



Universiteit
Leiden
The Netherlands

High fat diet induced disturbances of energy metabolism

Berg, S.A.A. van den

Citation

Berg, S. A. A. van den. (2010, October 6). *High fat diet induced disturbances of energy metabolism*. Retrieved from <https://hdl.handle.net/1887/16010>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/16010>

Note: To cite this publication please use the final published version (if applicable).

General introduction

The introduction to this thesis consists of two parts. The first part gives an overview of the current concepts and developments regarding obesity and insulin resistance, as well as a short introduction on lipids and their role in the development of pathology. The second part provides an in-depth description of the principles and calculations used in the techniques that are central tools for this thesis, namely the hyperinsulinemic-euglycemic clamp and indirect calorimetry.

Chapter 1, Part A: Biological background of obesity, insulin resistance and dyslipidemia

The definition of obesity and the obesity epidemic

Obesity is a medical condition in which body fat has accumulated to an extent that it may have adverse effects on health. Clinically, obesity is defined by the World Health Organization (WHO) as having a Body Mass Index (BMI) over 30. Within the obese population, three distinct sub-classes can be defined, based on the severity of obesity, ranging from class I obesity (BMI 30.0-34.9), class II obesity (BMI 35.0-39.9) and class III obesity (BMI over 40), which are also cumulative issues for public health action. Adverse consequences of obesity are a negative social image, cardiovascular disease and type 2 diabetes (49;96;256;279), as well as several cancers (193;207). In addition to these, obesity is associated with a number of other co-morbidities such as psychiatric and neurological disorders (16;95).

Currently, obesity is one of the most important risk factors contributing to disease burden worldwide, and the second leading preventable cause of death (after smoking) in the US (167). In 2005, 1.1 billion adults and 10% of children were classified as overweight or obese (96). In Europe, the incidence of obesity is rising, and maybe of even more concern; childhood obesity is becoming more and more prevalent (148).

Determinants of obesity – the role of genetic makeup

In a recent literature review by the Dutch National Institute for Public Health and the Environment (RIVM; report number 350020005), it was reported that about 40-70% of the total variation in body weight between individuals can be explained by genetic differences (150). For about 10% of the individuals with severe early onset obesity, a rare mutation in a single gene is known, for example leptin deficiency. This is the case for at most 2.5% of the individuals with the less extreme forms of obesity. In addition, for a limited number of gene variants convincing evidence supporting their involvement in determining BMI or obesity risk has been established (241;272)

For example, mutations in the melanocortin-4 receptor (MC4R) gene are currently the most prevalently found genetic cause for extreme obesity (94;151).

The MC4R is located in the hypothalamus and plays a key role in the regulation of food intake (87). In the hypothalamus, two types of neurons are involved in the positive and negative regulation of food intake. The MC4R is present on both and is activated by proopiomelanocortin (POMC) derived peptides alpha-MSH and beta-MSH and is antagonized by agouti-related peptide (AGRP). The activated receptor couples to the Gs/adenylyl cyclase system which leads to decreased food intake. To date, more than 70 different mutations in the MC4R gene have been associated with obesity in various study populations (235).

A second major genetic factor found to affect obesity is the fat mass and obesity associated gene (FTO) (196). The FTO gene encodes a nucleic acid demethylase, and is present in feeding-related nuclei of the brainstem and hypothalamus (81). In mice, it has been discovered that FTO knock out results in increased energy expenditure and reduced body weight (74), suggesting a direct role for FTO in metabolism, although this is currently under debate (31). The molecular mechanism underlying the role of FTO in energy metabolism is unknown.

Interestingly, so far, all genetics factors thought to be involved in the development of obesity are linked to neuronal pathways regulating food intake, satiety signaling and energy expenditure. In addition, while the estimated heritability for obesity is relatively high (up to 70%) only a small proportion of heritability can be explained by the combined effect of all currently identified susceptibility loci (less than 2%) (159). This indicates that rare variants with a high impact on obesity and interacting variants with a small effect exist. Either way, the genetics of obesity are extremely complex. In addition, the remaining 30-60% of obesity cases that can not be explained by heritability analysis result from environmental factors.

Determinants of obesity – the role of energy intake and energy expenditure

Obesity is by definition the result of an imbalance between the net intake and expenditure of energy. When net energy intake exceeds net energy expenditure, the remaining part is stored by the body, predominantly in the form of fat. Food consumption is the major determinant of energy intake, and therefore, may be the main determinant of obesity. Indeed, the development of obesity has been correlated to energy intake in a number of studies (232). Moreover, epidemiological data suggest a role for dietary fat intake in this process (147). A review of the results from 28 clinical trials (25) that studied the effects of a reduction in the amount of energy from fat in the diet showed that a reduction of 10% in the proportion of energy from fat was associated with a reduction in weight of 16 g/d. However, these data may be biased by carbohydrate intake, since increased fat intake is mostly paralleled by increased carbohydrate intake when consuming a mixed meal.

In humans, various mechanisms may explain excess food intake, and in particular the excess intake of food high in fat content. For example, the palatability of high fat foods (86;228) is very high, presumably associated with their high energy density (9 kcal/g) compared to carbohydrate (3.8 kcal/g) and protein (4 kcal/g) rich foods (227). In addition, high fat foods may fail to properly impact the central regulation of food intake (7).

In addition to excess food intake, low levels of energy expenditure have been proposed to predispose to the development of obesity (30;236), although the results obtained are not conclusive (253;264;265). Low levels of energy expenditure can be the result of a sedentary lifestyle, for example due to a highly mechanized working environment (26), excess television viewing (113;131) and low levels of sport participation (3). The extent to which imbalances in energy intake and energy expenditure can lead to obesity is included in the anecdote below.

In humans, the weight increase during the development of obesity is about 2/3 in the form of fat and 1/3 in the form of lean body mass. The energy content of fat equals 9,3 kcal/g and for lean mass equals 0,9 kcal/g, resulting in an average energetic content of 6,5 kcal/g of weight gain. If the energy balance is positive by 100 kcal per day, the equivalent of a small piece of lightly buttered toast, this would result in a weight gain of 1 kg in 65 days, or 5,5 kg per year. Yet, 100 kcal is less than 5% of the total daily energy requirements and almost impossible to measure with current energy balance equipment. Although this is an unlikely example, since increased adiposity is also associated with increased energy expenditure in humans, this simple example does explain that small imbalances may have major impacts on energy balance on the long term.

Mouse models of obesity

Although human intervention studies are being performed to study the effects of energy imbalances (109;267), there are limitations to the parameters that can be measured. For example, whole room indirect calorimetry can be performed in humans to study energy metabolism, although this is costly and laborious. In addition, data concerning *in vivo* organ specific nutrient processing is limited to studies using techniques such as venous catheterization (23;168). Venous catheterization experiments are performed by placing catheters before and after the organ of interest, and subsequent analysis of differences in nutrient uptake or formation. An excellent example of the use of this technique was performed by Kelley and Mandarino, who described that hyperglycemia normalizes insulin-stimulated skeletal muscle glucose oxidation and storage in noninsulin-dependent diabetes mellitus (127).

To overcome some of the practical problems associated with human intervention studies, a variety of rodent models of obesity are being used to study

environmental and gene-environment interactions. An important advantage of mouse models is the possibility to study the effects of modification of gene expression on the development of pathology. A wide variety of knockout and transgenic mouse models are available. For example, the leptin deficient *ObOb* mouse model has been used in a great number of studies concerning energy balance. Since leptin is an adipose tissue hormone that negatively regulates food intake, the *ObOb* model is characterized by a hyperphagic phenotype that is not compensated for by an increase in energy expenditure (100). A relatively less severe and perhaps more physiological model for obesity is the C57Bl/6 mouse. This inbred strain develops obesity and insulin resistance when fed a Western type or high fat diet.

High fat feeding is the most widely used intervention to generate obese, insulin resistant mouse models. In most studies, the diet has been used solely as a tool to obtain an obese mouse model, rather than to study the effect of dietary composition on the development of obesity and associated pathology. However, a number of studies have focused on the metabolic fate of certain lipid classes or even fatty acid species (28;108)

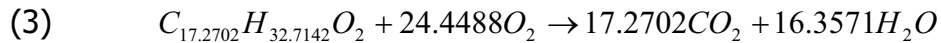
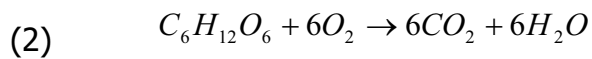
In vivo measurements of energy metabolism

Whereas the techniques and methods needed to measure energy intake are relatively easy, measuring energy expenditure is more complicated. Specialized techniques such as double-labeled water (48;208) and accelerometry (22;141;194) can be used to estimate energy expenditure in humans and rodents. However, techniques such as direct or indirect calorimetry are needed to calculate relative and absolute substrate energy expenditure levels. Indirect calorimetry is a non-invasive method to estimate substrate specific energy expenditure rates based on the amounts of oxygen consumed and carbon dioxide produced. In humans, whole room calorimeters are used, which enable accurate measurements of energy intake and energy expenditure, as well as activity patterns (202). Measurements spanning multiple days enable the calculation of energy expended while sleeping, resting or during high levels of physical activity (53;143).

In addition to estimates of absolute values of energy expenditure, a qualitative assessment can be made on the choice of metabolic substrates at a certain period moment. The respiratory exchange rate is a dimensionless parameter and represents a measure for the contribution of fat and carbohydrate oxidation to the total energy expenditure, and is calculated by the following formula.

$$(1) \quad RER = \frac{VCO_2}{VO_2}$$

For example, one can calculate the RER for glucose and a typical fatty acid by solving the stoichiometric equations below:



From the stoichiometric equation, one can calculate that the RER associated with glucose oxidation equals 1. For fat, it would equal approximately 0.7.

A more concise description of the measurements and calculations of relative and absolute energy expenditure is included in the chapter "Indirect calorimetry" in the second part of the introduction.

Insulin resistance

Insulin resistance can be defined as a state during which the normal physiological responses of tissues to insulin are disturbed, and higher levels of insulin than normal are required to evoke these responses. In insulin resistant subjects, the postprandial increase in insulin mediated glucose uptake by metabolically active tissues is disturbed (164). In addition, hepatic glucose production, which provides the body with glucose during fasting, is not efficiently repressed (126;164) by insulin in the postprandial period. As the pathology aggravates with time, more and more insulin is needed to overcome the insulin resistant state of the organs, leading to pancreatic hypertrophy, pancreatic cell death and ultimately Type 2 Diabetes.

As is the case for obesity, the incidence of insulin resistance/Type 2 Diabetes is rising (4;132). More disturbingly, and also in line with the obesity epidemic is the increased prevalence of early onset Type 2 Diabetes (224). In a comparative study, younger patients with T2DM were found to be more obese, more hypertriglyceridaemic, had lower HDL cholesterol and had a worse initial and ongoing glycaemic control than older patients (97).

Hyperinsulinemic-euglycemic clamp analysis

The gold standard to assess insulin sensitivity is the hyperinsulinemic-euglycemic clamp analysis. In the so-called clamp, the potency of insulin to repress hepatic glucose output and to simultaneously increase peripheral glucose uptake is measured. In brief, during a clamp, the mouse or human is made hyperinsulinemic by infusing insulin at a constant rate and the human/mouse is kept euglycemic by simultaneous infusion of glucose. The rate at which glucose is infused is one of the measures for insulin sensitivity. When combined with labeled tracers, tissue specific insulin sensitivity can be assessed, even at the organ level. Thus, the clamp is an artificial simulation of the postprandial response, when high levels of exogenous glucose enter the system after a period

of fasting, and insulin levels rise in order to maintain carbohydrate balance and to prevent damage to organs due to glucotoxicity.

A more concise introduction to measurements of insulin sensitivity by clamp analysis is included in the chapter "Hyperinsulinemic-euglycemic clamp analysis" in the second part of the introduction.

Obesity-associated insulin resistance

Various mechanisms have been recognized leading to insulin resistance, including age, gender, obesity and genetic make-up (79;189;204), as well as environmental factors such as dietary composition (78;211). The common denominator of insulin resistance and obesity may be the presence of plasma and tissue markers of inflammation. In both liver and adipose tissue, the presence of immune cells is associated with insulin resistance. Moreover, adipose tissue is now widely recognized as an endocrine organ in itself, and secretes signaling molecules which are collectively known as adipokines.

Adipokines are thought to be a key factor in the onset of obesity related insulin resistance (110;181;219). For example, adiponectin, the most abundant protein secreted by the adipose tissue, has been demonstrated to be reduced in animal models of obesity and insulin resistance (111;112) as well as in Type 2 Diabetes patients (93). Adiponectin-deficient mice are demonstrated to be insulin resistant (155). The effects of adiponectin may in part be explained by effects on the central nervous system, where adiponectin decreases food intake and increases energy expenditure (140).

A second adipokine known to be involved in energy metabolism is leptin. Central leptin receptor activation leads to changes in food intake via repression of orexigenic pathways while inducing anorexigenic pathways (195). With respect to glucose homeostasis, leptin is involved in insulin secretion by modulation of pancreatic β -cells function directly (170) and indirectly through central nervous system pathways (20).

In addition to adiponectin and leptin, the more recently discovered chemerin (273) and vaspin (99) are demonstrated to be directly involved in insulin mediated energy metabolism of adipose tissue. Whether dysregulation of adipokine expression is the cause or consequence of obesity, inflammation and/or insulin resistance remains hotly debated.

Dietary fatty acid composition and the development of obesity/insulin resistance

One of the causal links between obesity and inflammation/insulin resistance may be the obesity associated ectopic accumulation of lipid intermediates (39;40;254). These lipid intermediates include second messengers such as diacylglycerols and

ceramides (242), which have been demonstrated to affect insulin sensitivity (104;176). Of these second messengers, ceramides have been demonstrated to mediate the deleterious effects of saturated fat on insulin resistance (39). This is further illustrated by the observation that inhibition of ceramide synthesis or glucosylceramide synthesis ameliorates saturated fat induced insulin resistance (1;17;103)

Chemically, fatty acids can be divided into three major classes, based on the degree of saturation. Saturated fats have no double bonds within their chemical structure. Mono-unsaturated fats have one and poly-unsaturated fats may have up to 6 double bonds. In addition to the degree of saturation, fatty acids can be sub-divided based on carbon chain length. Short chain fatty acids have chain lengths up to 6 carbon units, medium chain fatty acids between 6 and 12, long chain fatty acids between 12 and 22 and very long chain fatty acids over 22 carbon units.

Dietary fat, and more specifically fatty acid composition, has been demonstrated to affect metabolism in both rodents (28;72) and humans (46;121;129;130;238). Since especially long chain saturated fatty acids have been demonstrated to have deleterious effects on postprandial insulin secretion, and to accumulate in ectopic tissues (27;226), this may thus link dietary intake of long chain saturated fatty acids with increased susceptibility to pathology as compared to short chain or unsaturated fatty acids (152).

The best-known fatty acids to positively affect health are the poly-unsaturated fatty acids, which are present in fish oil, bananas and sunflower seeds. Poly-unsaturated fatty acids have been demonstrated to have beneficial effects on blood lipid profiles and cardiovascular risk (36;50). In spite of this, poly-unsaturated fatty acids have also been demonstrated to have potential adverse effects on insulin sensitivity (2;70;172;206;222). The deleterious effects of long chain saturated fatty acids on insulin sensitivity have resulted in their designation "bad fat" (34;108;215;220). In part, these deleterious effects may be due to the differential metabolic fates of long chain fatty acids (56;144), in addition to their capacity to induce inflammation (41). Substitution of saturated fat with mono-unsaturated fat has been shown to reduce body weight (192), increase post-prandial fat oxidation rates (191) and to improve post-prandial glucose homeostasis in insulin resistant subjects (186). The Mediterranean diet, characterized by high amounts of monounsaturated fats has been demonstrated to be cardio protective (68;123;211).

Fatty acid metabolism

The oxidative fate of fatty acids is largely determined by chain length. For example, medium chain fatty acids, which can be found in coconut oil and dairy products, differ in several aspects from long chain fatty acids. Medium chain fatty

acids are water-soluble in the intestinal lumen and in the cytoplasm of target cells, and they are absorbed predominantly via the portal vein into the liver bypassing the lymphatic system. In addition, they are rapidly beta-oxidized due to their independence of mitochondrial carnitine palmitoyl transferase 1 (CPT1), which is a fatty acid transporter and one of the rate limiting steps for the beta oxidation of long chain fatty acids. Several studies showed an increased fat oxidation and whole body energy expenditure in rodents (246;263) as well as humans after 7-28 days diet intervention with medium chain fatty acids. In addition, medium chain fatty acids ameliorate insulin resistance in rodents, without affecting body weight (263).

For individual long chain fatty acids, the efficiency of oxidation of fatty acids decreases with increasing chain length and saturation level. In rats, after oral administration of labeled fatty acids, the efficiency of the oxidation of saturated FA has been demonstrated to be lauric acid (C12:0) > myristic acid (C14:0) > palmitic acid (C16:0) > stearic acid (C18:0)(144). Similar results have been found in a human study where the oxidation rate of stearic acid after a bolus administration was found to be poor in comparison to lauric acid (13% versus 41% oxidized within 9 hours after administration) (56).

These data demonstrate that long chain saturated fatty acids are not efficiently metabolized, and therefore may lead to adverse metabolic effects such as ectopic and intracellular fat accumulation.

Chapter 1, Part B: Practice and principles of indirect calorimetry and hyperinsulinemic-euglycemic clamp analysis.

Indirect calorimetry

Total energy expenditure as well as relative and absolute substrate specific absolute energy expenditure rates can be measured in great detail by indirect calorimetry. The substrates that are oxidized include protein, carbohydrates and fat. Since variation in protein oxidation contributes only to a small extent to the variation in total daily energy expenditure (268), protein oxidation is in general not included in the substrate specific calculations. Since fat and carbohydrate oxidation rates are most studied, they are discussed in more detail below.

To calculate relative and absolute substrate specific energy expenditure rates, one must start out by calculating the amount of oxygen needed to oxidize representative units of substrate. The chemical formula and structure of glucose and other monosaccharides is relatively simple, and is represented by: $(\text{CH}_2\text{O})_n$. In addition, all disaccharides and polysaccharides are nothing more than coupled monosaccharides. And thus, have the same basic formula.

The structure of fatty acids however is more complex. The average values for fatty acids have been calculated from the structure of the 13 most predominant fatty acids present in the human adipose tissue, accounting for 99% of the total fatty acid pool (15). The average fatty acid was determined to have the chemical formula $C_{17.2702}H_{32.7142}O_2$ and a molecular mass of 272.4051 grams. These data were used to generate the formulas needed to calculate fat specific energy expenditure.

From empirical data it has been determined that the energy potential of glucose at 37°C is 3.8683 kcal/g or 5.189 kcal/ lO_2 , whereas that of fat has been determined to be 9.746 kcal/g or 4.581 kcal/ lO_2 . Therefore, when a mixture of carbohydrates and fat is oxidized, the energetic output is between 4.581 and 5.189 kcal/ lO_2 , and thus, the measured oxygen consumption needs to be corrected for the substrate utilized in order to calculate energy expenditure.

Absolute substrate oxidation

By combining the stoichiometric equations for glucose and fat oxidation (equation 2 and 3) and the calculated volumes of oxygen and carbon dioxide at standard temperature and pressure, we can calculate the oxidation of volume of oxygen consumed and carbon dioxide produced per gram of substrate (equation 5, 6).

$$(4) \quad \text{Oxygen required (liters)} = \frac{1}{180.1577} * 6 * 22.3858$$

$$(5) \quad \text{Carbon dioxide produced (liters)} = \frac{1}{180.1577} * 6 * 22.2966$$

resulting in a volume of 0.7455 liters of oxygen and 0.7426 liters of carbon dioxide

For fat, the equations are

$$(6) \quad \text{Oxygen required (liters)} = \frac{1}{272.4051} * 24.4488 * 22.3858$$

$$(7) \quad \text{Carbon dioxide produced (liters)} = \frac{1}{272.4051} * 17.2702 * 22.2966$$

resulting in a volume of 2.0092 liters of oxygen and 1.4136 liters of carbon dioxide

Using these data we can calculate absolute substrate specific oxidation rates.

For example if a mixture of glucose (y gram) and fat (x grams) is oxidized. The calculated RER is

$$(8) \text{ RER} = \frac{VCO_2}{VO_2} = \frac{0.7426 * (y) + 1.4136 * (x)}{0.7455 * (y) + 2.0092 * (x)}$$

From this, oxygen consumption and carbon dioxide production can be isolated

$$(9) VO_2 = 0.7455 * (y) + 2.0092 * (x)$$

$$(10) VCO_2 = 0.7426 * (y) + 1.4136 * (x)$$

As both VO_2 and VCO_2 can be measured, this results in two equations and two unknowns. When expressing (x) and (y) in terms of VO_2 and VCO_2 we get

$$(11) (x) = 1.695 * (VO_2) - 1.701 * (VCO_2)$$

$$(12) (y) = 4.585 * (VCO_2) - 3.226 * (VO_2)$$

Since 1 gram of carbohydrates is oxidized generating 3.8683 kcal, and the amount of energy generated by 1 gram of fat oxidation equals 9.4760 kcal, the total amount of energy produced can be calculated by multiplication of these constants by the output of equations (11) and (12).

Hyperinsulinemic-euglycemic clamp

As was mentioned in the first part of the introduction, insulin sensitivity can be assessed by hyperinsulinemic-euglycemic clamp analysis. During the hyperinsulinemic-euglycemic clamp, plasma insulin levels are artificially elevated while keeping plasma glucose at euglycemic levels by a co-infusion of glucose. In addition to the "cold glucose" that is infused, "hot" (radioactively labeled) glucose is infused. The amount of radioactive glucose is low compared to the cold glucose, so that it can be regarded as a so called "tracer". After a certain period of time, depending on the kinetics of the system, the concentrations of tracee and tracer in the plasma will reflect their respective rates of appearance. At that time, the relative concentrations of tracee and tracer will not alter provided that the infusion of both is not altered.

During the clamp, the tracer is diluted in the plasma by the glucose that is produced by the liver and the glucose that is infused exogenously. To correct for the differences in dilution of the tracer that is infused during the clamp, the specific activity of glucose is calculated

$$1. SA = \frac{(Counts)_{plasma}}{[gluc]_{plasma}}$$

Where $(Counts)_{plasma}$ represents the radioactive counts measured per volume of plasma and $[gluc]_{plasma}$ is the concentration of glucose in the plasma. Under steady state tracer condition, the rate of disappearance of glucose can then be calculated by dividing the infused counts $(Counts)_{in}$ by the specific activity.

$$2. R_d = \frac{(Counts)_{in}}{SA}$$

Note that although there is a correction for the total plasma glucose pool in the equation used to calculate the specific activity, tracer steady state has to be assured to accurately and reliably calculate R_d . In case of fast fluctuations of the plasma glucose pool, tracer steady state can not be assured, and thus R_d can not be measured accurately.

In the hyperinsulinemic state, under clamped glucose conditions, the rate of disappearance of glucose is equal to the sum of the amounts of glucose produced by the liver and the glucose that is infused exogenously.

$$3. R_{d \text{ hyperinsulinemic}} = GIR + EGP_{\text{hyperinsulinemic}}$$

Since under basal conditions, both EGP and R_d have been determined (equation 1), the fractional repression of EGP (equation 3) and stimulation of R_d (equation 4) can be calculated.

$$4. \%repression = -\left(\frac{EGP_{\text{basal}} - EGP_{\text{hyperinsulinemic}}}{EGP_{\text{basal}}}\right) * 100$$

$$5. \%stimulation = \left(\frac{R_{d\text{basal}} - R_{d \text{ hyperinsulinemic}}}{R_{d\text{basal}}}\right) * 100$$

When using two tracers, insulin sensitivity can be determined at the organ level, although that is beyond the scope of this introduction. In this thesis, an unlabeled tracer clamp was performed in **chapter 2**, and a single label tracer is used in **chapters 3 and 4**.

Outline of thesis

During the development of diet induced obesity, an imbalance between energy intake and energy expenditure is by definition present. This imbalance may be due to impaired nutrient partitioning to and inside metabolically active tissues, as well as due to impaired satiety signaling.

The focus of this thesis is on the role of dietary fat acids on energy metabolism and insulin sensitivity. To gain more insight in the time course of the development of hepatic lipid accumulation and increase in plasma markers of inflammation, we studied ApoE*3-Leiden transgenic mice during a 16 week high fat diet intervention at 9 different intervals (**Chapter 2**). In **Chapter 3** we assessed the role of dietary fatty acid chain length on whole body metabolism and tissue specific insulin sensitivity, by enriching high fat diets in medium chain triglycerides or long chain triglycerides. In **Chapter 4** we studied the detrimental effects of stearic acid, one of the most abundant long chain saturated dietary fatty acids in everyday food, on metabolism and insulin sensitivity by comparing diets low and high in stearic acid content. In **Chapter 5** we studied the effect of pharmacological modulation of ceramide synthesis on energy metabolism, satiety signaling and obesity by administering leptin deficient *ObOb* mice the iminosugar derivative N-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojiricin (AMP-DNM). In **Chapter 6** we studied the role of mitochondria in fatty acid metabolism. In response to a high fat diet, we determined the association of mitochondrial uncoupling of fatty acid metabolism with food intake and total energy expenditure levels.

Chapter 7 starts with the discussion of the results found in **Chapter 2, 3, 4, 5, and 6**, in the context of the knowledge already available in literature. In **Chapter 7** all chapters are reviewed, individually and in relation to each other. In **Chapter 8**, suggestions and future perspectives are included which may aid in future studies of energy balance, and how the techniques and concepts of the current experiments may be improved.