Chapter 9
Gut Microbiota in Metabolic Syndrome

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Abstract The human gut is colonized by trillions of bacteria that complement our own human genes with 150-fold more microbial genes. Acting at the intersection between diet and host biology the gut microbiota can be considered a metabolic organ that affects host physiology. Here we explore evidence that supports the hypothesis that there is a dynamic interrelationship between our diet and gut microbiota that plays an important role in nutrition as well as modulating energy balance. Furthermore, the gut microbiota is altered in obesity and germ-free mice have reduced adiposity and are protected against diet-induced obesity. Accordingly, the gut microbiota may be considered an important environmental factor that contributes to obesity and metabolic diseases.

Keywords Gut microbiota · Metabolism · Metagenome · Obesity

9.1 Introduction

We humans are much alike and our genomes are $> 99\,\%$ identical and we all have approximately the same human cellular composition. However, despite this similarity we differ drastically in our microbial communities. The greatest variation is between body sites (Costello et al. 2009). For example, the difference in microbial communities between a person's mouth and gut is comparable to the difference in microbial communities that reside in soil and seawater. Even within a body site, the differences between people are not subtle and the gut communities can differ by 80–90 % at the species level (Turnbaugh et al. 2009; Costello et al. 2009).

The human gut microbiota is established at birth when our sterile gut is colonized by bacteria from our mothers and the environment (Reinhardt et al. 2009, Dominguez-Bello et al. 2010). This initial microbiota develops into a complex ecosystem in a predictable fashion determined by internal (e.g., oxygen depletion

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and the immune system) and external (e.g., mode of birth, impact of environment, diet, hospitalization, and application of antibiotics) factors. Whereas the initial gut microbiota exhibits relatively low diversity the adult microbiota is composed by > 1,000 bacterial species of which 160 are found in > 50 % of the study population (Qin et al. 2010). The adult gut microbiota is estimated to weigh 1.5 kg, which is similar to the liver, and the microbiome contains 150–200 times as many genes as we have in our own human genome (Qin et al. 2010, Xu and Gordon 2003). The gut microbiota should be considered a metabolic organ that can affect our metabolism and physiology function on the intersection between our genetics and our ingested food. Accordingly, obesity is associated with an altered gut microbiota and germ-free mice have reduced adiposity and resistant to diet-induced obesity (Bäckhed et al. 2004, 2007; Ley et al. 2005, 2006). However, the underlying mechanisms are just being clarified and will require a combination of clinical and animal studies.

9.2 Microbial Extraction of Energy and Storage in Adipose Tissue

The gut microbiota contains an impressive suite of glycosidases and lyases that are essential for degrading otherwise non-degradable polysaccharides from the diet and ferment them to short chain fatty acids (SCFA) (Xu et al. 2003). Interestingly, the microbiota of obese mice exhibit increased capacity to degrade complex polysaccharides and store these extra calories in as adipose tissue (Turnbaugh et al. 2006). Similarly, germ-free mice have reduced capacity to degrade polysaccharides and the reduced levels of SCFA reaching the liver from portal circulation in these mice is associated with reduced lipogenesis (Bäckhed et al. 2004). In addition to salvage energy by fermenting carbohydrates to SCFA the gut microbiota promotes glucose absorption from the small intestine by a yet unidentified mechanism (Bäckhed et al. 2004). The increased levels of circulating SCFA and glucose act as substrates for *de novo* lipogenesis in the liver by activating the key lipogenic transcription factors sterol regulatory element binding protein 1c (SREBP-1c) and carbohydrate element binding protein (ChREBP). These transcription factors in turn regulate the rate limiting lipogenic enzymes fatty acid synthase and acetyl-CoA caboxylase (Bäckhed et al. 2004). The increased lipogenesis was confirmed when we applied LC-MS based lipidomics to investigate how the gut microbiota affects the host lipidome. We identified ~ 100 triglyceride species that were increased in livers of conventionally raised mice (Velagapudi et al. 2010). Furthermore, colonization was associated with increased hepatic VLDL production which is transported to the adipose tissue for storage (Bäckhed and Crawford 2010).

The gut microbiota does not only promote lipogenesis and VLDL production it also facilitate storage in adipose tissue by increasing lipoprotein lipase (LPL) activity. In white fat, LPL is regulated posttranscriptionally by nutritional status: fasting reduces and refeeding increases enzyme activity. Angiopoietin-like protein (Angptl4, also known as fasting-induced adipose factor) is an important regulator

of LPL activity (Yoshida et al. 2002). Expression analysis revealed that the gut microbiota regulates the expression intestinal Angptl4 expression and analysis of germ-free *Angptl4-/-* mice revealed that the gut microbiota increase LPL activity by suppressing Angptl4 and that this suppression was associated with increased adiposity (Bäckhed et al. 2004, 2007). Accordingly, the gut microbiota has profound effects not only on energy extraction but also on energy partitioning and can thus be considered as an environmental factor that modulates adiposity.

9.3 Microbial Modulation of Host Metabolism

The gut microbiota does not only affect energy extraction but also affect host metabolism by other means. We found that germ-free mice are resistant to diet-induced obesity (Bäckhed et al. 2007), which suggests that the gut microbiota may have large effects on both central and peripheral metabolism. The observation that germ-free mice are protected from developing diet-induced obesity has recently been confirmed by several independent laboratories (Rabot et al. 2010; Fleissner et al. 2010; Ding et al. 2010) and some additional mechanistic insights are currently being made. Since 'Western' diet and other high-fat diets do not contain complex polysaccharides the gut microbiota promotes obesity by other means than carbohydrate fermentation and recent evidence suggested that the gut microbiota modulates several important pathways implicated in energy metabolism (Greiner and Backhed 2011).

The lean phenotype of germ-free mice is associated with increased levels of phosphorylated AMP-activated protein kinase (AMPK), and its downstream molecular targets involved in fatty acid oxidation (acetylCoA carboxylase; carnitinepalmitoyltransferase) in skeletal muscle and liver (Bäckhed et al. 2007). AMPK is an enzyme that functions as a "fuel gauge", which monitors cellular energy status (Kahn et al. 2005) and may thus be responsible for the ability of germ-free mice to increase fatty acid oxidation in skeletal muscle (Bäckhed et al. 2007). As mentioned above the gut microbiota suppresses the intestinal expression of Angptl4, which in addition to its effects on regulating LPL, also is a potent regulator of fatty acid oxidation (Mandard et al. 2006). In support of these findings we observed that germ-free mice lacking *Angptl4* lost their protection from developing diet-induced obesity (Bäckhed et al. 2007). Further phenotypic characterization revealed that genes involved in fatty acid oxidation were controlled by Angptl4 under germ-free conditions. Notably, Angptl4 did not signal via AMPK, which suggests that germ-free mice are protected against diet induced obesity by two complementary but independent mechanisms that result in increased fatty acid metabolism. In addition the gut microbiota may affect expression and function of other hormones that modulate host metabolism.

9.4 The Gut Microbiota and Obesity

The gut microbiota is altered in obesity but is there direct evidence that the gut microbiota may affect obesity? Elegant experiments revealed such link by transplanting the gut microbiota of lean and obese mice into germ-free recipients (Turnbaugh et al. 2006). Surprisingly, the lean and obese phenotypes were transferred to the recipients where mice transplanted with an obese microbiota gained significantly more adipose mass. A recent study identified that Toll-like receptor 5 (Tlr5), a pattern recognition receptor that recognizes the bacterial protein flagellin, as an important modulator of gut microbial ecology (Vijay-Kumar et al. 2010). Strikingly, the *Tlr5*-deficient mice are associated with inflammatory bowel disorder as well as obesity and the Metabolic Syndrome (Vijay-Kumar et al. 2007, 2010). Importantly, the obesity phenotype was transferrable to germ-free mice demonstrating that the phenotype was caused by the microbiota. Further analysis revealed that the altered gut microbiota in Tlr5-deficient mice was associated by increased food intake thus suggesting that the gut microbiota may affect the central nervous system. Furthermore, it is becoming increasingly clear that the gut microbiota modulates behavioral aspects of animal physiology. For example, the germ-free mice have increased locomotors activity and display altered anxiety behavior (Bäckhed et al. 2007; Heijtz et al. 2011). Accordingly, the gut microbiota does not only modulate peripheral metabolism but can also affect eating and behavioral phenotypes.

9.5 The Gut Microbiota as Modulator of Glucose Metabolism

In addition, to microbial effects on host adiposity the gut microbiota also contributes to metabolic abnormalities by promoting low-grade metabolic inflammation (Cani et al. 2007). Endotoxin is taken up from the gut together with chylomicrons or alternatively through increased gut permeability (reviewed in (Caesar et al. 2010)). Activation of TLR4 in macrophages that are recruited to the adipose tissue promotes inflammation that reduces insulin sensitivity (Saberi et al. 2009). Germ-free mice are exposed to very low levels of endotoxin from the diet but the levels are very low compared to those observed in colonized mice. As expected germ-free mice have reduced adipose inflammation and improved insulin sensitivity compared with colonized counterparts (Bäckhed et al. 2004; 2007; Rabot et al. 2010; Reigstad et al. 2009). Interestingly, antibiotic treatment of obese mice with antibiotics reduces plasma endotoxin levels, adipose inflammation, adiposity, liver triglycerides, and improves host glucose metabolism (Cani et al. 2008; Membrez et al. 2008). Taken together these findings suggest that the gut microbiota can contribute directly to host metabolism by affecting energy harvest from the diet and by modulating metabolic and/or inflammatory signaling pathways.

9.6 Systems Biology Approaches to Investigate Host Microbial Interactions

The gut microbiota has an immense propensity to affect the metabolism of ingested compounds such as dietary, drug, and xenobiotic components. Recent metabolomic and lipidomic studies of germ-free and colonized mice have revealed that the gut microbiota affects several important biotransformations that may have large effects on host physiology (Claus et al. 2008; Velagapudi et al. 2010; Wikoff et al. 2009). Untargeted mass spectrometry-based metabolomics of serum from germ-free and conventionally raised mice demonstrated that a vast majority of the serum metabolites were modulated by the gut microbiota (Wikoff et al. 2009). Many of these metabolites corresponded to increased xenobiotic metabolism in colonized mice and included metabolites that were sulfated, glucoronidated, or conjugated to glycine (Wikoff et al. 2009; Claus et al. 2008), which renders hydrophobic compounds water soluble. Tyrosine can be metabolized to *p*-cresol sulfate sulfatation by the gut microbiota and thus germ-free mice have reduced serum levels of *p*-cresol sulfate and corresponding elevated levels of tyrosine (Wikoff et al. 2009). The capacity of the gut microbiota to perform such modifications contributes to an individual's capacity to perform xenobiotic metabolism as individuals with high bacterially mediated p-cresol generation have reduced with O-sulfonation of widely used analgesic paracetamol (acetaminophen; (Clayton et al. 2009)). These findings may provide a mechanism for why pre-dose urinary composition can be used to predict the extent of liver damage sustained after paracetamol administration (Clayton et al. 2006).

Metabolism of other amino acids such as tryptophan is also modulated by the gut microbiota. Serum levels of tryptophan are accordingly reduced in colonized mice compared with germ-free counterparts (Velagapudi et al. 2010; Wikoff et al. 2009), which can be explained by that the gut microbiota expresses tryptphanase which converts tryptophan to indole, pyruvate, and ammonia. Further metabolism of indole to indole-3-propionic acid is exclusively microbially mediated and thus IPA was only detected in the serum of conventionally raised mice (Wikoff et al. 2009). IPA is further metabolized by host enzymes in the liver to indoxyl and indoxyl sulfate. Furthermore, serotonin is produced from tryptophan by enterochromaffin cells in the gut and accordingly germ-free rats have increased volumes of serotonin producing cells (Uribe et al. 1994). Taken together, microbial metabolism of amino acids may have profound effects on host physiology.

9.7 Using Metabolomics to Identify Microbial Metabolites in Disease

The above examples illustrate how the gut microbiota effects may affect host physiology by modulating metabolism of dietary components. Accordingly, metabolomic investigations of urine from mice predisposed or resistant to developing nonalcoholic

fatty liver disease revealed that susceptible mice were associated with increased microbial conversion of phosphatidylcholine to trimethylamines (TMA), which are excreted into the urine. Mechanistically, the reduced levels of phosphatidylcholines may promote steatosis and insulin resistance since phosphatidylcholines are required for VLDL secretion and impaired VLDL secretion could lead to triglyceride accumulation in the liver. However, recent findings revealed that metabolism of dietary choline to TMA is associated with cardiovascular disease and plasma levels of choline-derived metabolites are a excellent predictor of cardiovascular disease (Wang et al. 2011). Accordingly, dysbalanced choline metabolism by altered gut microbial structure/function may affect host metabolism and physiology.

9.8 Targeted Approaches to Determine Microbial Regulation of Specific Classes of Metabolites

9.8.1 Bile Acids

Metabolism of bile acids is another example of mammalian-microbial co-metabolism that may have great physiological effects (Claus et al. 2008; Martin et al. 2007; Swann et al. 2010). The gut microbiota is important for deconjugation, dehydrogenation, and dehydroxylation of bile acids, which results in secondary bile acids and increases the chemical diversity of these signaling molecules (Midtvedt 1974). Accordingly, germ-free animals have very simplified bile acids characterized by conjugated cholic and murocholic acids, which are absorbed before they reach the distal gut (Claus et al. 2008; Swann et al. 2010; Wostmann 1973). Bile acids have different capacity to induce the nuclear receptor FXR and the G-coupled receptor TGR5 (Thomas et al. 2008). Furthermore, bile acids have been shown to regulate not only their own synthesis and enterohepatic recirculation, but also triglyceride and cholesterol homeostasis through activation of FXR (Sinal et al. 2000). Interestingly, bile acids reduce diet-induced obesity and prevent hyperglycaemia in rodents, which suggests that they also have effects on energy homeostasis (Ikemoto et al. 1997; Prawitt et al. 2011; Watanabe et al. 2011).

In contrast, bile acid activation of the GPCR TGR5 in brown adipose tissue increases energy expenditure by producing active tri-iodothyronine (T_3), which subsequently increases metabolic rate and energy expenditure (Watanabe et al. 2006). Accordingly, stimulation of TGR5 prevents diet-induced obesity (Thomas et al. 2009). Recent data demonstrated that TGR5 also is expressed by L-cells in the colon and activation of TGR5 in L-cells induce secretion of the incretin GLP-1, which promotes improved pancreas function and glucose metabolism in obese mice (Reimann et al. 2008; Thomas et al. 2009). However, it is currently unclear whether this protection requires TGR5 activation in brown adipose tissue or in L-cells.

The immense capacity of the gut microbiota to modulate bile acid diversity clearly indicates that signaling through FXR and TGR5 are influenced by the gut microbiota.

This was recently illustrated by that the gut microbiota modulated FXR-regulated pathway transcripts in gnotobiotic rats (Swann et al. 2010) and suggests that new pharmacological approaches targeting bile acid signaling networks must take the person's gut microbiota into account.

9.8.2 Short Chain Fatty Acids

Fermentation of fibers in the distal gut produces SCFAs, which has important signaling functions in the gut through the GPCRs GPR41 and GPR43. Enteroendocrine cells express the SCFA receptor GPR41 and may thus be regulated through the fermentation capacity of the gut microbiota. There are no apparent differences in the body composition of germ-free wildtype and *Gpr41*-deficient mice (Samuel et al. 2008). However, colonized *Gpr41*-deficient mice were leaner, which was associated with decreased expression of the hormone PYY. GPR43 was first identified as a modulator of inflammatory responses in the gut as a chemoattractant receptor on neutrophiles (Maslowski et al. 2009). Besides its effects on host inflammation GPR43 has profound effects on host physiology. Bjursell et al., recently found that *Gpr43*-deficient mice were resistant to diet-induced obesity (Bjursell et al. 2010). Protection against developing diet-induced obesity was, at least in part, explained by increased energy expenditure in *Gpr43*-deficient mice and the reduced fat mass was accompanied by improved glucose tolerance. Despite no difference in adipocyte size *Gp43-/-* mice contained fewer macrophages in the white adipose tissue, which may explain the improved glucose metabolism.

9.8.3 Endocannabinoids

Anandamide (AEA) is an endogenous endocannabinoid (eCB) that is synthesized from membrane bound phosphatidylethanolamine. AEA binds the eCB receptor CB_1, which has profound effects on host metabolism. Genetic and pharmacological interference with the CB_1 receptor protects against the development of hepatic steatosis and diet-induced obesity (Osei-Hyiaman et al. 2005, 2008). Recent results using several animal models demonstrated that the gut microbiota modulates AEA levels in the colon but not in the small intestine and that the elevated levels correlated with reduced expression of fatty acid amide hydrolase (FAAH), which degrades AEA (Muccioli et al. 2010).

Obesity is associated with increased production of adipose derived eCB, increased CB_1 expression, and increased gut permeability (Cani et al. 2007; Di Marzo 2008). Pharmacologic inhibition of CB_1 in obese mice improved tight junction, reduced gut permeability, leading to reduced serum endotoxin levels (Muccioli et al. 2010). Thus it is possible that the gut microbiota controls gut permeability through modulating the endocannabinoid system. As indicated above the gut microbiota has extensive effects

on host adiposity, as well as on the eCB system in adipose tissue. Strikingly, prebiotic-mediated decreases in fat mass, reduced CB_1 mRNA expression, and reduced AEA levels were associated with increased expression of markers of adipocyte differentiation and lipogenesis suggesting a role of the gut microbiota. Importantly, these results were phenocopied by blocking CB_1 receptor signaling (Muccioli et al. 2010). Conversely, agonists of CB_1 increased adipogenesis in lean mice. Since CB_1 function is associated with plasma LPS levels Muccioli et al hypothesized that LPS could modulate eCB -induced adipogenesis. Accordingly, LPS reduced the expression of adipogenic and lipogenic markers in adipose tissue. Furthermore, LPS completely abolished the adipogenic effects of eCB receptor activation. Taken together, these data suggest that the eCB system regulates adipogenesis, which is controlled by LPS.

In summary, the gut microbiota affects several important signaling systems that contribute to controlling host metabolism these include, but are likely not limited to the SCFA, bile acid, and eCB system.

9.9 Conclusions

The recent identification of the gut microbiota as an environmental factor that contributes to metabolic diseases has spurred research all over the world. At present several studies have demonstrated an altered gut microbiota that is associated with obesity. However, the results are highly variable between studies, likely in part due to different analytical procedures, heterogeneous populations, and relatively small study groups. Accordingly, well controlled studies are required to parse out the relevant differences between the gut microbiota between lean and obese individuals, furthermore, the gut microbiota should also be studied in other metabolic diseases such as diabetes and cardiovascular disease. Accordingly, systems biology approaches to construct metabolic networks and putative functions encoded in the microbiome, which are associated with specific metabolic phenotypes in the host (including obesity, diabetes, and cardiovascular disease), will be of utmost importance to gain understanding in how the gut microbiota may affect host metabolism. However, one limitation with this approach is the lack of direct mechanistic understanding. An attractive tool to investigate mechanisms is to use ‘personalized’ gnotobiotics where germ-free mice are colonized with defined microbial communities and subsequently phenotyped. In these models interventions can be tested as well as the impact of specific macro- and micronutrients (Kau et al. 2011). The usage of genetically manipulated mouse models, especially tissue specific, will provide further evidence for host factors involved in specific signaling pathways or biological processes. However, to fully understand the intricate interplay between the gut microbiota, host, and metabolism we will need to integrate data from several ‘omics’ technologies such as metagenomics, transcriptomics, proteomics, and metabolomics

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