High fat diet induced disturbances of energy metabolism

Berg, S.A.A. van den

Chapter 8: Future perspectives

Obesity is associated with, and is often hypothesized to precede the development of insulin resistance, type 2 diabetes and cardiovascular disease. Therefore, it is important to study the processes involved during the development of obesity. During the development of obesity an imbalance between energy intake and energy expenditure is always present, and this is always followed by an increased flux of energy to white adipose tissue stores. In this respect, it does not matter whether EI and EE are not matched due to a low satiety signal, a low mitochondrial efficiency or impaired nutrient partitioning. In all studies included in this thesis, differences in the development of obesity between groups were virtually always associated with differences in EI. Since the energy balance is determined by both EI and EE, it is therefore difficult to determine whether a disturbance in oxidative metabolism is causally involved in the development of obesity if differences in EI are present.

Generally, differences in EI are tackled by performing paired fed studies (221). Although paired fed studies have been used in the past to match food intake between groups, this may have potential unwanted and unpredictable effects on body composition (243), liver glycogen content (107) as well as on the synthesis, secretion and stability of pancreatic enzymes (179). In addition to this, the irregular feeding pattern in paired fed animals may have profound effects on the development of obesity (6;212). Therefore, it is better to dissect the contribution of EI to excess weight gain by treating variability in EI as a co-variable rather than trying to correct for it by paired feeding. Mathematical modeling can be used to dissect the contribution of EI to obesity (69). By calculating the contribution of EI to EE, and subsequent stratification for EI, one can address whether differences in EE exist that are independent of EI, and are in fact causally related to the development of obesity.

A prerequisite for mathematical modeling of energy metabolism is that food intake, gas exchange and fecal/urinary energy excretion are monitored accurately and at high resolution. Surprisingly, it has been difficult to calculate a energy balance for rodents. For example, a recent publication (85) reported an EI rate of wild type mice of at least 17 kcal/d, whereas EE measured by indirect calorimetry was less than 5 kcal/hr/(kg BW)$^{0.75}$. Since these animals weighed at most 40 grams at the time of measurement, such a large positive energy balance would translate to a rate of weight change of at least 4.7 g/week. However, the measured weight gain was less than 1 g/week. This indicates that even careful indirect calorimetry and food intake measurements can lead to estimates of energy imbalance that are inconsistent with the weight gain measurements. In part, this discrepancy may be due to methodological/mechanical flaws, although it may also result from conceptual flaws in the calculation of EE.
To obtain a closed energy balance, it is important that the mathematical calculations which are used to estimate substrate specific energy expenditure are

improved. The current calculations (190) are based on the assumption that fatty acid oxidation of all fatty acids is similar to that of a hypothetical average fatty acid. A second methodological flaw is the calculation of the energy potential of glucose, which has been estimated from data obtained from the heat of combustion of glucose at 25°C.

In addition to the improvement of the calculations that are currently used in the calculation of EE in rodent experiments, measurements of protein balance are required in the calculation of energy balance. In humans, the protein balance over a period of time is essentially 0, as the body has no capacity to store protein and lean mass is stable. However, in rodents, even when fed a low fat diet, growth of the lean mass continues over time (91). In addition, in rodents fed a high fat diet, lean mass increases even more (91). Similar results have been found in humans where lean mass comprised approximately 25% of the total mass gained in periods of caloric excess (120). An increase in lean mass implicates that the protein balance is positive, and hence that protein intake may not reflect protein turnover.

As was mentioned before, the development of obesity directly results from an energy imbalance, and this imbalance is associated with an increased flux of fatty acids towards the adipose tissue. However, in some cases the capacity for the white adipose tissue to store energy is insufficient. This results in an excess flux of fatty acids to other organs, which may lead to ectopic fat accumulation. Since insulin resistance may, in part, be determined by the formation of DAG and CER from ectopically stored fatty acids, it is crucial to accurately measure these fluxes.

Currently, tissue specific carbohydrate and fatty acid fluxes are measured by radioactive tracer studies (18;239). These techniques enable the accurate calculation of the rate of uptake of fatty acids and carbohydrates by metabolically active organs, such as muscle, heart and spleen. However, the major drawback of radioactive isotope techniques is that it limits the possibility to acquire simultaneous information on pathway specific carbon fluxes. In addition, the use of radioactivity limits the potential re-use of experimental animals for repeated measure studies.

In light of this, novel experimental methods are being developed to study carbohydrate and fatty acid fluxes *in vivo*. For example, stable isotope Mass Isotopomer Distribution (MID) analysis has been successfully employed to study intermediary metabolism of nutrients (14). For example, MID analysis has been used to study the elongation of fatty acids (182), protein synthesis (274), cholesterol and bile synthesis (205) and the contribution of carbohydrate intermediates to glycogen synthesis (247). MID analysis is based on the use of stably labeled, rather than radioactive isotopes and detection is based on mass

as determined using a Mass Spectrometer. By labeling the precursor with a specific mass tag, the enrichment of the various intermediates in a pathway can be determined, based on combinatorial probabilities of labeled and unlabeled monomeric subunits. An extensive overview of MID analysis can be found in a review by Bequette et al (14).

The improvements in analytical techniques, as those mentioned above, may provide additional tools to investigate complex biological questions. For example, stearate was demonstrated to affect obesity and hepatic insulin sensitivity, independent of food intake. The low oxidative efficiency of stearic acid may directly result in obesity by an increased flow of energy to the white adipose tissue. However, the mechanisms involved in the selective induction of hepatic insulin resistance in stearic acid fed animals remain to be clarified. It may be that stearic acid is indeed accumulating in the liver due its low incorporation in VLDL and subsequently results in the formation of CER and DAG. MID analysis may be used to quantify whether stearic acid is indeed accumulating (165) and if ceramide synthesis is indeed higher in animals fed a diet rich in stearic acid (244). In addition to this, quantitative tissue specific energy flux data can be derived from the combination of radioactive lipid infusion studies and mathematical modeling. This would enable the determination of what organs have a low uptake of stearic acid, and therefore may contribute to continuously high levels of circulating stearic acid. Furthermore, the incorporation of circulating stearic acid into VLDL could than be addressed by MID analysis (276), which would provide more information on whether or not stearic acid is accumulating in the liver.

It is important, however, to keep in mind that mathematical modeling of whole body energy metabolism and substrate fluxes only provide the tools needed to study multifactorial pathologies such as DIO and IR, by giving the researcher the possibility to integrate data of different variables. However, the most important parameter will remain the experimental design. In this thesis, we demonstrated that the duration of energy imbalance determined, in part, the pathology that was present in high fat diet fed ApoE*3-leiden mice. This stresses the importance of the registration of the exact duration and consequences of the intervention. In addition, dietary fatty acid composition was demonstrated to have profound effects on the extend of the development of obesity and insulin resistance, and that major differences exist in the organ specificity of the insulin resistance. Third, and perhaps the most important conclusion is that when one studies the development of obesity and associated pathologies, it is imperative to keep in mind that obesity is always the result of a positive energy balance. It is therefore impossible to attribute an effect of an intervention on obesity if differences in EI or EE exist. Since matching food intake between animals leads to even more uncontrolled variables in the equation of energy balance, the most

important goal in future experiments is the calculation of the net effect of an intervention of energy intake and energy expenditure.