The metabolic response to fasting in humans: physiological studies

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English Summary

In this thesis, we investigated the metabolic adaptation to fasting. Investigating pathways, involved in protecting the body from energy depletion, may provide us with answers on the metabolic adaptation to overfeeding, i.e. the metabolic consequences of obesity. The background of this thesis is presented in **chapter 1**. General considerations (e.g. definitions) on fasting are discussed. Consequently, the adaptations to fasting of fatty acid and glucose metabolism are reviewed. In the section on the adaptation of glucose metabolism, much attention is paid to peripheral insulin stimulated glucose uptake and the role of fatty acids in this process. Finally, the thesis outline is presented.

In **chapter 2** we studied whether the relative protection from FFA-induced insulin resistance during fasting in women, is associated with lower muscle ceramide concentrations compared to men, since women have lower plasma glucose levels than men despite higher plasma FFA during fasting, suggesting protection from FFA-induced insulin resistance.

We studied lean men and women in hyperinsulinemic euglycemic clamp studies. After a 38 h fast, plasma glucose levels were significantly lower in women than men with a trend for a lower endogenous glucose production in women, while FFA and lipolysis were significantly higher. Insulin-mediated peripheral glucose uptake was not different between sexes. There was no gender difference in muscle ceramide in the basal state and ceramide did not correlate with peripheral glucose uptake. Muscle FAT/CD36 was not different between sexes in the basal state and during the clamp.

These data show that during fasting, women are relatively protected from FFA-induced insulin resistance possibly by preventing myocellular accumulation of ceramide. This is not explained by differences in total muscle FAT/CD36.

It has been demonstrated repeatedly that short-term fasting induces insulin resistance although the exact mechanism in humans is unknown to date. Muscle ceramide is suggested to induce insulin resistance by interfering with the insulin signaling cascade in obesity. In **chapter 3** we performed clamp studies to investigate peripheral insulin sensitivity together with muscle ceramide concentrations and protein kinase B/AKT phosphorylation after short-term fasting.

We found that insulin mediated peripheral glucose uptake was significantly lower after 62 h compared to 14 h of fasting. Intramuscular ceramide concentrations tended to

increase during fasting and that the phosphorylation of protein kinase B/AKT at serine^{473} in proportion to the total amount of protein kinase B/AKT was significant lower during the clamp. Muscle ceramide did not correlate with plasma free fatty acids.

This demonstrates that fasting decreases insulin mediated peripheral glucose uptake with lower phosphorylation of AKT at serine^{473}, which may play a regulatory role in fasting induced insulin resistance. The role of muscle ceramide remains elusive.

The transition from the fed to the fasted resting state is characterized by changes in lipid metabolism besides peripheral insulin resistance. Acylcarnitines have been suggested to play a role in insulin resistance besides other long-chain fatty acid metabolites. It is unknown whether muscle long-chain acylcarnitines increase during fasting or relate with glucose/fat oxidation and insulin sensitivity in lean healthy humans.

Chapter 4 discusses hyperinsulinemic euglycemic clamp studies after 14 and 62 hours of fasting. Hyperinsulinemia decreased long-chain muscle acylcarnitines after 14 but not after 62 hours of fasting. During the basal state and clamp, fatty acid oxidation was lower after 14 vs. 62 hours of fasting.Absolute changes in glucose and fat oxidation within clamps were not different. Muscle long-chain acylcarnitines did not correlate with substrate oxidation or insulin-mediated peripheral glucose uptake.

It was concluded that muscle long-chain acylcarnitines do not unconditionally reflect fatty acid oxidation. The adaptation in fatty acid oxidation suggests a different insulin-regulated set point.

The ketone body *D*-3-hydroxybutyrate (*D*-3HB) plays an important role in the adaptation to fasting. It has been suggested by at least two separate studies that *D*-3HB can be coupled to carnitine to form 3-hydroxybutyrylcarnitine. However, it is unknown whether and how *D*-3HB can be coupled to carnitine resulting in *D*-3HB-carnitine in humans.

To assess which stereo isomers of 3-hydroxybutyrylcarnitine are present in vivo, we performed pancreatic clamp studies in 12 healthy men and homogenate studies in liver and muscle of mice as described in **chapter 5**.

Muscle *D*-3HB-carnitine was approximately 7.5 fold higher compared to *L*-3HB-carnitine in ketotic men after 38 h of fasting. Muscle *D*-3HB-carnitine and *D*-3-HB turnover correlated significantly. The ACS pathway was active in liver and muscle homogenates though less than the SCOT pathway that was only active in muscle homogenates.

Therefore, *D*-3HB-carnitine, related with the *D*-3HB turnover, can be formed in muscle by mitochondrial SCOT and a carnitine acyltransferase. These data provide a

newly identified alternative pathway of ketone body metabolism. The purpose of *D*-3HB-carnitine synthesis and its fate remain to be elucidated.

The dose-response relationship between insulin and ketone bodies was demonstrated to be shifted to the right in type 2 diabetes mellitus patients. In contrast ketone body levels have also been reported to be decreased in obesity. To clarify this paradox, we investigated the metabolic adaptation to fasting with respect to glucose and ketone body metabolism in lean and obese men without non insulin dependent diabetes mellitus in **chapter 6**. We hypothesized ketone body production to be equal under equal plasma insulin levels thereby reflecting absence of insulin resistance on ketogenesis.

Pancreatic clamp studies were performed after 38 hours of fasting in lean and obese men. In the basal state, ketone body fluxes were higher in lean compared with obese men. During the pancreatic clamp, no differences were found in ketone body fluxes during similar plasma insulin levels. Peripheral glucose uptake was lower in obese men.

Obese subjectsare resistant to insulin's effect on stimulation glucose of glucose uptake, but not its inhibiting effect on ketogenesis, implying differential insulin sensitivity of intermediary metabolism in obesity.

Intermittent fasting has shown to increase insulin sensitivity but it is uncertain whether intermittent fasting selectively influences intermediary metabolism. Such selectivity might be advantageous when adapting to periods of food abundance and food shortage.

In **chapter 7** we investigated the effects of 2 weeks intermittent fasting vs. 2 weeks of a standard diet on glucose, lipid and protein metabolism in the basal state and during a two-step hyperinsulinemic euglycemic clamp with assessment of energy expenditure and muscle insulin signaling.

Peripheral glucose uptake during step 1, but not step 2, was significantly higher after intermittent fasting compared to SD but this was not the case for hepatic insulin sensitivity. Lipolysis was more inhibited by insulin after intermittent fasting. Proteolysis was only lower after intermittent fasting during step 2. Intermittent fasting decreased resting energy expenditure and tended to augment insulin signaling of AKT, whereas higher phosphorylation of glycogen synthase was found.

Intermittent fasting differentially affects glucose, lipid and protein metabolism. The decrease in REE after intermittent fasting is a potential cause for increasing weight during intermittent fasting when caloric intake is not adjusted. Whether intermittent fasting is beneficial in improving peripheral insulin resistance in obese insulin resistant subjects remains to be established.

The interpretation of the prolonged supervised fast (to detect an insulinoma) is troublesome in those patients who develop hypoglycemia with appropriate hypoinsulinemia during this test. In **chapter 8** we investigated in this group of patients whether abnormalities in intermediary metabolism, known for their relation with ketotic hypoglycemia (fatty acid oxidation and amino/organic acids) could be detected which might explain the hypoinsulinemic hypoglycemia.

We studied 10 patients with otherwise unexplained hypoglycemia during prolonged fasting in an extended metabolic diagnostic protocol based on stable isotope techniques after an overnight fast.

There were no hypoglycemic events. No abnormalities in fatty acid oxidation (FAO) or in amino acid/organic acids were found in this patient group.

We found no signs of metabolic derangements in these patients. Therefore, the previously observed low plasma glucose values during the supervised fast probably represent the lower tail of the Gaussian Curve of plasma glucose concentrations during fasting.

Finally, **chapter 9** is a perspective describing the integrated metabolic response to fasting regarding adaptive changes in lipid and glucose metabolism. Here we tried to elucidate the relevance and physiological aspects of a mechanism needed for survival of the organism.

Although the purpose of fasting may be simple (i.e. to safe energy), the exact reason for these adaptations is unknown. We shed light on the discrepancy between muscle FAO and lipid supply during short-term fasting.Moreover we discuss fasting induced peripheral insulin resistance. Although this is a highly regulated process as shown in this thesis, its explanation is currently lacking. The thought that this insulin resistance simply prevents hypoglycemia may be too feeble. Some suggestions are put forward for future studies that may increase our understanding of the physiological adaptation to fasting as well as pathophysiological states as obesity induced insulin resistance and type 2 diabetes mellitus.