Prebiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome

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Abstract

Prebiotic fibers are non-digestible carbohydrates that promote the growth of beneficial bacteria in the gut. Prebiotic consumption may benefit obesity and associated co-morbidities by improving or normalizing the dysbiosis of the gut microbiota. We evaluated the dose response to a prebiotic diet on the gut microbiota, body composition and obesity associated risk factors in lean and genetically obese rats. Prebiotic fibers increased Firmicutes and decreased Bacteroidetes, a profile often associated with a leaner phenotype. Bifidobacteria and Lactobacillus numbers also increased. Changes in the gut microbiota correlated with energy intake, glucose, insulin, satiety hormones, and hepatic cholesterol and triglyceride accumulation. Here we provide a comprehensive analysis evaluating the results through the lens of the gut microbiota. Salient, new developments impacting the interpretation and significance of our data are discussed. We propose that prebiotic fibers have promise as a safe and cost-effective means of modulating the gut microbiota to promote improved host:bacterial interactions in obesity and insulin resistance. Human clinical trials should be undertaken to confirm these effects.

Keywords

gut microbiota; prebiotic fibers; obesity; lipid metabolism; glucoregulation

Introduction

The human gastrointestinal (GI) tract contains an estimated 10^{14} microbial cells, primarily inhabiting the colon, and representing over 1,000 bacterial types.[1] Historically, the profile of the gut microbiota has been highly scrutinized, as it is intricately related to physical wellbeing; with some strains promoting health and others disease. In addition to its role in establishing and maintaining normal intestinal health, the gut microbiota has been found to improve or exacerbate a myriad of diseases ranging from colorectal cancer to autoimmune and allergic diseases.[2] Certain gut microbes have been found to be protective, for example,

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the higher levels of *Faecalibacterium prausnitzii* found in healthy subjects compared with patients with colitis are important given the protective effect of *F. prausnitzii* on gut mucosa.[3] Additionally, colonization of germ free mice with *Bacteroides fragilis* produces polysaccharide A (PSA), which positively modulates the host's T-cell dependent immune response.[4] Recently, however, the role of the gut microbiota in the development of obesity and its associated co-morbidities has come to the forefront. Dysbiosis of the gut microbiota is present in obesity with a reduction in Bacteroidetes and an increase in Firmicutes typically reported[5,6] although not consistently.[7,8] Ley et al.[6] also report that weight loss increases the proportion of Bacteroidetes relative to the Firmicutes. Within the Archaea, obesity is associated with a decrease in *Methanobrevibacter smithii*.[9] Whether the gut microbiota promotes obesity or the changes occur as a result of obesity requires further research. A prospective study in children found that Bifidobacterium spp numbers were higher and *Staphylococcus aureus* were lower in children that were normal weight at the outset of 7 y,[10] providing some support for the former hypothesis. Conversely, a high-fat, Western diet has been found to increase Firmicutes and decrease Bacteroidetes in the absence of weight gain.[11] It is entirely possible that both the obese phenotype and diet affect the gut microbiota. Furthermore, it has been proposed that changes at the microbial community level impact obesity, however, less data are available on these groups and this would be a fruitful area for future exploration.

The role of the gut microbiota, as it relates to obesity development, has not been fully defined; however, is evidenced by the elegant experiments of Turnbaugh et al.[12] who demonstrated colonization of gnotobiotic mice with microbiota extracted from obese animals resulted in increased fat mass compared with those colonized with microbiota from lean animals. It has been suggested that the gut microbiota from, an obese phenotype, extract the energy from foods more efficiently, resulting in increased adiposity. The energy extraction hypothesis is based on comparisons between germ free and conventionalized mice. Here the gut microbiota increased monosaccharide uptake in the gut resulting in increased production of short chain fatty acids (SCFA). One of the chief SCFA is acetate, which has been found to stimulate hepatic de novo lipogenesis, and consequently increase adipocyte fatty acid storage.[13] SCFA may also promote fat storage by acting as signaling molecules for the G-protein coupled receptors (GPR) 41 and 43. GPR43 is expressed in intestine, adipocytes, and immune cells and its deficiency protects mice from high fat diet-induced obesity.[14,15] Furthermore, germ free GPR41 knockout mice are leaner and weigh less than their wild type littermates, but only when colonized with a model fermentative community composed of *B. thetaiotaomicron* and *M. smithii*.[16] The gut microbiota has also been proposed to increase adipocyte fatty acid storage through the suppression of intestinal fasting-induced adipose factor (FIAF). Suppression of FIAF in the gut epithelium increases lipoprotein lipase (LPL) activity which, in turn, promotes triglyceride storage in adipocytes.[17] Conversely, a hypothesis has been proposed whereby the gut microbiota suppresses AMP-activated protein kinase (AMPK) and consequently fatty acid oxidation resulting in increased adiposity.[18] It should be noted that these two mechanisms are not mutually exclusive.

The gut microbiota is highly active and produces many metabolic byproducts resulting in both local and systemic effects. Research into the role of these metabolic byproducts on obesity and the associated inflammation is still in its infancy; however, it has been shown that lipopolysaccharide (LPS) levels negatively correlate with bifidobacteria numbers.[19] LPS is secreted by gram negative bacteria and has been found to increase levels of tumor necrosis factor (TNF-), a potent inflammatory cytokine linked to obesity and type 2 diabetes. This has been dubbed metabolic endotoxemia.[19] Importantly, the relationship between bifidobacteria and reduced LPS levels requires additional investigation to determine the specific mechanism(s) involved, however, it may be linked to improvements

in gut mucosal barrier functions.[19] On the other hand, antibiotic induced changes in the gut microbiota reduced metabolic endotoxemia in genetically obese and diet-induced, obese mice.[20]

Bacterial colonization begins at, or possibly before birth,[21] and can be influenced throughout the lifespan by a variety of factors, of which diet is a key contributor.[22] Prebiotic fibers have been defined by Roberfroid as "non-digestible food ingredients that benefit the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon."[23] The full effects of prebiotic fibers on the microbiota have yet to be determined; however, increases in lactobacilli and bifidobacteria are consistently reported.[24,25] Increases in Bifidobacterium are relevant to obesity, as high fat feeding reduced Bifidobacterium spp, and Eubacterium rectal-*Clostridium coccoides* groups in mice.[26]

Given the abnormalities associated with the obese gut microbiota and the ability of prebiotic fibers to alter its composition, we set out to examine the effects of increasing doses of prebiotic fiber, as compared with a control diet, on the gut microbiota and physiological parameters related to obesity. This is important given a growing need to enhance our understanding of how dietary choices can positively modify the gut microbiota and improve weight loss outcomes. Considering the complexity of achieving and maintaining weight loss, adding another tool to the arsenal is crucial.

Modulation of the Gut Microbiota by Prebiotic Fibers

Our article, published in the *British Journal of Nutrition*,[27] identifies several changes in the gut microbiota, of animals exposed to prebiotic fibers, and links these changes to alterations in physiology. We designed three diets: control 0% prebiotic, 10% prebiotic, and 20% prebiotic fiber by weight. In humans, doses greater than 30 g/day, which equates to an approximate 5% dose by weight, produce unfavorable gastrointestinal symptoms.[28,29] The majority of animal studies have evaluated a 10% dose by weight. By examining a range from 0–20% in our study, our goal was to determine dose-response effects and whether there exists a threshold above which no further benefits are identified. All diets provided an equal contribution of protein and fat to the total energy value. Subsequently, lean and genetically obese rats were exposed to the diets for 10 weeks. Cecal samples were obtained from the rats, total bacterial DNA was extracted and total bacteria, Bacteroides/Prevotella, *Clostridium coccoides*, *Clostridium leptum*, Lactobacillus, Bifidobacterium, and Enterobacteriaceae were quantified by qPCR.

This work supports previous reports of decreased numbers of Bacteroides and increased Firmicutes with obesity.[6] However, the Firmicutes phylum includes at least 250 genera and the Bacteroidetes at least 20; therefore, the impact of obesity at the division level is of interest[30] and requires further exploration. To this end, Million et al.[9] found lower levels of *B. animalis* in obesity and alterations in various strains of Lactobacillus compared with lean. Here we report that the presence of obesity, without any dietary intervention, reduced total bacteria, *Clostridium leptum* and Enterobacteriaceae numbers as compared with the lean animals. Others work supports reduced *Clostridum leptum* in humans with obesity.[8] The implications of reduced Enterobacteriaceae in obesity remain to be elucidated; however, these results are in opposition to others that found increased Enterobacteriaceae and *Escherichia coli* in overweight, pregnant women compared with normal weight, pregnant women.[31] The significance of these results to non-pregnant populations, however, may be limited.

Correlational relationships between body weight and body fat, as determined by dual energy X-ray absorptiometry (DXA), revealed Bacteriodes and total bacterial were negatively correlated with percent body fat and body weight; whereas, levels of Lactobacillus were

positively correlated with body weight and fat. Other accounts of increased Lactobacillus with obesity,[9] however, the genus Lactobacillus contains many different species with potentially differing effects. A recent study looked at seven different strains of Lactobacillus, in the lean and obese state. *L. paracasei* and *L. plantarum* were associated with a lean phenotype whereas *L. reuteri* was increased in obesity.[9] The implications of increased Lacto-bacillus are unclear, however, given that probiotic administration of *Lactobacilli gaseri* SBT2055 has been found to decrease visceral and subcutaneous fat mass in overweight adults (BMI 24.2–30.7 kg/m^2).[32] On the other hand, it has been suggested that *L. reuteri* can enhance nutrient absorption and processing by the intestine.[9,33] *L. reuteri* will also produce reuterin in the presence of glycerol which could impact obesity and inflammation given reuterins' antimicrobial properties and potential for modifying gut microbiota.[34] As evidenced by the Million et al.[9] study, the effects likely depend on the specific species involved.

In our experiment, we were also able to evaluate how the obese microbiota responds to a dietary intervention compared with the response by lean microbiota. The lean and obese animals in the 10% prebiotic fiber group, had lower levels of total bacteria, however, *Clostridium leptum* and Enterobacteriaceae numbers normalized, with no difference between the lean and obese groups. With the 20% prebiotic fiber group, levels of *Clostridium coccoides*, *C. leptum*, Lactobacillus, Bifidobacterium, and Enterobacteriaceae were all increased with obesity compared with the lean animals. Limited data are available regarding the Clostridium group in obesity. One study found a trend (p = 0.088) toward increased *C. coccoides* in obese pregnant women compared with lean[31] and another study showed no change with overweight or obesity.[8] *Faecalibacterium prausnitzii,* of the class Clostridia and member of the C. leptum cluster, were also found to be increased in obese children living in south India.[35] Importantly, however, in the absence of the dietary intervention we found reduced levels of *Clostridium leptum* and Enterobacteriaceae numbers. This discrepancy highlights the importance of the interaction between diet and physiology and the complexity of the gut microbiota. Others also report increased Bifidobacterium levels with prebiotic fibers in obesity.[19] Increases in Bifidobacterium are noteworthy as levels have been reported to be low in obesity[8] and bifidobacteria are associated with health improvements due to a reduction in inflammatory cytokines.[19] It is likely that changes at the smaller community level have a significant impact on disease progression and this remains relatively unexplored. With respect to our results, it is interesting to report that the response to dietary interventions is altered by the host physiology (i.e., genetically lean or obese).

When we evaluate the effects of diet independently of genetic grouping, supplementation with prebiotic fiber decreased the Firmicutes equally in the 10% and 20% dose; however, this pattern was reversed in the Bacteroidetes groups. Recently, Everard et al.[36] conducted a comprehensive evaluation of the effects of prebiotic fibers on the gut microbiota, in genetically obese and diet-induced, leptin-resistant mice. They support our results with increased Bacteroidetes and decreased Firmicutes. It is important to note that although the lean and obese animals were exposed to the same diets, the amount of food consumed by the obese animals was significantly greater than the lean animals. For example, the average food intake, over the ten weeks, for the obese 20% group was 1.82 kg of which 356 g was prebiotic fiber; whereas, in the lean 20% group the average food intake was 1.08 kg of which 210 g was prebiotic fiber. Thus, an obese animal on a 20% prebiotic diet is consuming a greater absolute amount of fiber because of increased overall food consumption. This makes it difficult to distinguish if the effects are related to the dose or the disease state or a combination of the two. A further limitation is that the energy values of the prebiotic fiber diets are lower due to the lower energy value of the fiber as compared with the cornstarch.

Overall, these results support an aberrant gut microbial profile in obesity and improvements with prebiotic fiber supplementation. The effects of prebiotic fibers were significantly altered by both the prebiotic dose and the disease state. This suggests the lean and obese physiology will respond differently to treatments designed to modulate the gut microbiota and this should be considered in future research and clinical practice. Furthermore, the effective dose and the feasibility of using prebiotic fibers as a treatment should be determined via human clinical trials.

Gut Microbiota and Enteroendocrine Hormone Secretion

The gut is an active enteroendocrine organ that secretes several hormones in response to food stimulus or lack of food. The foods eaten can alter the expression of these hormones which, in turn, act on the brain to signal hunger or satiety. To evaluate the effects of prebiotic fibers on satiety hormones, we provided the animals with 5 g of their diet and subsequently measured plasma levels of satiety hormones over 90 min. Glucagon-like peptide-1 (GLP-1) is an anorexigenic, appetite suppressing hormone, proposed to reduce GI transit time by acting as an ileal break.[37] Furthermore, GLP-1 stimulates insulin secretion from the pancreatic cells and inhibits glucagon secretion.[38] In our study, postprandial GLP-1 levels increased with the 20% prebiotic fiber diet. Given that we also measured select gut bacteria, we were interested to determine if changes in satiety hormones could be linked to alterations in the gut microbiota. Of note, Enterobacteriaceae increased in conjunction with GLP-1 total area under the curve (tAUC). Further research into the relationship between satiety hormones and the gut microbiota is needed; however, it has been reported that GI transit time can alter the profile of the gut microbiota.[39–41] In support of this theory, we report both dysbiosis of the gut microbiota and increased gastric emptying in the obese rats as compared with the lean rats. We did not find a significant reduction in GI transit time with prebiotic supplementation, however, our sample size was small (n = 6). We do report increased GLP-1 and an upregulation of peptide YY (PYY) gene expression with prebiotic supplementation. Given that PYY and GLP-1 slow GI transit time,[37,42] this might provide another mechanism whereby prebiotic fibers affect the composition of the gut microbiota. Prebiotic fibers also modulate the gut microbiota in a cyclic manner. When the bacteria metabolize prebiotic fibers,[43] they produce SCFA, which lowers the luminal pH. This affects the composition of the gut microbiota, as not all species can thrive in this environment.[8] Additionally, the gut microbiota influences satiety hormone production. Metabolism of non-digestible carbohydrates by the gut bacteria results in the production of SCFA, which have been shown to upregulate gene expression of proglucagon, the precursor to GLP-1,[44,45] and PYY[43] in the intestinal tract.

Ghrelin is an orexigenic hormone that stimulates appetite.[46] Here we found decreased plasma levels of des-acyl ghrelin with prebiotic fiber supplementation in the lean group. Ghrelin levels in the obese group did not change, however, others note that ghrelin levels are reduced in obesity and the meal response is attenuated, both of which we also observed.[47,48] In our study, Bacteroides and total bacteria were positively correlated with ghrelin tAUC. The interplay between gut endocrine functions and the microbiota and their metabolic byproducts requires further investigation.

We also assessed the gene expression of select hormones throughout the GI tract. Proglucagon is the precursor for GLP-1, discussed above, but also for glucagon-like peptide-2 (GLP-2). In this experiment, proglucagon levels increased with prebiotic supplementation. GLP-2 is a gut trophic hormone, involved in maintaining the gut epithelial barrier through the tight-junction integrity.[49,50] In mice, a high fat diet resulted in increased gut permeability and disruption of tight-junction proteins. This was associated with an increase in metabolic endotoxemia.[20,50] Prebiotic treatment, on the other hand, improved the

gut barrier and reduced LPS levels.[50] Prebiotic-stimulated GLP-2 secretion may improve host health by improving the gut barrier and allowing for tighter regulation of the transport of bacterial metabolic byproducts; thereby, reducing the systemic inflammation associated with obesity and related metabolic disorders.[2,50] This remains to be demonstrated in humans, however, and a study by Brignardello et al.[51] reports no differences in intestinal permeability in obese vs. lean individuals.

Gut Microbiota and Glycemic Regulation

Aberrant microbiota has also been linked to type 2 diabetes, with reduced numbers of the Firmicutes, Clostridia, *Bacteroides vulgates,* and Bifidobacteria in adults with type 2 diabetes.[2,52,53] Furthermore, the ratio of Bacteroidetes to Firmicutes is positively correlated with plasma glucose concentrations.[53] In support of gut microbial dysbiosis in type 2 diabetes development, we found Bacteroides and total bacteria were negatively correlated with fasting insulin and insulin incremental area under the curve (iAUC); whereas, Enterobacteriaceae increased in conjunction with glucose iAUC. Others report the ratios of Bacteroides/Prevotella to *C. coccoides–E. rectal* are positively correlated with plasma glucose concentrations.[53] The results of the few human clinical trials involving prebiotic fibers or probiotics on patients with overweight or diabetes mellitus has recently been reviewed by Delzenne et al.[54] The role of prebiotic fibers in glucoregulation is uncertain, as improvements, in humans, are not consistently reported[54] and the role of the gut microbiota is unknown, as these studies did not measure the profile of the gut microbiota. Several animal studies report improvements in glycemic regulation with prebiotic supplementation,[55,56] however, no changes in glucose or insulin iAUC were noted with prebiotic supplementation in our animals. Conversely, *Lactobacillus acidophilus* NCFM administration improved insulin sensitivity in a subset of patients with type 2 diabetes, impaired glucose tolerance, and normal glucose tolerance as compared with a placebo.[57] This data suggests modification of the gut microbial profile could reduce the risk of type 2 diabetes. The discrepancy in outcomes with prebiotic fiber supplementation between human and animal studies may be a result of dosage, as human studies typically administer a lower dose due to gastrointestinal discomfort experienced by patients with higher dosages. Differences between pre and probiotic effects are likely due to the differences in bacterial strains. The effects of prebiotics are broader than supplementation with a single probiotic and would be affected by the host's gut microbiota.

Gut Microbiota and Lipid Metabolism

The experiments to assess the effects of prebiotic fibers on obesity and associated risk factors were published in two separate papers. The second paper focused on the effects of prebiotic fibers on lipid metabolism.[58] Given that the same animals were used for both experiments we can report in this addendum the interactions between the gut microbiota and lipid metabolism. The gut microbiota stimulate monosaccharide uptake and transfer to the liver resulting in subsequent stimulation of de novo lipogenesis.[17] Furthermore, the gut microbiota may affect hepatic fatty acid storage through changes to bile acids and SCFA production.[59] In our study, Bacteroides numbers were negatively correlated with cecal triglyceride (TG), liver total cholesterol (TC), serum TC, and serum TG. Lactobacillus positively correlated with liver TC and serum TG and *C. coccoides* with liver TC whereas *C. leptum* was positively correlated with liver TC. Enterobacteriaceae was negatively correlated with serum TG. The role of the gut microbiota in response to liver disease has recently been reviewed.[60] There is significant evidence that the endotoxins produce by microbial metabolism play a role in non-alcoholic fatty liver diseases (NAFLD) as plasma endotoxin levels are elevated in NAFLD, a phenomenon linked to intestinal overgrowth and induction of toll-like receptor 4 (TLR4) in the hepatocytes.[61,62] The gut microbiota has been

clearly linked to NAFLD, however, dietary modulation of the gut microbiota, via prebiotics, for the treatment of NAFLD remains an untested possibility.

Conclusion

Dysbiosis of the gut microbiota is present in a number of metabolic disorders including obesity, type 2 diabetes, and NAFLD. Prebiotic fibers have the ability to alter the gut microbiota in a positive manner, indicating their promise as a dietary treatment. Several animal studies, including our recent publications, have been undertaken and provide promising results. Human clinical trials are now necessary to determine if these benefits are translatable. One major limitation to prebiotic fiber usage in humans is the effective dose. The current dose of 10% used in the majority of animal studies is not feasible in humans due to increased GI side-effects. Future research should evaluate the physiological benefits of prebiotic fibers, in human patients, with a variety of diseases associated with the metabolic syndrome and their relationship to the gut microbiota.

Acknowledgments

Abbreviations

AMPK	AMP-activated protein kinase
DXA	dual energy X-ray absorptiometry
FIAF	fasting-induced adipose factor
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
GLP-2	glucagon-like peptide-2
GPR 41	G-protein coupled receptor 41
GPR43	G-protein coupled receptor 43
iAUC	incremental area under the curve
LPL	lipoprotein lipase
LPS	lipopolysaccharide
NAFLD	non-alcoholic fatty liver disease
PYY	peptide YY
SCFA	short chain fatty acids
TLR4	Toll-like receptor 4
tAUC	total area under the curve
TC	total cholesterol
TG	triglyceride
TNF	tumor necrosis factor

References

1. Wallace TC, Guarner F, Madsen K, Cabana MD, Gibson G, Hentges E, et al. Human gut microbiota and its relationship to health and disease. Nutr Rev. 2011; 69:392–403. http://dx.doi.org/10.1111/j. 1753-4887.2011.00402.x. [PubMed: 21729093]

2. Prakash S, Rodes L, Coussa-Charley M, Tomaro-Duchesneau C. Gut microbiota: next frontier in understanding human health and development of biotherapeutics. Biologics. 2011; 5:71–86. [PubMed: 21847343]

3. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of Faecali-bacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis. 2009; 15:1183–9. http:// dx.doi.org/10.1002/ibd.20903. [PubMed: 19235886]

4. Troy EB, Kasper DL. Beneficial effects of Bacteroides fragilis polysaccharides on the immune system. Front Biosci. 2010; 15:25–34. http://dx.doi.org/10.2741/3603.

5. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005; 102:11070–5. http://dx.doi.org/10.1073/pnas. 0504978102. [PubMed: 16033867]

6. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444:1022–3. http://dx.doi.org/10.1038/4441022a. [PubMed: 17183309]

7. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, et al. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond). 2008; 32:1720–4. http:// dx.doi.org/10.1038/ijo.2008.155. [PubMed: 18779823]

8. Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, et al. Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring). 2010; 18:190–5. http://dx.doi.org/ 10.1038/oby.2009.167. [PubMed: 19498350]

9. Million, M.; Maraninchi, M.; Henry, M.; Armougom, F.; Richet, H.; Carrieri, P., et al. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. Int J Obes (Lond). 2011. In press. http://dx.doi.org/ 10.1038/ijo.2011.153

10. Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr. 2008; 87:534–8. [PubMed: 18326589]

11. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology. 2009; 137:1716–24. e1–2. http://dx.doi.org/10.1053/j.gastro.2009.08.042. [PubMed: 19706296]

12. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444:1027–31. http:// dx.doi.org/10.1038/nature05414. [PubMed: 17183312]

13. Delzenne NM, Williams CM. Prebiotics and lipid metabolism. Curr Opin Lipidol. 2002; 13:61–7. http://dx.doi.org/10.1097/00041433-200202000-00009. [PubMed: 11790964]

14. Bjursell M, Admyre T, Göransson M, Marley AE, Smith DM, Oscarsson J, et al. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. Am J Physiol Endocrinol Metab. 2011; 300:E211–20. http://dx.doi.org/10.1152/ajpendo. 00229.2010. [PubMed: 20959533]

15. Cani PD, Delzenne NM. The gut microbiome as therapeutic target. Pharmacol Ther. 2011; 130:202–12. http://dx.doi.org/10.1016/j.pharmthera.2011.01.012. [PubMed: 21295072]

16. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A. 2008; 105:16767–72. http://dx.doi.org/ 10.1073/pnas.0808567105. [PubMed: 18931303]

17. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004; 101:15718–23. http://dx.doi.org/10.1073/pnas.0407076101. [PubMed: 15505215]

18. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A. 2007; 104:979–84. http://dx.doi.org/10.1073/pnas.0605374104. [PubMed: 17210919]

19. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia. 2007; 50:2374–83. http://dx.doi.org/10.1007/s00125-007-0791-0. [PubMed: 17823788]

20. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008; 57:1470–81. http://dx.doi.org/10.2337/db07-1403. [PubMed: 18305141]

21. Mshvildadze M, Neu J. The infant intestinal microbiome: friend or foe? Early Hum Dev. 2010; 86 (Suppl 1):67–71. http://dx.doi.org/10.1016/j.earlhumdev.2010.01.018. [PubMed: 20116944]

22. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010; 107:14691–6. http://dx.doi.org/10.1073/pnas.1005963107. [PubMed: 20679230]

23. Roberfroid MB. Prebiotics and probiotics: are they functional foods? Am J Clin Nutr. 2000; 71(Suppl):1682S–7S. discussion 1688S–90S. [PubMed: 10837317]

24. Salvini F, Riva E, Salvatici E, Boehm G, Jelinek J, Banderali G, et al. A specific prebiotic mixture added to starting infant formula has long-lasting bifidogenic effects. J Nutr. 2011; 141:1335–9. http://dx.doi.org/10.3945/jn.110.136747. [PubMed: 21613452]

25. Costabile A, Kolida S, Klinder A, Gietl E, Bäuerlein M, Frohberg C, et al. A double-blind, placebo-controlled, cross-over study to establish the bifidogenic effect of a very-long-chain inulin extracted from globe artichoke (Cynara scolymus) in healthy human subjects. Br J Nutr. 2010; 104:1007–17. http://dx.doi.org/10.1017/S0007114510001571. [PubMed: 20591206]

26. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007; 56:1761–72. http://dx.doi.org/10.2337/db06-1491. [PubMed: 17456850]

27. Parnell, JA.; Reimer, RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats; Br J Nutr. 2011. p. 1-13.http://dx.doi.org/10.1017/S0007114511003163

28. Kaur N, Gupta AK. Applications of inulin and oligofructose in health and nutrition. J Biosci. 2002; 27:703–14. http://dx.doi.org/10.1007/BF02708379. [PubMed: 12571376]

29. Jenkins DJ, Kendall CW, Vuksan V. Inulin, oligo-fructose and intestinal function. J Nutr. 1999; 129 (Suppl):1431S–3S. [PubMed: 10395613]

30. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci U S A. 2009; 106:2365–70. http://dx.doi.org/10.1073/pnas.0812600106. [PubMed: 19164560]

31. Santacruz A, Collado MC, García-Valdés L, Segura MT, Martín-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr. 2010; 104:83–92. http://dx.doi.org/10.1017/S0007114510000176. [PubMed: 20205964]

32. Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, et al. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr. 2010; 64:636–43. http://dx.doi.org/10.1038/ejcn.2010.19. [PubMed: 20216555]

33. Casas IA, Dobrogosz WJ. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. Microb Ecol Health Dis. 2000; 12:247–85. http://dx.doi.org/10.1080/08910600050216246-1.

34. Cleusix V, Lacroix C, Vollenweider S, Le Blay G. Glycerol induces reuterin production and decreases Escherichia coli population in an in vitro model of colonic fermentation with immobilized human feces. FEMS Microbiol Ecol. 2008; 63:56–64. http://dx.doi.org/10.1111/j.1574-6941.2007.00412.x. [PubMed: 18028400]

35. Balamurugan R, George G, Kabeerdoss J, Hepsiba J, Chandragunasekaran AM, Ramakrishna BS. Quantitative differences in intestinal Faecalibacterium prausnitzii in obese Indian children. Br J Nutr. 2010; 103:335–8. http://dx.doi.org/10.1017/S0007114509992182. [PubMed: 19849869]

36. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GM, Neyrinck AM, et al. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes. 2011; 60:2775–86. http://dx.doi.org/10.2337/db11-0227. [PubMed: 21933985]

37. Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. Dig Dis Sci. 1993; 38:665–73. http://dx.doi.org/10.1007/BF01316798. [PubMed: 8462365]

38. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007; 132:2131–57. http://dx.doi.org/10.1053/j.gastro.2007.03.054. [PubMed: 17498508]

39. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, et al. The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res. 2010; 51:1101–12. http://dx.doi.org/10.1194/jlr.M002774. [PubMed: 20040631]

40. McConnell EL, Fadda HM, Basit AW. Gut instincts: explorations in intestinal physiology and drug delivery. Int J Pharm. 2008; 364:213–26. http://dx.doi.org/10.1016/j.ijpharm.2008.05.012. [PubMed: 18602774]

41. Abrams GD, Bishop JE. Effect of the normal microbial flora on gastrointestinal motility. Proc Soc Exp Biol Med. 1967; 126:301–4. [PubMed: 6066182]

42. Chelikani PK, Haver AC, Reidelberger RD. Comparison of the inhibitory effects of PYY(3-36) and PYY (1-36) on gastric emptying in rats. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R1064–70. http://dx.doi.org/10.1152/ajpregu.00376.2004. [PubMed: 15242829]

43. Karaki S, Mitsui R, Hayashi H, Kato I, Sugiya H, Iwanaga T, et al. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. Cell Tissue Res. 2006; 324:353–60. http://dx.doi.org/10.1007/s00441-005-0140-x. [PubMed: 16453106]

44. Delzenne NM, Cani PD, Daubioul C, Neyrinck AM. Impact of inulin and oligofructose on gastrointestinal peptides. Br J Nutr. 2005; 93(Suppl 1):S157–61. http://dx.doi.org/10.1079/BJN20041342. [PubMed: 15877889]

45. Tappenden KA, Drozdowski LA, Thomson AB, McBurney MI. Short-chain fatty acid-supplemented total parenteral nutrition alters intestinal structure, glucose transporter 2 (GLUT2) mRNA and protein, and proglucagon mRNA abundance in normal rats. Am J Clin Nutr. 1998; 68:118–25. [PubMed: 9665105]

46. Kojima M, Kangawa K. Ghrelin, an orexigenic signaling molecule from the gastrointestinal tract. Curr Opin Pharmacol. 2002; 2:665–8. http://dx.doi.org/10.1016/S1471-4892(02)00220-5. [PubMed: 12482728]

47. English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JPH. Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab. 2002; 87:2984–7. http://dx.doi.org/10.1210/jc.87.6.2984. [PubMed: 12050284]

48. Zwirska-Korczala K, Konturek SJ, Sodowski M, Wylezol M, Kuka D, Sowa P, et al. Basal and postprandial plasma levels of PYY, ghrelin, cholecystokinin, gastrin and insulin in women with moderate and morbid obesity and metabolic syndrome. J Physiol Pharmacol. 2007; 58(Suppl 1): 13–35. [PubMed: 17443025]

49. Drucker DJ. Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology. 2002; 122:531–44. http://dx.doi.org/10.1053/gast.2002.31068. [PubMed: 11832466]

50. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009; 58:1091–103. http://dx.doi.org/10.1136/gut.2008.165886. [PubMed: 19240062]

51. Brignardello J, Morales P, Diaz E, Romero J, Brunser O, Gotteland M. Pilot study: alterations of intestinal microbiota in obese humans are not associated with colonic inflammation or disturbances of barrier function. Aliment Pharmacol Ther. 2010; 32:1307–14. http://dx.doi.org/10.1111/j.1365-2036.2010.04475.x. [PubMed: 21050232]

52. Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, et al. Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol. 2010; 61:69–78. http://dx.doi.org/10.1007/s00284-010-9582-9. [PubMed: 20087741]

53. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One. 2010; 5:e9085. http://dx.doi.org/10.1371/journal.pone.0009085. [PubMed: 20140211]

54. Delzenne NM, Neyrinck AM, Bäckhed F, Cani PD. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat Rev Endocrinol. 2011; 7:639–46. http://dx.doi.org/10.1038/nrendo.2011.126. [PubMed: 21826100]

55. Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM. Involvement of endogenous glucagon-like peptide-1(7-36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrinol. 2005; 185:457–65. http://dx.doi.org/10.1677/joe.1.06100. [PubMed: 15930172]

56. Urías-Silvas JE, Cani PD, Delmée E, Neyrinck A, López MG, Delzenne NM. Physiological effects of dietary fructans extracted from Agave tequilana Gto. and Dasylirion spp. Br J Nutr. 2008; 99:254–61. http://dx.doi.org/10.1017/S0007114507795338. [PubMed: 17711612]

57. Andreasen AS, Larsen N, Pedersen-Skovsgaard T, Berg RM, Møller K, Svendsen KD, et al. Effects of Lactobacillus acidophilus NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. Br J Nutr. 2010; 104:1831–8. http://dx.doi.org/10.1017/S0007114510002874. [PubMed: 20815975]

58. Parnell JA, Reimer RA. Effect of prebiotic fibre supplementation on hepatic gene expression and serum lipids: a dose-response study in JCR:LA-cp rats. Br J Nutr. 2010; 103:1577–84. http://dx.doi.org/10.1017/S0007114509993539. [PubMed: 20021705]

59. Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med. 2011; 62:361–80. http://dx.doi.org/10.1146/annurev-med-012510-175505. [PubMed: 21226616]

60. Musso G, Gambino R, Cassader M. Gut microbiota as a regulator of energy homeostasis and ectopic fat deposition: mechanisms and implications for metabolic disorders. Curr Opin Lipidol. 2010; 21:76–83. http://dx.doi.org/10.1097/MOL.0b013e3283347ebb. [PubMed: 19915460]

61. Thuy S, Ladurner R, Volynets V, Wagner S, Strahl S, Königsrainer A, et al. Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. J Nutr. 2008; 138:1452–5. [PubMed: 18641190]

62. Sabaté JM, Jouët P, Harnois F, Mechler C, Msika S, Grossin M, et al. High prevalence of small intestinal bacterial overgrowth in patients with morbid obesity: a contributor to severe hepatic steatosis. Obes Surg. 2008; 18:371–7. http://dx.doi.org/10.1007/s11695-007-9398-2. [PubMed: 18286348]