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The gut microbiota as a therapeutic target for obesity: a scoping review

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Abstract

There is mounting evidence that microbiome composition is intimately and dynamically connected with host energy balance and metabolism. The gut microbiome is emerging as a novel target for counteracting the chronically positive energy balance in obesity, a disease of pandemic scale which contributes to >70 % of premature deaths. This scoping review explores the potential for therapeutic modulation of gut microbiota as a means of prevention and/or treatment of obesity and obesity-associated metabolic disorders. The evidence base for interventional approaches which have been shown to affect the composition and function of the intestinal microbiome is summarised, including dietary strategies, oral probiotic treatment, faecal microbiota transplantation and bariatric surgery. Evidence in this field is still largely derived from preclinical rodent models, but interventional studies in obese populations have demonstrated metabolic improvements effected by microbiome-modulating treatments such as faecal microbiota transplantation, as well as drawing attention to the unappreciated role of microbiome modulation in well-established anti-obesity interventions, such as dietary change or bariatric surgery. The complex relationship between microbiome composition and host metabolism will take time to unravel, but microbiome modulation is likely to provide a novel strategy in the limited armamentarium of effective treatments for obesity.

Key words: **Microbiome: Obesity: Obesity intervention: Faecal microbiota transplantation: Diet: Bariatric surgery: Probiotics**

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Introduction

The human gut microbiome comprises up to 100 trillion microbes, consisting of at least 150 times more genes than the whole human genome[1]. Recent decades have seen a surge in research surrounding the cross-talk between gut microbiota and their host, the interactions between which have been implicated in a multitude of physiological processes and pathologies, from regulation of appetite signalling[2] to gut barrier function[3] and modulation of host immune responses[4].

Gut bacteria can modulate the digestibility and absorbability of dietary substrates, thereby influencing energy-harvesting efficiency[5–8]. The emerging understanding of the intimate relationship between intestinal microbiota and host metabolism has sparked considerable interest in the gut microbiome as a novel target for counteracting the chronic, positive energy balance in obesity. Obesity is a disease that has reached pandemic levels globally in the last 50 years and is a major public health issue, contributing to >70 % of premature deaths[9].

This scoping review explores the potential for therapeutic modulation of gut microbiota as a means of prevention and/or treatment of obesity and obesity-associated metabolic disorders. This review focuses on interventional approaches which have been shown to affect the composition and function of the intestinal microbiome, including dietary strategies[10,11], oral probiotic treatment[12], faecal microbiota transplantation (FMT)[13,14] and bariatric surgery[15,16] (Fig. 1). It goes on to consider opportunities for perinatal intervention, given the high susceptibility of the microbiome to metabolic alterations as it is established and evolves during early life[17,18].

The obese microbiome – an overview

The link between gut microbiota and obesity became a subject of scientific interest when it was observed that germ-free mice, which lack gut microbiota, have reduced adiposity and better glucose and insulin tolerance than their conventional counterparts[7]. Germ-free mice are unable to process food efficiently,

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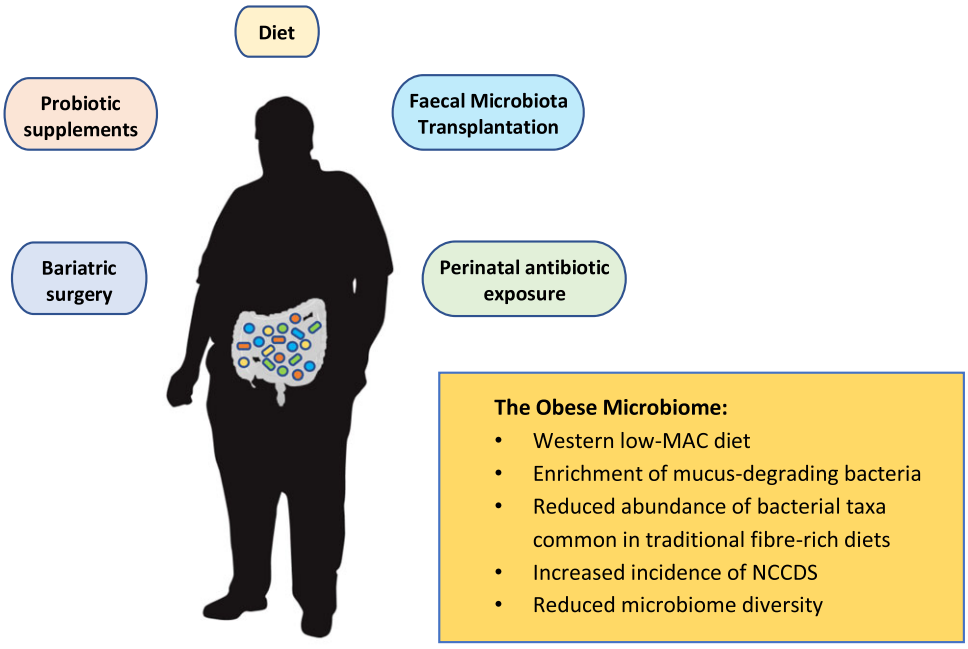

Fig. 1. Summary figure of interventions which demonstrate potential for altering the composition of the obese microbiome, and characteristic features of the obese microbiome. MAC, microbiota-accessible carbohydrates; NCCDs, non-communicable chronic diseases. Figure adapted from Sonnenburg and Sonnenburg 2019[45].

but gain weight following gut colonisation with almost any microbial population[1,7]. When germ-free mice are colonised with specified microbial populations (i.e. *gnotobiotic mice*), they gain weight despite *decreased* energy intake and increased energy expenditure relative to germ-free controls[7]. This counterintuitive phenomenon suggests that inoculation of germ-free mice with microbiota confers increased capacity to harvest calories from ingested food[1]. In 2006, pioneering work from Jeff Gordon's lab found that transfer of microbiota from obese to germ-free mice resulted in a significantly greater increase in body fat compared with gut colonisation with microbiota from lean donors[5]. This led to the suggestion that microbiome composition is a key driver of energy host balance, and that the apparent capacity of the 'obese microbiome' (i.e. microbiota from obese donors) to harvest more energy from the diet is a transmissible trait[5]. Since these preliminary findings suggested exciting potential for microbiome-related therapies to affect metabolic health, the past 15 years have seen a growing body of research which seeks to determine how our gut microbiota may alter the way we absorb, metabolise and store energy.

In humans and mice, over 90 % of the distal gut microbiota comprises species from two bacterial phyla: the Bacteroidetes and the Firmicutes[19]. The obese phenotype is often cited as being associated with an increased ratio of Firmicutes to Bacteroidetes phyla in the gut microbiome, as observed in both mice[20] and humans[21]. Though the association between obesity and the relative abundance of Bacteroidetes versus Firmicutes is often heralded as a robust finding, human and animal studies have yielded conflicting results about the precise nature of associations between obesity and microbiome composition[22–25]. An analysis comparing results of highly cited studies found that the variation in relative abundance of Bacteroidetes and Firmicutes was much greater between studies than between lean and obese subjects within any individual study[22]. Differing trends in Firmicutes:Bacteroidetes (F/B) ratios with increasing body mass index (BMI) have been reported in men versus women[26], and even in studies where obesity is associated with an increased F/B ratio, the association is not necessarily constant – F/B ratio has been seen to increase with BMI up to 33 and subsequently decrease when BMI > 33[26]. Important sources of inter-study variation include methodological differences such as bacterial sequencing methods, bacterial sample source (e.g. duodenum versus faeces) and differences in BMI categories in different countries[27]. Furthermore, the obese microbiome has been shown to have markedly reduced microbial diversity in faecal metagenome analysis comparing human twin pairs discordant for obesity[21], though this too is not consistently demonstrated[22]. As yet, it seems that there is no simple taxonomic signature of obesity in the human gut microbiota. Indeed, a taxonomic signature alone may be of little significance without accompanying functional analysis at the species level, since closely related taxa can have widely varying functions whilst distantly related taxa may function similarly[22].

Much of our current understanding of the role of host–microbe interactions in obesity is derived from studies on germ-free mice. Although germ-free mice can survive on a standard diet, the lack of an established gut microbiome is associated with a plethora of physiological abnormalities such as underdeveloped intestinal morphology[28], decreased basal metabolic rate[28] and reduced immune resistance to infection[29]. Immune-mediated components may play an important role in the mechanisms underlying microbiota-driven weight gain. Indeed, the introduction of gut bacteria in germ-free mice seems to trigger the formation of isolated lymphoid follicles and cause

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structural changes in intestinal epithelial cells which line the gut and act as a physical barrier between gut luminal contents and underlying cells of the immune system[29]. As well as triggering changes in intestinal morphology and physiology, the commensal gut bacteria themselves provide 'colonisation resistance' which intestinal pathogens must overcome in order to establish infection[29]. Colonisation of germ-free animals with some commensal bacteria species has been shown to be protective against intestinal bacterial pathogens[30,31]. Given the intimate and dynamic relationship between the immune system and the microbiota, it is likely that weight gain associated with gut colonisation is at least in part immune-mediated, especially in the context of gnotobiology.

One of the hallmarks of obesity is a state of chronic, low-grade inflammation. The presence of excess nutrients seems to drive activation of specialised immune cells and lead to an unresolved inflammatory response within adipose tissue[32]. Studies in conventional mouse models and humans suggest that the microbiota may play a role in modulating obesity-associated inflammation. Lipopolysaccharides (LPS) or 'endotoxins' are bacterial cell wall components thought to be involved in the initiation of obesity-associated inflammation[33]. Studies investigating LPS, which activates the host's innate immune system through macrophage surface receptor Toll-like receptor 4 (TLR4), demonstrate increased levels of circulating LPS in mice fed a high-fat diet[34] and increased TLR4 activation in obesity-prone rats with altered gut microbiota[35]. Similar findings have been shown in humans, with obese and type 2 diabetic subjects showing higher baseline circulating LPS levels than non-obese controls, as well as a greater rise in LPS levels after eating a high-fat meal[36]. Obesity-prone rats treated with antibiotics show decreased levels of LPS and TNF-α expression in the intestine, alongside a reduction in body weight and improvements in glucose tolerance[37,38], whilst TLR4-knockout mice seem to be protected against insulin resistance induced by a high-fat diet[39]. It has been suggested that obesity-associated endotoxemia may be in part driven by microbiota-induced increases in gut permeability, causing LPS translocation and a subsequent increase in circulating LPS levels[34].

Another potential key player in obesity-associated inflammation may be short-chain fatty acids (SCFAs). SCFAs are derived from gut microbial fermentation of indigestible dietary polysaccharides and serve as the main energy source for colonocytes as well as being substrates for lipid storage and regulating appetite via G-protein-coupled receptor signalling[1]. SCFAs have a well-characterised anti-inflammatory effect on colonic epithelium and immune cells, as demonstrated in a study by Maslowski *et al.* in which mice deficient in GPCR 43, a receptor known to be stimulated by SCFAs, showed exacerbating or unresolving inflammation in models of colitis, arthritis and asthma[40]. Germ-free mice produce almost no SCFAs[40], and it has been suggested that adiposity seen in gnotobiotic mice may be partly due to the increased availability of SCFAs brought about by the transplanted bacteria which could in turn increase energy harvesting from ingested foods[1]. SCFAs have also been shown to reduce the release of inflammatory cytokines[40] which may in turn enhance hypothalamic sensitivity to the satiety hormone leptin[41].

Despite a wide consensus that gut microbiota composition is linked to host energy balance, the host–microbe mechanisms underlying this complex process in humans remain elusive, as human studies have been primarily epidemiological[1]. Microbiome communities are complex networks of bacteria, archaea, fungi, viruses and protozoa – all of these components, together with their metabolites, could contribute to the obese phenotype, exerting either individual or synergistic effects[42].

Your microbiome is what you eat: gut populations are plastic

The gut microbiome is plastic and adaptable in the face of a changing nutritional environment. A large increase in fibre intake can substantially alter microbiota composition and function over 1–2 days, as might be expected for a complex microbial community that must adapt to rapid turnover during day-to-day dietary variation[10]. The influence of dietary alteration on microbiome composition has been recognised since the early twentieth century, when Herter and Kendall used culture-based methods to demonstrate that protein-dominated diets could shift the bacterial microbiota in monkeys and cats, increasing abundance of proteolytic bacteria and decreasing *Lactobacillus* and *Bifidobacterium* species[43,44]. Germ-free mice colonised with human microbiota and fed a fibre-rich diet show significant up-regulation of bacterial genes involved in polysaccharide metabolism[11]. However, when a no-fibre diet is given, enrichment of bacterial genes involved in degradation of glycans from the surrounding host mucus is observed[11]. This demonstrates the flexibility of the gut microbiota to undergo functional adaptations when confronted with dietary change.

Recent decades have seen the human microbiota undergo substantial remodelling in industrialised societies, coincident with increased antibiotic use and sanitation, and industrialisation of food production[45]. The modern 'Western diet' consists of processed foods rich in fat, sugar, protein and additives, with relatively sparse amounts of micronutrients or dietary fibre[46,47]. Dietary fibres which are indigestible by the host but can be broken down by gut bacteria-derived enzymes are termed microbiota-accessible carbohydrates (MACs)[48]. MACs serve as the major energy source for colonic bacteria[49]. MAC deprivation in modern Western diets seems to favour shifts in gut microbial compositions to enrichment of mucus-degrading microorganisms[45,49], emergence of antibiotic-resistant species[45], and loss of seasonally volatile species due to the stable homogeneity of the industrialised diet[50] (Fig. 1). This contrasts starkly with the microbiome of individuals from an isolated Yanomami Amerindian village with no known previous contact with Western people, whose faecal, oral and skin bacterial microbiome were found to exhibit the highest diversity of bacteria and genetic functions ever reported in a human group[51].

It has been proposed that we currently face a mismatch between our recently altered microbiota and the more slowly evolving human genome[45,52]. Molecular signals generated by microbial taxa which have become extinct in the 'industrialised' microbiome are likely to have influenced the evolution of the human genome[45]. It is speculated that loss of these microbial signals might explain physiological abnormalities including dysregulated immune function and a chronic baseline of

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inflammation which may drive the increased prevalence of non-communicable chronic diseases such as metabolic syndrome, atherosclerosis and autoimmune disease[45]. In a Colombian population in the midst of Westernisation, gene sequencing of stool samples found that microbiomes dominated by pathobionts such as *Escherichia coli* and *Enterobacter hormaechei* were associated with increased BMI and waist circumference, as well as an increased risk of obesity and cardiovascular disease, relative to individuals whose microbiomes comprised taxa associated with diets rich in fibre and complex carbohydrates[53]. Germ-free mice fed a low-fibre diet exhibit lower species diversity in their gut microbiota compared with their high-fibre-fed counterparts. This effect is partially reversible upon returning to a normal diet[54]. The loss of diversity is compounded with each subsequent generation of mice maintained on a low-fibre diet, and the ability to recover is reduced, suggesting extinction of some microbial species associated with low fibre intake[54,55].

In a 1-year dietary intervention study in which twelve obese people were assigned to either fat-restricted or carbohydrate-restricted low-calorie diets, the percentage weight loss of participants was correlated with increased abundance of Bacteroidetes and not with changes in dietary calorie content[56] (Table 1). These changes were phylum-wide and not due to increases or losses of specific bacterial species – indeed, bacterial species-level diversity within individuals' microbiota remained constant over time[56]. The association of obesity with profound, phylum-wide changes in microbiota composition despite inter-individual differences in species-level composition might suggest that the factors which drive phylum-wide microbiota shifts operate on highly conserved bacterial traits common to many species within each phylum[56]. Although dietary fibres can drive changes in microbiota composition, the baseline composition of an individual's microbiome will influence the extent of change possible. A study in which fourteen participants with metabolic syndrome were put on a standardised diet found that two of the participants demonstrated a markedly reduced ability to digest orally administered resistant starch[57]. It was suggested that the low starch fermentation rate in the two diet-unresponsive individuals could be partially attributed to markedly low numbers of *Ruminococcus bromii*-related taxa in their colonic microbiota at baseline[57]. Host genetics add a further layer of complexity to individual energy balance. Concordance rates of body adiposity between monozygotic twins is double that of dizygotic twins[58], despite showing similar degrees of co-variation in microbiota composition[21].

It has been proposed that re-establishment of 'compatibility' between the microbiome and the human genome might require a controlled 're-wilding' process in which microbial species and/or functions now absent or sparse in industrialised microbiomes are re-established through increased consumption of foods which support engraftment of these species in the gut[45]. Using mice colonised with different human microbiota, Shepherd *et al.* demonstrated that introduction of *Bacteroidetes ovatus* or *NB001*, a commensal species which utilises *porphyran* (a polysaccharide abundant in seaweed) resulted in a predictable rise in levels of NB001 when the mice were fed seaweed[59]. The introduction of porphyran into the diet rescued NB001 from undetectable levels even in mice with the most resistant microbiota[59]. Although the utility of this particular polysaccharide system may only apply to individuals colonised with competing porphyran users (e.g. a limited subset of the Japanese population), these results provide an intriguing proof-of-concept for controlling strain engraftment into the gut microbiome, using select pairs of nutrients and their cognate utilisation systems to stimulate blooming of select bacterial species[59].

Probiotics for weight loss: credible or a con?

Another potential means of microbiota manipulation is oral consumption of viable bacterial strains. So-called *probiotic* dietary supplements are a multi-billion dollar industry, claiming efficacy for ameliorating a wide range of diseases ranging from irritable bowel syndrome and sepsis to dermatitis and depression[60]. The rationale for therapeutic use of probiotics is to introduce selected bacterial strains associated with health benefits into the microbiome. Given the emerging links between obesity and associated shifts in microbiome composition[20,21], it has been speculated that probiotics may serve as therapeutic agents in the context of obesity and associated metabolic disorders[61]. However, the mechanisms of action underlying the capacity of administered microorganisms to colonise the host gastrointestinal mucosa remain poorly understood. This is in part due to the limitations of analyses such as stool assessment, or *in vitro* cell studies which are unlikely to represent the complex host–microbiome interactions underlying the colonisation process *in vivo*[60]. Despite the popularity of probiotic supplements, decades' worth of research on the efficacy of probiotics in treating disease have spawned conflicting claims which remain inconclusive[60].

Some randomised controlled trials (RCTs) have suggested that probiotics lower BMI and visceral fat mass. A multicentre RCT involving eighty-seven adults found that daily oral consumption of fermented milk containing *Lactobacillus gasseri* over a 12-week period was associated with significant reductions in abdominal visceral fat (4·6 % reduction) and waist circumference (1·8 % reduction)[61] (Table 1), both parameters known to be closely correlated with high risk of metabolic syndrome and cardiovascular disease[62]. In another RCT, twenty non-obese males were participants to receive either placebo or VSL#3 (a commercial multispecies probiotic) daily, alongside a high-fat, hyperenergetic diet over 4 weeks[63] (Table 1). The authors claimed that VSL#3 supplementation protected participants from gaining body mass and fat mass, based on a mean increase of 0·8 in BMI and 10·3 % increase in body fat mass in the placebo group versus an unchanged mean BMI and a 3·7 % increase in body fat mass in the probiotic-supplemented group[63]. However, the small sample size (placebo group $N = 11$, probiotic group $N = 9$) and inclusion of only young, lean male participants in the study means that such results can hardly be extrapolated to an obese population.

In 2018, Borgeraas *et al.* published a systematic review and meta-analysis of RCTs conducted to examine the effects of probiotic supplementation on body weight, BMI and fat mass, comprising fifteen studies, 957 participants (63 % women) all with a mean BMI > 25 (i.e. overweight or obese) and ranging from 18 to 75 years of age[64]. Meta-analyses including thirteen studies found that, compared with placebo, the probiotic-supplemented groups showed greater weight loss (weighted mean difference

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Table 1. Summary of characteristics of studies examining the effects of different interventions on metabolic outcomes such as body weight, fat mass and insulin sensitivity

Authors (year)	Study design	Study population characteristics	Intervention group	Control group	Significant findings
Ley *et al.* (2006)[56]	Obese participants randomised to either fat-restricted or carbohydrate-restricted low-calorie diets for 1 year Gut microbiota composition monitored over 1 year post-dietary intervention using 16S rRNA sequencing	Obese participants: 21–65 years old, BMI 30–43 kg/m^2, 9 female, 3 male	12 obese people Fat-restricted diet ($N = 6$) Carbohydrate-restricted diet ($N = 6$)	2 lean people on a 'control' diet	Over the 1-year dietary intervention, relative abundance of Bacteroidetes increases and abundance of Firmicutes decreased in obese participants, irrespective of diet type Percentage weight loss of obese participants correlated with increased abundance of Bacteroidetes and not with changes in dietary calorie content
Kadooka *et al.* (2010)[61]	Multicentre RCT Obese participants randomly assigned to receive fermented milk (200 g daily), with or without *Lactobacillus gasseri* over 12 weeks	87 participants with BMI 24·2–30·7 kg/m^2 Participants with diabetes excluded	43 participants (29 male, 14 female) with mean BMI 27·5 kg/m^2 receiving fermented milk with *Lactobacillus gasseri*	44 participants (30 male, 14 female) with mean BMI 27·2 kg/m^2 receiving fermented milk without *Lactobacillus gasseri*	Daily oral consumption of fermented milk containing *Lactobacillus gasseri* over a 12-week period associated with significant reductions in abdominal visceral fat (4·6 % reduction) and waist circumference (1·8 % reduction) relative to control group
Osterberg *et al.* (2015)[63]	Non-obese participants randomised to receive either placebo or VSL#3 (a commercial multispecies probiotic) daily, alongside a high-fat, hyperenergetic diet over 4 weeks	20 non-obese, healthy males (18–30 years old)	9 male participants with mean BMI 23·2 kg/m^2 receiving VSL#3	11 male participants with mean BMI 24·0 kg/m^2 receiving placebo	Mean increase of 0·8 in BMI and 10·3 % increase in body fat mass in the placebo group Unchanged mean BMI and a 3·7 % increase in body fat mass in the probiotic-supplemented group
Ridaura *et al.* (2013)[14]	Germ-free mice received FMT from human twins discordant for obesity All recipient mice fed *ad libitum* a low-fat, high-polysaccharide diet up to 35 d post-FMT	FMT donors: Four human twin pairs (1 MZ, 3 DZ pairs), discordant for obesity (obese twin BMI>30 kg/m^2) with a sustained multiyear BMI difference of ≥5·5 kg/m^2 Recipients were adult male germ-free C57BL/6J mice	$N = 3$–4 mice per donor microbiota sample per experiment; $N = 1$–5 independent experiments per microbiota	N/A	Mice receiving FMT from obese donors showed significantly greater body mass and adiposity compared with those receiving FMT from lean donors Mice transplanted with FMT from obese donors *and* co-housed with mice transplanted with FMT from lean donors showed less adiposity compared with their counterparts not housed with lean mice Weight gain prevention was only observed when mice were fed a low-fat/high-fibre diet, and was abolished in mice fed a high-saturated fat/low-fibre diet

Table 1. (*Continued*)

Authors (year)	Study design	Study population characteristics	Intervention group	Control group	Significant findings
Vrieze *et al.* (2012)[69]	Men with metabolic syndrome underwent bowel lavage followed by random assignment to receive a small intestinal infusion from either an allogenic lean male donor, or an autologous sample (i.e. reinfusion of their own faeces)	FMT donors: lean healthy Caucasian males (BMI < 23 kg/m^2) FMT recipients: 18 men with metabolic syndrome, aged ˜40–60 years old) Exclusion criteria included use of any medication, probiotics and/or antibiotics in the past 3 months	9 men receiving allogenic gut microbiota infusion	9 men receiving autologous gut microbiota infusion	Participants in the allogenic infusion group showed significant improvement in peripheral insulin sensitivity 6 weeks post-infusion, as well as a trend towards improvement in hepatic insulin sensitivity, compared with autologous infusion group
Kootte *et al.* (2017)[71]	Men with metabolic syndrome randomised to receive an autologous FMT or an allogenic FMT from one of 11 healthy lean donors	FMT donors: 11 lean healthy Caucasian males (BMI < 23 kg/m^2) FMT recipients: 38 Caucasian men with metabolic syndrome, aged 21–69 years old and BMI > 30 kg/m^2 Exclusion criteria included use of probiotics and/or antibiotics or any medication known to influence gut microbiota composition in the past 3 months	26 men receiving allogenic gut microbiota infusion	12 men receiving autologous gut microbiota infusion	Six weeks post-FMT, participants receiving allogenic FMTs had improved peripheral insulin sensitivity and a significant decrease in HbA1c (39·5 to 38·0 mmol/mol), whilst no significant changes were seen in the autologous FMT group No improvements in metabolic parameters observed at 18 weeks, by which time duodenal and faecal microbiota composition had returned to baseline
Liou *et al.* (2013)[15]	Obese mice fed a high-fat diet underwent either RYGB or sham operations Germ-free mice inoculated with caecal contents from RYGB donors or sham-operated donors	Microbiota donor mice: diet-induced obese male C57BL/6J mice Microbiota recipient mice: lean, germ-free male Swiss Webster mice, age-matched with donors	*N* = 10 germ-free mice inoculated with caecal contents from RYGB-operated mice	*N* = 10 germ-free mice inoculated with caecal contents from sham-operated mice *N* = 7 uninoculated germ-free mice	Germ-free mice inoculated with caecal contents from RYGB donor mice exhibited a significant decrease in body weight 2 weeks post-colonisation, whereas germ-free mice which were uninoculated or inoculated with caecal contents from sham-operated mice exhibited no significant weight change
de Groot *et al.* (2020)[78]	Obese men with metabolic syndrome received allogenic FMT either from post-RYGB donors or from donors with metabolic syndrome	FMT recipients: 22 obese Caucasian males aged 21–69 years old with BMI ≥ 30 kg/m^2	*N* = 10 men receiving FMT from donors with metabolic syndrome *N* = 12 men receiving FMT from post-RYGB donors	N/A	At baseline, RYGB donors were significantly more insulin-sensitive than recipients receiving FMT from RYGB donors There was a significant change in peripheral insulin sensitivity (Rd) on FMT from RYBG donors versus FMT from donors with metabolic syndrome – this difference was mainly driven by a significant decrease in Rd in the group receiving FMT from metabolic syndrome donors, while the group receiving FMT from post-RYGB donors showed a mild (but statistically insignificant) improvement in Rd 2 weeks post-FMT

Table 1. (*Continued*)

Authors (year)	Study design	Study population characteristics	Intervention group	Control group	Significant findings
Cox *et al.* (2014)[17]	Mice received low-dose penicillin (LDP) (1 μg/g body weight) continuously for life either from birth (LDP-b) or from weaning at day 28 of life (LDP-w)	C57BL/6J mice	*N* = 11 mice (5 male, 6 female) receiving LDP beginning at weaning at day 28 of life *N* = 9 mice (5 male, 4 female) receiving LDP from birth	*N* = 11 control mice (6 male, 5 female) had no penicillin exposure	Prior to weaning, male LDP-b mice had increased total mass and fat mass at 20 weeks of life, relative to controls. LDP-w male mice showed similar trends; however, later LDP exposure had lesser effects on body composition. Female LDP-b mice, but not LDP-w, had significantly elevated total mass but not fat mass at week 20 Feeding on a high-fat diet had an additive effect on LDP-induced weight gain
Mueller *et al.* (2015)[84]	Longitudinal observational study following 436 mother–child pairs up to 7 years post-partum Prenatal antibiotic use ascertained by a questionnaire administered late in the third trimester and delivery mode by medical record	436 mother–child pairs. 52·5 % of children female. Mean pre-gravid BMI of mothers = 25·8 kg/m^2 366 out of 436 children exposed to antibiotics during the second or third trimester 337 children delivered vaginally, 99 delivered by caesarean section	N/A	N/A	Children exposed to prenatal antibiotics during the second or third trimester had an 84 % higher risk of obesity compared with unexposed children
Luoto *et al.* (2010)[83]	Pregnant women randomised to receive daily *Lactobacillus rhamnosus* probiotics or placebo 4 weeks before expected delivery and for 6 months postnatally Growth patterns of infants monitored during a 10-year follow-up period	113 women completed the 10-year follow-up All children included had at least one close relative with atopic dermatitis, allergic rhinitis or asthma	*N* = 54 children in the probiotics group completed the 10-year follow-up 63 % male, mean gestational age at birth 39·3 weeks, 13 % caesarean-section delivery	*N* = 59 children in the placebo group completed the 10-year follow-up 58 % male, mean gestational age at birth 39·4 weeks, 12 % caesarean-section delivery	In children exposed to perinatal probiotics, the first phase of excessive weight gain (foetal period up to 48 months) seemed to be restrained, particularly in children who later became overweight

rRNA, ribosomal RNA; RCT, randomised controlled trial; FMT, faecal microbiota transplant; MZ, monozygotic; DZ, dizygotic; BMI, body mass index; LDP, low-dose penicillin.

−0·60 [95 % CI −1·19, −0·01] kg]) and greater reduction in BMI (weighted mean difference −0·27 [95 % CI −0·45, −0·08] kg m^{-2})[64]. Though these differences were statistically significant, the actual effect sizes were small[64]. In the seven studies reporting fat mass as an outcome, the effect of probiotic supplementation on fat mass was not significant (−0·42 [95 % CI −1·08, 0·23] kg)[64]. Sensitivity analyses showed that the effects of probiotic supplementation were reduced when restricting analyses to include only subjects clearly classified as overweight or obese – in these cohorts, BMI reduction was smaller (weighted mean difference −0·14 [95 % CI −0·45, −0·18] kg m^{-2}), as was weight loss (weighted mean difference −0·25 [95 % CI −1·06, 0·56] kg)[64]. One-third of the studies included in this review included two or multiple species of probiotics in the test supplement[64]. Every unique probiotic strain will have a different therapeutic potential, so meta-analyses that assess all bacterial strains together are of limited utility. Furthermore, many studies testing efficacy of probiotic supplements are appreciably underpowered[64].

A 2017 systematic review by Crovesy *et al.* analysed fourteen RCTs (1067 participants in total) examining the effects of *Lactobacillus* probiotic supplementation on body weight and/or fat mass in participants aged 19–60 years[65]. Two studies reported weight gain in the *Lactobacillus*-supplemented group, whilst three showed no difference in body weight between groups[65]. Nine studies did observe weight or fat loss in the probiotic-supplemented group, although some studies included additional weight loss interventions alongside probiotic supplementation including a hypoenergetic diet in two studies and Roux-en-Y gastric bypass surgery (RYGB) in another[65], making it difficult to attribute any clinical outcome to probiotics alone.

Most currently available probiotic preparations have not been formulated with a specific metabolic target, and most lack the density or complexity required to influence an established gut microbiota. It is therefore not surprising that currently available probiotic treatments have not conferred clinically significant health benefits for patients with obesity or associated metabolic disorders. A lack of understanding of the mechanisms of action underlying gut colonisation by administered probiotics makes it difficult to design probiotic studies that fulfil a particular therapeutic goal. Research efforts to develop rationally designed microbiome-targeted therapies by identifying candidate organisms that confer a metabolic benefit have a better chance of yielding clinically useful therapies.

Faecal microbiota transplantation: a future in obesity treatment?

FMT involves transferring the whole faecal microbial community from a healthy donor into the intestinal tract of a recipient, in order to modify intestinal microbial composition and function[13]. Arguably, FMT is the most persuasive experimental tool to demonstrate a causal role for gut microbiota in human disease[42]. Indeed, FMT showed the role of manipulating the gut microbiota in both treating and preventing disease associated with *C. difficile*[66] and is now an established treatment for patients with recurrent or refractory *C. difficile* infections[13,67]. Preclinical studies have shown that FMT can reverse obesity in mice[14], so it is an attractive microbiome-targeting therapy for obesity, although studies in humans remain few.

In a fascinating study by Ridaura *et al.*, germ-free mice received FMTs from human twins discordant for obesity[14]. Despite all mice being fed the same low-fat/high-fibre diet, mice receiving FMT from obese donors showed significantly greater body mass and adiposity compared with their counterparts receiving FMT from lean donors[14] (Table 1). Mice are coprophagic, so share the microbiome of cohabiting mice. The authors therefore carried out an experiment in which mice respectively transplanted with lean and obese microbiota were co-housed[14]. Mice transplanted with FMT from obese donors *and* co-housed with mice transplanted with FMT from lean donors showed less adiposity compared with their counterparts not housed with lean mice[14]. Faecal metagenome analysis pre- and post-co-housing showed that the microbiota of obese-phenotype mice were successfully invaded by bacteria (largely of the Bacteroidetes phylum) from the microbiota of the lean-phenotype mice when these mice were co-housed[14]. The invasion of obese mice with 'lean microbiota' was sufficient to reshape their microbiota and prevent the development of increased body mass and obesity-associated metabolic phenotypes[14]. Importantly, this effect was *diet-dependent* – weight gain prevention was only observed when mice were fed a low-fat/high-fibre diet, and was abolished in mice fed a high-saturated fat/low-fibre diet[14]. These findings support the concept that therapeutic establishment of a defined 'lean' microbiota could be an effective way of preventing obesity, with the important caveat that a concomitant healthy diet would be necessary[68].

In one of the few human studies investigating the efficacy of FMT in an obesity-related context, eighteen men with metabolic syndrome were randomly assigned to receive a small intestinal infusion from either an allogenic lean male donor, or an autologous sample (i.e. reinfusion of their own faeces)[69] (Table 1). Relative to the autologous group, subjects in the allogenic group showed significant improvement in peripheral insulin sensitivity 6 weeks post-infusion, as well as a trend towards improvement in hepatic insulin sensitivity[69]. Of note, men in the allogenic group showed significant increases in butyrate-producing bacteria in both faecal and duodenal samples, leading the authors to suggest a role for butyrate in contributing to improvement in insulin sensitivity[69], as observed in mice[70].

More recently, the same group carried out another study in which thirty-eight men with metabolic syndrome were randomised to receive an autologous FMT or an allogenic FMT from one of eleven healthy lean donors[71] (Table 1). At six weeks post-FMT, participants receiving allogenic FMTs exhibited metabolic improvements, including improved peripheral insulin sensitivity and a significant decrease in HbA1c (39·5 to 38·0 mmol/mol), whilst no significant changes were seen in the autologous FMT group. Although half the subjects receiving allogenic FMT showed clinically relevant improvements at six weeks, these metabolic improvements were transient – no metabolic changes were observed at eighteen weeks, by which time duodenal and faecal microbiota composition had returned to baseline[71]. The short-lived nature of the metabolic improvements seen in the allogenic FMT group is perhaps due to the host immune system developing resilience, which, together with

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lifestyle factors such as diet, may drive the intestinal microbiota composition back to baseline[72].

Whilst FMT is being increasingly used clinically to treat recurrent *Clostridium difficile* infections, it is worth noting that the data remain limited on the full range of possible adverse effects of this treatment[73]. A notable case report is that of a 32-year-old female who underwent FMT for recurrent *C. difficile* and reported unintentional rapid weight gain of 15·4 kg over 16 months, increasing her BMI to 33 from a baseline of 26, despite a medically supervised diet and exercise programme[73]. There are many confounding factors at play here, not least the recipient's long-standing diarrhoeal infection and treatment with an extensive cocktail of antibiotics before and after FMT, and the lack of any microbiome sequencing data comparing the patient and donor is a key limitation. Nevertheless, the observed increase in BMI to 34·5 at 36 months post-FMT (from a baseline BMI of 26) raises the possibility that the FMT played a causal role in this substantial and long-lasting weight change[73], which would align with Ridaura *et al.*'s findings in animal models[14].

So far, the apparent metabolic benefits of allogenic FMT seen in obese human cohorts show some promise, although human studies remain limited in the sample size and range of patient phenotypes included. Further randomised clinical trials should extend selection criteria to a range of obese phenotypes beyond those with metabolic syndrome and explore a broad range of clinically relevant outcomes, such as long-term glycaemic control, truncal weight loss, or onset of obesity-associated co-morbidities. Additionally, it is worth noting that the two RCTs cited above[69,71] included male participants only. Given evidence that microbiota composition differs by sex in a BMI-specific manner[26], it is important that we obtain sex-specific data on the efficacy of microbiome-targeted interventions for obesity.

Does microbiome modulation contribute to metabolic improvements after bariatric surgery?

Bariatric surgery, which results in malabsorption and improved satiety, is the ultimate therapeutic resort for morbidly obese patients and is superior to any other weight-loss intervention[62,74,75]. Within days after surgery, patients show improvement in metabolic parameters such as fasting glucose levels[74], before any significant change in weight. These early effects of bariatric surgery are thought to be driven by altered glucose homeostasis in the duodenum and by calorie restriction. Indeed, BMI- and HbA1c-matched patients undertaking either a very low-calorie diet or RYGB show insignificant differences in β-cell function and weight loss 3 weeks after intervention[76]. However, it is thought that the longer-term durability of weight reduction and glycaemic control post-gastric bypass is attributable to other factors, such as altered incretin hormone secretion or bile acid malabsorption[77], which induce significant alterations in microbiome composition[74].

In mouse models of RYGB, pre- and post-surgical faecal metagenome analysis found that RYGB led to a sustained alteration of the gut microbiota within 1 week of surgery[15] (Table 1). There were substantial increases in proportions of Verrucomicrobia and Proteobacteria phyla[15], mirroring microbiota changes seen in humans post-gastric bypass surgery[16]. Germ-free mice inoculated with caecal contents of RYBG-operated mice showed significantly greater reduction in fat mass relative to those inoculated with microbiota from sham-operated donors[15], suggesting that gastric bypass surgery confers a shift in microbiota composition which renders the host less predisposed to weight gain. In a human study, subjects with metabolic syndrome receiving allogenic FMT from post-RYGB donors (RYGB-D) showed a mild improvement in peripheral insulin sensitivity (33·9 to 36·2 μmol/kg/min) 2 weeks post-FMT[78] (Table 1). At baseline, RYGB donors were significantly more insulin-sensitive than RYGB-D FMT recipients[78]; hence, these data suggest that healthy metabolic characteristics can be successfully acquired through FMT.

Exciting as such findings may be, they reflect a short time frame and there remains a lack of data which capture microbiome evolution at several time points after surgical intervention[79]. Aron-Wisnewsky *et al.* followed twenty-four severely obese patients at 1, 3 and 12 months post-bariatric surgery and found that microbial gene richness (MGR) is only partially restored in most patients, who retain a low MGR despite exhibiting weight loss or major metabolic improvements[80]. MGR improvement seems to reach its peak at 1 year and shows no further improvement 5 years after surgery[80], leading the authors to suggest that additional interventions such as specialised diets or FMT should be considered before or after bariatric surgery in severely obese individuals, in order to boost MGR[80].

Some studies using RYGB mouse models have provided = insight into the mechanisms driving microbiome modulation post-bariatric surgery. RYGB-operated mice and gnotobiotic mice receiving RYGB microbiota show greater propionate and lower acetate production compared with sham-operated controls[15]. Propionate has the highest known affinity of any SCFA for the GPR41 receptor, and GPR41-knockout mice show reduced energy expenditure and increased adiposity[81]. Another proposed mechanism for microbiota-driven weight change points to the profound increase in total circulating bile acids that follows bariatric surgery. As well as aiding lipid digestion and absorption, bile acids contribute to regulation of several metabolic processes by binding to the *farnesoid X receptor (FXR)*. Obese FXR-knockout mice failed to maintain initial weight loss after vertical sleeve gastrectomy (a common bariatric surgical procedure), whilst their wild-type counterparts maintained weight loss over 11 weeks[75]. Caecal microbiota abundance of *Bacteroides* was reduced in wild-type gastrectomy mice compared with sham-operated controls, but did not differ among FXR-knockout mice[75]. The authors suggested that FXR signalling may link altered bile acid homeostasis to post-operative changes in gut microbial composition, potentially being an important mediator in the maintenance of weight loss following gastrectomy[75]. Further mechanistic studies such as these are warranted, as the discovery of metabolic pathways on which the complex microbiota network converge could reveal powerful therapeutic targets for microbiome modulation.

Nipping obesity in the bud: perinatal prevention of obesity

The establishment and maturation of the intestinal microbiota begins in pregnancy. The neonatal microbiota is highly

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susceptible to perturbations such as delivery route[82], antibiotic treatment[18] or dietary changes[18,83]. Much of an individual's founding microbiota is acquired at birth and matures gradually, reaching adult-like complexity by about 3 years of age[18]. Microbiota disturbances during these early years have been associated with negative metabolic effects such as obesity in later life. There has been speculation about whether medical advances such as caesarean section deliveries, antibiotics and formula milk feeding might contribute to the obesity pandemic[18].

Early life represents a window of metabolic vulnerability. Mice administered low-dose penicillin (LDP) at birth show greater weight gain than unexposed mice, or mice exposed to LDP at weaning[17] (Table 1). Feeding on a high-fat diet has an additive effect on LDP-related weight gain, demonstrating the synergistic effects of dietary excess and early microbiota disturbance[17]. Notably, LDP-related metabolic disturbance is sustained in adulthood beyond cessation of antibiotic treatment – LDP-treated mice develop adult-onset obesity, despite recovery of the microbiota 4 weeks after stopping antibiotic treatment[17]. These findings support the idea that even transient microbiome disturbances in early life can have long-lasting metabolic effects.

The long-term metabolic impact of early-life antibiotics has similarly been observed in humans. A longitudinal study following 436 mother–child pairs up to 7 years post-partum showed that children exposed to prenatal antibiotics during the second or third trimester had an 84 % higher risk of obesity compared with unexposed children[84] (Table 1). There were, however, significant limitations in this study, including lack of information about postnatal antibiotic use or medical indications for prenatal antibiotic use, as well as a high drop-out rate[84]. Similar studies which incorporate longitudinal faecal metagenome analysis would be informative in determining whether microbiota composition could in part explain the intriguing relationships observed between childhood obesity and prenatal antibiotic use or delivery method. Infancy may be a critical therapeutic window for prevention of obesity. In a perinatal probiotic intervention study which tracked growth patterns of infants during a 10-year follow-up, 113 women were randomised to receive *Lactobacillus rhamnosus* probiotics or placebo 4 weeks before expected delivery and for 6 months postnatally[83] (Table 1). In children exposed to perinatal probiotics, the first phase of excessive weight gain (foetal period up to 48 months) seemed to be restrained, particularly in children who later became overweight[83]. The authors concluded that probiotic-induced microbiota modulation in early life may restrain excessive weight gain in early infancy[83], although of course a multitude of hereditary and environmental factors are also likely to be at play. Large-scale longitudinal studies which take into account a range of confounding factors influencing weight development will be important for informing anti-obesity interventions during the perinatal window of opportunity.

Technical challenges and emerging technologies for microbiome research

Gnotobiotic mice may provide the best experimental system for interrogating the effect of microbiota on metabolic function, but they are not humans with pre-existing gut microbiota. The relationship between a newly introduced gut microbiome and a surrogate host is likely to differ from that of a host and microbiome which have co-evolved over millennia[42]. Human microbiota-associated models (i.e. germ-free mice which have been inoculated with human microbiota) are intended to replicate human microbiome phylogenetic composition[28], but many taxa fail to colonise in germ-free recipients[85]. As well as having several physiological abnormalities[28], human microbiota-associated mice lack human-specific factors such as diet, lifestyle, disease phenotype or human genotype which are integral influencers of microbiome composition[42].

Exciting developments for microbiome research include *in vitro* bioreactors which mimic the human gastrointestinal tract, and *ex vivo* organoid models derived from human intestinal tissues. These platforms enable *in vitro* culture of human gut microbes, enabling highly controlled investigation of gut–microbiota interactions in real time[86]. Human intestinal organoids resemble foetal intestines, providing a useful platform for studying early microbial colonisation and establishment, as well as the impact of nutrition or antibiotics on microbiome development during early life[86].

Gastrointestinal microbiomes are studied using metagenomic sequencing, typically of faecal samples. Traditional methods relied upon an amplicon sequencing strategy using the 16S rRNA gene as a phylogenetic marker. This method of analysis is largely limited to taxonomic classification at the genus level and provides almost no functional information[87]. Cutting-edge studies now use *shotgun metagenomics*, which can assess the entire genomic content of any microbiome sample, achieving precise strain-level classification and directly determining functional properties[88]. Computational methods of metagenomic sample analysis derive genomes from *de novo* assemblies. This approach has limited capacity for distinguishing closely related bacterial taxa and may include assembled genomes which are incomplete or represent chimeric species[88]. Given that multiple strains of the same bacterial species may exist in an individual's microbiome, there is clearly a need to optimise the resolution of metagenomic analyses[88]. The best available means of obtaining high-quality reference genomes is from pure cultures[88]. Progress is being made in this area, with reference-based metagenomic analysis being used to compile the Human Gastrointestinal Bacteria Culture Collection. This is a set of 737 whole-genome-sequenced bacterial isolates that has increased the previous collection of human gut-derived bacterial genomes by 37 % and revealed 105 novel species[88].

Conclusions

Whilst the gut microbiome may have an important role to play in the establishment and maintenance of the obese phenotype, it is only one among a multitude of biological, psychological and social factors driving a chronic, positive energy balance. The increased supply of cheap, calorie-dense foods, together with improved food distribution and pervasive food marketing have been major drivers of the obesity epidemic over the past four decades[89]. Since major contributors to the rise of obesity occur at a population level, it is probable that effective solutions will be

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beyond the scope of individual actions, such as banning unhealthy food marketing to children[90], reducing the cost of healthy foods[89], or implementing national food and agricultural policies that align with promotion of public health[91].

This review has explored the mounting evidence that microbiome composition is intimately and dynamically connected with host energy balance and metabolism. Interventional studies in obese populations have demonstrated metabolic improvements effected by microbiome-modulating treatments such as FMT, as well as highlighting the role of microbiome modulation in well-established anti-obesity interventions such as dietary change or bariatric surgery. However, with an evidence base that is largely derived from rodent studies and a lack of mechanistic insight into bacterial colonisation of the human gut, the question of how therapeutic manipulation of gut microbiota might effectively prevent or promote the reversal of obesity in humans is far from answered[92]. Shotgun metagenomics now enables characterisation of human microbiomes at strain-level resolution, yet there remains a pressing need for a continued, global effort to collate genome sequences of cultured gastrointestinal bacterial isolates from individuals across diverse communities. This will be critical to understanding bacterial *function* at the strain level, paving the way for rational design of microbiome-based therapeutics. Longitudinal efficacy studies will be needed, involving faecal metagenomic analysis of large cohorts. As the complex relationship between microbiome composition and host metabolism is unravelled, it appears probable that microbial manipulation will provide a novel strategy as an effective treatment for obesity.

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Stephanie Santos-Paulo is the primary author of this paper. She conducted the literature review and wrote the majority of the text.

Robert Bryant, Samuel Costello and Samuel Forster read several drafts of the paper, providing extensive feedback and suggestions as well as making minor edits to the content.

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