Chapter 1

1

Introduction

Fasting and energy-sensing

A prolonged fast has tremendous effects on metabolism. Mainly insulin, but also glucose levels quickly decline over the course of a couple of days. During the fed state energy is mainly derived from carbohydrate oxidation. This switches during fasting towards lipid oxidation. The amount of carbohydrate and lipid oxidation can be estimated by indirect calorimetry. This method uses the CO2 and O2 in the exhaled air to calculate substrate oxidation [1]. During a fast, the increase in lipid oxidation leads to the production of ketone bodies (beta-hydroxybutyrate and acetoacetae) in the liver, which can be used as fuel by the brain when glucose levels drop [2]. After 2-3 days of fasting, liver glucose stores (glycogen) are depleted. At this point, glucose is synthesized in the kidney and also in the liver from lactate, pyruvate, glycerol and amino acids such as alanine (gluconeogenesis) [3].

While fasting, the activity of hormonal axes such as the thyroid-axis – important for metabolism – and the reproductive axis are downregulated. With respect to the thyroid-hormone axis this is characterized by decreased thyroid-stimulating hormone (TSH) and triiodothyronine (T3) levels whereas T4 levels remain stable which is due to an altered deiodinase activity during fasting and leptin might be involved as well [4;5]. Likely, the reduction in T3 levels saves energy during fasting. The pituitary-gonadal axis is downregulated during fasting as well; plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone decrease during fasting whereas sex-hormone binding globulin (SHBG) levels increase [6]. Most likely, this is an evolutionary mechanism to prevent reproduction when food is sparse.

On a molecular level, so-called energy-sensing pathways are affected during fasting. The adenosine monophosphate-activated kinase (AMP-activated kinase, AMPK) is of special interest. This enzyme is phosphorylated by liver kinase B1 (LKB1), in response to energy depletion and the coinciding increase in AMP/ATP ratio. Generally speaking, the actions of AMPK aim to restore energy balance; AMPK inhibits anabolic processes and stimulates catabolic processes. AMPK is also involved in the metabolic shift towards lipid oxidation during fasting; AMPK phosphorylates and thereby deactivates acetyl-CoA carboxylase 1 (ACC). This results in an increased fatty acid oxidation and reduced lipid storage [7]. The most important effects of AMPK on glucose metabolism are that it increases glucose uptake [8;9] and inhibits the hepatic glucose production [10-12]. Besides AMPK, there are many upstream and downstream (in)direct targets that play a role during fasting with respect to either energy-sensing or the

metabolic shift such as the sirtuins (SIRT, from silent information regulators), histone deacetylase 4 (HDAC4), mammalian target of rapamycin (mTOR), Forkhead box O (FOXO), protein kinase B (PKB/Akt), pyruvate dehydrogenase kinase isozyme 4 (PDK4), glucose transporter type 4 (GLUT4), cluster of differentiation 36 (CD36) and ACC.

Obesity

The body mass index (BMI) is used to define obesity; the BMI is calculated by dividing body weight (in kilograms) by the square of height (in meters).The World Health Organization defines obesity as a BMI ≥ 30 kg/m^2 and overweight as a BMI between 25 and 30 kg/m^2. The prevalence of obesity has doubled since 1980. In 2008 there were 1.5 billion overweight and 500 million obese adults (>20 years) [13]. Obesity is a risk factor for cardiovascular diseases, diabetes, musculoskeletal disorders and many forms of cancer (e.g. endometrial, breast and colon cancer). Since it became clear that AMPK is important for sensing and repairing energy balance disturbances, and obesity is clearly a result from disrupted energy balance, the role of AMPK in type 2 diabetes mellitus and obesity has been investigated. Skeletal muscle is very flexible with respect to the metabolic shift from glucose towards fatty acid oxidation. Since the *musculus vastus lateralis* is easily accessible, energy-sensing pathways are often studied in human biopsy materials of this muscle. With respect to AMPK, thus far only few alterations in obesity and type 2 diabetes mellitus (T2DM) have been found. One study has shown that AMPK activity is reduced in muscle from obese subjects [14]. In contrast, other studies showed that skeletal muscle AMPK activity is similar between lean and obese individuals or obese subjects and T2DM patients [15;16].

Brain

The hypothalamus is probably the most important area of the brain involved in the homeostasis of metabolism, food intake and energy expenditure [17]. The hypothalamus is located in the ventral part of the diencephalon, below the thalamus. The hypothalamus has important hormonal effects, since it secretes factors and hormones that stimulate the pituitary to secrete hormones (with neuro-endocrine feedback). The hypothalamic arcuate nucleus receives information about the peripheral blood through the median eminence, where the blood-brain barrier is absent [18]. Claude

Bernard was the first to suggest a role for the hypothalamus in glucose homeostasis in 1855 [19]. It is now known that different hypothalamic areas have different roles in weight maintenance. Animal studies show that damaging the lateral hypothalamus induces weight loss whereas damaging the ventromedial part (VMH) of the hypothalamus induces obesity (3-6). On a neuronal level, food intake is regulated by hypothalamic neurons that either stimulate (orexigenic) or inhibit (anorexigenic) food intake. The orexigenic neurons contain NPY (neuropeptide Y) and AGRP (agouti-related protein), neurotransmitters that stimulate food intake [23-25]. Anorexigenic neurons contain CART (cocaine- and amphetamine-regulated transcript) and POMC (proopiomelanocortin), neurotransmitters that inhibit food intake [26-28].

Functional Magnetic Resonance Imaging

When a human body is placed in a magnetic resonance imaging (MRI) scanner its hydrogen nuclei get excitated by radiofrequency pulses in two directions; longitudinal and transversal. Contrasts on MR images are based on the relaxation of these excitated nuclei; longitudinal relaxation (T1) and transverse relaxation (T2). Functional MRI (fMRI) uses blood-oxygen-level dependent (BOLD) signals as a measure of brain activity. The basics behind this mechanism were described at first by Linus Pauling in 1936 which was later implemented by Seiji Ogawa [29;30]. Oxygenated hemoglobin (Hb) is diamagnetic, whereas deoxygenated Hb is paramagnetic. Brain activity in a certain brain region increases the blood flow to this region. This alters the ratio of oxygenated Hb versus deoxygenated Hb in favor of the oxygenated hemoglobin in the blood stream itself and in the surrounding tissues. Paramagnetic molecules reduce signal intensity (T2 value) and result in a dark image. When the amount of paramagnetic molecules in the blood decreases (during increased brain activity) the opposite occurs; the signal intensity increases. Since this was discovered, a lot of research has been performed in the neuropsychological field to correlate brain function with specific brain regions.

Within the brain, the hypothalamus is involved in the regulation of food intake and energy expenditure [17] whereas the rewarding effects of food are mainly regulated in the amygdala [31]. As reviewed, the brain – mainly the hypothalamus again - also plays a role in glucose homeostasis [32]. To date, however, there are not many functional MRI studies that looked at the effect of fasting on neuronal activity. A positron emission tomography (PET) study showed that a 36 hour fast (“hunger”), compared to the

satiated state, increased regional cerebral bloodflow (rCBF) in the hypothalamus, insula, (para)limbic areas (such as the ACC), thalamus, cadaute, precuneus, putamen and cerebellum [33]. Two other PET studies looked at the response to respectively the taste or ingestion of a meal after a 36 hour fast in lean and obese individuals. rCBF increased in the midbrain and insula and decreased in the PCC, temporal cortex and OFC in obese compared to lean participants in response to tasting a meal after the fast [34]. Meal ingestion, after a 36 hour fast, led to a higher increase in rCBF the prefrontal cortex in obese compared to lean individuals [35]. Besides, a larger rCBF decrease was seen in the (para)limbic areas in obese compared to lean individuals. Decreases in the hypothalamus, ACC and thalamus upon satiation were smaller in obese than lean participants [35].

Heart Rate Variability

During a short-term fast in humans, the activity of the sympathetic nervous system (SNS) increases [36;37]. A derivative of SNS activity, heart rate variability (HRV), can easily be measured in humans by electrocardiography. Indeed, heart rate is under control of the autonomic nervous system (as reviewed [38]). The autonomic nervous system consists of the sympathetic nervous system that increases heart rate and the parasympathetic nervous system that decreases heart rate. The balance between these autonomic branches is extremely dynamic and quickly adapts to environmental cues, such as fasting. The sympathetic nervous system mobilizes energy whereas the parasympathetic nervous system is important for the digestion of food and energy storage. Disbalances of the autonomic nervous system mostly result in hyperactivity of the sympathetic nervous system and a decrease in parasympathetic nervous system activity. In obesity, an increased SNS activity has been previously described [39].

Outline Thesis

The general aim of this thesis was to study the physiological adaptations to a prolonged-fast in different populations on several parameters. In these studies, we were mainly interested in the response of energy-sensing pathways in skeletal muscle. First, we hoped to contribute to the understanding of normal fasting physiology. Besides, a better understanding of the response of these energy-sensing pathways to a prolonged-fast - and the possible differences in this response between lean and

obese subjects - may lead to the identification of factors that are involved in the pathophysiology of obesity. A bit far stretched, baseline differences between lean and obese subjects in the functioning of their energy-sensing systems might give cues for therapeutic targets.
In the current thesis, we hypothesized that the response to fasting would be different between lean and obese subjects. Next to the energy-sensing pathways, we also studied the neuronal response with functional MRI scanning and we assessed the response of the autonomic nervous system by heart rate variability measurements. These studies are described in the "middle" chapters of this thesis. The thesis starts with a chapter that is dedicated to the time-course of energy-sensing adaptations during a 24 hour fast in healthy young men. At the very end of this thesis, we describe the result of a study in which we hypothesized that the adaptations to a 60 hour fast would be altered by the presence of food related odours during fasting.

In **chapter two**, we hypothesized that the response to a prolonged fast of 48 hours would be different between lean and obese individuals. We compared this response on several levels in 14 obese and 12 lean individuals: we made fMRI scans, took blood samples, performed an indirect calorimetry, an electrocardiogram to measure HRV and took muscle biopsies. All measurements were performed both before and after the 48 hour fast (the indirect calorimetry and blood sampling were also performed after 24 hours of fasting). The results of the HRV measurements, used to study the effect of fasting on SNS activity in lean and obese individuals, are described in **chapter three**. In **chapter four** we describe the differential effect of fasting on the hypothalamus, amygdala and posterior cingulate cortex functional connectivity networks (fMRI) in lean and obese individuals. In **chapter five**, we investigated the time course of metabolic adaptations in response to a 24 hour fast. We investigated this time course by taking blood samples, by performing an indirect calorimetry (to measure lipid and carbohydrate oxidation) and by taking muscle biopsies in a group of 12 healthy young male volunteers at 3 time points during a 24 hour fast (different in that respect compared to chapter two). In the muscle biopsies we were mainly interested in the so-called "energy-sensing" pathways. In **chapter six** we evaluated the hypothesis that the response to fasting would be influenced by the presence or absence of visual and odorous food cues, based on a study in *Drosophila Melanogaster* [40]. Twelve lean men fasted twice during 60 hours; once in the presence and once in the absence of food cues. We studied the effects of the presence and absence of the

food cues on blood parameters, lipid and glucose oxidation (measured by indirect calorimetry) and the hypothalamic BOLD signal (measured by fMRI). In **chapter seven** we discuss all chapters described above.

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