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Review

The gut microbiota, obesity and insulin resistance

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ABSTRACT

The human gut is densely populated by commensal and symbiotic microbes (the "gut microbiota"), with the majority of the constituent microorganisms being bacteria. Accumulating evidence indicates that the gut microbiota plays a significant role in the development of obesity, obesity-associated inflammation and insulin resistance. In this review we discuss molecular and cell biological mechanisms by which the microbiota participate in host functions that impact the development and maintenance of the obese state, including host ingestive behavior, energy harvest, energy expenditure and fat storage. We additionally explore the diverse signaling pathways that regulate gut permeability and bacterial translocation to the host and how these are altered in the obese state to promote the systemic inflammation ("metabolic endotoxemia") that is a hallmark of obesity and its complications. Fundamental to our discussions is the concept of "crosstalk", i.e., the biochemical exchange between host and microbiota that maintains the metabolic health of the superorganism and whose dysregulation is a hallmark of the obese state.

Differences in community composition, functional genes and metabolic activities of the gut microbiota appear to distinguish lean *vs* obese individuals, suggesting that gut 'dysbiosis' contributes to the development of obesity and/or its complications. The current challenge is to determine the relative importance of obesity-associated compositional and functional changes in the microbiota and to identify the relevant taxa and functional gene modules that promote leanness and metabolic health. As diet appears to play a predominant role in shaping the microbiota and promoting obesity-associated dysbiosis, parallel initiatives are required to elucidate dietary patterns and diet components (e.g., prebiotics, probiotics) that promote healthy gut microbiota. How the microbiota promotes human health and disease is a rich area of investigation that is likely to generate fundamental discoveries in energy metabolism, molecular endocrinology and immunobiology and may lead to new strategies for prevention of obesity and its complications.

Contents

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1. Introduction

The global obesity epidemic presents an unprecedented challenge to the public health and economies of 'westernized' societies due to the plethora and debilitating impacts of obesity comorbidities (Consultation, 2000; WHO, 2005). The etiology of obesity reflects complex interactions among genetic and environmental factors, with current high energy diets and sedentary lifestyle considered to be foremost among the latter (Kahn et al., 2006). Humans are superorganisms or "holobionts" (Mindell, 1992) composed of 10% human cells and 90% microbial cells (Lederberg, 2000). The human and microbial genomes (hologenome) have co-evolved, and their metabolism and survival is now inextricably intertwined. The gut is one of several sites that is densely populated by commensal and symbiotic microbes, with the majority of the constituent microorganisms being bacteria. Accumulating evidence indicates that the bacterial species of the gut (collectively referred to herein as the "gut microbiota") play a significant role in the development of obesity, obesity-associated inflammation and cardiometabolic complications. This rapidly-evolving field is the subject of the present review.

The gut microbiome (the collective genomes of the gut microbes) encodes 3.3 million non-redundant genes, which is 150 times larger than the human gene complement (Qin et al., 2010). This genetic richness enables the gut microbiota to perform diverse and active metabolic activities that are not encoded in the human genome, such as processing dietary polysaccharides (Gill et al., 2006; Zhao, 2010). In addition, the gut microbiota exchange metabolites with the host and interacts with host signaling pathways to modulate host bile acid, lipid and amino acid metabolism as well as host gene expression, etc. (Claus et al., 2008; Martin et al., 2007; Velagapudi et al., 2010; Wikoff et al., 2009). Human metabolism at the systemic level is therefore shaped by the host, the gut microbiota and by "crosstalk" between the two in response to the environment (Li et al., 2008; Zhao, 2010; Zhao and Shen, 2010).

In this review, we discuss our current knowledge of the mechanisms by which the gut microbiota influences host metabolism, adiposity and obesity complications. In Section 2 we provide a brief overview of the analysis and community composition of the human gut microbiome. In Section 3 we detail mechanisms by which the gut microbiota modulates both energy harvest (host ingestive behavior, intestinal absorption and energy recovery from the diet) and energy expenditure (the anabolic/catabolic balance). A key concept is that products of gut microbial metabolism, in particular short chain fatty acids (SCFAs) function via diverse host molecular mechanisms to regulate host energy intake, energy expenditure and storage.

Changes in the gut microbiota that typify obesity and/or overnutrition are now implicated in the impaired gut barrier function and associated inflammation of obesity ("metabolic endotoxemia"). Cell biological and molecular mechanisms regulating gut permeability and the response to endotoxemia are discussed in Section 4.

Central to our understanding of the role of gut microbiota in maintaining host health is the concept of "dysbiosis", i.e., the breakdown in the balance between gut bacteria that promote health and those that either provide no benefit or in fact are deleterious to the host. In Section 5 we discuss shifts in composition, gene contents and metabolic activities of the gut microbiota that are associated with obesity and potentially underlie its inflammatory and metabolic complications. In Section 6 we review evidence for the preventive and therapeutic efficacy of probiotics and prebiotics in weight management and metabolic health.

2. Analysis and composition of the human gut microbiome

Traditionally, studies of the gut microbiome identified taxonomic groups and individual species with microbiological culture-dependent methods or such methods in conjunction with polymerase chain reaction (PCR), fluorescence *in situ* hybridization (FISH) and gel-based approaches. More recently, relatively inexpensive high throughput methodologies including phylogenetic arrays and next generation sequencing have dramatically expanded both the breadth and precision of microbiome and metagenomic analyses (Claesson et al., 2009; Petrosino et al., 2009). For example, 454 pyrosequencing employs millions of single-stranded sequencing templates attached to individually-isolated beads to achieve massively parallel sequencing, 200 bp read lengths and >99% accuracy (Novais and Thorstenson, 2011). Barcoding of sequencing templates allows multiplexing within individual wells, further increasing throughput and decreasing the cost. Analysis of microbial community structure by pyrosequencing is based almost universally on amplicons (sequence reads) of the 16S rRNA gene, which is highly conserved between different species of bacteria and archaea. Amplicons of one or more of the nine variable regions of the 16S rRNA gene (Neefs et al., 1993) are quantified and subsequently assigned to microbial phylogenies. Most researchers cluster 16S rRNA gene sequences sharing 97–99% identity into the same operational taxonomic unit (OTU) that represents one species-level sequence cluster or "phylotype." Similarity of bacterial communities is evaluated using UniFrac analysis (Lozupone and Knight, 2005), which is based on the premise that similar communities share a greater fraction of the overall phylogenetic tree.

Sequencing-based approaches reveal that the human gut microbiota comprises more than 1000 phylotypes. In healthy individuals, these can be classified into six bacterial divisions/phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia (Eckburg et al., 2005; reviewed in Lozupone et al., 2012; Zhang et al., 2009). Bacteroidetes and Firmicutes account for >90% of the total gut microbiota. At the genus level, the major constituents of human gut microbiota are obligate anaerobes from genera *Bacteroides*, *Eubacterium*, *Clostridium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, *Bifidobacterium*, and *Fusobacterium*, as well as less predominant facultative anaerobes, such as *Escherichia*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Lactobacillus* and *Proteus* (Moore and Holdeman, 1974; Suau et al., 1999). Methanogenic archaea are also widely reported (Miller et al., 1984), but appear restricted to one species, *Methanobrevibacter smithii* (Eckburg et al., 2005).

The bacterial composition (species members and abundance) of the gut microbiota is unique for each person (Lozupone et al., 2012; Zoetendal et al., 1998). Moreover, the concept of a ubiquitous "core" microbiome at the species level appears unlikely (Lozupone et al., 2012), as previously-suggested core species can represent less than 1% of the total microbiota in some individuals (Turnbaugh et al., 2009). Despite wide variability in species composition (microbiota), functional gene profiles (microbiomes) are similar across healthy individuals (Consortium, 2012; Qin et al., 2010; Turnbaugh et al., 2009). However, this functional redundancy can reflect evolutionary convergence of unrelated taxa, further complicating our understanding of what constitutes healthy *vs* dysbiotic variation in microbial community structure.

The concept of "enterotypes" may be more useful in this regard. As described (Arumugam et al., 2011), enterotypes are based on co-occuring associations of specific genera and species. Shotgun metagenomic analysis of fecal bacterial composition of large cohorts of healthy people has suggested three enterotypes dominated by *Bacteroides*, *Prevotella*, and *Ruminococcus* (or other Firmicutes genera), respectively, with this primary bacterial framework suggested to regulate the composition and function of other 'secondary' species within the host gut (Arumugam et al., 2011; Zupancic et al., 2012). However, other studies have not confirmed the same pattern, instead suggesting a trade-off between *Prevotella* and *Bacteroides* within individuals (Consortium, 2012; Wu et al., 2011; Yatsunenko et al., 2012). Importantly, although differences in enterotype were initially reported to be independent of geography, gender, age or body mass index (Arumugam et al., 2011), substantial differences in bacterial assemblages and functional genes were recently reported in a comparative study of US residents and those in either the Venezuelan Amazon or rural Malawi (Yatsunenko et al., 2012). These differences may reflect (at least in part) differences in long-term diet pattern, which have been associated with human enterotypes (Wu et al., 2011). Specifically, the *Bacteroides* enterotype was associated with higher dietary consumption of protein and saturated fats, whereas the *Prevotella* enterotype was associated with low intake of protein and fats but high ingestion of carbohydrates and simple sugars (Wu et al., 2011).

3. Mechanisms by which the gut microbiota regulates host energy balance and storage

3.1. Increased energy extraction from the diet: evidence from mice and humans

Energy metabolism in mice and humans can be significantly impacted by the presence, composition and metabolic actions of the gut microbiota. Conventionally raised mice have higher levels of serum metabolites from glycolysis and the

tricarboxylic acid cycle (e.g., pyruvic acid, citric acid, fumaric acid, and malic acid) than germ-free (GF) animals, suggesting that hosts have higher energy availability and metabolism in the presence of gut bacteria (Velagapudi et al., 2010). The seminal studies of (Backhed et al., 2004) demonstrated that GF C57BL/6 mice consume more energy but are significantly leaner than their conventionally raised counterparts harboring normal gut microbiota. Within 14 days of 'conventionalization' of GF mice with the cecal microbiota of conventionally-raised mice fed a low-fat, polysaccharide-rich diet, formerly GF mice accrue 60% more adiposity and become insulin resistant, despite reduced food intake (Backhed et al., 2004). Moreover, in contrast to conventional mice, GF mice fed a high-fat and sugar-rich, "western" diet failed to develop obesity or insulin resistance (Backhed et al., 2007), thereby supporting an essential role for gut microbiota in the development of diet-induced obesity. Remarkably, GF mice that were conventionalized with microbiota from obese mice became significantly fatter than recipient GF mice that were conventionalized with microbiota from lean mice. Thus, the energy harvest phenotype could be transmitted by the gut microbiota (Turnbaugh et al., 2006, 2008). Employing a different mouse strain (C3H), Fleissner and colleagues (Fleissner et al., 2010) confirmed that germ-free mice were resistant to the obesigenic effects of a high sucrose, high palm oil 'western' diet. However, in these same studies GF mice were not resistant to the obesigenic effects of a low sucrose, lard-based high fat diet (HFD). These results suggest that diet composition and/or genetic background influence the protection from diet-induced obesity conferred by GF status.

To investigate the relationship between microbiota and energy harvest in humans, investigators (Jumpertz et al., 2011) placed 12 lean and 9 obese adult white males on either a 2400- or 3400-kcal/day diet for 3 days in a random crossover fashion, with each diet followed by a 3-day washout period with a weight-maintaining diet. The investigators monitored fecal bacterial composition by pyrosequencing and measured ingested and stool calories with bomb calorimetry during each of the three diet phases. The variation of dietary calorie load was rapidly (within 3 days) associated with changes in the bacterial composition of the gut microbiota. Specifically, the degree of 'overnutrition' (energy consumption as a percentage of weight-maintaining energy needs) on either the 2400- or 3400-kcal/day diet was associated with proportionally more Firmicutes and fewer Bacteroidetes. Notably, in lean subjects energy loss (i.e., stool calories as a percentage of ingested calories) was negatively associated with the proportional change in Firmicutes and was positively associated with the proportional change in Bacteroidetes. Overall, a 20% increase in gut Firmicutes and 20% decrease in Bacteroidetes was associated with ~150 kcal increase in energy harvest by the host, suggesting the potential impact of the gut microbiota on host energy harvest (Jumpertz et al., 2011).

3.2. Role of bacterial fermentation in host energy harvest

An essential mechanism by which the gut microbiota enhances host energy harvest is through the hydrolysis and fermentation of dietary polysaccharides that the host cannot otherwise digest. Microbial fermentation generates monosaccharides and short chain fatty acids (SCFAs), which can be absorbed and used as energy by the host. Conventionally raised mice absorb more monosaccharides from the gut than GF mice (Backhed et al., 2004). Consistent with this observation, colonization of GF mice with the gut microbiota of conventionally-raised mice induces the expression of sodium/glucose transporter-1 (SGLT1) in the small intestine (Hooper et al., 2001) and increases the density of capillaries underlying the small intestinal villus epithelium (Stappenbeck et al., 2002). Microbiota-generated SCFAs, predominantly acetate, butyrate and propionate are readily absorbed by colonocytes. A significant amount of acetate enters systemic circulation and reaches peripheral tissues, whereas butyrate and propionate are largely utilized by the colonic epithelium and liver, respectively (Lin et al., 2012). Considered together, SCFAs potentially contribute 6–10% of the basal energy requirements of people in developed countries, and the contribution could be higher in individuals who consume more dietary fiber (McNeil, 1984).

Generation of SCFAs by gut microbial fermentation additionally involves the metabolic actions of methanogenic Archaea (group Euryarchaeota), chemoautotrophs that use hydrogen gas as a source of electrons for reducing carbon dioxide. As hydrogen is the end product of various forms of fermentation (Thauer et al., 1977), methanogens in the human gut are postulated to increase the efficiency of bacterial fermentation and SCFA production, thereby promoting energy harvest and weight gain (Samuel and Gordon, 2006). Consistent with this concept, co-colonization of GF mice with *Methanobrevibacter smithii* (the principal methanogenic Archaea species in the human gut) and *Bacteroides thetaiotaomicron* (a human gut commensal that ferments dietary polysaccharides) greatly increased the efficiency of bacterial fermentation and SCFA production and promoted increased fat pad mass (Samuel and Gordon, 2006).

3.3. Modulation of host energy intake and metabolism by SCFAs

3.3.1. SCFA regulation of gut peptide secretion

Somewhat paradoxically, in addition to serving directly as energy substrates, SCFAs produced by bacterial fermentation function as regulators of energy intake and energy metabolism (reviewed in Conterno et al., 2011). It is well-established that non-digestible, fermentable polysaccharides such as oligofructose (inulin) enhance gut microbiota production of SCFAs, and that this enhanced production is associated with increased host satiety and reduced food intake (Archer et al., 2004; Cani et al., 2004, 2005, 2006, 2007b, 2009a,b; Delzenne et al., 2005; Tarini and Wolever, 2010; Whelan et al., 2006). Reduced food intake in part reflects increases in the gut peptide hormones glucagon-like peptide (GLP)-1 and peptide (P)YY, which decrease appetite and energy intake (Delzenne et al., 2010; Neary and Batterham, 2009), in conjunction with decreased secretion of the gut peptide ghrelin, which increases food intake through effects on hypothalamic, brainstem and reward-related

circuits (Alvarez-Castro et al., 2012). Thus, the actions of prebiotics to increase GLP-1 and PYY while reducing ghrelin are consistent with increased satiety and reduced energy intake.

These conclusions are largely supported by studies that directly assess the effects of SCFAs on gut peptides, appetite and energy metabolism. For example, intracolonic and ileal infusions of mixed SCFAs increase PYY secretion in rats and pigs (Cherbut et al., 1998; Cuche et al., 2000). In rodent models of obesity, initial supplementation studies with either acetate (Kondo et al., 2009; Yamashita et al., 2007) or butyrate (Gao et al., 2009) reported suppressed weight gain, but this was independent of appetite suppression. More recently, Lin and colleagues (Lin et al., 2012) demonstrated that dietary supplementation with either acetate, butyrate or propionate protected against HFD-induced obesity and insulin resistance in mice, but only butyrate and propionate stimulated gut hormone secretion and suppressed food intake. Studies in humans are limited, but suggest that propionate may reduce appetite in human subjects (Arora et al., 2011). In addition to effects on gut peptides, butyrate and propionate are reported to induce leptin expression from adipocytes (Soliman et al., 2011), providing a potential additional mechanism of appetite regulation. Overall, accumulating evidence indicates that SCFAs reduce appetite and/or alter energy metabolism to promote healthy weight.

3.3.2. Role of G protein-coupled Free Fatty Acid Receptors (FFARs)2 and -3 in SCFA-dependent metabolic regulation

The signaling actions of SCFAs are mediated by the G protein-coupled receptors Free Fatty Acid Receptor (FFAR)2 (GPR43) and FFAR3 (GPR41). Based on *in vitro* studies, acetate preferentially activates FFAR2, propionate activates FFAR2 and FFAR3, and butyrate preferentially activates FFAR3 (Brown et al., 2003; Le Poul et al., 2003). Accordingly, these receptors are implicated in the salutary effects of SCFAs on appetite and energy metabolism discussed in the previous section. However, knockout mouse studies indicate that the metabolic roles of FFAR2 and -3 are more complex and contextual, such that their global abrogation promotes leanness rather than obesity.

FFAR2 promotes energy storage by increasing adipogenesis (Dewulf et al., 2010; Hong et al., 2005), inhibiting adipocyte lipolysis (Ge et al., 2008) and by decreasing whole body energy expenditure (Bjursell et al., 2011). Consistent with these actions, FFAR2 is induced in subcutaneous adipose tissue of mice made obese by HFD (Dewulf et al., 2010). Moreover, FFAR2-deficient (*Gpr43*−/−) mice fed a HFD became less obese, with more lean mass, greater insulin sensitivity, and lower hepatic triglycerides and plasma cholesterol than wild type mice. These salutary effects reflected higher core body temperature and greater energy expenditure in the knockout animals (Bjursell et al., 2011).

Similarly, mice deficient in FFAR3 (*Gpr41*−/−) are significantly leaner than their wild-type (+/+) counterparts. Notably, unlike GF wild-type mice, GF *Gpr41*−/− mice do not develop increased adiposity following conventionalization (Samuel et al., 2008). These results indicate that FFAR3 signaling is required for the gut microbiota to promote host adiposity in conventional mice. The lean phenotype of mice lacking FFAR3 derives from the (expected) reduction in PYY secretion and the corresponding increase in intestinal transit rate. This increased intestinal transit rate results in decreased efficiency of energy absorption. These results suggest that the gut microbiota increases energy harvest by slowing intestinal transit via an SCFA (butyrate and propionate)–FFAR3–PYY signaling pathway (Samuel et al., 2008). Thus the combined actions of FFAR2 and FFAR3 in wild-type mice are expected to promote both increased energy harvest and increased triglyceride storage in adipose tissue. A current challenge is to mechanistically reconcile these effects of FFAR deletion with the demonstrated ability of SCFAs and precursor fermentable carbohydrates such as inulin to reduce appetite, increase energy expenditure and protect against diet-induced obesity (Section 3.3.1). More fine-grained studies with conditional FFAR2/3 knockout mice may help resolve this issue by uncoupling the metabolic effects of FFAR2/3 signaling in gut peptide-secreting enteroendocrine cells from the obesigenic effects of FFAR2/3 signaling in adipocytes and other metabolically important cells.

3.4. Appetite regulation by microbiota-Toll like Receptor (TLR) 5 interaction

TLRs are extracellular pattern-recognition receptors (PRRs) of the innate immune system that recognize invariant microbial motifs (pathogen-associated molecular patterns or PAMPS) on bacteria, viruses and fungi (reviewed in Kumar et al., 2011). Recognition of PAMPs by PRRs rapidly triggers multiple antimicrobial immune responses through the induction of inflammatory signaling pathways and the downstream transcription of cytokines and other inflammatory mediators. Given the extent of the intestinal microbiota, it is not surprising that PAMP detection by TLRs at the epithelium–luminal interface is vital to maintain mucosal immune tolerance and intestinal homeostasis (Cerf-Bensussan and Gaboriau-Routhiau, 2010) (see also Sections 4.3.1 and 4.4).

TLR5 specifically detects bacterial flagellin and is highly expressed in the mouse gut mucosa (Rhee, 2011). TLR5 recognition of extracellular flagellin induces nuclear factor (NF)-κB-mediated transcription of host defense genes and enhances cell survival in the face of the ensuing inflammation. Remarkably, TLR5−/− (T5KO) mice become hyperphagic and develop obesity and related metabolic syndrome pathologies including hyperlipidemia, hypertension and insulin resistance (Vijay-Kumar et al., 2010). Food restriction prevented obesity, but not insulin resistance in these mice, indicating that TLR5 deficiency is sufficient to cause insulin resistance in the absence of obesity.

Notably, the hyperphagic/obese phenotype of T5KO mice was correlated with alterations in the bacterial composition of the gut. UniFrac analysis (Section 2) revealed that the gut microbiota of T5KO and wild-type littermate mice were significantly different at the species level, with 116 bacterial phylotypes from various phyla stereotypically enriched or reduced in T5KO mice relative to wild-type mice. Critically, transplantation of the gut microbiota from TLR5−/− mice to GF wild-type

mice recapitulated the hyperphagia, obesity and metabolic dysregulation in the recipients, indicating that these alterations promote the hyperphagic/obese phenotype and are not merely an epiphenomenon. Moreover, antibiotic treatment of TLR5-deficient mice normalized food intake to wild type levels and significantly ameliorated metabolic syndrome (Vijay-Kumar et al., 2010). These observations suggest that the gut microbiota regulates host ingestive behavior and the development of insulin resistance and that this regulation is dependent on TLR5, perhaps through its impact on microbial composition of the gut.

3.5. Metabolic regulation by suppression of AMP-activated protein kinase (AMPK)

AMPK is an enzyme expressed in liver, brain and skeletal muscle that functions as a cellular energy sensor and metabolic regulator. It is activated by phosphorylation at Thr-172 of the catalytic α subunit, when intracellular AMP:ATP or NAD:NADH ratios increase in response to metabolic stress such as exercise or glucose deprivation. AMPK activation increases cellular energy levels by stimulating catabolic pathways (e.g., glucose transport, fat oxidation) and by inhibiting anabolic pathways (e.g., fatty acid, protein and glycogen synthesis), in part through inhibition of mammalian Target of Rapamycin (mTOR) (reviewed in Inoki et al., 2012). AMPK stimulates fatty acid β oxidation through inhibitory phosphorylation of acetyl-CoA carboxylase (Acc). The resulting decrease in cellular malonylCoA levels activates carnitine:palmitoyl transferase-1 (Cpt1), the rate-limiting enzyme in mitochondrial β-oxidation (Kahn et al., 2005).

Backhed and colleagues (Backhed et al., 2007) demonstrated that the resistance to HFD-induced obesity in GF mice was associated with 40% more phosphorylated AMPK and Acc and 15% less CPT1 activity in skeletal muscle as compared with conventional mice. Similarly livers of GF mice had twice the phospho-AMPK of livers from conventional mice, and this AMPK activation was reflected in substantially reduced levels of glycogen synthase and glycogen. Enhanced AMPK activation in GF *vs* conventional mice was associated with significantly elevated levels of AMP and NAD^+ in skeletal muscle and liver, respectively. The investigators concluded that the gut microbiota predisposes the host to obesity and insulin resistance in part by decreasing AMPK activity and fatty acid oxidation in peripheral tissues (Backhed et al., 2007). The mechanism of this suppressive effect of gut microbiota on AMPK activity is not elucidated. Moreover, this mechanism would need to counteract the reported actions of both butyrate and acetate to enhance AMPK activity (Gao et al., 2009; Sakakibara et al., 2006).

3.6. Regulation of fatty acid uptake by suppression of fasting-induced adipose factor (Fiaf/Angptl4)

Fiaf (also known as angiopoietin-like 4) is a protein secreted by adipose tissues, liver and intestine that inhibits the activity of Lipoprotein Lipase (LPL), a key enzyme in the hydrolysis of lipoprotein-associated triglycerides and the release of fatty acids for transport into cells. In adipocytes, fatty acids released by LPL are re-esterified into triglyceride and stored as fat. The increase in body fat observed upon conventionalization of GF mice is associated with a decrease in Fiaf expression in the ileum and a 122% increase in LPL activity in epididymal adipose tissue (Backhed et al., 2004). Consistent with a role for gut microbiota in clearance of circulating lipids (and increase in tissue lipids), conventionally-reared mice have reduced serum levels of triglyceride, chylomicrons, fatty acids and cholesterol as compared with GF mice (Velagapudi et al., 2010).

Fiaf may also protect against obesity by indirectly regulating lipid catabolism. This insight comes from studies of GF Fiaf-deficient mice, which unlike GF wild type mice, are not resistant to western diet-induced obesity (Backhed et al., 2007). Skeletal muscle of Fiaf-deficienct GF mice contains reduced levels of peroxisome proliferator activated receptor coactivator 1α (Pgc-1α) and attenuated expression of *Cpt1* and other Pgc-1α-regulated genes involved in fat oxidation. Although the mechanism by which Fiaf induces Pgc-1α expression is currently unclear, a model emerges in which the gut microbiota increases host sensitivity to overnutrition by suppressing Fiaf expression, thereby (1) increasing LPL activity and lipid storage in tissues, and (2) reducing catabolic pathways mediated by Pgc-1α-regulated gene products in peripheral tissues (Backhed et al., 2007).

3.7. Transcriptional regulation of hepatic lipogenesis via SREBP-1c and ChREBP

Germ-free mice receiving microbiota from conventional mice dramatically increase the synthesis of hepatic triglycerides and alter the levels of multiple hepatic triglyceride species (Backhed et al., 2004; Velagapudi et al., 2010). Enhanced triglyceride synthesis is coincident with upregulation of the lipogenic genes, acetyl-CoA carboxylase (*Acc1*) and fatty acid synthase (*Fas*). Both *Acc1* and *Fas* are transcriptional targets of the basic helix–loop–helix leucine zipper proteins, sterol response element binding protein 1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP), two transcription factors critical for hepatocyte lipogenesis in response to insulin and glucose, respectively (Backhed et al., 2004). In conventionalized mice, liver levels of ChREBP were significantly increased, and CHREBP nuclear translocation to the nucleus following dephosphorylation by PP2A was enhanced (Backhed et al., 2004). Notably, the PP2A activator, xylulose-5-phosphate (Xu5P) was significantly elevated in conventionalized mice, suggesting that elevated levels of this hexose monophosphate shunt intermediate promote the increased activation of hepatic ChREBP and hepatic lipogenesis in the conventionalized mice.

4. Role of gut microbiota in obesity-associated inflammation

Obesity is a state of chronic, low-grade inflammation, which is now recognized to play an underlying pathogenic role in the metabolic complications and negative health outcomes of obesity (reviewed in Lumeng and Saltiel, 2011; Shoelson and Goldfine, 2009; see also Lumeng, this volume; Snyder-Cappione and Nikolajczyk, this volume; Denis and Obin, this volume). The gut mucosal surface is the predominant site of pathogenic bacterial entry into the body. In this section we discuss (1) the role of increased gut permeability and resulting endotoxin and bacterial translocation in obesity-associated inflammation and (2) host molecular mechanisms by which host–microbiota interactions at the gut mucosal interface either maintain intestinal barrier function or promote bacterial translocation and obesity-associated inflammation.

4.1. Metabolic endotoxemia

Obesity is associated with elevated plasma levels of bacterial lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria (e.g., *Escherichia coli*) (Amar et al., 2011a,b; Brun et al., 2007; Creely et al., 2007; Sun et al., 2010). Energy intake, in particular HFD feeding increases gut permeability and increases plasma LPS levels 2- to 3-fold (i.e., 10–50 times lower than levels attained in septicemia or infections) (Amar et al., 2008; Brun et al., 2007; Cani et al., 2007a). LPS transits into the circulatory system reflects passage of bacterial fragments across the gut into systemic circulation, either through increases in diffusion due to intestinal paracellular permeability or through absorption by enterocytes during chylomicron secretion (Boroni Moreira and de Cassia Goncalves Alfenas, 2012). This phenomenon, termed "metabolic endotoxaemia" is typically associated with loss of gut *Bifidobacterium* spp., which are known to increase/maintain mucosal barrier function against bacterial antigens (Griffiths et al., 2004; Ruan et al., 2007; Wang et al., 2004, 2006; see Section 5.1). A direct role for LPS in obesity and its complications is supported by studies in which LPS was infused subcutaneously for 4 weeks into wild type mice maintained on a normal chow diet. The infusions led to increased whole body, liver and adipose tissue weights, adipose and liver inflammation (i.e., elevated TNF-α, IL-1, IL-6, and plasminogen activator inhibitor (PAI-1)) as well as fasted hyperglycemia and insulinemia. These effects of LPS were comparable to those induced by HFD (Cani et al., 2007a).

4.2. Metabolic bacteremia

In addition to bacterial fragments, the translocation of live bacteria to host tissues is also a feature of obesity ("metabolic bacteremia"). After only 1-week of HFD feeding (i.e., before the onset of HFD-induced hyperglycemia) 16S rRNA analysis revealed that the amount of total bacteria, Gram-negative bacteria, and *E. coli* DNA significantly increased in the intestinal lumen, ileal mucosa, blood and mesenteric adipose tissue of HFD-fed mice (Amar et al., 2011a). Moreover, gavage of ampicillin-resistant, green fluorescent protein-labeled *E. coli* (GFP-*E. coli*) confirmed that the translocation of live Gram-negative bacteria through the intestinal mucosa occurred prior to diabetes onset. HFD-fed mice had more GFP-*E. coli* adhering to the intestinal mucosa and cecum, within lamina propria, as well as within submucosa than gavaged chow-fed mice. Thus, HFD increased the adherence of Gram-negative bacteria to the intestinal mucosa and facilitated transmucosal bacterial translocation. Moreover, GFP-*E. coli* co-localized with dendritic cells in lamina propria, on the luminal side of the villi and in mesenteric lymph nodes, indicating both phagocytosis and immune activation.

To test the hypothesis that metabolic bacteremia is an early event in the pathogenesis of T2D, Amar and colleagues quantified the 16S rRNA gene in the blood of 3280 participants in a nine year longitudinal study of the insulin resistance syndrome (Amar et al., 2011b). Pyrosequencing of baseline samples indicated that all participants shared a core blood microbiota composed predominantly (85–90%) of phylum Proteobacteria. However, total 16S rRNA gene *concentration* at baseline was higher among study participants who presented with abdominal adiposity and developed diabetes after nine years than among those who did not. The authors concluded that the amount of 16S rRNA gene in the blood may provide a novel biomarker(s) of diabetes risk (Amar et al., 2011b).

4.3. Mechanisms regulating gut barrier function and metabolic endotoxemia/bacteremia: host–microbiota crosstalk

Clearly, compromised gut barrier function is an important underlying factor in metabolic endotoxemia and bacteremia (Amar et al., 2011a; Cani et al., 2007b, 2008). Studies now link obesity-associated changes in gut microbiota to specific host mechanisms that regulate gut permeability and bacterial translocation. These include mechanisms that promote intestinal epithelial function and tight junction integrity as well as mechanisms of bacterial surveillance/detection. Bacteria and bacterial cell wall components are recognized by host cells and activate host innate immunity through bacterial antigen receptors. These include the LPS receptor CD14, TLRs, which provide extracellular surveillance, and PRRs of the nucleotide-binding oligomerization domain (Nod)-like receptor family, which recognize intracellular/internalized pathogens (Kufer and Sansonetti, 2007; Lundin et al., 2008). It is not unexpected therefore that these receptors play important roles in preventing or promoting bacterial translocation into the host.

4.3.1. Mechanisms maintaining gut barrier function and preventing bacterial translocation

4.3.1.1. GLP-2. The metabolic endotoxemia of HFD-induced obesity is associated with reductions in *Bifidobacterium*, and both can be ameliorated with prebiotics such as inulin (Amar et al., 2011a; Cani et al., 2007b, 2009b). Intestinotrophic proglucagon-derived peptide (GLP)-2 is a peptide produced by L cells of the intestine that promotes intestinal growth (e.g., crypt cell proliferation, villus elongation) and barrier function via Insulin-like Growth Factor (IGF)-1 and β catenin pathways (Dube et al., 2006, 2008; Tsai et al., 1997). The salutary effects of prebiotics and *Bifidobacterium* in particular on barrier function are associated with enhanced production of GLP-2, coincident improvements in intestinal tight-junction integrity (i.e., increased mRNA of junctional proteins ZO-1 and occludin), and reduced gut permeability (decreased translocation of dextran–FITC) (Cani et al., 2009b). When obese *ob/ob* mice were treated with both prebiotics and a GLP-2 antagonist, most the beneficial effects of prebiotics on host inflammation were abolished. However, when the *ob/ob* mice were treated with GLP-2 alone, gut permeability, metabolic endotoxemia and systemic and hepatic inflammation were improved to a level comparable to that attained with prebiotic treatment. These results suggest that prebiotics increase gut barrier function through *Bifidobacterium*-induced increases in intestinal GLP-2 production. The specific mechanism(s) by which increases in *Bifidobacterium* promote L cell production of GLP-2 is presently undetermined.

4.3.1.2. TLR-2. TLR-2 is a cell membrane-associated PRR that recognizes diverse microbial molecules including cell wall peptidoglycan (PGN), lipoteichoic acid and lipoprotein from Gram-positive bacteria as well as lipoarabinomannan from mycobacteria (reviewed in Kawai and Akira, 2005). TLR2 maintains the barrier integrity of the intestinal epithelium, including the preservation of tight junctions at the frontline of host defense (Cario, 2008). TLR-2 signaling activates the anti-apoptotic PI3K/AKT pathway via the ubiquitous TLR adaptor protein, myeloid differentiation primary-response protein 88 (MyD88) (Cario et al., 2007). In so doing, TLR2 protects gut epithelial cells from apoptosis induced by stress or injury. This TLR2/MyD88/PI3K/AKT pathway reportedly mediates the salutory actions of the probiotic *Lactobacillus casei* to prevent TNF-α or IFNγ-induced impairments in the barrier function of Caco-2 cells (Eun et al., 2011). Surprisingly, although TLR2 is relatively insensitive to LPS (Tapping et al., 2000), TLR2 agonist prevents LPS-induced increases in paracellular permeability in primary colonic epithelial cultures (Hanson et al., 2011).

Consistent with these observations, TLR2−/− mice fed a normal diet have increased LPS absorption from the gut and develop subclinical inflammation, insulin resistance, glucose intolerance, and (after 4 months) obesity (Caricilli et al., 2011). Moreover, the microbiota of TLR2−/− mice are dramatically altered as compared with wild-type mice, with a 3-fold increase (to 40% of OTUs) in predominantly Gram-positive species (Firmicutes) but a corresponding 40-fold decrease (to 1% of OTUs) in predominantly Gram-negative species (Proteobacteria). Notably, the metabolic endotoxemia of TLR2−/− mice could be reproduced in conventional wild-type mice by microbiota transplantation and was reversed by antibiotics, directly implicating these changes in gut microbiota in the dysregulated metabolic phenotype of TLR2−/− mice (Caricilli et al., 2011). However, complicating the elucidation of the role of TLR2 in obesity-induced metabolic endotoxemia is the fact that TLR2-deficient mice are resistant to HFD-induced obesity due to elevated metabolic rate and preferential use of fat as a metabolic substrate (Davis et al., 2011; Ehses et al., 2010).

4.3.1.3. MyD88. The key signaling role of MyD88 downstream of TLRs identifies it as an important signaling node in the regulation of gut homeostasis. First, as discussed above (Section 4.3.1.2), MyD88 is required for the TLR-2-mediated protection of tight junctions and intestinal barrier function in response to inflammation and stress. In addition, MyD88-dependent upregulation of endogenous host antibacterial compounds is essential for controlling intestinal barrier penetration by commensal and pathogenic bacteria (Brandl et al., 2007, 2008; Vaishnava et al., 2008). A recent comprehensive study of the gut microbiome and transcriptional profiles of duodenum, jejunum, ileum and colon in GF and conventionally-raised wild-type and *Myd88*−/− mice revealed that MyD88 was essential for microbiota-induced expression of the antimicrobial genes *Reg3β* and *Reg3γ* in colonic epithelium (Larsson et al., 2012). In addition, Moreover, *Myd88* abrogation is associated with altered bacterial diversity in the distal gut (increased ratio of Firmutes/Bacteroidetes) (Wen et al., 2008) and a greater proportion of segmented filamentous bacteria in the small intestine (Larsson et al., 2012). Together, these observations point to MyD88 as an important mediator of microbiota-host interactions that maintain metabolic health.

Consistent with the function of MyD88 in controlling translocation of commensal and pathogenic bacteria, HFD-fed MyD88−/− mice developed more severe diabetes in association with hyperinsulinemia, hyperleptinemia and liver inflammation (Amar et al., 2011a). Not surprisingly, this sensitivity to HFD was associated with significantly increased bacterial translocation in MyD88−/− mice (Amar et al., 2011a). As expected, HFD-fed MyD88−/− mice expressed less TNF-α and serum amyloid (SAA)3 in colonocytes and/or adipose tissue (Amar et al., 2011a; Reigstad et al., 2009), inconsistent with the role of MyD88 in LPS-induced inflammatory signaling downstream of CD14/TLR4 (see Section 4.3.2.1 below). A current challenge is to disentangle and reconcile the metabolically-protective actions of MyD88 to prevent bacterial translocation with its role in LPS signaling during metabolic endotoxemia.

4.3.1.4. NLRC2 (NOD2). Phagocytosed (internalized) bacteria are detected by cytosolic PRRs, including the nucleotide-binding oligomerization domain (NOD)-like receptors (NLR). The approximately 20 mammalian NLR proteins are divided largely into two subfamilies: the NLRCs (formerly NODs) and the NLRPs (formerly NALPs) (Kumar et al., 2011). Although direct evidence is currently lacking, multiple lines of evidence suggest that NLRC2 (NOD2) may protect against obesity-associated dysbiosis and metabolic endotoxemia. NLRC2 is an important microbial sensor at the intestinal barrier that recognizes muramyldipeptide

(MDP), a peptidoglycan (PGN) component of virtually all bacterial cell walls. NLRC2 maintains microbial community structure and suppress *de novo* colonization of opportunistic pathogens in the terminal ileum by modulating cell-mediated immunity, including the production of endogenous antimicrobial compounds from Paneth cells (Kobayashi et al., 2005; Petnicki-Ocwieja et al., 2009; Rehman et al., 2011). Humans carrying the *Nod2* frameshift mutation (SNP13) have significantly increased loads of Bacteroidetes and Firmicutes (Rehman et al., 2011) and multiple *Nod2* polymorphisms confer greater risk for Inflammatory Bowel Disease (Abraham and Cho, 2006; Cuthbert et al., 2002). NLRC2 activation inhibits TLR4 in enterocytes, thereby conferring protection from the intestinal mucosal injury of necrotizing enterocolitis (Richardson et al., 2010). NLRC2 also mediates immunologic tolerance (induction of IL-10, tolerogenic $CD103^+$ DCs and $CD4^+Foxp3^+$ regulatory T cells) in response to the probiotic strain *Lactobacillus salivarius Ls33* and to specific MDP purified from this strain (Macho Fernandez et al., 2011). NLRC2 therefore emerges as a potentially beneficial PRR in the context of obesity-associated dysbiosis.

4.3.2. Host signaling mechanisms promoting bacterial translocation and its sequelae in obesity

4.3.2.1. CD14/TLR4. LPS induces cellular inflammatory responses by activating the CD14/TLR4/MD2 signaling complex (McGettrick and O'Neill, 2010). MyD88 functions downstream of TLR4 in this response to induce the transcription of inflammatory genes by both NF-κB (via TRAF6-TAK1 dependent activation of the IKK complex) and AP-1 (through TRAF6-TAK1-dependent activation of JNK and p38) (Brown et al., 2011; McGettrick and O'Neill, 2010). Both CD14−/− and TLR4−/− mice are protected from the obesigenic, inflammatory and metabolic effects of either HFD or LPS infusion (Cani et al., 2007a, 2008; Davis et al., 2008; Saberi et al., 2009). Importantly, protection from HFD-induced obesity and diabetes in CD14−/− mice is coincident with the prevention of bacterial translocation (Amar et al., 2011a). Thus recognition of LPS through the CD14/TLR4 is directly implicated in HFD- induced metabolic endotoxemia/bacteremia and its obesigenic effects.

4.3.2.2. NLRC1(NOD1). The cytosolic PRR, NLRC1 (formerly NOD1) detects bacterial cell wall PGN containing D-glutamyl-meso-diaminopimelic acid (meso-DAP) (Kufer and Sansonetti, 2007; Kumar et al., 2011). These PGNs are found primarily in Gram-negative bacteria. NLRC1 mediates MyD88-independent activation of NF-κB via homophilic interactions with RIP2 adaptor, a caspase recruitment domain (CARD)-containing protein kinase that interacts directly with the IκB kinase complex (Kobayashi et al., 2002; Magalhaes et al., 2011). Notably, NLRC1−/− mice are resistant to bacterial translocation and impaired glucose homeostasis induced by HFD (Amar et al., 2011a), suggesting that NLRC1-dependent recognition of meso-DAP in the PGN of Gram-negative bacteria activates downstream mechanisms that promote these HFD-associated pathologies. Thus, two non-redundant molecular mechanisms of Gram-negative detection by the host – one recognizing LPS (CD14/TLR4) and the other PGN (NLRC1)-promote bacterial translocation, obesity and diabetes in response to HFD. It is proposed that these two pathways synergize to drive the full inflammatory response to translocated bacteria (Amar et al., 2011a). In addition, as MyD88 emerges as a potentially important signaling node in protection from these effects (Section 4.3.1.3), an additional MyD88-associated PRR is implicated (Amar et al., 2011a).

4.3.2.3. Endocannabinoid (eCB) signaling. The endocannabinoid (eCB) system consists of the neuromodulatory lipids (endocannabinoids) anandamide (AEA) and 2-arachidonoylglycerol (2-AG), their receptors (CB_1 and CB_2, respectively), and the enzymes that degrade them (fatty acid amide hydrolase [FAAH] and monacylglycerol lipase [MGL], respectively) (reviewed in Duncan et al., 2005). The eCB system regulates diverse physiological processes, including appetite (Di Marzo et al., 2001) and gut motility (Duncan et al., 2005).The gastrointestinal tract contains an eCB system where endocannabinoids are synthesized locally.

Interaction between the gut microbiota and the eCB system regulates gut permeability and thus metabolic endotoxemia in obesity. This conclusion is based on assessment of intestinal eCB system activity in multiple mouse models used to study gut microbiota–obesity relationships (Muccioli et al., 2010). For example, changes in the gut microbiota which were associated with anti-obesity effects selectively decreased CB_1 receptor expression in the colon (but not jejunum), suggesting that the gut microbiota selectively modulate colonic CB_1 receptor expression. In addition, AEA levels and FAAH mRNA were elevated in colons of ob/ob mice that were fed prebiotics, consistent with downregulated CB_1 receptor activity and coincident with reduced plasma LPS. Subsequently obese mice were treated with the CB_1 receptor antagonist SR141716A for 12 days to reduce eCB system activity. This treatment (but not treatment with a CB_2 receptor antagonist) reduced gut permeability, plasma LPS, lower body weight gain and fasting glucose and improved liver inflammatory tone. Reciprocally, 4-week infusion of cannabinoid receptor agonist (HU-210) to lean mice increased gut permeability and plasma LPS level, consistent with the hypothesis that intestinal CB_1 receptor activity compromises gut barrier function and promotes metabolic endotoxemia.

This conclusion is challenged by more recent studies (Zoppi et al., 2012) in which gut permeability was assessed in CB_1 receptor knockout (CB1R−/−) mice and wild-type mice using an immobilization and acoustic (IA) stress paradigm. Both under basal conditions and after IA stress, CB1R−/− mice had *greater* colonic barrier dysfunction as compared with wild-type mice, based on greater paracellular permeability to $^{(51)}$Cr-EDTA, more bacterial translocation to mesenteric lymph nodes, significantly lower IgA secretion, and greater expression of inflammatory enzymes (COX2, NOS2) (Zoppi et al., 2012). Thus, although both studies implicate the eCB system in regulation of gut permeability, we currently do not understand the (patho)physiological contexts in which CB_1 receptor antagonism or agonism are appropriate therapeutic approaches.

4.3.2.4. Metabolic endotoxemia and adipogenesis: role of endocannabinoid (eCB) signaling. Mice receiving LPS injection develop increased adiposity, suggesting that metabolic endotoxemia or bacteremia can promote obesity *per se* (Cani et al., 2007a). A

hallmark of inflammatory and metabolically deleterious obesity is an increase in the size of existing adipocytes (hypertrophic obesity), coincident with impaired adipogenesis, i.e., impaired ability to recruit and differentiate new, smaller adipocytes (Gustafson et al., 2009). Recent evidence suggests that metabolic endotoxemia promotes hypertrophic obesity through specific interaction of LPS with the eCB system (Muccioli et al., 2010).

First, obese individuals have higher plasma and adipose levels of eCBs as well as elevated expression of the CB_1 receptor in adipose tissues (Cote et al., 2007; Engeli et al., 2005). Moreover, blockade of CB_1 receptor-mediated signaling protects animals from obesity, insulin resistance, and related complications (DeLeve et al., 2008; Gary-Bobo et al., 2007; Osei-Hyiaman et al., 2008). Reductions in fat mass and improved metabolic markers in obese *ob/ob* mice receiving prebiotics is associated with decreased CB_1 receptor mRNA expression and AEA levels in adipose tissues, coincident with increased expression of adipogenic markers (C/EBP-α , PPAR-γ, aP2) (Muccioli et al., 2010). Moreover, blocking of CB_1 receptor signaling in *ob/ob* mice produced results that were similar to those obtained with prebiotics. These results support the hypothesis that changes in gut microbiota that attenuate obesity and its complications do so in part by decreasing eCB system tone in adipose tissue.

Intriguingly, activation of the eCB system by a CB_1 receptor agonist in lean (*ob*/+) mice increased markers of adipogenesis and lipogenesis, and this activation resulted in an increase in the number of small adipocytes (i.e., adipocyte hyperplasia) (Muccioli et al., 2010). However, the simultaneous treatment of lean (*ob*/+) mice with both CB1 receptor agonist and LPS (thereby mimicking the increased eCB tone and metabolic endotoxemia of obesity) decreased the expression of adipogenic markers (Muccioli et al., 2010). As discussed in Section 4.3.2.3., the eCB system also regulates gut permeability, with gut CB1 signaling promoting loss of barrier function and metabolic endotoxemia. Thus, it is proposed that adipogenesis is under the control of an LPS–eCB regulatory loop that becomes dysregulated in obesity, thereby inhibiting adipogenesis and promoting hypertrophic obesity (Muccioli et al., 2010).

4.4. Sterile inflammation: fatty acids as PRR ligands

PRRs can also be activated by molecules of nonmicrobial origin (sterile inflammation), in particular by dietary lipids. TLRs (TLR2 and 4) and NLRCs can be activated by saturated fatty acids (SFAs) and antagonized by n-3 polyunsaturated fatty acids (PUFAs), respectively (Hsueh et al., 2011; Lee et al., 2010; Zhao et al., 2007). Importantly, SFA-dependent activation of TLRs 2 and 4 has been directly implicated in the development of obesity-associated inflammation and insulin resistance (Davis et al., 2009; Senn, 2006; Shi et al., 2006), suggesting that FFA-induced inflammation could exacerbate or synergize with metabolic endotoxemia. However, the role of SFAs and PUFAs in regulating gut-associated PRRs and the impact of this sterile (nonmicrobial) activation on HFD-induced gut inflammation remains to be elucidated. Complex lipids are present on the surface of bacteria, and as yet unidentified lipid species could also activate bacterial sensing responses through PRRs. NLRP3, which can sense lipotoxicity-associated increases in intracellular ceramide (Vandanmagsar et al., 2011), has been proposed as one such sensor (Amar et al., 2011a).

5. Obesity-associated changes in the composition, function and metabolism of the gut microbiome

5.1. Changes in community composition: microbiota as biomarkers of obesity and Type 2 diabetes

5.1.1. Phylum-wide changes

One goal of microbiome studies is to identify reliable biomarkers of disease risk, including the predisposition to obesity and Type 2 diabetes. Initial studies using 16S rRNA gene-based sequencing techniques reported that the gut microbiotas of obese Americans and obese (*ob/ob*) mice are distinguishable from the microbiotas of their lean counterparts based on a significantly greater ratio of phylum Firmicutes as compared to phylum Bacteroidetes (F/B ratio) in obesity. This higher F/B ratio reflects phylum-wide increase in Firmicutes and/or reduction in Bacteroidetes (Ley et al., 2005, 2006; Turnbaugh et al., 2006, 2009; Zhang et al., 2009). In addition, the obese microbiota has lower bacterial diversity than lean microbiota (Turnbaugh et al., 2008, 2009). The inverse association between obesity and the proportion of Bacteroidetes has been demonstrated in overweight pregnant women, infants who became overweight in childhood, in HFD-fed and genetically obese (*fa*/*fa*) rats, and following both diet- and bariatric surgery-induced weight loss (Armougom et al., 2009; Furet et al., 2010; Ley et al., 2006; Mozes et al., 2008; Santacruz et al., 2010; Waldram et al., 2009).

Intriguingly, when obese people lost weight with either a fat-restricted or carbohydrate-restricted low calorie diet, the proportion of gut Bacteroidetes increased (and the F/B ratio decreased) in association with the percent reduction in body weight (not caloric intake) (Ley et al., 2006). Moreover, changes in the proportion of Firmicutes and Bacteroidetes in the gut of HFD-fed and obese (*ob/ob)* mice was not associated with energy harvest capacity of the gut microbiota (fecal energy content measured with bomb calorimetry, and fecal SCFA amounts), suggesting that the abundance of Firmicutes and Bacteroidetes and the F/B ratio may not modify the energy extraction capacity of the gut microbiota (Murphy et al., 2010). These observations suggest that changes in the proportion of Firmicutes and Bacteroidetes impact host weight (gain or loss) by mechanisms independent of energy harvest.

However, the concept that obesity is associated with increased Firmicutes and decreased Bacteroidetes is contradicted by studies in humans and rodents that report either no difference in relative abundance of Firmicutes and/or Bacteroidetes in obese *vs* lean individuals, no effect of weight loss on the F/B ratio, or even *greater* relative abundance of Bacteroidetes in

obese individuals (Balamurugan et al., 2010; Collado et al., 2008; Duncan et al., 2008; Jumpertz et al., 2011; Schwiertz et al., 2010; Zhang et al., 2010, 2012). In conclusion it remains uncertain whether the relative abundance of Firmicutes and Bacteroidetes is a useful biomarker for overweight or obesity (Murphy et al., 2010).

5.1.2. Archaea methanogens

As previously discussed (Section 3.3), archaea methanogens in the human gut increase the efficiency of bacterial fermentation and SCFA production, potentially promoting energy harvest and weight gain (Samuel and Gordon, 2006). Accumulating evidence suggests that obese individuals have higher concentrations of archaea methanogens in the gut. Enrichment in H_2-producing *Prevotellaceae* and/or H_2-oxidizing methanogenic *Methanobrevibacter* is reported for the gastrointestinal tracts of obese humans (Zhang et al., 2009; Armougom et al., 2009). In addition, Turnbaugh et al. demonstrated that the cecal microbiome of obese (*ob/ob*) mice harbored more genes matching Archaea than the microbiomes of lean (*ob/+* and *+/+*) littermates (Turnbaugh et al., 2006). In contrast, a recent study reports decreased *Methanobrevibacter* in overweight (but metabolically healthy) persons (Schwiertz et al., 2010). More data are needed to confirm the role of gut archaea methanogens in promoting human obesity and associated metabolic complications.

5.1.3. Finer-grained changes: increases in opportunistic pathogens and decreases in SCFA-producers characterize obesity and Type 2 diabetes

In contrast to changes at the phylum level, changes in microbiota composition at the level of class and below have been associated with obesity and Type 2 diabetes, in particular, increases in known or potential opportunistic pathogens and reductions in select SCFA producers. The pathogenic Firmicute, *Staphylococcus aureus* is more abundant in the gut microbiota of overweight/obese pregnant women and infants who become overweight in childhood (Collado et al., 2008; Kalliomaki et al., 2008; Santacruz et al., 2010). Increased populations within the Gram-negative family Enterobacteriaceae (including *E. coli*) were detected in overweight compared with normal-weight pregnant women, and women who gained excessive weight during pregnancy had more *E. coli* than women who experienced normal weight gain (Santacruz et al., 2010). Dramatic overgrowth of Enterobacteriaceae was observed in a morbidly obese volunteer (BMI = 58.8 kg/m^2), with the *Enterobacter* genus, which includes many opportunistic pathogens, constituting 35% of the gut bacteria (Fei and Zhao, in press). Higher levels of opportunistic pathogens (e.g., *Clostridium hathewayi*, *Clostridium ramosum*, *Eggerthella lenta*, etc.) were detected in Type 2 diabetes patients than in non-diabetic individuals (Qin et al., 2012). The gut of obese HFD-fed mice had greater levels of the family Mollicutes (mycoplasm) together with concomitant reductions in other bacterial groups including the Bacteroidetes compared to lean, chow-fed mice (Turnbaugh et al., 2008). In contrast, the Zhao laboratory reported that the abundance of four groups within the Mollicutes was not uniformly altered by HFD, with some increasing and others decreasing (Zhang et al., 2010). In addition, sulfate-reducing, endotoxin-producing bacteria of the Proteobacterial family Desulfovibrionaceae, were enriched (Zhang et al., 2010). A *Halomonas* and a *Sphingomonas* species were uniquely present in the gut of genetically obese (*fa/fa*) Zucker rats when compared with wild-type rats (Waldram et al., 2009).

In contrast to opportunistic pathogens, SCFA- producing genera are reduced in obesity, including those conferring demonstrated health benefits. Cecal *Bifidobacterium* are reduced in genetic (*ob/ob*) and HFD-induced obesity, coincident with reduced gut barrier function and metabolic endotoxemia (Cani et al., 2007b, 2008). Small-scale human studies report that bifidobacterial abundance is reduced in overweight adults and children, and in women with greater weight gain during pregnancy (Collado et al., 2008; Kalliomaki et al., 2008; Santacruz et al., 2009, 2010). *Faecalbacterium prausnitzii* is a Firmicute that provides benefits to host metabolism by producing butyrate from unabsorbed carbohydrate (Li et al., 2008) and by secreting anti-inflammatory metabolites that block NF-kappaB activation (Sokol et al., 2008). Gut *F. prausnitzii* were significantly less abundant in morbidly obese western subjects with diabetes compared to lean healthy controls, and the level of *F. prausnitzii* was negatively associated with plasma levels of inflammatory cytokines (Furet et al., 2010). However, underscoring the impacts of diet, age and degree of obesity on microbiota composition and dynamics, *F. prausnitzii* was more abundant in obese Indian children than normal weight controls (Balamurugan et al., 2010), potentially contributing to greater energy harvest in the former. Importantly, a large scale metagenomic analysis has now confirmed that a gut dysbiosis characterized by increased opportunistic pathogens, greater capacity for sulfate reduction and decreased SCFA producers is a hallmark of Type 2 diabetes (Qin et al., 2012)(see Section 5.2). In this study, the gut microbiome of Type 2 diabetes patients harbored lower levels of butyrate-producing bacteria including *F. prausnitzii*, *Eubacterium rectale*, and *Roseburia intestinalis.*

5.1.4. Alterations in gut microbiota in response to weight loss

Consistent with altered gut microbiota in obese *vs* lean individuals, changes in gut microbial community composition are observed following weight loss. Fecal Enterobacteriaceae and sulfate-reducing bacteria were significantly decreased in obese adolescents who experienced substantial weight loss in response to an energy-restricted (30–40%) diet and a physical exercise program (Sotos et al., 2008). In the same study, those who lost a minor amount of weight had reduced populations of butyrate-producing *Roseburia–Eubacterium rectale* group. In a similar study with overweight adolescents, a calorie-restricted (10–40%) diet and increased physical activity regime over 10 weeks resulted in increased *Bacteroides fragilis* group and *Lactobacillus* group counts, and decreased *Clostridium coccoides* group, *Bifidobacterium longum*, and *Bifidobacterium adolescentis* counts (Santacruz et al., 2009). However, the same intervention program led to different changes in another group of overweight/obese adolescents. Salient features were reductions in the proportion of *Clostridium histolyticum* and *Eubacterium rectale–Clostridium coccoides* groups that were significantly correlated with weight loss, as well as an increase

in *Bacteroides–Prevotella* group in subjects ($N = 23$) who lost more than 4 kg (Nadal et al., 2009). Moreover individuals who lost >4 kg of weight had distinct gut microbiota before as well as after dietary intervention. Total fecal bacteria, *B. fragilis* group, *Clostridium leptum* group, and *Bifidobacterium catenulatum* group counts were significantly higher, whereas, *C. coccoides* group, *Lactobacillus* group, *Bifidobacterium breve*, and *Bifidobacterium bifidum* were significantly lower in the high weight-loss group than in the low weight-loss group both before and after weight loss intervention. This observation suggests that the composition of the gut microbiota in obesity can impact and perhaps predict weight loss (Nadal et al., 2009).

Somewhat surprisingly, rapid weight loss following roux-en-Y gastric bypass (RYGB) surgery has been associated with increases in pathogenic gut bacteria and loss of beneficial species. For example, RYGB was associated with a remarkable increase in Gammaproteobacteria (96.2% of which were members of the Gram-negative Enterobacteriaceae), a decrease in Firmicutes, and a loss of Archaea methanogens (Zhang et al., 2009). Similarly, it was reported that RYGB surgery increased *E. coli* and decreased putatively beneficial bacteria, including the *Lactobacillus*/*Leuconostoc*/*Pediococcus* group as well as *Bifidobacterium* (Furet et al., 2010). These diverse alterations in gut bacterial composition in response to weight loss interventions are likely to reflect complex metabolic, neuroendocrine and immune changes in hosts that occur differentially in response to lifestyle *vs* surgical weight loss interventions and which remain to be elucidated.

5.2. Shifts in functions of the gut microbiota in obese individuals revealed by metagenomic studies

In gut metagenomic studies, total gut microbial DNA is extracted and sequenced, so that the gene contents and potential metabolic contributions of all microbial members can be explored holistically without microbiological isolation or cultivation.

Shotgun sequencing of the gut metagenomes of obese and lean mice and humans has revealed that the obesity-associated gut microbiota has increased capacity to harvest energy from the diet (Turnbaugh et al., 2006, 2008, 2009). For example, compared to lean (*ob/+* or *+/+*) mice, the cecal microbiome of genetically obese *ob/ob* mice contains more genes encoding glycoside hydrolases involved in the initial hydrolysis of indigestible dietary polysaccharides (e.g., α-glucosidases) as well as genes encoding proteins that import the hydrolyte sugars (e.g., ATP-binding cassette [ABC] transporters), metabolize them (e.g., α-galactosidases) and generate short chain fatty acids (e.g., pyruvate formate-lyase). Overall, these observations demonstrate that the *ob/ob* microbiome has a higher capability to ferment dietary polysaccharides and extract energy from food (Turnbaugh et al., 2006). Similarly, in obese mice made by consuming a high sugar/high-fat 'western' diet, the gut microbiome is enriched for microbial genes responsible for importing and fermenting refined sugars and glycans, including phosphotransferase system (PTS) genes and genes involved in fructose and mannose metabolism (Turnbaugh et al., 2008).

The characterization of the fecal metagenome of 6 female adult monozygotic twin-pairs (3 obese twin-pairs *vs* 3 lean twin-pairs) confirmed that the obesity-associated gut microbiome was enriched for PTS genes required for microbial processing of simple sugars and carbohydrates. Among the obesity-associated microbial genes, 75% were from Actinobacteria (compared with 0% in lean-enriched genes), and the other 25% were from Firmicutes; among genes enriched in lean twins, 42% were from Bacteroidetes (compared with 0% in obesity-enriched genes) (Turnbaugh et al., 2009).

Recently, Greenblum and colleagues (Greenblum et al., 2012) studied obesity-associated topological shifts in the human gut microbiome by analyzing the fecal metagenomic sequences of 82 lean/overweight adults (BMI < 30), 42 obese adults (BMI > 30), and 6 obese and lean monozygotic twin pairs and their mothers. The sequences were annotated to 1610 specific enzymes using Encyclopedia of Genes and Genomes (KEGG) database, and resultant enzyme data were used to construct the metabolic network of the human gut microbiome. The authors identified obesity-associated enrichments in enzymes regulating membrane transport and coincident obesity-associated depletion of enzymes for cofactors and vitamin metabolism, nucleotide metabolism, and transcription. The topological distribution of the obesity-associated enzymes in the metabolic network indicated that these enzymes were peripheral enzymes representing either initial or terminal metabolic steps rather than core metabolic processes of the gut microbiome. Moreover, obesity-enriched enzymes generally represented metabolic inputs to the network, indicating that the metabolic interface between the gut microbiome and the host was changed in obesity. Overall, the metabolic network of the obese microbiome was less modular than that of the lean microbiome, suggesting that the diversity of the obese microbiome is relatively low.

Qin and associates developed a protocol for metagenome-wide association studies (MGWAS) based on deep shotgun sequencing of gut microbiotas and applied this protocol to compare the fecal microbial DNA of 345 Chinese Type 2 diabetic (T2D) patients and healthy controls (Qin et al., 2012). The abundance of 52,484 microbial genes and 6957 genetic orthologues were different between T2D patients and healthy individuals. Somewhat surprisingly, these differences accounted for only 3.8 ± 0.2% of total microbial gene abundance in individuals, indicating that T2D-associated dysbiosis was comparatively modest. As mentioned above (Section 5.1.3), the T2D gut microbiome harbored lower levels of butyrate-producing bacteria (e.g., *F. prausnitzii*, *E. rectale*, and *R. intestinalis*, etc.) and higher levels of opportunistic pathogens (e.g., *C. hathewayi*, *C. ramosum*, *E. lenta*, etc.). According to the differential orthologue data, T2D associated microbial functions were enriched for membrane transportation of sugars and branched-chain amino acids, methane metabolism, xenobiotics degradation and metabolism, sulfate reduction, and oxidative stress resistance, but were decreased for bacterial functions such as chemotaxis, flagellar assembly, butyrate biosynthesis, and metabolism of cofactors and vitamins. Quantification of the differential gene markers in an additional 11 T2D patients and 12 non-diabetic controls showed these markers could be used to identify individuals with high risk for T2D. Although this study needs to be confirmed in different study populations, the results

suggest that certain metagenomic markers can be used to classify type T2D patients, and that the dysbioses of obesity and T2D share salient features.

5.3. Metabolic activity changes in the gut microbiota of obese individuals

Consistent with metagenomic data suggesting that obese gut microbiota has a greater capacity to extract energy from food, energy loss (i.e., fecal energy content) was reduced and the amounts of cecal SCFAs available to the host were increased in obese (*ob/ob) vs* lean *(ob/+)* mice (Turnbaugh et al., 2006). However, a subsequent study (Murphy et al., 2010) reported reduced fecal energy and increased cecal SCFAs only in 7-week-old *ob/ob* mice, not in 11- or 15-week-old *ob/ob* mice or in mice made obese by HFD. Consistent with this result, fecal acetate diminished between ages 7- to 15-weeks in both *ob/ob* mice and HFD-fed mice. These observations demonstrate the potentially dynamic role(s) of the gut microbiota during murine development and suggest that energy harvest by the gut microbiota and its contribution to host obesity in the mouse may be more important during development and less so after maturation (Murphy et al., 2010).

Analysis of SCFA production in humans also suggests that the fermentation activity of the gut microbiota is increased in obesity (reviewed in Conterno et al., 2011). Schwiertz and collaborators (Schwiertz et al., 2010) compared the amounts of fecal SCFAs in lean (BMI = 18.5–24.9 kg/m^2, $n = 30$), overweight (BMI = 25–30 kg/m^2, $n = 35$) and obese (BMI > 30 kg/m^2, $n = 33$) human volunteers. The total concentration of SCFAs was significantly greater in obese (103.9 ± 34.3 mmol/l) and overweight subjects (98.7 ± 33.9 mmol/l) than in lean subjects (84.6 ± 22.9 mmol/l). These results were interpreted as reflecting greater SCFA production in obese and overweight individuals. Among SCFAs, propionate was preferentially increased from 15.9% in lean volunteers to 18.7% in overweight ($P = 0.02$) and 18.3% in obese ($P = 0.03$) volunteers (Schwiertz et al., 2010).

Urinary metabolite levels are influenced by differences in the intestinal microbiota, as both bacterial as well as shared host-bacterial metabolism (“co-metabolism”) generate specific metabolic products, such as hippurate (from aromatic compounds) and trimethylamine (from phosphatidylcholine) (Martin et al., 2007, 2008; Nicholson et al., 2005; Wang et al., 2011). Thus, metabolic shifts of the gut microbiota in response to obesity are indirectly reflected in changes of urine metabolites. For example, Calvani and colleagues (Calvani et al., 2010) reported that the urine of morbidly obese subjects contains lower concentrations of hippurate and trigonelline and higher levels of 2-hydroxyisobutyrate than the urine of normal weight individuals. However, following bariatric (RYGB) surgery, the urine concentrations of hippurate, trigonelline and 2-hydroxyisobutyrate in these individuals approximated those of lean subjects (Calvani et al., 2010). The urine of obese, leptin receptor-deficient (*fa/fa*) Zucker rats contains lower concentrations of hippurate and trimethylamine N-oxide (TMAO) and higher levels of acetate than the urine of lean wild-type rats (Waldram et al., 2009).

5.4. Changes in gut microbial composition: the cause or effect of obesity?

The argument has been made that obesity-associated changes in gut microbiota composition are not a cause of obesity *per se*, but merely reflect altered diet and/or metabolism (Fleissner et al., 2010). Resistin-like molecule β (RELMbeta) is a goblet cell-specific, cysteine-rich peptide previously shown to function as part of the mucosal innate immune response (Barnes et al., 2007). Unlike wild-type mice which become obese on HFD, RELMbeta KO mice remain comparatively lean on HFD. Importantly, similar HFD-induced changes in gut bacterial composition were noted for wild-type and RELMbeta KO mice (Hildebrandt et al., 2009), suggesting that diet, rather than the obese phenotype, plays a dominant role in shaping the bacterial composition of the gut microbiota.

To assess the contributions of altered gut microbiota and inflammation to diet-induced obesity (DIO), de La Serre and colleagues (de La Serre et al., 2010) compared intestinal inflammation and cecal microbial composition in three treatment groups of Sprague–Dawley rats: (1) rats prone (P) to develop DIO when fed a HFD (DIO-P), (2) rats resistant (R) to DIO on a HFD diet (DIO-R), and (3) rats fed a low-fat diet. Compared with the two groups of lean rats, DIO-P rats showed ileal epithelial inflammation, decreased intestinal alkaline phosphatase (IAP, a luminal enzyme that detoxifies LPS) activity, increased gut TLR4 activation, increased gut permeability and elevated plasma LPS. HFD significantly decreased the total cecal bacterial number and increased the relative proportion of Bacteroidales and Clostridiales in both DIO-P (obese) and DIO-R (non-obese) rats, suggesting that these changes in cecal bacteria result from HFD but are not associated with obesity. However, an increase in Enterobacteriales was observed in the microbiota of DIO-P rats only, indicating the association between this LPS-producing bacterial order and the development of obesity in obesity-prone SD rats. Based on these data, the authors propose that obesity is caused by HFD-induced changes in the gut microbiota that increase LPS levels and inflammation in the gut lumen (de La Serre et al., 2010).

Recent studies from the Zhao lab confirm and extend this important conclusion to humans (Fei and Zhao, in press). In these studies, an *Enterobacter* species isolated from the gut of a morbidly obese subject induced systemic endotoxemia, aggravated inflammation, obesity and insulin resistance in HFD-fed GF mice, which are normally resistant to HFD (see Section 3.1). This result supports the concept that overgrowth of endotoxin producers in the gut is a contributing factor to, rather than a consequence of, obesity and insulin resistance.

The causative role of gut microbiota in the insulin resistance of obese humans has been elegantly demonstrated by gut microbiota transfer from lean, healthy individuals to obese, insulin resistant individuals (Vrieze et al., 2012). After removing the endogenous microbiota of obese, insulin resistant male adults by bowel lavage, researchers assigned the volunteers to

either an allogenic infusion group, who were infused via the small intestine with the fecal microbiota from age- and sex-matched lean healthy individuals, or to an autologous infusion group, who were re-infused with their own gut microbiota. The allogenic infusion significantly increased the abundance of 16 fecal bacterial populations, including the butyrate-producer *R. intestinalis*, and altered the abundance of seven intestinal mucosal bacterial groups, including increases in the butyrate-producer *Eubacterium hallii*. Moreover, whereas the insulin resistance of those receiving autlogous infusion was unchanged, recipients of allogenic infusions experienced significant increases in peripheral insulin sensitivity and a tendency for improved hepatic insulin sensitivity.

In summary, obesity dose change the microbial composition of the gut microbiota, and these gut microbial alterations play an etiological role in obesity and insulin resistance, but now there is no consensus on the specific pattern(s) of gut bacteria that are involved in the etiology and thus can be a biomarker/therapeutic target for obesity due to the inconsistent and even controversial results of different studies. Additional clinical and preclinical studies are needed to illustrate the definite role of specific bacteria species/groups in obesity and insulin resistance.

6. Probiotics and prebiotics as obesity/metabolic therapies

6.1. Probiotics

The term, ‘‘probiotic’’ means ‘‘for life.’’ The Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) define probiotics as ‘‘live microorganisms which when administered in adequate amounts, confer a beneficial health effect on the host.’’ Select probiotic strains , in particular those of genera *Lactobacillus* and *Bifidobacterium* have been shown to ameliorate obesity, inflammation and associated metabolic complications through several mechanisms, including inhibition of pathogen adhesion to gut mucosa, ‘stabilization’ of microbial community structure, and through improvements in mucosal integrity and barrier function compromised by disease, injury, or stress (Amar et al., 2011a; Cani et al., 2007b; Ewaschuk et al., 2007; reviewed in Fioramonti et al., 2003; Guarner, 2007; Moreira et al., 2012). As discussed in Section 4.3.1.1, these improvements in mucosal integrity reflect the actions of SCFA products of bacterial fermentation. However, direct beneficial actions of *Lactobacilli* on epithelial cells and on the enteric nervous system regulating gut contractility have also been reported, as recently reviewed (Lakhan and Kirchgessner, 2011).

Rodent models have provided robust *in vivo* platforms for identifying human gut commensals that could be developed as probiotic therapies. The ability of *Bifidobacteria* to enhance gut barrier function was demonstrated *in vivo* by Griffiths and colleagues (Griffiths et al., 2004), who supplemented newborn Balb/c mice with *Bifidobacterium infantis* and *B. bifidum* for up to 28 days. Mice receiving probiotics had significantly reduced intestinal endotoxin as compared with mice that received vehicle alone. With respect to obesity, 8-week feeding of *Lactobacillus rhamnosus* PL60 to mice made obese by diet reduced body weight and white adipose tissue (epididymal and perirenal) without reducing energy intake (Lee et al., 2006). Similarly, oral supplementation with VSL#3 (a commercial probiotic mixture of three strains of *Bifidobacterium* and 4 strains of *Lactobacillus*) improved HFD-induced steatosis and insulin resistance in mice coincident with salutory effects on immunity (Ma et al., 2008).

Yin and colleagues (Yin et al., 2010) studied the effects of four different *Bifidobacterium* strains isolated from feces of healthy humans on HFD-induced obesity in rats. Compared with rats fed on HFD only, one strain decreased body weight, one strain increased body weight, and two strains had no significant effect on body weight, suggesting that the anti-obesity effects of *Bifidobacterium* are likely to be strain-specific. Intriguingly, however, all four strains were able to lower serum and liver triglyceride and alleviate lipid deposition in liver (Yin et al., 2010).

More recent studies (An et al., 2011) evaluated the effects of a combination of three species of *Bifidobacterium* isolated from the gut of healthy Koreans on obese, HFD-fed rats. The three strains were *B. pseudocatenulatum* SPM 1204, *B. longum* SPM 1205, and *B. longum* SPM 1207. After 7 weeks, rats receiving the HFD supplemented with the human bifidobacterial strains had reduced body and fat accumulation, coincident with improvements in lipid profiles and glucose-insulin homeostasis as compared to rats receiving HFD alone. The mechanism(s) underlying these improvements were not elucidated. These studies suggest that probiotic strains derived from the healthy human gut may prove effective in weight management and in metabolic therapies. Ironically, probiotics are used extensively to promote weight gain in livestock (including one of the four strains studied by Yin et al. (2010) and discussed above), leading some researchers to propose that the extensive consumption of foods containing probiotic bacteria by humans may be linked to the current obesity pandemic (Raoult, 2008).

Well-controlled studies in humans are less available, but suggested the potential for probiotic therapy in weight management. In a double-blind, randomized, placebo-controlled intervention trial 43 overweight human subjects (BMI, 24.2–30.7 kg/m^2) experienced significantly decreased abdominal visceral and subcutaneous fat area, body weight, and waist circumference following 12 weeks of consuming 200 g/day of fermented milk containing probiotic strain *Lactobacillus gasseri* SBT2055 (LG2055) as compared to fermented milk alone (Kadooka et al., 2010). The continuous perinatal intake of probiotic strain *L. rhamnosus* GG by mothers from 4 weeks prior to scheduled delivery to postnatal month 6 was associated with the prevention of excessive weight gain of their children during a 10 year follow-up (Luoto et al., 2010).

6.2. Prebiotics

Prebiotics are non-digestible polysaccharides that promote SCFA production and the growth of beneficial gut bacteria, especially *Bifidobacterium* and *Lactobacillus* (reviewed in Roberfroid et al., 2010). Studies in healthy humans and rodents demonstrate that prebiotic consumption reduces hunger and enhances satiety (Cani et al., 2009a; Parnell and Reimer, 2009), and as discussed in Section 3.3.1, this modulation of ingestive behavior is mediated in part by SCFA-induced changes in gut peptide secretion. In addition, by promoting *Bifidobacterium* populations, prebiotics promote gut barrier function (Cani et al., 2007b, 2009b), as discussed above.

The relevance of prebiotics to the management of obesity in humans is supported by only a few intervention studies. Increased satiety and dramatically decreased body weight, waist circumference and BMI were achieved in obese, pre-menopausal women (BMI > 30 kg/m^2) who consumed oligofructose-rich syrup of the Andean tuber Yacon (*Polymnia sanchifolia*) *vs* placebo (Genta et al., 2009). Participants receiving the fructan-rich syrup lost an average of 15 kg during the study, coincident with significant reductions in fasting insulin (~50%) and LDL–cholesterol (~30%). More recently, prebiotics (oligofructose) or placebo (maltodextrin) were administered to 48 overweight or obese adults (BMI > 25 kg/m^2) at 21 g per day for 12 weeks in a randomized, double-blind, placebo-controlled trial (Parnell and Reimer, 2009). Compared to placebo, prebiotic intake reduced body weight by 1.0 ± 0.4 kg, coincident with decreased secretion of ghrelin, increased circulating PYY levels, reduced calorie intake, and lowered plasma glucose and insulin.

7. Concluding remarks

Driven by advances in high throughput sequencing, computational power and bioinformatics, the impact of gut microbiota on obesity and metabolic health has emerged as an important focus of biomedical research. We have presented evidence that the gut microbiota is intricately involved with the host (us!) in regulating or dysregulating energy intake and metabolism, obesity-associated inflammation and glucose-insulin homeostasis. Thus, investigations of the gut microbiota are vital to understanding and intervening in the current obesity epidemic. Many unanswered questions remain. For example, given the substantial inter-individual differences in microbiota composition, can we identify the core features of a healthy and stable microbiota? What compositional or functional changes in the gut microbiota are most relevant to the onset and progression of obesity and its complications? What features of host–microbiota cross-talk are critical for maintaining a stable microbiota and gut homeostasis, and how can these be manipulated to restore equilibrium under conditions of dysbiosis?

Germ-free animal models, in particular those that are conventionalized with human gut microbes, provide a powerful preclinical platform for investigating these questions and for assessing the role of specific gut bacteria species/groups and their metabolites in obesity and its sequelae. As discussed in Sections 5.4 and 6.1, respectively, this approach has already identified a human gut *Enterobacter* species that promotes obesity in GF mice in addition to human bifidobacterial strains that protect mice against obesity and metabolic complications. The identification and/or engineering of therapeutic bacterial strains (“bacteriotherapy”) holds significant translational promise, given the recent demonstration of improved glucose–insulin homeostasis in obese subjects with metabolic syndrome following microbiota infusions from lean individuals (Vrieze et al., 2012). Manipulation of the gut microbiota with diet, such as with prebiotics and synbiotics (combining prebiotics and probiotics), provides an additional, readily-translatable approach to improve metabolic health.

In closing, we point out that elucidating the mechanisms and health implications of our molecular, cell biological and metabolic co-evolution with the gut microbiota presents significant challenges. Multiple and diverse competencies and investigative platforms are needed to meet these challenges. Thus, integrated collaborations among microbiologists, endocrinologists, nutritionists and cell, molecular, computational and systems biologists will be required at both the preclinical and clinical level. This is an exciting and rich area of investigation that will continue to generate fundamental discoveries in gastroenterology, molecular endocrinology, immunobiology and microbiology. These discoveries will drive the development of new preventive and therapeutic strategies for advancing human cardiometabolic health.

8. Conflict of interest

None.

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