



## Conference on ‘Carbohydrates in health: friends or foes’ Symposium 3: Non-digestible carbohydrates, gut microbiota and obesity

### Gut microbiota and energy balance: role in obesity

Michael Blaut

Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke,  
14558 Nuthetal, Germany

The microbial community populating the human digestive tract has been linked to the development of obesity, diabetes and liver diseases. Proposed mechanisms on how the gut microbiota could contribute to obesity and metabolic diseases include: (1) improved energy extraction from diet by the conversion of dietary fibre to SCFA; (2) increased intestinal permeability for bacterial lipopolysaccharides (LPS) in response to the consumption of high-fat diets resulting in an elevated systemic LPS level and low-grade inflammation. Animal studies indicate differences in the physiologic effects of fermentable and non-fermentable dietary fibres as well as differences in long- and short-term effects of fermentable dietary fibre. The human intestinal microbiome is enriched in genes involved in the degradation of indigestible polysaccharides. The extent to which dietary fibres are fermented and in which molar ratio SCFA are formed depends on their physicochemical properties and on the individual microbiome. Acetate and propionate play an important role in lipid and glucose metabolism. Acetate serves as a substrate for *de novo* lipogenesis in liver, whereas propionate can be utilised for gluconeogenesis. The conversion of fermentable dietary fibre to SCFA provides additional energy to the host which could promote obesity. However, epidemiologic studies indicate that diets rich in fibre rather prevent than promote obesity development. This may be due to the fact that SCFA are also ligands of free fatty acid receptors (FFAR). Activation of FFAR leads to an increased expression and secretion of enteroendocrine hormones such as glucagon-like-peptide 1 or peptide YY which cause satiety. In conclusion, the role of SCFA in host energy balance needs to be re-evaluated.

#### Gut microbiota: Dietary fibre: Energy extraction: SCFA: Obesity: Mouse studies

Obesity has become a worldwide problem and a major public health issue<sup>(1)</sup> because a large proportion of obese subjects sooner or later will become afflicted with various diseases such as coronary heart disease, type-2-diabetes and non-alcoholic fatty liver disease<sup>(2)</sup>. Typical symptoms, referred to as metabolic syndrome, include abdominal adiposity, hypertension, dyslipidaemia and insulin resistance. The micro-organisms residing in the gastrointestinal tract, the so-called gut microbiota, have been linked to obesity<sup>(3)</sup>. This leads to the suggestion that the intestinal microbiome, the collective genomes of the microbiota, may contribute to obesity development and symptoms of the metabolic syndrome<sup>(4)</sup>. These observations have triggered a great

number of animal studies. Even though mice and human subjects differ in various aspects of their physiology, animal models offer an opportunity to conduct experiments that cannot be done in human subjects. Major advantages include the use of mouse mutants, easy accessibility to intestinal contents and organs, and targeted association of germ-free mice with selected microorganisms. How intestinal bacteria in conjunction with nutritional factors affect host energy metabolism is the focus of this review. Mouse experiments demonstrated that transplantation of microbiota from obese mice or human subjects to germ-free mice made the recipient mice also obese, while they stayed lean when microbiota were transplanted from lean mice<sup>(5,6)</sup>. Based on

these and other observations it has been proposed that the microbiomes of human individuals differ in their capacity to extract energy from the diet<sup>(7)</sup>. The intestinal microbiome affects nutrient absorption and the host energy metabolism by its ability to convert fermentable dietary fibre into SCFA, which provides additional energy to the host. However, SCFA are also ligands of the free fatty acid receptors (FFAR)2 and FFAR3. Activation of FFAR affects the functions related to satiety and insulin sensitivity. Therefore, the role of dietary fibre and intestinal bacteria in the development or the prevention of obesity and metabolic disease is in part controversial.

### Intestinal microbiota

The intestinal tract is home to  $10^{13}$ – $10^{14}$  micro-organisms, the vast majority of which are bacteria, the remainder being methanogenic archaea and yeasts<sup>(8)</sup>. Intestinal bacteria belong to only few major phyla: Firmicutes (includes genera such as *Clostridium*, *Eubacterium*, *Faecalibacterium*, *Ruminococcus* and *Roseburia*), Bacteroidetes (includes genera such as *Alistipes*, *Bacteroides*, *Parabacteroides*, *Porphyromonas* and *Prevotella*), Actinobacteria (*Bifidobacterium* and *Collinsella*) Proteobacteria (mainly *Escherichia coli* and relatives), Verrucomicrobia (*Akkermansia*) and Fusobacteria (*Fusobacterium*). Firmicutes and Bacteroidetes account for up to 90 % of all bacterial cells in the human intestine. Colonisation of the intestinal tract by micro-organisms starts at birth following oral uptake of bacteria from the mother and from the environment. Usually, the microbial community in the gut ecosystem stabilises within the first 2 years of age. The microbiota in the digestive tract affect host physiology in many ways; for example, they confer colonisation resistance on the host and play an important role in the maturation of the innate and the adaptive immune system.

The gut microbiota may be considered as an additional organ whose metabolic potential exceeds that of the liver. Well-known catalytic activities include the conversion of host-derived substances such as bile acids, of non-nutritive dietary components such as secondary plant metabolites and of xenobiotics (drugs). One core activity of the microbiome relates to the degradation of carbohydrates that escape digestion in the small intestine. The metagenome, which encompasses all genes of all microbial community members, contains a large proportion of genes involved in the breakdown of carbohydrates<sup>(9)</sup>. Such genes are overrepresented in the human intestinal microbiome<sup>(10)</sup> indicating their importance for the ecosystem. These genes encode a large array of proteins that catalyse the depolymerisation of non-digestible polysaccharides and their subsequent conversion to SCFA, preferentially acetic acid, propionic acid and butyric acid. Non-digestible but fermentable carbohydrates may be extremely variable with respect to their structure as well as physical and chemical properties. This high variability is related to the large number of possible monomers and linkages. Intestinal bacteria express a

wide variety of enzymes that afford the depolymerisation of complex carbohydrates<sup>(11)</sup>. Bacterial population groups involved in this process may compete for a given substrate among each other but they may also catalyse complementary reactions. Important functions of the human gut microbiome are redundant, i.e. more than one species is capable of catalysing a given reaction in the breakdown of complex carbohydrates. Redundancy of metabolic functions confers stability on the ecosystem. Such functions may be considered as core activities of the microbiota. It is important to note that individuals may differ in the bacterial species that catalyse the degradation of a given substrate. Accordingly, the metagenomes, which reflect the metabolic functions of the respective microbiota, display a high degree of similarity among human subjects, while at the same time the corresponding microbial communities are highly variable in composition. In other words, taxonomic variability of the gut microbiota is greater than functional variability<sup>(12)</sup>. Genes encoding core activities are found in the microbiome of every individual. Other activities, for example, the ability to convert the isoflavone daidzein, a polyphenol found in soya, to equol is restricted to certain individuals<sup>(13)</sup> and may therefore not be considered a core activity.

### Bacterial degradation of dietary fibre in the intestinal tract

Dietary fibre refers to plant material that escapes digestion in the small intestine. The main components of dietary fibre are matrix polysaccharides that may be a part of complex structures, such as plant cell walls, but they may also serve as storage materials in the plant. Dietary fibre is found in whole grains, fruit and vegetables. Various types of dietary fibre can be distinguished based on their chemical structure (e.g. type of monomers and type of linkage), physical properties (e.g. solubility, viscosity and water-holding capacity) and on whether they can be fermented by intestinal microbiota<sup>(14)</sup>. Solubility of the respective dietary fibre is a major factor that affects their fermentability by intestinal bacteria, but is not sufficient to predict its physiologic effects. There is a discrepancy between the chemically determined amounts of fibre and its physiologic effects<sup>(15)</sup>. Cellulose, a  $\beta$ -glucan, is essentially insoluble and not or only weakly degraded by intestinal bacteria. It may nevertheless affect intestinal physiology, for example, by attenuating the rise in blood glucose after a meal or by exerting a laxative effect owing to its water-holding capacity. Other dietary fibres of relevance to human nutrition include hemicelluloses (a wide variety of hexosans and pentosans), pectins (chains of  $\alpha$ -(1–4)-linked D-galacturonic acid, the carboxyl groups of which may be esterified to varying degrees with methanol), guar gum (galactomannan) and fructans such as inulin and oligofructose. Depending on the individual nutrition pattern, such dietary fibres make up a smaller or larger part of a typical human diet and they may, to a smaller or larger extent serve as growth substrates for

intestinal bacteria. However, in human subjects who consume a typical Western-type diet, resistant starch is the most important substrate of intestinal microbiota<sup>(16)</sup>. In human nutrition, starch is the main carbohydrate source and normally readily digested in the small intestine. However, seeds, legumes and unprocessed whole grains may contain starch that is physically inaccessible and therefore not degraded by host enzymes. Starch which escapes digestion in the small intestine is referred to as resistant starch. Another form of resistant starch includes starch that occurs in its natural granular form and can be found in uncooked potatoes or unripe bananas. Resistant starch can also be formed during cooking and cooling of starch containing foods and is referred to as retrograded starch<sup>(17)</sup>.

The large variety of non-digestible but fermentable substrates is probably one reason for the high diversity of the microbial community in the intestine. The first step in the bacterial breakdown of dietary fibre (Fig. 1) involves de-branching and de-polymerising enzymes such as cellulase, xylanase, arabinofuranosidase, xylosidase, arabinogalactanase, arabinofuranosidase, galactosidase, pectinase, exopectate lyase, glucuronidase,  $\beta$ -glucanase,  $\beta$ -glucosidase,  $\beta$ -galactosidase, fructooligosaccharidase and pullulanase<sup>(9)</sup>. The resulting oligosaccharides and monosaccharides are either taken up by bacteria that contribute to their degradation or by bacteria that do not possess the required enzymes and therefore profit from bacteria that make the monomeric carbohydrates available. The latter process is referred to as cross-feeding<sup>(18)</sup>. Intestinal bacteria use different pathways for the degradation of the resulting monomeric or oligomeric carbohydrates. Altogether these pathways lead to the formation of SCFA, mainly acetate, propionate and butyrate; other fermentation products such as lactate, succinate and ethanol are intermediates, the majority of which are also degraded to SCFA<sup>(8)</sup>. Side products of bacterial fermentation in the digestive tract are carbon dioxide, molecular hydrogen and formate. The latter may be further converted to acetate by bacteria such as *Eggerthella lenta* or *Blautia producta*, or to methane by the archaeon *Methanobrevibacter smithii* (Fig. 1).

### SCFA are major products of bacterial fermentation of dietary fibre

The most important products arising from bacterial breakdown of fermentable carbohydrates in the colon are SCFA, 95 % of which are acetate, propionate and butyrate, which are present at a molar ratio of approximately 60:23:17. Total SCFA concentrations in the proximal and the distal colon are approximately 120 and 90 mM, respectively<sup>(19)</sup>. Even though the SCFA concentration in the distal colon is still relatively high (because fermentation of colonic contents during passage goes on), 95 % of the SCFA formed in the colon are absorbed<sup>(20)</sup>. Both the colonic SCFA concentrations and the molar SCFA ratios vary considerably in response to the type of dietary fibre ingested. The dietary fibre intake in European subjects was reported to be on average

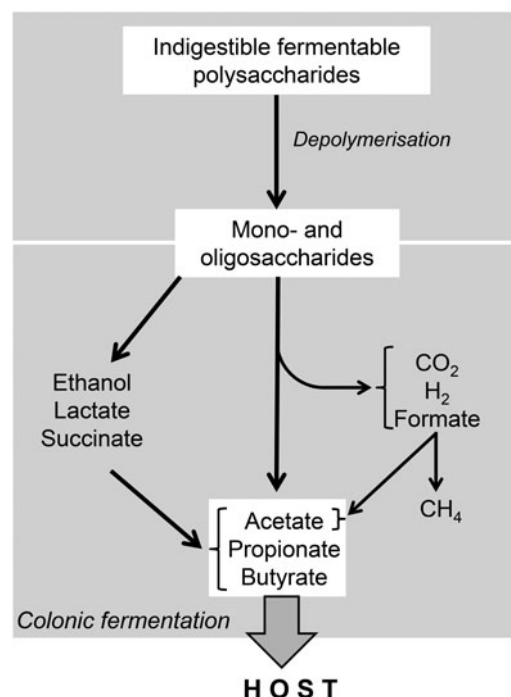


Fig. 1. Scheme depicting various steps in the fermentation of indigestible polysaccharides by intestinal microbiota.

20–25 g/d but could be as high as 60 g/d<sup>(21)</sup>. The latter value can probably be reached when a diet rich in whole-grain products, fruit and vegetables is consumed. Based on the analysis of ileal effluents from ileostomy patients and obtained by terminal ileal intubation, it has been estimated that up to 9 % of starch in a meal is malabsorbed and passes into the colon<sup>(22)</sup> where it undergoes bacterial fermentation. Accordingly, the total amount of SCFA produced in the human colon has been estimated to be 500–600 mmol/d with a total energy value of 600–750 kJ/d<sup>(22)</sup>, corresponding to 6.9–8.6 % of an assumed average adult diet of 8700 kJ. However, this estimation appears too high (mentioned later).

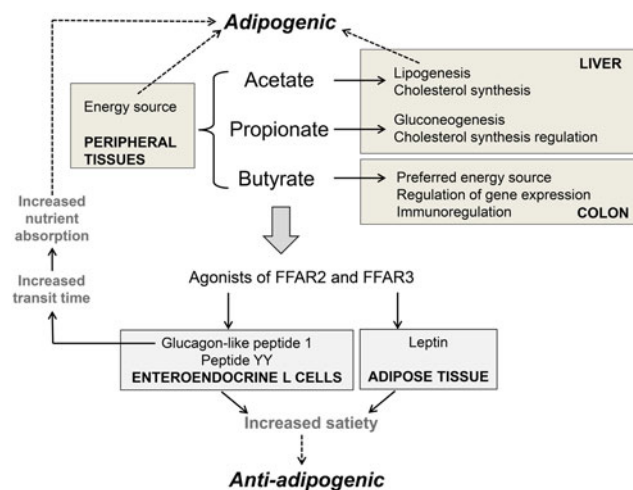
The energy content of monomers derived from indigestible but fermentable carbohydrates would be similar to that of glucose if the host possessed enzymes for cleavage and uptake of the resulting monomers. The energy content extracted from dietary fibre and available for the host is considerably smaller because approximately 40 % of the carbon from fermentable carbohydrates is converted into bacterial biomass which is excreted<sup>(23)</sup>. The remainder is converted to carbon dioxide (5–10 %), some lactate (3–5 %) and SCFA (45–50 %). The energy gained by the oxidation of SCFA, which are formed from bacterial fermentation of one hexose moiety from non-digestible carbohydrates such as oligofructose, has been calculated to be approximately 25–35 % of what would be gained if fructose was absorbed in the small intestine (approximately 15.7 kJ/g fructose)<sup>(23)</sup>. An assumed daily intake of 40 g fermentable carbohydrates, i.e. NSP (20 g) plus resistant starch (20 g), would contribute approximately 188 kJ to the daily energy requirement

(15.7 kJ/g  $\times$  40 g  $\times$  30 %), which corresponds to 2.2 % of an adult diet of 8700 kJ. However, it has to be noted that the amount of fermentable fibre entering the colon very much depends on dietary habits. For example, 2–6-year-old children from a rural area in Africa on a traditional diet ingested daily 14.2 g fibre at a total food intake of 445 g (3.2 %), whereas Italian children of the same age on a European diet ingested daily 8.4 g fibre at a total food intake of 925 g (0.9 %) <sup>(24)</sup>.

SCFA formed in the colon by bacterial fermentation are absorbed and subsequently used in various tissues (Fig. 2). Acetate and butyrate are preferentially used for energy generation in all body tissues, whereas propionate preferentially enters the gluconeogenic pathway into the liver <sup>(25)</sup>. However, acetate can also be used for lipogenesis. SCFA provide 60–70 % of the energy required by isolated colonocytes with butyrate being the preferred energy source for these cells <sup>(26)</sup>. However, besides serving as an energy source, butyrate influences gene expression by inhibiting histone deacetylase, which in colon cancer cells results in cell cycle arrest and activation of apoptosis <sup>(27)</sup>. Therefore, SCFA are considered important for keeping the colonic mucosa in a healthy state.

### Intestinal microbiota and obesity

The prevalence of obesity and associated diseases is worldwide on the rise. Even though genetic susceptibility plays an important role, this increase in obesity has been mainly attributed to considerable changes in lifestyle during the past decades. Important factors include excessive consumption of energy-rich diets, lack of physical activity, a sedentary lifestyle and unhealthy eating behaviour <sup>(28)</sup>. One decade ago, the intestinal microbiota was identified as another factor involved in obesity development <sup>(29)</sup>. This notion has mainly been based on two observations: (1) gut microbiota composition differed between lean and obese human subjects or mice <sup>(3,30)</sup>; (2) the obese phenotype could be transferred by transplantation of the microbiota from obese mice or human subjects to recipient germ-free mice <sup>(5,6)</sup>. These results were taken as an indication that the microbiome of obese donors has an increased capacity to extract energy from the diet compared with the microbiome of lean donors <sup>(30)</sup>. Several mechanisms have been proposed to account for these observations <sup>(31)</sup>: (1) the fermentation of indigestible carbohydrates by intestinal microbiota leads to an increased intestinal absorption of monosaccharides and SCFA followed by increased hepatic lipogenesis; (2) high-fat diets trigger an increased transfer of bacterial lipopolysaccharide (LPS) from the intestinal lumen to the blood causing metabolic endotoxaemia and low-grade inflammation <sup>(32)</sup>; (3) the gut microbiota suppress the formation of angiopoietin-like protein 4, also known as fasting-induced adipose factor, in gut epithelium. As angiopoietin-like protein 4 is an inhibitor of lipoprotein lipase, its suppression would lead to increased TAG storage in adipose tissue. Only the first two points will be discussed here. The intestinal microbiome affects glucose and lipid



**Fig. 2.** Simplified scheme showing the roles of SCFA in host metabolism. On the one hand, SCFA provide energy to the host and play a role in lipogenesis; thereby, SCFA could contribute to adiposity. On the other hand, by activating free fatty acid receptors (FFAR)2 and FFAR3, SCFA can trigger the increased release of peptide YY, glucagon-like peptide 1 and leptin, which would result in increased satiety which eventually may help to prevent/reduce adiposity. However, peptide YY also slows down gut transit time which would result in improved energy extraction and contribute to obesity. However, which of these effects is dominant, is still unclear.

metabolism as well as satiety. This may be concluded from a comparison of germ-free and conventional mice, which revealed that conventional mice display higher serum glucose and liver TAG levels compared with germ-free mice as well as higher levels of the hormones leptin and insulin in serum <sup>(29)</sup>. Leptin is produced by adipocytes and plays a major role in the regulation of body weight <sup>(33)</sup>. Leptin inhibits hunger and increases energy expenditure by means of activating leptin receptors in the brain. In agreement with the higher leptin levels found in conventional mice compared with germ-free mice oxygen consumption, which reflects energy expenditure, was 27 % lower in the latter mice <sup>(29)</sup>. Such a difference in total energy expenditure monitored over 24 h was also observed in another mouse study, but interestingly this difference was only observed during night time, when the mice are active <sup>(34)</sup>.

### Do SCFA contribute to or rather prevent obesity development?

SCFA formed through bacterial fermentation of indigestible carbohydrates provide additional energy to the host which potentially could contribute to obesity development (Fig. 2). However, epidemiological studies rather indicate that a diet rich in fibre correlates with a lower incidence of obesity and symptoms of the metabolic syndrome <sup>(15)</sup>. For example, a prospective cohort study involving 161 737 US women showed an inverse association between whole-grain intake and type 2 diabetes <sup>(35)</sup>. The results of intervention studies which investigated the

effects of dietary fibre on weight loss are inconsistent. Although a number of studies reported an improvement of weight loss in response to dietary fibre supplementation, others failed to show significant effects on weight loss<sup>(15)</sup>. These discrepancies might be due to differences in study design and the type of dietary fibre consumed by the study subjects. In principle, there are two opposing effects: on the one hand, SCFA derived from dietary fibre provide additional energy which could contribute to obesity development; on the other hand, dietary fibre decreases the energy density of the diet. Which of the two effects is of more importance probably depends on the type of dietary fibre that is ingested and possibly the duration of intake.

Theoretically, non-fermentable fibre reduces the energy density of a diet to a larger extent than a fermentable fibre because only the latter can be converted to SCFA. This consideration is supported by a mouse study in which the effect of guar gum, which is soluble and fermentable, was compared with that of a largely (93 %) insoluble and non-fermentable oat fibre fraction<sup>(36)</sup>. The study investigated in obesity prone C57BL/6J mice whether long term feeding (45 weeks) of a high-fat diet (macronutrient energy: protein 18 %; carbohydrates 41 %; fat: 41 %) supplemented with either 10 % guar gum or 10 % insoluble fraction of oat fibre affected body weight, liver fat, insulin sensitivity and gene expression of metabolic markers in liver and adipose tissue. Body weight increased to a similar extent in both the mouse groups during the first 10 weeks. Thereafter the mice on the guar gum supplemented diet gained significantly more body weight compared with the mice fed on the diet supplemented with the non-fermentable oat fibre fraction (42 g v. 33 g after 45 weeks). Although dietary intake was comparable, the body fat content did not differ between the groups after 15 weeks, but after 43 weeks, it was 35 % lower in the oat fibre group than in the guar gum group (9 g v. 14 g). Markers of insulin resistance were higher in the mice fed the guar gum containing diet than in the mice fed the diet containing oat fibre. In agreement with a role of SCFA in lipogenesis, liver TAG levels in the guar gum group were elevated compared with the oat fibre group. Furthermore, gene expression analysis in liver indicated increased fatty acid oxidation in the mice fed the non-fermentable oat fibre. Excreted hydrogen as a marker of bacterial fermentation was significantly elevated in the guar gum group (24 ppm) compared with the oat fibre group (2 ppm). The latter indicates that the oat fibre was poorly fermented and accordingly, hardly any SCFA were produced. In contrast, guar gum was well fermented leading to increased SCFA formation, which in turn contributed to an increase in digested energy. Hence, in a direct comparison of a fermentable and a non-fermentable fibre, the latter resulted significantly in lower weight gain and improved insulin sensitivity in mice fed a high-fat diet. However, the difference between the two fibres became evident only after long-term feeding.

Several short-term animal studies reported that soluble fibres such as guar gum and psyllium were more effective

in improving insulin sensitivity than insoluble cellulose<sup>(37,38)</sup>. However, beneficial effects observed in rats after short-term feeding of guar gum disappeared after 67 weeks, whereas rats fed cellulose still displayed lower pancreatic insulin and glucagon concentrations and a slightly reduced body weight after 67 weeks<sup>(39)</sup>. Taken together these results indicate that short- and long-term effects of dietary fibres may differ.

Besides serving as energy source acetate, propionate and butyrate have been recognised as ligands of G-protein-coupled receptors FFAR2 and FFAR3 (formerly G-protein-coupled receptor 43 and G-protein-coupled receptor 41<sup>(40)</sup>). These receptors are expressed in enteroendocrine L cells of ileum and colon as well as in adipocytes and immune cells. Activation of FFAR2 in adipocytes triggers the release of leptin from adipocytes<sup>(41)</sup> and the secretion of peptide YY (PYY) from enteroendocrine cells<sup>(42)</sup>. Both leptin and PYY are known for their anorexigenic effects, i.e. their ability to reduce appetite<sup>(43)</sup>. Interestingly, mice deficient in FFAR3 (*Ffar3*<sup>-/-</sup>) and maintained on a polysaccharide-rich low-fat chow diet, gained 30 % less body weight and had 25 % less body fat than the corresponding wild-type mice even though they did not differ in the amount of chow consumed. These results suggest that FFAR3 activation in these mice led to an increased nutrient extraction from the diet<sup>(44)</sup>. This was attributed to elevated PYY serum levels as a result of SCFA-mediated FFAR3 activation, because PYY not only has anorexigenic effects but also slows down gut transit time<sup>(45)</sup>. Prolonged transit time in turn enhances the efficiency of energy extraction. Accordingly, a comparison of the energy content of faeces from *Ffar3*<sup>-/-</sup> mice and wild-type mice using bomb-calorimetric analysis revealed a more efficient extraction of energy in the *Ffar3*<sup>+/-</sup> mice<sup>(44)</sup>. PYY concentrations in germ-free mice were 40 % lower compared with conventional mice supporting a role of intestinal SCFA in FFAR activation<sup>(44)</sup>. It appears that in this experiment the effect of PYY on gut motility dominated over its anorexigenic effect. Since FFAR activation enhances leptin formation by adipocytes, it is not surprising that *Ffar3*<sup>-/-</sup> mice displayed significantly lower leptin levels than the corresponding wild-type mice. This could only in part be attributed to the lower fat mass of these animals.

Acetate and propionate enhance the secretion of glucagon-like peptide 1 (GLP-1) in primary murine colonic cell cultures<sup>(46)</sup>. Similar to PYY, GLP-1 is produced by enteroendocrine L cells. Besides stimulating the production of insulin in pancreatic  $\beta$ -cells, GLP-1 also promotes insulin sensitivity and satiety. Mice deficient in FFAR2 or FFAR3 exhibit low levels of circulating GLP-1 and impaired glucose tolerance suggesting that, SCFA play an important role in glucose homeostasis<sup>(46)</sup>. In agreement with this finding, increased wheat fibre intake by hyperinsulinaemic human subjects resulted in higher postprandial plasma SCFA and GLP-1 concentrations compared with a control group, but this effect became only detectable after 9 and 12 months of intervention<sup>(47)</sup>. However, it has to be noted that some results concerning the role of FFAR2 are in part

controversial: in contrast to Tolhurst *et al.*<sup>(46)</sup>, Bjursell *et al.*<sup>(48)</sup> did not find a significant difference in glucose tolerance in *Ffar2*<sup>-/-</sup> mice fed a chow diet compared to *Ffar2*<sup>+/+</sup> mice. On a high-fat diet, these *Ffar2*<sup>-/-</sup> mice had a lower body fat mass in spite of a higher food intake compared with wild-type controls. The lower food intake in the *Ffar2*<sup>+/+</sup> mice compared with the *Ffar2*<sup>-/-</sup> mice is in agreement with an SCFA-dependent FFAR2 activation which triggered an increase in anorexigenic GLP-1 and PYY. However, the remaining discrepancies between the studies have not yet been resolved and need clarification. A very recent investigation showed that *Ffar2*<sup>-/-</sup> mice fed a normal diet become obese, while mice overexpressing FFAR2 remained lean<sup>(49)</sup>. However, these two mouse strains did not show their respective phenotypes when kept germ-free. Further analyses revealed that FFAR2 activation in adipocytes suppressed insulin signalling, which in turn reduced fat accumulation in adipocytes and the utilisation of lipids and glucose in other tissues. Based on their study the authors proposed that FFAR2 serves as a sensor that contributes to energy homeostasis<sup>(49)</sup>.

#### Role of oligofructose in the amelioration of diet-induced obesity and symptoms of the metabolic syndrome

The consumption of high-fat diets is associated with increased plasma LPS levels in human subjects<sup>(50)</sup> and mice<sup>(32)</sup> referred to as endotoxaemia. The latter is associated with elevated levels of inflammatory markers, indicative of low-grade inflammation, and with the onset of symptoms of the metabolic syndrome. Increased plasma LPS levels have been attributed to diets rich in fat which lead to increased gut permeability facilitating the transfer of LPS from the gut lumen into the blood stream. Interestingly, oligofructose, a non-digestible fermentable carbohydrate, was reported in mice to reduce gut permeability, decrease plasma LPS levels and ameliorate symptoms of the metabolic syndrome<sup>(51)</sup>. Since these changes coincided with an increase in faecal bifidobacteria, the health-promoting effects were attributed to this bacterial population group. However, experimental evidence for a role of bifidobacteria in oligofructose-dependent amelioration of metabolic disease symptoms has not been presented. We investigated the potential role of bifidobacteria in a gnotobiotic mouse model. The mice were either colonised with a microbial community encompassing eight bacterial species, including *Bifidobacterium longum* or with the same community without this bacterium. Germ-free mice served as a control group. All three groups were fed either a high-fat diet or a high-fat diet supplemented with 10 % oligofructose. After 4 weeks feeding, mice fed the diet supplemented with 10 % oligofructose gained significantly less body weight and had significantly less body fat than the mice fed the high-fat diet without oligofructose supplementation (unpublished results). Since this effect was independent of the microbial status, the idea that bifidobacteria mediate the health-promoting effect of oligofructose has to be questioned.

#### Conclusions

In healthy individuals, intake and expenditure of metabolic energy are balanced. Recent evidence suggests that the gut microbiome affects host energy metabolism. The intestinal microbiome shows high individual variability, utilises dietary fibre as the primary energy source and influences the harvest, storage and expenditure of energy. Available evidence indicates that high-fat diets favour the development of a microbiome that affords an increased energy harvest from the diet promoting obesity and metabolic diseases. However, the mechanisms by which intestinal bacteria influence host energy metabolism are incompletely understood. Fermentable dietary fibre undergoes bacterial fermentation in the colon and conversion to SCFA, which on one hand provide additional energy to the host and can be used for lipogenesis and gluconeogenesis (Fig. 2). On the other hand, dietary fibre decreases the energy density of the diet and, based on their ability to activate FFAR2 and FFAR3, SCFA may also lead to increased satiety and insulin sensitivity. Which of these effects predominates, and how they are affected by the type of dietary fibre, needs clarification. Long- and short-term effects of dietary fibre may differ. Not all effects of gut microbiota on host energy metabolism are necessarily related to SCFA formation. Recent mouse studies identified intestinal bacteria that enhance obesity development. *Enterobacter cloacae* isolated from the gut of an obese patient induced obesity and insulin resistance when introduced into the gut of germ-free mice because of its ability to produce enterotoxins<sup>(52)</sup>. *Clostridium ramosum*, which is increased in obese human subjects, promoted obesity in gnotobiotic mice, probably by enhancing the absorption of nutrients in the small intestine<sup>(53)</sup>. The mechanisms underlying such effects need to be elucidated in more detail. In particular, it is necessary to identify the bacterial molecules that mediate these effects as well as their targets in the host. Even though most studies referred to in this review have been done in animals, there are a number of links suggesting that the results obtained in animals are of relevance to human subjects. In particular, the fact that it is possible to transfer the obese phenotype from obese human subjects by transplanting their microbiota argues in favour of a common mechanism in human subjects and mice. Identification of bacteria that promotes obesity could help to develop strategies that reduce their intestinal cell number in order to minimise their obesogenic effects.

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## Conflicts of Interest

None.

## Authorship

M. B. was the sole author of the manuscript.

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