Obesity is one of the major current public health problems because of its increasing prevalence and association with important chronic disorders\(^1\). These include type-2 diabetes mellitus, atherosclerosis, CVD, non-alcoholic fatty liver disease and cancer. Obesity is the result of a long-term positive imbalance between energy intake and expenditure, which is regulated by multiple pathways involving metabolites, hormones and neuropeptides. High-fat diet-induced obesity and metabolic disorders are also associated with a state of chronic low-grade inflammation and increased susceptibility to infection, due to malfunction of the immune system. Obese individuals have increased macrophage infiltration in the adipose tissue along with the production of inflammatory adipokines, cytokines and associated immune factors. Inflammatory immune mediators (e.g. C-reactive protein, TNF\(\alpha\), IL-6 and monocyte chemotactic protein 1) and some adipokines (e.g. leptin) are usually elevated in obese mice and human subjects, whereas the production of the anti-inflammatory and insulin-sensitizing adipokine adiponectin is reduced\(^2\).

In fact, chronic activation of the innate immune system is regarded as a risk factor for the development of obesity and associated disorders; this activation might partly depend on the immunomodulatory effects exerted by dietary compounds in the gut and beyond\(^3\).

The human intestinal tract is populated by a vast number of bacterial species that reach concentrations ranging from \(10^7\) to \(10^{12}\) cells/g intestinal content, from the small
Obesity, weight loss and gut microbiota composition

Obesity is associated with phylum and group-specific changes in the microbiota, and with reduced bacterial diversity (6,7). Increases in the relative abundance of Firmicutes and reductions in Bacteroidetes have been associated with obesity by comparisons between the distal gut microbiota of genetically obese ob/ob mice (leptin deficient) and their lean (ob/+ or +/+ ) littermates using DNA sequencing techniques (8). A higher proportion of Archaea was also found on the caecal microbiota of these genetically obese mice in comparison with their lean littermates (9). Diet-induced obesity in mice has also been associated with an increased proportion of Eubacterium dolichum, belonging to the Firmicutes division (10). Compared to lean rats, obese Zucker rats (fa/fa) showed reduced Bifidobacterium counts quantified by fluorescence in situ hybridization and increased abundance of Halomonas and Sphingomonas, detected by PCR and denaturing gradient gel electrophoresis (7). Obesity induced by a high-fat diet was also associated with lower Bifidobacterium numbers in caecal content in mice (11).

Similar alterations in the relative proportions of Firmicutes and Bacteroidetes in faeces have been associated with human obesity (12). In addition, obese human adults submitted to a hyperenergetic diet (either low carbohydrate- or low-fat diet) showed significant increases in the faecal proportions of Bacteroidetes parallel to weight loss over a 1-year-long intervention (12). Furthermore, a lower proportion of Bacteroidetes and a higher proportion of Actinobacteria have been associated with obesity by comparisons between the faecal microbiota of obese and lean twin human subjects (6). A larger-scale intervention trial has recently demonstrated that both an energy-restricted diet and increased physical activity induce changes in the gut microbiota structure of obese adolescents, correlated with weight loss and BMI Z-score reductions (13,14). Clostridium histolyticum, Clostridium lituseburensense and Eubacterium rectale–Clostridium coccoides proportions dropped significantly, while those of the Bacteroides–Prevotella group increased after the intervention in those adolescents that experienced significant weight reduction (81% of their body weight) as determined by fluorescence in situ hybridization (15). When the microbiota was analysed by quantitative real-time PCR, increased Bacteroides fragilis and Lactobacillus group numbers and reduced C. coccoides and Bifidobacterium longum group numbers were detected in those adolescents that experienced important weight loss after the intervention (14). Moreover, the effectiveness of lifestyle intervention on body-weight loss seems to be influenced by the composition of the individual’s microbiota (14). Alterations in the faecal microbiota composition also seem to precede overweight in children, early in life. Children maintaining normal weight showed an increased number of Bifidobacterium, whereas children becoming overweight showed an increased number of Staphylococcus aureus in faeces during infancy (15).

Perturbations in the composition of gut microbiota associated with genetic or diet-induced obesity seem to be reversible by oral transfer of the gut microbiota from lean or obese mice to a germ-free recipient (10,16) or by the administration of prebiotic substrates to animal models at least over short-term periods (17). Studies on the evolution of mammals and their gut microbes by DNA sequencing also indicate that the diet is a fundamental promoter of change in gut bacterial diversity (18). Altogether, this evidence supports the hypothesis that the modulation of gut microbiota via dietary intervention is a potential strategy to help manage obesity and metabolic-associated disorders (10,16,17), although actual proof is still limited.

Role of the gut microbiota in nutrient metabolism and energy storage

The intestinal microbiota develops an important biochemical activity within the human body by providing additional metabolic functions (4) and regulating the diverse aspects of cellular differentiation and gene expression via host–microbe interactions (19). In fact, comparisons between germ-free mice and mice colonized by the conventional distal gut microbiota showed that the microbiota, as a whole, increases the host’s ability to extract energy from the diet and store this energy in adipocytes, contributing to body-weight gain (20). The intestinal microbiota provides enzymes involved in the utilization of non-digestible carbohydrates and host-derived glycoconjugates, deconjugation and dehydroxylation of bile acids, cholesterol reduction and biosynthesis of vitamins (K and B group), isoprenoids and amino acids (e.g. lysine and threonine) (4,19). In particular, the ability of the commensal microbiota to utilize complex dietary polysaccharides which would otherwise be inaccessible to human subjects and to generate SCFA seems to contribute to the ability of the host to harvest energy from the diet (20). This may represent 10% daily energy supply in omnivores and up to 70% in herbivores (21). Specific components of the commensal microbiota also regulate serum lipids and cholesterol by taking part in bile-acid recycling and metabolism. Bacterial enzymes mainly catalyse the deconjugation and dehydroxylation of bile acids, which alter the solubilization and absorption of dietary lipids throughout the intestine (22). Faecal commensal bacteria also reduce cholesterol to coprostanol and, thus, increase its excretion in faeces (23).

In addition, the commensal microbiota and its metabolites regulate the expression of genes involved in the processing and absorption of dietary carbohydrates and...
complex lipids in the host, favouring fat storage\(^{(20,24)}\). The expression of a monosaccharide transporter (Na+/glucose co-transporter) has been induced in \textit{Bifidobacterium thetaiotaomicron} mono-colonized mice, leading to increased absorption of dietary monosaccharides and SCFA and, thereby, promoting \textit{de novo} synthesis of lipids in the liver\(^{(25)}\). In fact, the colonization of germ-free mice by the conventional microbiota leads to increased liver expression of key enzymes (acyl-CoA carboxylase and fatty acid synthase) involved in \textit{de novo} fatty acid biosynthetic pathways and the transcriptional factors (carbohydrate response element-binding protein and sterol regulatory element-binding protein-1) involved in hepatocyte lipogenic responses to insulin and glucose\(^{(20)}\). Furthermore, microbial colonization reduces the levels of circulating fasting-induced adipose factor in the gut, skeletal muscle and liver levels of phosphorylated AMP-activated protein kinase, which jointly contribute to reducing fat oxidation and enhancing fat storage\(^{(25)}\).

\section*{Role of the gut microbiota in neurohormonal function}

The gut microbiota could also interact with the production and function of hormones and neuropeptides synthesized by the nervous system and enteroendocrine cells of the gastrointestinal tract mucosa and peripheral organs (adipose tissue, pancreas and liver), which are critical to the regulation of energy balance. Colonization of the germ-free intestine of mice by conventional microbiota stimulates adipokine leptin synthesis, with a proportional increase in body fat and insulin resistance\(^{(26)}\). Although leptin is the dominant long-term signal informing the brain of energy stores and inhibiting food intake, leptin deficiency is not a common cause of obesity but leptin resistance is\(^{(26)}\). Obese subjects usually have increased serum leptin levels associated with increased hunger and reduced energy expenditure. Increased leptin levels could also induce the production of pro-inflammatory T-helper 1-type cytokines and contribute to the inflammatory tone associated with obesity\(^{(26)}\). SCFA, which are mainly produced by the gut microbiota, act as ligands for G protein-coupled receptors, such as Gpr41, expressed in the intestine, colon and adipocytes, which upon activation stimulate the expression of peptide hormones (e.g. leptin and peptide tyrosine–tyrosine) involved in appetite and energy metabolism\(^{(27)}\). In particular, Gpr41-deficient mice show a reduced expression of peptide tyrosine–tyrosine, which modulates gut motility and reduced harvest of energy from the diet, in a microbiota-dependent manner. Autoantibodies against key appetite-regulating neuropeptides and peptide hormones (e.g. alpha-melanocyte-stimulating hormone, neuropeptide Y, agouti-related protein, ghrelin and leptin) have also been detected in the sera of human subjects and rats\(^{(28)}\). The sequence homology found between these neuropeptides and proteins from some members of the intestinal microbiota would suggest that the microbiota could influence their production and, therefore, eating behaviour. Mice infected with \textit{Helicobacter pylori} showed delayed gastric emptying, increased visceral perception and abnormal feeding patterns\(^{(29)}\). Feeding behaviour remained altered for up to 2 months post-infection, possibly due to altered gastric mechanosensitivity, increased postprandial cholecystokinin release inducing satiety and increased TNF\(\alpha\) expression in the central nervous system\(^{(30)}\). However, the administration of \textit{Lactobacillus} strains after \textit{H. pylori} eradication normalized the feeding behaviour\(^{(31)}\).

Interactions between the gut microbiota composition and stress-related hormones, which affect energy balance, have also been identified. Stress at late stages during pregnancy, parallel to elevated cortisol plasma levels, was found to lead to reductions in faecal \textit{Bifidobacterium} counts in infant monkeys\(^{(32)}\). Stress induced in male rat pups by maternal separation early in life also led to increased plasma corticosterone and the systemic immune response with alterations in the faecal microbiota compared to the control group\(^{(33)}\). In germ-free mice, higher plasma adrenocorticotrophic hormone and elevated corticosterone were detected in response to restraint stress as compared to conventional mice\(^{(34)}\). However, the excessive hypothalamic–pituitary–adrenal stress response in germ-free mice was reversed by inoculation with a \textit{Bifidobacterium infantis} strain. Glucocorticoids are well known for their critical role in metabolism and, in particular, alterations in tissue-specific cortisol levels influencing lipogenic and gluconeogenic pathways in fat and liver, associated with obesity and the development of insulin-resistance\(^{(35)}\).

\section*{Role of the gut microbiota in immune function}

Obesity induced by high-fat diets and the associated metabolic disorders are characterized by a state of low-grade inflammation which has been related to alterations in the gut microbiota composition and increased plasma lipopolysaccharide (LPS) levels\(^{(11,36)}\). Mice fed a high-fat diet exhibited a significant increase in plasma LPS, which was termed ‘metabolic endotoxemia’, associated with changes in the gut microbiota (reductions in \textit{Bifidobacterium} and \textit{E. rectale/C. coccoides}). A mouse model chronically infused with a dose of LPS to reach the same plasma LPS levels as those measured in the high-fat-diet-fed mice also mimicked the phenotype of high-fat-fed mice. This was characterized by fasting hyperglycaemia, obesity, steatosis, adipose tissue macrophages infiltration, hepatic insulin resistance and hyperinsulinaemia. Furthermore, mice knocked out in CD14, a key molecule in Toll-like receptor 4 signalling, were completely resistant to the development of inflammation induced by both high-fat feeding and chronic LPS administration in the visceral and subcutaneous adipose depots, the liver and the muscle\(^{(36)}\). In contrast, the inhibition of the gut microbiota by antibiotic administration (norfloxacin and ampicillin) in two different mouse models of insulin resistance resulted in reduced serum LPS levels, low-grade inflammation, obesity and type-2 diabetes\(^{(15)}\). Altogether, these findings demonstrate the link between the gut microbiota, LPS and certain metabolic disorders. In human subjects, increased LPS plasma levels have also been associated with an elevated BMI and high-fat feeding\(^{(37,38)}\). These increased LPS...
concentrations were sufficient to activate the synthesis of inflammatory cytokines (e.g. TNFα) by monocytes in vitro. Therefore, metabolic endotoxemia has also been considered a possible factor contributing to the postprandial inflammatory state, which could favour certain chronic disorders, including type-2 diabetes and atherosclerosis in human subjects (38).

The colonization of the germ-free mouse intestine also regulates the expression of serum amyloid A proteins, which are mediators of inflammation and metabolism and whose serum levels are increased in subjects with obesity, chronic hyperglycaemia, insulin resistance and CVD (39). The serum amyloid A3 protein expression was significantly augmented in adipose and colonic tissues by the presence of intestinal microbes, when comparing germ-free and conventionally raised mice. The authors propose that LPS, and potentially other products of gut bacteria, activate Toll-like receptors and mediate signalling through MyD88 and NF-kB to promote increased serum amyloid A3 and pro-inflammatory cytokine expression (e.g. TNFα), thereby exacerbating the chronic low-grade inflammation associated with obesity (39).

Effects of probiotics and prebiotics on obesity and metabolic disorders in animals

A summary of trials evaluating different modes of action of classical probiotics (Lactobacillus and Bifidobacterium strains), prebiotics or a combination thereof synbiotics, on diverse biomarkers of body-weight balance, immunity and metabolism in conventional animals and animal models of obesity, diabetes and hyperlipidemia is shown in Table 1. For example, feeding rats with skim milk fermented by Lactobacillus gasseri SBT2055 led to reduction in adipocyte size and increased numbers of small adipocytes in white adipose tissue, also reducing serum leptin concentrations compared with control rats, suggesting that the probiotic plays a role in regulating adipose tissue growth (40). Dietary supplementation of high fructose-induced diabetes and streptozotocin-induced diabetes in rats with a probiotic product (dahi) containing Lactobacillus acidophilus NCDC14 and Lactobacillus casei NCDC19 improved the biomarkers of glucose and lipid metabolism and delayed or suppressed glucose intolerance, hyperglycaemia, hyperinsulinaemia, dyslipidaemia and oxidative stress (41,42). The administration of either Lactobacillus paracasei NCC2461 or Lactobacillus rhamnosus NCC4007 to germ-free mice colonized with human baby microbiota also decreased plasma concentrations of VLDL and LDL and stimulated glycolysis (43). Similarly, when the same murine model was administered galactosyl oligosaccharides combined with L. rhamnosus NCC4007 as a synbiotic, the levels of plasma lipoproteins, hepatic TAG and kidney lipids were reduced (44). It seems that the reduction in TAG in the liver was mainly due to the probiotic, while the decrease in plasma lipoproteins was mainly due to the probiotic L. rhamnosus. This synbiotic also induced a remarkable stimulus to both growth and activity of bifidobacteria and, in particular, of B. longum (44).

The oral administration of the probiotic product VSL#3 to wild-type male C57BL/6 mice fed a high-fat diet significantly improved their insulin resistance, hepatic natural killer T cell depletion and hepatic steatosis induced by the high-fat diet. This effect was natural killer T cell dependent, resulting from the attenuation of the TNFα and IκB kinase inflammatory signalling and leading to improved sensitivity in insulin signalling (45).

Inulin-type prebiotics have also been demonstrated to modulate lipid and glucose metabolism in different animal models. Oligofructose decreases food intake, fat mass development and hepatic steatosis in normal and obese rats and mice; moreover, it exerts an anti-diabetic effect in streptozotocin-treated rats and high-fat-fed mice (46,47). The positive effects of oligofructose on diverse metabolic parameters are partly explained by its ability to regulate the expression of anorexigenic peptides, such as GLP-1 that promotes satiety, as well as other gastrointestinal peptides (such as peptide tyrosine-tyrosine and ghrelin), which could jointly be involved in controlling food intake as detected in rats (46). Moreover, the administration of oligofructose to high-fat-fed mice increased the intestinal Bifidobacterium numbers and normalized the endotoxemia and inflammatory tone associated with the high-fat diet (17). Furthermore, the administration of oligofructose to genetically obese mice (ob/ob) induced specific changes in the gut microbiota, characterized by increases in Lactobacillus, Bifidobacterium and C. cocoides–E. rectale groups, which led to reductions in intestinal permeability and an improvement in tight-junction integrity and inflammatory markers (plasma LPS and cytokines) (48). These models were associated with increases in portal plasma GLP-2 levels and its precursor (the proglucagon mRNA), in the jejunum and colon.

Effects of probiotics and prebiotics on obesity and metabolic disorders in human subjects

A summary of human clinical trials that have evaluated different effects of probiotic, prebiotic and synbiotic intake on biomarkers of lipid and glucose metabolism, blood pressure and body weight is shown in Table 2. Supplementation of hypercholesterolemic patients with the probiotic bacteria Lactobacillus plantarum 299v significantly lowered serum concentrations of LDL cholesterol and fibrinogen (49). A functional food product containing the same strain, L. plantarum 299v, also decreased different biomarkers of CVD risk in heavy smokers (49), (Table 2). Monocytes isolated from the subjects treated with L. plantarum 299v also showed significantly reduced adhesion to native and stimulated human umbilical vein endothelial cells, suggesting that the probiotic product could reduce CVD risk (49). A yoghurt supplemented with L. acidophilus 145, B. longum 913 and oligofructose increased HDL cholesterol concentrations and decreased the ratio of LDL: HDL cholesterol in comparison with control yoghurt in women (50). However, the administration of other probiotic strains did not exert significant effects on serum lipids, cholesterol or lipoproteins (51,52). The effects of probiotic supplementation together with dietary counselling exerted positive effects on glucose metabolism in normoglycaemic pregnant women (53). Blood glucose...
concentrations were the lowest in the diet/probiotic group during pregnancy and over the 12 months’ postpartum period. Glucose tolerance was also better in the diet/probiotic group compared with the control/placebo group. Glucose tolerance was also better in the diet/probiotic group compared with the control/placebo group.

<table>
<thead>
<tr>
<th>Probiotic/prebiotic/dose</th>
<th>Animal model</th>
<th>Duration</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. paracasei</em> NCC2461 and <em>L. rhamnosus</em> NCC4007 (10⁸ cfu/d)</td>
<td>Female germ-free mice C3H colonized with human baby flora</td>
<td>14 d</td>
<td>↓ Food intake; ↑ Glucose tolerance and insulin secretion; ↑ Portal and colonic GLP-1(7–36)</td>
<td>46</td>
</tr>
<tr>
<td><em>L. paracasei</em> NCC2461 or <em>L. rhamnosus</em> NCC4007 (10⁸ cfu/d) with GOS (3%)</td>
<td>Female germ-free mice C3H colonized with human baby flora</td>
<td>14 d</td>
<td>↓ Propionate and butyrate in caecum with <em>L. rhamnosus</em>; ↓ Isobutyrate in caecum with <em>L. paracasei</em>; ↓ Liver TAG and ↑ glycogen with <em>L. paracasei</em>; ↓ Hepatic lipids and serum lipoproteins; ↑ <em>Bifidobacterium</em> and <em>B. longum</em></td>
<td>45</td>
</tr>
<tr>
<td><em>VSL#3</em> (<em>B. breve, B. lactis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus</em> and <em>S. thermophilus</em>) (1.5 × 10⁹ cfu/d)</td>
<td>Male C57BL6 mice with steatosis and insulin resistance induced by a high-fat diet</td>
<td>28 d</td>
<td>↑ Hepatic NKT cell numbers and ↓ inflammatory signalling improving steatosis and insulin resistance.</td>
<td>44</td>
</tr>
</tbody>
</table>

cfu, colony-forming units; GOS, galactosyl-oligosaccharides; LPS, lipopolysaccharide; NKT, natural killer T cells; ↑, increase; ↓, decrease.

with mild hypercholesterolemia⁵⁶. An infant formula containing galactosyl-oligosaccharides and long chain fructo-oligosaccharides in a ratio of 9:1 did not exert significant effects on total cholesterol and LDL cholesterol in infants compared with those receiving a control infant formula⁵⁷. Daily consumption of oligofructose decreased the basal hepatic glucose production in healthy subjects, without any effect on insulin-stimulated glucose metabolism⁵⁸. However, this prebiotic had no effect on glucose and lipid metabolism in type-2 diabetics⁵⁹. In a pilot study with 10 human subjects, oligofructose treatment also increased satiety following breakfast and dinner, reduced hunger and prospective food consumption following dinner⁶⁰. A long-term study (12 months) including 100 subjects revealed that those who received the prebiotic supplement had a smaller increase in BMI, BMI.
Gut microbiota, obesity and metabolic disorders

Conclusions

A number of ecological studies have uncovered the association between the composition of the gut microbiota and body weight and the prominent role played by the diet in these interactions. Mechanistic studies have also revealed that the gut microbiota may perform specific functions in the metabolic, neurohormonal and immune dysfunction associated with obesity. In this scenario, the use of dietary strategies targeting the gut ecosystem emerges as an additional tool to control metabolic disorders. A small number of trials have demonstrated that the administration of probiotics, prebiotics and their combination (synbiotics) exert positive effects in vivo, which are often more remarkable in animals than in human subjects. Nevertheless, the findings indicate that advances in this field could be of value to improve intervention strategies to manage obesity and its associated metabolic disorders.

Acknowledgements

This work was supported by grants AGL2008-01440/ALI and Consolider Fun-C-Food CSD2007-00063 from the Spanish Ministry of Science and Innovation and AP 124/09 from Consejerı´ a de Sanidad (Valencia, Spain). The scholarships to A. S. from CONACYT (Mexico) and to P. G. from CONICET (Argentina) are fully acknowledged. This review was drafted by Y. S. and discussed and approved by all authors. Authors declare no conflict of interest.

References


