

Energy flow and metabolic efficiency attributed to brown adipose tissue

Gabriella von Essen

Academic dissertation for the Degree of Doctor of Philosophy in Physiology at Stockholm University to be publicly defended on Friday 7 April 2017 at 10.00 in Vivi Täckholmsalen (Q-salen), NPQ-huset, Svante Arrhenius väg 20.

Abstract

The large capacity of brown adipose tissue (BAT) to expend energy as heat makes it an interesting potential player in weight regulation and other metabolic conditions. This is of particular interest as it has been recognized that adult humans possess BAT. The protein responsible for the heat production is uncoupling protein 1 (UCP1), which, as the name implies, uncouples the respiratory chain from ATP production; instead heat is produced. Cold is the strongest recruiter and activator of BAT. However, also obesogenic food has a low but nonetheless significant effect on the recruitment and activation of UCP1, although the significance of this has been discussed.

In the present thesis, I have studied the effect of diet on BAT and the possibilities for it to be obesity-protective. This can be done by comparing responses in wild-type mice and in UCP1-ablated mice. Since the effect of diet on BAT is low, it is of importance to control the temperature and maintain thermoneutrality. Other confounding factors to keep in mind are differences in actual energy and composition of food and also cohort differences. When controlling all the parameters mentioned and giving the mice the same obesogenic diet, the mice possessing UCP1 compared to UCP1-ablated mice had higher energy expenditure, and lower weight gain, despite eating more. This confirms the presence of a UCP1-dependent diet-induced thermogenesis. Thus, the conclusion must be that possessing UCP1 does result in obesity protection at thermoneutrality. However, the relevance for human energy balance is still not established.

Keywords: *Brown adipose tissue, BAT, UCP1, diet-induced thermogenesis, DIT, obesity, high-fat diet, energy expenditure.*

Stockholm 2017

<http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-140190>

ISBN 978-91-7649-734-0
ISBN 978-91-7649-735-7

Department of Molecular Biosciences,
The Wenner-Gren Institute



Stockholm University, 106 91 Stockholm

DOCTORAL THESIS

Energy flow and metabolic efficiency
attributed to brown adipose tissue

Gabriella von Essen

Energy flow and metabolic efficiency attributed to brown adipose tissue

Gabriella von Essen

©Gabriella von Essen, Stockholm University 2017
Cover image by Thea von Essen

978-91-7649-734-0 (Print)

978-91-7649-735-7 (PDF)

Print: Universitetservice US-AB, Stockholm, Sweden, 2017

Distributor: Department of Molecular Biosciences, The Wenner-Gren Institute

“True knowledge exists
in knowing that you know
nothing.”

Sokrates (470 f.Kr.-399 f.Kr.)

The present thesis is based on the following enclosed papers:

Paper I

Adaptive Facultative Diet-induced Thermogenesis in Wild-type but not in UCP1-ablated Mice

Gabriella von Essen, Erik Lindsund, Barbara Cannon and Jan Nedergaard

Submitted for publication

Paper II

Highly recruited brown adipose tissue does not in itself protect against obesity

Gabriella von Essen, Elaina Maldonado, Erik Lindsund, Barbara Cannon and Jan Nedergaard

Under revision for Cell Metabolism

Paper III

At thermoneutrality, medium-chain fatty acids totally protect against diet-induced obesity in a UCP1-independent manner

Gabriella von Essen, Petter Englund, Barbara Cannon and Jan Nedergaard

Submitted for publication

Paper IV

No insulating effect of obesity

Alexander W Fischer, Robert Csikasz, Gabriella von Essen, Barbara Cannon and Jan Nedergaard

Am J Physiol Endocrinol Metab. 2016 Jul 1;311(1):E202-13.

Table of Contents

Abbreviations	x
Preface	11
1. Why Brown Adipose Tissue	13
2. Brown adipose tissue	15
2.1 The BAT, the WAT and the Brite/Beige	15
2.2 Sympathetic β -adrenergic stimulation	16
2.3 UCP1, thermogenesis and lipolysis	17
2.4 Diet-induced thermogenesis	18
3. Temperature	19
3.1 Housing temperature and metabolic rate	19
3.2 Parameters affecting perceived temperature	20
3.3 Confounding factors in determining housing temperature	22
4. Energy Metabolism	23
4.1 Weight balance – Gaining or losing weight	23
4.2 Energy flow from ingested food	25
4.3 Storing – Metabolic efficiency	26
4.4 Energy expenditure	27
4.4.1 <i>Compartments of energy expenditure</i>	27
4.4.2 <i>Measuring energy expenditure</i>	29
5. Food	30
5.1 Energy in food	30
5.2 Consequences of different energy factors	33
5.3 Fatty acids affecting BAT	35
5.3.1 <i>Dietary fatty acids</i>	35
5.3.2 <i>Short-chain fatty Acids (SCFA)</i>	36
5.3.3 <i>Medium-chain triglycerides (MCT)</i>	38
5.3.4 <i>Long-chain fatty acids (LCFA)</i>	41
5.3.5 <i>Polyunsaturated fatty acids (PUFA)</i>	44
5.3.6 <i>Conjugated linoleic acid (CLA), C18:2</i>	47
5.4 Some other diet points	48

6. Is UCP1 obesity-protective?	52
6.1 Fluctuations in Ucp1 expression	52
6.2 Does UCP1 matter for obesity?	53
6.2.1 <i>Body weight</i>	54
6.2.2 <i>Metabolic efficiency</i>	56
6.2.3 <i>Obesity Index (OBI) as a mouse BMI</i>	58
6.2.4 <i>Body fat, oxygen consumption and UCP1 activity</i>	59
6.2.5 <i>UCP1 in outbred NMRI mice</i>	62
6.2.6 <i>UCP1-dependent metabolic efficiency</i>	64
7. Concluding remarks	66
Svensk sammanfattning	70
Acknowledgements	71
References	73

Abbreviations

ALA = α -linolenic acid
BAT = Brown adipose tissue
CLA = Conjugated linoleic acid
DE = Digestible energy
DHA = Docosahexaenoic acid (22:6n-3)
DIT = Diet-induced thermogenesis
e% = energy percent
EE = Energy expenditure
EPA = Eicosapentaenoic acid (20:5n-3)
EFA = Essential fatty acid
eWAT = Epididymal white adipose tissue
FA = Fatty acid
GE = Gross energy (= ingested food)
HFD = High-fat diet (i. e. high-fattening diet)
ingWAT = Inguinal white adipose tissue
IBAT = Interscapular brown adipose tissue
LA = Linoleic acid
LBM = lean body mass
LCFA = Long-chain fatty acid
LCT = Long-chain triglyceride
LC-PUFA = Long-chain polyunsaturated fatty acid
MCFA = Medium-chain fatty acid
MCT = Medium-chain triglyceride
ME = Metabolizable energy
MEff = Metabolic efficiency
MUFA = Monounsaturated fatty acid
NE = Norepinephrine
NE = Net energy
NME = Net metabolizable energy
PUFA = Polyunsaturated fatty acid
SCFA = Short-chain fatty acid
SFA = Saturated fatty acid
TG = Triglyceride
TNZ = Thermoneutral zone
UCP1 = Uncoupling protein 1
WAT = White adipose tissue

Preface

On my way to Stockholm University I pass 3 kiosks, 3 grocery stores (open until 10 pm) and 1 hot-dog man. On the subway platform there are 2 vending machines for snacks. In addition, aggressive and sophisticated advertising encourages me that I need or deserve certain (unhealthy) food items (Figure 1). Thus, within 10 minutes I have access to and possibility to – at low cost and no hard work at all – ingest my whole daily energy demand. This reality really puts a pressure on us to cope with both resisting the temptations and, if not doing that, handling all the ingested food items in our bodies.

Obviously, it is not strange, being constantly exposed to extremely palatable, energy-dense, highly processed food in combination with a low amount of movement and furthermore a warm environment, that metabolic problems increases. Rather, it is strange that we do not have more problems. As I see it, we have three directions to go, but of course all three can be considered in parallel, and the question is where to put the focus.

One is to go back at least to as we lived 100 years ago. This will not happen and is not even possible. Another option is to find new treatments. Absolutely. However, we have normalized that more and more people have metabolic problems and that we then need more and more treatments. I must say I was a little chocked when I read that new clinical guidelines published this year by leading international diabetes organizations (including the International Diabetes Federation), suggested that bariatric surgery, involving surgery of (not sick) stomach or intestine, should be considered a standard treatment option for type 2 diabetes. This is a very drastic action, and can maybe be a complement or an option in rare cases.

A third way to go is to learn more about metabolism to at least have a possibility to change attitudes or approaches to make other choices for both society, as well as for individuals, of rather preventing metabolic problems than treating them once they are a fact. To be able to resist aggressive advertisements and commercial interests.

This is what I think is one of the most important ways to go. The years spent working with physiology has increased my understanding of the extreme complexity of metabolism and of brown adipose tissue, but also has generated more questions and the realization of how much more is needed to be known. Apart from learning more about energy expenditure in general and diet-induced thermogenesis in particular, I have gained insight into the importance of spending years and years of maybe not so glamorous, not so ex-

travagant and not so spectacular and often tedious work to insights. However, there is no short-cut to understanding. I really want to stand up for basic science. I prefer to work on understanding to at least have the possibility to prevent rather than treat diseases. I am thankful and proud if I just can contribute a tiny piece of understanding to that complicated puzzle of knowledge in metabolism. Although, and with all respect: “The truth is rarely pure and never simple” (Oscar Wilde, 1895).

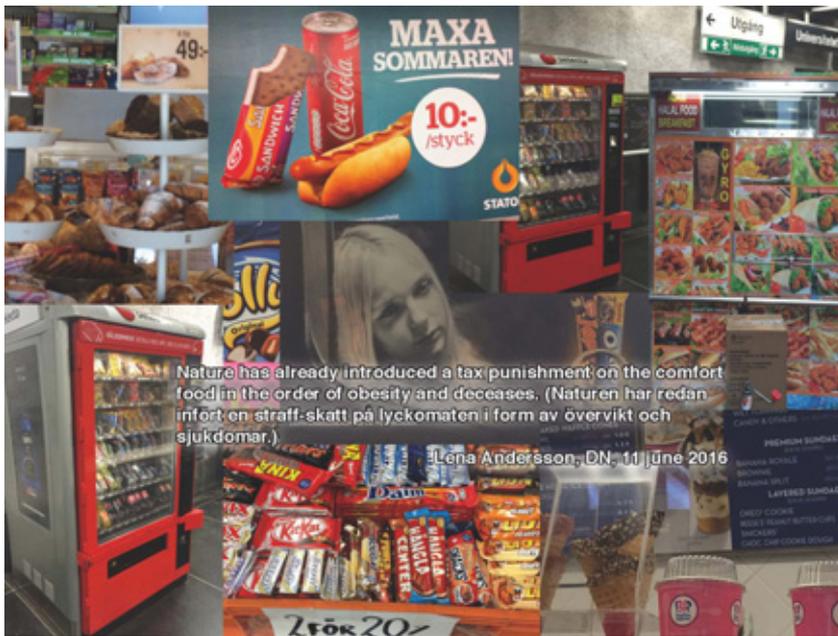


Figure 1. It is flavoursome, it is accessible, it is energy-dense, it is cheap. Being surrounded by an obesogenic world 24/7 is a big challenge for both our bodies, minds and society.

1. Why Brown Adipose Tissue

Metabolic problems are rising in the world, with increasing prevalence of diabetes in almost all countries, with a predictive increase of 55 % worldwide in 20 years (IDF diabetes, 2016). The incidence of obesity (BMI \geq 30) has doubled in Sweden in the last 25 years, from 5% to 10% (The National Board of Health and Welfare, Sweden). Even a worldwide rise in blood glucose has been seen during the last 30 years, with an increase in serum glucose by 0.07 mM (Danaei et al., 2013). All this implies a shift towards a more unhealthy metabolism.

Despite work to counteract obesity and diabetes by many researchers, and by guidelines (often confusing though) from health authorities (National Food Agencies, WHO), as well as treatment by physicians, metabolic diseases have not decreased; on the contrary metabolic disorders are increasing. Treatment and prevention of the development of obesity and other metabolic conditions have obviously not been successful, which focuses the question on understanding and investigating the nature of metabolism further.

The metabolic condition associated with and preceding type II diabetes and obesity is the metabolic syndrome. It consists of at least three of the five following symptoms: high blood glucose or insulin resistance, central obesity, dyslipidemia in serum (high triglycerides and low HDL), high blood pressure, and microalbuminuria (IDF, WHO).

Brown adipose tissue (BAT), with its main function to generate heat to defend body temperature during cold, is – when active – a highly energy-expending tissue. The Uncoupling protein 1 (UCP1) is responsible for this and, when active, BAT/UCP1 has the capacity to modify at least the first three parameters in the metabolic syndrome. Since the re-emerged interest in brown fat due to the discovery of its presence in adult humans (Nedergaard et al., 2007), it has been shown that humans with lower BMI do have more brown fat than obese people (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2014, 2009; Zingaretti et al., 2009). Furthermore, BAT has a high capacity of glucose uptake (Matsushita et al., 2014), and BAT-positive compared to BAT-negative humans have lower fasting blood glucose and triglyceride levels in the blood (Wang et al., 2015). In mice, BAT has a capacity to clear triglycerides from the blood stream (Bartelt et al., 2011). In addition, mice lacking UCP1 are more prone to obesity compared to wild-type (Feldmann et al., 2009; Rowland et al., 2016; Paper I). Altogether, this makes BAT an interesting tissue to study for its potential

involvement in metabolism and energy expenditure, as well as a promising target for counteracting metabolic imbalances.

The first question addressed in the present thesis is: what is the BAT-dependent contribution to energy expenditure depending on diet, so-called diet-induced thermogenesis. And secondly: can this BAT-dependent diet-induced thermogenesis protect against obesity. The approach has been to analyse patterns and consequences of active or not active BAT on obesity and energy expenditure, rather than to investigate molecular mechanisms.

2. Brown adipose tissue

For endothermic mammals, brown adipose tissue (BAT) has developed during evolution as a thermogenic organ specialized in adaptive non-shivering thermogenesis (NST) to maintain and defend body temperature at temperatures below thermoneutrality. Thermoregulation can also be achieved by shivering, but for obvious reasons this is associated with less comfort. To cope with challenges such as seasonal environmental temperature fluctuations, complex mechanisms and strategies have evolved such as daily torpor and hibernation in some mammals (Cannon and Nedergaard, 2004; Jastroch et al., 2016). These energy saving strategies and NST to lower, increase and maintain body temperature to cope with environmental stress, involve BAT and require a dynamic regulation controlled by the sympathetic nervous system and mediated by norepinephrine. Since the 1960s, 88 eutherian mammal species have been tested for their NST-capacity by injecting norepinephrine (summarized in a review of Oelkrug et al. (2015)), revealing that NST capacity is inversely correlated to increases in body mass (Oelkrug et al., 2015). That is why it is not surprising that BAT is particularly important for smaller mammals, their newborns and also human babies (Cannon and Nedergaard, 2004; Oelkrug et al., 2015). Human BAT was mentioned for the first time over 100 years ago (Hatai, 1902), but was thought to disappear with age, since it visibly disappeared and adults were later shown to have much lower concentrations of uncoupling protein compared to young children (Lean, 1989). However, since ten years ago, it has become clear by positron emission tomography (PET), that BAT is even present in adult humans (Cypess et al., 2009; Nedergaard et al., 2007; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009), which has increased interest in whether BAT can contribute to whole body energy expenditure and therefore have an impact on weight regulation in humans, as it does in mice (Paper I).

2.1 The BAT, the WAT and the Brite/Beige

Traditionally, the view was that there are in principle two types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). Although brown adipose tissue implies adiposity through its name, it can be distinguished from white adipose tissue (WAT), which *stores* energy as tri-

glycerides, whereas BAT *expends* energy as heat. Cells in WAT contain only one large lipid droplet, which is an appropriate way to store triglycerides, whereas the lipids in BAT are multilocularly stored to facilitate rapid mobilization of the triglycerides. Furthermore, BAT is, in contrast to WAT, highly innervated and vascularized, has high lipolytic capacity and high mitochondrial density and a large amount of uncoupling protein 1 (UCP1).

Recently, another type of adipose tissue has been recognized, “brite” (brown-like in white) or “beige” cells. Certain cells within some white adipose depots have a capacity to become more thermogenic, a phenomenon referred to as browning, first described already 30 years ago (Young et al., 1984). This inducible ability to become thermogenic (i.e. to express UCP1 and incorporate it into mitochondria) is now well accepted (Carey and Kingwell, 2013; Dempersmier and Sul, 2015; Kajimura and Saito, 2014; Nedergaard and Cannon, 2014; Peirce et al., 2014; Petrovic et al., 2010; Seale et al., 2008; Wu et al., 2012). Thus, adipose tissue has high plasticity and consists of at least three types of cells, the white, the beige and the brown, where some of them can (under certain circumstances) visually transform into each other, referred to “browning” (i.e. increased thermogenic capacity) and “whitening” (i.e. decreased thermogenic capacity). In attempts to distinguish and identify the different types of fat tissues, especially the “new” brite/beige tissues, a number of marker genes have been proposed (de Jong et al., 2015; Wu et al., 2012). However, the magnitude and the significance of such inducibility or browning capacity in WAT are still under debate. Nonetheless, UCP1 is the strongest marker of high thermogenic capacity in all fat depots. For simplicity, I will refer to BAT when discussing thermogenic capacity. Furthermore, in terms of total UCP1 content, BAT has far higher amounts compared to Brite/Beige (i.e. inguinal WAT), implying its superior thermogenic capacity (Paper I, II, III).

2.2 Sympathetic β -adrenergic stimulation

BAT is under adrenergic control via norepinephrine, which, similarly to epinephrine, also underlies the fight-or-flight response during stress, affecting smooth muscle, cardiac muscle, glands and other non-motor organs such as white and brown adipose tissue. The sympathetic nervous system, being part of the autonomic nervous system, is always active at a basal level, a sympathetic tone exists, to maintain all aspects of homeostasis, such as body temperature and blood pressure.

When released from nerves, norepinephrine binds to α_1 -, α_2 - and β -adrenergic receptors (β ARs), where the β_3 ARs are the most significant in rodents in mature brown adipocytes (Cannon and Nedergaard, 2004). β ARs are G-protein coupled receptors, that, when stimulated by norepinephrine, activate a downstream pathway via adenylyl cyclase, cyclic AMP and protein kinase A, which stimulates lipolysis by activating perilipin and adipose tri-

glyceride lipase (ATGL) to release free fatty acids (FFAs) from the triglyceride droplets. FFAs are activated to acyl-CoA and enter the mitochondria via a carnitine shuttle system, supplying the electron transport chain with substrate for thermogenesis. Furthermore, norepinephrine stimulates expression of Ucp1 mRNA, also via PKA.

Stimulation by norepinephrine thus stimulates both gene transcription and cell differentiation, as well as thermogenesis in BAT.

2.3 UCP1, thermogenesis and lipolysis

The protein responsible for the heat production in BAT (and Brite) is the uncoupling protein 1 (UCP1), which is part of the mitochondrial carrier protein family. UCP1 catalyses a proton leak in the inner mitochondrial membrane whereby ATP production is bypassed and heat is released instead.

Fatty acids are presumably involved in the direct regulation and/or function of UCP1 (Azzu et al., 2010; Cannon and Nedergaard, 2004; Shabalina et al., 2004). The generally accepted model is that purine nucleotides (experimentally usually GDP, but probably ATP *in vivo*) constantly inhibit UCP1 activity, and when stimulated through norepinephrine and possibly with a direct interaction of fatty acids, UCP1 is activated (Shabalina et al., 2010). However, the exact mechanism of how fatty acids, in addition of being a substrate, are involved in regulation of UCP1 is not yet clarified. It is not clear whether they are just overcoming the inhibition (by nucleotides) or whether they are involved in the functional mechanism of UCP1. Although the 32 kDa UCP1 protein responsible for heat production was identified already in 1970s (Nicholls and Rial, 1999), it has not been fully understood in terms of the exact regulation and function. Nonetheless, the β -adrenergic receptor system, together with fatty acid stimulation and nucleotide inhibition, plays a critical role in regulating lipolysis and thermogenesis. In BAT, lipolysis and thermogenesis are dependent on each other; if lipolysis is occurring, heat is always produced and vice versa (Cannon and Nedergaard, 2004; Collins and Surwit, 2001). Stimulation of the β_3 ARs thus increases lipolysis and thermogenesis, thereby increasing energy expenditure, implying a possible participation of β -adrenergic stimulation in regulating body weight. In fact, impaired adrenergic signaling in adipose tissue is linked to obesity (Collins and Surwit, 2001).

BAT activity thus needs to be controlled to regulate the amount of heat that needs to be produced in order to defend normothermic body temperature when exposed to the cold. Both the recruitment of the tissue and the activation of UCP1 are mainly under adrenergic control. Inactive brown adipocytes have a low amount of free fatty acids and high purine nucleotide concentrations in the cytosol, resulting in low UCP1 activity. Sympathetic stimulation of lipolysis leads to increased free fatty acid levels and activated UCP1. The

degree of heat dissipated at a certain time-point and condition depends on the state of recruitment and the level of activity (Cannon and Nedergaard, 2004).

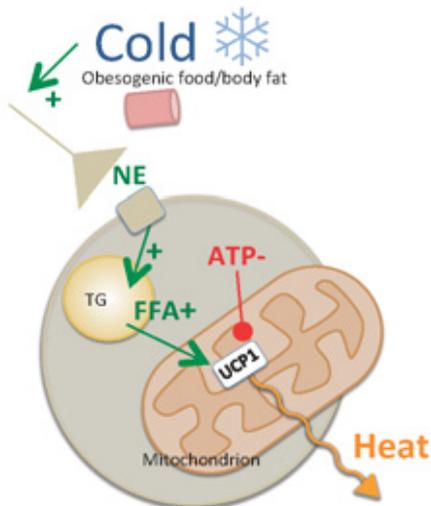


Figure 2. Cold, and to a lesser extent obesogenic food, perceived by the brain, stimulates norepinephrine (NE) release from nerves going to the brown adipose cells. NE binds to receptors on the cell membrane, which in a downstream signaling pathway, stimulates degradation of the triglycerides (TG) in the lipid droplets. The released free fatty acids (FFA) interact with UCP1 and the inhibition of UCP1 from cytosolic ATP is overcome. This event leads to that ATP synthesis is bypassed and instead heat is dissipated. Stimulation of the receptors on the cell membrane, can also be achieved by injecting NE or an agonist into the body. This is often done in experiments to mimic sympathetic action. (Adapted from Cannon and Nedergaard 2010).

2.4 Diet-induced thermogenesis

Apart from the well established non-shivering thermogenesis, brown fat is believed to have one more physiological function. The second task is more controversial, and implicates an increase in energy expenditure to ensure that sufficient nutrient intake is achieved (Cannon and Nedergaard, 2004). That is, by decreasing metabolic efficiency (i.e. dissipating extra energy intake as heat instead of storing it), adequate intake of essential nutrients can be supplied when overeating a nutrient-poor diet without excessive weight gain. This part of metabolism is referred to as adaptive thermogenesis or facultative diet-induced thermogenesis (DIT). In fact, already 1979, overfeeding a so-called cafeteria diet was shown to activate BAT (Rothwell and Stock, 1979), implying the activity of UCP1 to be affected by food, in addition to cold. Since then, a numerous compounds have been shown to induce adaptive thermogenesis: caffeine, certain fatty acids, chili, olive oil, fish oil to mention a few (Bonet et al., 2013; Dulloo et al., 2012).

3. Temperature

Thermoneutrality refers to an environmental temperature zone of a few degrees when no extra heat production is required for homeothermic organisms to maintain core temperature. The lower critical temperature in this range represents a threshold when the thermoregulatory system has to produce additional heat, through either shivering or non-shivering thermogenesis. The upper critical temperature is when metabolic rate increases to cool down the body by evaporative water loss. For humans, the thermoneutral zone is 28 – 30 °C when naked and 22 – 25 °C when wearing clothes (Lodhi and Semenkovich, 2009). For mice, thermoneutrality is around 30°C or more under most conditions, that is when the mice are not gestational or lactating (Gordon, 1985).

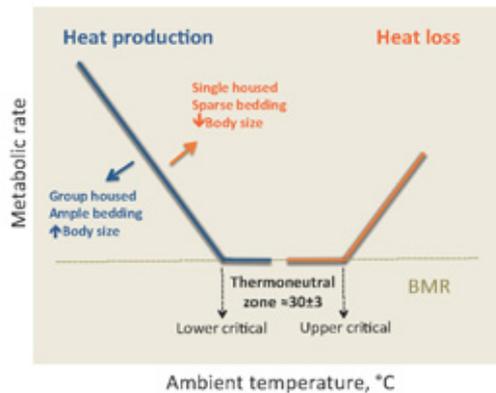


Figure 3: Ambient temperature and metabolic rate. Metabolic rate is dependent on ambient temperature. However, there is a zone where metabolic rate is as low as it can get and no extra heat is needed to keep eutherms – the thermoneutral zone. Below this zone, the body has to increase metabolism to heat up. Above this zone, the body has to increase metabolism to cool down so as to avoid hyperthermia. (Adapted from Cannon and Nedergaard, 2004; Gordon 2012)

3.1 Housing temperature and metabolic rate

Guidelines for animal vivariums are usually recommended to have an ambient temperature of 20 – 24 °C (room temperature), but this temperature places rodents into a chronic mild cold stress to uphold core temperature. Rats and mice have approximately the same lowest critical temperature to avoid

elevated metabolic rate, about 30 °C. However, when having a choice they spend time in a large range of ambient temperatures but rats prefer a lower temperature, an average of 24.9 °C for Sprague-Dawley rats (Gordon, 1987), whereas mice select an average of 30.9 °C (Gordon, 1985). When rats are placed in environmental temperatures where metabolic rate is the lowest, they reduce physical movement, probably to reduce their own heat production to avoid heat stress (Gordon, 1987). Mice are ten times smaller than rats, consequently their own heat production by, for instance, movement, does not affect ambient temperature to the same extent thus higher environmental temperature is both preferred and sustained. Rats have to increase metabolic rate 20 % when lowering the housing temperature from 30 °C to 22 °C (Gordon, 1987). Mice, on the other hand, have to increase their metabolic rate by 50 percent when lowering the temperature from 30 °C to 20 °C (Golozoubova et al., 2004; Paper IV). Mice are, therefore more sensitive to housing temperature than rats.

The Scholander plot¹ in Paper IV illustrates the importance of every degree for energy expenditure. Even a housing temperature of 27 °C versus 30 °C results in an increase in metabolic rate by as much as 10 to 20 % (Paper IV). This clearly illustrates the importance of being aware of how sensitive mice are to ambient temperature. This is also what Gordon et al. (2012) conclude that mice have a more narrow zone of thermoneutrality than do rats, about 30–32 °C. When the use of laboratory animals was changed from rats (most used rodents up to 1997) to mice, the housing temperature is of major importance compared to when rats were used. Furthermore, mice are especially used in the fields of obesity and diabetes, where measurement of metabolism is central and crucial. Just a few degrees can alter the outcome and therefore the interpretation.

Furthermore, by measuring BAT temperature, a 3-fold greater increase was shown when lowering housing temperature from 31 °C to 26 °C, and a 5-fold greater increase between 31 °C and 21 °C in BAT in C57Bl/6 mice (David et al., 2013), also clearly illustrating the need for increased thermogenesis when using lower housing temperatures.

3.2 Parameters affecting perceived temperature

Many parameters affect the thermoneutral zone, for instance size, strain, bedding, nesting material, number of animals in the cage. Interestingly, hair coat and insulation also affect the metabolic rate, but not when mice are housed in the thermoneutral zone (Paper IV).

¹ A study design performed by Scholander et al. in the 1940s, where metabolic rate is plotted as a function of ambient temperature for a large number of species (Scholander et al., 1950). In our study, we measured mice in metabolic chambers by lowering the temperature in steps for two hours each in a temperature range from 33 °C to 7 °C (Paper IV).

The lower critical temperature is well studied, and shown to be highly variable but clearly body-mass-dependent (Riek and Geiser, 2013), whereas the upper critical temperature is more difficult to establish and define. Despite difficulties in measurements, it is reasonable to assume that the upper limit for a mouse to keep normothermy is above 32 but below 34 °C (Gordon, 2012). The lower critical temperature is maybe of more importance in mice, due to the sensitivity of mice to cold stress, which, as mentioned, has a great impact on metabolic rate. Furthermore, the breadth of the thermoneutral zone is somewhat correlated to body size (broader in larger animals), but is also influenced to a large degree to insulation (Riek and Geiser, 2013). Summarizing a large number of studies performed between 1940 and 1987 Gordon concluded a lower critical temperature between 24.6 (42.5 g mice) and 32 °C (22.8–32 g) for a number of strains with different weights and sex (Gordon, 2012).

C57Bl/6 (Black 6) mice, which are one of the most common used strains, have been shown to when given a choice, to prefer 28 °C at night and 33 °C at day (Leon et al., 2010). In an old study from the 40s, C57 mice had their lowest metabolic rate at around 32 °C in the daytime (Herrington, 1940).

Importantly, cage conditions have a high impact for the temperature actually perceived by the mouse. At an air temperature of 22 °C and giving mice different bedding material and conditions (as a possibility to be buried, pine shavings, filter etc.) and measuring the temperature near the mouse, the operative temperature differed from 20.5 °C when mice had no bedding and a wire bottomed cage to 30.0 °C for mice buried in wood shavings (Gordon et al., 1998). Thus, it is extremely important to be aware of the enrichments and conditions inside the animal cages.

Another parameter that affects the operating housing temperature is the number of mice in the cage. Mice housed at 28 °C with a density of 1, 2 or 6 mice per cage showed a significant depression (in the range of 1/3 and 5/6 less compared to single caged, respectively) of BAT, mitochondrial GDP binding and UCP concentrations with increased mice density (Himms-Hagen and Villemure, 1992), clarifying that social thermoregulation (huddling) clearly reduces the need for BAT thermogenesis.

Housing temperature: the set ambient temperature in the vivariums

Operating temperature: the actual ambient temperature perceived by the mouse. Dependent on housing conditions, cage density, size, insulation

Thus, huddling, housing conditions such as bedding and nesting material are just as important as the selected housing temperature, and affect what the actual perceived housing temperature is, thereby influencing the outcome of an experiment.

Nonetheless, mice always choose thermoneutrality when having a choice, and to be able to approximate human conditions to mice, thermoneutral temperature is therefore recommended (Cannon and Nedergaard, 2011; Gordon, 2012; Overton, 2010). Furthermore, if the objective is to study BAT in any context other than cold, thermoneutrality is also preferred since every degree cooler than thermoneutrality has a larger impact on BAT activity than for instance food. In addition, cooler housing temperature affects most physiological parameters associated with the metabolic syndrome (Cannon and Nedergaard, 2011; Overton, 2010).

3.3 Confounding factors in determining housing temperature

Thus, every degree matters for metabolism in the tiny temperature-sensitive mouse, possibly a lot more than for rats and humans. That is one of the reasons that I compiled all my studies in Figures 6 to 14, to be able to ascertain any the patterns, despite unconscious differences in the execution of the studies.

It cannot be excluded that the papers included in present thesis and all the studies on high-fat fed male C57Bl6 mice presented in Figures 6 to 14, as well as in Papers I–IV, could have had slightly different operating temperatures. Firstly, it is difficult to have an exact control over the heating system in every spot in vivariums, it may certainly vary a degree or two. This is probably why it is common in articles, to specify the housing temperature in a range, for instance 21–22 °C rather than one exact temperature. Secondly, the mice could have had different amounts of nesting material. Thirdly, the mice differ in size, for instance the UCP-ablated mice are smaller (in average 2 g less than the wild-type, see Table 2). All this could be confounding factors affecting the results. Especially the UCP1-ablated mice that are cold-sensitive (Enerbäck et al., 1997; Liu et al., 2003) are possibly very affected by every the slightest cooler temperature.

Nevertheless, despite such a potential confounding mild cold-stress in some/any of the cohorts in Figures 6 to 14, which would protect them against weight gain, the UCP1-ablated mice still had higher weight gain and metabolic efficiency than wild-type mice, and this was accompanied by lower energy expenditure (Paper I).

4. Energy Metabolism

Metabolism (from Greek: μεταβολή *metabolē*, "change") is chemical transformations within the body to sustain life. The energy originally comes from food and then flows through the system. Basically the energy from food has three fates; use for body functions, loss as heat and storage. Wilbur Olin Atwater (1844–1907), who worked with energy content in food and energy expenditure over a hundred years ago expressed it as: “Food may be defined as material which, when taken into the body, serves to either form tissue or yield energy, or both” (Atwater, 1905). This basically captures the purpose of these conversions: to generate energy for cellular processes and produce building blocks for molecules and tissues from the ingested food.

Furthermore, the balance between intake and expenditure determines the storage, which can be referred to metabolic efficiency (i.e. the amount of energy from food which is stored as body fat). Like most functions in the body, energy metabolism is regulated and dynamic but not so easily captured. Measuring the energy content in food, the energy expended and the energy stored are more complicated than first thought of, and will be further discussed. Brown adipose tissue is one of many factors involved in regulating energy metabolism. Since brown fat – when active – contributes to a significant degree to energy expenditure, it has the capacity to be significantly involved in weight homeostasis and thereby possibly in counteracting overweight.

4.1 Weight balance – Gaining or losing weight

During evolution humans have developed complex genetic and physiological systems to protect against starvation and defend stored body fat rather than the opposite. The last century of industrialization has provided highly accessible mass-produced, high-calorie, readily digestible foods, and simultaneously we have adopted a more sedentary lifestyle. Starvation, cold and heavy manual work, which used to protect us against weight gain, are practically abolished in our part of the world. In this modern obesogenic (warm, hyperphagic and sedentary) environment, individuals having the appropriate combination of ancestral energy-conserving genes are at greater risk for overweight and obesity (Bellisari, 2008).

Apart from the obvious causes of obesity such as hyperphagia and low activity (which can be both genetically and environmentally determined), also low resting metabolic rate, low rates of fat oxidation or high respiratory exchange ratio, low spontaneous physical activity and impaired sympathetic nervous activity have been found to be connected to susceptibility to weight gain in both animals and humans (Galgani et al., 2008).

When having the same food intake, the tendency to gain or lose weight varies more between pairs of identical twins than within pairs, indicating that genetics plays a major role (Bouchard and Tremblay, 1997). Low resting metabolic rate, also a genetic factor in, for example, the Pima Indians, is associated with obesity (Tataranni et al., 2003). In fact, from studies with monozygotic twins, it has been shown that as little as one-third of the variability in body weight is attributable to non-genetic factors (Galgani et al., 2008).

Hormones play a major role in regulating fat storage, where for instance, high levels of insulin in the blood, are tightly connected to obesity (e.g. Collins and Surwit, 2001).

To further aggravate the interpretation of causes of obesity and weight regulation it is also surrounded by a lot of myths and presumptions both from society and researchers (Casazza et al., 2013). The well-spread belief that small sustained changes in energy intake and energy expenditure would lead to large long-term weight changes, is a long-lived myth. When humans are assigned to weight-loss programs in either research or under health authorities, they are commonly referred to the 3500-kcal rule (Wishnofsky, 1958) with the goal to lose one pound a week (body fat contain roughly 3500 kcal/pound or 32 MJ/kg²). Even under very controlled conditions, when comparing predicted weight loss against actual weight loss, most subjects lost much less than predicted (Thomas et al., 2013). The idea that it is possible to exact calculate and predict a certain weight loss or gain, by only counting the calories in and out, is a myth because such simplistic calculations ignore physiological compensatory mechanisms that limits changes in body weight.

Thus, many variables affect the energy balance involuntarily – far beyond any conscious control or theoretical calculations on energy in and out of the body. Even though in theory positive or negative weight balance is determined by the difference between energy in and energy out, both measurements in themselves consist of many problems, which will be further discussed.

² 1 pound equals 0.454 kg and 1 kcal equals 4.18 kJ. Pure triglycerides contains ≈ 37 MJ/kg, but body fat also contain other components, thus body fat contain less energy.

4.2 Energy flow from ingested food

The energy from ingested food flows through the system with losses of energy along the way. These energy steps are summarized in Figure 4. To estimate the actual energy available for body functions, these losses have to be considered. By putting food in a bomb calorimeter, the total energy can be measured when a substance undergoes complete combustion under standard conditions. This total energy is referred to gross energy (GE), but does not represent the energy, that is actually available to the body. Wilbur Olin Atwater (1844–1907) and colleagues did a vast amount of experimental studies with Calorimetry and different food items in the late 19th century and early 20th century, and from that developed a system to calculate the available energy content in macronutrients and food items. The term Available energy that Atwater used is equivalent to the modern term metabolizable energy (ME), which is gross energy minus the losses in gastric tract, kidneys and surfaces. Basically these losses are physical matter not incorporated or absorbed in the body (i.e. feces, passing wind, urine, skin and hair), thereby not available for the body.

Losses in secretions and gases are usually ignored. Metabolizable energy can thus be defined as “food energy available for heat production (= energy expenditure) and body gains” (Atwater and Bryant, 1900). However, the remaining energy (ME), after subtracting the physical losses from the gross energy, has a high variability depending also on the composition of the food, for example the content of fibers (Merrill and Watt, 1973).

Not even all the metabolizable energy is available for the body for maintenance and physical activity. Some energy is utilized during the metabolic processes associated with absorbing and digesting the food and can be measured as heat production. This can be considered an indispensable part in energy expenditure and is referred to as obligatory diet-induced thermogenesis, or thermic the effect of food, and varies with the type of food ingested. A small part of the energy is also lost as heat due to microbial fermentation.

These obligatory heat losses are subtracted from the ME and result in net metabolizable energy (NME).

Furthermore, extra energy can also be lost in the heat produced by other metabolic processes, such as the effects of cold (thermoregulation), hormones, and drugs etc. In addition, when processing of the food a non-obligatory heat producing part can occur referred to facultative diet-induced thermogenesis. In these cases the amount of heat produced is not dependent on the type of food alone and consequently these energy losses are not taken into consideration when assigning energy factors to foods. When subtracting also the optional heat losses, the energy remaining is referred to as net energy for maintenance (NE), which is the energy that can be used by the body for maintenance = basal metabolism, physical activity and the energy needed for growth, storage, fetus, lactation etc. (Livesey, 2001; Warwick and Baines,

2000). Cold, together with facultative diet-induced thermogenesis are associated with brown adipose tissue activity.

Thus, the steps between ME and NE include the heat losses which are used by the body to maintain euthermia and facultative diet-induced thermogenesis, which are associated with BAT. Nonetheless, the issue of what the food actually contains in terms of energy is a very complicated matter.

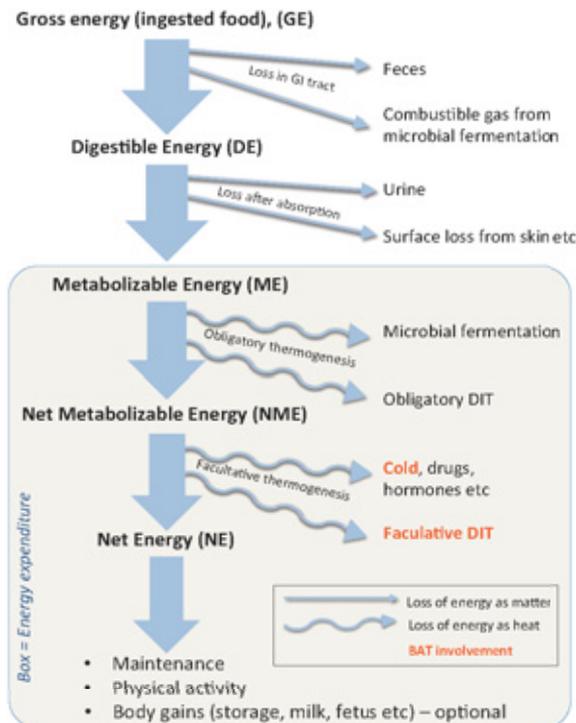


Figure 4. Overview of the food energy flowing through the body. After losses in the form of physical matter and heat, the available energy for basal metabolism, physical activity and tissue gains remains. Total energy expenditure, which can be measured in metabolic chambers, includes the Net Metabolizable Energy and all the heat losses. Based on and expanded from figures in FAO 77 (2002), Livesey (2001) and Warwick & Baines (2000).

4.3 Storing – Metabolic efficiency

When energy from food flows through the system, it can be more or less efficient (i.e. more or less can be lost as heat). The amount of the energy from ingested food which is stored as body fat determines the metabolic efficiency. High values mean more is stored and less is lost as heat. This also clearly illustrates that counting calories is difficult, since we do not know the efficiency or rather whether calories go to ATP or go to heat, or when in

excess are stored. Low metabolic efficiency is largely correlated to possession of UCP1 (Paper I) and to temperature (Paper II).

4.4 Energy expenditure

When subtracting the loss of energy in feces, urine and gas, the rest resembles the energy expenditure that can be measured in metabolic chambers. Many parameters contribute to the total energy expenditure, where the amount of activity and the ambient temperature have the largest impact. Lean mass, genotype (UCP1^{+/+} or ^{-/-}) and type of food also affect total energy expenditure to a not small degree (Wanders et al., 2015).

4.4.1 Compartments of energy expenditure

Total daily energy expenditure can be subdivided into different compartments. When dealing with humans, because we live in a thermoneutral environment usually, only three components are used: basal metabolic rate (BMR), thermic effect of food, and physical activity (Westerterp, 2004). When referring to smaller mammals, one more compartment is added in total energy expenditure, the thermoregulation. In addition, some of the compartments can be subdivided into an obligatory part and to a facultative part (Clapham, 2012; Dulloo et al., 2012; van Marken Lichtenbelt and Schrauwen, 2011). It is in the facultative compartments that energy expenditure can be affected the most, whereas the obligatory parts, BMR, food digestion and lowest possible involuntary physical movement (such as posture and fidgeting) are more inflexible.

The most obvious facultative compartment is physical activity, which can be altered consciously by exercise in humans or encouraged in rodents by for instance access to a running wheel. The degree of physical activity has been shown not to contribute to the differences between wild-type and UCP1-ablated (Wanders et al., 2015), indicating that the higher energy expenditure in wild-type in Paper I is not due to physical movement. Furthermore, physical movement does not differ between 23 °C and 28 °C housing temperature either (Wanders et al., 2015). Thus, we can probably exclude differences in physical activity between the wild-type and the UCP1-ablated mice in our studies (Paper I and III).

In a thermoneutral environment, heat production from all reactions in the body is sufficient to maintain core temperature without additional heat production. When not staying in a thermoneutral housing temperature, thermoregulation can have a large effect on total energy expenditure. Mice spend 50 % more energy when kept in mild cold such as room temperature (Golozoubova et al., 2004). Even though cold has greater capacity to recruit BAT, it is not a feasible method for humans, therefore putting interest in the

compartment of diet-induced thermogenesis as a more realistic target. The search for a way or a compound to affect facultative diet-induced thermogenesis would be of great interest because of its possible involvement in counteracting obesity. The different compartments of energy expenditure are summarized in Figure 5.

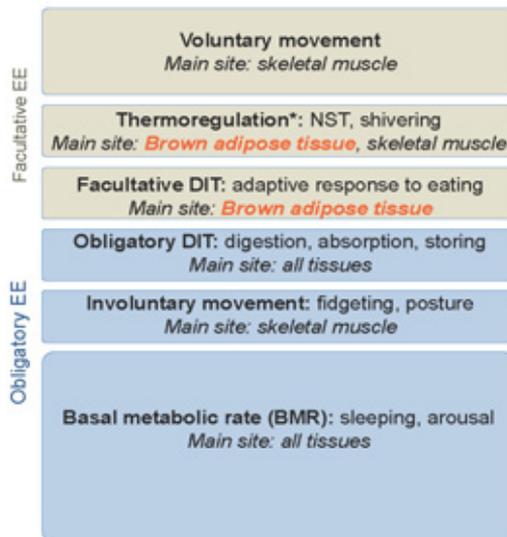


Figure 5. The compartments of energy expenditure. All reactions in the body release heat in the process of converting substrates to ATP and can be divided into two categories: obligatory and facultative. The obligatory (blue) energy expenditure is the heat generated from all processes necessary for life. The facultative (brown) energy expenditure occurs mainly in skeletal muscle and brown adipose tissue and is referred to as the regulated part of thermogenesis in response to diet and temperature changes in the environment. * Adaptive thermogenesis below or above thermoneutral zone. Adapted from Clapham, 2012; Dulloo et al., 2012; van Marken Lichtenbelt and Schrauwen, 2011.

The capacity of BAT thermogenesis

In cold-acclimated rodents BAT may dissipate heat about 300–400 W/kg wet weight of the tissue (Foster and Frydman, 1979; Heldmaier and Buchberger, 1985; Klingenspor et al., 2008; Puchalski et al., 1987), which for a mouse with 200 mg interscapular BAT would correspond to about 0.15–0.3 W. Considering a mouse expend about 0.4–0.6 W (see for instance Paper II, figure 4A) in thermoneutrality and the double in the cold, BAT has the capacity to contribute to a vast amount of total energy expenditure. That is of course, only if it is active. For BAT to reach its fully potential of heat release (energy) it would need dense sympathetic innervation and vascularisation, high lipolytic capacity and mitochondrial density and a high amount of UCP1, which takes about 4 weeks.

4.4.2 Measuring energy expenditure

In the 17th century it was known that bodies releases heat in some way and the first direct calorimeters was developed by placing objects in chambers surrounded by ice and the melting time was measured (Frankenfield, 2010). This method, direct calorimetry (measuring heat), resembles energy expenditure very well (see Figure 4 and the heat losses), but was back then, and still is a difficult method to execute. Antoine Lavoisier (1743 – 1794) was one of the first to recognize the connection between oxygen consumption and combustion, although he did not know what this “something from the air³” was (Scott, 2014). From this knowledge the indirect calorimetry⁴ (measuring gas exchange) was developed, which is the most common method to measure energy expenditure today. Indirect calorimetry is based on the fact that heat (energy) corresponds to oxygen consumption. However, this is true for aerobic combustion, but indirect calorimetry cannot capture anaerobic combustion, which is one reason that direct calorimetry could be recommended (Kaiyala and Ramsay, 2011). The anaerobic part of energy expenditure is usually very low and can basically be ignored. However, it cannot be excluded that it might influence the measurement. Some studies when studying both methods in parallel have concluded that there is a risk of overestimating HFD-feeding compared to chow-feeding in indirect calorimetry (Burnett and Grobe, 2014, 2013). But it can also be interpreted that direct calorimetry failed to detect the difference (Speakman, 2014).

Because measurements of energy expenditure can have some confounding issues depending on type of macronutrients and compositions, it is at least “safe” to compare mice eating the same diet, as we do in Paper I and II.

Nonetheless, it should be awareness of, that the method used for measuring energy expenditure, might or might not be totally true. It has been shown that medium-chain triglycerides (MCT) compared to long-chain triglycerides (LCT) are associated with increased microbiota fermentation (anaerobic) (Rial et al., 2016). Thus it could be that we did not catch a difference between the MCT-fed versus LCT-fed mice in Paper III, which could be due to heat produced by gut microbiota in MCT-fed mice (although this part of heat losses are very low). However, long-term even very small numbers add up and can result in large differences.

³ In the 17th century the theory was that materials when combusted released phlogiston, an odorless and weightless substance, which generated heat. Later carbon dioxide (1754) and oxygen (1774) was discovered, but the connection between the components was not clear. Lavoisier proposed that something was taken up from the air (which turned out later to be O₂) rather than released to the air (phlogeston), to dissipate heat. (Frankenfield, 2010; Scott, 2014)

⁴ The first indirect Calorimetry experiment on animals was performed 1783 and on humans already 1790 by Lavoisier. (Frankenfield, 2010)

5. Food

In diets for research, as well as food items from grocery stores, the labels tell us the energy content in the food. I had not earlier been thinking of how these energy values are actually determined, and was a little surprised when I realized that the most common way to assign diet and food items with energy content is to calculate the macronutrients by weight and use general energy factors. In the next section, I will discuss this issue and the problems with that. Furthermore, I will discuss some components in food associated with BAT.

5.1 Energy in food

About a hundred years ago, as mentioned, Wilbur Olin Atwater came up with the factors for the metabolizable part of the macronutrients containing energy. These factors are based on the heats of combustion of the macronutrients, and corrected for energy losses. It uses a single factor (a mean) for each of the energy-yielding substrates, regardless of the food in which they are found. The energy values Atwater came up with are 16.7 kJ/g for protein, 16.7 kJ/g for carbohydrates and 37.4 kJ/g for fat (Atwater, 1916), which are referred to as “Atwater’s general factors”, and resemble metabolizable energy (ME, see Figure 4). These factors are easy and convenient to use, but in some cases inaccurate. For instance, when humans were served high amounts of almonds, the Atwater factors resulted in 32 % overestimation compared to their actual energy content (Novotny et al., 2012). Furthermore, the Atwater general factors were less accurate in high-fibre, low-fat diets compared to a highly refined diet, in that they overestimate the energy in fibre-rich food (Zou et al., 2007). Possible implications of using the general Atwater factors would be that shorter fatty acids (as in Paper III) and carbohydrates are often overestimated, whereas longer fatty acids and protein are often underestimated.

To further confuse the matter, there are also other factors around with slightly different conversion factors, for instance the Rubner factors 17.2/17.2/38.9, which presumably overestimate the ME (Livesey, 1995). Also, it is common to round off the factors to 17, 17 and 37 (Atwater) or 39 kJ/gram (Rubner).

The Atwater general factors are now uniformly used in nutrient labeling and diet formulation, in part because of their obvious simplicity: protein 16.7; carbohydrates 16.7 and fat 37.4 kJ/gram. In addition, they are usually rounded off and recommended as 17 for protein and carbohydrates, and fat 37 kJ/gram (FAO, 2002). This rounding off could also interfere with what numbers end up being used. One kcal equals 4.184 kJ and one kJ equals 0.239 kcal (FAO, 2002). Sometimes these values are rounded off to 4.18 or 4.2 and when recalculating from kcal to kJ can then affect what numbers are used.

Furthermore, these general factors do not take into account that the macronutrients differ in composition and also do not have the same availability in different food, that, for instance, protein differs in amino acid composition, yielding different energy contents (Ferrer-Lorente et al., 2007). Thus, in the 1950s Merrill and Watt re-examined the Atwater system and came up with the “Atwater specific factor system”. This new system integrated 50 years of research and derived different factors for proteins, fats and carbohydrates, depending on the foods in which they are found. They emphasized that there are rather ranges in the heats of combustion, and in the coefficients of digestibility of different proteins, fats and carbohydrates, and these should be reflected in the energy values applied to them (Merrill and Watt, 1973). To mention a few discrepancies: the heat of combustion of protein in potatoes is 10.2 kJ/g whereas it is 18.2 kJ/g for eggs; digestibility in carbohydrates due to the fibre content affects the available energy content significantly, 10.4 kJ/g in lemon and 17.4 kJ/g in polished rice; for fat the differences are smaller, but still significant, from 35 kJ/g in barley, 36.8 in milk products and 37.7 kJ/g in meat (FAO, 2002; Merrill and Watt, 1973). These ranges for protein, fat and carbohydrate are 44, 7 and 35 percent differences respectively, which are not negligible values. However, in practice, the difference is not as large. Application of the specific factors to a common mixed diet in the United States would result in values that are on average about 5 percent higher than those obtained with general factors (FAO, 2002). Thus, the most commonly used general factors (Atwater), may underestimate the actual energy in food but not so much.

Even though the specific factors are more accurate than the general factors, they are not so user-friendly, which led to another system for determining the energy content in food, the net metabolizable energy (NME) proposed by Livesey 2001. The NME system takes into account that especially protein and fibre content influence the availability of the food energy.

Protein is particularly thermogenic and the net metabolizable energy (NME) is actually only 13 kJ/g (Livesey, 2001). Reported values for thermic effects (obligatory diet-induced thermogenesis) of macronutrients are 0–3% for fat, 5–10% for carbohydrate, and 20–30% for protein (Tappy, 1996).

Thus, depending on the larger thermic effect of protein and the lower energy content in fibre-rich carbohydrates, the actual energy in food is difficult to determine.

Table 1. Models for predicting energy in macronutrients (alcohol is excluded). Rounded values. (Atwater, 1916; FAO, 2002; Livesey, 2002, 2001; Merrill and Watt, 1973).

	GE kJ/g	DE, kJ/g	ME as Atwater general factors, kJ/g	ME as Atwater specific factors, kJ/g	ME as modified general factors, kJ/g	NME factors, kJ/g
Protein	23.6	21.5	16.7	8–18	17	13.3
Fat	39.3	37.6	37.4	35–38	37	36.6
Carbohydrates, total			16.7	11–17	17	
-Monosaccharides (available)					16	16
-Available by differ- ence (minus fat and protein)	17	17	16.7		17	17
Dietary fibre						
-Fermentable	17	11			11	8
-Non-fermentable	17	11			0	0
-In conventional foods (assuming 70% is fermentable)					8	6

GE = Gross energy (from combustion in bomb calorimeter); DE = Digestible energy; ME = Metabolizable energy; NME = Net metabolizable energy.

Atwater (1900) defined metabolizable energy (ME) as “food energy available for heat production (= energy expenditure) and body gains”. A hundred years later, ME is still valid and defined by Livesey (2001) as “the amount of energy available for total (whole body) heat production at nitrogen and energy balance”. ME then includes all energy needs plus heat production and this is what is measured in a metabolic chamber.

On the other hand, net metabolizable energy (NME) is based on the ATP-producing capacity of food components, rather than on the total heat-producing capacity of foods, i.e. “food energy available for body functions that require ATP”, which means that food components differ in the efficiency in with which they are converted to ATP, and therefore their ability to fuel energy needs of the body (FAO, 2002; Livesey, 2002; Warwick and Baines, 2000).

Depending on the lower efficiency of converting proteins and fermentable carbohydrates into ATP, it has been suggested that they should be assigned lower energy values, as proposed in the NME system (Livesey, 2001), which thus can be argued to be superior to the ME system. However, when measuring energy expenditure, all heat losses are included which thus is comparable with energy in food, and furthermore it is difficult to determine the exact amount of heat loss, which would favor the ME system (Warwick and

Baines, 2000). Whether the energy content in food should be assigned to ME or NME is still under debate.

Thus, currently there is no way to exactly determine how much energy an individual actually obtains from 1 g of protein, carbohydrate, fibre, or fat. The general factors 17/17/8/37 kilojoule/gram, respectively for ME are usually all we have to go on, or in some cases the specific factors, but it is important to keep in mind that the numbers do not always reflect reality.

5.2 Consequences of different energy factors

The discrepancy in estimating energy in food can be avoided at least by using the same kind of diet in experiments. A common diet used for obesity studies is the semi-synthetic high-fat diet (HFD, D12451) from Research Diets (New Brunswick, US), which is specified in all the ingredients and does not differ between batches. We used this diet and a chow diet in all our studies in Paper I-IV (plus another diet in paper III).

Chow versus high-fat diet

In Paper I, we see both a clear genotype effect (wild-type versus UCP1-ablated) and a diet effect (HFD versus chow). The HFD-fed wild-type expend more, eat more but gain less compared to UCP1-ablated, therefore the wild-type have lower metabolic efficiency (expressed as % of food which is stored as body fat). This result of a lower metabolic efficiency is robust and not biased since the two genotypes eat the same food (HFD). However, the metabolic efficiency is also clearly higher, when mice eat HFD compared to chow, which is a little more ambiguous. We cannot know for certain that we use the correct energy content for the two diets. The chow diet is high-fibre and low-fat, which contains plant-derived ingredients and it is not processed, and can furthermore vary depending on season and batch. Fibre content (Livesey, 2002) and crudeness (Zou et al., 2007) of a diet are inversely correlated to available energy in the food. Thus, even though the supplier of the chow we use (R70, Labfor) takes into account that not all energy is available for metabolism, it could still differ from the actual energy content. The supplier of the HFD diet uses Atwater general factors (17/17/37) for estimating the energy content in the diet, which also possibly do not reflect the exact actual energy content in the diets. However, the metabolic difference was still so large that we can assign the HFD a larger fattening effect than chow.

Implication of energy in fatty acids

This food (energy) issue was also apparent when I was working with Paper III with the two high-fat diets containing either long-chain triglycerides or medium-chain triglycerides. The product data sheets contained the same val-

ues for energy in both diets, even though it is known that fatty acids differ in energy content (Marten et al., 2006). Both high-fat diets used in Paper III were obtained from Research Diets and had a stated macronutrient composition of 45 energy% fat, 20 energy% protein and 35 energy% carbohydrates. The standard high-fat diet contained 40 % lard, mainly long-chain triglycerides (abbreviated LC), whereas the diet high in medium-chain triglycerides (abbreviated MC) contained 40 % medium-chain triglycerides (see also 5.3.3). According to the manufacturer's product data, the energy content of all these high-fat diets is 19.8 kJ/g, which is based on 24 g protein, 41 g carbohydrates and 24 g lipid source per 100 g food, and an estimated energy content of the individual macronutrients of 17, 17 and 37 kJ/g (Atwater general factors), respectively. However, depending on chain length and saturation, different fatty acids differ in energy content.

To account for difference in energy content for the different composition of fatty acids, I recalculated the energy values by multiplying the amount of the fatty acids in the high-fat diets with values for different fatty acids from Livesey et al. (1988). The calculations yielded different energy values for the lipid part of the diets: 39.6 kJ/g fat for LC and 36.0 kJ/g fat for MC. These values were used together with 17 kJ/g protein and carbohydrates for the different diets, generating new energy contents for the diets: 20.6 kJ/g for LC and 19.7 kJ/g for MC.

The LC mice in Paper III had slightly higher food intake, but gained significantly more body fat, about 5 g. When using the Atwater general factors for energy in food, the difference between the groups was 67 kJ in three weeks, which corresponds to 1.8 grams body fat ($67 \text{ kJ} \times 37^5 \text{ kJ/g body fat}$). This explains then 36 % of the body fat differences. However, when using the recalculated specific factors for the energy, the difference was 118 kJ, which would correspond to 3.2 g. This difference of 1.4 grams between the two diets explains 64 % of the body fat differences. For the other assigned energy factors, the values might be wrong, but at least they are systematically wrong in the same magnitude. Nonetheless, possibly biased values for energy in the two diets in Paper III cannot be ignored. By recalculating the food factors due to the differences in fatty acid composition, thus explains a major part of the body fat differences.

Thus, when studying specific components of food, the difficulties with assigning food energy can have large consequences, as in Paper III when we actually masked 1.4 g body fat by using the same general factors for estimating food energy. This can really impact the results and furthermore the interpretation. The remaining about 2 gram body fat difference between groups, which cannot be simply explained by differences in food intake could be caused by differences in metabolic efficiency. Indeed, the metabolic efficiency (kJ body fat stored divided by kJ eaten) was notably low for the MC mice,

⁵ When measuring body composition, the fat part is actually pure triglycerides and can thus be assigned 37 kJ/g.

10 % versus 25 % for the LC mice. So, the LC mice being more metabolically efficient, store a larger part of the food ingested, which explains the higher body fat gain.

Thus, greater food intake and higher metabolic efficiency explain the higher body fat gain in LC mice. This should also be reflected and further-more explained by a higher energy expenditure in the MC mice. This is also a reason to use the ME system, because it correspond to the expenditure. However, we could not detect any significant difference in energy expenditure between MC and LC (although a tendency was visible). Nor did we see higher amounts of UCP1 protein, which supposedly could explain the lower metabolic efficiency in the MC-fed mice. An objection is of course that the energy expenditure was measured for a too short period (only 22 hours). The UCP1 content was in fact quite low in the MC mice, rather it correlated significantly to the amount of body fat, see Figure 12A.

5.3 Fatty acids affecting BAT

Fatty acids have many functions in the body. They can be oxidized for energy, be stored as triglycerides, be incorporated in membranes and work as ligands in signaling. Fatty acids have also been shown to have direct effects on UCP1 in isolated mitochondria from BAT (Shabalina et al., 2008). This part has been more elaborated my licentiate thesis “The effect of various dietary fatty acids on adaptive thermogenesis” (von Essen, 2014).

5.3.1 Dietary fatty acids

Dietary lipids contain a mixture of fats, where the most abundant structure is fatty acids, usually in the form of triglycerides (90-95% of total dietary fat content). Fatty acids are a diverse group of molecules containing a hydrocarbon chain that terminates with a carboxylic acid group. Human diets contain at least 20 different types of fatty acids with different lengths and degree of saturations (Surette, 2008). The most abundant dietary fatty acids contain an even number of carbons from 8 to 22 and have 0–6 double bonds (Innis, 2011). Short chain fatty acids ($\leq 6C$), are mainly produced by bacteria from non-digestible carbohydrates in the colon, but small amounts are also found in the diet.

Depending on the chain-length of the fatty acids, they are absorbed and metabolized differently, basically through two metabolic fates. Short- and medium-chain fatty acids (SCFAs and MCFAs), 12 carbon and shorter, are readily absorbed in the intestine and transported bound to albumin via the portal vein directly to the liver, where they are metabolized or further transported to other tissues. Long-chain fatty acids (LCFA), ≥ 14 carbons are absorbed by enterocytes, and transported in chylomicrons via the lymph system

to the circulation. By the action of lipoprotein lipase (LPL), chylomicrons deliver fatty acids to peripheral tissues and eventually, as chylomicron remnants, enter the liver.

Fatty acids can be saturated or have different amounts of double bonds in different configurations. The double bonds in monounsaturated and polyunsaturated fatty acids can have *cis* or *trans* configurations. The *cis* form is when hydrogen atoms are on the same side, whereas the *trans* form is when hydrogen atoms are on opposite sides of a double bond. The *Cis* configuration is the naturally occurring fatty acid in the diet and in the body. The *trans* form occurs when liquid oils are processed through hydrogenation to make oils solid in room temperature to increase shelf-life in commercial products (Iqbal, 2014). This form has lately been considered unhealthy, due to increased risk of heart- and coronary disease (Iqbal, 2014; Remig et al., 2010). Fatty acids can also occur both as *cis* and *trans* forms as in naturally occurring conjugated fatty acids, only having one carbon between the double bonds.

All these different dietary fatty acids, from the 2-carbon short fatty acid acetate, to polyunsaturated docosahexaenoic, DHA (C22:6-n3) and different conjugated linoleic acid isomers (CLA), affect the body differently, apart from supplying the body with almost the same energy content. One aim of this thesis is to present how lipids in the diet affect brown adipose tissue and the implication on body weight, adiposity and other features related to adaptive thermogenesis.

5.3.2 Short-chain fatty Acids (SCFA)

Increasing evidence implies that short-chain fatty acids (SCFAs) are important in gut microbiota health, as well as being involved in the regulation of lipid, glucose and cholesterol metabolism in various tissues (den Besten et al., 2013; Tan et al., 2014). SCFA have a chain length of two to six carbons where C2-C4 are most abundant ($\geq 95\%$) and can provide up to 10% of daily energy intake in humans (Bergman, 1990; den Besten et al., 2013). Dietary fibre including non-starch polysaccharides, resistant starches and oligosaccharides are resilient to digestion in the small intestine and reach the colon undigested, where they are fermented by colonic bacteria producing SCFA (Tan et al., 2014; Ziegler et al., 2003). Fermentation of complex carbohydrates is important in all vertebrates, as no enzymes break down these carbohydrates, and SCFA contribute to energy demand from 5 % in rat to 70 % in sheep (Bergman, 1990). Furthermore, a small portion is also found in dairy products from cows (Neveu et al., 2014) as well as in goat, buffalo and sheep. SCFAs are taken up by transporters, and to a lesser extent by passive diffusion, to the colonocytes, supplying them with energy. SCFAs not used by the colonocytes reach the liver via the portal vein. How SCFAs are taken up from the blood by tissues is largely unknown.

Mice exposed to high-fat diets where 5 % of the LCFAs were exchanged to different ratios of acetate and propionate did not differ in body weight and body fat, but the mice showed healthier lipid profile and improved insulin sensitivity, indicating an importance of especially propionate on health (Weitkunat et al., 2016).

GPR receptors

In addition to their role as energy substrates, SCFAs have also been recognized to bind to some receptors. The G protein-coupled receptors, GPR41, GPR43 and GPR109a, are highly expressed in the gastrointestinal tract, immune, nervous system and white adipose tissue, and have been found to be activated by SCFA (Brown et al., 2003; Tan et al., 2014). So far, only mRNA expression of GPR43 has been found in brown adipose tissue (Regard et al., 2008). Recently, it has also been confirmed by Western blotting that even the receptor GPR43 is present in BAT culture (Hu et al., 2016). Furthermore, the same group showed that acetate increased levels of UCP1 protein by 2-fold, indicating a physiological role of acetate by activation of GPR43 in BAT. This was confirmed by inhibition of the acetate-effect in GPR43 knock-down brown adipocytes.

The effects of SCFA binding to GPR43 and the involvement in lipid metabolism can be studied by knocking down the *Gpr43* gene. One study showed that GPR43 KO mice (C57Bl5 background) gained less weight, had lower adiposity and higher energy expenditure when high-fat fed in a 30-week study (Bjursell et al., 2011). These results contradict another study performed by Kimura et al. (2013), where GPR43 knockout mice (129 background) became obese when HFD-fed, but not in a germfree environment (no SCFA production). Thus, GPR43 KO mice on a 129 background showed weight gain on a normal and a high-fat diet (Kimura et al., 2013) and also impaired glucose tolerance (Kimura et al., 2013; Tolhurst et al., 2012) whereas GPR43 KO mice on a C57Bl6 background had a lean phenotype on a high-fat diet (Bjursell et al., 2011). This discrepancy in the effects of knocking down GPR43 receptors could depend on genetic background, 129 versus Bl6 (Kim et al., 2014).

The field of GPRs and BAT function is not yet very explored, however increasing evidence shows a link to BAT thermogenesis. Another recent paper found that the GPR3 receptor is involved in the thermogenic function of BAT. GPR3 knockout mice had lower amounts of both UCP1 mRNA and protein, lost their thermogenic capacity and had higher body fat with aging compared to wild-type littermates (Godlewski et al., 2015).

Butyrate

Since SCFAs are involved in lipid metabolism and have receptors in brown fat could indicate an effect on BAT activity. Not many studies have been

performed in this context: only one by Gao et al. (2009) added butyrate to the diet. The result showed improved insulin sensitivity, higher energy expenditure and increased mRNA and protein levels of UCP1 in mice, which could indicate a slight thermogenic effect. However some data were presented in a biased way, for instance division of food intake and energy expenditure by total body weight.

Altogether, SCFAs have metabolically beneficial features, probably partly through GPR43 and GPR41 receptors. Not many studies have been conducted studying the involvement of SCFA on activated brown adipose tissue; however, butyrate as indicated above, did seem to increase UCP1 protein in interscapular BAT (Gao et al., 2009). Furthermore, mRNA for SCFAs receptors has been detected in BAT (Regard et al., 2008; Zaibi et al., 2010), which could be a promising target for further investigations, but more studies need to be performed.

5.3.3 Medium-chain triglycerides (MCT)

Medium-chain triglycerides (MCTs) have been shown to reduce adiposity and have other metabolic beneficial effects in both animals and humans (Marten et al., 2006; Mumme and Stonehouse, 2015; Nagao and Yanagita, 2010; Paper III). Lately, MCTs have also arised an interest in improving gut microbiota and possibly turning unhealthy obese into a more healthy obese phenotype (Rial et al., 2016).

Medium-chain fatty acids (MCFA) are saturated fatty acids, having a chain length of 8–10 carbons; sometimes C6 and C12 are also included in the MCFAs. Most dietary fats contain fatty acid carbon length of 14 and more, but there are some natural sources of shorter fatty acids such as coconut oil and palm kernel oil, where MCFA (C8–C10) make up more than 10 % of total fatty acid content; when C12 is included it is almost 60 %. Even bovine milk contain small amount of MCFA, 4–12 % depending on lactating stage. The most used MCFAs in trials are octanoic (C8) and decanoic (C10) oils, in ratios of 50:50 to 80:20. Compared to long-chain fatty acids (LCFA), MCFAs have a lower melting point, lower molecular weight, are liquid at room temperature, and have slightly less energy content, 33.4 kJ/g for C8, 35.5 kJ/g for C10 versus 39.2 kJ/g in LCFA (Bach et al., 1996; Marten et al., 2006). Since MCFA are shorter, they have a slightly different ratio of CO₂ production and O₂ consumption when oxidized, giving a slightly higher RER, 0.727 for C8 versus 0.696 for C16 (Bach et al., 1996). These properties make them more efficiently absorbed in the intestine and faster hydrolyzed compared to LCFA. Most MCFA are transported via the portal vein to the liver, as opposed to LCFA, which are transported via the lymph in the form of chylomicrons; consequently, the MCFAs do not stimulate bile acid and cholecystokinin secretion as much as LCFAs (Marten et al., 2006).

Furthermore, MCFAs produce more ketone bodies (Maki et al., 2009) and are not stored in triglyceride droplets in fat cells or ectopically in liver and skeletal muscle (De Vogel-van den Bosch et al., 2011a, 2011b), to the same extent as LCFAs. Similar to SCFAs, MCFAs are readily oxidized primarily in the liver but also transported to other tissues for oxidation.

Apparently contradictory results

The lower body fat gain by feeding of MCT is associated with higher energy expenditure, which has been suggested to be due to activation of brown adipose tissue. Indeed, already in 1987 Rothwell et al. showed that rats intubated by gavage with MCT compared to water, gained less weight, had higher energy expenditure and lower metabolic efficiency, accompanied by a 71 % increase of thermogenic capacity estimated as GDP-binding in isolated mitochondria from interscapular BAT (Rothwell and Stock, 1987). The same year Baba et al. (1987) concluded that the increased thermogenesis shown in MCT-fed rats (Baba et al., 1982) was not due to activated interscapular brown adipose tissue (IBAT). The increased thermogenesis still occurred despite excising IBAT (Baba et al., 1987). However, further study by the original authors showed that when surgically removing 40 % of BAT in rats, a complete compensation by other BAT depots occurred within 13 days (Rothwell and Stock, 1989). Hence, the six-week study by Baba et al (1987) cannot rule out involvement of activated BAT caused by MCT feeding.

Different types of diets

The above mentioned studies added purified MCT of only C8 and C10 fatty acids to the diets. Naturally occurring oils, high in MCT, such as coconut oil, contain only about 15 % of these fatty acid, but also high amounts of lauric acid (47 %), as well as smaller amounts of longer fatty acids.

When rats were fed a semisynthetic high-fat diet (60 % fat) containing a large amount of coconut oil, the rats gained only slightly but significantly more weight than when fed semisynthetic low-fat diet (12 % fat) (Portillo et al., 1998). These findings were associated with significantly higher levels of UCP1 protein in the coconut oil-fed rats. The same group performed another study where rats were first fed the high-fat coconut diet followed by two energy restricted (40 % reduced) diets with different lipids. Despite eating and losing the same in the two energy-restricted groups, the coconut-fed rats had higher amounts of UCP1 protein (Portillo et al., 1998). The results of the studies are difficult to interpret, when comparing “normal-fat olive oil energy-restricted diet” with a “high-fat coconut oil energy restricted diet”, there are many parameters including the amount of fat content and sucrose and type of fat, which makes it difficult to assign the effect to amount of fat or type of fat or some other differences in ingredients such as sucrose. Furthermore, when diets are not similar in macronutrients, the energy content has a

high risk of being miscalculated (see section 5.2), causing errors in interpretation.

Low MCT

Whether a MCT-induced increase in UCP1 is due to the fatty acids affecting brown fat directly or indirectly through sympathetic stimulation is not completely clear, although it is suggested that the increased energy expenditure in MCT-fed animals is, partly at least, due to sympathetic activation of brown fat thermogenesis. In experiments by Liu et al. (2012), male C57Bl6 mice were assigned to one of two semisynthetic high-fat diets, with either 2 % MCT or 2 % of LCT yielding approximately 4 energy percent of MCT or LCT. The MCT oil contained only C8 and C10 in a ratio of 75:25, while the LCT contained fatty acids ≥ 16 carbons, including essential fatty acids (LA and ALA). During 12 weeks, both groups gained weight, and from week 8 the MCT-fed weighed slightly (1.2 grams) less. Even though the amounts of MCT were quite low, the MCT-fed mice versus LCT-fed had higher levels of UCP1 protein in BAT and in inguinal WAT. Furthermore, mRNA of ATGL and β_3 -adrenergic receptors were higher in iWAT and IBAT, as well as HSL in IBAT, indicating increased lipolysis (Liu et al., 2012). Metabolic variables in serum were also more favorable in MCT-fed mice with lower TG, FFA and LDL, along with higher levels of HDL and norepinephrine. A similar study was performed by same group a few years later with same results (Zhang et al., 2015). Both these studies indicate that even a very low level of MCT can have a large effect on body weight.

Altogether, this indicates that MCT stimulate the sympathetic nervous system to increase lipolysis in BAT, which stimulates the activity of UCP1. A mechanism for this could be that MCT, as opposed to LCT, lead to more ketone body production (Bach et al., 1996), and these have been showed to stimulate the firing rate of sympathetic efferent fibers to BAT (Sakaguchi et al., 1988).

Diet-induced thermogenesis from MCT feeding

The increased energy expenditure (EE) by MCT has also been shown to increase EE directly after feeding (Noguchi et al., 2002). Fasting rats were starved for 18 hours and then administered 1 gram of either MCT (C8 and C10) or LCT ($\geq 16C$); thereafter oxygen consumption was monitored for 6 hours. After administration of the oils, the EE was much higher in the MCT group, indicating that the effect of MCT is in the diet-induced thermogenesis (DIT) compartment of EE (Noguchi et al., 2002).

Paper III

Taken together, the positive effects on adiposity and energy expenditure by medium-chain fatty acids might very well be mediated by a stimulatory ef-

fect in BAT. The above studies showed increased UCP1 when MCT fed. This may be seen as surprising considering the fact that the longer the fatty acid chain is, the more oxygen the mitochondria consume (Shabalina et al., 2008), which would then fit with less UCP1 in MCT fed mice in Paper III. But the study in Paper III showed clear obesity protection when mice were fed MCT.

However, the effect of MC on UCP1-ablated mice was not qualitatively different from that in wild-type, also demonstrating that the obesity-protective effect of medium-chain triglycerides is not dependent on UCP1. The study was coherent with other studies in showing an anti-obesity effect of medium-chain triglycerides versus long-chain triglycerides for the mice. Notably, the effect was much larger when the mice are kept at thermoneutral-ity (Paper III) than at room temperature (most other studies).

Another consideration is that the relative absorption in the gut may differ. We did not follow fecal energy content. But it seems that medium-chain fatty acids are actually more easily absorbed than long-chain fatty acids. In one study, rats were fed high-fat diets either high in LCT or MCT. The LCT fed rats did eat more as in our present study, but they also lost more energy in feces, resulting in a non-significant difference in net energy intake (Hoeks et al., 2003).

The novel findings in Paper III are that even at thermoneutrality, MCT protects (even more) against obesity. However, disappointingly, the effect is essentially not due to UCP1. It cannot be excluded that a small part is UCP1-dependent, due to the fact that the difference in metabolic efficiency between the two diets was larger in the wild-type than in the UCP1-ablated mice. Thus, the difference in weight balance could not be totally explained, and we must conclude that this issue needs further investigation.

5.3.4 Long-chain fatty acids (LCFA)

Fatty acids longer than 12 carbons, or sometimes 14 carbons, are considered long-chain fatty acids (LCFA). Since long-chain fatty acids differ in structure depending on length and degree and placement of double bonds, they have different effects in the body.

It has recently been discovered that LCFAs are involved in regulation of energy metabolism (Nakamura et al., 2014) and therefore some effect on brown adipose tissue (BAT) is likely. Furthermore, it has been shown that different dietary fatty acids activate the norepinephrine (NE) turnover rate in interscapular brown adipose tissue (IBAT) (Young and Walgren, 1994).

Lipid pool

A factor to keep in mind, Young et al. (1994) point out, is that for longer dietary fatty acids to affect sympathetic nervous system (SNS), the lipids in the diet have to be ingested some considerable time prior to measurement in

order to alter the endogenous fatty acid pool in the brain and possibly in peripheral tissues. Rats receiving 50 % of energy from safflower-oil (high in LCTs) and 50 % from chow did not show any alteration in the SNS activity after 5 days, but did so after 14 days, compared to only chow fed. In contrast, when rats were fed coconut oil (high in MCFAs) instead, no effect on turnover rate of NE in IBAT was seen within 14 days, indicating that chain-length and double bonds of fatty acids have different effects on the SNS in IBAT. Furthermore, depending on chain-length and oxidation rate, the metabolic fate of the fatty acids is different. When ingestion of either 30 g LCT or 30 g MCT/LCT in humans, the LCT were totally oxidized to a lower extent than MCT, 20 % versus 61 %, indicating that more of LCFAs compared to MCFAs end up in the endogenous pool, in lipoproteins, NEFAs and lipid stores (Binnert et al., 1998). Interestingly, obese subjects had a lower oxidation rate of LCT compared to lean controls, but showed no difference in MCT oxidation rate, indicating defective dietary LCT oxidation.

C18 fatty acids

An 18-carbon fatty acid chain, readily supplied in diet, can have 0-4 double bonds in different configurations, for instance oleic acid (C18:1), alpha-linolenic acid (18:3n-3) and linoleic acid (18:2n-6).

The C18 fatty acids can have several structures, which could have different physiological effects. Vögler et al. (2008) gave different C18-oils by gavage for 7 days to chow-fed 16 week old male rats. The oils used were oleic acid (C18:1*cis*; OA), elaidic acid (C18:1*trans*; EA), stearic acid (C18:0; SA), linoleic acid (C18:2; LA) and a synthetic derivate of oleic acid with an OH-group in α -position (C18:1OH; 2-OHOA). Compared to the other C18 fatty acids, only the 2-OHOA rats showed an increase in UCP1 mRNA and protein in white adipose tissue (WAT), but not in BAT. This was accompanied by the highest weight reduction for the 2-OHOA-fed rats; however, oleic acid-fed rats also reduced their weight, but the others did not. This indicates that even the same length of fatty acids, but with different configurations, can affect weight and UCP1 expression, possibly by enhanced energy expenditure (Vögler et al., 2008). However, when looking at food intake, the 2-OHOA ate less, followed by the oleic acid-fed, implying that weight reduction follows lower food intake, though it is difficult to see whether the net effect would give lower metabolic efficiency since this is not accounted for. Nonetheless, the 2-OHOA fed rats did increase the UCP1 content in WAT, which indicate a possibility to manipulate fatty acids to make them more thermogenic. However, the comparison in both WAT and BAT was to the control set to 1, which could mean that in absolute values the increase in WAT might be very low, since the control is very, very low. Whether this would have an impact on weight homeostasis – a 20-fold increase of mRNA in WAT compared to other groups – is not clear, since the metabolic efficiency might be similar in the different C18 fatty acid-fed rats. Also, the

UCP1 mRNA and protein levels in WAT are initially very low so that the total amount would still be very low.

Dietary fat sources

Since both MCT and PUFA have been shown to increase diet-induced thermogenesis, Rodríguez et al. (2002) wanted to investigate the further effect of different fat sources. Adult male rats were fed different high fat diets (40 e%) namely: beef tallow (rich in animal-derived SFA), palm oil (rich in plant-derived SFA), olive oil (rich in MUFA) and sunflower oil (rich in PUFA). After 4 weeks of ad libitum feeding, the groups did not show any difference in weight gain, IBAT and pWAT wet weights, but the olive oil-fed did have a slightly higher whole body energy expenditure and upregulated mRNA expression of UCP1 in IBAT, indicating that olive oil had some thermogenic effect (Rodríguez et al., 2002). However, no difference was seen in the protein content of UCP1 between groups. The fact that no difference in weight gain was seen might be explained by a too short study (4 weeks) such that the differences would not yet have become visible.

When a compound such as a long fatty acid has a very weak effect at the whole animal level, but still some effect, the influence on, for instance weight gain, has possibly to be observed during a longer exposure. This is evident in a 12-week study, where the difference between two high-fat (different lipid profile)-fed groups did not appear until after 7 weeks (Ludwig et al., 2013). Furthermore, it is also possible that other components in olive oil than oleic acid give the mild thermogenic indication. Olive oil contains, in addition to high levels of oleic acids also phenolic compounds, which have been shown to increase UCP1 protein in IBAT and norepinephrine secretion in rats (Oi-Kano et al., 2008, 2007).

Monoacylglycerides

Capsaicin (CAP), the ingredient in red chili pepper, binds to the TRPV1 receptor, leading to an activation of the sympathetic nervous system and up-regulation of UCP1 (Sharma et al., 2013). It has been shown that monoacylglycerol (MG) can function as a novel TRPV1 agonist (Iwasaki et al., 2008). Two experiments were performed with 18:1 fatty acids in a triacylglyceride (TG) diet and 18:1 or 18:1 plus 18:2 plus 18:3 in a MG diet. Two doses of monoacylglycerol were used, 15 e% and 30 e%, and the TG diet contained 30e% TG. No difference in food intake and body weight were seen in the 4-week study. However, by supplying the high-fat diet with 30 % monoacylglycerol, UCP1 protein in BAT was upregulated by two-fold in IBAT versus TG and 15% monoacylglycerol, suggesting a possible adrenergic stimulation in IBAT by monoacylglycerol intake (Iwasaki et al., 2011). Body weight and IBAT weight did not differ, but the distribution of fat depots showed small differences with significantly lower epididymal white adipose tissue (eWAT)

in rats fed the high amount of monoacylglycerol, which might be beneficial since eWAT is a visceral fat depot associated with metabolic syndrome. The effect of high amounts of monoacylglycerides is modest, but might be more pronounced long-term. However, long-term feeding of high amounts of a synthetically derived monoacylglycerides seems to be not so feasible.

5.3.5 Polyunsaturated fatty acids (PUFA)

Two families of fatty acids cannot be synthesized in the body and have to be supplied by the diet: α -linolenic acid (ALA) and linoleic acid (LA), therefore considered essential fatty acids (EFA). The EFAs are divided into two groups, the omega-3 and the omega-6, where omega number marks the starting point of the first double bond counting from the methyl-group. ALA (n-3) and LA (n-6) can be further metabolized in the body to their longer derivatives, (which have many important functions in the body), for instance eicosapentenoic acid, EPA (20:5n-3) and docosahexaenoic acid, DHA (22:6n-3). EPA and DHA are of animal origin and are referred to as long-chain PUFA (LC-PUFA) as they are longer than 18 carbons, whereas short-chain PUFA (SC-PUFA) are up to 18 carbons, usually originating from plants. The conversion from the short chain n-3 PUFA (ALA), to long chain n-3 PUFA (EPA and DHA), seems to be efficient in mice (Pauter et al., 2014). Whether this is true also for humans is discussed, and the conversion capacity might not be totally sufficient, although not lead to deficiency (Ruxton et al., 2007).

Short-chain and long-chain PUFA

Polyunsaturated fatty acids (both n-3 and n-6) have been shown to increase thermogenesis or thermogenic markers in rodents (Le et al., 2012; Ludwig et al., 2013; Oudart et al., 1997; Takahashi and Ide, 2000). The effect of PUFA on thermogenesis is most pronounced when long-chain-n-3 PUFA are used (LC-n-3-PUFA). Fish-oil and fatty fish contain high amounts of EPA (C20) and DHA (C22), which are LC-n-3-PUFAs, as opposed to plant-derived n-3-PUFAs, which are shorter, such as ALA (18C). Exchanging part of the lipid content in a high-fat diet for EPA and DHA did not decrease body weight, but reduced adiposity in rats together with increased thermogenic markers (Oudart et al., 1997; Takahashi and Ide, 2000). In both studies, there was a tendency to lower weight in EPA/DHA-fed rats, however, this was not significant, which maybe could change if studies were performed longer than 3-4 weeks.

Takahashi et al. (2000) also studied the effect of short-chain-n-3 PUFAs (SC-n-3 PUFA), supplying the diet with 39 e% perilla oil (high in ALA) and compared it to long-chain- n-3 PUFA (LC-n-3-PUFA) fish-oil (high in EPA and DHA) and short-chain-n-6 PUFA (SC-n-6-PUFA) safflower oil (high in LA). The tendency in terms of eating was SC-n-6-PUFA > SC n-3-PUFA >

LC-n-3-PUFA, with significant differences only between the first and the last. In terms of weight gain, it was the reversed pattern, however not significant. The feeding efficiency might therefore be similar in the groups. Nonetheless, perirenal and epididymal white adipose tissue weights were higher in n-6-PUFA group than in both n-3-PUFA groups, and these latter also showed higher mRNA UCP1 levels in BAT, indicating some protection for visceral fat increase from n-3-PUFA fatty acids, possibly due to activation of UCP1.

Flachs et al. (2005) also compared short-chain (flax seed) and long-chain n-3 (EPA/DHA) PUFA, but in contrast to Takahashi, Flachs et al. saw a small difference with lower delta body weight and eWAT in LC-n-3-PUFA. This study was performed in mice, as opposed to rats in the Takahashi et al. study. Furthermore, increased fatty acid oxidation in the gonadal WAT depots were observed, indicating that part of the adipose reduction in these depots was due to a metabolic switch in the adipocytes (Flachs et al., 2011, 2005).

Mice fed low levels (≈ 5 e%) of long-chain n-3 PUFA (LC n-3 PUFA) under long-term (30 weeks) thermoneutral conditions, compared to n-6-PUFA-fed mice, did not differ in weight and energy expenditure, but n-3 PUFA-fed showed decreased fat content in a visceral depot, the eWAT (Janovská et al., 2013). Together with Flach et al. (2005, 2011) studies showing no change in UCP1 mRNA expression in long-chain n-3 PUFA versus n-6 PUFA, Janovska et al. concluded that the anti-obesity effect from LC n-3 PUFA is not UCP1-dependent, which is the opposite conclusion from Oudart et al. (1997) who concluded that increased GDP-binding in BAT mitochondria, together with lower eWAT, is due to adaptive thermogenesis in BAT; again however, these were rats. Furthermore, Oudart et al. seeing a BAT-dependent effect compared LC n-3 PUFA to MUFA, whereas Flachs and Janovska seeing no BAT-dependent effect compared LC n-3 PUFA to other PUFA. This may convey that a possible effect is dependent on what the comparison is against.

When replacing 35 percent of dietary lipids with EPA/DHA in a high-fat diet (48 energy %), the mice had gained less body fat after 12 weeks despite eating more than the control group eating high-fat diet (Ludwig et al., 2013). Furthermore, these mice showed reduced visceral fat and ectopic fat accumulation, as well as improved insulin sensitivity, indicating beneficial effects from LC n-3 PUFA, including increased thermogenic capacity.

Whereas most studies on PUFA show apparently more effect on browning of WAT than increased activity in BAT, pine nut oil, high in PUFA, has been shown to increase UCP1 protein in BAT (Le et al., 2012). Pine nut oil from Korean pine is used in many countries. It contains high amounts of PUFA and MUFA, as the control diet high in soybean oil also did. However, pine nut oil contains pinolenic acid, an unusual n-6 fatty acid with 3 double bonds, which the control diet did not contain. After long-term feeding (12 weeks), pine nut oil-fed mice had significantly lower body weight, increased mitochondrial biogenesis and increased thermogenesis in BAT. The authors sug-

gest that this possibly, at least partly, was mediated by activating PPAR α and/or PPAR δ . A limitation to the study is that food intake is not presented, which could, if lower than soy bean oil, explain the lower body weight. However, the UCP1 protein is higher in pine nut oil-fed mice, which indicates a clear thermogenic effect.

An aspect to keep in mind is not only weight, but also a state of health, which is rarely mentioned. Rats fed both low (0.3 %) and high (10 %) amounts of a mixture of linoleic acid and α -linolenic acid (n-6/n-3 PUFA) had some health issues in a study by Nedergaard et al. (1983). They were described as being scrubby and low-weight, compared to rats fed an intermediate dose (3 %). Note though, that the rats had been on the different diets for three generations, which could affect the results and further worsen the deficiency or surplus of the fatty acids. The higher dose was, furthermore, associated with increased BAT activity and lower weight (Nedergaard et al., 1983). However, the lower weight might not only be beneficial if health is decreased. The dose issue is therefore an aspect to keep in mind.

Oxidative stress

Polyunsaturated fatty acids (PUFA) while being essential for healthy, are also more sensitive to oxidation, due to the double bonds. Processing, heating and storing food, procedures common in modern diets since we eat more fried and refined foods, can hasten this process, forming unhealthy peroxides and dienes. Penumetcha et al. (2013) showed that C57BL/6 J mice fed a diet containing heated (causing mild oxidation) soybean oil for 16 weeks increased body fat, despite decreased weight gain compared to mice pairfed soybean oil. Furthermore, together with higher epididymal WAT weight, also interscapular BAT weight was increased, but not UCP1 protein in the heated soybean oil fed mice. A limitation, the authors argue, is that energy expenditure was not measured, to see whether there was an effect on thermogenesis associated with the oxidized lipids (Penumetcha et al., 2013). Nonetheless, it is interesting to see that mild oxidized lipids can alter fat pads without weight gain, attributing the concept that there is far more to health than total body weight.

Duration

Another factor influencing the outcome of studies is their duration. The effects of diet is most often quite low, and might need some time to become detectable. However, this varies with type of fatty acid. MCTs seem to have a quite rapid effect, illustrated by an acutely higher diet-induced thermogenesis in MCT versus LCT when these were given by gavage (Noguchi et al., 2002). Even though longer fatty acids have a direct stimulatory effect when administered directly on mitochondria (Shabalina et al., 2008), they need weeks to enter the endogenous lipid-pool to have an effect on the sympathet-

ic nervous system (Young et al., 1994). Actually, this seems to be the case in PUFA feeding, where the largest effects on thermogenic markers were seen in the long-term studies of 12–30 weeks (Janovska et al., 2013; Le et al., 2012; Ludwig et al., 2013).

Weak dietary inducers of thermogenesis, such as PUFA and MUFA, may need thermoneutrality and a longer duration to be more pronounced.

5.3.6 Conjugated linoleic acid (CLA), C18:2

Conjugated linoleic acids (CLA) are naturally occurring fatty acids primarily found in products from ruminants. They are a family of isomers of linoleic acid (C18:2), with two double bonds separated by a single bond between them. 17 isomers have been found to occur naturally in milk, dairy, beef, human milk and human adipose tissue (Dhiman et al., 2005). The most abundant naturally occurring isomers, which also possibly possess the most biological activity, are the cis-9, trans-11 isomer (c9t11-CLA) and the trans-10, cis-12 isomer (t10c12-CLA). The c9t11-CLA is the most abundant of food from ruminant origin (73–94%), whereas in synthetic CLA, for instance used in clinical studies and research, the t10c12-CLA accounts for half the content or more, together with c9t11-CLA (Dhiman et al., 2005). CLAs have been shown to be anti-carcinogenic (Ha et al., 1987) and reduce adiposity in rodents and humans (reviewed in Kennedy et al., 2010), where the anti adiposity effect was first described 1997 (Park et al., 1997).

Browning effect

The reduction of body fat when CLA (especially t10c12-CLA) is added to the diet is suggested to be a combination of increased energy expenditure and apoptosis, decreased pre-adipocyte differentiation and lipogenesis, and increased fatty acid oxidation in white adipose tissue (House et al., 2005). Several studies have shown an increase of UCP1 mRNA and protein in white adipose tissue when mice are fed CLA (House et al., 2005; LaRosa et al., 2006; Peters et al., 2001; Wendel et al., 2009). This effect is not seen in brown adipose tissue, which suggests that the anti-obesity effect is at least partly due to browning of white adipose tissue. Wendel et al. (2009) show that the induced UCP1 in WAT is possibly not through β_3 -adrenergic signaling, since no increase in β_3 ARs and down-stream markers were seen. However, the authors argue, this effect cannot be ruled out, due to increased levels of serum norepinephrine two hours after CLA administration, as seen in another study performed by Ohnuki et al. (2001), which could mean that the β_3 ARs are stimulated at an earlier stage (Ohnuki et al., 2001). Bonet et al. (2012), when referring to Wendel's study, discuss that the simultaneous down-regulation of the RIP 140-gene, being a co-regulator suppressing UCP1 expression (Nautiyal et al., 2013) could explain the CLA effect as an inhibitor of the inhibitory effect of RIP140 on UCP1 gene expression.

Dose-dependent

The robust anti-obesity effect of primarily t10c12-CLA administration in the diet is dose-dependent and yields larger effect, the higher the dose. However, at higher doses, apart from reducing fat mass, t10c12-CLA also induces side effects, such as increased storage of lipids in the liver and chronic inflammation (Letona et al., 2011; Poirier et al., 2005). The content of naturally occurring CLA in milk is 0.34–1.07 % of total fat and slightly less in meat (Dhiman et al., 2005) which is less than usually used in trials, where mice can be fed up to 1.5 % CLA of total diet (Shen et al., 2013).

Thus, the dose is important; when giving mice different doses of CLA, the intermediate dose was most beneficial in terms of reduced adiposity, whereas the higher dose caused lipid storage in liver. Interestingly, the UCP1 content in epididymal and inguinal white adipose depots were also higher in the intermediate dose versus the higher dose, and notably UCP1 mRNA in iWAT was highest in the lowest dose with c9t11-CLA. The most abundant isomer, c9t11-CLA, naturally occurring in food, has not been shown to be as anti-fattening as the t10c12-CLA isomer. However, c9t11-CLA has shown other benefits, such as having anti-diabetic and anti-inflammatory effects in white adipose tissue (Moloney et al., 2007).

Taken together, CLA has been shown to have an anti-obesity effect; however, the dosage and isomers have to be investigated further to obtain the most beneficial effects without unwanted side-effects.

5.4 Some other diet points

Fructose

In Paper II, all mice were fed high-fat diet but housed at two temperatures, 4 °C and 30 °C. The mice in the cold were obesity-protected to some degree, but when transferred to the warm, they gained weight very rapidly, despite a 50-times higher UCP1 content compared to warm-acclimated mice. Thus, UCP1 is not the least active, which is of course disappointing. The obesity protection from UCP1 seen in cold-acclimated mice is shut down, but the question is why did the mice gain body fat at an even faster rate than the constantly warm-acclimated mice. Some of the explanation was due to higher food intake, but not all. We do not know why they eat slightly more, but possibly there is still a habit of high energy intake. However, it is also remarkable how fast they actually do adjust to lower energy demands.

Yet another explanation could also be that the larger amount of fructose ingested in the cold (166 % more) could affect the metabolism. The ordinary high-fat diet (D12451, Research Diets), commonly used in obesity studies, is not only high in fat (45 energy%), but also high in sucrose (17%), which makes it highly palatable and obesogenic. Fructose, as opposed to glucose, is

not highly regulated. Throughout history, the low intake of fructose from fresh fruit has not put any evolutionary pressure on taking care of large amounts of fructose, such as the diet contains today, from sucrose and the recent considerable increase of high fructose corn syrup (HFCS). Fructose, like glucose, is readily taken up from the intestine and transported via the portal vein to the liver. While glucose is then spread to all tissues and is taken up and metabolized, fructose is mainly metabolized in the liver. Excess fructose bypasses the regulatory steps of glycolysis and provides additional substrate for glycogenesis and more importantly *de novo* lipogenesis and triglyceride synthesis (Basciano et al., 2005). Consequently, hepatic accumulation of triglycerides and formation of VLDL for export will increase. Furthermore, not only is there lipotoxicity as a consequence of high intake of fructose, mice also become leptin resistant, which affects appetite and energy expenditure (Shapiro et al., 2008). It could be possible that mice eating a high fructose diet in the cold are more vulnerable to the effect of fructose, since the total ingestion of the poorly regulated fructose is higher compared to mice in warmer temperatures. The ability of fructose to induce leptin resistance, insulin resistance and features of the metabolic syndrome (Basciano et al., 2005; Lustig, 2010; Tappy et al., 2010) might indirectly counteract brown fat activity and, in this case, promote subsequent higher metabolic efficiency when the brown-fat-activator (cold) is withdrawn, causing highly increased fat storage.

That the type of diet, not only the energy content, matters for the results is also clear when comparing the result for the chow-fed mice in Paper II. The chow-fed cold-acclimated mice did not show any difference in terms of body fat gain, food consumption and metabolic efficiency when moved from the cold to the warm, compared to warm-acclimated, thus, clarifying again, that the type of diet matters.

Low-Fat Control diets

To exclude one confounding factor when comparing the chain-length of fatty acids, most studies compare diets with the same dietary composition, with the only difference being exchanging one fatty acid or oil. This is apparent when discussing the Portillo et al. study (1998), where high-fat (HF) diet containing 50 energy percent coconut oil is compared to a low-fat (LF) diet containing 12 e% of olive oil. UCP1 protein in BAT is upregulated, along with a lower body weight, in coconut oil versus olive oil, which was interpreted such that the coconut oil induces the thermogenic effect; however it could be the higher fat amount *per se*, which has been showed to increase UCP1 in BAT (Feldmann et al., 2009; LeBlanc et al., 2003; Mercer and Trayhurn, 1984). Furthermore, when comparing a low-fat diet with a high-fat diet, it is also important to consider which type of low-fat diet is used. A natural mix of grain (chow) can show different results compared to a low-fat semi-synthetic control diet. Benoit et al. (2013), showed that semi-synthetic

high-fat fed mice, compared to semi-synthetic low-fat fed, did not differ in body weight gain, BAT weight and some WAT depots, in contrast to a semi-synthetic high-fat diet versus a chow-fed where the same parameters (Benoit et al., 2013) showed difference in mentioned parameters. Thus, to both be able to repeat an experiment and to exclude variability it can be recommended to use a semi-synthetic control diet, at least it is of importance to be aware of the importance of the type of control diet (Hoevenaars et al., 2012). Rothwell and Stock (1987) used a stock diet (which could be chow), and tube-fed rats with either water or MCT oil. Despite the same energy intake, MCT-fed rats increased energy expenditure, possibly due to increased BAT activity, leading to decreased body weight and metabolic efficiency. Whether this is an MCT effect or a lipid effect *per se* is not completely clear. However, when taken together with other results on MCT, it seems likely that MCT do have an effect on BAT. Nonetheless, to get a clear result concerning whether the studied fatty acid, and not the fat content, is responsible for the results, it is recommended to use the same composition and ratio of macronutrients.

Essential nutrients

When studying effects of nutrient compounds, it is important to have control over the amounts of essential nutrients. Both low protein diets (Rothwell et al., 1983), deprivation of certain amino acids (Cheng et al., 2010; Hasek et al., 2010; Plaisance et al., 2010) and low amounts of essential fatty acids (EFA) (Yazbeck et al., 1989) or high amounts of EFA (Nedergaard et al., 1983) show increased thermogenic activities in BAT or browning of WAT in rodents.

In the study of Yazbeck et al. (1989), the rats (Long Evans rats) weighed only 35 grams at the start, which means a very young age. When feeding the rats an EFA-deficient diet, they gained less weight, had lower metabolic efficiency (body fat gain/energy food intake), higher resting metabolic rate, which was suggested to be due to the higher thermogenic capacity in BAT. The increased thermogenesis could evolutionarily been a way for the body to cope with low nutritive density to have a means to allow overeating to obtain essential nutrients without obesity. However, rats in this study did not overeat despite increased energy expenditure, probably giving them severe problems, including growth retardation. In contrast, Nedergaard et al. (1983) did not see any increased thermogenesis in low EFA-fed rats, but saw an increase in high EFA-fed rats, indicating diet-induced thermogenesis.

As with many essential and non-essential nutrients, the amount that is optimal is not clarified. By giving EFA-deficient rats different amounts of linoleic acid, the intermediate dose, 2.5 % opposed to 1 % and 5 %, was most beneficial in terms of lipolysis and insulin sensitivity (Harant-Farrugia et al., 2014), indicating that EFA do not have to be supplied in high amounts. This was also observed in the Nedergaard study, where the intermediate dose (3

%) was most beneficial in health parameters such as skin condition and body weight, as opposed to both low (0.3 %) and high (10 %) doses of EFA.

Indirect effects on BAT by handling food-producing animals

A little sidetrack into dietary fatty acids affecting BAT is that how we feed our cattle can indirectly affect the thermogenic properties of the animal-derived products. When cattle were fed a grass-based diet compared to a grain-based diet, the muscle tissue contained 2.3 fold more LC-n-3 PUFA (Cherfaoui et al., 2013). Another interesting study showing that how we treat and feed animals affects the composition of, for instance, fatty acids in our food, which, in turn, affects how we respond metabolically, is the following. Ruminant products such as milk and beef contain up to 500 % more CLA when cows are fed all pasture feed (fresh grass) versus being grain fed (Dhiman et al., 1999). To take it further and speculate: when we feed our cows grain instead of grass, we might remove a large fraction of beneficial fatty acids, CLA and LC n-3 PUFA, resulting in reduced adaptive thermogenesis.

Diet diversity

Despite a seemingly vast diversity of food items in the grocery shops, the truth is that we have decreased the number of species we ingest. From about 150 species being ingested per week a thousand years ago, we now only consume about 20 today, of which most are processed (Spector, 2015). This might also be one of the issues in laboratory diets. Chow contains many ingredients and complex food components, whereas the semi-synthetic high-fat diets contain only a few, highly purified ingredients.

Thus, it cannot be excluded that some of the metabolic problems today arise not only from what we eat, but also from what we do not eat.

6. Is UCP1 obesity-protective?

As the main function of brown adipose tissue is to produce heat by uncoupling protein 1 (UCP1), low temperature is the strongest inducer of brown fat recruitment, as well as of its activity. The difference in UCP1 protein between warm-acclimated (30 °C) and cold-acclimated (4 °C) mice is in the order of 10–20-fold (Fischer et al., 2016; Mattsson et al., 2010). However, food (i. e. obesogenic food) also has the capacity to recruit brown adipose tissue, which was first demonstrated in 1979 by Rothwell and Stock in rats fed a cafeteria diet. Both mRNA and protein of UCP1 are increased by HFD versus chow, although the variation is large (Fromme and Klingenspor, 2011; García-Ruiz et al., 2015).

That certain diets have a not inconsiderable capacity to increase UCP1-dependent thermogenesis is referred to as facultative diet-induced thermogenesis. To unmask the lower effect from diet versus cold (even mild, as in room temperature), the animals are preferably housed in a thermoneutral temperature. In contrast to the greatly increased total UCP1 protein levels by cold (Paper II), the high-fat diet induced UCP1 levels are moderately increased (Paper I and III). Recruitment in cold is well studied, whereas the diet recruitment of UCP1 needs further investigation. The latter issue will be discussed in this section.

At thermoneutrality, diet-dependent UCP1 recruitment has not (yet) been studied to a large extent. However, we have robust indications that obesogenic diets versus chow increase both *Ucp1* mRNA (unpublished) and UCP1 protein about 5-fold compared to chow (Feldmann et al., 2009, Paper I). Thus, this diet effect on UCP1 has the potential to be significant for energy expenditure and accompanying weight homeostasis, even at thermoneutrality. Interestingly, combining cold and obesogenic food gave a 50-fold increase in UCP1 protein when mice had been in the cold for 4 weeks compared to being in a thermoneutral temperature (Paper II).

6.1 Fluctuations in *Ucp1* expression

Recruitment of *Ucp1* mRNA takes only hours, whereas full recruitment of UCP1 protein rather takes several weeks (Nedergaard and Cannon, 2013). Thus, the mRNA level of UCP1 can be a sign of activation, since the process of recruitment starts with transcription. The rather quick response to a stimu-

lus is apparent when measuring mRNA in BAT from mice where the food was removed 7 hours before euthanizing the mice, showing a 25 % decrease (Figure 6A). Furthermore, Ucp1 mRNA seems to have a diurnal variation (Figure 6B), which coincides with wakefulness and possibly eating, and also body weight changes (Figure 6C). The circadian rhythm of Ucp1 has been observed earlier with higher values during the night, which also correlated with the highest BAT temperatures (Gerhart-Hines et al., 2013). Gerhart-Hines et al. (2013) had the highest values of Ucp1 mRNA later at night and in the early morning, whereas we had higher values early in the night (Fig. 5B). Nonetheless, some diurnal variation of Ucp1 mRNA seems likely (Gerhart-Hines et al., 2013; Figure 6B), and there is also a suggested clock mechanism in BAT that could be pharmacologically targeted to even counteract obesity (Nam et al., 2015), but all this needs further investigation.

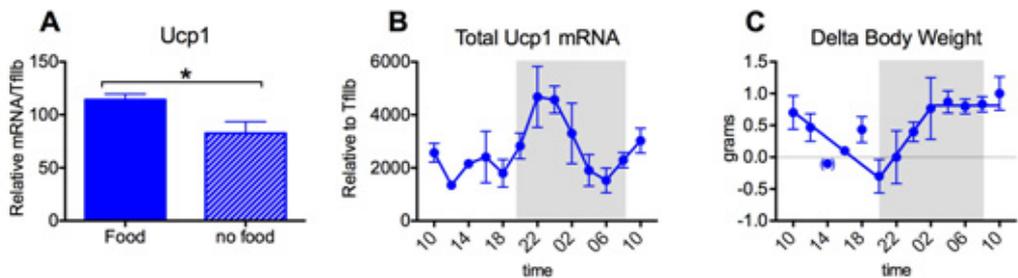


Figure 6. Ucp1 mRNA in IBAT in chow-fed 12 weeks old mice. A: Mice were euthanized at 10 pm. Half of them had the food removed 7 hours before. n=7. BC: Diurnal study with mice euthanized every other hour for 24 hours. n = 3. (von Essen, unpublished data).

6.2 Does UCP1 matter for obesity?

High-fat diet-dependent increases in UCP1 protein are highly variable, but the effect seems to be independent of fat content and duration (Fromme and Klingenspor, 2011). There is nonetheless a clear UCP1 increase following HFD (Feldmann et al., 2009; Fromme and Klingenspor, 2011; Paper I, III). The question then is; does this diet-recruited UCP1 protein have an impact on protection against obesity. One way to study this is to compare wild-type with UCP1-ablated mice at thermoneutrality (to distinguish the effect from that of cold), and indeed the wild-type mice are to some extent protected against obesity (Feldmann et al., 2009; Rowland et al., 2016; Paper I). However, some data show that UCP1-deficient mice at thermoneutrality do not gain more weight but do have a tendency to higher adiposity (Liu et al., 2003; Rowland et al., 2015) or show no effect or the opposite result (Paper III). Since the diet effect depending on genotype is absent sometimes and the effect is at any case not very large, I compiled a number of studies from our lab, using both wild-type and UCP1-ablated mice, to be able to reveal a pat-

tern. I only used studies with male C57Bl/6 mice that had been fed high-fat diet (HFD) for 4 weeks at thermoneutrality. Furthermore, only studies where both genotypes had been studied together were included. I collected 10 cohorts and about 75 wild-type and 70 UCP1-ablated mice.

This overview of C57Bl/6 male mice fed high-fat diet for 4 weeks at thermoneutrality showed varied and not completely consistent results. Starting weights and n-numbers of the cohorts from Figures 7 to 12 are compiled in Table 2.

Table 2. C57Bl/6 wild-type and UCP1-ablated male mice, fed HFD for 4 weeks at thermoneutrality. All studies are on male mice at the age of 8–16 weeks at the start of the study, housed at thermoneutrality and fed high-fat diet (D12451, Reserach Diets) for 4 weeks. UCP1(+/+) mice were C57Bl/6 from our own breeding or obtained from Scanbur (Europe). Mice from Scanbur are indicated with "S" in the table. UCP1(-/-) mice were from our own breeding and descendents of the mice described in Enerbäck et al. (1997) and then backcrossed to the C57BL/6 strain for more than ten generations.

Cohort	UCP1(+/+) Initial weight \pm SEM	UCP1(-/-) Initial weight \pm SEM	Reference
1	24.6 \pm 0.5 g (n = 6)	24.9 \pm 0.1 g (n = 6)	Feldman et al. 2009
2	27.9 \pm 0.5 (n = 6)	25.7 \pm 0.4 (n = 6)	Feldman et al. 2009
3	28.1 \pm 0.6 g (n = 7); all S	23.2 \pm 0.8 g (n = 5)	Paper II
4	25.7 \pm 0.6 g (n = 8); 4 S	27.6 \pm 0.8 g (n = 8)	Paper I
5	30.8 \pm 0.8 g (n = 10)	26.5 \pm 0.6 g (n = 12)	Paper I
6	25.0 \pm 0.6 g (n = 10)	22.1 \pm 0.2 g (n = 6)	Paper I
7	27.0 \pm 0.1 g (n = 8); all S	24.6 \pm 1.0 g (n = 6)	Paper III
8	25.7 \pm 0.4 g (n = 10)	23.2 \pm 0.4 g (n = 9)	Unpublished
9	27.9 \pm 1.0 g (n = 6)	25.7 \pm 0.4 g (n = 6)	Unpublished ¹
10	28.78 \pm 0.5 g (n = 6)	27.2 \pm 0.5 g (n = 7)	Unpublished ²

¹ Monitored by Gustavo Abreu-Vieira

² Monitored by Anastasia Kalinovich

6.2.1 Body weight

In all cohorts except number 4, the UCP1-ablated mice were smaller at the start (Table 2 and Figure 7A). This resulted in no weight differences after 4 weeks of HFD feeding (Figure 7B). However, the weight gain in the UCP1-ablated mice was significantly larger when compiling all the 70 mice from each genotype (Figure 7C). Considering separate cohorts, only number 7 had higher body weight gain in the wild-type compared to the UCP1-ablated mice. However, only three cohorts showed significantly higher body weight gain in the UCP1-ablated mice (Figure 7D), which increased to six cohort when calculating percent body weight gain, depending on smaller starting weights for the UCP1-ablated mice (Figure 7E). Instead of looking at all the individual data, the means for all mice show significance (Figure 7F). Note

that in both genotypes the variability is large, but in fact almost all the high values in the wild-type mice belong to cohort number 7 indicated in pink. Thus, the weight gain is not only genotype-dependent, in that the UCP1-ablated mice gained more weight both on an individual level (Figure 7G) or when comparing all studies together (Figure 7G), but there is also a cohort-dependent effect (Figure 7DE). Most of the time, the UCP1-ablated mice gain more weight, sometimes no difference and seldom the wild-type gain more. In all figures, cohort 7 (indicated in pink) is conspicuous and it would be a little tempting to exclude this cohort, since it could be considered an outlier in differing so much from the other wild-type groups. However, this is how it sometimes looks (the world does contain outliers) and by compiling many cohorts, the impact from “outliers” is not so large and thus, the “real” effect can still be revealed.

Thus, it is important to be aware of this; since when referring only to one study, the results can differ markedly.

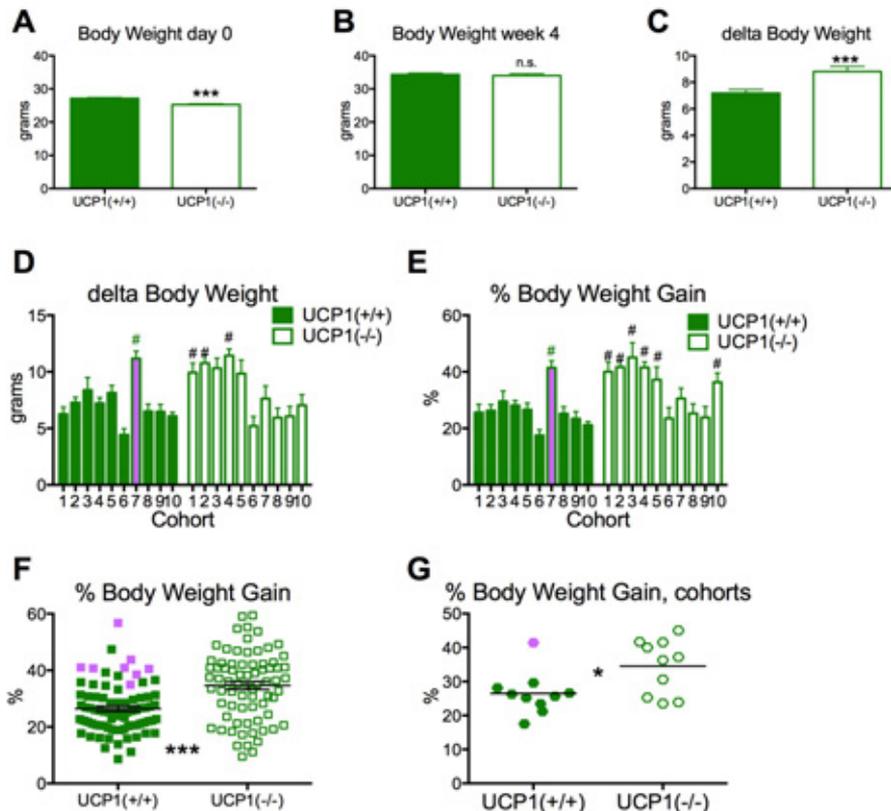


Figure 7. Body weight for of C57Bl/6 mice (UCP1^{+/+} and UCP1^{-/-}) listed in Table 2. A–C: Mean for all individual mice from the 10 cohorts, n=73-79. D–E: means for each cohort respectively, n=5-12. F: each dot resembles one mouse. G: Each dot is one cohort (from E). Cohort 7 indicated in pink. Significance indicated as * ≤ 0.05 ; ** < 0.01 ; *** < 0.001 when comparing wild-type with UCP1-ablated; # (black) when UCP1-ablated exceeds wild-type; # (green) when wild-type exceeds UCP1-ablated.

Furthermore, some of the studies did not show any difference between the genotypes, for instance cohort 6, which was used in Paper I for the part where the food was removed (Paper I, Figure 4). However, I happened to keep these mice, and for the first 4 weeks, both genotypes had identical body weight gain and body fat gain. But, after leaving them for 16 more weeks, the UCP1-ablated mice had significantly higher body weight and body fat (Figure 8AB). Thus, the UCP1-ablated mice clearly gained more weight, but the effect did not appear until after some weeks. This is also in accordance with another study, where the effect of HFD feeding was only seen after 3 months (Kontani et al., 2005) and not after normally 4 weeks. Furthermore, even the chow fed UCP1(-/-) gained more weight after 20 weeks, despite a lower starting weight compared to wild-type mice. Although Feldman et al. (2009) already saw this in a 4-weeks study, these results had never been repeated for chow-fed mice, only for HFD-fed mice (Feldmann et al., 2009; Rowland et al., 2016; Paper I).

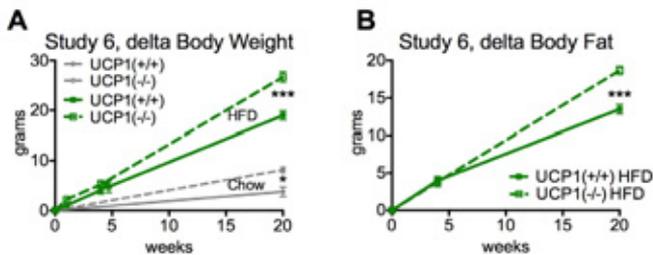


Figure 8. Cohort 6. (Paper I until 4 weeks, thereafter not published). Significance indicated as in Figure 6.

6.2.2 Metabolic efficiency

For most of the cohorts, body composition has been measured, but not for all. In some cases, I only had body weight, but nevertheless, most of the weight gain is body fat, see Figure 9. This example is from Paper I, but the course of events usually follows the same pattern, most body weight gain is body fat. Thus, body weight gain, body weight gain per food intake and percent body weight gain are almost as good measurements as body fat in obesity studies (unless, of course, the mice exercise, but that is not the case in these contexts). Thus, metabolic efficiency can also be indicated as grams of body weight gain per ingested food energy.

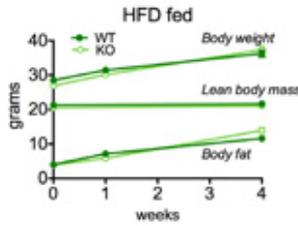


Figure 9. Body composition during 4 weeks of HFD feeding (Paper I).

In all cohorts except one, food intake was measured and then the metabolic efficiency can be calculated. In all studies, the food intake was similar between the two genotypes or in some cases lower for the UCP1-ablated (Paper 1). Thus, the metabolic efficiency follows the same pattern as in the case of body fat gain. Only one cohort, again number 7 (Paper III), showed higher fat-storing capacity for the wild-type compared to the UCP1-ablated (Figure 10A), resulting in significantly higher body weight gain per ingested food energy totally (Figure 10BC).

In those cases where the body composition was obtained, the pattern is more pronounced (Figure 10DEF). The outlier wild-type cohort number 7 was not measured. Thus the individual pattern was clearly that a larger fraction of the UCP1-ablated mice are more efficient in storing food as body fat (Figure 10F). Furthermore, the variability in UCP1-ablated mice is larger in that some of the mice do have as low metabolic efficiency as the wild-type mice, but a larger proportion of the UCP1-deficient mice have higher metabolic efficiency.

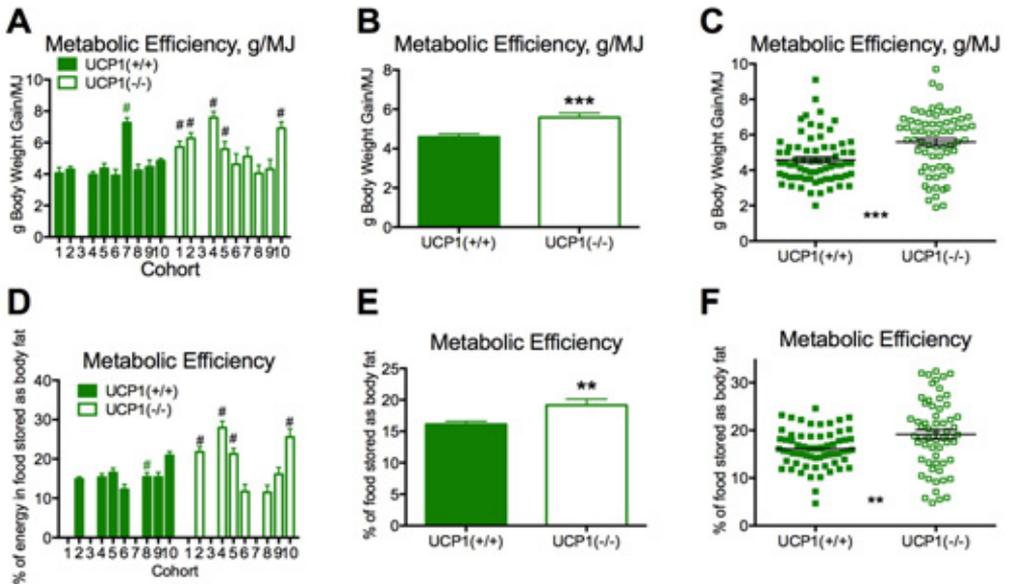


Figure 10. Metabolic efficiency in C57Bl/6 mice from Table 2. Significance indicated as in Figure 6.

6.2.3 Obesity Index (OBI) as a mouse BMI

In humans, we often use body mass index (BMI) to categorize weight imbalances (or normal weight). By taking the square of body mass (kilogram) and dividing by the body height (meter), we can define normal weight (20-25), underweight (>20), overweight (25–30) and obesity (<30). Interestingly, high BMI is inversely correlated to low levels of BAT (Cypess et al., 20010; van Marken Lichtenbelt et al., 2014, 2009; Zingaretti et al., 2009). In mice, we instead have an obesity index (which I will abbreviate OBI), by dividing body fat with lean body mass, as an indicator of excess lipid storing. Also this measure resulted in higher values for the UCP1-ablated mice compared to wild-type mice (Figure 11ABC), indicating higher adiposity. Furthermore, most cohorts showed a higher obesity index for the UCP1-ablated mice and so did the individual mice. Cohort 7 (Paper III) was measured week 3 instead of week 4, which resulted in no higher values for the wild-type, as was shown for the other parameters in that particular cohort.

Furthermore, to see the development of the obesity index over time by diet and temperature, I compiled some complementary studies together with B6 mice, in Figure 11DE. We can consider 12 week old wild-type housed in room temperature (RT, ≈ 21 °C) as the “standard” mouse. The mice from Paper IV, Figure 7, had this condition at the start, with a mean OBI of 0.15. After 4 weeks, the OBI had increased to 0.21, which indicates a slight age-dependent increase (Figure 11D, two right columns). The rest of the mice had been at thermoneutral temperature and for chow-fed mice the increase was slightly higher and more so for the HFD-fed mice. Furthermore, the UCP1-ablated mice showed slightly higher OBI on chow than the wild-type mice, and much more in the HFD fed mice.

Thus, the obesity index shows age-dependent, temperature-dependent and genotype-dependent effects. To make it a little more useful, I suggest some limits for categorizing adiposity as follows: up to 0.25 in obesity index, the mice can be considered normal weight, 0.25–0.45 overweight and > 0.45 as obese. This would thus indicate that both high-fat feeding and high temperature leads to obesity, which is worsened for the UCP1-ablated mice.

There also seems to be an aging effect, at least at thermoneutrality. Sometimes the mice are kept for further studies and we have the opportunity to study them at older ages. In wild-type mice, they increase the OBI slightly with age even when chow-fed at thermoneutrality, where some individuals even become obese, although this particular cohort at the age of 35 weeks did not (Figure 11E, 4th column). However, mice lacking UCP1 are more prone to obesity, where half of the male 40 week-old mice were obese (over 0.45), and a small group of females had an OBI over 1.0 (right column), notably when chow fed (Figure 11E).

Among cohorts and studies, there is some significant variability. However, taken together the clear tendency is that at thermoneutrality the UCP1-ablated mice are more susceptible to obesity, even when chow fed.

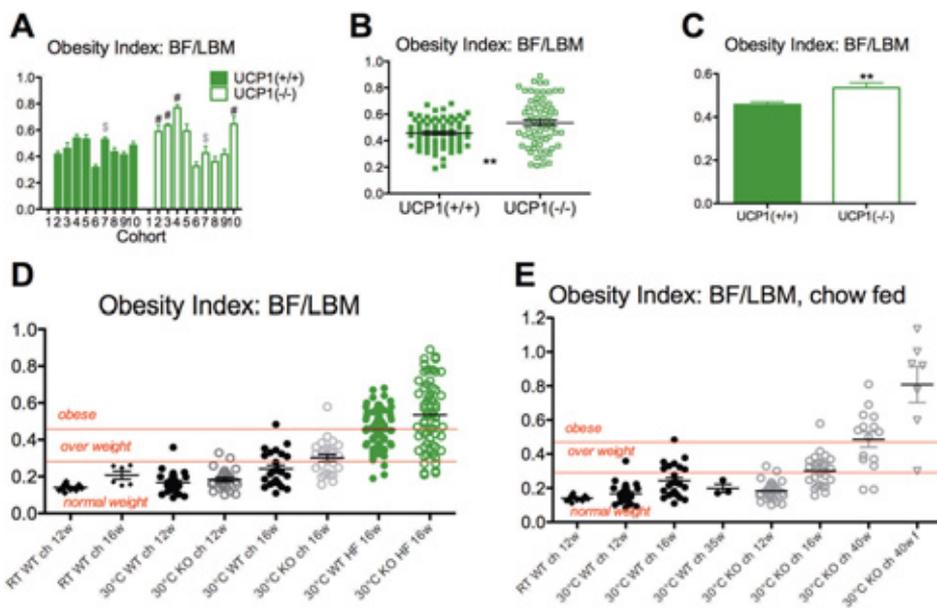


Figure 11. Obesity index (body fat/lean body mass) ABC: Measured before and after 4 weeks of HFD. DE: Measured at indicated age of mice. RT = room temperature ($\approx 22^\circ\text{C}$); w = weeks of age; WT = wild-type mice; KO = UCP1-ablated mice. Significance indicated as in Figure 6. \$ = measured at 3 weeks.

6.2.4 Body fat, oxygen consumption and UCP1 activity

Even though UCP1 amount is correlated to some protection from obesity when wild-type mice are compared to UCP1-ablated mice (Feldmann et al., 2009; Kontani et al., 2005; Rowland et al., 2016), UCP1-amount is also correlated to amount of body fat (Figure 12AB, from Paper I and III). This concomitant body fat gain and recruitment of UCP1 might seem somewhat contradictory. However, it has been suggested that BAT also is involved in increasing energy expenditure to allow for uptake of sufficient protein and other nutrients when overeating a nutritionally poor diet (Cannon and Nedergaard, 2004). Since serum leptin correlates to body fat (e.g. Iwamoto et al., 2011; Figure 12C) and to *Ucp1* mRNA levels (Figure 12D), it could be suggested that this UCP1 recruitment could be leptin-mediated. However, this has been shown to not be the case (Fischer et al., 2016a). Thus, the question of why UCP1 correlates with body fat and/or HFD, remains to be further investigated.

Rats have been shown to have higher levels of circulating norepinephrine when they are fed lard compared to chow, as a result of increased SNS activity (Young et al., 1994). Furthermore, the same group showed that when adding sucrose to the chow diet, the rats also had an increased SNS activity in IBAT. The HFD we use contains both lard (40 %) and sucrose (17 %), which

could thus fit with higher SNS activity in IBAT with a subsequent higher NE in the circulation, thus a slightly higher SNS tone, which could contribute to some recruitment/activation of UCP1.

Increased sympathetic tone has been shown to be significantly higher by circulating norepinephrine (NE) in both obese and HFD-fed rats (Olea et al., 2014) twice the concentration versus chow-fed. One study in mice showed a slight increase in plasma NE in HFD-fed versus chow-fed mice (Male C57Bl/6) as well as higher concentrations of NE in hypothalamus (D'Souza and Abraham, 2016). Thus, obesity and HFD-feeding is associated with increased sympathetic tone, which likely affect BAT.

Furthermore, mice eating a high-fat diet have higher levels of fatty acids and triglycerides in serum compared to chow (Glastras et al., 2016). Since fatty acids have a direct effect on UCP1 in isolated mitochondria from BAT, (Shabalina et al., 2008), the higher amount of fatty acids *per se* in the serum could contribute to a low activation of BAT. However, this is not confirmed *in vivo*.

In Paper I oxygen consumption was measured and UCP1 protein was determined, in parallel. A clear correlation was seen between amount of UCP1 and 4 weeks of HFD feeding, especially at eating time (7–12 pm) (Figure 12E). It has been shown that most of the food is consumed during the first period of the night, starting one hour before lights off (Mukherji et al., 2015; Tempel et al., 1989; Tsai et al., 2013), which also coincides with the largest energy expenditure (Tsai et al., 2013). Thus, it is reasonable to assume that the higher energy expenditure in HFD-fed wild-type mice occurring mainly between 7 pm to midnight is eating-dependent and furthermore UCP1-dependent. This diet- and UCP1-dependent higher energy expenditure can thus be termed facultative diet-induced thermogenesis.

One objection could be that the UCP1-ablated mice had lower food intake (Paper I), which could explain the lower energy expenditure due to decreased demand for obligatory DIT in the UCP1-ablated. However, even though the wild-type mice did eat 17 % more than the UCP1-ablated mice (50.7 kJ/day versus 42.0 kJ/day, $p = 0.007$) in the metabolic chambers, the difference in energy cost of handling the ingested food (obligatory DIT) would not be large. Based on calculations using different thermic effect of macronutrients⁶ yields 4.0 kJ/day for wild-type mice and 3.3 kJ/day for UCP1-ablated to digest the food eaten. A difference of 0.7 kJ/day extra in expenditure would be difficult to detect when measuring oxygen consumption. To estimate energy expenditure from oxygen consumption and respiratory exchange ratio (RER), the Weir equation can be used, yielding a difference of 4.2 kJ in daily energy

⁶ fat (2.5 %), carbohydrates (7.5 %) and protein (25 %) respectively (Food and Nutrition Board, 2005), together with the composition of the HFD would yield 8.7 % thermic effect of measured food intake.

expenditure between HFD fed wild-type and UCP1-knockout mice⁷. This is a higher value than the calculated difference in obligatory DIT (0.7 kJ/day) between the genotypes when HFD-fed. Thus, most of the extra oxygen that the wild-type mice consume is not due to slightly higher demand for obligatory digestion.

Furthermore, the difference in body fat gain in Paper I is 3.5 grams in 4 weeks between the two HFD-fed genotypes, which equals 130 kJ (3.5 x 37 kJ/g fat). Dividing this number with 28 days yields 4.6 kJ/day in body fat gain for the UCP1-ablated. It fits remarkably well (assuming this difference was the same for the whole period of 4 weeks, which we do not know, although it seems likely). Wild-type mice expend 4.2 kJ (whereof 0.7 kJ is for digesting the extra food) more per day and the UCP1-ablated mice gain 4.6 kJ more body fat per day. This thus explains the higher metabolic efficiency in the UCP1-ablated mice. The facultative DIT can furthermore be estimated to be around 10 %.⁸

Taken together, the data from Papers I, III and Figure 12 indicate that HFD feeding is associated with UCP1 recruitment. This rather clear recruitment of UCP1 from HFD is likely to result in a low activation or slight increase in sympathetic tone, which explains the higher energy expenditure (\approx 10% termed facultative diet-induced thermogenesis) giving partial obesity protection.

⁷ (16.3 J/ml x ml/min O₂ used) + (4.6 J/ml x ml/min O₂ used x RER) (Weir, 1949), yielding J/min (or energy per some chosen time unit). In theory the RER for the high-fat diet is 0.84 calculated by using %carbohydrates + %protein + %lipids in the high-fat diet and an RER of 1, 0.85 and 0.7 respectively (Livesey and Elia, 1988). Taking the total oxygen consumption for 24 hours day and using the Weir equation would yield a difference of 5 kJ in daily energy expenditure (37 kJ for WT and 32 for KO, p = 0.007).

⁸ By taking the calculated higher energy expenditure of 4.2 kJ/day minus obligatory DIT of 0.7, which is about 3.5 kJ/day, and dividing it by total energy expenditure of 37 KJ/day for wildtype.

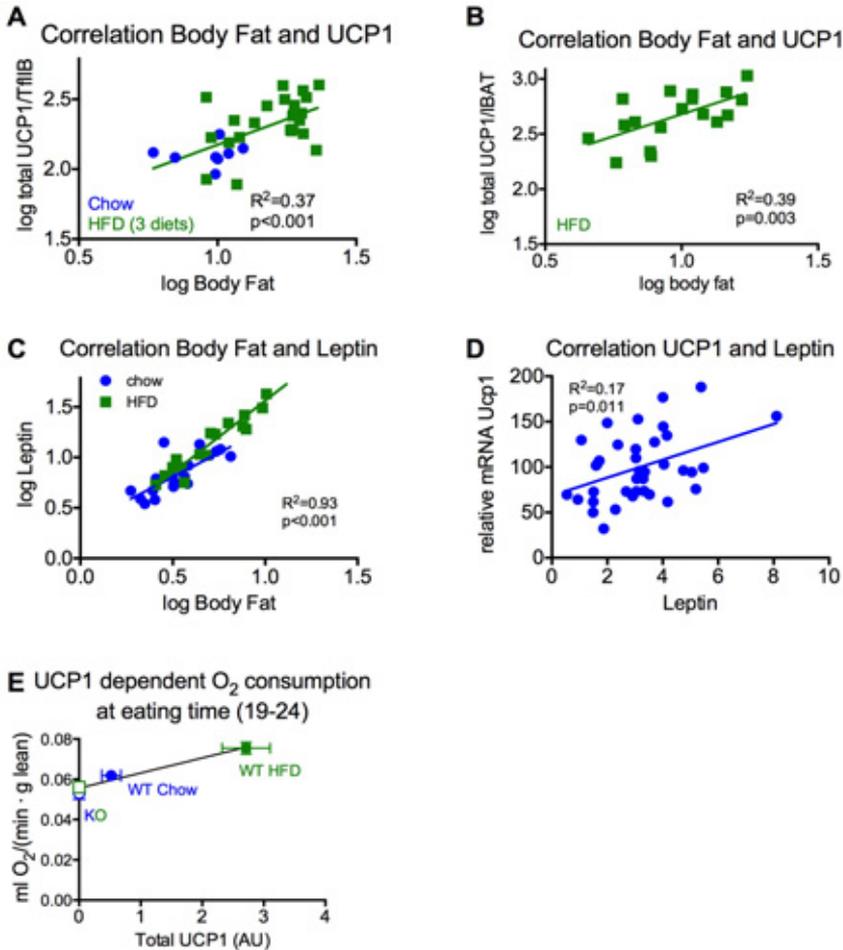


Figure 12. UCP1 correlates with body fat, leptin and oxygen consumption. A: Mice from Paper III fed chow and three types of high-fat diets. B: Mice from Paper I fed high-fat diet. C: Mice fed chow or high-fat diet for 4 weeks, 16 weeks old (unpublished). D: Same mice as in D. E. Wild-type (filled circles) and UCP1-ablated (open circles), Oxygen consumption at eating time correlates with total UCP1 (Paper I).

6.2.5 UCP1 in outbred NMRI mice

The previous figures (Figure 7 to 12) have concerned male C57BL/6 (B6) mice at thermoneutrality. B6 mice are inbred and obesity-prone and have been shown to be sensitive to UCP1 deficiency (i.e. gaining excess of lipids). To broaden the picture a bit, I have also studied outbred NMRI mice. We constructed a UCP1-ablated NMRI stock by breeding the wild-type with UCP1-ablated mice on an FVB-background. Thus, we got a first generation of heterozygotes (UCP1+/-) and by breeding these mice for several generations we obtained 3 genotypes: UCP1+/, UCP1+/- and UCP1-/. To keep

the stock outbred, new mates were obtained from outside (Scanbur, Europe) and bred in some of the generations. By doing this, we obtained a unique (semi)outbred stock of UCP1-ablated mice, which thus more resembles humans (as we are outbred) in having larger variability. Since the goal of using outbred stocks is to maintain maximum heterozygosity to minimize systematic genetic change (Chia et al., 2005), the stock is also less amenable to show differences when effects are low. Furthermore, the NMRI stock is not obesity prone, which could also mean that the presence or not of UCP1 would not have as large an effect as in B6. Nonetheless, I monitored about 200 mice from 19 litters, males and females, the 3 genotypes, a few on chow and most on HFD (Table 3).

Table 3. The NMRI study.

Wildtype (WT), UCP1+/+:	22 males	15 females
Heterozygot (HZ), UCP1+/-:	37 males	36 females
Knockout (KO), UCP1-/-:	42 males	30 females
81 female (23.3–44.8 g, mean 30.1 g)		
122 male (29.1–46.8 g, mean 37.8 g)		
19 litters		

NMRI mice, being outbred, show larger variation than for instance B6, which is obvious looking at the starting weights (Table 3). However, in terms of body weight gain, the NMRI do not gain as much weight as the obesity prone B6 strain (Figure 13AB). Sometimes the mean values can be somewhat meaningless, due to high variation, and the metabolic efficiency did not show any significant difference between the groups (Figure 13C). However, plotting the individual values reveal an interesting, larger dispersion in males (Figure 13D). This is also the case for the females, but here the “high-gainers” were very few (Figure 13EF). Grouping all males and females for only wild-type and UCP1-ablated mice showed no significant differences between groups, but a small group of outliers gaining larger amounts than the others in the UCP1-ablated group was evident (Figure 13G).

Altogether, wild-type and heterozygotes show the same effect, and UCP1-ablated mice did not differ very much, but had some individuals being more susceptible to obesity. Despite monitoring about 200 mice, the number could still be too few in each group, due to the large variation, which is expected, since the mice are outbred. However, there is a risk of masking a real effect. Furthermore, there was a larger difference between litters than between genotypes in that only a few litters contained the “high-gainers”.

Nonetheless, there seems to be a small group of more obesity-prone individuals in the UCP1-ablated group, an issue that would be interesting to investigate further. Since the NMRI are more different from each other and in

that sense a little more like humans, it cannot be excluded that there is a small UCP1-negative group of individuals, which are more obesity-prone.

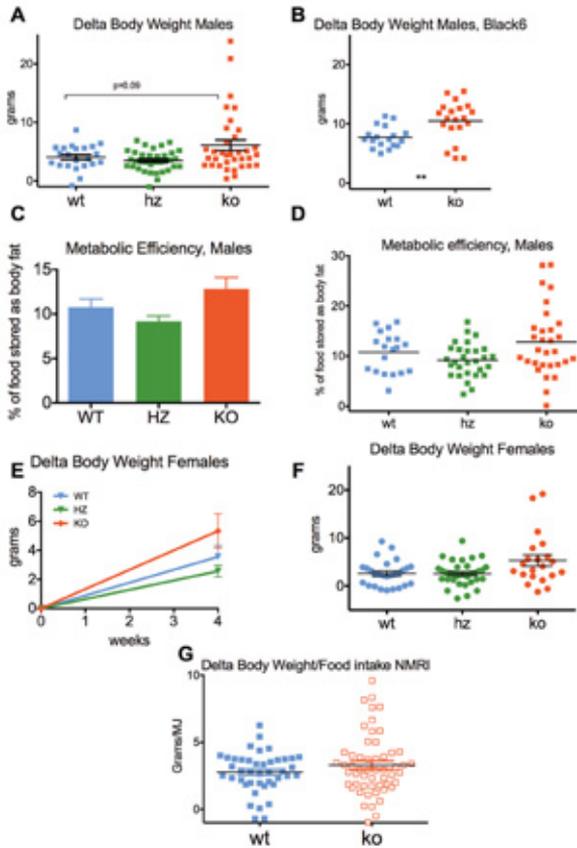


Figure 13. NMRI mice from Table 3. WT=UCP1+/+; HZ=UCP1+/-; KO=UCP1-/-.

6.2.6 UCP1-dependent metabolic efficiency

So far, I have concluded that in B6, UCP1 has a significant effect on body weight in that the UCP1-ablated mice had significantly higher metabolic efficiency than the wild-type mice (Paper 1, Figures 6 to 11). Furthermore, UCP1 protein amount in wild-type mice correlates to body fat and HFD-fed mice have higher energy expenditure. In addition, there is a possible disadvantage even in the outbred NMRI stock for some individuals not possessing UCP1. To draw some conclusions even further on the advantages of having UCP1 or not, I will also include a few other cohorts, including the 129sv strain (inbred, obesity resistant).

The 129sv mice are only from one study and, as discussed before, even an inbred strain can vary to a large degree, but I wanted to include as many mice, genders and strains as possible. The overview in Figure 14A shows

firstly that chow-fed mice as expected, have lower metabolic efficiency than HFD-fed mice. Secondly, C57Bl/6 mice (obesity-prone) as a group have the highest metabolic efficiency. Thirdly, female mice and UCP1-ablated mice (both males and females) show the largest variation. Also, 129sv mice, considered obesity-resistant, do actually eat much less and thus the metabolic efficiency is high anyway, at least in males and UCP1-ablated females.

Compiling all mice eating HFD (males, females, strains) in a wild-type (n=126) group and in an UCP1-ablated (n=132) group demonstrated that UCP1-ablated mice are significantly more efficient in storing ingested energy as body mass (Figure 14BC).

On average, UCP1-ablated mice gain almost 1 gram more body weight per ingested mega joule food (although, as discussed before, we cannot know the exact food energy, but all these mice at least ate the same diet) for 4 weeks (Figure 14C). Even though a higher rate gain of body fat does not last for ever, this higher gain might be rather large over time, as seen in the chow-fed 40-week old UCP1-ablated mice in Figure 11E.

So, back to the question, does UCP1 protect the mice from obesity? Yes, at least for a good fraction of the population.

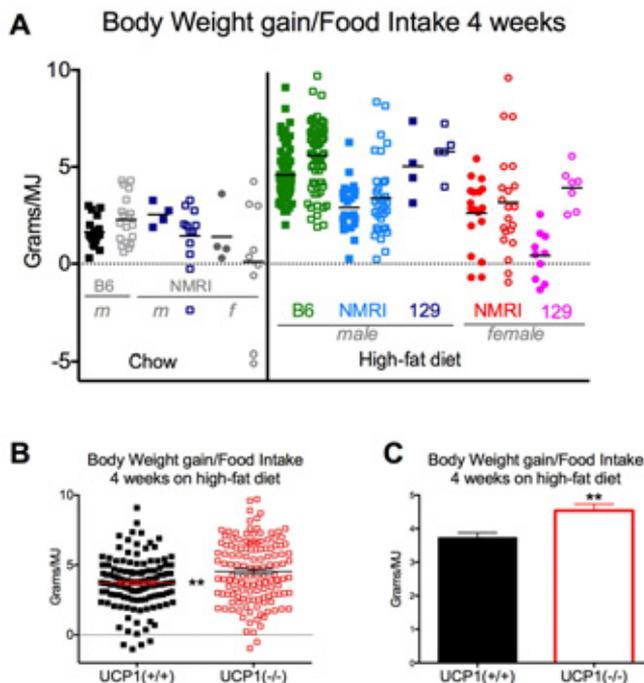


Figure 14. Wild-type versus UCP1-ablated mice – the outcome. Mice from Paper I and III, Table 2 plus from some unpublished studies (von Essen) compiled showing grams of body mass per ingested food energy. BC: wild-type, n=126; UCP1-ablated, n=132. Significans indicated as * when p<0.5 in wild-type versus UCP1-ablated.

7. Concluding remarks

The large capacity of brown adipose tissue (BAT) to expend energy as heat makes it as an interesting player in weight regulation and other metabolic conditions. Cold is the strongest recruiter and activator of BAT, illustrated in Paper II with 50-fold higher UCP1 levels in cold-acclimated versus warm-acclimated. Cold is also obviously accompanied by higher energy expenditure (Paper IV), thus protecting against weight gain (Paper II). However, also food and/or high amount of body fat at thermoneutrality had to a low but nonetheless significant recruitment and activation of UCP1 (Paper I, III).

It is somehow strange that we (and mice) do not fully compensate increased energy demand with increased food intake and subsequent weight gain. The “low-gainer groups” included in this thesis did not eat more to gain as much as the “high-gainer groups”: the wild-type versus UCP1-ablated mice (Paper I) or the cold-exposed versus the warm-exposed (Paper II), or the medium-chain fatty acid fed versus the long-chain fatty acid fed (Paper III). Increasing the energy expenditure to even a small extent (long-term though) in any way (e.g. by physical activity or cold) is not fully compensated by increased food intake. It is not fully known why mice (and humans) reduce food intake less than they reduce energy expenditure at thermoneutrality, resulting in increased adiposity (Xiao et al., 2015). This is one reason why increased energy expenditure is desirable and why BAT is interesting (to increase energy expenditure without physical movement and cold and to thus permit higher food intake).

There is a clear effect of possessing UCP1 in low amounts at thermoneutrality, in that both UCP1-positive mice (et al., 2009; Rowland et al., 2016; Paper I) and UCP1/BAT-positive humans (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Zingaretti et al., 2009) have lower body weight. There is also a clear indication that just having high levels of UCP1 protein does not in itself protect against obesity at all (Paper II). Recruitment is one side of the story and activation is the other. To have the beneficial effects from UCP1 (increased energy expenditure) both need to be present.

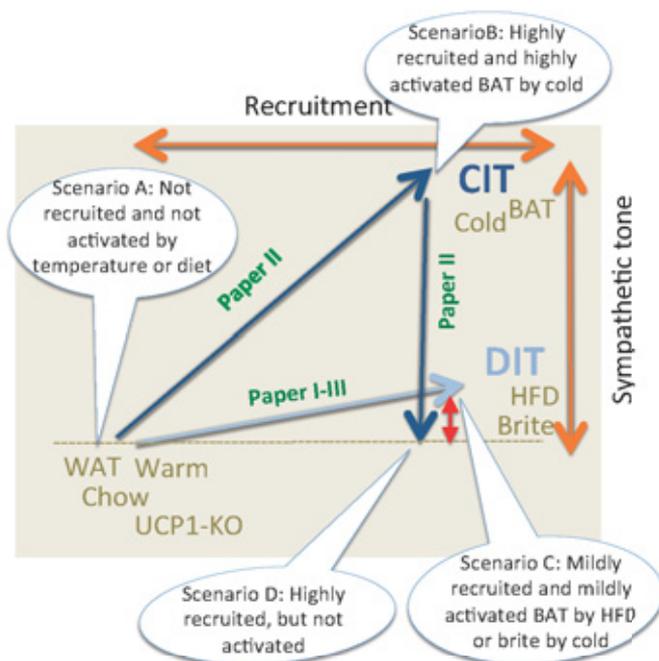


Figure 15. A summary of the degree of recruitment and activation of UCP1 (or degree of sympathetic tone) discussed in the Papers in present thesis. Details are discussed in the main text.

Figure 15 is an attempt to discuss some of the scenarios involved in Papers I-III (i.e. UCP1) and to summarize the present thesis. The two main states of thermogenic outcome are the degree of recruitment and the degree of sympathetic tone (i.e. activation of UCP1) (Cannon and Nedergaard, 2004). Noteworthy is also that the discussion in this thesis is mainly the effects of diet (and temperature) on BAT activity. It is also of course possible to achieve thermogenesis by chemical uncouplers or targeting expression of UCP1 by pharmacological stimuli in BAT and even in other tissues (Ost et al., 2017), but this will not be discussed here.

Scenario A resembles when both states are basically absent or very low as in *thermoneutrality* and in *chow-feeding* (e.g. Paper I, Figure 7). Furthermore, *white adipose tissue (WAT)* is not associated with thermogenesis, nor obviously are *UCP1-ablated mice* (e.g. low energy expenditure for UCP1-ablated mice in Paper I). All these parameters are therefore placed under the dotted line in Figure 15, which means very low or no recruitment, nor activation of UCP1 (= very low sympathetic tone). The strongest inducer of thermogenesis is *cold* as in Paper II, which takes the mice to Scenario B (or chronic cold-induced thermogenesis, CIT). Only cold and only *brown adipose tissue (BAT)* are involved in the highest amount of thermogenesis or the strongest browning (= high sympathetic tone). A lower amount of browning,

Scenario C, can be obtained by high-fat diet (or high amount of body fat⁹), which then can be termed diet-induced thermogenesis (DIT). Furthermore, it is facultative since this does not happen in UCP1-ablated mice. Browning can also occur in certain WAT depots, which by cold (and only by cold) become more thermogenic, then referred to as beige/brite or inducible WAT.

It is important to distinguish between absolute level of thermogenesis and relatively high increases in thermogenesis. This is nicely described in Kalinovich et al. (2017), where the degree of browning from UCP1 depends on what the starting point is. Transferring mice from 20 °C to 4 °C (a common experimental procedure to study thermogenic capacity) showed a low relative increase in BAT (due to already recruited state at room temperature), but a high relative increase in brite. In contrast, when transferring mice from 30 °C to 20 °C (a possibly more relevant step for humans) showed a high relative increase of UCP1 in BAT but no increase in brite (Kalinovich et al., 2017). Nonetheless, in absolute values, the levels of UCP1 in brite/beige are much lower compared to BAT no matter the degree of recruitment.

In Paper II, the highly cold-recruited mice were moved to the warm and the thermogenic activity immediately disappeared and the mice turned obese (despite functional UCP1, Paper II, CL-injection), which is of course expected since there is no need for thermogenesis. This resembles Scenario D and is an acute effect of the temperature decrease. What was a little surprising was that the thermogenic outcome seems to go *below* DIT (Scenario C). The cold-acclimated mice increased body fat gain to an even higher extent than the warm-acclimated mice after transfer from the cold to the warm. One could expect that the sympathetic tone would be the same as in the warm-acclimated mice (a slight increase).

It is not exactly known what causes the adaptive thermogenesis or DIT from HFD-feeding (first shown 1979 by Rothwell and Stock), but a clue was when Feldmann et al. (2009) demonstrated that DIT was UCP1-dependent by showing that UCP1-ablated mice had higher adiposity (Virtue and Vidal-Puig, 2013). Furthermore, the relationship in terms of regulation between cold-induced thermogenesis (CIT) and diet-induced thermogenesis (DIT) is not totally clear either. It has been concluded in a human study that CIT and DIT are probably differently regulated (Peterson et al., 2016). This might be the case.

The nervous regulation of NE (by sympathetic nervous system, SNS) is fast and very effective to regulate the amount of heat produced, which is important because environmental temperature can change fast. On the other hand, overeating and/or adiposity is a state that evolutionary did not have to be regulated. Thus, it is possible that overfeeding results in a low increase in sympathetic tone which happen to affect BAT if BAT happens to be present.

⁹ It is not yet clarified whether UCP1 expression is mainly due to an obesogenic diet or a high amount of body fat.

Thus, in a fully recruited thermogenic state (Scenario B), it is crucial to be able to turn off the activity, so that the mice do not get hyperthermic¹⁰, whereas the regulation and “fine tuning” of thermogenesis would not be as important in Scenario C. In a very low thermogenic state, it could be that the regulation is somehow “sloppy” because there is no need for turning it on or off. It is possible that the low recruitment and activity is secondary to the slight increase in sympathetic tone associated with high-fat feeding.

The little discrepancy between a mildly increased sympathetic tone (as in HFD-feeding) and the totally shut down of thermogenesis (where both conditions actually do not need heat production) is indicated as a red arrow in Figure 15 and is thus the UCP1-dependent diet-induced-thermogenesis.

The first question addressed in the present thesis was: what is the BAT-dependent (i.e. UCP1) contribution to energy expenditure depending on diet. This issue has been questioned (Kozak, 2010; Maxwell et al., 1987), since it does not seem logical in an evolutionary perspective to have a mechanism to increase energy expenditure. However, as discussed above, this could just be a side effect from obesogenic food, resulting in a very low (but still in a long-term perspective) significant increase of sympathetic tone. HFD-feeding leads to increased levels of UCP1 (Paper I and III), accompanied of higher energy expenditure at eating time (Paper I). This can be considered facultative (due to UCP1) diet-induced (due to high-fat diet) thermogenesis. Furthermore, why have higher amounts of UCP1 protein, if it is not used? Thus, this facultative diet-induced thermogenesis is UCP1-dependent. Noteworthy, this slight increase of sympathetic tone is also most desirable when eating an obesogenic diet, as we do.

And secondly: Can this UCP1-dependent diet-induced thermogenesis protect against obesity even at thermoneutrality. Since the effect of diet on BAT is low, it is of importance to control the temperature and keep thermoneutrality. Furthermore, other confounding factors to keep in mind are differences in actual energy and compositions of food and also cohort differences. However, when controlling all mentioned parameters and giving the mice the same obesogenic diet, the presence of UCP1 or not, had a larger effect on metabolic efficiency than did strain and sex (Figure 14A). This confirms the presence of UCP1-dependent diet-induced thermogenesis.

Thus, the conclusion must be that possessing UCP1 does result in obesity protection, even at thermoneutrality, at least for a good portion of a population.

¹⁰ Actually, this was painfully evident when we injected the cold-acclimated mice with CL 316 243 (a β 3-agonist) after transfer to 30 °C, and most of them died within 24 hours. It is thus extremely important to instantly be able to totally shut down the activation when not needed to avoid hyperthermia.

Svensk sammanfattning

Metabola sjukdomar som fetma och typ 2 diabetes har ökat markant de senaste 30 åren. Aktivt brunt fett har visat sig kunna ha en positiv inverkan på dessa tillstånd. Eftersom det visat sig att även vuxna personer har brunt fett, har intresset för fysiologin och regleringen av brunt fett ökat, främst för dess potential att reducera vikt. Det bruna fettets funktion är att producera värme till skillnad från det vita fettets som lagrar fett. Proteinet UCP1 (uncoupling protein 1) som gör detta sitter i mitokondriernas innermembran och fränkopplar andningskedjan från ATP produktion, vilket resulterar i värme produktion. Detta är viktigt för att hålla värmen vid kalla temperaturer, men det har även visat sig att fettande mat kan ge en viss ökning av brunt fett.

I denna avhandling har jag studerat matens inverkan på det bruna fettets och dess påverkan på fetma i möss. Främst har tre parametrar studerats: matintag, energiomsättning och huruvida närvaro eller frånvaro av UCP1 påverkar mössens vikt. Detta kan göras genom att jämföra vildtyps-möss med möss där man tagit bort genen för UCP1 (UCP1-knockout). Eftersom bruna fettets funktion är att producera värme är det mer aktivt i kyla, därför måste studier utföras i termoneutral zon (den temperatur där djuret inte behöver öka sin energiomsättning för att hålla värmen).

Både mätning av energi i mat och mätning av energiförbrukning innebär vissa svårigheter som också diskuteras i avhandlingen. För att underlätta tolkningen av resultaten har jag sammanställt många olika studier och bara jämfört möss (vildtyp och UCP1-knockout) i termoneutral temperatur och vid samma ålder som fått exakt samma mat. Sammanställningen och artiklarna i denna avhandling visar att vildtypsmössen har lite högre energiomsättning och att de har lägre vikt än UCP1-knockout mössen. Detta är med stor sannolikhet kopplat till närvaron av brunt fett, samt att detta bruna fett är lite aktiverat. Dock slås aktiveringen omedelbart av om kyl-anpassade möss tas in i värme.

En slutsats från denna avhandling är att även ett så lite aktiverat brunt fett kan ha betydelse för vikten, men att det också är viktigt att ha konstant aktivering för att upprätthålla de positiva effekterna av det bruna fettets.

Acknowledgements

First of all I am so thankful, and I want to thank you all, for having had the opportunity to spend a not small amount of years in the **physiology corridor**. I have, of course, learned a lot about brown fat, and other things. However, I have also learned so much about the nature of scientific thinking and education. All the incredible discussions at seminars, extremely high academic standards, but also a lot of joy. This place is the perfect combination of deep intelligent thoughts and fun times. The brain wants to have fun to be able to achieve. And, at least my brain has had great fun. I thank you all for this fantastic, inspiring period in my life.

I am so thankful to have been in Jan and Barbara's BAT-group. It has been interesting, it has been super instructively, it has been inspiring, it has been difficult, it has been frustrating, but always worthwhile.

I want to thank you **Jan**, my supervisor, for all your support and our nice work together. I also want to thank you for your extreme sense of telling a story. For seeing a drama, which is there but not always totally apparent. Science is not anything if it's not told, or if it's told in an uninteresting way. I am thankful to have been able to have that insight from you. I want to thank you **Barbara**, my co-supervisor, for always being interested and always have something extra to add to the scientific work. You have this incredible skill for seeing the details and to like magic turning the language from okay to smooth, accessible and graceful. I thank you for sharing that insight too. You both put science first. Always. I thank you for having the possibility to be part of that.

BAT-group: Thank you **Irina** Sha. for knowledgeable discussions and valuable advice. **Natasa** for always helping and always knowing everything, always being knowledgeable and nice. **Anastasia** for all the help with Western blots and always being kind and clever. **Jasper** for being a great room-mate and for stimulating discussions. **Petr** for also being a great room-mate and nice talks. **Ineke** for being nice and enlightened. **Erik** for being helpful and doing a lot of my Western Blots and being astute. **Natalie** for being so nice and adding the French touch. **Antonia** and **Ankita** for being sweet and clever.

Glut-group: Thank you **Tore** for all the great energy and cheerfulness. **Anna** for being a great room-mate, and all the interesting talks, you are not only a nice person, you also became a dear friend. **Jessica** for all the nice fika moments and great and inspiring talks. **Alice, Mia, Carina, Natalia, Jelena** and **Nodi** for lots of cosy fika and interesting discussions about this and that. **Benjamin** and **Muhamed** for being nice.

Elovl-group: Thank you **Anders** for always being so kind and caring. **Emanuela, Amanda, Anna P, Abbe** and **Hoi** for making the lab cheerful.

Thank you **Bo** for taking care of stuff and being so nice. **Robert** for all the great food and fun discussions.

Animal house: Thank you **Birgitta, Ellie, Pia, Nina, Raad, Stina, Eva, Lisa, Emilie, Sofie, Solveig** and **Irina Sab.** for taking care of the little fellows so well, and always being helpful and pleasant.

Thank you **Administration** for seeing that everything runs smoothly.

I also want to thank some old colleagues for so many good times, discussions and thoughts. Thank you **Gustavo, Alex, Tomas, Lotta, Damir, Birgitta, Anette, Yanling, Masa, Petter, Claudia, Victor, Tanja** and **Ida** (really miss our nice fika moments, hope you enjoy all your brown pottery)

Stort tack **mamma** och **pappa** för ert stöd på alla sätt och vis. **Syskon** med familjer för att ni finns. Ett speciellt tack till **Annerose** för att du tagit så mycket hand om Indra. Tack alla vänner för ert stora stöd. Speciellt tack till **Suzanne** för ditt lite oväntade stora intresse för brunt fett, men framför allt för allt ditt stöd och många härliga diskussioner och hundpromenader. **Indra** för alla avkopplande promenader för att tanka energi och tänka. Vem kan vara stressad kring en Labrador? Sist men inte minst, tack kära dotter **Thea**, för att du tagit så väldigt mycket ansvar denna tid, speciellt tycker jag att det var väldigt roligt att du gjorde omslaget till avhandlingen. Det blev en helt perfekt bild.

Again, thank you all for this extremely worthwhile period. Now I know so much, so I do not know anything.

References

- Atwater, W.O., 1916. Principles of nutrition and nutritive value of food. United States Farmers' Bull. 1910;142. 60.
- Atwater, W.O., Bryant, A.P., 1900. Dietary studies of University boat crews. Washington : Government printing office, 1900.
- Azzu, V., Jastroch, M., Divakaruni, A.S., Brand, M.D., 2010. The regulation and turnover of mitochondrial uncoupling proteins. *Biochim. Biophys. Acta - Bioenerg.* 1797, 785–791. doi:10.1016/j.bbabi.2010.02.035
- Baba, N., Bracco, E.F., Hashim, S.A., 1987. Role of brown adipose tissue in thermogenesis induced by overfeeding a diet containing medium chain triglyceride. *Lipids* 22, 442–444. doi:10.1007/BF02537276
- Baba, N., Bracco, E.F., Hashim, S.A., 1982. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. *Am. J. Clin. Nutr.* 35, 678–682.
- Bach, A., Ingenbleek, Y., Frey, A., 1996. The usefulness of dietary medium-chain triglycerides in body weight control: fact or fancy? *J. Lipid Res.* 37, 708–726.
- Bartelt, A., Bruns, O.T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M.G., Tromsdorf, U.I., Weller, H., Waurisch, C., Eychmüller, A., Gordts, P.L.S.M., Rinninger, F., Bruegelmann, K., Freund, B., Nielsen, P., Merkel, M., Heeren, J., 2011. Brown adipose tissue activity controls triglyceride clearance. *Nat. Med.* 17, 200–5. doi:10.1038/nm.2297
- Basciano, H., Federico, L., Adeli, K., 2005. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab* 2, 5. doi:10.1186/1743-7075-2-5
- Bellisari, A., 2008. Evolutionary origins of obesity. *Obes. Rev.* 9, 165–180. doi:10.1111/j.1467-789X.2007.00392.x
- Benoit, B., Plaisancié, P., Awada, M., Géloën, A., Estienne, M., Capel, F., Malpuech-Brugère, C., Debard, C., Pesenti, S., Morio, B., Vidal, H., Rieusset, J., Michalski, M.-C., 2013. High-fat diet action on adiposity, inflammation, and insulin sensitivity depends on the control low-fat diet, *Nutrition Research*. doi:10.1016/j.nutres.2013.07.017
- Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 70, 567–590.
- Binnert, C., Pachiaudi, C., Beylot, M., Hans, D., Vandermander, J., Chantre, P., 1998. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols 595–601.
- Bjursell, M., Admyre, T., Göransson, M., Marley, A.E., Smith, D.M., Oscarsson, J., Bohlooly-y, M., 2011. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* 211–220. doi:10.1152/ajpendo.00229.2010.
- Bonet, M.L., Oliver, P., Palou, A., 2013. Pharmacological and nutritional agents promoting browning of white adipose tissue. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* 1831, 969–985. doi:10.1016/j.bbalip.2012.12.002

- Bouchard, C., Tremblay, A., 1997. Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. *J. Nutr.* 127, 943S–947S.
- Brown, A.J., Goldsworthy, S.M., Barnes, A.A., Eilert, M.M., Tcheang, L., Daniels, D., Muir, A.I., Wigglesworth, M.J., Kinghorn, I., Fraser, N.J., Pike, N.B., Strum, J.C., Steplewski, K.M., Murdock, P.R., Holder, J.C., Marshall, F.H., Szekeres, P.G., Wilson, S., Ignar, D.M., Foord, S.M., Wise, A., Dowell, S.J., 2003. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278, 11312–11319. doi:10.1074/jbc.M211609200
- Burnett, C.M.L., Grobe, J.L., 2014. Dietary effects on resting metabolic rate in C57BL/6 mice are differentially detected by indirect (O₂/CO₂ respirometry) and direct calorimetry, *Molecular Metabolism*. doi:10.1016/j.molmet.2014.03.003
- Burnett, C.M.L., Grobe, J.L., 2013. Direct calorimetry identifies deficiencies in respirometry for the determination of resting metabolic rate in C57BL/6 and FVB mice. *Am. J. Physiol. - Endocrinol. Metab.* 305, E916–E924. doi:10.1152/ajpendo.00387.2013
- Cannon, B., Nedergaard, J., 2011. Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J. Exp. Biol.* 214, 242–253. doi:10.1242/jeb.050989
- Cannon, B., Nedergaard, J., 2004. Brown Adipose Tissue : Function and Physiological Significance 277–359. doi:10.1152/physrev.00015.2003
- Carey, A.L., Kingwell, B.A., 2013. Brown adipose tissue in humans: Therapeutic potential to combat obesity. *Pharmacol. Ther.* 140, 26–33. doi:10.1016/j.pharmthera.2013.05.009
- Casazza, K., Fontaine, K.R., Astrup, A., Birch, L.L., Brown, A.W., Bohan Brown, M.M., Durant, N., Dutton, G., Foster, E.M., Heymsfield, S.B., McIver, K., Mehta, T., Menachemi, N., Newby, P.K., Pate, R., Rolls, B.J., Sen, B., Smith, D.L., Thomas, D.M., Allison, D.B., 2013. Myths, presumptions, and facts about obesity. *N. Engl. J. Med.* 368, 446–54. doi:10.1056/NEJMsal208051
- Cheng, Y., Meng, Q., Wang, C., Li, H., Huang, Z., Chen, S., Xiao, F., Guo, F., 2010. Leucine deprivation decreases fat mass by stimulation of lipolysis in WAT and upregulation of UCP1 in BAT. *Diabetes* 59, 17–25. doi:10.2337/db09-0929
- Cherfaoui, M., Durand, D., Bonnet, M., Bernard, L., Bauchart, D., Ortigues-Marty, I., Gruffat, D., 2013. A grass-based diet favours muscle n-3 long-chain PUFA deposition without modifying gene expression of proteins involved in their synthesis or uptake in Charolais steers. *Animal* 7, 1833–40. doi:10.1017/S1751731113001432
- Chia, R., Achilli, F., Festing, M.F., Fisher, E.M., 2005. The origins and uses of mouse outbred stocks. *Nat Genet* 37, 1181–1186. doi:ng1665 [pii]r10.1038/ng1665
- Clapham, J.C., 2012. Central control of thermogenesis. *Neuropharmacology* 63, 111–123. doi:10.1016/j.neuropharm.2011.10.014
- Collins, S., Surwit, R.S., 2001. The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog. Horm. Res.* 56, 309–328. doi:10.1210/rp.56.1.309
- Cypess, A.M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A.B., Kuo, F.C., Palmer, E.L., Tseng, Y.-H., Doria, A., Kolodny, G.M., Kahn, C.R., 2009. Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–17. doi:10.1056/NEJMoa0810780
- D'Souza, S.S., Abraham, A., 2016. High-fat simple carbohydrate feeding impairs central and peripheral monoamine metabolic pathway triggering the onset of metabolic syndrome in C57BL/6J mice. *Neurol India* 64(5), 923–33. doi:doi: 10.4103/0028-3886.190261
- Danaei, G., Singh, G.M., Paciorek, C.J., Lin, J.K., Cowan, M.J., Finucane, M.M., Farzadfar,

- F., Stevens, G.A., Riley, L.M., Lu, Y., Rao, M., Ezzati, M., 2013. The global cardiovascular risk transition: Associations of four metabolic risk factors with national income, urbanization, and western diet in 1980 and 2008. *Circulation* 127, 1493–1502. doi:10.1161/CIRCULATIONAHA.113.001470
- David, J.M., Chatziioannou, A.F., Taschereau, R., Wang, H., Stout, D.B., 2013. The hidden cost of housing practices: Using noninvasive imaging to quantify the metabolic demands of chronic cold stress of laboratory mice. *Comp. Med.* 63, 386–391.
- de Jong, J.M.A., Larsson, O., Cannon, B., Nedergaard, J., 2015. A stringent validation of mouse adipose tissue identity markers. *Am. J. Physiol. Endocrinol. Metab.* ajpendo.00023.2015. doi:10.1152/ajpendo.00023.2015
- De Vogel-van den Bosch, J., Hoeks, J., Timmers, S., Houten, S.M., van Dijk, P.J., Boon, W., Van Beurden, D., Schaart, G., Kersten, S., Voshol, P.J., Wanders, R.J.A., Hesselink, M.K., Schrauwen, P., 2011a. The effects of long- or medium-chain fat diets on glucose tolerance and myocellular content of lipid intermediates in rats. *Obesity (Silver Spring)*. 19, 792–9. doi:10.1038/oby.2010.152
- De Vogel-van den Bosch, J., van den Berg, S.A.A., Bijland, S., Voshol, P.J., Havekes, L.M., Romijn, H.A., Hoeks, J., van Beurden, D., Hesselink, M.K.C., Schrauwen, P., van Dijk, K.W., 2011b. High-fat diets rich in medium- versus long-chain fatty acids induce distinct patterns of tissue specific insulin resistance. *J. Nutr. Biochem.* 22, 366–371. doi:10.1016/j.jnutbio.2010.03.004
- Dempersmier, J., Sul, H.S., 2015. Shades of brown: a model for thermogenic fat. *Front. Endocrinol. (Lausanne)*. 6, 71. doi:10.3389/fendo.2015.00071
- den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.-J., Bakker, B.M., 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* 54, 2325–2340. doi:10.1194/jlr.R036012
- Dhiman, T.R., Helmink, E.D., McMahon, D.J., Fife, R.L., Pariza, M.W., 1999. Conjugated Linoleic Acid Content of Milk and Cheese from Cows Fed Extruded Oilseeds. *J. Dairy Sci.* 82, 412–419. doi:10.3168/jds.S0022-0302(99)75247-1
- Dhiman, T.R., Nam, S.-H.H., Ure, A.L., 2005. Factors affecting conjugated linoleic acid content in milk and meat. *Crit. Rev. Food Sci. Nutr.* 45, 463–482. doi:10.1080/10408390591034463
- Dulloo, A.G., Jacquet, J., Montani, J.P., Schutz, Y., 2012. Adaptive thermogenesis in human body weight regulation: More of a concept than a measurable entity? *Obes. Rev.* 13, 105–121. doi:10.1111/j.1467-789X.2012.01041.x
- Enerbäck, S., Jacobsson, A., Simpson, E.M., Guerra, C., Yamashita, H., Harper, M.-E., Kozak, L.P., 1997. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature*. doi:10.1038/387090a0
- FAO, 2002. Food energy – methods of analysis and conversion factors. *Fao Food Nutr. Pap.* 77 93. doi:ISSN 0254-4725
- Feldmann, H.M., Golozoubova, V., Cannon, B., Nedergaard, J., 2009. UCPI Ablation Induces Obesity and Abolishes Diet-Induced Thermogenesis in Mice Exempt from Thermal Stress by Living at Thermonutrality. *Cell Metab.* 9, 203–209. doi:10.1016/j.cmet.2008.12.014
- Ferrer-Lorente, R., Fernández-López, J.A., Alemany, M., 2007. Estimation of the metabolizable energy equivalence of dietary proteins. *Eur. J. Nutr.* 46, 1–11. doi:10.1007/s00394-006-0623-x
- Fischer, A.W., Hoefig, C.S., Abreu-Vieira, G., de Jong, J.M.A., Petrovic, N., Mittag, J., Cannon, B., Nedergaard, J., 2016a. Leptin Raises Defended Body Temperature without

- Activating Thermogenesis. *Cell Rep.* 14, 1621–1631. doi:10.1016/j.celrep.2016.01.041
- Fischer, A.W., Shabalina, I.G., Mattsson, C.L., Abreu-Vieira, G., Cannon, B., Nedergard, J., Petrovic, N., 2016b. UCP1 Inhibition in Cidea-Overexpressing Mice is Physiologically Counteracted by Brown Adipose Tissue Hyperrecruitment. *Am. J. Physiol. - Endocrinol. Metab.* ajpendo.00284.2016. doi:10.1152/ajpendo.00284.2016
- Flachs, P., Horakova, O., Brauner, P., Rossmeisl, M., Pecina, P., Franssen-Van Hal, N., Ruzickova, J., Sponarova, J., Drahota, Z., Vlcek, C., Keijer, J., Houstek, J., Kopecky, J., 2005. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce ??-oxidation in white fat. *Diabetologia* 48, 2365–2375. doi:10.1007/s00125-005-1944-7
- Flachs, P., Ruhl, R., Hensler, M., Janovska, P., Zouhar, P., Kus, V., MacEk Jilkova, Z., Papp, E., Kuda, O., Svobodova, M., Rossmeisl, M., Tsenov, G., Mohamed-Ali, V., Kopecky, J., 2011. Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids. *Diabetologia* 54, 2626–2638. doi:10.1007/s00125-011-2233-2
- Food and Nutrition Board, 2005. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. *Natl. Acad. Press. Washington, DC* (2005), pp. 107–264.
- Foster, D.O., Frydman, M.L., 1979. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol.* 57, 257–270. doi:10.1139/y79-039
- Frankenfield, D.C., 2010. On heat, respiration, and calorimetry. *Nutrition* 26, 939–950. doi:10.1016/j.nut.2010.01.002
- Fromme, T., Klingenspor, M., 2011. Uncoupling protein 1 expression and high-fat diets. doi:10.1152/ajpregu.00411.2010.
- Galgani, J.E., Moro, C., Ravussin, E., 2008. Metabolic flexibility and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* 295, E1009-17. doi:10.1152/ajpendo.90558.2008
- Gao, Z., Yin, J., Zhang, J., Ward, R.E., Martin, R.J., Lefevre, M., Cefalu, W.T., Ye, J., 2009. Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice 1–14. doi:10.2337/db08-1637.
- García-Ruiz, E., Reynés, B., Díaz-Rúa, R., Ceresi, E., Oliver, P., Palou, A., 2015. The intake of high fat diets induces the acquisition of brown adipocyte gene expression features in white adipose tissue. *Int. J. Obes.* 39, 1619–1629. doi:10.1038/ijo.2015.112
- Gerhart-Hines, Z., Feng, D., Emmett, M.J., Everett, L.J., Loro, E., Briggs, E.R., Bugge, A., Hou, C., Ferrara, C., Seale, P., Pryma, D.A., Khurana, T.S., Lazar, M.A., 2013. The nuclear receptor Rev-erb α controls circadian thermogenic plasticity. *Nature* 503, 410–413. doi:10.1038/nature12642
- Glastras, S.J., Chen, H., Teh, R., McGrath, R.T., Chen, J., Pollock, C.A., Wong, M.G., Saad, S., 2016. Mouse models of diabetes, obesity and related kidney disease. *PLoS One* 11, 1–15. doi:10.1371/journal.pone.0162131
- Godlewski, G., Jourdan, T., Szanda, G., Tam, J., Resat Cinar, Harvey-White, J., Liu, J., Mukhopadhyay, B., Pacher, P., Ming Mo, F., Osei-Hyiaman, D., Kunos, G., 2015. Mice lacking GPR3 receptors display late-onset obese phenotype due to impaired thermogenic function in brown adipose tissue. *Sci. Rep.* 5, 14953. doi:10.1038/srep14953
- Golozoubova, V., Gullberg, H., Matthias, A., Cannon, B., Vennström, B., Nedergard, J.,

2004. Depressed thermogenesis but competent brown adipose tissue recruitment in mice devoid of all hormone-binding thyroid hormone receptors. *Mol. Endocrinol.* 18, 384–401. doi:10.1210/me.2003-0267
- Gordon, C.J., 2012. Thermal physiology of laboratory mice: Defining thermoneutrality. *J. Therm. Biol.* 37, 654–685. doi:10.1016/j.jtherbio.2012.08.004
- Gordon, C.J., 1987. Relationship between preferred ambient temperature and autonomic thermoregulatory function in rat. *Am J Physiol.*
- Gordon, C.J., 1985. Relationship between autonomic and behavioral thermoregulation in the mouse. *Physiol. Behav.* 34, 687–690. doi:10.1016/0031-9384(85)90365-8
- Gordon, C.J., Becker, P., Ali, J.S., 1998. Behavioral thermoregulatory responses of single- and group-housed mice. *Physiol. Behav.* 65, 255–262. doi:10.1016/S0031-9384(98)00148-6
- Ha, Y.L., Grimm, N.K., Pariza, M.W., 1987. Anticarcinogens from fried ground beef: altered derivatives of linoleic acid. *Carcinogenesis* 8, 1881–887.
- Harant-Farrugia, I., Garcia, J., Iglesias-Osma, M.C., Garcia-Barrado, M.J., Carpené, C., 2014. Is there an optimal dose for dietary linoleic acid? Lessons from essential fatty acid deficiency supplementation and adipocyte functions in rats. *J. Physiol. Biochem.* 70, 615–627. doi:10.1007/s13105-014-0315-6
- Hasek, B.E., Stewart, L.K., Henagan, T.M., Boudreau, A., Lenard, N.R., Black, C., Shin, J., Huypens, P., Malloy, V.L., Plaisance, E.P., Krajcik, R. a, Orentreich, N., Gettys, T.W., 2010. Dietary methionine restriction enhances metabolic flexibility and increases uncoupled respiration in both fed and fasted states. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R728–R739. doi:10.1152/ajpregu.00837.2009
- Hatai, S., 1902. On the presence in human embryos of an interscapular gland corresponding to the so-called hibernating gland of lower mammals. *Ann Anat* 21369–373.
- Heldmaier, G., Buchberger, A., 1985. Sources of heat during nonshivering thermogenesis in Djungarian hamsters: a dominant role of brown adipose tissue during cold adaptation. *J. Comp. Physiol. B* 156, 237–245. doi:10.1007/BF00695778
- Herrington, L.P., 1940. The heat regulation of small laboratory animals at various temperatures. *Am. J. Physiol* 129, 123–139.
- Himms-Hagen, J., Villemure, C., 1992. Number of mice per cage influences uncoupling protein content of brown adipose tissue. *Proc Soc Exp Biol Med* 200, 502–506.
- Hoeks, J., Hesselink, M.K.C., Van Bilsen, M., Schaart, G., Van Der Vusse, G.J., Saris, W.H.M., Schrauwen, P., 2003. Differential response of UCP3 to medium versus long chain triacylglycerols; Manifestation of a functional adaptation. *FEBS Lett.* 555, 631–637. doi:10.1016/S0014-5793(03)01343-7
- Hoevenaars, F.P.M., Van Schothorst, E.M., Horakova, O., Voigt, A., Rossmeisl, M., Pico, C., Caimari, A., Kopecky, J., Klaus, S., Keijer, J., 2012. BIOCLAIMS standard diet (BIOsd): A reference diet for nutritional physiology. *Genes Nutr.* 7, 399–404. doi:10.1007/s12263-011-0262-6
- House, R.L., Cassady, J.P., Eisen, E.J., McIntosh, M.K., Odle, J., 2005. Conjugated linoleic acid evokes de-lipidation through the regulation of genes controlling lipid metabolism in. *In Vivo (Brooklyn).* 247–258.
- Hu, J., Kyrou, I., Tan, B.K., Dimitriadis, G.K., Ramanjaneya, M., Tripathi, G., Patel, V., James, S., Kawan, M., Chen, J., Randeve, H.S., 2016. Short-chain fatty acid acetate stimulates adipogenesis and mitochondrial biogenesis via GPR43 in brown adipocytes. *Endocrinology* 157, 1881–1894. doi:10.1210/en.2015-1944
- Innis, S.M., 2011. Dietary Triacylglycerol Structure and Its Role in Infant Nutrition. *Adv. Nutr. An Int. Rev. J.* 2, 275–283. doi:10.3945/an.111.000448.Table

- Iqbal, M.P., 2014. Trans fatty acids - A risk factor for cardiovascular disease. *Pakistan J. Med. Sci.* 30, 194–197. doi:10.12669/pjms.301.4525
- Iwamoto, J., Takeda, T., Sato, Y., Matsumoto, H., 2011. Serum leptin concentration positively correlates with body weight and total fat mass in postmenopausal Japanese women with osteoarthritis of the knee. *Arthritis* 2011, 580632. doi:10.1155/2011/580632
- Iwasaki, Y., Saito, O., Tanabe, M., Inayoshi, K., Kobata, K., Uno, S., Morita, A., Watanabe, T., 2008. Monoacylglycerols activate capsaicin receptor, TRPV1. *Lipids* 43, 471–483. doi:10.1007/s11745-008-3182-5
- Iwasaki, Y., Tamura, Y., Inayoshi, K., Narukawa, M., Kobata, K., Chiba, H., Muraki, E., Tsunoda, N., Watanabe, T., 2011. TRPV1 Agonist Monoacylglycerol Increases UCP1 Content in Brown Adipose Tissue and Suppresses Accumulation of Visceral Fat in Mice Fed a High-Fat and High-Sucrose Diet. *Biosci. Biotechnol. Biochem.* 75, 904–909. doi:10.1271/bbb.100850
- Janovská, P., Flachs, P., Kazdová, L., Kopecký, J., 2013. Anti-obesity effect of n-3 polyunsaturated fatty acids in mice fed high-fat diet is independent of cold-induced thermogenesis. *Physiol. Res.* 62, 153–161. doi:932464 [pii]
- Jastroch, M., Giroud, S., Barrett, P., Geiser, F., Heldmaier, G., Herwig, A., 2016. Seasonal Control of Mammalian Energy Balance: Recent advances in the understanding of daily torpor and hibernation. *J. Neuroendocrinol.* 1–10. doi:10.1111/jne.12437
- Kaiyala, K.J., Ramsay, D.S., 2011. Direct animal calorimetry, the underused gold standard for quantifying the fire of life. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 158, 252–264. doi:10.1016/j.cbpa.2010.04.013
- Kajimura, S., Saito, M., 2014. A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. *Annu. Rev. Physiol.* 76, 225–49. doi:10.1146/annurev-physiol-021113-170252
- Kalinovich, A. V., de Jong, J.M.A., Cannon, B., Nedergaard, J., 2017. UCP1 in adipose tissues: two steps to full browning. *Biochimie.* doi:10.1016/j.biochi.2017.01.007
- Kennedy, A., Martinez, K., Schmidt, S., Mandrup, S., LaPoint, K., McIntosh, M., 2010. Antiobesity mechanisms of action of conjugated linoleic acid. *J. Nutr. Biochem.* 21, 171–179. doi:10.1016/j.jnutbio.2009.08.003
- Kim, S., Kim, J.-H., Park, B.O., Kwak, Y.S., 2014. Perspectives on the therapeutic potential of short-chain fatty acid receptors. *BMB Rep.* 47, 173–8. doi:10.5483/BMBRep.2014.47.3.272
- Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., Terasawa, K., Kashihara, D., Hirano, K., Tani, T., Takahashi, T., Miyauchi, S., Shioi, G., Inoue, H., Tsujimoto, G., 2013. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* 4, 1829. doi:10.1038/ncomms2852
- Klingenspor, M., Fromme, T., Hughes, D.A., Manzke, L., Polymeropoulos, E., Riemann, T., Trzcionka, M., Hirschberg, V., Jastroch, M., 2008. An ancient look at UCP1. *Biochim. Biophys. Acta - Bioenerg.* 1777, 637–641. doi:10.1016/j.bbabo.2008.03.006
- Kontani, Y., Wang, Y., Kimura, K., Inokuma, K.I., Saito, M., Suzuki-Miura, T., Wang, Z., Sato, Y., Mori, N., Yamashita, H., 2005. UCP1 deficiency increases susceptibility to diet-induced obesity with age. *Aging Cell* 4, 147–155. doi:10.1111/j.1474-9726.2005.00157.x
- Kozak, L.P., 2010. Brown Fat and the Myth of Diet-Induced Thermogenesis. *Cell Metab.* doi:10.1016/j.cmet.2010.03.009
- LaRosa, P.C., Miner, J., Xia, Y., Zhou, Y., Kachman, S., Fromm, M.E., 2006. Trans-10, cis-12

- conjugated linoleic acid causes inflammation and delipidation of white adipose tissue in mice: a microarray and histological analysis. *Physiol. Genomics* 27, 282–294. doi:10.1152/physiolgenomics.00076.2006
- Le, N., Shin, S., Tu, T., Kim, C., Kang, J., Tsuyoshi, G., Teruo, K., Han, S., Yu, R., 2012. Diet Enriched with Korean Pine Nut Oil Improves Mitochondrial Oxidative Metabolism in Skeletal Muscle and Brown Adipose Tissue in Diet-Induced Obesity. *J Agric Food Chem*.
- Lean, M.E.J., 1989. Brown adipose tissue in humans. *Proc. Nutr. Soc.* 48, 243–256.
- LeBlanc, J., Lupien, D., Diamond, P., Macari, M., Richard, D., 2003. Thermogenesis in response to various intakes of palatable food 1–8. doi:10.1103/PhysRevE.67.011707
- Leon, L.R., Gordon, C.J., Helwig, B.G., Rufolo, D.M., Blaha, M.D., 2010. Thermoregulatory, behavioral, and metabolic responses to heatstroke in a conscious mouse model. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R241–R248. doi:10.1152/ajpregu.00309.2009
- Letona, A.Z., Niot, I., Laugerette, F., Athias, A., Monnot, M., Portillo, M.P., Besnard, P., 2011. CLA-Enriched Diet Containing t10, c12- CLA Alters Bile Acid Homeostasis and Increases the Risk of Cholelithiasis in Mice 1 – 3 6–13. doi:10.3945/jn.110.136168.
- Liu, X., Rossmeisl, M., McClaine, J., Kozak, L.P., 2003. Paradoxical resistance to diet-induced obesity in UCP1-deficient mice. *J. Clin. Invest.* 111, 399–407. doi:10.1172/JCI200315737.
- Liu, Y., Zhang, Y., Xu, Q., Yu, X., Zhang, X., Wang, J., Xue, C., Yang, X., Zhang, R., Xue, C., 2012. Increased Norepinephrine by Medium-Chain Triglyceride Attributable to Lipolysis in White and Brown Adipose Tissue of C57BL/6J Mice. *Biosci. Biotechnol. Biochem.* 76, 1213–1218. doi:10.1271/bbb.120079
- Livesey, G., 2002. Thermogenesis associated with fermentable carbohydrate in humans, validity of indirect calorimetry, and implications of dietary thermogenesis for energy requirements, food energy and body weight. *Int. J. Obes. Relat. Metab. Disord.* 26, 1553–1569. doi:10.1038/sj.ijo.0802168
- Livesey, G., 2001. A perspective on food energy standards for nutrition labelling. *Br. J. Nutr.* 85, 271–287. doi:10.1079/BJN2000253
- Livesey, G., 1995. Metabolizable energy. *Am J Clin Nutr* 62, 1135S–42S.
- Livesey, G., Elia, M., 1988. Estimation of energy expenditure, and net fat oxidation calorimetry: evaluation of errors to the detailed composition of net carbohydrate and synthesis by indirect with special reference. *Clin. Nutr.* 47, 608–28.
- Lodhi, I.J., Semenkovich, C.F., 2009. Why We Should Put Clothes on Mice. *Cell Metab.* 9, 111–112. doi:10.1016/j.cmet.2009.01.004
- Ludwig, T., Worsch, S., Heikenwalder, M., Daniel, H., Hauner, H., Bader, B.L., 2013. Metabolic and immunomodulatory effects of n-3 fatty acids are different in mesenteric and epididymal adipose tissue of diet-induced obese mice. *Am. J. Physiol. Endocrinol. Metab.* 304, E1140–56. doi:10.1152/ajpendo.00171.2012
- Lustig, R.H., 2010. Fructose: Metabolic, Hedonic, and Societal Parallels with Ethanol. *J. Am. Diet. Assoc.* 110, 1307–1321. doi:10.1016/j.jada.2010.06.008
- Maki, K.C., Mustad, V., Dicklin, M.R., Geohas, J., 2009. Postprandial metabolism with 1,3-diacylglycerol oil versus equivalent intakes of long-chain and medium-chain triacylglycerol oils. *Nutrition* 25, 627–633. doi:10.1016/j.nut.2008.11.028
- Marten, B., Pfeuffer, M., Schrezenmeir, J., 2006. Medium-chain triglycerides. *Int. Dairy J.* 16, 1374–1382. doi:10.1016/j.idairyj.2006.06.015
- Matsushita, M., Yoneshiro, T., Aita, S., Kameya, T., Sugie, H., Saito, M., 2014. Impact of

- brown adipose tissue on body fatness and glucose metabolism in healthy humans. *Int. J. Obes.* 38, 812–817. doi:10.1038/ijo.2013.206
- Mattsson, C.L., Csikasz, R.I., Shabalina, I.G., Nedergaard, J., Cannon, B., 2010. Caveolin-1-ablated mice survive in cold by nonshivering thermogenesis despite desensitized adrenergic responsiveness. *Am. J. Physiol. Endocrinol. Metab.* 299, E374–E383. doi:10.1152/ajpendo.00071.2010
- Maxwell, G.M., Nobbs, S., Bates, D.J., 1987. Diet-induced thermogenesis in cafeteria-fed rats: a myth? *Am. J. Physiol.* 253, E264–70.
- Mercer, S., Trayhurn, P., 1984. Effect of high fat diets on the thermogenic activity of brown adipose tissue in cold-acclimated mice. *J. Nutr.* 114, 1151–8.
- Merrill, A.L., Watt, B.K., 1973. *Energy Values of Food: Basis and Derivation*. Agric. Handbook No. 74.
- Moloney, F., Toomey, S., Noone, E., Nugent, A., Allan, B., Loscher, C.E., Roche, H.M., 2007. Antidiabetic effects of cis-9, trans-11-conjugated linoleic acid may be mediated via anti-inflammatory effects in white adipose tissue. *Diabetes* 56, 574–582. doi:10.2337/db06-0384
- Mukherji, A., Kobiita, A., Chambon, P., 2015. Shifting the feeding of mice to the rest phase creates metabolic alterations, which, on their own, shift the peripheral circadian clocks by 12 hours. *Proc. Natl. Acad. Sci.* 1519735112-. doi:10.1073/pnas.1519735112
- Mumme, K., Stonehouse, W., 2015. Effects of medium-chain triglycerides on weight loss and body composition: A meta-analysis of randomized controlled trials. *J. Acad. Nutr. Diet.* 115, 249–263. doi:10.1016/j.jand.2014.10.022
- Nagao, K., Yanagita, T., 2010. Medium-chain fatty acids: Functional lipids for the prevention and treatment of the metabolic syndrome. *Pharmacol. Res.* 61, 208–212. doi:10.1016/j.phrs.2009.11.007
- Nakamura, M.T., Yudell, B.E., Loor, J.J., 2014. Regulation of energy metabolism by long-chain fatty acids. *Prog. Lipid Res.* 53, 124–144. doi:10.1016/j.plipres.2013.12.001
- Nam, D., Yechool, V.K., Ma, K., 2015. Molecular clock integration of brown adipose tissue formation and function. *Adipocyte* 3945, 1–8. doi:10.1080/21623945.2015.1082015
- Nautiyal, J., Christian, M., Parker, M.G., 2013. Distinct functions for RIP140 in development, inflammation, and metabolism. *Trends Endocrinol. Metab.* 24, 451–459. doi:10.1016/j.tem.2013.05.001
- Nedergaard, J., Becker, W., Cannon, B., 1983. Effects of dietary essential fatty acids on active thermogenin content in rat brown adipose tissue. *J.Nutr.* 113, 1717–1724.
- Nedergaard, J., Bengtsson, T., Cannon, B., 2007. Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, 444–452. doi:10.1152/ajpendo.00691.2006.
- Nedergaard, J., Cannon, B., 2014. The browning of white adipose tissue: Some burning issues. *Cell Metab.* doi:10.1016/j.cmet.2014.07.005
- Nedergaard, J., Cannon, B., 2013. UCP1 mRNA does not produce heat. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* 1831, 943–949. doi:10.1016/j.bbalip.2013.01.009
- Neveu, C., Baurhoo, B., Mustafa, A., 2014. Effect of feeding extruded flaxseed with different grains on the performance of dairy cows and milk fatty acid profile. *J. Dairy Sci.* 97, 1543–1551. doi:10.3168/jds.2013-6728
- Nicholls, D.G., Rial, E., 1999. A history of UCP1: the first uncoupling protein. *J. Bioenerg. Biomembr.* 31, 399–406. doi:10.1023/A:1005436121005
- Noguchi, O., Takeuchi, H., Kubota, F., Tsuji, H., Aoyama, T., 2002. Larger Thermogenesis and Less Body Fat Accumulation in Rats Fed Medium-Chain Triacylglycerols than in

- Those Fed Long-Chain Triacylglycerols. *J. Nutr. Sci. Vitaminol. (Tokyo)*. 524–529.
- Novotny, J.A., Gebauer, S.K., Baer, D.J., 2012. Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets. *Am. J. Clin. Nutr.* 96, 296–301. doi:10.3945/ajcn.112.035782
- Oelkrug, R., Polymeropoulos, E.T., Jastroch, M., 2015. Brown adipose tissue: physiological function and evolutionary significance. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 185, 587–606. doi:10.1007/s00360-015-0907-7
- Ohnuki, K., Haramizu, S., Oki, K., Watanabe, T., Yazawa, S., Fushiki, T., 2001. Administration of capsiate, a non-pungent capsaicin analog, promotes energy metabolism and suppresses body fat accumulation in mice. *Biosci. Biotechnol. Biochem.* 65, 2735–2740. doi:10.1271/bbb.65.2735
- Oi-Kano, Y., Kawada, T., Watanabe, T., Koyama, F., Watanabe, K., Senbongi, R., Iwai, K., 2008. Oleu- ropein, a phenolic compound in extra virgin olive oil, increases uncoupling protein 1 content in brown adipose tissue and enhances noradrenaline and adrenaline secretions in rats. *J Nutr Sci Vitaminol* 54, 363–370.
- Oi-Kano, Y., Kawada, T., Watanabe, T., Koyama, F., Watanabe, K., Senbongi, R., Iwai, K., 2007. Extra virgin olive oil increases uncoupling protein 1 content in brown adipose tissue and enhances noradrenaline and adrenaline secretions in rats. *J. Nutr. Biochem.* 18, 685–692. doi:10.1016/j.jnutbio.2006.11.009
- Olea, E., Agapito, M.T., Gallego-Martin, T., Rocher, A., Gomez-Niño, A., Obeso, A., Gonzalez, C., Yubero, S., 2014. Intermittent hypoxia and diet-induced obesity: effects on oxidative status, sympathetic tone, plasma glucose and insulin levels, and arterial pressure. *J. Appl. Physiol.* 117, 706–719. doi:10.1152/jappphysiol.00454.2014
- Ost, M., Keipert, S., Klaus, S., 2017. Targeted mitochondrial uncoupling beyond UCP1 – The fine line between death and metabolic health. *Biochimie*. doi:10.1016/j.biochi.2016.11.013
- Oudart, H., Groscolas, R., Calgari, C., Nibbelink, M., Leray, C., Le Maho, Y., Malan, a, 1997. Brown fat thermogenesis in rats fed high-fat diets enriched with n-3 polyunsaturated fatty acids. *Int. J. Obes. Relat. Metab. Disord.* 21, 955–62.
- Overton, J.M., 2010. Phenotyping small animals as models for the human metabolic syndrome: thermoneutrality matters. *Int. J. Obes. (Lond)*. 34 Suppl 2, S53–S58. doi:10.1038/ijo.2010.240
- Park, Y., Albright, K.J. et al, Pariza, M.W., 1997. Effect of conjugated linoleic acid on body composition in Mice. *Am J Clin Nutr* 79, 352–361.
- Pauter, A.M., Olsson, P., Asadi, A., Herslöf, B., Csikasz, R.I., Zdravec, D., Jacobsson, A., 2014. Elovl2 ablation demonstrates that systemic DHA is endogenously produced and is essential for lipid homeostasis in mice. *J. Lipid Res.* 55, 718–28. doi:10.1194/jlr.M046151
- Peirce, V., Carobbio, S., Vidal-Puig, A., 2014. The different shades of fat. *Nature* 510, 76–83. doi:10.1038/nature13477
- Penumetcha, M., Schneider, M.K., Cheek, H.A., Karabina, S., 2013. A diet containing soybean oil heated for three hours increases adipose tissue weight but decreases body weight in C57BL/6 J mice. *Lipids Health Dis.* 12, 2–4. doi:10.1186/1476-511X-12-26
- Peters, J.M., Park, Y., Gonzalez, F.J., Pariza, M.W., 2001. Influence of conjugated linoleic acid on body composition and target gene expression in peroxisome proliferator-activated receptor α -null mice. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* 1533, 233–242. doi:10.1016/S1388-1981(01)00155-X
- Peterson, C.M., Lecoultre, V., Frost, E.A., Simmons, J., Redman, L.M., Ravussin, E., 2016.

- The thermogenic responses to overfeeding and cold are differentially regulated. *Obesity* 24, 96–101. doi:10.1002/oby.21233
- Petrovic, N., Walden, T.B., Shabalina, I.G., Timmons, J.A., Cannon, B., Nedergaard, J., 2010. Chronic peroxisome proliferator-activated receptor γ (PPAR γ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* 285, 7153–7164. doi:10.1074/jbc.M109.053942
- Plaisance, E.P., Henagan, T.M., Echlin, H., Boudreau, A., Hill, K.L., Lenard, N.R., Hasek, B.E., Orentreich, N., Gettys, T.W., 2010. Role of beta-adrenergic receptors in the hyperphagic and hypermetabolic responses to dietary methionine restriction. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R740-50. doi:10.1152/ajpregu.00838.2009
- Poirier, H., Niot, I., Clément, L., Guerre-Millo, M., Besnard, P., 2005. Development of conjugated linoleic acid (CLA)-mediated lipotrophic syndrome in the mouse. *Biochimie* 87, 73–79. doi:10.1016/j.biochi.2004.11.006
- Portillo, M.P., Serra, F., Simon, E., del Barrio, A.S., Palou, A., 1998. Energy restriction with high-fat diet enriched with coconut oil gives higher UCP1 and lower white fat in rats. *Int. J. Obes. Relat. Metab. Disord.* 22, 974–9. doi:10.1038/sj.ijo.0800706
- Puchalski, W., Böckler, H., Heldmaier, G., Langefeld, M., 1987. Organ blood flow and brown adipose tissue oxygen consumption during noradrenaline-induced nonshivering thermogenesis in the Djungarian hamster. *J. Exp. Zool.* 242, 263–271. doi:10.1002/jez.1402420304
- Regard, J.B., Sato, I.T., Coughlin, S.R., 2008. Anatomical Profiling of G Protein-Coupled Receptor Expression. *Cell* 135, 561–571. doi:10.1016/j.cell.2008.08.040
- Remig, V., Franklin, B., Margolis, S., Kostas, G., Nece, T., Street, J.C., 2010. Trans Fats in America: A Review of Their Use, Consumption, Health Implications, and Regulation. *J. Am. Diet. Assoc.* 110, 585–592. doi:10.1016/j.jada.2009.12.024
- Rial, S.A., Karelis, A.D., Bergeron, K.F., Mounier, C., 2016. Gut microbiota and metabolic health: The potential beneficial effects of a medium chain triglyceride diet in obese individuals. *Nutrients* 8, 1–19. doi:10.3390/nu8050281
- Riek, A., Geiser, F., 2013. Allometry of thermal variables in mammals: Consequences of body size and phylogeny. *Biol. Rev.* 88, 564–572. doi:10.1111/brv.12016
- Rodriguez, V.M., Portillo, M.P., Pico, C., Macarulla, M.T., Palou, A., 2002. Olive oil feeding up-regulates uncoupling protein genes in rat brown adipose tissue and skeletal muscle 1 – 3. *Am. J. Clin. Nutr.* 1–8.
- Rothwell, N.J., Stock, M.J., 1989. Surgical removal of brown fat results in rapid and complete compensation by other depots. *Am J Physiol* 257, R253-8.
- Rothwell, N.J., Stock, M.J., 1987. Stimulation of thermogenesis and brown fat activity in rats fed medium chain triglyceride. *Metabolism* 36, 128–130. doi:10.1016/0026-0495(87)90005-9
- Rothwell, N.J., Stock, M.J., 1979. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 1–5. doi:10.1007/s13398-014-0173-7.2
- Rothwell, N.J., Stock, M.J., Tyzbir, R.S., 1983. Mechanisms of thermogenesis induced by low protein diets. *Metabolism* 32, 257–261. doi:10.1016/0026-0495(83)90190-7
- Rowland, L.A., Bal, N.C., Kozak, L.P., Periasamy, M., 2015. Uncoupling protein 1 and sarcolipin are required to maintain optimal thermogenesis, and loss of both systems compromises survival of mice under cold stress. *J. Biol. Chem.* 290, 12282–12289. doi:10.1074/jbc.M115.637603
- Rowland, L.A., Maurya, S.K., Bal, N.C., Kozak, L., Periasamy, M., 2016. Sarcolipin and

- uncoupling protein 1 play distinct roles in diet-induced thermogenesis and do not compensate for one another. *Obesity* 24, 1430–1433. doi:10.1002/oby.21542
- Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., Millington, K.J., 2007. The Health Benefits of Omega-3 Polyunsaturated Fatty Acids: A Review of the Evidence. *Adv. Clin. Insights Bioceuticals* 20, 275–285.
- Saito, M., Okamatsu-ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., Tsujisaki, M., 2009. High Incidence of Metabolically Active Brown Adipose Effects of Cold Exposure and Adiposity. *Diabetes* 58, 1526–1531. doi:10.2337/db09-0530.
- Sakaguchi, T., Arase, K., Bray, G.A., 1988. Effect of intrahypothalamic hydroxybutyrate on sympathetic firing rate. *Metabolism* 37, 732–735. doi:10.1016/0026-0495(88)90006-6
- Scholander, P.F., Walters, V., Hock, R., Irving, L., 1950. Body Insulation of Some Arctic and Tropical Mammals and Birds. *Biol. Bull.* Vol. 99, N, 225–236.
- Scott, C.B., 2014. Combustion, Respiration and Intermittent Exercise: A Theoretical Perspective on Oxygen Uptake and Energy Expenditure. *Biology (Basel)*. 3, 255–263. doi:10.3390/biology3020255
- Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scimè, A., Devarakonda, S., Conroe, H.M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M.A., Beier, D.R., Spiegelman, B.M., 2008. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–7. doi:10.1038/nature07182
- Shabalina, I.G., Backlund, E.C., Bar-Tana, J., Cannon, B., Nedergaard, J., 2008. Within brown-fat cells, UCP1-mediated fatty acid-induced uncoupling is independent of fatty acid metabolism. *Biochim. Biophys. Acta - Bioenerg.* 1777, 642–650. doi:10.1016/j.bbabi.2008.04.038
- Shabalina, I.G., Jacobsson, A., Cannon, B., Nedergaard, J., 2004. Native UCP1 displays simple competitive kinetics between the regulators purine nucleotides and fatty acids. *J. Biol. Chem.* 279, 38236–38248. doi:10.1074/jbc.M402375200
- Shabalina, I.G., Ost, M., Petrovic, N., Vrbacky, M., Nedergaard, J., Cannon, B., 2010. Uncoupling protein-1 is not leaky. *Biochim. Biophys. Acta - Bioenerg.* 1797, 773–784. doi:10.1016/j.bbabi.2010.04.007
- Shapiro, A., Mu, W., Roncal, C., Cheng, K.Y., Johnson, R.J., Scarpace, P.J., 2008. Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R1370--1375. doi:10.1152/ajpregu.00195.2008
- Sharma, S.K., Vij, A.S., Sharma, M., 2013. Mechanisms and clinical uses of capsaicin. *Eur. J. Pharmacol.* 720, 55–62. doi:10.1016/j.ejphar.2013.10.053
- Shen, W., Chuang, C.-C., Martinez, K., Reid, T., Brown, J.M., Xi, L., Hixson, L., Hopkins, R., Starnes, J., McIntosh, M., 2013. Conjugated linoleic acid reduces adiposity and increases markers of browning and inflammation in white adipose tissue of mice. *J. Lipid Res.* 54, 909–22. doi:10.1194/jlr.M030924
- Speakman, J.R., 2014. Should we abandon indirect calorimetry as a tool to diagnose energy expenditure? Not yet. Perhaps not ever. Commentary on Burnett and Grobe (2014). *Mol. Metab.* doi:10.1016/j.molmet.2014.04.003
- Spector, T., 2015. Matmyten: vetenskapen om vad vi äter och hur det påverkar oss. Volante 2016, CPI, Leck, Gemany.
- Surette, M.E., 2008. The science behind dietary omega-3 fatty acids. *Cmaj* 178, 177–180. doi:10.1503/cmaj.071356
- Takahashi, Y., Ide, T., 2000. Dietary n-3 fatty acids affect mRNA level of brown adipose

- tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. *Br. J. Nutr.* 84, 175–184.
- Tan, J., McKenzie, C., Potamitis, M., Thorburn, A.N., Mackay, C.R., Macia, L., 2014. Chapter Three – The Role of Short-Chain Fatty Acids in Health and Disease, in: *Advances in Immunology*. pp. 91–119. doi:10.1016/B978-0-12-800100-4.00003-9
- Tappy, L., 1996. Thermic effect of food and sympathetic nervous system activity in humans. *Reprod. Nutr. Dev.* 391–397. doi:10.1051/rnd:19960405
- Tappy, L., Lê, K.A., Tran, C., Paquot, N., 2010. Fructose and metabolic diseases: New findings, new questions. *Nutrition* 26, 1044–1049. doi:10.1016/j.nut.2010.02.014
- Tataranni, P.A., Harper, I.T., Snitker, S., Del Parigi, A., Vozarova, B., Bunt, J., Bogardus, C., Ravussin, E., 2003. Body weight gain in free-living Pima Indians: effect of energy intake vs expenditure. *Int. J. Obes. Relat. Metab. Disord.* 27, 1578–83. doi:10.1038/sj.ijo.0802469
- Tempel, D.L., Shor-Posner, G., Dwyer, D., Leibowitz, S.F., 1989. Nocturnal patterns of macronutrient intake in freely feeding and food-deprived rats. *Am. J. Physiol.* 256, R541–548.
- Thomas, D.M., Martin, C.K., Lettieri, S., Bredlau, C., Kaiser, K., Church, T., Bouchard, C., Heymsfield, S.B., 2013. Can a weight loss of one pound a week be achieved with a 3500-kcal deficit? Commentary on a commonly accepted rule. *Int. J. Obes.* 37, 1611–3. doi:10.1038/ijo.2013.51
- Tolhurst, G., Heffron, H., Lam, Y.S., Parker, H.E., Habib, A.M., Diakogiannaki, E., Cameron, J., Grosse, J., Reimann, F., Gribble, F.M., 2012. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 61, 364–371. doi:10.2337/db11-1019
- Tsai, J.-Y., Villegas-Montoya, C., Boland, B.B., Blasler, Z., Egbejimi, O., Gonzalez, R., Kueht, M., McElfresh, T.A., Brewer, R.A., Chandler, M.P., Bray, M.S., Young, M.E., 2013. Influence of dark phase restricted high fat feeding on myocardial adaptation in mice. *J. Mol. Cell. Cardiol.* 55, 147–155. doi:10.1016/j.yjmcc.2012.09.010
- van Marken Lichtenbelt, W., Kingma, B., van der Lans, A., Schellen, L., 2014. Cold exposure – an approach to increasing energy expenditure in humans. *Trends Endocrinol. Metab.* doi:10.1016/j.tem.2014.01.001
- van Marken Lichtenbelt, W.D., Schrauwen, P., 2011. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R285–96. doi:10.1152/ajpregu.00652.2010
- van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M., Kemerink, G.J., Bouvy, N.D., Schrauwen, P., Teule, G.J., 2009. Cold-activated brown adipose tissue in healthy men 674. *N.Engl.J Med.* 360, 1500–1508.
- Virtanen, K.A., Lidell, M.E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N.-J., Enerbäck, S., 2009. Functional Brown Adipose Tissue in Healthy Adults. *N Engl J Med* 360, 1518–1525. doi:10.1056/NEJMoa0808949
- Virtue, S., Vidal-Puig, A., 2013. Assessment of brown adipose tissue function. *Front. Physiol.* 4, 128. doi:10.3389/fphys.2013.00128
- von Essen, G., 2014. The effect of various dietary fatty acids on adaptive thermogenesis. Licent. Thesis.
- Vögler, O., López-Bellan, a, Alemany, R., Tofé, S., González, M., Quevedo, J., Pereg, V., Barceló, F., Escriba, P. V, 2008. Structure-effect relation of C18 long-chain fatty acids in the reduction of body weight in rats. *Int. J. Obes. (Lond).* 32, 464–473. doi:10.1038/sj.ijo.0803768

- Wanders, D., Burk, D.H., Cortez, C.C., Van, N.T., Stone, K.P., Baker, M., Mendoza, T., Mynatt, R.L., Gettys, T.W., 2015. UCP1 is an essential mediator of the effects of methionine restriction on energy balance but not insulin sensitivity. *FASEB J.* 29, 2603–2615. doi:10.1096/fj.14-270348
- Wang, Q., Zhang, M., Xu, M., Gu, W., Xi, Y., Qi, L., Li, B., Wang, W., 2015. Brown adipose tissue activation is inversely related to central obesity and metabolic parameters in adult human. *PLoS One* 10, 1–13. doi:10.1371/journal.pone.0123795
- Warwick, P.M., Baines, J., 2000. Point of view: energy factors for food labelling and other purposes should be derived in a consistent fashion for all food components. *Br. J. Nutr.* 84, 897–902.
- Weir, J.B.D. V., 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* 109, 1–9. doi:PMCID: PMC1392602
- Weitkunat, K., Schumann, S., Nickel, D., Kappo, K.A., Petzke, K.J., Kipp, A.P., Blaut, M., Klaus, S., 2016. Importance of propionate for the repression of hepatic lipogenesis and improvement of insulin sensitivity in high-fat diet-induced obesity. *Mol. Nutr. Food Res.* 2611–2621. doi:10.1002/mnfr.201600305
- Wendel, A.A., Purushotham, A., Liu, L.-F., Belury, M.A., 2009. Conjugated linoleic acid induces uncoupling protein 1 in white adipose tissue of ob/ob mice. *Lipids* 44, 975–982. doi:10.1007/s11745-009-3348-9
- Westertep, K.R., 2004. Diet induced thermogenesis. *Nutr. Metab. (Lond)*. 1, 5. doi:10.1186/1743-7075-1-5
- Wishnofsky, M.M.D., 1958. Caloric Equivalent of Gained or Lost Weight. *Am. J. Clin. Nutr.* 6, 542–546.
- Wu, J., Boström, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., Khandekar, M., Virtanen, K.A., Nuutila, P., Schaart, G., Huang, K., Tu, H., Van Marken Lichtenbelt, W.D., Hoeks, J., Enerbäck, S., Schrauwen, P., Spiegelman, B.M., 2012. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150, 366–376. doi:10.1016/j.cell.2012.05.016
- Xiao, C., Goldhof, M., Gavrilova, O., Reitman, M.L., 2015. Anti-obesity and metabolic efficacy of the beta3-adrenergic agonist, CL316243, in mice at thermoneutrality compared to 22 °C. *Obesity* 23, 1450–1459. doi:10.1002/oby.21124
- Yazbeck, J., Gubern, M., Senault, C., Chapey, M.F., Portet, R., 1989. The effects of essential fatty acid deficiency on brown adipose tissue activity in rats maintained at thermal neutrality. *Comp. Biochem. Physiol. Part A Physiol.* 94, 273–276. doi:10.1016/0300-9629(89)90548-3
- Young, J.B., Daly, P.A., Uemura, K., Chaouloff, F., 1994. Effects of chronic lard feeding on sympathetic nervous system activity in the rat. *Am J Physiol* 267, R1320-8.
- Young, J.B., Walgren, M.C., 1994. Differential effects of dietary fats on sympathetic nervous system activity in the rat. *Metabolism* 43, 51–60. doi:10.1016/0026-0495(94)90157-0
- Young, P., Arch, J.R.S., Ashwell, M., 1984. Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS Lett.* 167, 10–14. doi:10.1016/0014-5793(84)80822-4
- Zaibi, M.S., Stocker, C.J., O'Dowd, J., Davies, A., Bellahcene, M., Cawthorne, M.A., Brown, A.J.H., Smith, D.M., Arch, J.R.S., 2010. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids, *FEBS Letters*. doi:10.1016/j.febslet.2010.04.027
- Zhang, Y., Xu, Q., Liu, Y.H., Zhang, X.S., Wang, J., Yu, X.M., Zhang, R.X., Xue, C., Yang, X.Y., Xue, C.Y., 2015. Medium-Chain Triglyceride Activated Brown Adipose Tissue and Induced Reduction of Fat Mass in C57BL/6J Mice Fed High-fat Diet. *Biomed.*

- Environ. Sci. 28, 97–104. doi:10.3967/bes2015.012
- Ziegler, T.R., Evans, M.E., Fernández-Estívariz, C., Jones, D.P., 2003. TROPHIC AND CYTOPROTECTIVE NUTRITION FOR INTESTINAL ADAPTATION, MUCOSAL REPAIR, AND BARRIER FUNCTION. *Annu. Rev. Nutr.* 23, 229–261. doi:10.1146/annurev.nutr.23.011702.073036
- Zingaretti, M.C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., Cinti, S., 2009. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120. doi:fj.09-133546 [pii]r10.1096/fj.09-133546
- Zou, M.L., Moughan, P.J., Awati, A., Livesey, G., 2007. Accuracy of the Atwater factors and related food energy conversion factors with low-fat, high fiber diets when energy intake is reduced spontaneously. *Am. J. Clin. Nutr.* 86, 1649–1656.