Chapter 7

Discussion

The prevalence of obesity has been increasing now for several decades [1]. Obesity is associated with an increased morbidity and an increased risk for type 2 diabetes mellitus, cardiovascular disease and cancer. To date, there is no effective non-invasive treatment for obesity. Lifestyle interventions often fail in the long run. Therefore, a better understanding of the (patho)physiological alterations that occur in patients with obesity will be useful for the development of adequate therapies. Besides, this could lead to a decrease in the stigmatization of obesity. Finally, a better understanding of this disease could lead to an improved political recognition of this problem that cannot simply be solved on an individual level.

Seemingly paradoxical, studying the physiology of fasting might contribute to a better understanding of obesity pathology. Fasting has profound effects on metabolism. The plasma levels of several hormones decrease, lipid oxidation increases, liver glycogen stores get depleted and insulin resistance develops [2-4]. In skeletal muscle, energy-sensing factors react to maintain homeostasis. A better understanding of the function of these energy-sensing factors, and the possible differences between lean and obese subjects, might lead to new targets for the treatment of obesity. With respect to energy sensing, we mainly focused on the AMP-activated kinase (AMPK). This enzyme is activated by upstream liver kinase B1 (LKB1) in response to the increased AMP/ATP ratio caused by energy depletion [5].
In this thesis, we compared the skeletal muscle, neuroendocrine, autonomic nervous system and metabolic adaptations to a 48 hour fast in obese compared to lean subjects. Besides, we investigated the time course of adaptations to a 24 hour fast in healthy young men. Finally, we performed an experimental study to determine whether the presence of odors would affect the hormonal and neuronal adaptations to a 60 hour fast.

COMPARING THE RESPONSE TO A 48 HOUR FAST BETWEEN LEAN AND OBESE SUBJECTS

Skeletal muscle

In **chapter two**, we hypothesized that the whole-body and skeletal muscle metabolic adaptations to a 48 hour fast would be different between lean and obese individuals. More specifically, we hypothesized that AMPK phosphorylation would be increased after 48 hours of fasting and that the increase would be attenuated in obese subjects. In contrast to our expectations we found that AMPK was slightly but significantly reduced after the 48 hour fast, but only in lean individuals. As expected from previous studies, we did demonstrate that the response of insulin, leptin, growth hormone and substrate oxidation to the fast is attenuated in obese subjects. Indeed, obese subjects are characterized by metabolic inflexibility [6]. We also found that the expression of mitochondrial respiratory-chain protein subunits was significantly reduced in obese individuals. This might have a causal link with the reduced metabolic flexibility in obese individuals. Future studies are necessary to determine the precise role of AMPK and the molecular pathways of the mitochondrial protein downregulation in the metabolic inflexibility that exists in obese subjects.

Heart rate variability

In **chapter three**, we describe the effects of a 48 hour fast on heart rate variability (HRV) parameters in lean and obese subjects. Heart rate variability is under control of the autonomic nervous system [7] and has been shown to be decreased in obesity, mainly due to an increase in sympathetic nervous system (SNS) activity [8]. Fasting has previously shown to increase SNS activity [9;10].

Contrasting our expectations, we did not observe any differences in HRV between lean and obese subjects at baseline. This may be due to our study design; we performed HRV measurements in the postprandial state (75 minutes after a standardized breakfast), which might have affected HRV, since meal ingestion increases the sympathetic tone [11;12]. We demonstrated that fasting decreased both vagal tone as well as the sympathetic tone in the lean subjects. In obese subjects, the fast induced a slightly smaller decrease in vagal tone and in fact increased the sympathetic tone. However, the differences between groups did not reach statistical significance. Finally

we show that the 8 week weight-loss intervention increased the postprandial sympathetic tone in the obese subjects. Taken together, our results suggest that the response to fasting might be attenuated in obese subjects but since statistical analysis of the difference of this response between groups did not reach significance our study may have been underpowered. Finally we showed that weight-loss increased the sympathetic response to meal ingestion. Future studies are necessary to investigate how long this adaptation is maintained and what the physiological meaning is.

Neuronal; functional magnetic resonance imaging

The effects of a 48 hour fast on three different "functional connectivity" brain networks, supposedly involved in the control of energy metabolism and mapped by functional magnetic resonance imaging (fMRI), were studied in 12 lean and 12 obese individuals. The results of this study are described in **chapter four**. fMRI scans were made at baseline (after an overnight fast) and after 48 hours of fasting. Functional connectivity (FC) is used to study how different brain regions interact. It can be either 'positive' or 'negative' [13;14]. FC is considered positive when brain regions show a similar response (increase or decrease) simultaneously. FC is considered negative when brain regions show an opposite neuronal response at the same time (one region increases activity whereas the other decreases neuronal activity or vice versa). We studied FC networks of the amygdala, the posterior cingulate cortex (PCC, default-mode network) and most importantly the hypothalamus. At baseline, lean individuals demonstrated increased amygdala functional connectivity with the ventromedial prefrontal cortex (vmPFC) and the superior temporal gyrus. Mainly the vmPFC is of interest, since previous studies suggest that this region might be involved in reward sensation [15]. Notably, the response to fasting in amygdala connectivity was not significantly different between groups.

PCC connectivity (default-mode network) with the brainstem was stronger in lean subjects, whereas PCC connections with the bilateral frontal opercular cortices, extending into the insula, were stronger in obese individuals at baseline possibly reflecting alterations in satiety/gustation [16;17]. PCC connectivity in response to fasting was not significantly different between groups.

Our most important findings were in hypothalamic connectivity. Connectivity between hypothalamus and left insula decreased to a greater extent in response to fasting in the obese subjects. This effect is probably the result of a (non-significant) stronger

connectivity with the left insula in the obese subjects at baseline. Furthermore, we found that the connectivity between the hypothalamus and the dACC turned from negative to positive in lean subjects, whereas it turned from positive to negative in the obese subjects upon fasting. Since the hypothalamus, insula and dACC are all part of the so-called 'salience' network [18] which has been proposed to perceive both internal and external cues to adapt behavior and/or physiology accordingly [19]. This possibly difference in the salience network's response to fasting may indicate a different neuronal perception of calorie-imbalance between lean and obese subjects. However, before strong conclusions are drawn, the physiological and behavioral ramifications need to be established.

In **chapter five**, we investigated the effects of 24 hours of fasting on skeletal muscle in healthy young individuals on three different time points. We found that the well-known shift from glucose towards lipid oxidation and the downregulation of insulin levels coincided with a decreased activation state of PKB/Akt and mTOR pathways in skeletal muscle. We had expected that AMPK, as an important energy sensor, would be activated upon fasting. However, AMPK activity was not affected at any of the studied time points. We cannot rule out that AMPK is activated at an earlier moment after meal termination (at the beginning of the fast). Future studies are therefore necessary to identify if and exactly when AMPK is activated upon fasting/ meal termination. On our skeletal muscle biopsies, transcriptome analysis identified 23 genes that were significantly affected by fasting at both 10 and 24 hours of fasting. Some of these genes had already previously been shown to be up (ANGPLT4, CITED2, PDK4, PFKFB3, TXNIP and UCP3) or down-regulated (SLC25A25) in response to fasting and are probably implicated in the shift from glucose to lipid oxidation. With respect to several other genes, results have to be confirmed in other studies and their precise role has to be elucidated since only few previous data is available.

7

THE EFFECTS OF ODORS ON METABOLIC, HORMONAL AND NEURONAL ADAPTATIONS TO A 60 HOUR FAST

In **chapter six** we translated a study performed in *Drosophila Melanogaster* to humans. Libert et al. showed that the caloric restriction induced increase in life span was attenuated when fruit flies were exposed to food odors during caloric restriction [20]. Food odors have been shown to impact on hypothalamic function in humans. We

hypothesized that the human adaptations to a short-term fast would be affected by the presence of food odors during fasting. We were mainly interested whether the hypothalamic response, measured by fMRI, to glucose ingestion would be affected. To study this hypothesis, we exposed 12 healthy young men to a 60 hour fast on two occasions in random order. During one fasting intervention, the volunteers were refrained from visual and odorous food cues. During the second fasting intervention volunteers were exposed to visual and odorous food stimuli at regular time points.
In contrast with our expectations, we found that the hypothalamic signal was similar between the fasting conditions. The well-known downregulation of pituitary-hypothalamus and pituitary-gonadal axes upon fasting was also similar in both conditions. Finally, the development of insulin resistance (another well-known effect of fasting) was not affected by the presence of food cues. In summary, the presence of odors during a short-term fast did neither affect the hypothalamic signal nor the hormonal and metabolic adaptations that occur in response to fasting.

OVERALL CONCLUSIONS

In this thesis we examined the hormonal; metabolic; molecular and neuronal effects of fasting in both lean and obese individuals. As expected, fasting induced a shift from glucose towards lipid oxidation. As expected, both the hormonal response as well as the metabolic shift from glucose towards lipid oxidation was impaired in obese individuals. With respect to the molecular pathways we investigated, our most striking finding was that – at baseline - the mitochondrial protein content in skeletal muscle of obese subjects was significantly reduced when compared to that of lean individuals.
The shift towards fat oxidation coincided with a time-dependent decrease in hormonal levels of insulin as well as a decreased activation state of PKB/Akt and mTOR pathways in skeletal muscle of lean young men. Interestingly, AMPK – an important energy-sensing kinase - was not affected by 24 hours of fasting. However, we found a trend ($p=0.08$) for a decreased AMPK activity and its downstream target ACC upon 48 hours of fasting in the lean subjects but not in obese subjects. Since this finding did not reach significance, it needs to be confirmed and further investigated in future studies.
We also assessed the neuronal response to fasting by performing fMRI scanning. We demonstrated that the neuronal response to fasting was significantly different in

lean compared to obese individuals in terms of functional connectivity between the hypothalamus and respectively the dACC and insula. Since these regions are part of the saliency network, these differences may reflect distinct perception of calorie-imbalance between lean and obese subjects. The physiological and behavioral implications of this finding need to be established.
Then, the effects of fasting on sympathetic tone (estimated by heart rate variability) were studied. Our data suggests that fasting decreases sympathetic tone in lean subjects, whereas it increases sympathetic activity in obese individuals. However, the group interaction statistic test did not reach significance. We also showed that weight-loss in the obese individuals significantly increased HRV parameters that reflect the postprandial sympathetic tone.
Finally, we studied the effects of fasting in the presence and absence of food-odors since this has been shown to reduce the fasting-induced increase in life span in fruit flies. However, we show that fasting physiology (hormonal, metabolic and neuronal responses) was not affected by the presence of food-related odors in a group of healthy young volunteers.

FUTURE PLANS

Our main plan for the future, directly related to the work described in this thesis, is to study the effects of weight-loss in obese subjects on gene expression as well as on energy-sensing pathways in skeletal muscle. The study design was already described in **chapter three**. Of course it would be interesting to investigate gene expression in this study as well. Besides, we think it would be very interesting to perform a study similar to **chapter five** but with biopsies performed at time points closer to meal termination. We think it is possible that we missed AMPK activation in our studies due to sampling error, we still think it is likely that AMPK is activated during fasting. By studying at earlier time points during fasting we would hope to find exactly when AMPK is activated during fasting.

References

1. World Health Organization. Obesity and Overweight, Fact Sheet No 311. 2011.
2. Cahill GF, Jr. Fuel metabolism in starvation. *Annu Rev Nutr* 2006; 26:1-22.: 1-22.
3. Heemstra KA, Soeters MR, Fliers E, Serlie MJ, Burggraaf J, van Doorn MB, van der Klaauw AA, Romijn JA, Smit JW, Corssmit EP, Visser TJ. Type 2 iodothyronine deiodinase in skeletal muscle: effects of hypothyroidism and fasting. *J Clin Endocrinol Metab* 2009; 94(6): 2144-2150.
4. Bergendahl M, Aloi JA, Iranmanesh A, Mulligan TM, Veldhuis JD. Fasting suppresses pulsatile luteinizing hormone (LH) secretion and enhances orderliness of LH release in young but not older men. *J Clin Endocrinol Metab* 1998; 83(6): 1967-1975.
5. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP, Alessi DR, Hardie DG. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2003; 2(4): 28.
6. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol* 1999; 277(6 Pt 1): E1130-E1141.
7. Dampney RA. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 1994; 74(2): 323-364.
8. Muscelli E, Emdin M, Natali A, Pratali L, Camastra S, Gastaldelli A, Baldi S, Carpeggiani C, Ferrannini E. Autonomic and hemodynamic responses to insulin in lean and obese humans. *J Clin Endocrinol Metab* 1998; 83(6): 2084-2090.
9. Chan JL, Mietus JE, Raciti PM, Goldberger AL, Mantzoros CS. Short-term fasting-induced autonomic activation and changes in catecholamine levels are not mediated by changes in leptin levels in healthy humans. *Clin Endocrinol (Oxf)* 2007; 66(1): 49-57.
10. Webber J, Macdonald IA. The cardiovascular, metabolic and hormonal changes accompanying acute starvation in men and women. *Br J Nutr* 1994; 71(3): 437-447.
11. Lu CL, Zou X, Orr WC, Chen JD. Postprandial changes of sympathovagal balance measured by heart rate variability. *Dig Dis Sci* 1999; 44(4): 857-861.

12. Tentolouris N, Tsigos C, Perea D, Koukou E, Kyriaki D, Kitsou E, Daskas S, Daifotis Z, Makrilakis K, Raptis SA, Katsilambros N. Differential effects of high-fat and high-carbohydrate isoenergetic meals on cardiac autonomic nervous system activity in lean and obese women. *Metabolism* 2003; 52(11): 1426-1432.
13. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U S A* 2001; 98(2): 676-682.
14. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A* 2003; 100(1): 253-258.
15. Stoeckel LE, Weller RE, Cook EW, III, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *Neuroimage* 2008; 41(2): 636-647.
16. Berthoud HR. The vagus nerve, food intake and obesity. *Regul Pept* 2008; 149(1-3): 15-25.
17. Scott TR, Yaxley S, Sienkiewicz ZJ, Rolls ET. Gustatory responses in the frontal opercular cortex of the alert cynomolgus monkey. *J Neurophysiol* 1986; 56(3): 876-890.
18. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci* 2007; 27(9): 2349-2356.
19. Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct* 2010; 214(5-6): 655-667.
20. Libert S, Zwiener J, Chu X, Vanvoorhies W, Roman G, Pletcher SD. Regulation of Drosophila life span by olfaction and food-derived odors. *Science* 2007; 315(5815): 1133-1137.