Review

MOLECULAR METABOLISM

Obesity and the gut microbiome: Striving for causality

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ABSTRACT

The gut microbiome has been proposed to play a causal role in obesity. Here, we review the historical context for this hypothesis, highlight recent key findings, and critically discuss issues central to further progress in the field, including the central epistemological problem for the field: how to define causality in the relationship between microbiota and obesity phenotypes. Definition of such will be critical for the field to move forward.

Keywords Microbiome; Microbiota; Obesity; Type 2 diabetes; Non-alcoholic fatty liver disease; Innate immunity; Segmented filamentous bacteria

INTRODUCTION

Obesity is a major risk factor for common, serious medical conditions, including type 2 diabetes, atherosclerotic cardiovascular disease, non-alcoholic fatty liver disease, Alzheimer's disease and diverse cancers [2,13,34,98,106,126]. As such, obesity has become a public health problem of the first order. By 2008, some 1.5 billion adults worldwide were estimated to be overweight, 502 million of them being obese [37]. The unfolding worldwide obesity pandemic of the 21st century thus threatens to overwhelm the public health gains made against infectious diseases in the 20th century. Obesity prevention and treatment remain problematic. Novel preventive and therapeutic approaches to obesity may well depend on better definition of the causes of obesity and its metabolic and end organ complications.

Obesity results from an imbalance between energy intake and expenditure. While genetic factors clearly contribute to control of the physiologic response to caloric excess, and hence to the development and maintenance of obesity [102], the dramatic rise in obesity prevalence over the past decades has, appropriately, turned attention towards the environment. Greater control of ambient temperature, increased sedentariness and the ubiquitous presence of cheap, high-calorie foods have all been implicated as important causal factors [83,89]. While these conditions are likely contributory to a greater or lesser extent, the twinned observations that obesity may be associated with gut microbiome configuration in humans [122] and that obesity phenotypes can be transmitted via the gut microbiota in rodent models of obesity [123] have focused attention on the role of the gut microbiome in the development of obesity. Curiously, the microbiome shares properties with both the environment (it is, perforce, an intimate part of the human environment) and genes (it is heritable and contains genetic material). Indeed, some have proposed that the microbial genetic material that we carry with us effectively represents an extension of our genome – a "meta-genome" [129]. In this context, it will be noted that alterations in this meta-genome occur on time-scales consonant with the observed, rapid increase in obesity prevalence. The gut microbiome thus represents a compelling candidate for being an important contributor to the current increase in obesity rates. Further, accumulating evidence supports a role for the gut microbiome as a modifier of some of the metabolic and end organ complications of obesity.

Here, we review some of the findings that have linked the gut microbiome with obesity and its complications, addressing key outstanding questions about the relationship between the microbiome and obesity, and critically discussing the ability of current methodological approaches to define causality and mechanism.

HISTORICAL PERSPECTIVE

The interplay between diet, gut flora ("microbiota" in modern parlance) and human health has been appreciated for over a century. Attempts to exploit this relationship go just as far back. Acceptance of the germ theory of disease led to early attribution of a number of human ailments to microbial sources, including conditions that succeeding generations of scientists and physicians have considered to be non-infectious. An initial proponent of such theories, now claimed as the father of probiotics, was the immunologist Nobel Laureate Elie Metchnikoff. In his 1907 treatise, "Essais optimistes" (published in translation as "The Prolongation of Life: Optimistic Studies" [86]), Metchnikoff proposed microbial origins for senility and hypothesized that products of intestinal putrefaction by microbes were responsible. He further suggested that lactate produced by bacteria in fermented milk products provided a means to avoid such putrefaction and senility, an idea based on the observation that certain Bulgarian centenarians consumed large quantities of yoghurt. It will be noted that many of today's approaches (e.g., functional metabolomics, in the form of testing for urinary metabolites as markers of intestinal fermentation), themes (e.g., the implication of butyrate as a key modulator of host pathophysiology) and issues (e.g., a paucity of culture conditions for intestinal bacteria) have apparently been part of the field for a long time [86].

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Study of interactions between the gut flora and organismal biology over the past century has demonstrated the influence of the former on numerous extra-intestinal phenotypes, particularly those which modulate immune responses to infection [30,58,63,113]. However, while the field has largely focused on immunity, examples of bacterial product-mediated regulation of broader host physiology have cropped up as well. Notable findings have included data supporting a somnogenic role for muramyl dipeptide [59] and an analgesic role for lipopolysaccharide [53]. More general roles for the microbiome in host physiology and health have been reviewed elsewhere [22].

RECENT KEY FINDINGS

Under normal conditions, bacterial cells in the gut vastly outnumber the cells of their host. Given this numerical dominance, the fact that these commensals modulate host physiology is not surprising. Recent investigation into these issues has been facilitated by technological advances that allow for robust molecular characterization of the microbiota, which has overcome the barrier posed by the fact that many if not most bacterial species in the gut have not been culturable [42]. In particular, molecular profiling has facilitated interrogation of the bacterial communities of the distal gut and, in doing so, has fundamentally advanced the fields of microbial ecology and functional genomics. Much remains unknown even at a descriptive level, however. In particular, attention to viruses, fungi and protozoa in the gut has lagged [87,96,107].

The ability to characterize gut microbiota in depth led to descriptive support for the hypothesis that the gut microbiome could regulate (and/or be regulated by) the development of obesity. Specifically, descriptive profiling revealed associations between obesity phenotypes and microbial class representation in the gut in both humans and rodent models [7,72,73,109,123]. Further, it was demonstrated that gut microbiota could alter adiposity in rodent studies involving transfer of a two species system consisting of *Bacteroides thetaiotamicron* and *Methanobrevibacter smithii* [109], or bulk cecal contents, into germ-free recipients [7]. However, none of these studies afforded a clear bridge between causal manipulations in rodent models and descriptive data from obese humans. Two studies from J. Gordon's lab did just this. Describing a gut microbiota-dependent, transferrable obese phenotype in rodents, along with phylum-level correlates in humans, the Gordon lab established that the gut microbiome *could*, in certain contexts, causally contribute to obesity in model systems with relevance to humans [73,123]. Thus, the field was opened to dissection of the cellular and molecular mechanisms by which the gut microbiome could contribute to obesity and its complications. The field has only expanded since. On the one hand, rodent studies have provided further examples of apparently causal associations between the microbiome and obesity, and have highlighted the potential contribution of microbiome-modulated immune responses to the development of obesity and its complications [14–16,49,127]. On the other hand, large-scale human studies have indicated that enrichment or induction of metagenomic modules (that is, induction of potentially species-independent genes or pathways with common molecular functions) may be associated with the development of obesity [5,122]. Further studies have indicated that such metagenomic modules cluster into discontinuous groupings (known as enterotypes) that associate with long-term dietary choices and are stable to short-term dietary change [5,134]. Direct association of specific enterotypes with obesity has not yet been observed, however. Details of these and other key studies are outlined in Table 1, and discussed below.

In the following sections, we address five interrelated questions: (i) Capability (can the gut microbiome modulate obesity?); (ii) Actuality (does the microbiome actually regulate obesity, and how would we know if this were true?); (iii) Identity and specificity (if the gut microbiome drives obesity, which specific phyla/classes/genera/species/microbial products – or conserved microbial metagenomic qualities – are responsible?); (iv) Mechanistic possibilities (if specific components of the gut microbiome drive obesity, how might they do this?) and (v) Therapeutic applicability (how might the gut microbiome be manipulated to prevent or treat obesity?).

CAPABILITY (CAN THE MICROBIOME REGULATE OBESITY?)

Support for capability hinges on biological plausibility, and on the quality of the available data. Several plausible, potentially overlapping biological mechanisms have been proposed and are discussed in detail below, including: (1) microbiota with augmented capacity for energy harvest; (2) direct alteration of host energetics (energy uptake, utilization and/or storage) by microbial metabolites; and (3) alteration of host energetics through mediate effects on the host immune system. In terms of the latter class of mechanism, it will be noted that more than half of the lymphocytes in the body are part of the enteric immune system, in close apposition to the gut microbiome [19].

Given reasonable plausibility, how good is the evidence that the microbiome can modulate obesity and its complications in experimental systems? The strongest evidence has come from comparing germ-free mice with both conventionalized mice (that is, germ-free mice colonized with microbiota from conventionally-raised mice) and conventionally raised mice. In general germ-free mice exhibit less adiposity, despite eating more food [7], than their conventionally raised counterparts. Reciprocally, increased adiposity has been reported in conventionalized mice (and conventionally-raised mice) in comparison to germ-free mice, during brief feeding on an autoclaved chow diet [7] as well on a high fat diet (HFD) [8]. The latter finding was not replicable in another lab, however, although differences in both mouse strain and diet between the studies complicates direct comparison [38]. Further complicating matters is the observation that in chickens, the presence of gut microbiota leads to reduced absorption of energy [91]. Nonetheless, increased weight gain has been reported after reductive colonization of germ-free mice with a two species system consisting of *Bacteroides thetaiotamicron* and *Methanobrevibacter smithii* [109,110], something that may well be a function of total intestinal bacterial load, as mice colonized with both *B. thetaiotamicron* and *M. smithii*–in comparison to mice colonized with either species alone or maintained in a germ-free state – had both increased bacterial burdens and adiposity [109]. Given the profound baseline physiologic abnormalities exhibited by germ-free mice [76–78,103,105,118], the fact that provision of microbiota alters gut physiology, energy handling and the obesogenic potential of such mice is neither surprising nor particularly informative as to whether specific properties of the microbiome play an important role in regulating obesity.

A related approach towards defining causality has employed the transfer of fecal microbiota from lean and obese mice into matched groups of germ-free recipients. Three such studies have demonstrated that the recipients of microbiota from obese mice develop greater

Author(s) [Ref.]	System	Phenotype	Microbes implicated in obese state	Proposed mechanism	Caveats
Backhed et al. [7]	mm	Increased adiposity	Whole microbiome	↓Angptl4 and ↑energy storage	Mixed strain background of knockout mice
Backhed et al. [8]	mm	Increased weight gain	Whole microbiome	↓AMPK activity and ↓energy expenditure	
Ley et al. [72]	mm	Microbiota associated with obesity—*Lep*$^{ob/ob}$	↑Firmicutes: Bacteroidetes		Leptin's newly recognized role in gut immunity
Ley et al. [73]	hs	Microbiota associated with obesity/weight loss	↑Firmicutes: Bacteroidetes	Increased energy harvest	small sample size ($n < 15$/group)
Turnbaugh et al. [123]	mm	Increased adiposity	↑Firmicutes: Bacteroidetes	Increased energy harvest	Leptin's newly recognized role in gut immunity
Fleissner et al. [38]	mm	No difference in obesity	↑Firmicutes: Bacteroidetes		Mouse strain choice
Hildebrandt et al. [50]	mm	Microbiota changes related to diet, not obesity	↑Firmicutes: Bacteroidetes		Use of purified diets versus natural ingredient diet
Turnbaugh et al. [121]	mm	Increased obesity	↑Firmicutes: Bacteroidetes		
Murphy et al. [92]	mm	Microbiota associated with obesity	↑Firmicutes: Bacteroidetes	SCFA production and fecal energy uncoupled from obesity	
Schwiertz et al. [114]	hs	Microbiota associated with obesity, obesity	↑Bacteroidetes	↑SCFA (propionate) production	
Turnbaugh et al. [122]	hs	Microbiota associated with obesity	Reduced diversity ↓Bacteroidetes, ↑Actinobacteria		
Duncan et al. [33]	hs	Microbiota associated weight loss/diet	No difference in Bacteroidetes, ↑Firmicutes		Relatively small sample size ($n < 30$/group)
Vijay-Kumar et al. [127]	mm	Increased obesity	Whole microbiome	Immune-mediated dysbiosis	
Henao-Mejia et al. [49]	mm	Increased obesity—*Lepr*$^{db/db}$, liver damage	Whole microbiome	Immune-mediated dysbiosis	
Samuel and Gordon [109]	mm	Increased epidymal fat pad weight	*Bacteroides thetaiotamicron* + *Methanobrevibacter smithii*	↑SCFA production	
Samuel et al. [110]	mm	Increased adiposity/weight gain	*Bacteroides thetaiotamicron* + *Methanobrevibacter smithii*	GPR41-mediated SCFA sensing	Mixed strain background of knockout mice
Caricilli et al. [17]	mm	Increased insulin resistance	Whole microbiome	Immune-mediated dysbiosis	
Shin et al. [117]	dm	Increased insulin resistance	*Acetobacter pomorum*		
Cani et al. [15]	mm	Increased insulin resistance	Whole microbiome	Metabolic endotoxemia	
Serino et al. [115]	mm	Increased insulin resistance	↓Firmicutes: Bacteroidetes	Metabolic endotoxemia	
Zhang et al. [138]	hs	Microbiota changes after gastric bypass	↑Firmicutes, ↓Gammaproteobacteria		small sample size ($n < 15$/group)
Li et al. [74]	rn	Microbiota changes after gastric bypass	↑Firmicutes and Bacteroidetes, ↓Proteobacteria		
Collado et al. [24]	hs	Microbiota associated with overweight/weight gain	↑*Bacteroides*, *Staphylococcus aureus*		Relatively small sample size ($n < 30$/group)
Kalliomaki et al. [61]	hs	Microbiota associated with overweight—children	↑*Staphylococcus aureus*, ↓Bifidobacteria		Relatively small sample size ($n < 30$/group)
Santacruz et al. [112]	hs	Microbiota changes associated with weight loss—adolescents	↓*Bacteroides*, Lactobacilli		Relatively small sample size ($n < 30$/group)
Nadal et al. [93]	hs	Microbiota associated with weight loss—adolescents	↑*Clostridium histolyticum*, *Eubacterium rectale–Clostridium coccoides*, ↓*Bacteroides–Prevotella*		Relatively small sample size ($n < 30$/group)
Sabate et al. [108]	hs	Hepatic Steatosis Severity	Whole microbiome	Small intestinal bacterial overgrowth	
Turnbaugh et al. [124]	hs/mm	Increased adiposity	↑Erysipelotrichi, ↑Bacilli, ↓Bacteroidetes		
Arumugam et al. [5]	hs	Normalized BMI	Correlation with Functional Modules consisting of ATPase complex and ectosine biosynthesis	Increased energy harvest	Relatively small sample size ($n < 30$/group)

Table 1: Summary of studies linking the gut microbiome to obesity phenotypes. Abbreviations: hs—*Homo sapiens*; mm—*Mus musculus*; dm—*Drosophila melanogaster*; rn—*Rattus norvegicus*; SCFA—short chain fatty acids; *Lep*$^{ob/ob}$—leptin-mutant obese mouse strain; *Lepr*$^{db/db}$—leptin receptor mutant obese/diabetic mouse strain.

adiposity on a HFD than do recipients of microbiota from lean mice. These studies have employed transfer from: (1) obese *Lep*$^{ob/ob}$ mice [123]; (2) HFD-induced obese wild type mice [121]; and (3) HFD-induced obese *tlr5*$^{-/-}$ mice [127]. Moreover, studies of humanized germ-free mice (that is, mice colonized with human microbiota) have demonstrated mirroring of donor adiposity in recipients that is capable of vertical transmission into the next generation [124]. It should be noted that the prevalent use of germ-free mice in these studies appears to be due, in part, to a theoretical concern over the efficiency of transfer of microbiota into mice in which microbial community structure has already been established. That said, a recent study clearly demonstrated the transmissibility of an obesogenic dysbiosis (that is, of an altered gut microbiota associated with the development of obesity) into conventionally raised *Lepr*$^{db/db}$ mice [49]. The use of antibiotics to deplete microbial constituents promises to complement such transfer approaches. However, the broad activity of current antibiotics, and the fact that their use can dramatically and irreversibly alter gut microbial ecology [27,28], hampers their potential utility in addressing such questions [125].

Examples of microbiota-mediated modulation of metabolic and end-organ complications of obesity are also beginning to accumulate. First, numerous examples of microbiome-mediated modulation of insulin resistance and type 2 diabetes exist, something seen in a number of the studies associating transfer of gut microbiota with rodent obesity [7,8,49,127]. Notably, this increase appears to be driven by proinflammatory microbial products in some contexts [15,17,115], and the

ability of microbiota to alter metabolic homeostasis is apparently conserved all the way back to *Drosophila* [117]. Second, the microbiota has long been recognized to play a role in the progression from non-alcoholic steatosis to steatohepatitis [1]. Consonant with this, a rodent study recently demonstrated transmissibility of liver damage severity via the microbiota [49].

Taken together, these rodent model studies indicate that alterations in the gut microbiome are capable of causally altering the development and maintenance of obesity and its complications.

ACTUALITY (DOES THE MICROBIOME REGULATE OBESITY?)

This does not mean that the microbiome actually does modulate obesity in humans. Despite strong experimental evidence that the microbiome can play a robust causal role under experimental conditions in rodent models, it may not materially affect the development of obesity in humans. Importantly, examples exist of dramatic differences in genetic susceptibility to infection in model systems, which translate into only minor differences in humans [130].

The correlation of microbiome alterations in rodent model systems, in which a causal link with obesity had been established, with altered microbiome configurations in humans with and without obesity promised to address such concerns [73,123]. In a groundbreaking initial study, twelve obese humans were shown to have relatively decreased Bacteroidetes and increased Firmicutes in their feces – similar to germ-free mouse recipients of obesogenic microbiota – in comparison to four fecal samples taken at two separate times from two lean individuals. Further, the relative abundance of Firmicutes to Bacteroidetes in these obese individuals decreased after dietary interventions resulting in weight loss [73]. Efforts to replicate these descriptive findings in humans have, however, failed, as detailed in Table 1 [33,114,122]. Further, the presence of similar shifts between Firmicutes and Bacteroidetes with aging has raised the possibility of uncontrolled-for confounders in the initial study [21,80]. These discrepant findings highlight the need for large-scale, well-controlled studies to robustly, rigorously and formally test associations between microbiome representation and architecture and obesity.

While data associating phylum-level shifts in the microbiome and obesity have not been replicable, associations between metagenomic modules and obesity phenotypes have been observed in large-scale follow-up studies, suggesting that enrichment or induction of (potentially species-independent) genetic modules may modulate the development of obesity. Alternately, such modules might themselves be enriched by obesity [5,122]. Such data suggest that *bona fide* associations may exist between microbiota and obesity in humans, although causality remains to be addressed. Whether these associations will hold up to large-scale replication has yet to be determined. This situation is reminiscent of genetic association studies done in the pre-genome-wide association scan era, during which many candidate associations were found using sample sizes which at the time were considered large, but were rather small in retrospect [54]. Very few of these earlier associations have held up to replication in the modern era, where the threshold for association is more stringent and requires sample sizes orders of magnitude larger [55]. It seems reasonable to postulate that causal contributions from the gut microbiome to the development of human obesity have effect sizes on the order of common genetic variations implicated in complex diseases. If this is the case, much larger studies will be necessary before we have clear evidence of association. Such studies may need to be carried out in parallel with genetic association studies, using analytic approaches to control for potential confounding due to population structure [99] and to control for the considerable influence of host genetics on the architecture of the microbiome, an influence which has been documented in controlled settings [9]. As is the case with genome-wide association studies, the technology and analytical capacity [67] for carrying out such large-scale analysis of the microbiome are moving forward at a rapid pace. Both cross-sectional and prospective studies on the scale of tens of thousands of individuals, with enough power to detect moderate effect sizes and control for an abundance of confounding factors in outbred human populations will be essential to adequately address this question. Fortunately, such studies seem likely to be feasible in the near future [66]. Such studies would certainly benefit from the experience gained from ongoing large-scale endeavors including MetaHIT [101] and the Human Microbiome Project [100]. Of course, the interpretation of such studies would necessarily be informed by smaller scale, more in-depth studies aimed at addressing whether perturbations in the gut microbiota are capable of modulating energy balance in the short term [60].

Large-scale descriptive studies may well provide solid evidence of correlations between microbiome architecture, functional genetic modules or specific species and human obesity. Demonstration of causality in humans will require experimental interventions that both perturb the gut microbiota and modulate obesity, however, thus fulfilling some form of Koch's postulates. In Box 1, we propose an adaptation of Falkow's molecular Koch's postulates for microbial pathogenicity to cover issues of causality in the relationship between the microbiome and obesity [36]. Such studies would require several years for their data to mature, even after the identification of obesogenic microbial culprits.

Fortunately, novel experimental approaches promise to bridge the gap between questions of causality in rodent model systems and

Box 1–Adapting Falkow's molecular Koch's postulates [36] to microbiome-mediated modulation of obesity.

(1) The phenotype should be associated with a microbial culprit, which could include:
  (a) a microbial species or group of species
  (b) a microbial metagenomic module
  (c) a microbe-derived molecule or set of molecules
(2) Specific inactivation or depletion of the microbial culprit, by
  (a) specific depletion of the microbial species or group of species
  (b) deletion of the metagenomic module
  (c) neutralization or removal of the molecule(s)
  should lead to a robust change in the associated phenotype.
(3) Introduction of the microbial culprit, by
  (a) colonization or recolonization with the depleted subset
  (b) re-induction of the metagenomic module
  (c) reintroduction of the molecule(s)
  should restore the phenotype.

It should be noted that persistent alterations of physiological states, not easily reversible by removal of the suspected organism(s)/metagenomic module(s)/microbial product(s), might occur. The development of immunological memory and tolerance serve as examples.

descriptive correlates in humans. Employing transfer of defined subsets of the human microbiome to germ-free mice, with vertical transmission to subsequent generations, such approaches promise to more effectively model the gut microbiome of humans (and its contribution to obesity) in rodents, by controlling for potential confounders [42,124]. Such approaches will need to be paired with human studies in order to draw meaningful correlates [84]. Of course, the ultimate goal of these studies is to accurately model microbiome dynamics, variability, and their effects on the development of obesity in a way that can be translated back to humans. This may not be possible for three primary reasons: (1) the possibility that the species or microbiomic parameters which are crucial for microbiome-mediated modulation of obesity display host specificity; (2) the presence of differences between obesity in recombinant inbred rodents in the lab and in humans in "the wild"; and (3) the fact that modulation of microbiome-mediated obesity may only be fully evident when both the microbes and the molecular mechanisms by which they alter the development of obesity are laid bare. While the latter possibility is discussed in further detail below, all three concerns underscore the need for interventional studies of the obese human microbiome. Suffice it to say, whether the microbiome actually modulates obesity and its complications remains to be established in humans.

MICROBIAL IDENTITY AND SPECIFICITY

With the demonstration of obesogenic microbiota in rodent systems and correlative evidence in human obesity, the focus of the field has shifted towards the identification of microbial culprits. Causal implication of specific commensal bacteria has not yet occurred, although compelling data associating phylum-level shifts in mice [72,123] and metagenomic differences in humans [73], discussed above, have been generated. In the case of the former, transmissibility studies do suggest causality [121,123].

It remains possible that the culprits are specific microbial species, a congeries of functionally similar species from diverse microbial classes, or even a functional set of gene expression modules [5,122,134]. Defining this may be contingent on defining the responsible mechanism(s), a paradoxical situation that underscores one of the central problems of the field. Presently, we have limited reductionist understanding of the mechanism(s) of microbiome-mediated modulation of obesity phenotypes because we have limited reductionist understanding of the microbial/molecular species involved. And *vice versa*. Until traction is gained on one side or the other, we may not find a satisfying answer to either question. That is, if we could successfully model obesity driven by a single microbial member, identifying the mechanism by which this occurred would likely prove easier. Similarly, if we understood the mechanism(s) by which the microbiome can alter obesity, identifying the causally responsible player(s) might be a more straightforward undertaking.

To some, the notion that a single commensal (or group of commensals) could single-handedly accelerate obesity may seem far-fetched. Examples exist of commensals which are capable of reprogramming host (patho-)physiology. Considering one such example may provide a clearer picture of how a single species might alter the development and maintenance of obesity. Segmented filamentous bacteria (SFB), classified as a commensal – although perhaps more accurately classified as a pathobiont, is capable of single-handedly having a major effect on programming of the local and systemic immune environment after weaning. It does so by polarizing the immune response towards increased total IgA production, increased intestinal antimicrobial peptide production and increased conversion of naïve CD4+ T-helper cells into a variety of mature effector populations. SFB has further been demonstrated to be capable, by itself in many cases, of driving significant extra-intestinal pathophysiology in immune-mediated disease models [57,65,70,82,135]. Curiously, one of the immune responses that SFB specifically induces is the polarization of naïve CD4+ T-helper cells to IL-17-producing effector cells in the gut [40,56]. Importantly, IL-17 has recently been implicated as a negative regulator of adipogenesis, obesity and glucose dysmetabolism [139]. Thus, given: (1) the role of SFB in driving Th17 cell conversion in the gut; (2) the influence of IL-17 on obesity and its metabolic and end organ complications; and (3) the established role of SFB-mediated exacerbation of extra-intestinal pathophysiology in a variety of settings, it seems plausible that a single species of bacteria might very reasonably modulate the development of obesity and its metabolic and end organ complications, though this remains to be determined.

Beyond commensal bacteria, a number of examples of the apparent influencing of adiposity and obesity by pathogenic viruses have been described [18,46,88]. One such virus, human adenovirus 36 was both causally implicated in obesity in rodent model systems and found to be associated with obesity in humans [6,97]. However, subsequent investigation failed to replicate the association, highlighting potential disconnects between capability in rodent model systems and actuality in outbred human populations [43].

To date, the focus of the field has been on descriptive studies aimed at identifying the particular microbial culprits involved in the development and maintenance of obesity. Such studies are a necessary precondition for progress on the other questions (actuality, mechanism and therapy), which we review.

MECHANISTIC POSSIBILITIES

Regardless of the underlying microbial culprits, identification of the mechanisms through which they act to regulate obesity may well lead to identification of novel targets for obesity prevention and/or therapy. A number of mechanisms have been proposed. Evidence, for each has been gathered, as detailed below. A few key points will aid our consideration of these varied mechanistic possibilities. First, there may be a multiplicity of mechanisms at play in the microbiome-dependent contribution to obesity phenotypes. Second, such mechanisms are likely not mutually exclusive (e.g., immune effects could select for microbiota with an altered capacity for energy harvest). Third, as a necessary consequence of involving the microbiome, some of these mechanisms may be context-dependent (e.g., a particular configuration of the microbiome may be obesogenic in the dietary, immunologic and genetic context of one host and not in another).

(a) *Energy harvest*: The microbiologists among us are keen to remind us that our gut microbes are among the first cells to encounter and process our food. As such, the microbiota are uniquely situated to modify the extent to which nutrients can be extracted. In this light, differential energy harvest capacity by microbiota was proposed as a mechanism for the increased adiposity of the recipients of microbiota from obese mice [73,123]. Subsequent investigation has revealed that the relation of obesity to markers of energy harvest capacity, including quantitative measures of short-chain fatty acids, butyrate, propionate and acetate production, is likely more complex than originally suspected [92]. Furthermore, it is unclear how such a difference in energy intake might bypass the homeostatic systems that regulated energy uptake and storage [111]. While examples of gut

microbiomes with differential capacity for energy harvest have been described [29], further study will be necessary to define the contribution of microbial energy harvest capacity to weight regulation. Ideally, studies of monocolonized (or conventionalized) gnotobiotic mice differing only in the presence of wild-type and mutant species lacking specific energy harvesting capacities would represent a major step towards evaluating the validity of this potential mechanism. However, the complex interdependence between metabolic markers of energy harvest and host physiology may hamper reductive evaluation of this mechanism. Specifically, microbe-derived short chain fatty acids regulate host physiology and immune function via gut-expressed receptors [81,120], suggesting a complementary mode of action.

(b) *Energetics*: Many proposed mechanisms fit within the rubric of a microbial metabolite or product modulating energy balance. Metabolomics studies have sought to identify such factors; promising candidates have been identified [51]. As above, short chain fatty acids have received particular attention. On the energy intake side of the equation, mice deficient in ffar3 (GRP41), a receptor for a variety of short chain fatty acids [11], are protected from obesity in the absence of microbiota, but not in the presence a conventionalized microbiota or a reductive model thereof [110]. The proposed mechanism of protection is microbiota-induced peptide YY expression, which has been reported to alter food intake [69]. However, this may be due to basal defects in short chain fatty acid-driven leptin production in white adipose tissue [136], as GPR41-deficient mice do not produce normal levels of serum leptin in the presence of microbiota [110]. As for microbiota-mediated regulation of energy storage, the lack of microbial induction of small intestinal expression of *fiaf* (angptl4), a negative regulator of a lipoprotein lipase that regulates lipid uptake into tissues, has been proposed to underlie the protection of germ-free mice from adiposity, though this finding is controversial (see above in "Capability") [7]. Finally, on the energy expenditure side of the equation, comparison of HFD-induced obesity in germ-free and conventionalized mice has suggested a role for differential AMPK activity [8]. The responsible bacterial product, or bacterially driven pathway, has not been identified. Further studies exploring the links between specific bacterial products the relevant host receptors and physiological pathways will be important to dissect the microbe-mediated contribution to each of these component parts of energy balance in microbiome-mediated modulation of obesity.

(c) *Inflammation*: Mirroring microbiologists, immunologists are keen to remind us that our immune systems devote a plurality of adaptive immune cells towards patrolling the mucosal barrier of the gastrointestinal tract, responding to and regulating relationships with commensals and pathogens alike. That said, while there are attractive hypotheses for linking gut immune responses to commensals with altered energy homeostasis, there is a paucity of mechanistic data. Potential mechanisms include (1) the local induction of cytokines that alter intestinal permeability [16,79,90]; (2) the systemic induction of cytokines—due, perhaps, to the increase in bacterial products observed in the circulation of mice and men on HFD ("metabolic endotoxemia" [14,16])—that alter energy balance; and (3) immune-mediated alteration of microbiome architecture or physiology [62].

In terms of the latter mechanisms, genetic deficiency in TLR5, which signals the presence of bacterial flagellin, was reported to foster a microbial environment that facilitates obesity as well as microbiota capable of transmitting obesity into germ-free mice [127]. The phenomenon was, however, not replicable in other labs [71]. Studies dissecting the relative contribution of such mechanisms will require careful analysis, as the balance between low-level and frank inflammation is delicate. For example, a subset of *tlr5*-deficient mice develops colitis, severe enough to result in rectal prolapse [128]. Similarly, mice deficient in *asc*, a key inflammasome component, selected a dysbiotic microbiota with the capability of accelerating disease in liver damage models, as detailed below. This dysbiosis also contributed to increased obesity in co-housed $Lepr^{db/db}$ mice [49]. Curiously, recent reports indicate that, in addition to its role as a regulator of food intake and adiposity [39], leptin plays an important role in mucosal immunity in the gut. Specifically, (1) mice carrying a hypomorphic allele of *ATG16L1*, a risk allele for Crohn's disease, exhibit increased leptin expression in small intestinal paneth cells, similar to patients with Crohn's disease who are homozygous for the risk allele; (2) this leptin receptor mutation is associated with *Entamoeba histolytica* infection in humans; and (3) intestinal epithelial cell expression of leptin-receptor is necessary for protection in a mouse model of such infection [12,32,47] Given this, the fact that leptin-deficient, $Lep^{ob/ob}$, mice were the source of microbiota able to transfer increases in energy harvesting capacity into germ-free mice [123] suggests the possibility that microbiome differences resulting directly from the genetic lesion in leptin may contribute to obesity, although the overall contribution of the microbiota to obesity in $Lep^{ob/ob}$ mice is likely quite small [23,64].

While the cellular and molecular details of the potential causal connections between inflammation and the development of obesity remain to be revealed, it is abundantly clear that inflammation plays a critical role in the promotion and exacerbation of the metabolic and end-organ complications of obesity [41,52,68,75,131,132]. Low-grade tissue inflammation in response to both nutrient overload and metabolic stress is thought to result in elaboration of cytokines, which directly alter insulin signaling in the periphery [45,95]. Importantly, TLRs and inflammasomes have been implicated in the exacerbation of non-alcoholic steatohepatitis, insulin resistance and atherosclerotic coronary artery disease in mouse models [1,25,31,49,116,119]. Some of these innate immune sensors are likely to exert their effects via alteration of the gut microbiota. Mice deficient in TLR5 and ASC, described above, exhibit gut microbiome-transmissible glucose dysmetabolism and acceleration of liver damage in the methionine-deficient, choline-deficient model of liver damage, respectively [49]. In both studies, key roles for pro-inflammatory microbial products were described.

Thus, a number of competing (though not necessarily mutually exclusive) potential mechanisms for microbiome-mediated modulation of obesity development and maintenance have been put forth. The field is currently in a somewhat data-impoverished zone. Nonetheless, microbiota-driven inflammation plays a clear role in exacerbating the complications of obesity, at least in animal models.

THERAPEUTIC APPLICABILITY

Despite the difficulty of task ahead, the field is spurred on by its ultimate goal – therapeutic manipulation of the microbiome to prevent or treat obesity and its discontents. Such novel approaches are clearly needed [85,133]. A multitude of microbiome manipulations, ancient and modern, have been proposed. Among the former, the use of probiotics such as yogurt has thousands of years of safety data along with some recent apparent efficacy data, at least with regards to long-term weight loss in humans [89]. The use of probiotics as growth promoters for livestock warrants cautious application of probiotics as a therapy for obesity, however [4]. Importantly, other dietary interventions, such as prebiotics (host-indigestible substrates for microbial fermentation, which alter the microbiota) may be of use [94], but likely require a more nuanced understanding of microbiome–diet interactions [134]. The antibiotic revolution of the past century has left some suspicion in its wake, as antibiotic use in early life has been associated with increased risk of overweight later, in children of normal weight

mothers [3]. Curiously, though, antibiotics also appear to decrease the risk of overweight in children of overweight mothers [3].

However, antibiotic approaches may also provide a solution. While the wholesale changes in microbial ecology induced by antibiotics, [28,125] have been discussed above, the use of species-specific morpholinos [44], species-specific bacteriophages [48] or bacteriocins [104] may allow finer targeting of obesogenic microbial culprits—inter alia allowing for experimental imputation of their causal role. Finally, recent excitement has arisen surrounding the concept of the fecal transplant therapy, used for antibiotic-refractory *Clostridium difficile*-associated colitis [10]. However, the observation that a colitic dysbiosis can be transferred from genetically susceptible to wild-type murine hosts suggests that, without the use of defined microbial communities, such an approach poses serious safety concerns [35]. Further, this observation suggests that if the microbiome does make an etiologic contribution to obesity, an obesogenic dysbiosis might similarly be casually transferrable amongst humans, an idea that is not entirely far-fetched in light of the observed propagation of obesity within a social network over several decades [20]. Despite these caveats, numerous approaches to the therapeutic manipulation of the microbiome exist, and one or more of them seem amenable to use for modulating potentially obesogenic microbiota.

FUTURE DIRECTIONS: THE STREAM AHEAD IS AWASH WITH COMPLEXITY

This review has highlighted what we consider some key steps in moving towards the goal of therapeutic manipulation, including: (1) large-scale studies to robustly implicate microbial culprits in relation to obesity phenotypes in humans; (2) parallel studies using a variety of approaches in rodent model systems to establish causal capability; (3) demonstration of Koch's postulates for microbial culprits thus identified via interventional studies; and (4) thorough, systematic, interdisciplinary evaluation of relative competing mechanistic proposals. The latter underscores the hard road ahead for the field. A key problem in attempts to define the molecular mechanisms underlying microbiome-mediated modulation of obesity is our present limited understanding of the complex dynamics of host–microbe and microbe–microbe interactions [9,26]. The true level of complexity may be daunting. Nevertheless, the field is progressing apace. In addition to providing insights into obesity, a better understanding of the relationship between microbiome, energy balance and obesity may well provide needed insights into its critically important obverse: malnutrition [137].

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