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## Gut Microbiota: The Neglected Endocrine Organ

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The concept that the gut microbiota serves as a virtual endocrine organ arises from a number of important observations. Evidence for a direct role arises from its metabolic capacity to produce and regulate multiple compounds that reach the circulation and act to influence the function of distal organs and systems. For example, metabolism of carbohydrates results in the production of short chain fatty acids (SCFAs), such as butyrate and propionate, which provide an important source of nutrients as well as regulatory control of the host digestive system. This influence over host metabolism is also seen in the ability of the prebiotic inulin is to influence production of relevant hormones such as glucagon like peptide-1 (GLP-1), peptide YY (PYY), ghrelin and leptin. Moreover, the probiotic *L. rhamnosus* PL60, which produces conjugated linoleic acid, has been shown to reduce body weight gain and white adipose tissue without effects on food intake. Manipulating the microbial composition of the gastrointestinal tract modulates plasma concentrations of tryptophan, an essential amino acid and precursor to serotonin, a key neurotransmitter within both the enteric and central nervous systems. Indirectly and through as yet unknown mechanisms, the gut microbiota exerts control over the hypothalamic-pituitary-adrenal axis (HPA). This is clear from studies on animals raised in a germ-free environment, who show exaggerated responses to psychological stress, which normalises following monocolonisation by certain bacterial species including *B. infantis*. It is tempting to speculate that therapeutic targeting of the gut microbiota may be useful in treating stress-related disorders and metabolic diseases.

**M**icrobiome science has prospered in recent years and the exciting discoveries arising from this surge in interest have forced a re-evaluation of our perception of the trillions of microorganisms which reside in the human gastrointestinal (GI) tract in particular. The gut microbiota performs a number of essential protective, structural and metabolic functions for host health including food processing, digestion of complex host-indigestible polysaccharides, pathogen displacement and synthesis of vitamins (1–3). As well as a direct action on the gut mucosa and the enteric nervous system, the metabolic output of the gut microbiota gives it a reach well beyond the local GI compartment. Thus, considering the ability to influence the function of distal organs and systems, in many respects the gut microbiota resembles an endocrine organ (4, 5).

Through this lens, the microbiota produces numerous chemicals of a hormonal nature which are released into the blood stream and act at distal sites (see Table 1). The targets for these substances are not just the local enteric nervous system but many other organs including the brain. It releases its hormonal products into interstitial tissue, to be picked up by blood and lymph capillaries and these secretions are usually effective in low concentrations on target organs or tissues remote from the enteric microbiota. As well as providing an important direct source of humoral agents, both active compounds and precursors, which act at distal sites (see Figure 1), the microbiota also plays an indirect role in regulating complex endocrine networks. Moreover, specific members of the overall microbial community can respond to hormones secreted by the host (6, 7). Taken together with the

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Abbreviations:

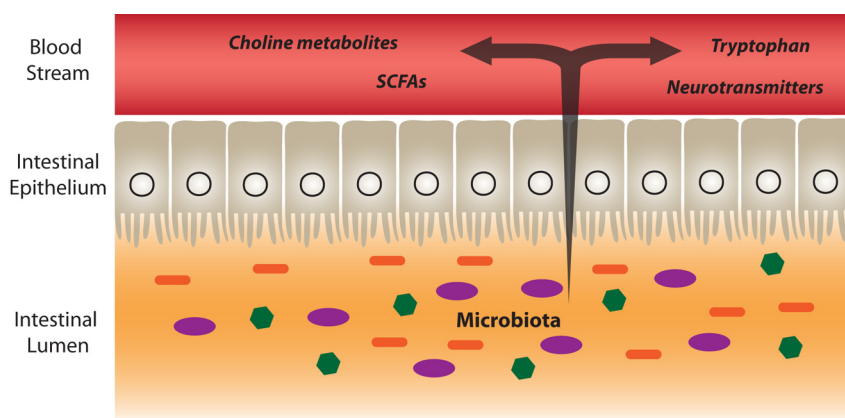
**Table 1.** Candidate Hormones of the Gut Microbiota

Class	Example (s)	Function (s)	Comment	Reference
SCFAs	Acetate	Energy Source	Directly produced by bacteria; Epigenetic and receptor-mediated effects; CNS effects linked to autism-like behaviours	(63, 84, 94)
Neurotransmitters	Butyrate	Host Metabolism	Can be directly produced by bacteria (see table 2) or indirectly regulated.	(61, 188, 200)
	Propionate	Signalling Molecules		
	Serotonin	Mood, emotion, cognition, reward (CNS);		
Precursors to Neuroactive Compounds	Dopamine	Motility/Secretion (ENS)	Kynurenine is itself a metabolite of tryptophan, production subject to regulation by microbiota	(135, 137, 138, 194)
	Noradrenaline			
	GABA			
Bile Acids	Tryptophan	Precursor to: 5-HT	Some effects mediated by bile acid receptors	(5, 101)
	Kynurenine	Kynurenic acid, quinolinic acid Dopamine		
Choline Metabolites	L-Dopa	Host Metabolism	Metabolised in the liver to trimethylamine-N-oxide, linked to cardiovascular disease	(37, 102, 103)
	Secondary bile acids	Lipid Metabolism (Choline)		
HPA Hormones	Cortisol	Stress Response	<u>Indirect regulation</u> ; HPA endocrine abnormalities prominent in stress-related psychiatric disorders	(177)
		Host Metabolism Anti-inflammatory Wound-healing		
Gastrointestinal Hormones	Ghrelin	Host Metabolism	<u>Indirect regulation</u> ; Possibly mediated by SCFAs via enteroendocrine cells	(70, 128)
	Leptin	Appetite Regulation		
	Glucagon like peptide-1 (GLP-1)	Gastrointestinal Motility/Secretion		
	Peptide YY (PYY)			

specialized functions performed by the gut microbiota and despite the obvious physical dissimilarity, its ability to function as a collective to influence other organs within the host and, in turn, to be responsive to the secretions of other host organs satisfies the most important conditions of any conceptual definition of an organ (8). It is these qualities which have seen the term ‘virtual organ’ assigned to the gut microbiota (5).

In this review, we consider the main features of the gut microbiota which enable this endocrine capacity and ren-

der it susceptible to modification, focusing on development, structure and function as well as the key hormonal mediators produced. Using specific examples, we explore the regulation of glucose metabolism and obesity together with the impact on the stress system, especially on the hypothalamic-pituitary-adrenal axis (HPA), and the influence on brain and behavior. Having examined the key endocrine functions this collection of microorganisms regulates in the host, we also consider the implications for health and disease.



**FIGURE 1. Hormone-like metabolites produced or regulated by the Gut Microbiota** Microbial metabolites, such as short chain fatty acids (SCFAs), with signaling functions are secreted into the gut lumen, transported across the epithelial barrier and transported to the effector organs, including the brain, via the blood stream. The gut microbiota is also capable of producing or releasing neurotransmitters such as serotonin or regulating the availability of precursors such as tryptophan. The microbiota also regulates the bioavailability of choline and its metabolites.

### The Diverse Gut Microbiome: A Complex Endocrine Organ

Unlike other endocrine systems or organs (see **Figure 2**) which secrete a single or at most a small number of humoral agents, the gut microbiota has the potential to produce hundreds of products. From a morphological and biochemical perspective, it is far larger and more biochemically heterogeneous than any other endocrine organ in man (9). In fact, the biochemical complexity of the gut microbiota even exceeds that of the brain and many of the hormones produced by the microbiota are also

neurotransmitters within the central nervous system (CNS). For example GABA, the most important inhibitory transmitter in the brain is produced by several *Lactobacilli* (10), while monoamines such as noradrenaline, dopamine and serotonin are also produced by certain strains of bacteria (11, 12) (See Table 2)

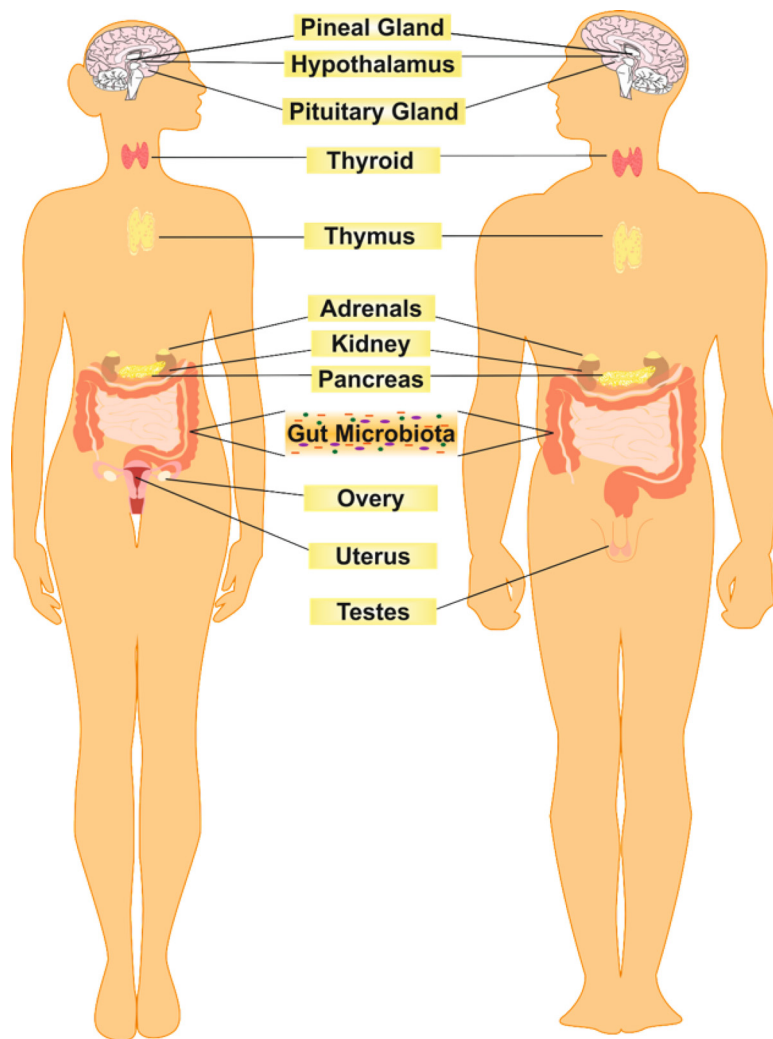
This biochemical capacity arises from the vast and diverse array of microbial cells, with an approximate weight of 1–2 kg in an average adult (13). The number of cells is far greater than the number of host cells such that an estimated 90% of cells found in the human body are actually of prokaryotic origin, derived from at least 40,000 bacterial species in 1800 genera (14, 15). The genomic complexity of this ‘virtual’ endocrine organ is enormous and in the adult, approximately eight million genes are represented in a small number of phyla (16). However, most of our gut bacteria are not readily culturable

in the laboratory and there is potential for selective growth bias towards specific organisms (17). Thus, in many ways, our appreciation of the functional repertoire of the gut microbiota has been constrained and distorted by the culture-based techniques which have dominated microbiology until relatively recently (18). An increased understanding of the key microbiota genes involved in its various functions is essential to parse and beneficially exploit the endocrine role of the gut microbiota.

### Structure and Development of the Gut Microbiome

Our knowledge of the gut microbiome is rapidly expanding and this is due to the emergence and rapid technological advances in culture-independent techniques, particularly genomic approaches (19, 20). This is probably best exemplified by large-scale metagenomic-based studies such as NIH funded Human Microbiome Project (HMP, <http://commonfund.nih.gov/hmp>), the European funded Metagenomics of the Human Intestinal Tract (MetaHIT, <http://www.metahit.eu>) consortium and the International Human Microbiome Consortium (IHMC, <http://www.human-microbiome.org>). Together, these studies have helped determine the breadth of microbial variation and function across large populations, revealed a high interindividual diversity, stability over time and established associations between microbiota alterations (both in terms of composition and stability) and disease states (21–23).

In adults, there is a stable and diverse gut microbiome, dominated by Bacteroidetes and Firmicutes phyla, and there seems to be a high metagenomic abundance of a housekeeping core with specific functional capabilities (21). These features of compositional stability and diversity are considered essential for health but are also readily amenable to potentially deleterious alterations. Moreover, the developmental trajectory from birth, which may prime for health later in life, is also fragile (24). Interestingly and in contrast to the elaboration of other endocrine



**FIGURE 2. The Gut Microbiota as an Endocrine Organ** The endocrine system is primarily composed of glands that each produce a single or at most a small number of humoral agents. However, the microbiota produces numerous chemicals of a hormonal nature which are released into the blood stream and act at distal sites. This biochemical capacity arises from the vast and diverse array of microbial cells which constitute the gut microbiota.

**Table 2.** Examples of Neurotransmitter-producing or releasing bacterial strains

Neurotransmitter	Bacterial Strain	Reference	
Serotonin	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> (MG 1363)	(201)	
	<i>L. lactis</i> subsp. <i>lactis</i> (IL1403)	(201)	
	<i>Lactobacillus plantarum</i> (F18595)	(201)	
	<i>Streptococcus thermophilus</i> (NCFB2392)	(201)	
	<i>Escherichia coli</i> K-12	(156)	
	<i>Morganella morganii</i> (NCIMB, 10466)	(202)	
	<i>Klebsiella pneumoniae</i> (NCIMB, 673)	(202)	
	<i>Hafnia alvei</i> (NCIMB, 11999)	(202)	
	Dopamine	<i>Bacillus cereus</i>	(203)
		<i>B. mycooides</i>	(203)
<i>B. subtilis</i>		(203)	
<i>Proteus vulgaris</i>		(203)	
<i>Serratia marcescens</i>		(203)	
<i>S. aureus</i>		(203)	
<i>E. coli</i>		(203)	
<i>E. coli</i> K-12		(156)	
<i>M. morganii</i> (NCIMB, 10466)		(202)	
<i>K. pneumoniae</i> (NCIMB, 673)		(202)	
Noradrenaline	<i>H. alvei</i> (NCIMB, 11999)	(202)	
	<i>B. mycooides</i>	(203)	
	<i>B. subtilis</i> ,	(203)	
	<i>P. vulgaris</i>	(203)	
	<i>S. marcescens</i>	(203)	
GABA	<i>E. coli</i> K-12	(156)	
	<i>L. brevis</i> DPC6108	(204)	
	<i>B. adolescentis</i> DPC6044	(204)	
	<i>B. dentium</i> DPC6333	(204)	
	<i>B. dentium</i> NFB2243	(204)	
Acetylcholine	<i>B. infantis</i> UCC35624	(204)	
	<i>L. rhamnosus</i> YS9	(205)	
	<i>L. plantarum</i>	(206)	
	Histamine	<i>L. lactis</i> subsp. <i>cremoris</i> (MG 1363)	(201)
		<i>L. lactis</i> subsp. <i>lactis</i> (IL1403)	(201)
<i>L. plantarum</i> (F18595)		(201)	
<i>S. thermophilus</i> (NCFB2392)		(201)	
<i>M. morganii</i> (NCIMB, 10466)		(202)	
	<i>K. pneumoniae</i> (NCIMB, 673)	(202)	
	<i>H. alvei</i> (NCIMB, 11999)	(202)	

organs, colonization of the infant gut only commences at birth when the infant is first exposed to a complex microbiota and its initial microbiome is seeded from maternal sources. Existing data indicate that babies born by caesarean section (C-section) develop a different microbiota to their vaginally-delivered counterparts with aberrant short-term immune responses and a greater long-term risk of developing immune diseases (25–27). Whether or not C-section delivery impacts on the subsequent endocrine capacity of the gut microbiota is uncertain but likely and higher rates of diabetes in such children have been established (26). The microbiome of unweaned infants is simple with alterations dictated by, for example, weaning or infection (28). After 1–3 years of age, a complex adult-like microbiome is evident and this adult-type microbiome is functionally specialized for energy harvest from particular diets (29). Homeostatic mechanisms within the

microbiota become less effective in the elderly and it is clear that the microbiota diverges between those who age healthily and those whose health deteriorates with age (30, 31).

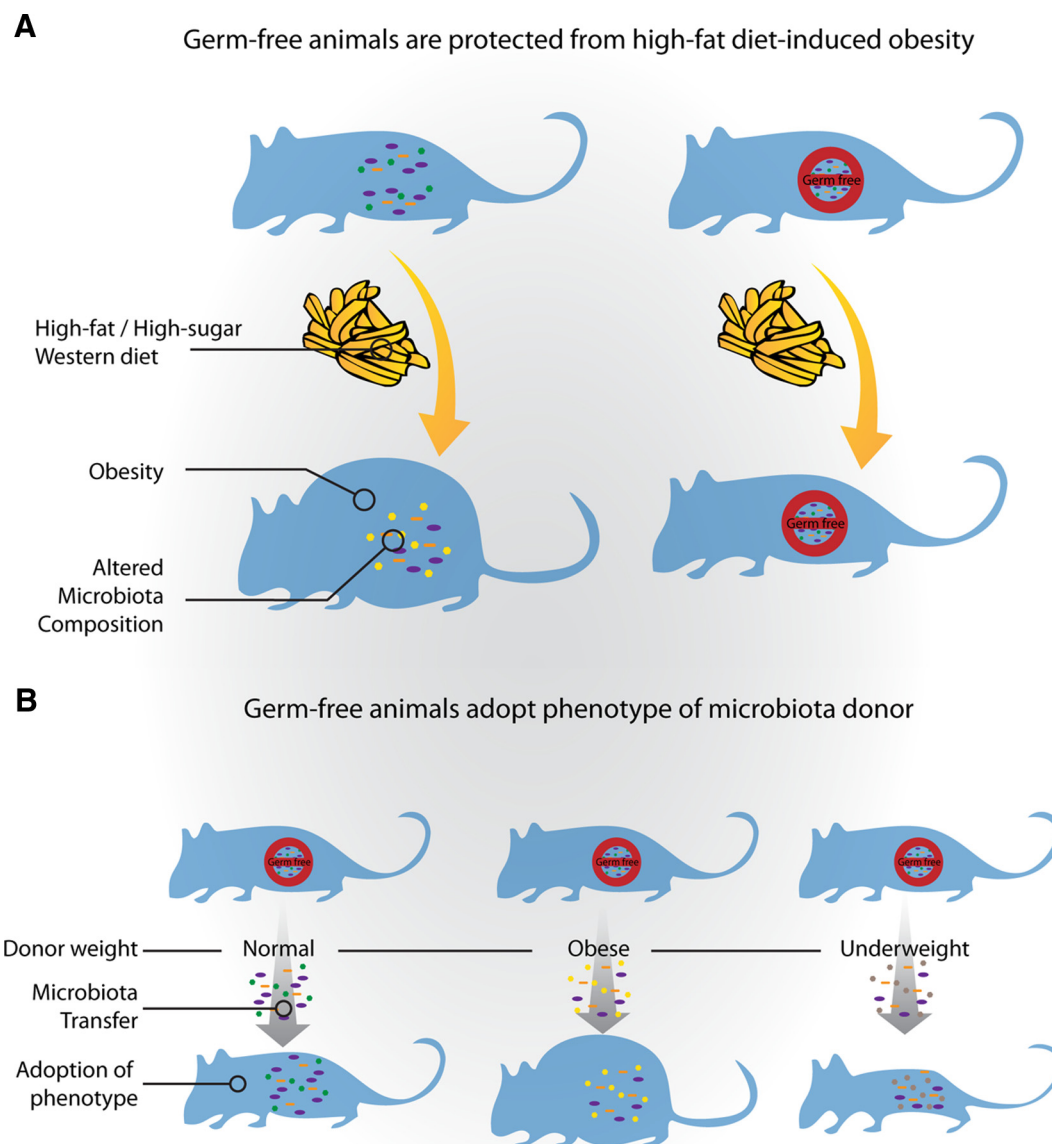
The concept of bacterial enterotypes within the gut ecosystem has recently emerged (32) and these alternative stable states of the gut microbiota have differential functional capabilities and by extension, potentially differential endocrine capabilities. Driven by species composition, these enterotypes are marked out by a relatively high abundance of either *Bacteroides*, *Prevotella* or *Ruminococcus* species independently of body mass index (BMI), age or gender (33). The *Bacteroides* and *Prevotella* enterotype have subsequently been associated with diets enriched for protein/animal fat and carbohydrate respectively and appear to be somewhat resilient to short-term dietary interventions (34). However, caution is advisable

and a debate persists regarding the clarity of these enterotypes and whether they might more accurately be considered gradients (35). This view is supported by a study based on HMP data in which a proportion of intermediate subjects were identified without such discrete boundaries (36). Nevertheless, the concept of an endocrine profile dependent on specific microbiome states is appealing and warrants further investigation.

### Microbiota and Host Metabolism

The endocrine range of the gut microbiota may at least partially explain the links between this microbial commu-

nity and the development of obesity, cardiovascular disease and metabolic syndromes (37). The functional interactions allowing such an important contribution to host metabolism and energy homeostasis are manifold and allow for a key role in regulating insulin sensitivity, fat storage, adiposity and body weight (see **Figure 3**) (38). Bäckhed and coworkers showed that mice raised in absence of microorganisms, termed germ-free (GF), had less total body fat than mice raised conventionally (CONV-R), despite the fact that GF animals had higher caloric intake (39). When GF mice are colonized with the microbiota of a conventional mouse they show a robust in-



**FIGURE 3. The Gut Microbiota and Host Metabolism 3A: Germ-free animals are protected from high-fat diet-induced obesity**

A number of important studies have demonstrated that germ-free animals gain less weight than their conventionally colonized counterparts when fed a high-fat/high-sugar western style diet. Germ-free animals also have less total body fat than mice raised conventionally and when colonized with the microbiota of a conventional mouse, they show a robust increase in body fat. Moreover, obesity is associated with alterations in the composition of the microbiota. **3B: Germ-free animals adopt phenotype of microbiota donor** Previously germ-free mice display an obese phenotype on receipt of a microbiota transplant from obese mice or when humanised with a microbiota from an obese individual. Similarly, germ-free animals lose weight upon receipt of a microbiota transfer from animals who have exhibited rapid weight loss following gastric bypass surgery

crease in body fat and become glucose intolerant even with a reduction in food intake. Bäckhed and coworkers also demonstrated that GF mice fed a high-fat, high-carbohydrate Western diet for eight weeks gained significantly less weight than CONV-R mice and were protected against diet-induced glucose intolerance and insulin resistance (40).

Interestingly, previously GF mice display an obese phenotype on receipt of a microbiota transplant from obese mice (41, 42) and lose weight upon receipt of a microbiota transfer from animals who have exhibited rapid weight loss following gastric bypass surgery (43). In line with this, a recent study showed transmission of the donor's metabolic phenotype using GF mice that received a microbiota transfer from monozygotic twins discordant for obesity. Notably, when cohousing mice who had received an obese twin's microbiota with mice containing the lean cotwin's microbiota, the development of increased body mass and obesity-associated metabolic profile was prevented, possibly due to the invasion of specific *Bacteroidetes* species (44). Taken together with the evidence that GF animals appear to be protected against metabolic diseases, these studies suggest a possible causal relationship between the gut microbiota and the emergence of obesity (45).

Whether changes in microbiota composition are the cause or the consequence of obesity and what particular community configurations or members are important to the development of obesity have been studied intensively. In genetically obese leptin deficient *ob/ob* mice, this characteristic has been associated with a reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes, two of the major bacterial phyla in the gut microbiota (46). Similar changes in microbiota composition have been observed in wild-type mice fed a high-fat/high-sugar Western diet (41). The functional consequences of these compositional shifts might well be reflected in differential biochemical profiles and functional metabolic outcomes, with the shift towards Firmicutes, for example, promoting more efficient absorption of calories and subsequent weight gain (47).

In line with these principles, Turnbaugh and coworkers identified a 'core microbiome' of microbial genes shared among individuals and found that variations from that core are associated with obesity (Turnbaugh and Gordon, 2009). However, comparisons of the abundance of the two major bacterial phyla in the obese vs. lean microbiome in animals and humans, have often produced conflicting results. While several studies have reported similar increases in the ratio between Firmicutes and Bacteroidetes in obese individuals (48–50), other studies have not observed a change in the Firmicutes/Bacte-

roidetes ratio (51–53). For example, one study reported reductions in *Bifidobacterium* spp. and increases in specific members of Firmicutes (eg, *Staphylococcus*) and Proteobacteria (eg, *Enterobacteriaceae*) in overweight pregnant women (49) while another study found enrichment in *Prevotellaceae*, a group within the Bacteroidetes phylum, in obese individuals (51). These disparities highlight the importance of considering the multitude of potential confounding factors such as diet, age, degree of obesity, demographic geography and population size, as well as technique and methodology used to profile the gut microbiota, when comparing studies (19). Indeed, high-fat diets can influence the murine microbiome composition independently of obesity (54). Thus, although much of the preclinical data is convincing, it remains unclear to date from clinical studies if the altered microbiota precedes the development of obesity or is a consequence of dietary intake and differences in host physiology (55).

In this context, some important observations can be noted. Olanzapine, an atypical antipsychotic which causes weight gain and type II diabetes, has been shown to alter the composition of the gut microbiota in rats as assessed by pyrosequencing technology (56). Administration of vancomycin (a broad spectrum antibiotic) to mice with diet-induced obesity resulted in changes in Firmicutes, Bacteroidetes and Proteobacteria with an improvement in metabolic abnormalities (57). Furthermore, a fatty acid-based dietary intervention (using an oleic acid derived compound) has been shown to offset a high-fat diet-induced dysbiosis of the gut and improve weight gain measures in obese mice (58). Conversely, transfer of the microbiomes from women in the third trimester of pregnancy, characterized by reduced microbial diversity and enrichment in Proteobacteria and Actinobacteria, to germ-free mice induced symptoms of metabolic syndrome in the mice (59). It seems likely then that at least in some cases of obesity, the microbiota might be a causal factor and a therapeutic target for symptom improvement.

However, it is important to acknowledge that obesity is likely multifactorial, with both central and peripheral mechanisms underlying its pathogenesis, including hypothalamic dysfunction (60). It is currently unclear whether CNS control of food intake is subject to microbial influences, endocrine or otherwise, at relevant brain centers (61). Future studies geared towards identifying important microbial genes instead of focusing solely on characterizing gut microbiota composition are required in order to more accurately describe the obese microbiome and predict its response after therapeutic intervention. Nevertheless, there are a number of candidate hormones, either produced or regulated by the gut microbiota, which might

mediate the impact which the gut microbiota can have on host metabolism.

### Candidate Hormones of the Gut Microbiota: Focus on Short Chain Fatty Acids

Short chain fatty acids (SCFA) are the major products of the bacterial fermentation of carbohydrates and proteins in the gut (62, 63). In many ways, they represent the signature hormones of the microbiota and may mediate many of the functions assigned to the microbiota through classical endocrine signaling. Receptors and transporters for SCFAs are expressed in the GI tract, where they appear to be of relevance to GI function (64–69). For example, SCFAs may modulate both enteroendocrine 5-HT secretion (5) and peptide YY release, an important neuropeptide at multiple levels of the gut-brain axis (70).

Fiber metabolized by the gut microbiota can increase the concentration of circulating SCFAs (71). These circulating SCFAs, such as butyrate and propionate, travel to sites far removed from their site of production and can be carried by monocarboxylate transporters which are abundantly expressed at the blood-brain-barrier (BBB) (72–75). This provides a plausible mechanism through which they can cross the BBB and enter the CNS. Once available in the CNS, they can be taken up via these same monocarboxylate transporters on glia and neurons (76–78) and they are thought to comprise a major energy source in cellular metabolism, particularly during early brain development (79). Interestingly, they can modulate intracellular calcium levels in neutrophils suggesting a role in cell signaling (80–82) and, via regulation of tyrosine hydroxylase gene expression, can potentially impact on neurotransmitter synthesis (83). Of note, intraventricular administration of propionic acid in rats induces a variety of behavioral alterations of relevance to autism although it is unclear if this occurs via similar mechanisms to the periphery (84). It is worth noting that GPR41, a receptor activated by propionic acid, is highly expressed in rat brain tissue (85). However, it remains to be definitively established if alterations in intestinal microbiota-derived SCFAs are actually reflected at physiologically relevant concentrations in the CNS.

Although there is patently a role for these microbial metabolites beyond the local regulation of energy homeostasis (86), this is the context in which they have been most studied. The main SCFAs are acetic, propionic and n-butyric acids, occurring roughly in molar ratios of 60:20:20 in the colon (87). Through their absorption and metabolism, the host is able to salvage energy from foodstuffs, particularly resistant starch and fibers that are not digested in the upper part of the GIT. The main site for SCFA production and absorption is the proximal large

intestine, where the fermentation of the undigested food by colonic bacteria occurs at high rates. Bacteria that produce SCFA include, but are not limited to, *Bacteroides*, *Bifidobacterium*, *Propionibacterium*, *Eubacterium*, *Lactobacillus*, *Clostridium*, *Roseburia* and *Prevotella* (63).

SCFA have a multiplicity of other effects in the body, and affect epithelial cell transport and metabolism, epithelial cell growth and differentiation, and hepatic control of lipid and carbohydrates, while providing energy sources for muscles and kidneys, as well as the heart and brain (88). Epithelial cells in the distal colon derive 60–70% of their energy requirements from bacterial fermentation products (89). As indicated above, SCFA also act as signaling molecules. Propionate, acetate, and to a lesser extent butyrate, are ligands for at least two G protein-coupled receptors (GPCRs), Gpr41 and Gpr43, which are broadly expressed, including in the distal small intestine, colon and adipocytes (90, 91). SCFA interaction with Gpr43 can profoundly affect inflammatory responses. For example, mice treated with oral acetate showed a substantial decrease in inflammation. This protection was mediated by acetate binding to Gpr43, because acetate had no effect in Gpr43-deficient mice. Furthermore, it was shown that Gpr43 exhibited enhanced expression in neutrophils and eosinophils, suggesting that SCFA-Gpr43 signaling is one of the molecular pathways whereby commensal bacteria regulate immune and inflammatory responses (92). Gpr43 is also induced during adipocyte differentiation and exhibits increased levels during high-fat feeding in rodents, indicating that Gpr43 may also affect adipocyte function (93). Hong et al (2005) demonstrated that acetate and propionate act on lipid accumulation and inhibition of lipolysis mainly through Gpr43 (94). Gpr41 has been shown to be implicated in microbiota-dependent regulation of host adiposity and leptin production (95).

Butyrate is known to exhibit many important physiological functions in eukaryotic cells (63). One of the most recognized cellular mechanisms for the action of butyrate is its effects on histone acetylation (96), where the inhibition of histone deacetylase facilitates hyperacetylation of histone proteins to occur, thus facilitating the access of DNA repair enzymes. Interestingly, sodium butyrate has been demonstrated to elicit an antidepressant effect in the murine brain (97). When injected systemically, sodium butyrate induced a short-lasting, transient acetylation of histones in frontal cortex and hippocampus, in conjunction with dynamic changes in expression of the brain-derived neurotrophic factor (BDNF), thereby resulting in an antidepressant-like behavioral response (97). Antidepressants can suppress cortisol in patients with melan-



cholic depression but whether butyrate has an impact on the HPA is not known.

Enhanced carbohydrate utilization from nondigestible plant polysaccharides is known to result in higher production of SCFAs that contribute to total energy in the host. In GF mice monoassociated with the probiotic strains *Lactobacillus plantarum* WCFS1 (98) and *Lactobacillus johnsonii* NCC533 (99), an upregulation of genes involved in carbohydrate transport and metabolism was observed. While increased efficiency to hydrolyze different complex sugars in *L. plantarum* WCFS1 resulted in higher production of fumarate and alcohol (98), it led to a longer colonization period of *L. johnsonii* NCC533 in the mouse gut (99). Consumption of fermented milk containing multiple probiotic strains led to enrichment of enzymes catalyzing carbohydrates into propionate in both monozygotic twins and gnotobiotic mice containing a model human microbiota community (100).

#### **Other Candidate Hormones of the Gut Microbiota: Relevance for Host Metabolism and Disease**

The gut microbiota is involved in the transformation of primary bile acids and by doing so, may control the host profile of agents which are involved in lipid and glucose metabolism (5, 101). Interestingly, bile acids have an antimicrobial function and GF animals have more bile acids than conventionally raised counterparts (37). Secondary bile acids produced microbially may also enter the circulation to interact with similar receptors that mediate the effects of the parent compounds (eg, farnesoid X receptor  $\alpha$ ) on glucose homeostasis (5). Microbial metabolic activities also include the metabolism of choline, important for lipid metabolism, to trimethylamine (37). Once synthesized by the intestinal microbiota, trimethylamine can be further metabolized in the liver to trimethylamine-N-oxide which, when present in the circulation at sufficient concentrations, can contribute to the development of cardiovascular disease (102, 103). Moreover, reducing the bioavailability of choline can contribute to nonalcoholic fatty liver disease (NAFLD) and altered glucose metabolism, both in mice and humans (104–106). These and other mechanisms implicate the hormone-like secretions of the intestinal microbiota in liver disease, through what has been termed the gut-liver axis (107).

#### **Manipulation of the Gut Microbiota: Probiotics, prebiotics and Host Metabolism**

If the composition of the gut microbiota changes, then theoretically, the endocrine output should also be modified. This does seem to be the case when membership of the microbiota is directly modified, albeit transiently, by probiotic strains or indirectly via prebiotics which then

promote the growth of specific beneficial strains over others. Strain-specific biochemicals may then boost the endocrine capacity of the endogenous gut microbiota and act like hormones to influence host metabolism. Alternatively, the transient alteration of the microbiota composition may nonspecifically engender an alteration in the overall endocrine output from this virtual organ or produce beneficial effects by alternative, possibly unknown, mechanisms.

Conjugated linoleic acid (CLA) is a naturally occurring derivative of linoleic acid found in foods and dairy products and has been shown to increase metabolic rates in mice (108, 109). The CLA-producing probiotic strain, *L. rhamnosus* PL60, has been reported to reduce body weight gain and white adipose tissue mass with no effect on food intake in high-fat diet fed mice. The effect was coupled to higher expression of uncoupling protein-2 (UCP2), while expression of fatty acid synthase (*fas*) and serum leptin and glucose levels were reduced (110). Another probiotic strain that produces CLA, *L. plantarum* PL62, also resulted in reduced body weight gain and glucose levels in diet-induced obese mice (111). Other CLA producing strains such as *L. paracasei* NFBC 338 (112) and *B. breve* NCIMB 702258 (113) can also modulate the fatty acid composition of host adipose tissue.

Probiotics have been shown to reduce adipocyte size in different adipose depots (114, 115) which is considered an important parameter in assessing their antiobesity potential. The putative mechanisms put forth are increased faecal excretion of neutral sterols and bile acids, decreased lymphatic absorption of triglycerides, phospholipids and cholesterol (116), or increased lipolysis (117). In the 3T3-L1 cell line, incubation with *L. plantarum* KY1032 cell free extract resulted in reduced adipogenesis (118), and incubation with the insoluble fraction from fermented kefir resulted in reduced adipocyte differentiation (119). Administration of *L. paracasei* NCC2461 to rats increased sympathetic nerve activity in white and brown adipose tissue, resulting in higher thermogenesis in brown adipose tissue and increased lipolysis in white adipose tissue (120). Supplementation with *L. paracasei* F19 resulted in reduced total body fat and decreased triglyceride levels in different lipoprotein fractions in mice fed high-fat diet (121). In addition, both GF and CONV-R mice supplemented with *L. paracasei* F19 had increased serum levels of Angiopoietin-like 4 (ANGPTL4), a microbially regulated lipoprotein lipase inhibitor that regulates lipid deposition into adipocytes (121, 122). Administration of *L. paracasei* F19 and *L. acidophilus* NCFB1748 to GF mice resulted in enrichment of probiotic strains in the ileum compared to the colon and upregulation of insulin-sensitizing hormones, adiponectin and adiponectin. De-

creased expression of resistin-like  $\beta$ , known to induce insulin resistance, was also reported (123). *ApoE*<sup>-/-</sup> mice that were supplemented with *L. reuteri* ATCC exhibited reduced body weight gain, reduced adipose and liver weights, and increased expression of carnitine palmitoyl transferase 1a (*cpt1a*), suggesting higher hepatic  $\beta$ -oxidation (124).

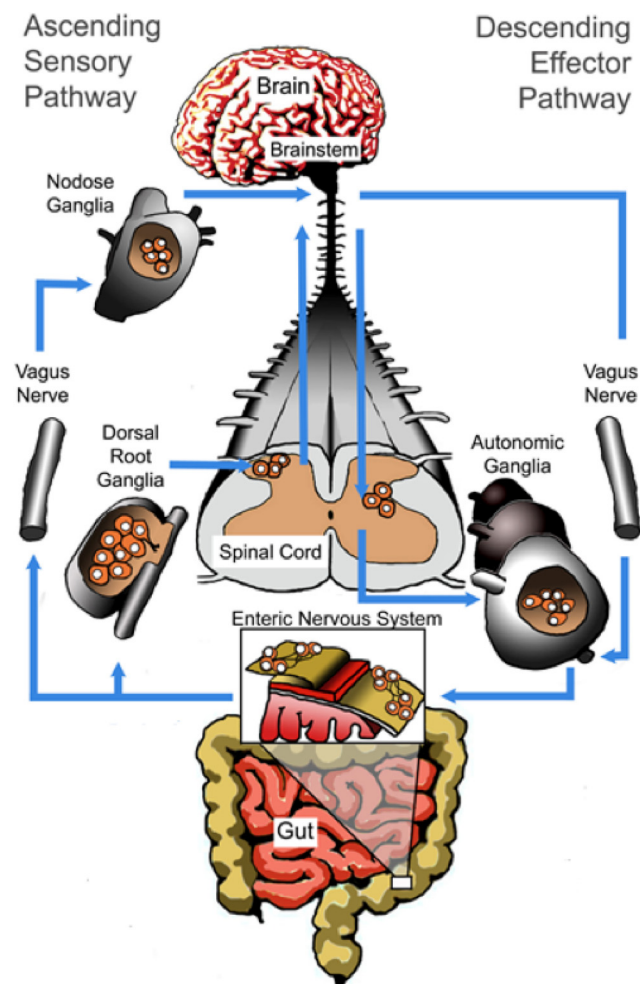
Inulin or inulin-type prebiotics, when taken orally, reach the colon intact where they undergo bacterial fermentation and stimulate the growth of *Bifidobacteria* species (125). These types of prebiotic can influence production of relevant GI hormones, possibly via SCFAs, such as glucagon like peptide-1 (GLP-1), peptide YY (PYY), ghrelin and leptin (126–128). Ghrelin in particular has attracted much attention as a hunger hormone (60). This allows the microbiota to exert an influence over not just host metabolism but also food intake and appetite regulation (129).

### Candidate Hormones of the Gut Microbiota: Focus on Tryptophan Supply

The ability of the gut microbiota to influence brain and behavior is considered a new frontier of research (61, 130). Although this crosstalk can occur via a number of mechanisms which take advantage of gut-brain axis scaffolding, including neural routes (see Figure 4), humoral options also exist (3). In this context, circulating concentrations of tryptophan, the amino acid precursor for the signaling molecule serotonin (5-HT), appears to be under the influence of the gut microbiota. 5-HT is a key neurotransmitter in the gut-brain axis, both at the level of the enteric nervous system (131) and in the CNS (132). Moreover, its physiological repertoire is extensive with a widespread receptor distribution and extends to cardiovascular function including blood pressure (BP) regulation (133), bladder control and platelet aggregation (134).

Studies in GF animals indicate that the peripheral availability of tryptophan in the circulation, critical for CNS 5-HT synthesis, is regulated by the gut microbiota and these elevated plasma tryptophan concentrations can be normalized following colonization of the GF animals (135) (135). Augmentation is possible following administration of the probiotic *B. infantis* (136). The mechanism underpinning the regulation of circulating tryptophan concentrations by the bacteria in our gut is unclear but may involve control over degradation of tryptophan along an alternative and physiologically dominant metabolic route, the kynurenine pathway (137, 138). Indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO), the enzymes that catalyze the initial metabolic step in this pathway, are subject to immune and glucocorticoid control respectively. A decreased ratio of

kynurenine to tryptophan, indicating decreased IDO/TDO activity, in GF animals connects this pathway to the reported alterations (135). Moreover, increased IDO activity is observed following the associated chronic GI inflammation that arises following infection with *Trichuris Muris*, (139). Importantly, metabolites produced downstream of kynurenine are neuroactive, such as kynurenic acid and quinolinic acid (137). The ability of the microbiota to regulate influential precursors such as trypto-



**FIGURE 4. Neuronal Pathways of the Gut-Brain Axis** In addition to hormonal crosstalk, the gut microbiota can influence brain and behavior by recruiting the neuronal pathways of the gut-brain axis. This term describes the bidirectional communication network between the gut and the brain. The CNS and enteric nervous system (ENS) communicate along vagal and autonomic pathways to modulate many GI functions. Mood and various cognitive processes can mediate top-down/bottom-up signaling. Vagal afferent nerves transmit to central brain regions in response to numerous signaling molecules in the gut which can be regulated or secreted by the gut microbiota. This vagal innervation is essential for homeostasis and provides both motor and sensory innervation for several key functions including satiety, nausea, sensations of visceral pain, sphincter operation and peristalsis. Spinal afferent nerves from the GI tract also project to the dorsal horn of the spinal cord. Not shown in this diagram are the HPA and the immune systems which are also important components of the gut-brain axis.

phan and kynurenine likely contributes significantly to its ability to sway CNS and ENS neurotransmission.

The relevance of these preclinical findings to the clinical situation is probably best gauged from studies in irritable bowel syndrome (IBS), a functional GI disorder which has been linked to alterations in the gut microbiota (140). Accordingly, increased IDO activity has been reported in both male and female IBS populations (141–143). Mechanistically, degradation of tryptophan can ensue following activation of Toll-like receptors (TLR) receptors (144, 145), which have altered expression and activity in both clinical IBS populations (146, 147) and animal models of the disorder (148). A differential TLR-specific pattern of kynurenine production in IBS has also been reported (142).

Other explanations are also possible and in addition to the utilization of tryptophan to satisfy the growth requirements for bacteria (149), tryptophan is also recruited by a bacteria-specific tryptophanase enzyme for indole production (150, 151). *Bacteroides fragilis* harbors this enzyme and has recently been linked to GI abnormalities in autism spectrum disorders (152). Certain bacteria are capable of tryptophan biosynthesis via enzymes such as tryptophan synthase (153, 154) while some bacterial strains are also proficient in serotonin production from tryptophan, at least *in vitro* (11, 155, 156).

### Microbiota, Stress and hypothalamic-pituitary-adrenal axis (HPA)

The use of GF animals has provided one of the most significant insights into the role of the microbiota in regulating the development of the HPA. Subsequent comparison with their conventionally colonized counterparts allows inferences to be drawn regarding the morphological and physiological parameters that may be under the influence of the developing microbiota. However, in the absence of the resident enteric microbiota, key members of the TLR family have low or absent expression profiles in the gut, thus compromising appropriate neuroendocrine responses to pathogens (157). For example, intact TLR4 signaling is required for plasma corticosterone release (158) and the TLR4 knockout mouse does not respond appropriately to lipopolysaccharide (LPS) from gram negative bacteria with an activation of the HPA (159).

Pivotal studies by Sudo and colleagues (160) provide insight into the role of the intestinal microbiota in the development of the HPA axis. In GF mice, a mild restraint stress induces an exaggerated release of corticosterone and ACTH compared to the specific pathogen free (SPF) controls. This has since been replicated independently, again following a mild stressor, in both mice (135) and

rats (161). This aberrant stress response in GF mice is partially reversed by colonization with faecal matter from SPF animals and fully reversed by monoassociation with *B. infantis* in a time dependant manner (160). This study clearly demonstrated that the microbial content of the gut is critical to the development of an appropriate stress response later in life and also that there is a narrow window in early life where colonization must occur to ensure normal development of the HPA.

Other aspects of this relationship need to be teased apart and additional indices of the HPA assessed for a more detailed characterization of the microbial influence on the stress response. This includes evaluating corticosterone output following different grades of stressor as well as recording not just peak cortisol concentrations but also recovery from the stressful insult. In this regard, the sustained HPA response to an acute stress, the trier social stress test (TSST), in subjects with IBS may be informative (162, 163). Although it is unclear if this is specifically related to the proposed alteration in the gut microbiota in this disorder, it will nevertheless be interesting to interrogate in GF animals whether the microbiota affects not just the ability to mount an appropriate response but also the ability to turn that response off and indeed whether these animals habituate to repeated stressors.

Endocrine abnormalities have long been associated with psychiatric illness, with hyperactivity of the HPA axis a prominent feature of at least some subtypes of major depression (164–166). In IBS, a significant comorbidity, such abnormalities are more inconsistent but still regularly reported (140, 163, 167). It is currently unknown if alterations in the microbiota are associated with psychiatric illness such as depression although that prospect is increasingly coming into focus. However, it is long known that stress and the HPA can influence the composition of the gut microbiome. The functional consequences of such changes are only now being understood.

Maternal separation, an early life stressor which can result in long-term HPA changes also has long term effects on the microbiome (168, 169). Analysis of the 16S rRNA diversity in adult rats exposed to maternal separation for three hours per day from post natal days 2 to 12 revealed a significantly altered faecal microbiome when compared to the nonseparated control animals (169). A study using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP) demonstrated that the community structure of microbiota from mice exposed to a prolonged restraint stressor was significantly different from the community structure found in nonstressed control mice (170). More recently, using the same approach, repeated social stress has been shown to decrease the relative abundance in bacteria of the genus *Bacteroides*, while increasing the

relative abundance of bacteria in the genus *Clostridium* in the cecum (171). The stressor also increased circulating levels of IL-6 and MCP-1, which were significantly correlated with stressor-induced changes to three bacterial genera (ie, *Coprococcus*, *Pseudobutyrvibrio*, and *Dorea*).

Demonstrating changes in baseline glucocorticoid levels in either rodents or man based on single time point serum samples, which can be highly variable (172), is notoriously unreliable. This may contribute to the lack of consensus across different studies although the stress-induced corticosterone response in rodents is considered more robust and reliable. In a maternal separation model, Desbonnet et al (173) found behavioral changes with *B. infantis* treatment but no reduction in corticosterone. Using a similar model, Gareau et al (174) found that feeding a *Lactobacillus* strain reduced corticosterone levels and McKernan et al (175) found that *B. infantis* administration reduced corticosterone levels, though the reduction did not reach statistical significance.

In a recent clinical study, healthy volunteers were given *L. helveticus* R0052 and *B. longum* R0175 in combination or placebo in a double-blind, randomized parallel group study for 30 days (176). Twenty-four hour urinary

free cortisol (UFC) output was reduced with probiotic treatment. No other HPA axis measures were taken. However, no in-depth analysis in man of HPA activity in response to a course of any putative probiotic has been undertaken either in healthy subjects or patient populations. Nonetheless, based on the currently available data Dinan and Cryan (177) concluded that developmental studies and those involving stress-related disorders should include the gut microbiota as an important regulator of the HPA and failure to do so can result in the introduction of a significant confounding variable.

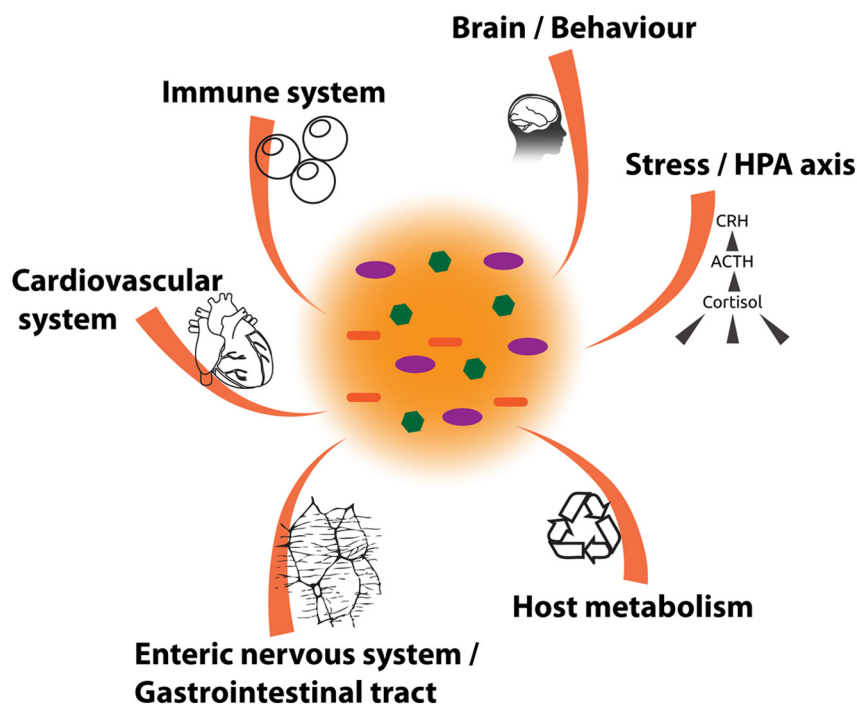
### Microbial Endocrinology

Not only do bacteria in the gut produce hormone-like substances and regulate hormonal output, they can also potentially respond to the hormonal secretions of the host. Lyte and colleagues have termed this area of study 'Microbial Endocrinology' (6, 7, 12) and although it has mostly focused on hormonally-driven bacterial growth in the context of pathogens and infectious processes, it is also of relevance to the composition and distribution of the intestinal microbiota (7). Elevations in noradrenaline concentrations following acute stress can, for example, stimulate the growth of nonpathogenic commensal *E.*

*Coli* (178) as well as other gram-negative bacteria (179). This has implications for stress-related disorders and as we have emphasized, could have knock-on consequences for the hormonal output of the microbiota which in turn might modify host behavior and perpetuate microbiota-driven pathological states. Clearly, the host-microbiota cross-talk is complex and we should not consider the contribution of either partner in isolation.

### Implications and Perspectives

Long neglected, the gut microbiota can no longer be considered a bystander in health and disease. We have described the clear links between the composition of our intestinal microbiota, its functional endocrine interactions and the development of obesity, cardiovascular disorders and metabolic syndromes as well as stress-related psychiatric disorders (see Figure 5). Integrating these important observations is a demanding proposition



**FIGURE 5. Implications of Endocrine Output of the Gut Microbiota in Health and Disease** The hormonal output of the gut microbiota is vital for host health and wellbeing. In addition to the local impact in the GI tract and enteric nervous system, it is also critical for control of host metabolism and the normal development of the immune system. Through as yet unknown mechanisms, the gut microbiota also seems to be able to regulate glucocorticoid production in the HPA. Similarly, through controlling the availability of the serotonin precursor, tryptophan, the gut microbiota can potentially influence brain and behavior. Metabolites produced by the gut microbiota from choline can also impact on the cardiovascular system.

but an extremely worthwhile venture with many potential benefits to society.

There is much current interest in the diagnostic potential of assessing alterations in the microbial ecology of the gut. One of the most frequently reported findings reported in the literature in both clinical and preclinical studies are alterations in the Firmicutes/Bacteroidetes ratio in disease states such as obesity and IBS (41, 48, 180). Although a consensus has still to become apparent on the reliability of these alterations as a biosignature, there is broad agreement that we need to move towards establishing a causal role for any alterations in composition which are present (181). In addition, preclinical studies suggest the presence of ‘melancholic microbes’ with the ability to influence psychiatric disorders such as depression and anxiety (182, 183). Tracking a compositional dysbiosis is a complex process and the repercussions in terms of a functional endocrine output remains to be defined. Given the broad span of impact exerted by the candidate hormones of the gut microbiota, urgent attention should be devoted to this topic which could also spawn novel therapeutic targets.

An important consideration to bear in mind when evaluating microbiota data from clinical studies is the caveat that much of what we understand is based on the assessment of faecal specimens. It is likely that faecal, luminal and mucosal bacterial populations represent distinct ecological niches in addition to the considerable variation along the known gradient of bacteria in the GI tract (18). The microbial communities in the small intestine, for example, have consequently received much less attention yet are likely to be proportionally much more affected following probiotic consumption (184). Moreover, the relationship between an altered microbiota composition in the faecal compartment and microbe-mucosa interactions remains to be fully defined. Clearly, there are many practical logistical reasons which favor faecal sampling protocols but reliance on this source may need to be reconsidered if we are to capture the true complexity of the gut microbiota and the clear distinction between the mucosa-associated and lumen residing microbiota (19). This has implications for our understanding of the gut microbiota as an endocrine organ and by extension its role in health and disease which are as yet not fully understood.

Deliberately manipulating the gut microbiota is an appealing therapeutic strategy and in the light of our discussion, could be considered as a viable option to treat stress-related psychiatric illnesses as well as metabolic dysfunction and eating disorders. Certainly, it seems that the gut microbiota is very plastic and the composition can be rapidly altered when the diet is changed. For example, Turnbaugh and colleagues showed that switching from a

low-fat, polysaccharide-rich diet to a high-fat, high-sugar diet shifted the structure of the microbiota within a single day and changed the representation of metabolic pathways in the microbiome (185). Probiotics have also shown utility in the treatment of some features of IBS for example (186) and boast a favorable safety profile (187). An extension of this concept is the promise of ‘Psychobiotics’ to exert beneficial psychotropic effects (188). As indicated above, prebiotics can modulate hormonal output and have also been considered in the treatment of GI disorders (189, 190). Faecal microbiota transplants are currently attracting much attention for the treatment of recurrent *Clostridium difficile* infections and this approach may ultimately filter into the treatment of other disorders as well (191–193). One could argue, based on the likely vast metabolic capacity in such a collection of bacteria, that this is akin to a large scale hormone replacement therapy.

This strategy is not without its challenges. We should bear in mind that individual bacteria or community structures can exert pleiotropic effects while the concept of functional redundancy also applies. It will also be interesting to see if we can artificially produce specific gut microbiota compositions that accurately deliver, for example, precise circulating or regional tryptophan concentrations. Similarly, it may be possible to use this approach to control the bioavailability of L-Dopa with implications for the treatment of Parkinson’s disease (194). We do not yet know if such a strategy might prove a double-edged sword such that beneficial effects in one domain might engender deleterious consequences in another, altered choline availability for example.

Outside of antibiotics, probiotics and prebiotics, less consideration has been given to the potential impact of currently available therapeutics on the intestinal microbiota. As indicated above, recent preclinical studies suggest that olanzapine may also alter the microbiota, reducing the levels of *Proteobacteria* and *Actinobacteria* by day 21 of treatment with a parallel trend towards an increase in the *Firmicutes* levels (56). Importantly, many of these effects can be attenuated by concurrent antibiotic administration in rats (195). Some members of the selective serotonin reuptake inhibitors (SSRIs) may also possess antimicrobial activity (196).

It is interesting to note some apparent contradictions, arising from studies in GF animals in particular, pertaining to the view of the gut microbiota as an essential endocrine organ. Chief among these is the fact that however abnormal these animals are, not only do they survive in the absence of this ‘virtual’ organ, they also live longer than their conventionally colonized counterparts (197). This contrasts with the potentially life threatening nature

of endocrine organ malfunction. For example, adrenocortical insufficiency in Addison's disease requires lifelong glucocorticoid and mineralocorticoid replacement therapy for survival and well-being (198). We should however point out that the diets of GF animals are customised and supplemented with various factors usually provided by the microbiota and essential to the survival of the host such as vitamin K (199). That the entire hormonal repertoire of the microbiota does not need to be replaced in GF animals is likely due to the fact that many of the intestinal secretions of the microbiota are not bacteria-specific while other hormones at distal sites are under the influence of the microbiota but host-derived. The exaggerated stress-response of GF mice in terms of corticosterone output is also somewhat at odds with their fearless nature in various behavioral tests such as the light/dark box which are interpreted as indicating reduced anxiety (135). Adding to this complexity is that GF rats do display increases in anxiety-like behaviors that seem to better correspond with their exaggerated stress-response (161). However, such contradictions probably emphasize some of the difficulties inherent in translating from the preclinical arena to the clinical domain rather than contradicting the endocrine nature of the microbiota.

## Conclusions

The microbiota has the capacity to produce a diverse range of compounds which play a major role in regulating the activity of distal organs including the brain. The influence of the microbiota in regulating metabolic activity is now recognized with increasing evidence suggesting involvement in glucose and weight regulation. Microbial signatures which increase the risk of diabetes mellitus and obesity have been put forward. Likewise, a role for the microbiota in the regulation of the HPA has been established as well as the serotonergic system via modulation of tryptophan availability. Unlike other endocrine organs, the microbiota has intense plasticity and can alter dramatically and rapidly in response to diet. With increased understanding of the key microbiota genes involved in endocrine regulation it may be possible to use probiotics or other modulatory means to treat or prevent the metabolic syndrome and stress-related disorders.

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## Search Strategies

A literature review was performed for English language publications using Pubmed, Science Direct, Google Scholar and other online sources using words such as: 'bacteria', 'endocrinology', 'microbiome', 'host metabolism', 'behavior', 'gastrointestinal microbiota', 'hormone', 'HPA', 'short chain fatty acid', 'neurotransmitter', 'tryptophan', and 'serotonin'. The bibliographies of relevant studies and systematic reviews were also considered. Studies were excluded if the abstract and title contained sufficient information to render it irrelevant. All potentially eligible studies were discussed in detail by all authors prior to selection for inclusion.

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