Title of the thesis: ***"The transcriptomic signature of fasting"***

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Summary of the "The transcriptomic signature of fasting"

Adaptation of the body to the total food deprivation occurs in a number of phases, described in **Chapter 1** of this thesis. The death occurs when 40-50% of the original body weight is lost. Due to relatively high metabolic rate in small animals comparing to the large ones, mice can survive maximum 4 days of food deprivation, rats around 14 days, and in humans – children 4 weeks and adults 2 months. Based on the rate of body weight loss, three phases is fasting can be distinguished. During the first, post-absorptive phase the rate of body weight loss is relatively high and fast (25% in mouse, 10% in rat, 3-4% in human). The intermediate, "coping" phase, is characterized by just one third of the weight loss seen in the first phase, thus it is assumed that the physiology of the animal is reasonably well armed against the lack of food at this time. During the preterminal, final phase, the body weight loss increases again and is followed by death. According to the current theory, during the first phase of food deprivation the carbohydrates are predominantly used, during the second mainly the fats, and during the last one the proteins. The data presented in this thesis question this assumption, and indicate that in the gut and liver these three metabolic processes are simultaneously activated in response to fasting.

The gut is one of the most energy consuming organs in the body. Still, not much is known about the consequences of fasting to the intestine, especially about the ways of adaptation to fasting in this organ. Therefore, in **Chapter 2** we examined the changes in gene expression on mRNA level, using microarray approach in a mouse model. The mice were fasted for 12, 24 and 72 hours. They stood the challenge without problems, given that the environment was warm enough. After ascertaining the changes in mRNA level in the extracts from the total small intestine, we analysed which metabolic routes showed the highest alteration. In addition, using the immunohistochemical techniques, we studied to what extent has the architecture of the small intestine undergo changes. The highest changes in the intestinal weight (~40% on the daily basis) occurred during the first 12 hours of fasting. In the following 36 hours this weight loss was ~7%, and ~30 during the last 24 hours, making the total loss of the gut weight about 50% after 72 hours of fasting. In spite this significant decrease, it seems that the weight loss was proportionally divided between the components of the small intestine (epithelium, smooth muscles and binding tissue), so that the tissue basis ("denominator"), that the changes in gene expression should be related to, remained unchanged. After 12, 24 and 72 hours of fasting 1495, 1336 and 3008 transcripts changed the expression ≥ 1.4 fold, respec-

tively. Furthermore, the unsupervised hierarchical clustering method revealed that the changes in expression profiles are clearly different between the time points. The biggest chges occurred during the first 12 hours of fasting. Surprisingly, most of these changes faded away, so that after 24 hours the changes were more prominent than after 12 hours of fasting. In the following 48 hours the changes have gradually increased again. The early changes in gene expression point to a shortage of glutamine and metabolic adaptations directed to limit its effects, by slowing down the burning of pyruvate, stimulation of glutamate breakdown via aspartate and phosphoenolpyruvate to lactate, and increased burning of fatty-acids and production of ketone-bodies. Also, the expression of genes involved in cell proliferation and programmed cell death was suppressed. Finally, the changes in the small intestine after prolonged fasting pointed out that the gut became able to produce glucose instead of lactate, that the burning of fatty-acids was slowed down, and that the respiratory chain was very strongly suppressed. Cell turnover also remained suppressed. These observation lead us to a conclusion that the small intestine looses its weight in a very high place, in order to produce ketone bodies, lactate, and eventually glucose. In spite the fact that these quantitavely significant adaptations in gene expression indicate radical changes in function, the architecture of the small intestine was kept intact, by reduction of the speed of cell turnover. We think that it points to a capability of the small intestine to quickly re-establish its primary function (the digestion) once the food is available again.

To study to which level the changes in the mRNA content described in Chapter 2 are reflected in the changed levels of the corresponding proteins, we carried out a proteomics study of these intestines described in **Chapter 3**. The proteins were separated with help of two-dimensional electrophoresis and studied for the changes in concentration in response to fasting. The proteins with different concentrations between the time-points were then identified using mass spectrometry. In total, 80 of such proteins were found. Those changed in response to 12 hours of fasting were mainly part of glycolysis and energy metabolism, while proteins involved in protein synthesis and amino-acid metabolism were decreased after 24 hours. Proteins with a cell protective role, like glutathione peroxidase and carbonic anhydrase, increased in concentration. After 72 hours of fasting, ezrin, a protein involved in the formation of intestinal vili, was increased. These results indicate that the RNA and protein expression patterns, with currently available technology, cannot yet be directly related to each other.

To work out the level of similarity between gut and liver in their adaptation to food withdrawal, we have also investigated the changes in RNA expression pattern of the fasted liver in **Chapter 4**. The studied livers came from the same animals from which the small intestines were analyzed in Chapter 2. We have also used the same approach and techniques as in Chapter 2. The liver weights decreased ~ 45%, 5% and 10% on the daily basis, during the first 12 hours, between 12 and 24, and after 24 hours of fasting, respectively. Just like suggested for the small intestine, the liver architecture remained unchanged, despite the 50 % weight loss. When compared with the fed group, 201, 504 and 119 transcripts were more than 1.4-fold changed after 12, 24 and 72 hours of fasting, respectively. This number was

considerably lower than that in the small intestine, as was the time-pattern of the changes. Hierarchical clustering showed further that expression patterns after 12 and 24 hours were not much different between themselves, but notably differed from the fed and 72 hours fasted animals. The changed expression of enzymes involved in amino-acid, carbohydrate, lipid and energy metabolism was most prominent after 24 hours, and pretty much declined after 72 hours. The strong stimulation of the urea cycle and tricarboxylic-acid cycle enzymes, and those involved in oxidative phosphorylation, points out to an increased burning of amino-acids during the first 24 hours. At this time point, fatty-acid burning and ketone-body production were also induced. Furthermore, the genes that protect the cell against the proteins which finished up miss-folded, due to the high pressure of strongly increased energy metabolism, were also upregulated. The sporadic processes that were still regulated after 72 hours of fasting, contained genes involved in the urea cycle, malate-aspartate shuttle through the mitochondrial membrane, and the gluconeogenic enzyme Pepck. At the same time, we noticed that the sugar storing product glycogen, which was totally gone from the liver after the first 12 hours of fasting, started slowly reappearing after 24 hours, to be present in high concentrations again after 72 hours in the pericentral cells of the liver. We interpreted these changes as indicative of glucose production in the periportal cells, and, as a consequence of glucose-6-phosphatase absence, of glycogen production in the pericentral cells. The changes in gene expression in the livers of fasted mice show therefore that the liver function in these circumstances is primarily directed to the production of fuel for other organs, like the brain. The storage of glycogen should in this setting be a consequence of a broadening of this function from periportal cells only to the total liver, where only the last step, namely the transformation of glucose-6-phosphate into glucose, is not sufficiently active in the pericentral liver cells, and therefore leads to the storage of glycogen in these cells.

In **Chapter 5** we studied the changes in lipid metabolism, and especially cholesterol metabolism, in the fasting gut and liver. The changes in gene expression leaned on the data described in Chapters 2 and 4, and indicate an increase of cholesterol trafficking in the liver and a decrease in the small intestine in response to fasting. We further studied the changes in lipid profiles of plasma, bile, liver and intestinal tissue, intestinal perfusate and faeces. Cholesterol concentration in plasma significantly increased and triglycerides decreased in response to both short and prolonged fasting. Triglyceride levels remained constant in the tissues - liver and small intestine, but the intestinal cholesterol even increased after prolonged fasting. Surprisingly, we determined an increase in production of bile salt, cholesterol and phospholipids in bile. They were accompanied by an increase in lipid turnover in liver and ntestinal, and increased cholesterol excretion through the intestinal wall into the lumen in prolonged fasting. Since the total cholesterol loss through faeces strongly declined, the excreted cholesterol obviously had to be reabsorbed in the small intestine. These data show that, in spite the absence of food, the lipid and especially cholesterol concentrations, remain remarkably stable, even in prolonged fasting, and that the bile production, so far mainly linked to feeding, paradoxically increases during fasting as well. Based on these data we hypothe-

sised that the fats released from bile into the intestinal lumen serve as a fuel for the intestinal epithelial cells, in order to stabilize the transport systems necessary for the quick recovery of the gut function once the food becomes available again.

In addition to a challenge to the current theory of "sugar-fat-protein" sequence in fuel utilisation in fasting, this research presents a remarkable fact that the organ architecture remains preserved even in prolonged fasting. Fasting, with its strong catabolic effect, induces huge changes in gene expression profile of the organs. However, it is also regulated in such a way that the organ architecture remains preserved for a very long time, enabling a quick recovery of function when food becomes available again.

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