five years, it is possible that fat distribution is an important regulator of metabolic health primarily in the initial phase of the development of obesity. In **chapter 3**, we have determined inter-depot differences regarding adipocyte size and WAT depot expansion during the development of obesity in male C57Bl/6J mice. There we found an association between limited gWAT adipocyte expansion capacity and insulin resistance. This indicates that capacity of the gWAT depot to expand determines the development of metabolic disorders in mice. Whether humans have such a WAT depot expansion capacity threshold for the development of metabolic disorders requires further investigation. As humans have high inter-individual variability due to genetic and environmental variation, a general threshold for the development of metabolic disorders may be challenging to find.

Interestingly, the limited capacity of subcutaneous adipocytes to expand has recently been suggested as a determinant of metabolic disease and adipocyte dysfunction in humans (13). Comparable to what we showed in the gWAT depot in mice (**chapter 3**), in humans the limited expansion capacity of subcutaneous adipocytes seems to result in increased hypertrophy of visceral adipocytes and adipose tissue dysfunction. A previous study determined increased visceral adipose tissue and liver fat as the best predictor for insulin resistance (14). The expansion capacity of adipocytes from the human sWAT depot might therefore be an important factor in the development of metabolic disorders. To address the role of adipose tissue expansion, inflammation, and function in the development of metabolic disorders, longitudinal studies in humans during the development of obesity are crucial.

Adipocytes as a potential therapeutic target to treat metabolic disorders

Increasing the adipocyte plasticity and the adipose tissue expansion capacity might be a way to decrease WAT dysfunction and inflammation during obesity. There are several potential means to improve adipocyte and WAT functioning. The first option would be to increase the expandability of the individual adipocyte, which would lead to increased lipid storage capacity of the adipose tissue, without associated stress. Adipocyte expandability is limited by multiple factors, including intracellular lipid droplet size, oxygen supply and extracellular matrix remodelling (15). Adipocyte specific proteins like Plin1 and Fsp27 have been shown to be crucial for metabolic flexibility and cell growth of the adipocyte (16, 17). For instance, mice deficient for Fsp27 have small adipocytes and associated impaired lipid storage capacity, which leads to hepatic steatosis and insulin resistance upon HFD feeding (18). Fsp27 interacts with adipocyte triglyceride lipase (ATGL) and thus plays a role in the regulation of TG-storage. By increasing adipocyte specific proteins like Fsp27, adipocytes might be able to grow larger.

Another way to increase the lipid storage capacity of the adipose tissue, is to increase the number of adipocytes in the adipose tissue. Adipocyte hyperplasia can only be facilitated by adipogenesis, and thus increasing the number of pre-adipocytes differentiating into adipocytes. Adipocytes are typically derived from adipose tissue resident mesenchymal progenitor cells. Recently, it has been suggested that adipocytes could also be derived by de novo production from bone marrow progenitor cells,

which indicates an alternative lineage involved in adipogenesis (19). These new findings contribute to the possibilities to increase the availability of pre-adipocytes for recruitment in the expanding adipose tissue depots. Both adjustments would improve fat storage in the adipose tissue rather than in peripheral tissues, and additionally would lower the obesity induced adipocyte stress, which both improve metabolic health.

Obesity-induced inflammation in the development of metabolic disorders

In humans, the effect of obesity-associated inflammation has been extensively studied in the adipose tissue itself. Systemic inflammation has primarily been assessed by measuring circulating cytokine levels during obesity. However, the composition of the circulating immune cell pool and activation status thereof has not been examined thoroughly thus far. We performed an extensive analysis of immune cell composition and activation status in the circulation as described in **chapter 2**. Furthermore, metabolically healthy versus unhealthy obesity is poorly understood. We specifically addressed the hypothesis that obesity-induced inflammation is a determinant of the difference in metabolic health between obese individuals. In this thesis, we indeed showed that metabolic health is associated with obesity-related inflammation, as both adipose tissue and systemic inflammation was higher in obese women with T2DM as compared to metabolically healthy obese women (**chapter 2**). However, the factors that lead to the differences in inflammatory status of obese woman with and without T2DM have not been revealed yet.

Previous studies in humans showed increased adipose tissue as well as systemic inflammation during obesity (20-22). In mice, the effect of obesity on adipose tissue inflammation has been extensively studied, however, obesity-induced systemic inflammation has been under-investigated so far, as also reviewed by Ip et al (23). In **chapter 2 and 4** we showed that obesity is associated with increased lymphocyte activation and increased numbers of monocytes in the circulation of both humans and mice. Additionally, we showed higher numbers of T cells, B cells and macrophages in the adipose tissue of mice with increasing obesity (**chapter 3**). It thus seems that systemic inflammation reflects the inflammatory status of the adipose tissue. The local inflammatory response in the adipose tissue during obesity may result in a spillover of inflammatory mediators into the circulation. Cytokines and chemokines from the adipose tissue can be released into the circulation, thereby inducing low-grade systemic inflammation and also promoting inflammation in other tissues.

In addition to inflammatory cytokine from hypertrophic adipocytes, other possible inducers of inflammation are released. Stressed and hypertrophic adipocytes have a disturbed lipid metabolism that can directly contribute to a pro-inflammatory state. Saturated fatty acids derived from or poorly stored by adipocytes are able to increase inflammation by binding to Toll like receptors (24). Furthermore, saturated fatty acids have been shown to increase reactive oxygen species (ROS), which also can stimulate inflammatory pathways, like the inflammasome complex, which in turn results in increased levels of the pro-inflammatory cytokine IL-1 β (25).

Recently, the role of the gut microbiota in the development of metabolic disorders received much

attention. It has been shown that obese human subjects have an altered bacterial composition in their gut, compared to lean human subjects (26, 27). Conversely, altering the microbiota of obese humans and mice by transfer from a healthier, lean phenotype, results in a better metabolic phenotype with increased insulin sensitivity (26, 28). This supports a role for the microbiota in the development of obesity induced inflammation and the development of metabolic disorders. Another gut related hypothesis that might contribute to inflammation is the leaky-gut syndrome. It is known that the altered gut microbiota in obesity are associated with changes in the digestion and absorption of food (29). This gut dysbiosis is also thought to lead to increased intestinal permeability, allowing endotoxins from the microbiota to enter the blood stream inducing systemic inflammation.

Antibodies in the development of metabolic disorders

In this thesis, we specifically focused on the role of IgG antibodies in the development of metabolic disorders. We showed in **chapter 2** an increased activation status of circulating B cells in obese women compared to age matched lean women. Furthermore, we found increased levels of IgG antibodies in the adipose tissue with increasing obesity (**chapter 6**). However, we could not prove that IgG antibodies are causative in the development of IR, as described in **chapter 5 and 6**. In addition, it remains unclear to which antigens these obesity-induced antibodies are directed. Stressed and dying adipocytes are suggested as a source of the antigens during obesity. It is generally known that dying adipocytes are surrounded by macrophages, characterized as CLS that phagocytose the cell debris. We showed that IgG antibodies co-localise with CLS, and are thus surrounding dying adipocytes. This co-localization consists of FcR mediated IgG-macrophage interaction, but is also partly FcR independent as we showed in **chapter 6**. This suggest that these antibodies are sampling antigens from these dying adipocytes to induce immune responses. We and others tried to reveal the antigens to which the obesity induced IgG antibodies respond (**chapter 6, 30**), however we were not able to identify specific obesity antigens yet. Winer did show increased IgG antibodies targeting general intracellular components in the circulation of insulin resistant versus insulin sensitive subjects. If the obesity related antigens originate from the AT, it might be a valuable approach to search for the antigens specifically in the human AT rather than in the circulation.

The underlying mechanisms driving obesity induced low-grade chronic inflammation are still poorly understood. Both the adaptive and innate immune responses have been recognized as key players in these inflammatory responses. Since autoantigens have been discovered to be present during obesity, the question arises if T2DM is possibly an autoinflammatory disease and thereby innate immune response driven, or an autoimmune disease and adaptive immunity driven. The presence of obesity related autoantibodies indicates that T2DM and insulin resistance may be autoimmune diseases. T2DM might thus be more similar to type 1 diabetes than originally thought, which is generally considered as a typical autoimmune disease. The innate immune system has also clearly proven its role in obesity induced inflammation. Especially macrophages have been implicated, which are known to take up and process excessive TG as well as to phagocytose dying and dead adipocytes. Both processes are able to

induce immune responses that can be recognized as autoinflammatory. With the current knowledge, I would not consider T2DM as a typical autoimmune nor a typical autoinflammatory disease, as it combines aspects of both.

Inflammation as a potential therapeutic target to treat metabolic disorders

As obesity-induced inflammation is known to contribute to the development of insulin resistance, inflammatory pathways seem promising targets to treat insulin resistance and T2DM. The anti-inflammatory drug aspirin seems to improve multiple metabolic measurements in T2DM patients (32), it may improve insulin sensitivity by preventing serine phosphorylation of the IRS proteins (33). However, the therapeutic value of high-doses of aspirin is limited by its side effects, including gastric ulcer formation and increased risk of bleeding and stroke. Salsalate, which similarly as aspirin belongs to the salicylate class of drugs, is not associated with these side effects. Salsalate is therefore recognized as a useful option in the treatment of insulin resistance and T2DM (34). Since 1876 salsalate has already been suggested as possible treatment for T2DM, and a recent study showed improved metabolic functioning in T2DM patients by salsalate (35). However, until now, salsalate has received limited study as potential treatment for T2DM, and larger clinical trials are needed.

Statins, generally used as lipid-lowering drugs, have been tested in clinical trials for a potential improvement of insulin sensitivity. Treatment with statins reduced cytokines and pro-inflammatory markers in the circulation, though no beneficial effect on glucose metabolism could be observed (36). In contrast, high-dose statin use is associated with increased risk for T2DM (37). Furthermore, blocking the IL1b signaling pathway by administration of neutralizing antibodies or administration of IL1 receptor antagonist does show beneficial effects on glucose metabolism in both obese humans and DIO-mice (38-42). Improved blood glucose levels after treatment of T2DM patients was attributed to enhanced pancreatic β -cell function, however, no effect on insulin sensitivity could be observed (43). TNF blockers did reduce blood glucose levels when used to treat patients with rheumatoid arthritis, as well as marginal effects in obese individuals (44-46). However, anti-TNF treatment has not been effective in improving glucose metabolism in T2DM patients thus far (47-50).

Another anti-inflammatory compound is dexamethasone, a member of glucocorticoid family of drugs which is considered the most effective compound to treat inflammatory diseases. However, dexamethasone therapy was associated with deteriorated insulin sensitivity in a clinical trial with healthy young males (51), likely due to systemic side effects. A strong reduction of inflammation may not only block the adverse, but also the beneficial effects of inflammation on energy metabolism and insulin sensitivity. It is suggested that inflammation regulates energy metabolism in a feedback manner by increasing energy expenditure (52). In a recent study, we studied the glucocorticoid receptor (GR) modulator C108297. This selective GR modulator combines both GR antagonism, which is known to reduce diet-induced obesity and GR agonism, which is known to reduce diet-induced inflammation (53). Selective GR modulation might thereby be a potential strategy to reduce obesity and related insulin resistance.

Anti-inflammatory therapies fail to improve insulin sensitivity in both animal models and clinical trials thus far (31). Failure of these drugs to treat insulin resistance is probably primarily caused by the complexity and comprehensiveness of obesity induced inflammation, with numerous inflammatory signals and immune responses involved. Targeting inflammatory pathways to treat T2DM seems more difficult than suggested, which may be explained by the fact that inflammatory pathways are involved in almost all physiological processes and diseases. Furthermore, it could also be risky to alter inflammatory responses, as the capacity to destroy invading pathogens may also be affected by anti-inflammatory agents. To circumvent interfering with general inflammatory processes in the body, targeting the inducer of obesity related inflammation instead of the inflammatory pathways itself, might be a relevant manner in the treatment of metabolic disorders. In this way, the obesity induced inflammation and thus the development of metabolic disorders can be diminished without affecting general inflammatory processes. This strengthens the value for identifying the antigens or other inducers of obesity related inflammation. Future studies should focus on unravelling the source and type of the inducers of obesity related inflammation.

Diet-induced obese mouse models to study metabolic disorders in humans

In this thesis, we have addressed mouse to human translatability, studying to what extent the situation in mice mimics the situation in humans in terms of WAT inflammation and development of insulin resistance during obesity. The expansion and immune cell composition of the different WAT depots during the development of obesity in mice has been extensively described in **chapter 3**. We found that the composition of the different WAT depots was very different, however, with increasing obesity, all depots had increasing numbers of macrophages, primarily of the pro-inflammatory M1 type. It is difficult to determine the immune cell populations in human adipose tissue over time with progressing obesity. We were therefore not able to perform a one to one comparison of the effect of obesity on the AT expansion and composition. However, we did compare systemic inflammation between lean and obese humans and mice (**chapter 4**). Even though the immune cell composition of the circulation is different between humans and mice, they both show increased lymphocyte activation and higher numbers of monocytes in the circulation in the obese condition. This indicates a similar effect of obesity on the immune cells, and thus a comparable obesity-induced inflammatory response in humans and mice. DIO mouse models can therefore be considered as a useful tool in the field of obesity research regarding obesity related inflammation.

We showed in **chapter 3** that gWAT is the depot to be primarily affected as well as the depot that shows an increase in all immune cell subtypes during the development of obesity. According to these data, the gWAT depot is most suitable to be analysed when studying the underlying mechanisms behind obesity induced WAT inflammation in mice. When studying the effect of immunological pathways on obesity-related disorders, immune deficient mouse models and immunological modulators might, next to changing immunological pathways, also affect body weight. This makes it very difficult to interpret the obesity-related results. When studying WAT inflammation and insulin resistance in mice,

differences in body weight should be recognized and if possible corrected for as this is an important confounding factor.

In this thesis, we have specifically focussed on the role of immunoglobulins (Ig) during obesity and primarily on IgG antibodies and related pathways, including the Fc-receptor pathway and complement system. For this purpose, we have used genetically modified mouse models as a tool to study the role of immune cells or immunological pathways in the development of obesity related inflammation. It has been shown that circulating IgG antibodies increase with obesity in mice (30). We additionally showed increased IgG in the adipose tissue of mice with increasing body weight (**chapter 6**). We found that mice have a much higher number of B cells in the circulation and the adipose tissue compared to humans (**chapter 4**). As IgG antibodies are produced by B cells, it is possible that the IgG effect induced by obesity is mouse specific, and may be of less importance in the human situation. However, although the number of B cells in the circulation is lower in humans, we did observe increased numbers of activated B cells in the human circulation with obesity (**chapter 2**). Furthermore, it has been shown that obesity increases systemic IgG levels in humans as well (54). Whether IgG plays a role in both mice and humans during obesity thus requires further investigation.

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Concluding remarks

Thus far, the mechanisms driving obesity-induced inflammation and the contribution thereof to metabolic disorders are not fully understood. This thesis described obesity-induced systemic and WAT inflammation in mice, and showed comparable effects of obesity on circulating immune cells between humans and mice. Furthermore, we determined that inflammatory status is associated with metabolic health in obese human subjects. Despite the fact that human studies are more difficult to carry out, costly and time consuming, they are crucial for a proper understanding of human pathology and to find potential targets to treat metabolic diseases. Therefore, more effort should be put into performing large scale longitudinal clinical trials focussing simultaneously on systemic and WAT inflammation in lean and obese subjects, with and without metabolic disorders. Longitudinal studies might eventually provide us with insight in the starting point of adipose tissue dysfunction and metabolic disorders in humans and they might provide clues towards the driver(s) of these processes. Nevertheless, mouse models have contributed greatly to our current knowledge. The data described in this thesis indicates a similar response of immune cells from humans and mice in obesity, which highlights the value and relevance of animal models in obesity research. In my view, mechanistic animal studies and human clinical trials are highly complementary and should be performed side by side to improve relevance and applicability for human therapeutic target discovery.

In conclusion, although ideally diet and exercise would be the best way to prevent obesity and metabolic disorders, this is obviously not effective in reducing obesity in our current society. Immune cells as well as inflammatory factors that have been shown to contribute to obesity related pathologies seem attractive targets in the fight against obesity and metabolic disorders. However, as the immune system is a very delicate and complex system comprising interaction and compensation from other

inflammatory pathways, obesity-induced inflammation might be too ambitious as a target. Therefore, I suggest that instead of broadly decreasing inflammatory responses, targeting the inducers of the inflammation would be a more promising approach to decrease obesity related inflammation and metabolic disorders. Our data suggest that obesity related antigens represent novel targets to treat obesity related insulin resistance.

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