Proceedings of the Nutrition Society (2010), **69**, 434–441 doi:10.1017/S0029665110001813
 First published online 14 June 2010

The 3rd International Immunonutrition Workshop was held at Platja D'Aro, Girona, Spain on 21–24 October 2009

3rd International Immunonutrition Workshop

Session 8: Probiotics in the defence and metabolic balance of the organism
Gut microbiota in obesity and metabolic disorders

Yolanda Sanz*, Arlette Santacruz and Paola Gauffin

Microbial Ecophysiology and Nutrition Group, Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Valencia, Spain

Obesity is a major public health issue as it is causally related to several chronic disorders, including type-2 diabetes, CVD and cancer. Novel research shows that the gut microbiota is involved in obesity and metabolic disorders, revealing that obese animal and human subjects have alterations in the composition of the gut microbiota compared to their lean counterparts. Moreover, transplantation of the microbiota of either obese or lean mice influences body weight in the germ-free recipient mice, suggesting that the gut ecosystem is a relevant target for weight management. Indigenous gut microbes may regulate body weight by influencing the host's metabolic, neuroendocrine and immune functions. The intestinal microbiota, as a whole, provides additional metabolic functions and regulates the host's gene expression, improving the ability to extract and store energy from the diet and contributing to body-weight gain. Imbalances in the gut microbiota and increases in plasma lipopolysaccharide may also act as inflammatory factors related to the development of atherosclerosis, insulin resistance and body-weight gain. In contrast, specific probiotics, prebiotics and related metabolites might exert beneficial effects on lipid and glucose metabolism, the production of satiety peptides and the inflammatory tone related to obesity and associated metabolic disorders. This knowledge is contributing to our understanding of how environmental factors influence obesity and associated diseases, providing new opportunities to design improved dietary intervention strategies to manage these disorders.

Gut microbiota: Obesity: Chronic inflammation: Type-2 diabetes: Probiotics: Prebiotics

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, TNFα, 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[2].

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

Abbreviation:LPS, lipopolysaccharide.

***Corresponding author:** Yolanda Sanz, fax +34 96 363 63 01, email yolsanz@iata.csic.es

intestine to the colon. This microbial community develops with its host throughout life by establishing mutualistic symbiotic relationships which favour their co-existence[3]. The collective genome (microbiome) of the gut microbiota contains at least 100 times as many genes as the human genome, providing additional features and contributing to human physiological diversity[4,5]. In particular, the gut microbiota has been considered to be a possible causative factor of metabolic conditions as well as a therapeutic target in recent years[5]. Herein, the proposed modes of action of the gut microbiota in obesity and associated-metabolic disorders and the effects of interventions with the probiotic, prebiotics and synbiotics are reviewed.

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 hypoenergic 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 lituseburense* 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 (8·1% of their body weight) as determined by fluorescence *in situ* hybridization[13]. 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

https://doi.org/10.1017/S0029665110001813 Published online by Cambridge University Press

complex lipids in the host, favouring fat storage[20,24]. The expression of a monosaccharide transporter (Na+/glucose co-transporter) has been induced in *Bifidobacterium thetaiotaomicron* mono-colonized mice, leading to increased absorption of dietary monosaccharides and SCFA and, thereby, promoting *de novo* synthesis of lipids in the liver[24]. In fact, the colonization of germ-free mice by the conventional microbiota leads to increased liver expression of key enzymes (acetyl-CoA carboxylase and fatty acid synthase) involved in *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].

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[20]. 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 *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 TNFα expression in the central nervous system[30]. However, the administration of *Lactobacillus* strains after *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 *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 adrenocorticotropic 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 *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 gluconeogenetic pathways in fat and liver, associated with obesity and the development of insulin-resistance[35].

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 *Bifidobacterium* and *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 hyperinsulinemia. 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

https://doi.org/10.1017/S0029665110001813 Published online by Cambridge University Press

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-κB 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, hyperinsulinemia, dyslipidemia 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 prebiotic, 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 C57BL6 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. coccoides–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 effects 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

https://doi.org/10.1017/S0029665110001813 Published online by Cambridge University Press

Table 1. Effects of probiotics, prebiotics and synbiotics on biomarkers of body weight, immunity and metabolism in animals

Probiotic/prebiotic/dose	Animal model	Duration	Outcome	Reference
L. gasseri SBT2055 milk fermented with 6×10^7 cfu/g	Male Sprague–Dawley rats	28 d	↓ Adipocyte size in mesenteric white adipose tissue, ↑ number of small adipocytes in mesenteric and retroperitoneal adipose tissues and ↓serum leptin	40
L. acidophilus NCDC14 and *L. casei* NCDC19 in dahi product (10^8 cfu/g)	High fructose-induced diabetes in male Wistar rats	8 weeks	↓ Plasma glucose, glycosylated haemoglobin, insulin, total cholesterol, TAG, LDL-cholesterol, VLDL-cholesterol and free fatty acids and liver glycogen. ↓ Thiobarbituric acid-reactive substances and ↑ reduced glutathione in liver and pancreas.	41
L. acidophilus NCDC14 and *L. casei* NCDC19 in dahi product ($7{\cdot}3 \times 10^9$ cfu/g)	Streptozotocin-induced diabetes in Wistar rats	28 d	↓ Incremental peaks and delayed reduction of insulin secretion during oral glucose tolerance test ↓ Oxidative damage in pancreatic tissues by inhibiting lipid peroxidation and formation of nitric oxide and ↑ glutathione content and activities of superoxide dismutase, catalase and glutathione peroxidase	42
L. paracasei NCC2461 and *L. rhamnosus* NCC4007 (10^8 cfu/d)	Female germ-free mice C3H colonized with human baby flora	14 d	↓ Plasma VLDL- and LDL-cholesterol; ↑ TAG ↓ Faecal excretion of bile acids ↓ Acetate and butyrate in the caecum ↓ Acetate/propionate in the liver	43
L. paracasei NCC2461 or *L. rhamnosus* NCC4007 (10^8 cfu/d) with GOS (3%)	Female germ-free mice C3H colonized with human baby flora	14 d	↓ Propionate and butyrate in caecum with *L. rhamnosus* ↓ Isobutyrate in caecum with *L. paracasei* ↓ Liver TAG and ↑ glycogen with *L. paracasei*	62
L. rhamnosus GG (10^8 cfu/d) with GOS (3%)	Female germ-free mice C3H colonized with human baby flora	14 d	↓ Hepatic lipids and serum lipoproteins ↑ *Bifidobacterium* and *B. longum*	44
VSL#3 (*B. breve, B. lactis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus* and *S. thermophilus*) ($1{\cdot}5 \times 10^9$ cfu/d)	Male C57BL6 mice with steatosis and insulin resistance induced by a high-fat diet	28 d	↑ Hepatic NKT cell numbers and ↓ inflammatory signalling improving steatosis and insulin resistance.	45
Oligofructose (10%)	Streptozotocin-treated diabetic male Wistar rats	6 weeks	↓ Food intake ↑ Glucose tolerance and insulin secretion ↑ Portal and colonic GLP-1(7–36)	46
Oligofructose (10%)	High fat diet fed male C57bl6/J mice	4 weeks	↓ Energy intake, epididymal fat mass and body-weight gain; ↓ Glycaemia	47
Oligofructose (10 g%)	High fat fed male C57bl6/J mice	14 weeks	↓ Endotoxemia and plasma and adipose tissue pro-inflammatory cytokines ↑ Glucose tolerance and glucose-induced insulin secretion	17
Oligofructose (10%)	*ob/ob* mice C57BL/6	4 weeks	↓ Intestinal permeability ↓ Inflammatory markers (LPS, cytokines, etc.) ↑ Portal plasma GLP-2 levels and the jejunum and colon precursor proglucagon mRNA.	48

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

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 during the last trimester of pregnancy and over the 12-month postpartum period[53]. In human subjects, inulin-type fructans have also generally been found effective on normalization of metabolic disorder biomarkers, although the results have not been as consistent as those reported in animals[54]. Supplementation of inulin to subjects under a moderately high-carbohydrate, low-fat diet led to decreased hepatic lipogenesis and plasma TAG concentrations, suggesting an effect on the reduction of atherosclerosis risk factors[55]. Oligofructose intake also led to slightly significant effects on postprandial insulin response, but not on lipid metabolism in individuals with mild hypercholesterolemia[56]. 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 formula[57]. Daily consumption of oligofructose decreased the basal hepatic glucose production in healthy subjects, without any effect on insulin-stimulated glucose metabolism[58]. However, this prebiotic had no effect on glucose and lipid metabolism in type-2 diabetics[59]. In a pilot study with 10 human subjects, oligofructose treatment also increased satiety following breakfast and dinner, reduced hunger and prospective food consumption following dinner[60]. A long-term study (12 months) including 100 subjects revealed that those who received the prebiotic supplement had a smaller increase in BMI, BMI

https://doi.org/10.1017/S0029665110001813 Published online by Cambridge University Press

Table 2. Effects of probiotics, prebiotics and synbiotics on biomarkers of body weight regulation and metabolic disorders in human subjects

Probiotic/prebiotic (dose/d)	Administration pattern/ duration	Study-design*	Outcome	Reference
L. plantarum 299v ($5{\cdot}0 \times 10^7$ cfu/d fermented milk)	Hypercholesterolaemic patients/6 weeks	CRDB	↓Plasma LDL-cholesterol and fibrinogen	49
	Heavy smokers/6 weeks	CRDB	↓Systolic blood pressure, leptin and fibrinogen, F(2)-isoprostanes and IL-6	63
L. acidophilus 145 (10^{6-8} cfu/g), *B. longum* 913 (at least 10^5 cfu/g) and oligofructose (1%) in yoghurt with *S. thermophilus* and *Lactococcus lactis* (300 g/d)	Healthy women, 15 normocholesterolemic and 14 hypercholesterolemic Three periods of 7 weeks: (1) control for all, (2) and (3) control–symbiotic exchange	CO	↑Plasma HDL-cholesterol and ↓ LDL/HDL cholesterol ratio Total cholesterol and LDL-cholesterol NS	50
L. fermentum PCC (2×10^9 cfu/capsule; 2 capsules/d)	Hypercholesterolemic patients/10 weeks	CDB	Plasma total cholesterol, HDL-cholesterol, and TAG and liver enzymes NS	51
L. acidophilus DDS-1 *B. longum* UABL-14 (10^9 cfu) plus oligofructose (10–15 mg) per capsule; 3 capsules/d	55 normocholesterolemic subjects 2 months or 2 menstrual cycles	CRSB	Plasma concentrations of total cholesterol, HDL-cholesterol, LDL- cholesterol and TAG NS	52
L. rhamnosus GG and *B. lactis* Bb12 (10^9 cfu each/d) plus dietary counselling	Intake by women from first trimester of pregnancy onwards	CRSB/	↓Blood glucose concentrations and ↑glucose tolerance during pregnancy and over the 12-month postpartum period	53
Inulin (10 g/d)	Non-obese healthy subjects for 3 weeks	CRDB	↓Plasma TAG, ↓ Hepatic lipogenesis	55
		CO	Plasma cholesterol NS	
Oligofructose ($10{\cdot}6$ g/d)	Subjects with hypercholesterolemia for 2 months	CRDB	↓Postprandial insulin response	56
		CO	Lipids NS	
GOS and lcoligofructose (9:1) ($0{\cdot}6$ g/100 ml)	Infants till 6 months of age	CRDB	Plasma cholesterol and LDL cholesterol NS	57
Oligofructose (20 g/d)	Healthy subjects for 4 weeks	DB CO	↓Basal hepatic glucose production Insulin-stimulated glucose metabolism NS	58
Oligofructose (20 g/d)	Type 2 diabetics for 4 weeks	DB	Glucose and lipids NS	59
Oligofructose (16 g/d)	Healthy, non-obese subjects for 2 weeks	CRSB	↑Satiety following breakfast and dinner ↓Hunger and prospective food	60
Oligofructose (8 g/d)	Healthy, non-obese subjects for 12 months	CRDB	↓BMI BMI *Z*-score and total fat mass	61

cfu, colony-forming units; GOS, galactosyl-oligosaccharides; lcoligofructose, long-chain insulin type; NS, no significant effects; ↑, increase; ↓, decrease.
*C, placebo-controlled; R, randomized; DB, double-blind; SB, single-blind trial; CO, cross-over.

Z-score and total fat mass, compared with the control group[61].

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 Consejería 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

1. James WP (2008) The epidemiology of obesity: the size of the problem. *J Intern Med* **263**, 336–352.
2. Zeyda M & Stulnig TM (2007) Adipose tissue macrophages. *Immunol Lett* **112**, 61–67.
3. Xu J, Mahowald MA, Ley RE *et al.* (2007) Evolution of symbiotic bacteria in the distal human intestine. *PLoS Biol* **19**, e156.

4. Gill SR, Pop M, Deboy RT *et al.* (2006) Metagenomic analysis of the human distal gut microbiome. *Science* **312**, 1355–1359.
5. Turnbaugh PJ, Ley RE, Hamady M *et al.* (2007) The human microbiome project. *Nature* **449**, 804–810.
6. Turnbaugh PJ, Hamady M, Yatsunenko T *et al.* (2009) A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484.
7. Waldram A, Holmes E, Wang Y *et al.* (2009) Top-down systems biology modelling of host metabotype-microbiome associations in obese rodents. *J Proteome Res* **8**, 2361–2375.
8. Ley RE, Bäckhed F, Turnbaugh P *et al.* (2005) Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* **102**, 11070–11075.
9. Samuel BS, Hansen EE, Manchester JK *et al.* (2007) Genomic and metabolic adaptations of *Methanobrevibacter smithii* to the human gut. *Proc Natl Acad Sci USA* **104**, 10643–10648.
10. Turnbaugh PJ, Bäckhed F, Fulton L *et al.* (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* **17**, 213–223.
11. Cani PD, Amar J, Iglesias MA *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772.
12. Ley RE, Turnbaugh PJ, Klein S *et al.* (2006) Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023.
13. Nadal I, Santacruz A, Marcos A *et al.* (2008) Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. *Int J Obes* **33**, 758–767.
14. Santacruz A, Marcos A, Wärnberg J *et al.* (2009) Interplay between weight loss and gut microbiata composition in overweight adolescents. *Obesity* (Silver Spring) **17**, 1906–1915.
15. Kalliomäki M, Collado MC, Salminen S *et al.* (2008) Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* **87**, 534–538.
16. Turnbaugh PJ, Ley RE, Mahowald MA *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031.
17. Cani PD, Neyrinck AM, Fava F *et al.* (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**, 2374–2383.
18. Ley RE, Lozupone CA, Hamady M *et al.* (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol* **6**, 776–788.
19. Hooper LV, Midtvedt T & Gordon JI (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* **22**, 283–307.
20. Bäckhed F, Ding H, Wang T *et al.* (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* **101**, 15718–15723.
21. Flint HJ, Bayer EA, Rincon MT *et al.* (2008) Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol* **6**, 121–131.
22. Ridlon JM, Kang DJ & Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. *J Lip Res* **47**, 241–259.
23. Norin E (2008) Intestinal cholesterol conversion in adults and elderly from four different European countries. *Ann Nutr Metab* **52**, 12–14.
24. Hooper LV, Wong MH, Thelin A *et al.* (2001) Molecular analysis of commensal host-microbial relationships in the intestine. *Science* **291**, 881–884.
25. Bäckhed F, Manchester JK, Semenkovich CF *et al.* (2007) Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* **104**, 979–984.
26. Matarese G & La Cava A (2004) The intricate interface between immune system and metabolism. *Trends Immunol* **25**, 193–200.
27. Samuel BS, Shaito A, Motoike T *et al.* (2008) 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 USA* **105**, 16767–16772.
28. Fetissov SO, Hamze-Sinno M, Coëffier M *et al.* (2008) Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulation by gut microflora. *Nutrition* **24**, 348–359.
29. Bercik P, Verdú EF, Foster JA *et al.* (2009) Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of *Helicobacter pylori* infection. *Am J Physiol Regul Integr Comp Physiol* **296**, R587–R594.
30. Verdu EF (2009) Probiotics effects on gastrointestinal function: beyond the gut? *Neurogastroenterol Motil* **21**, 477–480.
31. Verdu EF, Bercik P, Huang XX *et al.* (2008) The role of luminal factors in the recovery of gastric function and behavioral changes after chronic *Helicobacter pylori* infection. *Am J Physiol Gastrointest Liver Physiol* **295**, G664–G670.
32. Bailey MT, Lubach GR & Coe CL (2004) Prenatal stress alters bacterial colonization of the gut in infant monkeys. *J Pediatr Gastroenterol Nutr* **38**, 414–421.
33. O'Mahony SM, Marchesi JR, Scully P *et al.* (2009) Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry* **65**, 263–267.
34. Sudo N, Chida Y, Aiba Y *et al.* (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J Physiol* **558**, 263–275.
35. Simonyte K, Rask E, Näslund I *et al.* (2009) Obesity is accompanied by disturbances in peripheral glucocorticoid metabolism and changes in FA recycling. *Obesity* (Silver Spring) **17**, 1982–1987.
36. Cani PD & Delzenne NM (2009) Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Curr Opin Pharmacol* **9**, 737–743.
37. Lajunen T, Vikatmaa P, Bloigu A *et al.* (2008) Chlamydial LPS and high-sensitivity CRP levels in serum are associated with an elevated body mass index in patients with cardiovascular disease. *Innate Immun* **14**, 375–382.
38. Erridge C, Attina T, Spickett CM *et al.* (2007) A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am J Clin Nutr* **86**, 1286–1292.
39. Reigstad CS, Lundén GO, Felin J *et al.* (2009) Regulation of serum amyloid A3 (SAA3) in mouse colonic epithelium and adipose tissue by the intestinal microbiota. *PLoS One* **4**, e5842.
40. Sato M, Uzu K, Yoshida T *et al.* (2008) Effects of milk fermented by *Lactobacillus gasseri* SBT2055 on adipocyte size in rats. *Br J Nutr* **99**, 1013–1017.
41. Yadav H, Jain S & Sinhá PR (2007) Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. *Nutrition* **23**, 62–68.
42. Yadav H, Jain S & Sinha PR (2008) Oral administration of dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* delayed the progression of streptozotocin-induced diabetes in rats. *J Dairy Res* **75**, 189–195.

NS Proceedings of the Nutrition Society

43. Martin FP, Wang Y, Sprenger N *et al.* (2008a) Probiotic modulation of symbiotic gut microbial–host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* **4**, 157.
44. Martin FP, Sprenger N, Yap IK *et al.* (2009) Panorganismal gut microbiome-host metabolic crosstalk. *J Proteome Res* **8**, 2090–2105.
45. Ma X, Hua J & Li Z (2008) Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *J Hepatol* **49**, 821–830.
46. Cani PD, Daubioul CA, Reusens B *et al.* (2005) Involvement of endogenous glucagon-like peptide-1(7–36) amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. *J Endocrinol* **185**, 457–465.
47. Delmée E, Cani PD, Gual G *et al.* (2006) Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. *Life Sci* **79**, 1007–1013.
48. Cani PD, Possemiers S, Van de Wiele T *et al*. (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091–1103.
49. Bukowska H, Pieczul-Mróz J, Jastrzebska M *et al.* (1998) Decrease in fibrinogen and LDL-cholesterol levels upon supplementation of diet with *Lactobacillus plantarum* in subjects with moderately elevated cholesterol. *Atherosclerosis* **137**, 437–438.
50. Kiessling G, Schneider J & Jahreis G (2002) Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. *Eur J Clin Nutr* **56**, 843–849.
51. Simons LA, Amansec SG & Conway P (2006) Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. *Nutr Metab Cardiovasc Dis* **16**, 531–535.
52. Greany KA, Bonorden MJ, Hamilton-Reeves JM *et al*. (2008) Probiotic capsules do not lower plasma lipids in young women and men. *Eur J Clin Nutr* **62**, 232–237.
53. Laitinen K, Poussa T & Isolauri E (2008) The Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota Group. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. *Br J Nutr* **19**, 1–9.
54. Reimer RA & Russell JC (2008) Glucose tolerance, lipids, and GLP-1 secretion in JCR:LA-cp rats fed a high protein fiber diet. *Obesity* **16**, 40–46.
55. Letexier D, Diraison F & Beylot M (2003) Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in human subjects. *Am J Clin Nutr* **77**, 559–564.
56. Giacco R, Clemente G, Luongo D *et al.* (2004) Effects of short-chain fructo-oligosaccharides on glucose and lipid metabolism in mild hypercholesterolaemic individuals. *Clin Nutr* **23**, 331–340.
57. Alliet P, Scholtens P, Raes M *et al.* (2007) Effect of prebiotic galacto-oligosaccharide, long-chain fructo-oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. *Nutrition* **23**, 719–723.
58. Luo J, Rizkalla SW, Alamowitch C *et al.* (1996) Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucosa production but had no effect on insulin-stimulated glucose metabolism. *Am J Clin Nutr* **63**, 939–945.
59. Luo J, Van Yperselle M, Rizkalla SW *et al.* (2000) Chronic consumption of short-chain fructooligosaccharides does not affect basal hepatic glucose production or insulin resistance in type 2 diabetics. *J Nutr* **130**, 1572–1577.
60. Cani PD, Joly E, Horsmans Y *et al.* (2006) Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr* **60**, 567–572.
61. Abrams SA, Griffin IJ, Hawthorne KM *et al.* (2007) Effect of prebiotic supplementation and calcium intake on body mass index. *J Pediat* **151**, 293–298.
62. Martin FP, Wang Y, Sprenger N *et al.* (2008b) Top-down systems biology integration of conditional prebiotic modulated transgenomic interactions in a humanized microbiome mouse model. *Mol Syst Biol* **4**, 205.
63. Naruszewicz M, Johansson ML, Zapolska-Downar D *et al*. (2002) Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers. *Am J Clin Nutr* **76**, 1249–1255.

https://doi.org/10.1017/S0029665110001813 Published online by Cambridge University Press