High fat diet induced disturbances of energy metabolism

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Chapter 7: General discussion

Obesity and insulin resistance (IR) are multifactorial pathologies, characterized by a complex etiology. In addition to genetics, age and sex, environmental factors such as dietary composition and lifestyle have profound effects on the development of both pathologies. Excess dietary energy intake (EI) *per se* has effects on energy homeostasis through physiological systems involved in maintenance of substrate balances (75;76;216). Moreover, the composition of the diet itself may contribute and aggravate the phenotype in time by acting as a second hit. In particular, high levels of saturated long chain fatty acids have been associated with the development of obesity and IR. In this thesis, we have addressed a number of important aspects that play a role in the development of diet-induced obesity and insulin resistance.

Energy intake and energy expenditure

Theoretically, at constant EI, low levels of EE would be expected to lead to weight gain. However, there is discrepancy in the literature regarding the correlation of EE and the development of obesity in humans. Low levels of basal EE were positively correlated to weight gain in a number of studies (30;203;234) whereas in other studies high levels of total EE were positively correlated to weight gain (32;265). The discrepancies in the observations described above might be due to the conceptual differences between the measurements of basal EE and total EE. Whereas basal EE is determined in resting, fasted subjects, total EE is determined in free moving, fed individuals. Moreover, most of the studies assessing the relation between EE and weight gain try to stratify for individual differences in EI and EE by studying subjects at "energy balance" in a activity controlled laboratory environment (58;269). In these studies, subjects are regarded to be in energy balance if weight is stable for a certain period of time. Given the resolution of the measurements used, and the fact that even minute discrepancies between EI and EE disturb the energy balance, measuring subjects at exact energy balance is virtually impossible.

In **chapter 6** the intrinsic variation in EI and EE in free moving, ad libitum fed mice was studied. High levels of EI were correlated to high levels of total EE and a low degree of muscle mitochondrial coupling for fatty acid substrates. No correlation was found between EI and the degree of coupling on glycolitic substrates, indicating that the absolute contribution of carbohydrate oxidation to ATP synthesis rises with the intake of a mixed meal. This is in line with the potency of EI to simulate carbohydrate oxidation (75;76), but not fat oxidation (217).

From a physiological perspective, EI may be related to the mitochondrial degree of coupling in two ways. First, a low degree of coupling may result in higher EI in a supply and demand fashion. If mitochondrial degree of coupling is low, a greater amount of metabolic substrate per time unit is needed to generate the

same amount of ATP per time unit, requiring an increase in EI to meet ATP demand. As food is taken in as a mixed meal, carbohydrates, fat and proteins are consumed in greater amounts, and since higher levels of food intake per sé failed to induce fatty acid oxidation, this leads a more positive fat balance (75).

Second, uncoupling of fatty acid oxidation from ATP synthesis may protect animals with a high EI from diet induced obesity. EI is known to be variable between animals, and to be a good predictor for future weight gain (61). Mitochondrial uncoupling may function as a route to metabolize excess energy independent of ATP synthesis, thereby preventing a positive energy balance.

The relation between EI and the development of obesity was studied in more detail in **chapter 5**, where we demonstrate the effect of the iminosugar AMP-DNM on whole body energy homeostasis in ObOb mice. AMP-DNM treatment was associated with an increased release of peptide YY (PYY), a potent inhibitor of food intake. Indeed, AMP-DNM treated animals exhibited decreased food intake. EE was also reduced in AMP-DNM treated animals. Since EI was reduced by 26%, whereas EE was reduced by only 12%, AMP-DNM treated animals had a less positive energy balance. In accordance with the lower food intake, substrate specific energy expenditure calculations demonstrated that carbohydrate oxidation was lower and fat oxidation was higher in AMP-DNM treated animals compared to controls. This is in agreement with the findings described above, where higher food intake levels could be linked to a more positive fat balance via the same mechanism.

Etiology of energy imbalance

The development of obesity and insulin resistance is dependent on the extent of the positive energy balance itself and the duration of this energetic imbalance. As was mentioned before, even small disturbances in energy balance can lead to adiposity in the long term (43). As the average lifespan of a human being is measured in decades, "long term" interventions are measured in weeks or months, and thus, are relatively short. In rodents however, with an average lifespan measured in years or even months, long term experiments can be performed in a controlled laboratory setting. For example, the male C57Bl/6 mouse is considered adult at an age of 12 to 14 weeks, and has an average lifespan of 2.5 years (278). If started at an age of 14 weeks, and running for 16 weeks, an intervention runs for about 15% of the total adult life of the animal. Compared to humans, this would require a study continuously running for a period of 5 to 6 years. Therefore, mouse studies are invaluable to assess the long term effects of an intervention

In **chapter 2**, we demonstrated that during a 16 week high fat diet intervention, an early phase, (day 1 to week 1), a mid phase (week 2 and week 4) and a late phase (week 8 to 16) could be identified. These phases were associated with

changes in hepatic lipid profile, plasma markers of systemic inflammation (240) as well as changes in the hepatic transcriptome (197). In a separate study, we found additional evidence for a multi-phased response to high fat diet intervention in wild type C57Bl/6 mice (52). These data clearly indicate that the duration of the high fat diet intervention determines the pathology that is observed in the model.

In addition to changes in the hepatic lipidome, plasma triglyceride levels, as well as a number of plasma inflammatory markers were shown to be transiently elevated immediately after the switch to the high fat diet. The inflammatory markers C-Reactive Protein (CRP), Monocyte chemotactic protein-1 and 3, (MCP-1 and MCP-3, respectively), Tissue inhibitor of metalloproteinases-1 (TIMP-1) and Macrophage inflammatory protein-1 gamma (MIP-1gamma) showed significantly increased concentrations in high fat diet fed animals compared to controls. This may represent an acute phase inflammatory response to the intake of a high fat diet, and may in part be due to a short period of hyper caloric food intake (283). Temporal changes have also been identified in the regulation of neuronal and hormonal markers associated with food intake (146;171;277). The hypothalamic activity of neurons for NeuroPeptide Y (NPY), Agouti Related Protein (AgRP), Pro-opiomelanocortin (POMC) and CART (Cocaine and Amphetamine-Regulated Transcript prepropeptide) have all been shown to be time dependent (283).

Most high fat diet studies use an established obesity and insulin resistance phenotype as a starting point for an intervention. However, our findings stress the importance of the accurate registration of the duration of dietary intervention when studying changes in metabolism and systemic inflammation. Our data indicate that the development of diet induced pathology is not a linear response but is characterized by dynamic phases of response. This implies that the timing of an experiment needs to be carefully considered and chosen, since this will affect the processes that will be occurring. It is likely that the temporal phasing is present in other mouse models, although the exact timing may be dependent on the model used and the type of tissue studied.

Dietary composition

Since long chain saturated FA have been specifically associated with the development of pathology, the effect of fatty acid chain length on both metabolism and IR was studied in mouse models. In **chapter 3** we studied the effect of dietary fatty acid chain length in general. In **chapter 4** we studied the effect of a specific fatty acid, stearic acid. In these studies we performed a relatively short term intervention of 5 (**chapter 4**) and 8 weeks (**chapter 3**) to assess metabolic adaptations independent of the overt pathological effects associated with long term high fat diet intervention (197).

We demonstrated that feeding mice a diet rich in long chain triglycerides (LCT) resulted in increased adiposity, compared to mice fed a diet rich in medium chain triglycerides (MCT). Metabolic cage analysis revealed that EI was higher, and EE was lower in LCT fed animals. The lower EE in LCT fed animals was characterized by a lower oxidation of fatty acids, which is in agreement with previous findings (56;144;225). The changes in both EI and EE are consistent with a higher fat accumulation.

The low oxidative efficiency of LCT could potentially result in IR due to ectopic fat accumulation, in addition to the effects on the development of obesity. In our study, ectopic lipid accumulation in liver and muscle was indeed higher in LCT fed animals. The higher liver lipid accumulation was associated with reduced hepatic insulin sensitivity, as was demonstrated by hyperinsulinemic-euglycemic clamp. However, while liver insulin sensitivity was reduced in LCT fed animals compared to MCT fed animals, the opposite was true for muscle. This contradiction argues that different mechanisms are involved in the development of muscle and liver insulin resistance.

The ectopic accumulation of triglycerides has been associated with an increased formation of diacylglycerols (DAG) and ceramides (CER) (242). However, the accumulation of DAG and CER rather than triglyceride accumulation itself has been proposed to be causally involved in the development of insulin resistance (106;242;245). Studies in lipid-pretreated cells have shown that different types of fatty acids can lead to differences in the content of fatty acid intermediates. LCT reduced insulin sensitivity and elevated DAG and ceramide levels, whereas incubation with MCT had no effect on insulin sensitivity or accumulation of DAG or ceramides (41;169). Although these in vitro results are in line with the hepatic phenotype of the LCT/MCT fed animals, it is clear from the muscle phenotype that the *in vivo* situation is more complex and likely involves additional and/or alternative mechanisms.

A phenotype similar to the LCT fed animals was found in mice fed a diet naturally high in stearic acid. Animals fed a lard based diet, which is high in stearic acid, had higher EI and a lower EE compared to animals fed a diet low instearic acid content. Stearic acid fed animals became more obese and developed severe hepatic insulin resistance. Since stearic acid is poorly oxidized in the liver (185), elevating the level of dietary stearic acid could potentially lead to hepatic IR in a similar manner as the LCT diet. Interestingly, a second diet, artificially enriched in stearic acid, also resulted in the same adverse metabolic phenotype, as well as hepatic IR, although EI did not differ significantly between groups. Therefore, the increased adiposity in the stearate fed group may be a direct effect of a low oxidative efficiency of stearic acid (56), which in turn may affect peripheral insulin sensitivity in a similar manner as the LCT diet.

The higher degree of insulin sensitivity found in peripheral tissues of the LCT fed animals, as well as in the high stearic acid fed animals is interesting, and may directly be due to the lower insulin sensitivity of the liver. The low insulin mediated repression of hepatic glucose production in these groups may indicate that glucose production is not repressed effectively in the postprandial situation. Therefore, hepatic glucose production may continue to contribute significantly to the total pool of glucose available after a meal. Since food intake is higher (LCT, HFL) or at least similar (HFPS), the total amount of carbohydrate that is available is higher, peripheral tissues must remain insulin sensitive in order to facilitate the uptake and metabolism of carbohydrates.

In summary, an important take home message from the work in this thesis is that not all high fat diets are equal in their potency to induce obesity and insulin resistance, and therefore can not be regarded as a simple tool to gain a phenotype. In addition to the effect of the diet itself, the time span chosen to administer the diet is a major determinant in the type of pathology that is induced and will affect the conclusions that are drawn from the analyses.