REVIEW

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Gut peptides and the microbiome: focus on ghrelin

Natasha K. Leeuwendaal[a,b], *John F. Cryan*[a] *and Harriët Schellekens*[a,b]

Purpose of review
In this review, we present recent insights into the role of the gut microbiota on gastrointestinal (GI) peptide secretion and signalling, with a focus on the orexigenic hormone, ghrelin.

Recent findings
Evidence is accumulating suggesting that secretion of GI peptides is modulated by commensal bacteria present in our GI tract. Recent data shows that the gut microbiome impacts on ghrelinergic signalling through its metabolites, at the level of the ghrelin receptor (growth hormone secretagogue receptor) and highlights concomitant changes in circulating ghrelin levels with specific gut microbiota changes. However, the mechanisms by which the gut microbiota interacts with gut peptide secretion and signalling, including ghrelin, are still largely unknown.

Summary
The gut microbiota may directly or indirectly influence secretion of the orexigenic hormone, ghrelin, similar to the modulation of satiety inducing GI hormones. Although data demonstrating a role of the microbiota on ghrelinergic signalling is starting to emerge, future mechanistic studies are needed to understand the full impact of the microbiota-ghrelin axis on metabolism and central-regulated homeostatic and non-homeostatic controls of food intake.

Keywords
appetite, ghrelin, gut peptides, metabolism, microbiota

INTRODUCTION

Scattered throughout the epithelial cells of the gastrointestinal (GI) tract, enteroendocrine cells (EECs) are responsible for regulation of appetite, digestion, intestinal absorption and motility [1]. Despite the fact that these cells only constitute 1% of the total gut epithelial population, they comprise the largest endocrine system of humans and express receptors capable of sensing and responding to luminal contents [1]. EECs are capable of secreting up to ~20 specific GI peptides depending on cell type and location (Table 1). GI peptides are subsequently distributed in the GI mucosa to control digestion, appetite and metabolism [2,3]. EECs can be either open type, located in the intestinal epithelium at the GI lumen surface with extended microvilli structures, or closed type, near the basal membrane that lack microvilli [4]. Locations of EECs differ, with X/A, G, D and EC cells populating the stomach, G, D, I, K, L and EC cells being present in the small intestines, and L and EC cells being found in the large intestines [4]. The release of GI peptides by EECs throughout the digestive tract is mediated in response to nutrient availability and ultimately serves to communicate metabolic and nutrient status to the central nervous system (CNS). The brain subsequently determines and directs appetite and decisions on food seeking, food intake and food choice to balance the body's energy needs. This dynamic two-way communication of the gut–brain axis determines ongoing eating behaviour and impacts on overall energy homeostasis.

An increasing number of studies highlight a key role for the GI microbiota in host metabolism and energy balance (for reviews, see [5–8]). In addition, the gut microbiota is capable of modulating gut peptide (including Protein YY and Glucagon-like peptide-1) secretion and signalling (for reviews, see [9,10]). Here, we examine the current literature for interactions between the GI microbiota and gut hormone secretion, with particular emphasis on the orexigenic ghrelinergic system. Moreover, we will

[a]Department of Anatomy and Neuroscience and [b]APC Microbiome, Ireland University College Cork, Cork, Ireland

Correspondence to Dr Harriët Schellekens, Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland.
Tel: +353 (0)21 420 5429; e-mail: h.schellekens@ucc.ie

Curr Opin Endocrinol Diabetes Obes 2021, 28:243–252

DOI:10.1097/MED.0000000000000616

KEY POINTS

- Specific gastrointestinal microbiota and their metabolites are able to modulate ghrelin receptor (GHSR) signalling.
- Microbial derived SCFAs, AA, formyl peptides, LPS and H_2S are likely able to regulate circulating ghrelin levels via the direct or indirect modulation of ghrelin secretion from EECs.
- The gut microbiota represents a rich source for bioactive metabolites able to specifically modulate gut peptide secretion, including the orexigenic hormone ghrelin.
- Future mechanistic studies should explore the influence of the microbiota metabolome on ghrelinergic signalling and function across the gut–brain axis.

discuss the underlying mechanisms by which the microbiome may modulate ghrelinergic signalling in the gut–brain axis.

GASTROINTESTINAL PEPTIDES AND THE GUT–BRAIN AXIS

GI peptides play diverse roles and are secreted from EECs in response to different nutrients, meal anticipation, microbial metabolites, and other circulating factors [11], typically following the modulation of EEC-expressed G protein-coupled receptors (GPCRs) [12]. The GI peptides secreted by EECs depend on the local rates of nutrient absorption but is primarily determined by the EEC type and location in the GI tract (Table 1). EECs may secrete 1 type of hormone, such as K cells that produce glucose-dependent insulinotropic polypeptide (GIP), whereas others secrete several, such as the L cell that secretes glucagon-like peptide 1 (GLP-1), Peptide YY (PYY), and GLP-2 [12]. Secretion of the anorexigenic peptide CCK, is stimulated by amino acids (the most effective of which are L-phenylalanine and L-tryptophan) and fatty acids [13]. Ghrelin is primarily produced in the stomach from X/A-like oxyntic gland cells (P/D_1 cells in humans), but also by EECs of the small intestines, and circulating ghrelin levels are modified following secretion, via acylation and degradation [14]. CCK, GLP-1 and PYY interact with their respective GPCRs to promote satiety and inhibit food intake, whereas ghrelin secretion has the opposite effect and stimulates appetite [15]. GPCRs for CCK (CCKR) are located in the gallbladder, pancreas and stomach, GLP-1 GPCRs (GLP1R) are primarily located in the pancreas and stomach, and PYY receptors are scattered throughout the body [16–18]. As well as their effects locally, most gut peptides are essential for proper signalling crosstalk between the GI tract and the CNS [2]. This interaction is facilitated via the gut–brain axis, a complex communication network that incorporates both neural and hormonal signalling pathways for regulation of metabolic function and homeostasis [10].

Many of these gut peptides, including CCK, GLP-1 and PYY, have been shown to participate in the gut–brain axis communication via indirect mechanisms through modulation of GPCR signalling on afferent fibres of the vagus nerve (VN) (the primary element of the parasympathetic nervous system) or directly through traversing the blood-brain barrier and stimulating endogenous receptors in different parts of the brain [19–21]. The arcuate nucleus (ARC) of the hypothalamus is primarily targeted, specifically at the median eminence, a circumventricular organ with more permeable capillaries and is the brain region primarily involved in controlling homeostatic feeding behaviour and balance hunger and satiety [15,22]. Hormones CCK, GLP-1 and PYY have been shown to directly stimulate the VN via GPCRs on nerve endings [23]. Additionally, a recent discovery in mice revealed that EECs can interact physically with enteric glial cells via neuropod structures [24–26]. Neuropods are basal processes of EECs that contain large, dense vesicles of gut peptides and small, clear vesicles containing neurotransmitters [27,28]. These features extend into the lumen and interact with vagal neurons via release of vesicle contents for rapid signal transduction to the brain [29]. Thus, the interaction of gut peptides with the gut–brain axis orchestrates metabolic function, communicates nutrient status to the brain and modulates central-regulated appetite and homeostatic processes, which together drive food seeking and eating behaviour, to maintain energy balance.

MICROBIOTA AND GUT PEPTIDES

The GI tract is colonized by an enormous collection of microorganisms comprising niche populations that increase in density from the stomach (low microbial density of $\approx 10^1$–10^3 colony forming units [CFU]/mL) to the colon (very densely populated, $\approx 10^{11}$CFU/mL) [30,31]. Noteworthy, the microbiota of the stomach, the site of ghrelin secretion, is less diverse and fewer in numbers than populations found throughout other parts of the GI tract; culture-independent methods have identified the dominant phyla as being Bacteroidetes, Firmicutes, Actinobacteria, Fusobacteria and Proteobacteria [32]. It is estimated that between 10^1 and 10^3 CFU /mL of microbes are found in the stomach, which

Table 1. Common gastrointestinal (GI) peptides and the microbiome

GI Peptide	EEC Type	Location of secretion	Evidence of microbial metabolite influence	Evidence of microbiome influence	Reference
Cholecystokinin (CCK)	I	Proximal Small Intestine	Lys restriction – decreased intestinal CCK expression Acetate and Butyrate – increased plasma CCK	N/A	[11,12]
Gastrin	G	Stomach (Pyloric Antrum)	Increases Acetate – increased plasma Gastrin	N/A	[13]
Ghrelin	X/A	Stomach	Acetate, Propionate, Butyrate – decreased ghrelin secretion and attenuated ghrelin-mediated GHR1a stimulation LPS – decreased plasma ghrelin Gln, Glu, Lys, Thr, Val – increased ghrelin release Cys – reduced plasma acyl ghrelin H_2S – inhibited ghrelin secretion Formyl Peptides – decreased ghrelin secretion	Total Bacteria, *Clostridium*, *Ruminococcus* – positively associated with ghrelin Increased Bacteroidetes/Firmicutes, *Faecalibacterium*, *Prevotellaceae* – negatively associated with ghrelin *Bacteroides*, *Bifidobacterium*, *Lactobacillus* – both positively and negatively associated with ghrelin	[14–38]
Glucagon-like peptide-1 (GLP-1)	L	Distal Small Intestine and Colon	Acetate, Butyrate, Propionate – trigger GLP-1 secretion Indole – short exposures and long exposures increased and decreased GLP-1 secretion, respectively LPS – metabolic changes mediated by LPS attenuated in GLP-1R knock-out mice	Decreased Firmicutes, Bacteroidetes – increased serum GLP-1 Increased Proteobacteria – increased GLP-1	[39–42]
Glucagon-like peptide-2 (GLP-2)	L	Distal Small Intestine and Colon	N/A	Increased total bacteria count, *Lactobacillus*, *Bifidobacterium*, *C. coccoides-E. rectale* due to prebiotics – increased GLP-2 production	[43]
Glucose-dependent insulinotrophic polypeptide (GIP)	K	Proximal Small Intestines	Butyrate, Propionate – increased plasma GIP	N/A	[44]
Leptin	P	Stomach	Lys restriction – reduced mesenteric vein leptin concentration	N/A	[12]
Motilin	M	Proximal Small Intestines	N/A	N/A	N/A
Nesfatin	X/A	Stomach	N/A	N/A	N/A
Neurotensin	N	Distal Small Intestines and Large Intestines	N/A	N/A	N/A
Obestatin	X/A	Stomach	N/A	N/A	N/A
Oxyntomodulin	L	Distal Small Intestines and Colon	N/A	N/A	N/A
Peptide YY (PYY)	L	Distal Small Intestines and Colon	Butyrate, Propionate – increased basal levels, expression and secretion of PYY	Antibiotic-induced fluctuations in enterococci, coliforms, bifidobacteria, aerobic/facultative anaerobic bacteria– increased PYY secretion	[45,46]
Secretin	S	Proximal Small Intestines	N/A	Presence/absence of faecal microbiome – secretin degraded within 5 min/no degradation	[47]
Somatostatin	D	Stomach, Small Intestines	N/A	Presence/absence of faecal microbiome – somatostatin degraded within 5 min/no degradation	[47]

have the potential to influence host gastric cells and functions [33,34]. The host and the gut microbiome form a symbiotic relationship whereby the host serves as a habitat to the microorganisms and provides it with necessary nutrition, whereas the microbiome metabolises macronutrients into smaller subunits for absorption, that would otherwise remain indigestible [30]. Although human gut microbiomes show variation between individuals, 'healthy' adults will display certain 'typical' community aspects, such as the dominant phyla being Bacteroidetes and Firmicutes [35].

The interaction between the microbiome, the GI tract and the CNS is facilitated by the microbiota-gut-brain axis [36]. The microbiome influences the brain through production of bioactive molecules and metabolites, including short-chain fatty acids (SCFAs) and neuroactive signalling molecules, such as γ aminobutyric acid (GABA), serotonin and dopamine (for reviews, see [37,38]. In addition, the gut microbiota significantly impacts the production and secretion of gut peptides by EECs [39]. Changes in microbial composition and diversity have been correlated with changes in gut peptide secretion, and specific gut bacteria have been shown to be capable of modulating EECs (Table 1). For example, EECs of germ-free (GF) mice exhibit altered hormone secretion and functionality in comparison to mice with conventional diets. One study demonstrated that GF mice had a higher concentration of K-, L- and enterochromaffin cells, with higher corresponding GLP-1 serum concentrations, than the microbial-colonised counterpart mice [40▪]. A separate study showed decreased expression of CCK, PYY, GLP-1, ghrelin and leptin, lower ileal EEC concentrations and higher colonic EEC counts in GF over control mice fed the same fat emulsions [41]. This highlights that targeting the EEC-microbiota interaction may have potential as novel therapeutic strategies in metabolic disorders such as type 2 diabetes, where hormone secretion levels between sufferers and healthy patients differs [42]. Nevertheless, the mechanisms by which the microbiota regulates gut hormone levels are still poorly understood.

GHRELIN AND THE GHRELIN RECEPTOR

Ghrelin is a 28 amino acid GI hormone, discovered 2 decades ago by Kojima *et al.*, and is primarily released from the empty stomach by X/A-like oxyntic gland cells (P/D_1 cells in humans) [43,44]. Ghrelin is also expressed in the small intestines, kidneys, pancreas, heart, lungs, and placenta [45,46]. Although previous reports hypothesise the production of ghrelin in the brain, this opinion remains still somewhat controversial [47,48]. The ghrelin hormone is cleaved from preproghrelin (117 amino acids) to give proghrelin [44]. Next, the ghrelin O-acyltransferase (GOAT) enzyme acylates or, more specifically, octanoylates proghrelin at the serine-3 residue, and the mature, 28-amino acid ghrelin is secreted [43]. Acyl ghrelin (AG) and desacyl ghrelin (DAG) can both be found in the blood stream, but only AG is capable of high-affinity binding to the growth hormone secretagogue receptor (GHSR) [49,50].

The GHSR (also known as the ghrelin receptor) is a 41 kDa GPCR consisting of seven transmembrane domains that exists in 2 forms, GHSR-1a and GHSR-1b [49]. AG is the endogenous ligand of only the GHSR-1a variant, with GHSR-1b being constitutively expressed in certain tissues and appearing to serve as a regulator of GHSR-1a [49]. The ghrelin receptor has been found to regulate a wide array of functions from energy homeostasis, muscle atrophy, cardiac functions, bone metabolism, neurogenesis, and immune function in the periphery, to central appetite mechanisms that regulate both homeostatic and hedonic feeding [51–54]. The recently described role of ligand-dependent and ligand-independent actions of the GHSR in the mesocorticolimbic pathway highlights the impact of this receptor on reward-related behaviour [55]. Ghrelin mainly acts as an orexigenic hormone involved in energy homeostasis, with serum levels being elevated prior to feeding and falling postmeal [56]. Ghrelin modulates food intake, appetite, and weight regulation, and abnormal ghrelin plasma levels are associated with metabolic disorders including obesity and Prader–Willi Syndrome [57]. Moreover, ghrelin plays a role in regulation of glucose homeostasis and metabolic stress [51]. Ghrelin has recently also been explored for its therapeutic potential in stress-related psychiatric disorders [58].

GHSR-1a is expressed ubiquitously in both the central and peripheral nervous system, in line with its breath of signalling functions on expression [59,60]. Communication between ghrelin and the brain is essential for the correct regulation of metabolic function and energy storage [51]. Ghrelin is able to interact with homeostatic appetite centres directly and impact on non-homeostatic reward centres of the brain [57,61]. Additionally, ghrelin can interact with the VN for regulation of feeding behaviour and metabolism [62]. Rats with ghrelin receptor knockdown on the vagal afferent nerve displayed metabolic disturbances, meal pattern disruption, and hippocampal-dependant memory impairment [63].

Thus, ghrelin is a key hormone that relays important nutritional information along the gut–brain axis [64]. An imbalance or dysregulation of the ghrelin-gut–brain axis will result in damaging outcomes for the host, that therefore likely go beyond metabolic and homeostatic dysfunction. Interestingly, a significant overlap exists between the ghrelinergic system and the gut microbiome in the regulation of metabolic and central homeostatic processes across the gut–brain axis, which suggest a potential interaction between microbiota and ghrelin. The following paragraph will highlight the recent data on microbiota and ghrelinergic system and discuss proven and potential mechanisms of this interaction.

MICROBIOTA-DRIVEN MECHANISMS OF GHRELINERGIC SIGNALLING

The interactions between the ghrelinergic system and the GI microbiota are only just beginning to emerge [65]. Positive associations between ghrelin and total bacteria, *Clostridium*, and *Ruminococcus* have been identified [66–68]. Additionally, a negative association has also been observed between an increased Bacteroidetes/Firmicutes ratio, *Faecalibacterium*, Prevotellaceae and ghrelin levels [69–71]. However, some associations are unclear and differ among studies, with *Bacteroides*, *Bifidobacterium* and *Lactobacillus* being both positively and negatively associated with ghrelin [66,67,72–74]. GF models have also been used to examine ghrelin signalling, although not all studies provide specific data on microbial group differences. One study observed a 10-fold higher ghrelin concentration in ex-GF mice fed a high fat diet in comparison to the GF control mice, attributed to the increased acetate production of the microbiome in the former group [75]. A separate group confirmed lower levels of ghrelin in GF mice fed an intralipid emulsion in comparison to the normal controls, although (similar to the Perry *et al.* (2016) study) no microbiome data was provided for this study [41]. Conversely, GF mice and GF mice infected with *Helicobacter pylori* exhibited significantly higher ghrelin concentrations than the pathogen-free and infected microbiome control groups at the final experimental timepoint [76]. One study implicated lactate-producing bacteria, specifically lactobacilli, as the primary cause of elevated plasma ghrelin levels in GF rats that received faecal transplants from human patients with short-bowel syndrome [77].

Additionally, the use of antibiotics for perturbation of the mouse microbiome and downstream effects on ghrelin have also been examined. One such study showed that serum ghrelin concentrations were significantly lower in sub-therapeutic antibiotic treatment mice on a high fat diet than control mice on the same diet [78]. Conversely, higher plasma AG levels were observed in mice that had received a fat-free diet and a cocktail of antibiotics in comparison to control mice on the same diet, although neither plasma DAG nor the AG/total ghrelin ratio differed significantly between groups [79]. The differences seen here may be explained by the different antibiotics and differing diets administered in each study, however, both show that microbial perturbation by antibiotics can have an effect on ghrelinergic signalling. Although this highlights a potential microbiota-mediated regulation of ghrelin, very little is understood with regards to the putative mechanism by which the gut microbiota may affect circulating ghrelin levels and ghrelinergic signalling.

Microbiota and ghrelin receptor signalling

A recent study has shown that specific bacteria strains common to the human GI tract (primarily strains of *Bifidobacteria* and *Lactobacillus*) are able to attenuate ghrelinergic signalling [80▪▪]. Specifically, addition of *Bifidobacterium* supernatants prior to ghrelin exposure increased the potency of ghrelin on downstream signalling kinases, and *B. longum* APC1472, *Lb. rham*nosus DPC6118 and *Lb. gasseri* DPC6106 significantly reduced ghrelin-mediated GHSR internalisation [80▪▪]. This highlights for the first time the ability of specific bacterial strains to affect the complex signalling cascades of the ghrelin receptor, via the direct modification of ghrelin-mediated activation of the GHSR-1a (Fig. 1). Noteworthy, it was subsequently shown that *B. longum* APC1472 positively impacted on markers of obesity and stress in a preclinical mouse model of obesity as well as in healthy overweight and obese individuals (Schellekens *et al.*, '*Bifidobacterium longum* Counters the Effects of Obesity: Partial Successful Translation from Rodent to Human', EBIOMEDICINE, unpublished).

Microbiota-derived short chain fatty acids and ghrelin signalling

SCFAs are produced by gut microbes through fermentation of ingested, otherwise undigestible complex carbohydrates, with the most common including acetate, butyrate and propionate [81]. SCFAs play a role in gut health maintenance and present an energy source for colonic cells and are also implicated in CNS homeostasis [37,81,82]. SCFAs play a role in maintaining blood brain barrier integrity, the maturation of microglia, and regulating levels of neurotransmitters and neurotrophic factors, and administration of these compounds in experimental models of various CNS disorders have shown therapeutic potential [83]. Although *in vivo* studies exist that have monitored concentration changes of SCFAs in conjunction with ghrelin levels in the body, the molecular mechanisms are not yet fully understood.

SCFAs are negatively associated with ghrelin concentrations [84] and microbial SCFAs can target free fatty acid receptor 2 (FFAR2), a GPCR which has a downstream inhibitory effect on ghrelin secretion [85–87]. Interestingly, recent evidence has shown that SCFAs regulate ghrelin in a more direct manner, through antagonism of its primary GHR1a receptor [80▪▪]. *In vitro* experiments showed that cells expressing the GHR1a exhibited a significant decrease in ghrelin-mediated calcium influx when cotreated with ghrelin and propionate or butyrate. Additionally, ghrelin-mediated GHR1a internalization was

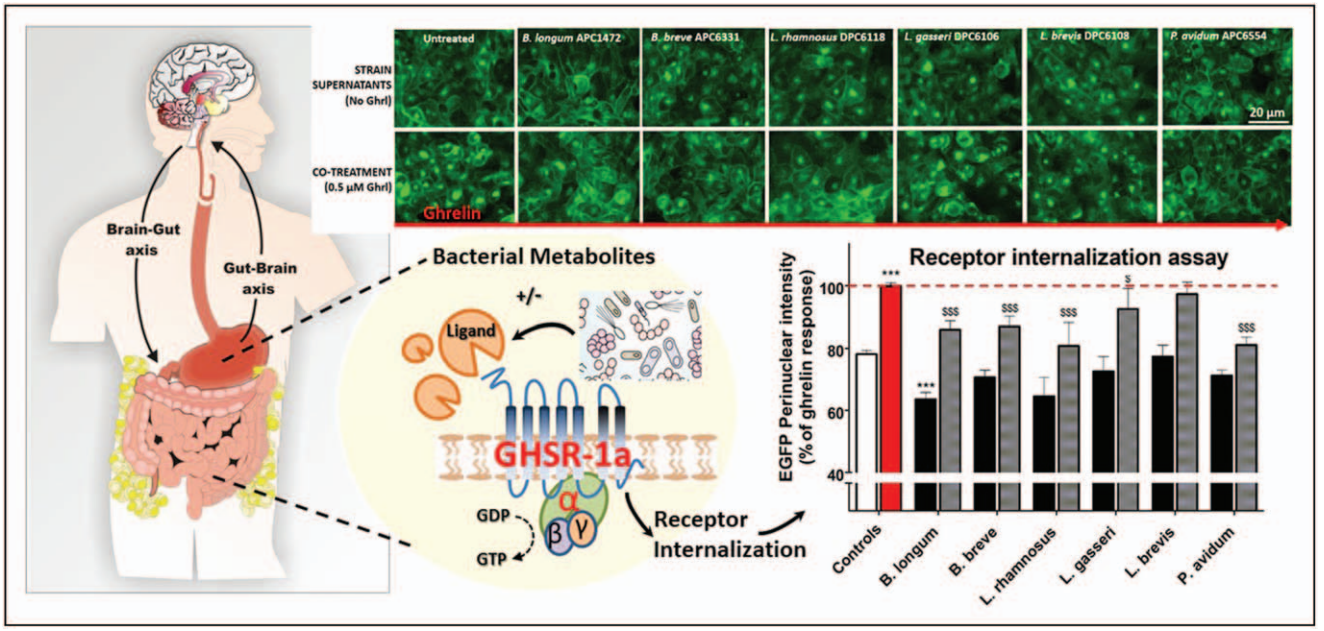

FIGURE 1. Bacterial strains supernatants attenuate ghrelin-mediated GHSR-1a signalling. Representative images of GHSR-1a-EGFP internalization in HEK293 cells and quantified fluorescence intensity in bar graph following treatment with bacterial supernatants (upper panel, black bars) and following cotreatment with 0.5 μM ghrelin (lower panel, light grey bars). Ghrelin-mediated GHSR-1a-EGFP internalization is depicted by the red bar. Bacterial strains: *B. longum* APC1472, *B. breve* APC6331, *L. rhamnosus* DPC6118, L. gasseri DPC6106, *L. brevis* DPC6108, and *P. avidum* APC6544. ***P # 0.001 vs. untreated control; $$$ P # 0.01 vs. ghrelin control, demonstrates attenuation of ghrelin-mediated GHSR-1a internalization. (adapted from [80▪▪]).

decreased in the presence of sodium acetate, sodium butyrate and sodium propionate [80▪▪]. Overall, gut microbes that produce SFCAs may influence ghrelinergic signalling, either indirectly via FFAR2-mediated ghrelin regulation or via direct antagonism or allosteric modulation of the ghrelin-specific receptor, GHR1a (Fig. 2). Together, this highlights a novel putative mechanism by which the microbiota may interact on the ghrelinergic axis, and future studies are warranted.

Microbiota-derived lipopolysaccharides and ghrelin signalling

Ghrelin signalling may also be affected by endotoxins produced by gut microbes. Gram negative bacteria-derived Lipopolysaccharide (LPS) stimulate TLR-4 and inflammation responses in the host [88,89]. I*n vivo* studies have shown that LPS administration alters circulating levels of ghrelin, albeit with some inconsistency, namely a decrease in fasting plasma ghrelin in rats and a biphasic change in healthy humans [90–92]. Exogenous ghrelin administration was shown to interact with the VN, resulting in a reduction of LPS-induced colonic hyperpermeability [93▪]. The LPS produced during a *H. pylori* infection was shown to stimulate TLR-4 in the stomach, which led to a signal cascade ultimately resulting in unwanted production of urease and ammonia and loss of proper epithelial permeability [94]. Specifically, *H. pylori* LPS and subsequent colonisation in the stomach induced secretion of proinflammatory cytokines (including IL-1β), and ghrelin-mediated activation of the GHR1a, resulting in a convergence of the ghrelin and TLR-4 signal pathways at MAPK and PLC/PKC/PI3K pathways [94]. Thus, the inflammatory response following *H. pylori* LPS is modulated via direct TLR-4 stimulation of LPS and via ghrelin-mediated GHR1a activation [94].Interestingly, *H. pylori*-infected obese individuals were shown to have less ghrelin-producing cells [95▪]. A separate study demonstrated an upregulation in GHSR mRNA and protein levels signalling in human osteoblast-like cells in the presence of a periodontopathogen and the IL-1β cytokine, which was enhanced further in the presence of ghrelin [96▪]. Noteworthy, EEC Toll-like receptor (TLR) 9 were shown to bind to CpG hexamers of typical enteropathogenic bacteria, resulting in the secretion of CCK [97▪]. Thus, evidence is accumulating demonstrating that the gut microbiome and specifically enteropathogens

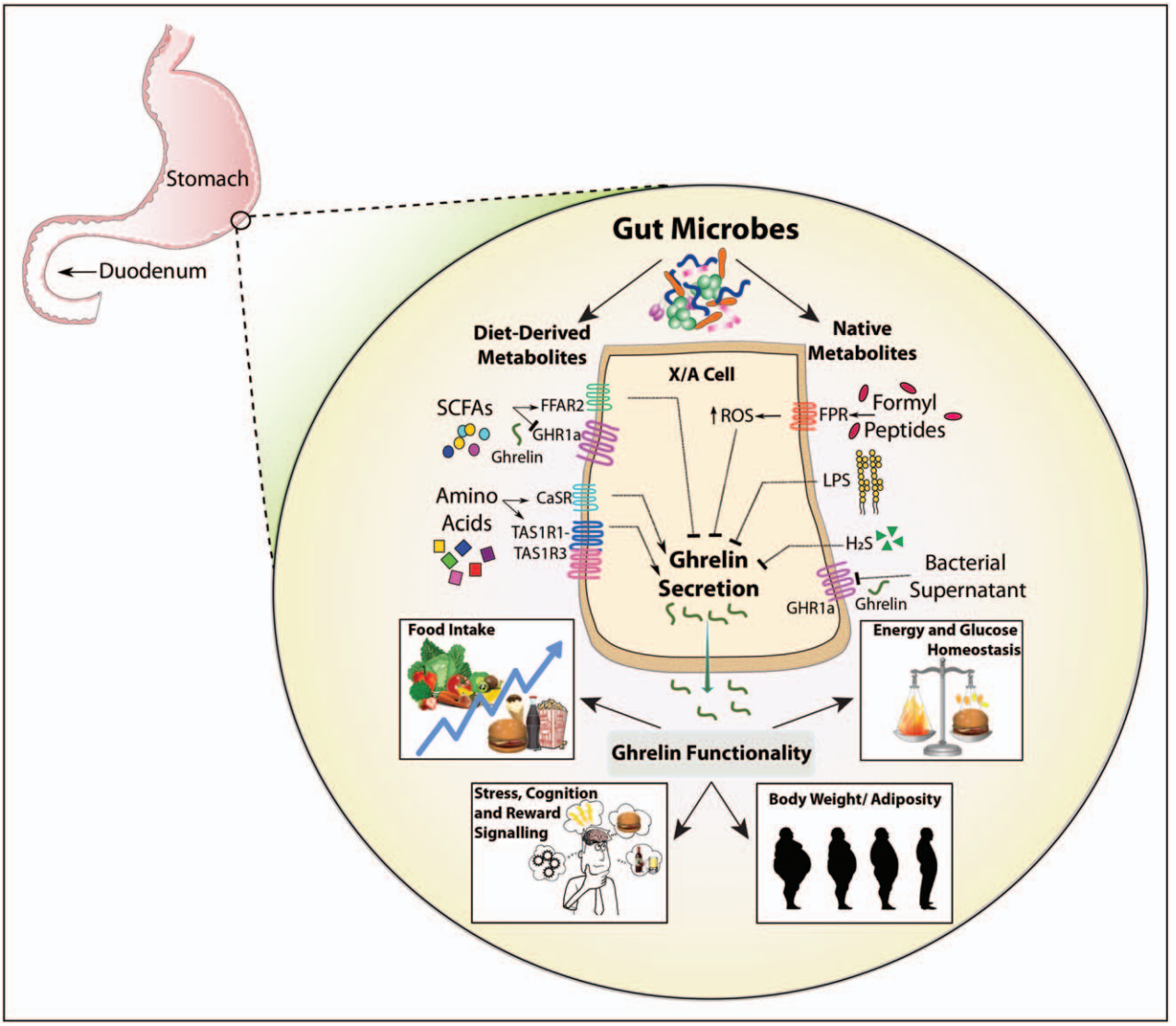

FIGURE 2. Putative mechanisms by which the microbiota influences ghrelinergic signalling.

modulate gut peptide secretion and ghrelin signalling via TLRs expressed on EECs (Fig. 2).

Other microbiota-derived metabolites and Ghrelin signalling

The microbiota may mediate ghrelin expression through the generation of reactive oxygen species (ROS) in host epithelial cells. Endogenous ROS are produced in the host cell by mitochondria, through conversion of O_2 to energy or as a response to exposure of cells to microbial factors [98]. Commensal gut microbes can produce formylated peptides capable of stimulating ROS generation in host cells through activation of epithelial GPCR formyl peptide receptor 1 (FPR1) [99]. Several studies have confirmed that the concentration of plasma ghrelin levels is increased by systemic oxidative stress, and that ghrelin may exert anti-inflammatory properties [100]. Finally, activation of antioxidant pathways in ghrelin cells decreases intracellular ROS, which increases ghrelin secretion [101▪]. Thus, microbial generation of ROS in X/A cells has the potential to modulate the concentration of secreted ghrelin (Fig. 2).

A significant quantity of the gaseous signalling Hydrogen Sulphide (H_2S) is produced by the gut microbiome, and it has been demonstrated that the bound sulfane-sulfur fraction concentration is decreased by 50–80% in GF mice in comparison to their conventional counterparts, highlighting the importance of the gut microbiome in H_2S bioavailability and metabolism [102]. Interestingly, a role for H_2S in ghrelin secretion has been reported, whereby H_2S inhibited ghrelin secretion in rat stomach cells *in vitro* and the administration of the same

H_2S donor molecule compound in mice exhibited delays in postprandial ghrelin secretion, as well as a reduced appetite [103▪]. This may suggest that microbial-produced H2S regulated ghrelin levels via modulation of its secretion (Fig. 2).

The gut microbiota also digest large dietary proteins into specific amino acids (AA), following catabolism by bacterial extracellular proteases and peptidases [104]. The colonic microbiota phyla of firmicutes have been reported to produce glutamine, glutamic acid, lysine, tryptophan and leucine [105]. The digestibility of threonine and valine by the microbiota in the small intestines was shown to be >50%, whereas cysteine degradation is a primary pathway for production of hydrogen sulphide by gut microbes [106,107]. Interestingly, specific AA have been shown to affect ghrelin levels *in vivo* with L-glutamine, L-glutamic acid, L-lysine, L-threonine and L-valine increasing [108,109,110], and L-cysteine, L-tryptophan and L-leucine reducing ghrelin plasma levels [111,112]. Gastric ghrelinoma cells secrete ghrelin in response to specific classes of amino acids via taste receptors mediated mechanism [113], which are affected in obesity [114] (Fig. 2).

In addition, the gut microbiota may be able to produce metabolites, yet to be discovered, that can either activate receptors on EECs for ghrelin secretion or act as allosteric modulators or ghrelin mimetics for GHSR modulation [80▪▪]. Overall, recent literature is highlighting the immense potential in exploration of microbiota-derived metabolites for GPCR modulation in the gut–brain axis [115, 116,117▪].

CONCLUDING REMARKS

Evidence shows that the gut microbiome produces metabolites that can interact with GI peptide-producing EECs and indirectly regulate metabolism, appetite and satiety systems. Ghrelin is an important gut peptide produced in the stomach and primarily involved in peripheral metabolism, glucose homeostasis, energy balance and central homeostatic as well as hedonic mechanisms that regulate appetite and food intake. Ghrelin is one of several important appetite regulating proteins produced by EECs but is unique in that it is the only known peripherally produced orexigenic modulator. Ghrelin is an important signalling peptide in the brain–gut axis, either indirectly interacting with the CNS via the VN or directly by traversing the blood-brain barrier and stimulating its target receptor, the GHSR-1a, expressed in the periphery and various parts of the brain. Correlation studies demonstrate changes in levels of the GI peptide ghrelin in the presence of defined microbial compositions (Table 1). However, evidence for the exact mechanisms of how ghrelin is modulated by the microbiome or microbial metabolites are still largely unexplored, with the majority of the studies examining plasma ghrelin levels overall.

This review has identified the potential role of the GI microbiota and its metabolites to directly or indirectly interact with the ghrelin signalling system. Specifically, we highlighted the ability of specific bacterial strains and SCFAs to directly modify ghrelin-mediated activation of the GHSR [80▪▪] (see (Fig. 1). In addition, we highlighted the potential of microbiota-derived metabolites to modulate the ghrelinergic system and the specific biochemical pathways of these interactions now need to be investigated further (see (Fig. 2).

The gut microbiota plays a key role that extends beyond digestion and metabolic function, to central-regulated processes, which govern homeostatic and non-homeostatic mechanism controlling eating behaviour. Understanding how the gut microbiota contributes to GI peptide secretion, including ghrelin, will significantly contribute to the development of microbiota-targeted strategies to modulate metabolism, appetite and eating behaviour.

Acknowledgements

The authors would like to thank Ken O'Riordan for assistance with graphic work.

Financial support and sponsorship

Their research is funded by Science Foundation Ireland Research Centre Grant SFI/12/RC/2273 to the APC Microbiome Institute Ireland. They have research partnerships with a number of food and pharma companies including Cremo, Pharmavite, Dupont and Nutricia.

Conflicts of interest

John F. Cryan & Harriët Schellekens are inventors on a patent based on the activity of bacterial strains at the ghrelin receptor. The remaining author has no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:
- ▪ of special interest
- ▪▪ of outstanding interest

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