Impact of gut-derived metabollites on substrate metabolism and metabolic health

Summary

In the last two decades, evidence for a link between the gut microbiome with the host metabolism and metabolic disorders like insulin resistance and type 2 diabetes mellitus has constantly increased. The gut microbiota can ferment indigestible carbohydrates (saccharolytic fermentation) and produce the major short chain fatty acids (SCFA) acetate, propionate and butyrate mainly in the proximal colon. Of relevance, SCFA have been linked to improvements in substrate handling at the tissue level contributing to an amelioration in insulin sensitivity and metabolic health. Additionally, fermentation of peptides and proteins (proteolytic fermentation), mainly occurring in the distal colon, yields products such as branched chain fatty acids (BCFA), which may have adverse effects on gut and metabolic health. However, most literature is based on animal studies and only a limited amount of human studies are available. Hence, in this thesis we focused on the role of gut-derived metabolites (SCFA and BCFA) in *in vivo* metabolic health in human cross-sectional and intervention studies with overweight and obese individuals as well as the role of SCFA in human adipose tissue and skeletal muscle derived in *vitro* models.

In **chapter 2,** we extensively reviewed findings on the most abundant SCFA acetate in the context of body weight control and insulin sensitivity. We discuss that circulating acetate may have tissue-specific metabolic effects (liver, skeletal muscle, adipose tissue and pancreatic beta cells) and potentially impact whole-body energy and substrate metabolism (satiety, energy expenditure, fat oxidation) resulting in an improved metabolic health and insulin sensitivity. In addition, we discuss that the effectiveness of interventions (pre- probiotics, synbiotics) aiming to increase acetate concentrations in the colon as well as in the circulation may depend on colonic production site. In this respect, differences in the site of acetate production (proximal vs distal colon) as well as the differences in colonic acetate release into the circulation have to be taken into account. Furthermore, we discuss that future interventions (using prebiotics, probiotics, synbiotics and/or vinegar administrations) with the aim to increase circulating acetate should be more personalized and thereby consider both metabolic and microbial phenotype of participants.

Interestingly, it has been shown that the relationship between gut microbiota and insulin sensitivity may be sex-specific in humans. Therefore, in **chapter 3**, we investigated whether the associations between circulating acetate with insulin sensitivity/resistance indices (Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), circulating insulin and Matsuda index) were different in male and female participants (BMI >27 kg/m^2, n=478) of the Diet, Obesity and Genes (DiOGenes) study before and after a low-calorie diet (LCD, 800 kcal/d). We found that acetate was positively associated with insulin resistance in females, but not in males, even after adjustment for age, BMI and fat free mass.

Of note, these findings warrant further investigations to understand the sex-specific differences in circulating acetate and their potential role in metabolic health during weight loss. In particular, factors contributing to circulating fasting acetate such as hormonal status, endogenous liver production (ketogenesis) and/or the gut-derived production of acetate should be considered. Furthermore, future studies should investigate acetate dynamics to fully elucidate the role of circulating acetate in metabolic health.

So far, in human studies gut microbial-derived SCFA have been mainly measured in feces. However, fecal SCFA production should be interpreted with caution, since they are the net result of colonic production, absorption rates and release into the circulation, which are intricately influenced by microbial composition, cross-feeding mechanisms and whole gut transit time among other factors. Therefore, in **chapter 4**, we investigated associations between fecal and plasma SCFA (acetate, propionate and butyrate) with metabolic health markers including circulating metabolites, gut hormones, substrate oxidation, inflammatory parameters and markers of insulin sensitivity in a well-phenotyped group of individuals with a large range in BMI and glucometabolic status. Interestingly, we found that plasma, not fecal, SCFA (acetate, propionate and butyrate) associated negatively with circulating markers of lipid metabolism and positively with circulating GLP-1 levels. The negative associations with circulating lipids (glycerol, triglycerides, free fatty acids, respectively) are in line with previous literature, suggesting a relationship between SCFA and adipose tissue metabolism and possible improvements in adipose tissue lipid buffering capacity. An interesting (novel) finding was the positive association of circulating SCFA with fasting GLP-1 levels possibly indicating a link of plasma SCFA with gut-hormone secretion. In a subgroup analysis, we found that insulin sensitivity (as measured by the golden standard hyperinsulinaemic-euglycemic clamp technique) associated with circulating SCFA but that the direction of this association depended on type of SCFA (acetate negatively and propionate positively). Although controversial associations of circulating acetate and insulin sensitivity have been found (in **chapter 3** and **chapter 4**), findings should be interpreted with caution taking into consideration that circulating acetate is affected by various factors including liver endogenous production (ketogenesis), gut-derived production as well as metabolic and microbial phenotype.

Besides saccharolytic fermentation and SCFA production, proteolytic fermentation may yield various metabolites such as BCFA (i.e. isobutyrate, isovalerate), which role in human substrate and energy metabolism remains largely unclear. In addition, research on other fecal metabolites derived from saccharolytic fermentation (i.e. valerate, lactate) is largely lacking. Therefore, in **chapter 5,** we investigated fecal BCFA (isobutyrate and isovalerate) and other fecal microbial metabolites (valerate, lactate, succinate and caproate) in insulin sensitive and insulin resistant individuals. Additionally, we assessed the relationship of fecal microbial metabolites with circulating metabolites, substrate oxidation, and

markers of insulin sensitivity (circulating insulin and HOMA-IR). Here, we found no significant differences in fecal BCFA concentrations between insulin sensitive and insulin resistant individuals. Additionally, there were no associations between BCFA and markers of insulin resistance and metabolic health. Further human studies are warranted to investigate the role of these microbially-derived fermentation products and their kinetics in metabolic health and insulin sensitivity.

In **chapter 6,** we investigated whether our previously observed increments in *in vivo* fat oxidation after colonic acetate administration in humans were mediated through direct effects of circulating acetate on the skeletal muscle fat oxidation. Therefore, we investigated the effects of sodium acetate on fat oxidation in human primary muscle cells (HSkMC) derived from a healthy insulin sensitive donor. We observed no direct sodium acetate-mediated effects neither on endogenous nor on exogenous fat oxidation in our human skeletal muscle cell model using physiologically relevant sodium acetate concentrations. Additionally, we observed no dose and time effect of sodium acetate on total and phosphorylated AMPK levels. However, we cannot exclude that our previously observed *in vivo* human effects on fat oxidation after colonic acetate administrations occur in tissues other than skeletal muscle (i.e. liver fat oxidation) or are mediated by other gut-derived metabolites or hormones (i.e. PYY). Additionally, the lack of effect on *in vitro* muscle fat oxidation may be donor-dependent, muscle fiber type-specific as well as species-specific and warrants further investigation.

In several animal as well as human *in vivo* studies an anti-lipolytic effect of SCFA was demonstrated, which may reduce systemic lipid overflow and ultimately result in improvements in insulin sensitivity. Therefore, in **chapter 7**, we used the human multipotent adipose-derived stem (hMADS) cell model to study the effects of SCFA on (cytosolic) lipolysis and to elucidate the potential underlying mechanisms. We found that SCFA differentially affect adipose tissue lipolysis, and in particular that acetate is the major SCFA responsible for the decreased basal and β-adrenergically stimulated glycerol release in our *in vitro* hMADS cell model. The latter anti-lipolytic effect was mediated through a GPR-dependent reduction in phosphorylation of hormone sensitive lipase (HSL). Thus, these data suggest that circulating acetate affect adipose tissue lipolysis, thereby possibly limiting lipid overflow and improving insulin sensitivity.

In conclusion, this doctoral thesis provides an increased insight in the role of saccharolytic (major SCFA as well as other less studied metabolites) and proteolytic fermentation products (BCFA) in insulin sensitivity and substrate metabolism in human cohorts as well as in *in vitro* studies. The main findings indicate that gut-derived metabolites (SCFA) in the circulation might be a better indicator of their effects on metabolic health as compared to fecal concentrations.

Furthermore, the present data suggest that the sex-specific relationship of circulating acetate with insulin sensitivity exist, which may depend on various

factors such as endogenous liver metabolism, hormonal profile, and microbial and metabolic phenotype.

Lastly, our *in vitro* experiments provided valuable mechanistic insight on the relation between acetate and skeletal muscle fat oxidation and adipose tissue lipolysis, which needs to be investigated in more detail in human *in vitro* and *in vivo* studies. Additionally, future studies need to consider SCFA and BCFA dynamics (production, absorption and release into the circulation), colonic production site as well as the role of these gut-derived metabolites in tissue and whole-body metabolism and metabolic health.