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Review

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Questioning the foundations of the gut microbiota and obesity

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The role of the gut microbiota in determining body fatness has been a prominent area of research and has received significant public attention. Based largely on animal studies, recent attempts to translate these findings into interventions in humans have not been successful. This review will outline the key mouse research that initiated this area of study, examine whether those results warranted the initial enthusiasm and progress into human studies, and examine whether later follow-up research supported earlier conclusions. It will look at whether the absence of a gut microbiota protects germ-free mice from obesity, whether microbiota can transfer obesity into germ-free mice, the evidence for the role of immune system activation as a causal mechanism linking the gut microbiota to body weight, and consider the evidence for effects of individual bacterial species. Finally, it will examine the outcomes of randomized controlled trials of microbiota transfer in human participants that have not shown effects on body weight. With a more critical reading, early studies did not show as large an effect as first appeared and later research, including human trials, has failed to support a role of the gut microbiota in shaping body weight.

This article is part of a discussion meeting issue 'Causes of obesity: theories, conjectures and evidence (Part II)'.

1. Introduction

The role of the gut microbiota as a causal factor in obesity has generated much research and public interest over the past two decades. Early studies reported differences in the composition of the gut microbiota in obesity, with an increased ratio of Firmicutes to Bacteroidetes, two of the major bacterial phyla in the gut, in obese mice compared to lean mice [1] and a smaller proportion of Bacteroidetes and higher proportion of Firmicutes in individuals with obesity compared to lean controls in a small human trial involving 12 individuals [2]. These findings led to the proposal that obesity itself may have a microbial component and that manipulating the composition of the gut microbiota may be helpful for regulating energy balance in individuals with obesity [1,2]. However, later reanalysis of a number of studies failed to find a difference in the ratio of Bacteroidetes and Firmicutes and, although differences in microbial diversity were noted, these were relatively weak and confounded by small sample sizes and a high degree of variation between individuals [3].

While many studies have reported associations between the gut microbiota and obesity, this review will focus on key studies that indicated a causal role for the gut microbiota in obesity. Much of this research has been conducted using mice, which eventually led to research involving human volunteers taking part in randomized controlled trials. Despite many years of research, the role for the gut microbiota in obesity remains uncertain and has not resulted in any new ways to understand, treat, or prevent obesity. This review critically weighs up the several different strands of evidence that have supported a causal role for the gut microbiota in obesity and considers whether this evidence was as strong as it appeared.

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2. Germ-free mice and resistance to obesity

Germ-free mice are born and raised in sterile microbe-free conditions. They have no gut microbiota and can reproduce and remain healthy while maintained within a sterile incubator. Their lack of a gut microbiota has been used by researchers to attempt to understand the role of the gut microbiota in obesity. Five studies described here have used germ-free mice to investigate whether the absence of a gut microbiota in germ-free mice is protective against obesity when feeding high-fat diets and to determine whether a gut microbiota is required for the development of obesity [4–9].

Interest in using germ-free mice as a model to study obesity was triggered by the observation that mice colonized with a gut microbiota from birth had 42% more total body fat than germ-free mice [5]. Additionally, transferring a typical laboratory mouse microbiota into adult germ-free mice resulted in a rapid increase and normalization of body fat levels to that of a typical laboratory mouse. This evidence that the gut microbiota could act as an environmental factor regulating fat storage stimulated a large amount of attention, which was further increased with the observation that these germ-free mice were resistant to diet-induced obesity when fed a high-fat diet typically used to cause obesity in mice [4]. These key observations provided evidence for the gut microbiota being necessary for body fat gain, in mice at least. This was supported in 2010 by research showing a reduced body weight gain in germ-free mice fed a high-fat mouse diet when compared to mice with a gut microbiota [9]. The germ-free mice showed reductions in food efficiency and higher levels of lipids in their faeces, suggesting that poor absorption of dietary fat contributed to this protection against weight gain.

A second study in 2010 complicated this story by reporting that this protection from developing diet-induced obesity in germ-free mice depended on the particular type of diet used [6]. Their results confirmed that germ-free mice were protected against obesity when fed a high-fat diet similar to those previously used based on hydrogenated vegetable shortening and beef tallow. But they showed that germ-free mice gained significantly more body fat than conventional mice when fed a high-fat mouse diet based on coconut oil instead. A different body weight response based on the fats used in the mouse diet was also reported in 2016, with germ-free mice that were fed a high-fat diet containing palm oil gaining excess weight while those fed the same diet in which palm oil was substituted with lard were resistant to weight gain [7]. The explanation for these divergent findings resulting from using high-fat diets containing different sources of fat remains uncertain.

Finally, an attempt was published in 2021 to replicate one of the original studies [8]. This study used the same mouse strain and the same high-fat diet used in 2007 [4] with the only difference that the mice remained on the diets for 16 weeks instead of only eight weeks. After 16 weeks, the germ-free mice showed similar body weight and percentages of body fat and visceral fat to the conventional mice [8]. This failure to replicate earlier research suggests that the absence of a gut microbiota does not consistently provide protection against obesity.

While initial studies on germ-free mice helped trigger nearly two decades of interest in the requirement of a gut microbiota for the development of obesity, any such role remains uncertain. More recent research has shown that the absence of the gut microbiota does not necessarily provide resistance to obesity, with unexplained conflicting responses based on the source of dietary fat used. Finally, a recent replication study of one of the initial pieces of research did not reproduce the same results and so the effects of the absence of a gut microbiota on obesity remain uncertain.

3. Gut microbiota transplantation as evidence of causality

A second line of research investigated whether the gut microbiota has a causal role in altering body fat by transferring an entire gut microbiota into germ-free mice from either lean or obese mice. Due to the absence of a gut microbiota, germ-free mice have been used in research as a model in which to transplant and compare the effects of the gut microbiota from different donor individuals. Three studies described here [10–12] transplanted the gut microbiota from lean or obese donors into germ-free mice and two studies [13,14] transplanted the gut microbiota from lean or obese donors into conventional mice that were not germ-free.

A key study in 2006 sparked considerable interest, with results showing that when the gut microbiota from mice with a genetic form of obesity was used to colonize germ-free mice, those recipient mice gained more body weight than when transplanted with gut microbiota from lean mice [12]. The microbiota composition of recipient mice resembled that of their donor, indicating that the transplant had been successful. The germ-free mice receiving a gut microbiota from genetically obese mice showed a 47% increase in body fat after 14 days, compared to only a 27% increase when receiving a gut microbiota from lean mice. However, these percentage increases were from the initial baseline body fat levels. While the initial and final weights of the mice were not reported, making it difficult to interpret the magnitude of the changes, the average gain of fat was reported as 1.3 g for the germ-free mice receiving the gut microbiota from a genetically obese mouse and 0.86 g for the germ-free mice receiving the gut microbiota from a lean mouse. This enabled calculation of the initial and final weights of body fat, with the calculated final weight of body fat of 4.07 g for mice receiving a gut microbiota from obese mice and 4.05 g receiving a gut microbiota from lean mice. These similar final amounts of body fat resulted from a 0.4 g difference in the initial amount of fat between the two groups. This highlights the limitations of only reporting percentage gain and does not suggest that the obesity was transferred with the microbiota from obese mice.

This initial study was followed by another transferring the gut microbiota from either mice with diet-induced obesity or lean control mice, resulting in a 68.5% increase in fat and a 34.5% increase in fat, respectively, in the recipient germ-free mice after 14 days [11]. Final weights were not provided but can again be calculated from other data, resulting in a final body fat of 3.9 g and 3.1 g in the two groups of mice. While in this case the body fat of mice was higher after gut microbiota transfer from an obese mouse, the differences were relatively small and the experiment did not continue long enough to determine whether the mice would become obese. The short 14 day length of this study limits our ability to infer whether these body weight differences would persist with time.

The largest of these studies was reported in 2013 and involved a total of 103 germ-free mice being transplanted with human gut microbiota from identical twins where one twin was classified as having obesity and the other twin was not [10]. The transplants were reported to be successful, with the recipient mouse microbiota reproducing the composition of the human donor microbiota. The obesity was reported to be transmissible, with the germ-free mice that received a gut microbiota from a twin with obesity showing a greater increase in body fat, although again this study was limited to only 15 days and only percentage increases in body fat were reported. Data included in electronic supplementary material, table s14 state that when these changes were normalized to initial body mass the germ-free mice receiving a gut microbiota from a twin with obesity showed a change in body fat of 1.8% compared to −0.07% for germ-free mice receiving a microbiota transplant from a twin without obesity. As the normalized data went unreported in the study they may have escaped the note of readers. While these differences remained statistically significant, these results represent small changes in total body fat.

While germ-free mice have been a popular model for investigating causality, conventional mice more closely replicate human subjects who are not germ-free. One 2014 study did transfer the gut microbiota from lean or obese donors into conventional mice following antibiotic treatment to deplete their resident microbiota [13]. The gut microbiota from lean or obese mice was transferred into adult recipient mice at eight weeks of age and into infant mice at three weeks of age to determine whether the age of recipient mice influenced potential effects. The gut microbiota transplant was successful in both infant and juvenile mice, although in adult mice the differences between transplant groups tended to converge over time. In both cases, there were no differences in body weight or body fat between those mice receiving a lean or obese microbiota over a relatively long follow-up period of 20 weeks. Although this research design was potentially more relevant to human subjects, this study and its null results did not attract as much attention as previous research.

Another similar study in 2017 also used conventional mice as the recipient of gut microbiota transfers from either lean or obese donor mice, this time without antibiotic treatment first [14]. This study followed two protocols, the first transferring gut microbiota from lean mice or mice with diet-induced obesity and the recipient mice fed chow diet for three weeks, the second transferring gut microbiota from lean mice, mice with diet-induced obesity, or genetically obese mice and the recipient mice fed a high-fat diet for six weeks. In both experiments, despite the transfer of obese-mouse microbiota changing the gut microbiota of the recipient mice, there were no differences reported in body weight or body fat between the groups of mice.

No study has shown that the transfer of mouse gut microbiota from a mouse or human with obesity into germ-free mice has resulted in those mice becoming obese. The small size of reported changes questions the relevance of these effects, and the limited duration of studies raises uncertainty about how persistent such effects would be. The lack of published reports of positive results from studies with a duration longer than two weeks suggests that these may be difficult to achieve. The limited number of studies, the common lack of reporting of weights or raw data and the lack of direct replication limit the certainty of results. The lack of reported effects in conventional mice with their own resident gut microbiota that may better represent human subjects could have raised more questions about whether these results could be applied to human subjects. Although gut microbiota transplants into germ-free mice have been highly influential, it remains unclear what (if any) degree of body fat change is transmissible via a gut microbiota transplant.

4. Microbiota transplantation following gastric bypass surgery

Another strand of investigation has been whether a gut microbiota transplant could reduce weight gain in mice, in contrast to whether a transplant from an obese mouse could increase weight gain. Gastric bypass surgery is a surgical method of inducing reductions in body weight and body fat and it results in significant changes in the composition of the gut microbiota [15]. This led to the hypothesis that this altered microbial composition may itself contribute to the resulting weight loss and that this effect could be transferrable with the gut microbiota.

Support for this was published in 2013 with the findings that Roux-en-Y gastric bypass (RYGB) surgery in mice produced rapid changes in the composition of the gut microbiota [16]. The transfer into lean germ-free mice of gut microbiota from mice following RYGB resulted in weight loss compared to those receiving microbiota transferred from mice following a sham surgery [16]. The transfer resulted in persistent differences in the gut microbiota of recipient mice receiving transplants from mice after RYGB. It is worth noting that the changes in body weight and body fat were presented as percentage change, while data included in electronic supplementary material, figure S6 indicated that body weights were not significantly different between the two groups.

Another study in 2015 supported the hypothesis, with lean germ-free mice transplanted with gut microbiota from humans after they had undergone RYGB surgery accumulating 43% less body fat over 14 days than when transplanted with patient microbiota from before the RYGB surgery [17]. Again these data were presented as a percentage change, and results contained in electronic supplementary material, figure S5 in the supplementary data show that there were no significant differences between body weight and body fat percentage between the two groups. The composition of the microbiota of recipient mice after the transplant was not determined in this study.

More recent research has not supported the hypothesis; a transfer of gut microbiota from the caecum of rats following RYGB surgery into germ-free mice failed to produce a difference in percentage body fat gain despite the successful establishment of transplanted microbiota [18]. Another similar study in 2021 transferred colonic microbiota from rats six weeks after RYGB surgery into germ-free mice and reported that body weights were similar between recipient groups [19]. A well-described recent study in 2022 transplanted the faecal microbiota from human patients before and after two different types of gastric weight loss surgery into mice with differences in particular bacterial genera between different groups of recipient mice [20]. In this study, the microbiota was transferred into both germ-free and conventional laboratory mice and there were no differences in weight gain or in fat mass between any of the groups.

A role for the altered composition of the gut microbiota in the resulting weight loss following gastric bypass surgery represented an appealing hypothesis but this has not been supported by later research. The results of early research appearing to support the hypothesis are less compelling after a more careful reading. Additionally, the exclusive use of lean germ-free mice in experiments to receive transplants represents a missed opportunity to investigate whether the transfer of post-bariatric surgery causes any change in body weight in mice that already have a high body fat.

5. The immune system as a causal pathway

The activation of the immune system by components of bacteria originating in the gut has been proposed as a mechanistic causal pathway linking the gut microbiota and obesity. Proteins that form components of bacterial structures such as bacterial cell membranes can function as antigens that are recognized by specific receptors of the immune system and trigger responses that can include chronic low-grade inflammation, which could lead to increased fat gain. Seven studies investigated the role of cell receptors and their function in stimulating inflammation and on body weight when activated or the effects mice genetically modified to lack these receptors [21–27].

In 2007, lipopolysaccharide (LPS) was identified as a potential mechanistic link between the gut microbiota, chronic inflammation and weight gain. LPS is a component of the cell membrane of gram-negative bacteria and it binds to toll-like receptor-4 (TLR4) in human immune cells, resulting in inflammation [21]. Feeding mice a very high-fat diet that resulted in increased body weight reportedly caused LPS from bacteria in the gut to leak into the circulation and increase levels of LPS in the blood. When LPS was chronically infused into mice over 28 days to reach the same levels as from a very high-fat diet, this reportedly resulted in similar increases in body weight and body fat to those as caused by the high-fat diet itself [21]. It was also reported that mice lacking the TLR4, and thereby unable to respond to LPS, were protected from this LPS-induced increase in body fat [21]. This formed an appealing mechanistic link between a component of gut bacteria and body fat gain in mice via a mechanism involving low-grade chronic inflammation.

These findings do not appear to have been replicated. The authors published a study in 2013 in which infusion of LPS did not increase body weight, body fat, or food intake in mice fed on either low-fat or high-fat diets [24]. This null result was not emphasized and appears to have remained unnoticed. Any protection from high-fat diet-induced weight gain due to the absence of TLR4 in mice could not be replicated in two different mouse models lacking TLR4 signalling [23]. However, the potential role for LPS in causing obesity had remained prominent in the literature for many years.

Another potential receptor is toll-like receptor-5 (TLR5), which specifically recognizes flagellin, a component of the tail-like structure that certain bacteria use for propulsion. A role for TLR5 was first proposed in 2010, with the observation that mice lacking TLR5 showed increased food intake and higher body fat [26]. Similar outcomes were seen later, with mild chronic inflammation and increased body weight in mice lacking functional TLR5 [22]. However, these results have not been replicated and other studies did not find any differences in body weight or body fat in the same mice lacking functional TLR5 [25]. The first findings were also not repeated in a new mouse model in which the TLR-5 receptor gene had been deleted. When fed a high-fat diet these TLR5 knockout mice showed no differences in body weight or body fat [27].

The suggestion by initial high-profile studies of links between the gut microbiota and body weight via components of gut bacteria such as LPS and flagellin and receptors of the immune system including TLR4 and TLR5 has not been confirmed by later research. This lack of replication is not easily appreciated from reading the literature, where the citations of early high-profile findings have remained prominent and influential.

6. Individual bacterial genera and obesity

While the previously discussed research has looked at the gut microbiota as a whole, there has also been interest in a potential role for individual bacteria that live within the gut to influence body weight and body fat. Two examples that have received particular attention are the bacteria *Christensenella minuta* described here in three studies [28–30] and *Akkermansia muciniphila* detailed here in two studies [31,32], which have investigated whether these individual bacteria can directly influence body weight.

The abundance of the bacterial family *Christensenellaceae* represented by the species *C. minuta* has been reported to negatively correlate with human body weight in several twins studies [33,34]. Evidence was first reported in 2014 for a causal effect of *Christensenellaceae* on body weight in mice [28]. Twelve germ-free mice were inoculated at six weeks of age with faecal microbiota from a human donor with obesity but lacking *Christensenellaceae* plus 100 million *C. minuta* cells; twelve mice were inoculated with donor stool without *C. minuta*. After 21 days, the body weights of those mice inoculated with *C. minuta* had increased by around 10% versus 15% for mice without it, with a final body fat percentages of 21.5% and 24.5%, respectively [28]. However, the result of a replication of this experiment shown in the electronic supplementary material of that study, (figure S6) appears to show no differences in the percentage body weight increases between mice inoculated with *C. minuta* and those without. As this supplementary figure was not mentioned in the study it required detailed reading of the supplementary data and raises uncertainty about the repeatability of the results.

These results attracted the interest of a biotech company that in 2021 carried out a study feeding mice a high-fat diet and giving half of these mice a daily supplement with *C. minuta* [29]. Daily administration of *C. minuta* for four weeks was reported to prevent high-fat diet-induced weight gain in mice [29]. This was followed by a Phase 1 clinical trial (ClinicalTrials.gov Identifier: NCT04663139) by the same company, giving 20 human volunteers either a daily dose of live *C. minuta* or a placebo for twelve weeks [30]. However, although completed in June 2021, the results of this trial have not been published and no further trials involving *C. minuta* are currently underway. Evidence for *C. minuta* having a causal role in determining body weight or body fat in mice remains limited and further evidence of beneficial effects in humans has not been published.

The bacterium *A. muciniphila* is a normal inhabitant of the mucosal lining of the human gut and its abundance in the gut has been inversely correlated with body weight in humans [35,36]. Supplementing mice with live *A. muciniphila* reduced gain in body fat when fed a high-fat diet [32], leading to interest in this bacterium as a potential treatment for obesity. However, a randomized, double-blind, placebo-controlled pilot study in overweight/obese volunteers supplementing with either live or pasteurized *A. muciniphila* for three months did not result in changes in body fat [31]. A larger study (ClinicalTrials.gov Identifier: NCT05417360) supplementing with only pasteurized *A. muciniphila* bacteria to aid with weight maintenance after weight loss is still currently recruiting. Two further randomized controlled trials in humans evaluating live *A. muciniphila* against placebo with body weight as a primary outcome are still currently recruiting (ClinicalTrials.gov Identifier: NCT04797442, NCT05720299). While the outcomes of these ongoing research trials remain to be seen, current evidence does not support a causal role for *A. muciniphila* in influencing body weight in humans.

While a potential causal role for *Christensenella* and *Akkermansia* in reduced body weight has attracted considerable attention, later research does not yet appear to have confirmed this. Some initial animal research appears less conclusive with more detailed reading and human supplementation trials have yet to produce supporting results. Interest in the supplementation with *Akkermansia* remains ongoing and may still result in evidence for meaningful effects on human body weight. Current research does not appear to provide good evidence for individual members of the human gut microbiota acting as causal agents in the development or prevention of obesity.

7. Trials in human participants

Highly publicized research indicating that the gut microbiota could be both a cause of obesity and transplantable in mice led to microbiota transplants being tested in a series of clinical trials in human volunteers. These include six studies that have directly tested the hypothesis that if the gut microbiota were contributing to obesity then transferring the gut microbiota from a lean individual into individuals with obesity would lead to weight loss [37–42]. A further four studies investigated the question from different angles using transplants from post-bariatric surgery patients to aid weight loss, testing whether transplants from individuals with obesity could treat cachexia in patients with cancer, the unintended effects of transplanting microbiota from individuals with obesity to treat *Clostridium difficile* infection, or testing the influence of antibiotics on body weight [43–46]. Finally, four other studies have included a faecal transplant as part of other interventions such as bariatric surgery, with different dietary interventions, or to prevent weight regain [47–50].

The first study published in 2012 was a pilot randomized controlled trial involving 18 individuals with obesity [41]. Participants received either faecal microbiota from lean male donors or a placebo in the form of their own collected faecal microbiota; they were then followed-up for six weeks. Data included in table S1 from the electronic supplementary material to article [41] showed that neither weight, BMI, nor body fat mass percentage were changed after six weeks. However, changes to body weight were not mentioned in the paper, despite weight being listed as a primary outcome when the trial was preregistered as the 'FATLOSE trial: Faecal Administration To LOSE weight' (Dutch Trial Register: NTR1776) [41]. This was followed-up in 2017 with a similar but larger randomized control trial in which 26 participants received a faecal microbiota transplant from a lean donor and 12 received their own as a control and were then followed-up for 18 weeks [38]. A randomized subgroup of half of the participants in the experimental group received a second microbiota transplant after six weeks from the same lean donor. Results showing that body weight and BMI at six weeks remained unchanged in both groups were only included in the article's electronic supplementary material (table S3). Body weight and BMI outcomes at 18 weeks follow-up were not provided. These null results were noted but neither emphasized nor discussed in the paper, leaving the implications of these results potentially unrecognized by readers.

A further four randomized control trials were then published between 2019 and 2022 with more prominently reported null results. In a pilot randomized controlled trial involving 22 adult participants with obesity, 11 received a faecal microbiota transplant from a healthy donor while 11 received placebo capsules [37]. Capsules of either donor faecal microbiota or placebo were given at baseline, with further capsules at week four and week eight. This did not affect body mass index after 12 weeks despite alterations in the gut microbiota composition in those receiving the microbiota transplant [37]. Another randomized placebo-controlled pilot trial of a faecal microbiota transplant contained in oral capsules involved 24 adults who received weekly donor faecal microbiota transplants from healthy lean donors or placebo capsules containing no faecal material [42]. Participants were given these capsules for two consecutive days, followed by capsules once per week for the next five weeks. Measurements taken after six and twelve weeks showed no difference in body fat [42]. Another larger randomized controlled trial in 2020 included 86 adolescent participants with obesity, who received either encapsulated faecal microbiota from lean donors or placebo capsules. Follow-up after 26 weeks showed no changes to BMI, waist circumference, or body fat percentage [39]. A final large controlled trial included 61 adult subjects with obesity who were randomized to receive either a microbiota transplant from lean donors, lean microbiota transplant plus a lifestyle intervention, or a sham transplant every four weeks for up to 12 weeks [40]. No significant weight loss was observed after the transplant despite analysis of the microbiota composition indicating that the repeated transplants resulted in successful colonization of the microbiota from lean individuals.

Taking a different approach, a study in 2020 transferred the faecal microbiota from donor individuals following Roux-en-Y gastric bypass surgery for weight loss into 12 participants with obesity [45]. There were no changes in body weight after follow-up four weeks later. Another study in 2021 took an opposite approach by transferring the gut microbiota from individuals with obesity into 24 patients suffering from weight loss caused by cachexia due to cancer [43]. Twelve patients received their own microbiota stored from before they began chemotherapy, and 12 patients received the microbiota from an individual with obesity. After 12 weeks, there were no differences in BMI, body weight, or body fat percentage [43]. Another study made use of a

combination of observational and randomized control trial data from 173 participants involved in research using microbiota transplants to successfully treat *C. difficile* infection [44]. The trends of body mass index changes up to 48 and 52 weeks after the microbiota transplants were not different between patients receiving donor microbiota from normal weight, overweight, or obese donors [44]. Finally, in another approach to test the role of the gut microbiota in human body weight 57 men with obesity were randomized to receive either amoxicillin or vancomycin antibiotics, or placebo for seven days to suppress their own resident microbiota [46]. Body weight remained unchanged for all treatment groups despite vancomycin treatment significantly reducing the bacterial diversity in the gut and altering the microbial composition for the duration of the eight-week follow-up.

Other studies have included a microbiota transplant as a part of other interventions. In one study, 41 participants received either a faecal microbiota transplant from a lean donor or their own faecal microbiota as a control and administered via a tube into their small intestine six months before undergoing bariatric surgery [48]. Weight loss was not different between microbiota transplant groups either in the six months before bariatric surgery or 12 months after. As this study focused on clinical outcomes, investigation of microbiota colonization was not reported [48]. A different and more complex intervention involved randomizing 70 patients with severe obesity and metabolic syndrome to receive either a faecal microbiota transplant from lean healthy donors or a placebo [49]. These two groups were then randomized to receive daily either a high-fermentable fibre or low-fermentable fibre supplement. *Post hoc* analysis of the participants' gut microbiota composition showed that successful colonization of the donor microbiota was only seen in the group receiving a faecal microbiota transplant combined with a low-fermentable fibre supplement. However, body weight and waist circumference remained unchanged in transplant groups over 12 weeks [49]. Another study compared the effect of a faecal transplant from either a lean donor or a placebo on its own in patients with obesity and then combined that with a Mediterranean diet. Despite the colonization by some donor bacterial strains, the microbiota transplant did not result in any difference in body weight [47]. While these studies suggest that specific changes in diet may support the establishment of a transplanted microbiota, it does not show that this resulted in any effect on body weight.

Finally, with a novel approach to preventing regain after weight loss, faecal microbiota was collected from participants who had successfully lost 3.5% of their body weight after a six-month weight loss intervention [50]. The participants then later received either their own microbiota or a placebo during the period of weight regain with the aim of reducing the amount of body weight gained back. This involved receiving a microbiota transplant of their own previous microbiota on ten separate occasions over a six-month period starting two months after faecal samples were collected. While no differences were seen among those on Mediterranean diet alone without the polyphenol supplements, when combined with a Mediterranean diet and polyphenol supplements the personal microbiota transplant reduced the amount of weight regained after weight loss from 50% to 17%, equalling 1.6 kg and 3.7 kg of weight regain, respectively [50]. This suggests that a gut microbiota shaped by weight loss may then influence the host's ability to regain body weight. However, the numbers of participants in each diet group were small and further research will be needed to confirm this result.

These studies have used a diverse range of study designs and methods, with various sources of donor microbiota, different ways of introducing the donor microbiota into recipients, and differing numbers of transplants given to recipients. While some study designs or methods may be more effective at successfully transplanting a gut microbiota than others, these studies do not provide evidence to suggest that this resulted in any greater effect on body weight. The trials in human volunteers have not produced evidence that the gut microbiota is a transferable factor that influences obesity in humans.

8. Limitations of previous research

There are a number of limitations related to study design and methodology among the published research discussed in this review. A common problem in research into the gut microbiota is a lack of standardization in study design. There are few agreed-upon standardized protocols or optimal methods for conducting microbiota transplants, as well as certain inherent limitations based on the source of transplants. Research involving gut microbiota transplants from mice has often used the contents of the caecum [11,12], but this is not possible for samples donated by human volunteers where faecal microbiota represents the only viable option; additionally, human samples often involve freezing and storage of donated faecal material before use [10], although in some limited cases fresh donated material has been used [45]. Human trials have used different routes to introduce microbiota transplants including using an oral tube into the small intestine [40,41,45], or using encapsulated faecal material in capsules taken by mouth [37,39,42] and designed to survive the passage through the stomach to release their contents in the intestine. In some studies, whole bowel irrigation has been used to wash out the contents of the gut prior to a microbiota transplant [39,41,45], while in others no bowel preparation was used [42]. How much these various sources of donor material and storage conditions may have affected the results of the research described in this review is unknown as they have not been directly compared against each other. As microbiota research has focused on bacteria in the gut, any role for fungi or viruses during microbiota transplants in obesity research has been neglected. More in-depth analysis of the composition of the donor gut microbiota could be valuable as the identities of the microbes that are donated from lean healthy donors are not easily comparable between studies. However, if the gut microbiota is playing a role in shaping body weight, then a microbiota from a lean human might be expected to contain factors contributing to that leanness, even if the exact composition is poorly understood. While the superiority of any donor source or method of transplantation for this type of research remains uncertain, none appear to have resulted in different outcomes on body weight.

Introducing a new microbiota into the human gut that is already in possession of a gut microbiota is not as straightforward as transplanting a microbiota into germ-free mice with no resident microbes to compete with the incomers. The potential lack of survival of donor microbes during transfer and the degree to which the donor microbiota establishes

itself in the gut of the recipient become more important questions when no effect is seen after a microbiota transfer. While a change towards donor microbiota was reported after six weeks in one study, this had returned to baseline composition after 12 weeks [41]. The degree of transplanted colonization is also unknown, with some studies in human volunteers investigating microbiota changes after the transplant, while others have focused on clinical outcomes rather than reporting changes in the recipients' gut microbiota. Additionally, while some studies only used a single microbiota transplant [41], more recent research has often used repeated transplants to maintain colonization. For example, four donor microbiota transplants spread over 12 weeks reportedly led to successful colonization and maintenance of donor microbiota in human recipients [40]. A different study carrying out microbiota transplants on two consecutive days, followed by transplants once a week for the next five weeks, also led to the successful transfer and maintenance of donor microbiota in humans [42]. Although it has been shown that it is possible for donor gut microbiota to colonize the recipient and be maintained through repeated transfers, findings from these studies did not show an effect of the transplanted gut microbiota on body weight. This suggests that failure of the that colonization and maintenance of the donor gut microbiota is not the cause of the lack of an effect.

Another element of variability in the reported studies was the hypothesis being tested. Research questions in mouse experiments have tested whether a gut microbiota transplant from an obese donor could cause body weight gain in lean mice, whereas human trials have generally tested the opposite: whether a gut microbiota transplant from a lean donor can cause body weight loss in participants with obesity. While the development and reversal of obesity both represent effects on body weight, the removal of factors that may induce the development of obesity cannot be assumed to effectively treat it. Better-designed human trials may produce new results, but at present it can only be concluded that current evidence does not support a causal role for the microbiota in influencing obesity.

The results of the studies outlined in this review indicate that a more critical reading of published research could have identified more research gaps requiring attention, particularly before moving to human trials. Improvements to the way in which research has been reported could have allowed greater independent scrutiny of prominent early studies into the effect of the gut microbiota on obesity that have had a significant influence on later research. This includes making the raw data available, placing important data in the paper and not in supplementary data, including measurements of weight instead of only percentage change, and considering whether the size of any reported change was physiologically meaningful; including these would have helped provide a more realistic assessment of many high-profile results. Additionally, it would have been beneficial to require the direct replication of high-profile research studies in mice, long-term follow-up of mice after faecal microbiota transplants, and replication of effects reported in germ-free mice in conventional mice with a microbiota prior to initiating human trials. Finally, no lean mice have been shown to become obese and no obese mice have been shown to have significant weight loss as a result of a gut microbiota transplant. It could have been expected that such results would be required before assuming that these effects could be induced in human participants.

9. Conclusion

Several independent strands of evidence have been explored in the literature, focusing on germ-free mice, whole microbiota transplants, immunological mechanisms and individual bacterial genus. This has given the appearance of a robust area of research with several pillars of support. However, when each strand of evidence is critically, it becomes evident that key initial data have often been presented in ways that amplified small effects, single findings have not been later replicated, and key results have been included only in supplementary material. Research showing no effects can be difficult to publish and it remains unknown how many null findings on this topic remain unpublished. When they are published, conflicting results and null results have not attracted the same degree of publicity nor citations in the scientific literature and so have remained less visible. This leaves initial positive research prominent in the published literature, giving an undue impression of the evidence. While this resulted in a scientific literature that appeared robust, on closer inspection each individual part appears less convincing. A number of randomized controlled trials with human participants have now been published that fail to show a clinical effect on body weight from microbiota transplantation. This may dampen enthusiasm for the subject, but currently it remains an area of research and public interest. A critical reading of the literature now suggests that this lack of an effect in human volunteers may have been predictable from previous research in mice and that more robust evidence in mice should have been required before moving to human trials. Alterations of the gut microbiota composition seen in obesity may be associated with or result from obesity, rather than being a cause of this condition. However, the evidence presented in this review should not be taken to imply that the gut microbiota has no role in the effects of obesity, as this research question is beyond the scope of this review. In conclusion, high-profile research indicating links between the gut microbiota and excess body weight were not based on robust and repeatable results and have not been supported by later research.

Data accessibility. This article has no additional data.

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

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