The transcriptomic signature of fasting

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Citation for published version (APA):
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A short review of the effects of food deprivation
Chapter 1
The effects of fasting

Background and definitions

Throughout evolution, quality and abundance of food fluctuated, causing recurrent periods of feast and famine. Endotherms, with their high cost for thermoregulation, should be least tolerant to fluctuating food supplies and deficits in food intake [1]. Animals have developed the ability to store and control the distribution of energy during extreme resource limitations. This capacity to survive food deprivation is undoubtedly of substantial survival value [2]. Numerous biochemical and physiological adaptations support prolonged survival and physiological functions that sustain existential behavior (such as avoidance of predators or foraging) [3]. To ensure that digestive and metabolic processes can resume when food becomes available again is of equal importance. Energy saving occurs through suspending non-essential processes. Cessation of ovulation and suppression of reproductive behavior, for instance, is a natural physiological response to food shortage [4, 5].

A separate set of adaptations helps to sustain the storage of reserves. This latter set of adaptations can become disadvantageous under conditions of unlimited abundance, like in the modern Western society, generating obesity in preparation for scarcity that never comes [6].

The magnitude and consequences of fasting vary and depend on the levels, content and duration of insufficient energy intake, ranging from malnutrition to death of starvation. A feeling of discomfort from not eating is described by the term hunger, which has also been used to describe undernutrition, particularly in reference to food insecurity. Malnutrition encompasses overnutrition and obesity as one, and undernutrition, comprising stunting, wasting, and deficiencies of micronutrients, as another form [7]. Wasting represents a weight-for-height that is more than 2 standard deviations below the reference standard, while stunting describes a height-for-age in children that is more than 2 standard deviations below the reference standard [8]. In addition, malnutrition can be divided into two other conventional categories: kwashiorkor and marasmus. Kwashiorkor refers to a condition commonly thought to occur when carbohydrates are the major dietary energy source for a prolonged period of time, with protein being underrepresented in the diet. Clinical symptoms include hypoalbuminemia, edema, ascites, dermatitis, fatty liver with hepatomegaly, anorexia and muscle wasting [9-11]. Early weaning from mother’s milk to a protein-deficient oral diet is often associated with this condition in the non-industrialized countries. Marasmus refers to chronic deprivation of adequate dietary energy to maintain body weight, and can be, in severe cases, characterized by extreme weight loss and cachexia (with anorexia nervosa being a classic example in wealthy Western societies) [9]. The liver normally remains functional in marasmus, because hepatic serum protein levels are not affected until very late in the process [12]. Death from marasmus is typically caused by loss of respiratory muscle function and respiratory failure.

The World Health Organization (WHO) global database on child growth suggests that in the developing countries 1/5th of children younger than 5 years are underweight, 1/3rd are stunted and 1/30th suffer from severe wasting (the data are for 2005). Africa, Asia and Latin America are most affected, with the highest prevalence in South Central Asia and Eastern Africa [7]. The World Food
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Program (WFP) Annual report for 2006 indicates that almost 90 million people in 78 countries in the world are starving (87% are women and children), while 800 million or 16% of the total population in the developing countries, are undernourished [13]. Inadequately nourished children are usually lethargic, nonresponsive and impulsive. They display non-goal directed behavior, do not develop normal social interactions, and cannot cope with stress or high daily demands. Inadequately nourished adults develop behavior patterns similar to those of children. Malnutrition in childhood reduces adult body weight, which in turn restricts the capacity to work [14]. Malnutrition also adversely affects fertility [14, 7].

Biology of fasting

The response to total food deprivation proceeds in number of stages and ends with death. The successive stages are, however, not precisely defined. The initial period involves fasting and is followed by starvation [3]. Fasting in humans commonly refers to abstinence from food, often with religious or political motives, while starvation represents a state of extreme hunger, resulting from a prolonged lack of nutrients. Some authors define the first 24 h of food deprivation in humans as fasting, several (up to 4) days as starvation, and more than 7 days as undernutrition-underfeeding [15]. The transition between the stages is not the same for all species and neither are the maximal periods of starvation. The ability to tolerate deficits does decline with body size: in the mouse the maximum duration of starvation period is 4 days [16], in rat 12-15 days [17], in children 4 weeks and in adult humans 8-9 weeks [18, 19].

Due to the lack of consensus and to avoid further confusion, we will use the terms fasting and starvation (or short and prolonged fasting) to distinguish the severity of impact of total food deprivation. In this review, we will focus on complete food withdrawal. Cachexia and caloric restriction will be discussed as special cases of nutrient deprivation.

Body weight. As recently reviewed [20], Chossat noted already in 1843 [21] that mammals and birds died of starvation after losing 40-50% of their initial body weight. It was suggested by Krieger in 1921 [22] that the lethal levels of weight loss in humans vary between 40% and 50%, depending whether it results from complete or semi-starvation. Obese individuals, however, may lose up to 65-80% in some cases of therapeutic fasting [23]. In famine situations, a BMI of 10 kg/m2 can still be compatible with life, but hunger strikers usually die before such emaciation develops, demonstrating a broad variation in the tolerance of starvation [24, 25].

Resting metabolism and body temperature. The ever cited report of Benedict et al. from 1919 [26] revealed a moderate increase in resting energy expenditure (REE) in humans during the first 12 days of a prolonged period of restricted food intake, followed by the decrease over the next 30 days. Short-term total fasting is also accompanied by increased resting energy expenditure, to a certain extent probably due to the energy costs of gluconeogenesis, ketogenesis and fatty acid-triacylglycerol recycling [15, 27, 28]. As fasting proceeds, REE declines below the initial values [29-31] (after 4 days in humans [32, 33]). REE remains low during starvation [32, 33] and marasmic malnutrition [34, 35]. The reason for this decline may be the decrease in the fat-free mass as a re-
sult of the weight loss and a better metabolic efficiency that comes with a low energy intake. The gradual depletion of energy stores may explain the sensitivity to hypothermia during prolonged fasting [3, 36]. Hypothermia is more pronounced in small than in large animals [37]. Upon refeeding, REE increases in concert with diet-induced thermogenesis [34, 38], demonstrating high adaptability of energy metabolism.

*Physical activity and behavior.* The reduction in physical activity and the decrease in body temperature probably contribute more to energy sparing and, thereby, to tolerance of starvation, than the reduction in basal metabolic rate [3]. Hibernation and torpor are the most powerful means of endotherms to reduce their energy consumption [39]. In torpid states, organisms drop their metabolic rate to just a fraction of the normal resting metabolic rate [40]. Nonetheless, animals may forage more actively in conditions of food scarcity at the expense of increased energy usage or, just as commonly, decrease their activity and reduce energy expenditure. The ‘choice’ will depend on the foraging behavior, the reason of food deprivation, and other aspects of the animal’s biology. During the later and more critical phases of starvation many animals exhibit a marked stimulation of activity [17, 3].

*Modules/phases of fasting*  

Based on the rate of weight loss upon fasting, nitrogen excretion, concentration of plasma metabolites and resting metabolic rate, the body passes through three successive adaptive phases during fasting [41]. In mammals, these phases have been associated with the primary fuel that is putatively available to the tissues (e.g [42-45]), as determined by daily nitrogen excretion, amounts of fasting plasma metabolites, and total body nitrogen and neutral lipid composition.

During the first, postabsorptive fasting phase (*phase I*), the rate of weight loss is relatively high (~24% per day in mice [46], ~10% per day in rats [47, 48], and 3-4% in humans). These authors hypothesize that metabolism is largely fueled by (liver) glycogenolysis, which maintains blood sugar levels. Lipids are mobilized and glycerol and free fatty acids are released into circulation. Fatty acids can generate energy in tissues that can oxidize them (skeletal muscle, myocardium, liver and kidney), while glycerol can be converted into glucose in the liver [49].

During the intermediate *phase II* the loss of body mass is slower (~7% per day in mice, ~6% per day in rats [47], and ~1% in humans). In humans, this state can be maintained for several weeks and has been referred to as a coping phase. Liver glycogen stores are thought to be depleted and gluconeogenesis supplies the requirements of glucose-requiring organs such as the brain. Amino acids from proteolysis in muscle are the initial fuel for gluconeogenesis, but their input could diminish as lipolysis in fat tissue increases glycerol availability. Fatty-acid oxidation and ketone-body synthesis are thought to be increased [45, 50].

The preterminal *phase III* increases the rate of loss of body weight again in some animals (~9% in rats [47]). The adipose stores are depleted and the muscle is rapidly broken down for gluconeogenesis. The rapid loss in muscle mass cannot be sustained long and eventually kills the animal.
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Biochemistry of short fasting

During fasting, adipose tissue, muscle, liver, and kidneys work jointly to provide, convert, and preserve energy for the body. During the brief postabsorptive period, blood fuel homeostasis is maintained primarily by hepatic glycogenolysis and adipose tissue lipolysis [51, 52]. At the whole-body level, this adaptive response is accompanied by a decline in circulating insulin, glucose, triglycerides and cholesterol levels [48]. By the second or third day of starvation in humans, hepatic glycogen contribution to blood glucose has basically stopped [19]. As fasting progresses, muscle proteolysis supplies glycogenic amino acids for heightened hepatic gluconeogenesis for a short period of time. After about three days of starvation in humans, when the coping phase begins, protein degradation declines [41] and alternate fuels, such as ketone bodies [45] and free fatty acids [50] are utilized to maintain energy needs [51]. UCP2 and UCP3 protein levels were reported to increase in the mitochondria of skeletal muscle after food deprivation [53, 54], which may protect against oxidative stress during fat oxidation [55]. The ability of the kidney to conserve ketone bodies prevents the loss of large quantities of these fuels in the urine.

The amino-acid carbon skeletons are oxidized directly in the citric acid cycle, or indirectly after conversion to glucose, which is subsequently oxidized in the citric acid cycle of other cells [56]. The nitrogen from amino acids is excreted from the body by the kidneys as urea, ammonium, creatinine, and small quantities of other guanidine compounds [57]. Urea and ammonia excretion reflect the majority of the energy derived from aminogen catabolism. After a few days of starvation, total urinary nitrogen excretion diminishes as the major nitrogenous excretory compound, urea, decreases [58]. Autophagy is another well-known adaptive response to nutrient deprivation. It has been reviewed in relation to starvation, e.g. in [59-61], and will not be addressed in detail in this review.

Biochemistry of prolonged fasting

Prolonged fasting is accompanied by an increase in circulating corticosterone (in rodents; cortisol in humans) and plasma urea levels and a further decline in whole-body protein synthesis [47, 45, 42]. Total splanchnic glucose production after several weeks of starvation sums to approximately 80 grams daily in humans. About 13% arise from glucose synthesis from ketone bodies, 47% from recycled lactate and pyruvate, 20% from fat-derived glycerol, and the remaining 20 % from protein-derived amino acids [62-64, 19]. Although the glucose metabolism in the brain remains significant after several weeks of starvation in obese subjects, β-hydroxybutyrate and acetoacetate oxidation accounts for > 65% of fuel consumption in the brain [62, 19].

On the opposite page

Figures 1 and 2: Phase transition biomarker pathway in the fasting murine small intestine (1) and liver (2), created in the GenMAPP suite, to show the main metabolic changes in response to fasting. Warm colors (from yellow to red) represent down-regulation, while cold colors (light blue to dark green) indicate an induction (see scale on the right border of the figure). Gray indicates no significant change. Genes not coupled to reporters on the array are shown in white. Genes represented by more than one sequence on the array are shown in dash-lined boxes, with the level of change depicted by the colored line surrounding the field. Each gene-box is split into 3 units, representing (from left to right) a change in expression after 12, 24 and 72h of fasting comparing to fed mice. The data from Chapters 2 [46] and 4 in this thesis were used.
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The oxidation of amino acids remains essential, no matter how big the fat reserves are [56]. Branched-chain amino acids are preferentially catabolized in muscle. The nitrogen is released into the bloodstream primarily as glutamine [65] and alanine [66]. Glutamine is mainly metabolized by kidney to produce ammonium ions and the remaining carbon goes to glucose via the gluconeogenic pathway [67, 68]. Ammonia excretion increases late in fasting, with ammonia becoming the principal nitrogenous excretory product [63] (a condition that is highly correlated with ketonuria [69, 70]).

The kidneys produce ~40% of new glucose in starvation, while the liver produces produces ~60% [19]. A recent, but controversial series of experiments suggest that, in addition to the liver [63, 71, 72] and kidney [63, 73], the small intestine also has the capacity to produce glucose upon prolonged fasting [74, 46]. It contributes indirectly, by providing lactate and alanine to the liver in short-term fasting [75, 76], and directly by the production of glucose [46] (perhaps up to 27% of whole-body glucose production in extended fasting in the rat [77]). The concept is controversial, since other studies were unable to detect glucose formation from glutamine in the isolated small intestine of 72h fasted rats [78]. Furthermore, phosphoenolpyruvate kinase (Peck1) expression in the small intestine was reported to amount to only 0.05% of that in the liver after 12h hours of fasting in mouse [79], also arguing against intestinal gluconeogenesis. Our own data, however, suggest that the Pepck mRNA concentration in the intestine increases up to ~50 % of that in the liver after 72h of starvation in mice [Chapter 4 in this thesis]. The issue of intestinal gluconeogenesis in prolonged fasting therefore deserves additional study.

Regulation

During starvation, the low level of glucose in the blood leads to a decreased secretion of insulin and an increased secretion of glucagon. Glucocorticoids from the adrenal glands further induce gluconeogenesis, reduce glucose utilization by the muscles, and increase lipolysis and protein breakdown [80]. Chronic starvation such as seen in anorexia nervosa is also associated with changes in thyroid hormone metabolism, viz. a decrease in total and free T4 and T3, and an increase in rT3 [81].

Transcriptional regulation of genes involved in lipid metabolism and ketone body synthesis is known to be under peroxisome proliferator-activated receptor alpha (PPARα) coordination [82] [26]. It was recently reported that fibroblast growth factor 21 (FGF21) is a mediator of the pleiotropic actions of PPARα. It stimulates lipolysis in white adipose tissue and ketogenesis in liver, reduces physical activity and promotes torpor [83].

Organ-specific patterns

Based on the whole body energy expenditure, the “sugars-fats-proteins” succession of energy substrates during fasting was proposed [41, 84]. This model was then implicitly expanded to all organs separately. However, microarray studies in rodents that have prospected the adaptive response to fasting of the small intestine [46], liver [85] [Chapter 4 in this thesis], and muscle [86-88], and more limited studies in kidney [73], reveal a different scenario. Muscle and kidney respond to fasting
with a progressive change over time in mRNA concentrations of enzymes involved in protein, carbohydrate and fat metabolism. The response in liver peaked at 24-48 hours of fasting in mouse, while most adaptive changes had abated by 72 hours. The intestine showed a pronounced peak of adaptive metabolic changes at 12 hours of fasting, while many of the early adaptive changes had subsided by 24 hours. Thereafter, the expression of amino-acid catabolizing and gluconeogenic enzymes gradually increased. Figures 1 and 2 summarize the vital part of the metabolic response to fasting in the small intestine and liver. Early changes in intestinal gene expression (Figure 1) were associated with glutamine conservation, inhibition of pyruvate oxidation, stimulation of glutamate catabolism via aspartate and phosphoenolpyruvate to lactate, and enhanced fatty-acid oxidation and ketone-body synthesis. Major changes upon continued fasting implied the production of glucose rather than lactate from carbohydrate backbones, and downregulation of fatty-acid oxidation. The liver expression profile (Figure 2) differs from that in the small intestine in the (increased) expression of enzymes of the TCA cycle peaking at 24 hours, indicating a stimulation of amino-acid oxidation. In this period, fatty-acid oxidation and ketone-body formation were also induced. The continuous high expression of enzymes of malate-aspartate shuttle, and the gluconeogenic enzyme Pepck (as well as the re-appearance of glycogen in the pericentral hepatocytes [Chapter 4 in this thesis]) indicate that amino-acid oxidation yields to glucose and glycogen synthesis during prolonged fasting. The difference in patterns, size and direction of gene expression change in different organs could be used as a biomarker of phase transition and/or organ specificity.

Special cases of nutrient deprivation

Cachexia, definition and background

Severe or chronic disease can lead to wasting syndrome or cachexia, which involves weight loss and muscle wasting, and contributes significantly to disease morbidity and mortality. The degree of cachexia is inversely correlated with the survival time and always implies a poor prognosis [89]. The abnormalities associated with the condition are progressive weight loss, anorexia, asthenia, and anemia [90].

The weight loss of cachexia is thought to be caused by a reduction in food intake (due to pain, depression, side effects of therapy, etc), an increase in energy expenditure, a high catabolic state, or combination of these. There is evidence, however, that even in the presence of an increased resting metabolic rate, the total energy expenditure is reduced because of a decreased physical activity. Wasting is often the result of endocrine disorders accompanying the disease process itself. This, coupled with reduced energy intake, is often the primary cause of wasting [91].

Biology, biochemistry and regulation of cachexia

A variety of changes in nutrient metabolism have been described in patients with cancer cachexia, but thus far the exact mechanism remains unknown. Cachexia is characterized by hyperglycaemia, hyperinsulinaemia, hyperlactataemia, hypoalbuminemia, increased gluconeogenesis and decreased glycogen production [92, 93]. The associated metabolic disturbances in carbohydrate, lipid, and protein metabolism increase energy deficiency [90]. Patients often suffer from glucose
intolerance and insulin resistance, and in particular in relation to the liver, with increased activity of the Cori cycle [90]. Adipocyte lipolysis is increased, probably because of the enhanced expression and function of adipocyte hormone-sensitive lipase [94].

An acute-phase protein response is seen in a significant proportion of patients with cancer with disease progression. The cancer affects protein turnover in different tissues of the body in a different manner. These patients seem to lose a larger proportion of skeletal muscle mass than individuals subjected to starvation. The lysosomal system, cytosolic proteases and the ubiquitin (Ub)-proteasome pathway contribute to muscle protein degradation in cachexia. The Ub-proteasome pathway, which accounts quantitatively for the majority of skeletal muscle degradation, is stimulated by cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, interferon-γ and proteolysis-inducing factor [89].

Cachexia-induced hypoalbuminemia, which facilitates excessive transudation of fluids into extravascular spaces (edema and ascites), is a major contributor to the morbidity of the cachectic patients. It is mediated by TNFα, which provokes the export of C/EBP-β from the nucleus, preventing it to act as a transcriptional factor on the albumin promoter [93]. Stress-induced hypoalbuminemia occurs following a traumatic event or acute illness, despite an adequate intake of nutrients prior to the illness or injury [9]. In contrast to what is often assumed, serum protein levels of hepatic origin (albumin, prealbumin, and transferrin) do not reflect the condition of malnutrition per se; but rather the body’s physiologic response to injury and infection. The degree of illness can, however, influence appetite, gastrointestinal function and hemodynamic stability, which can, in turn, negatively affect the patient’s nutritional status.

The metabolism of critical illness is a combination of starvation and stress. There is an increased production of cortisol, catecholamines, glucagon and growth hormone and increased insulin-like growth factor-binding protein-1 [92]. Phagocytic, epithelial and endothelial cells increase reactive oxygen and nitrogen species, chemokines, pro-inflammatory cytokines and lipid mediators, so that antioxidant depletion develops. TNF-α stimulates oxidative stress and NO synthesis. Aside from potential humoral mediators of cachexia, tumor-derived biologically active molecules have been reported recently.

Caloric restriction, definition and background

Caloric restriction (CR) is defined as a moderate reduction in caloric intake (20–40%, without malnutrition) and may slow ageing, reduce age-related chronic diseases, decrease the incidence of malignancies and extend lifespan [80, 95-97]. The effect of CR on longevity was discovered in rats over 70 years ago [98]. Thanks to the interest in factors and mechanisms that can extend the life span, CR is also widely studied in primates, including humans [99-101, 96, 95]. However, the results of human studies attempting to determine the effects of CR on health have been difficult to interpret, due to the difficulties in establishing a long term (or even a life-long) control of experimental conditions.
Biology, biochemistry and regulation of caloric restriction

Calorie-restricted diets cause a decrease in body temperature, in heart rate and blood pressure, and in glucose and insulin levels [80, 102]. In mice, glucose and insulin levels were 20 and 80% lower after a meal, respectively, and the insulin sensitivity was 3.3-fold greater in the calorie-restricted than in the ad libitum-fed group [103]. CR decreases the IGF-1 concentration in blood, lowers the triglycerides level and increases the HDL-cholesterol level [104].

The metabolic rate as normalized for body weight does not decline in CR mice and the lifetime metabolic output of these animals is larger than that of the ad libitum-fed cohorts [105]. CR has also been reported to stimulate neurogenesis and enhance synaptic plasticity, which might increase the ability of the brain to resist cognitive decline [106]. CR may also stimulate UCP expression, but the current data are not yet entirely consistent [107-110]. Changes on the gene expression level in multiple tissues [111] were associated with upregulation of genes involved in lipid metabolism and the metal-ion response, and with downregulation of those involved with immunity and protein folding. The latter indicates a possible decrease in oxidative stress induced by CR.

CR upregulates stress hormones and downregulates thyroid hormones, possibly by reducing leptin levels [112]. Adiponectin levels rise during CR [113], suggesting that it might also have an important role in the physiological shift to insulin sensitivity in these animals.

In yeast, the extension of lifespan by CR requires Sir2 and is accompanied by an increase in respiration, which, in turn, increases Sir2 activity [114]. Sir2 was shown to possess a remarkable NAD-dependent histone-deacetylase activity [114, 115]. Mammalian sirtuin (SIRT1) is also a NAD-dependent deacetylase [114]. CR also attenuates the age-related upregulation of nuclear factor (NF)-κB [80, 116], which induces the expression of TNFα in white adipose tissue and the production of inflammatory cytokines in immune cells [117]. Furthermore, SIRT1 was shown to deacetylate and downregulate NF-κB [118], leading to the hypothesis that the upregulation of SIRT1 plays a role in the increase in insulin sensitivity and reduction in inflammation in CR.

Conclusions

The outcome of fasting depends on the amount of energy intake, duration of nutrient deprivation, and quality of food, and ranges from mild to harsh, from unpleasant feeling of hunger to death. Summarizing such a wide range of effects, without subdividing the fasting phenomenon, is intricate. Most of the changes induced by short to medium-term fasting are reversible with food. Malnutrition during pregnancy and childhood, however, entails enduring consequences. If food is not administered in time and starvation persists into the preterminal phase of fasting, some of the consequences are no longer reversible. The biochemical and physiological characteristics of severe starvation, though induced by different factors, are comparable to those of cachexia. We believe that the extended fasting can be used as a valuable model for studying the wasting syndrome. Finding an approach to either postpone, or avoid entering into the preterminal phase is, in our view, the goal that the current fasting research field should be aiming for. Applying the ‘omics’
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techniques will provide the scientific community with additional targets, especially regarding the transcriptional and signaling regulation of the process.

A deeper understanding of mechanisms underlying the adaptation to fasting will thus provide more proficient intervention in starvation, malnourishment and cachexia, but the patients suffering from diabetes and metabolic syndrome could also benefit from this knowledge. The treatment of diseases not in direct correlation to fasting, like epilepsy, might also be enhanced, if the mechanism of the weight-loss inducing ketogenic diets [119] becomes understood. Additionally, the animal husbandry, with 30-50% neonatal mortality rate, and starvation as one of the main causes of death [120], could benefit if entering the phase III of fasting could be avoided. Moreover, the mechanisms of life extension by caloric restriction, a hot topic in the scientific community [80], need a better mechanistic understanding.

Aim of the thesis

The biology of fasting, whether induced by disease or caused by food scarcity, is far from being completely elucidated. In particular, the understanding of the contribution of different organs to the overall adaptive response to fasting is lacking. The aim of this thesis is to obtain a deeper insight into the changes in gene expression profiles and the underlying molecular mechanisms of the adaptive response to food deprivation in two different organs. We studied the small intestine and the liver, organs that are intimately associated with digestion and metabolism, respectively.

The response to fasting of the small intestine has, despite its major role in nutrient processing, only attracted attention recently when compared to muscle, liver, or kidney. To obtain a more comprehensive understanding of the temporal effects of food deprivation on the mouse small intestine, we report a genome-wide transcriptomics study of the effects of fasting in Chapter 2. Gene-expression profiling, pathway analysis, and immunohistochemistry were carried out after 0, 12, 24, and 72 hours of fasting. This study showed that the expression of genes involved in metabolism and cell turnover changed in a highly significant, coordinated manner, with a remarkably discontinuous transition between short-term (12 hours) and prolonged (more than 24 hours) fasting.

To substantiate our genomics findings, we performed a comprehensive analysis of changes occurring on the level of protein expression, using tissues from the same animals as studied in Chapter 2. Chapter 3 therefore contains a comparative proteomics study of the small intestine. It combines two-dimensional gel electrophoresis, with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, to identify the intestinal proteins with changed expression levels. In total, the expression of 80 protein spots changed significantly between the different groups, with phasespecific pattern of change.

The liver response to fasting, by increasing gluconeogenesis and lipid utilization, is well documented at the physiological level. We report now, in Chapter 4, the gene-expression profiling, pathway, network and gene-set enrichment analysis and immunohistochemistry of the liver of mice fasted for 12, 24 and 72 hours. Additionally, this study contains a report on changes in metabolite concentrations (ammonia, glucose, lactate and amino-acids) in the plasma of fasted mice.
The collective data show that the response to fasting in the liver markedly differed from the response pattern seen in the small intestine in that it started already at 12 hours of fasting and peaked at 24-48 hours of fasting, while most adaptive changes had abated by 72 hours. Our comparison of small intestine and liver suggest that each organ mounts its own specific response to fasting with distinct temporal patterns.

In Chapter 5, finally, we focused on the physiology of lipids during fasting and, in particular, on the response of cholesterol metabolism. The rationale for this study is the fact that, although cholesterol plays an important role in triglyceride transport, its role in fasting is not established. We extended our previous transcriptomics study (Chapters 2 and 4) by performing a comparative analysis of the expression profiles of the liver and the small intestine, with the aim to gain insight into the regulation of cholesterol metabolism under conditions of food deprivation. Chapter 5, therefore, surveys the differences in expression of cholesterol-metabolizing enzymes in liver and small intestine, and reports about alterations in lipid profiles of plasma, bile, liver, small intestine and faeces, all after 12, 24 and 48 hours of fasting. We were able to show an increase in biliary lipid output, hepatic and intestinal lipid turnover, and hepatic cholesterol trafficking in the absence of luminal nutrients, while the loss of metabolites, by means of faecal sterol excretion, was strongly suppressed.
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