

Endocrine impact of *Helicobacter pylori*: Focus on ghrelin and ghrelin *o*-acyltransferase

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Abstract

Ghrelin is predominantly produced by the gastric enteroendocrine cell compartment and is octanoylated by the recently discovered ghrelin *o*-acyltransferase (GOAT) before secretion into the bloodstream. This octanoylation is essential for many of the biological properties of ghrelin including appetite stimulation and anti-inflammatory properties as only the acylated form of ghrelin binds to the ghrelin receptor, the growth hormone secretagogue receptor (GHS-R). Given the gastric location of ghrelin production, it is perhaps not surprising that insult to the gastric mucosa affects circulating ghrelin levels in humans. *Helicobacter pylori* (*H. pylori*) infects more than fifty percent of the world's population and once established within the gastric mucosa, can persist for life. Infection is associated with chronic gastritis, gastric atrophy and ulceration, reduced appetite and a lower body mass index (BMI). The large majority of studies investigating levels of circulating ghrelin and ghrelin expression in the stomach in patients with *H. pylori* infection

indicate that the bacterium has a negative impact on ghrelin production and/or secretion. Eradication of infection restores ghrelin, improves appetite and increases BMI in some studies, however, a causative relationship between *H. pylori*-associated serum ghrelin decline and food intake and obesity has not been established. Most studies measure total ghrelin in the circulation although the measurement of the ratio of acyl/total ghrelin gives a clearer indication that the ghrelin acylation process is altered during infection and atrophy. GOAT is essential for the production of biologically-active, acyl ghrelin and the impact of *H. pylori* on GOAT expression and activity will be highly informative in the future.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection of the gastric mucosa in humans has been shown to alter the normal gastric physiology. Ghrelin is a 28 amino acid peptide hormone, primarily produced in, and secreted from, the gastric mucosa, that has been demonstrated to be involved in appetite, food intake and energy homeostasis. *H. pylori* colonization has been negatively associated with circulating ghrelin levels although this finding is the subject

of much controversy. The following review seeks to address differences in the published studies regarding the impact of *H. pylori* infection on ghrelin and introduces a putative role for the newly-discovered ghrelin acylating enzyme, ghrelin *o*-acyltransferase (GOAT).

THE GUT AS AN ENDOCRINE ORGAN

In addition to classical endocrine tissues such as the pituitary, thyroid and adrenal glands, peripheral organs are a rich source of hormones, some of which act in an intracrine/autocrine/paracrine manner. Amongst these organs, the gut is the largest and most diffuse endocrine organ producing an array of peptide hormones important for both enteric and non-enteric physiology. Enteroendocrine cells are distributed throughout the gastrointestinal tract, however, a significant proportion of endocrine hormones involved in appetite regulation are produced and secreted by the gastric mucosa.

The gastric mucosa

In the stomach, the surface epithelium is connected *via* the foveolae and neck region to the deeper gastric glands (Figure 1). Undifferentiated stem cells are located in the neck region and migrate outwards along the foveolar axis differentiating into mucous cells, and inwards differentiating into mucous, parietal, chief and endocrine cells^[1]. Histologically, the stomach is divided into three different regions - cardia, corpus/fundus and antrum/pylorus (Figure 1). The cardia comprises the proximal 0.5-2 cm of the stomach, and is mainly composed of mucus-producing cells and scattered parietal cells. In the corpus and fundus, most cells in the upper part of the glands are acid-secreting parietal cells, whereas the lower half of the glands is dominated by chief (peptic) cells that secrete digestive enzymes (pepsinogen and lipase). Most of the endocrine cells are found in the lower third of the glands. The antrum/pylorus occupies the distal quarter of the stomach, is mainly composed of mucus producing cells, with sparse parietal and endocrine cells located in the connecting neck region, and the endocrine cells are dominated by gastrin-producing (G) cells^[2]. Maintenance of gastric secretory functions is achieved through endocrine and paracrine mediators. Acid secretion is induced by a variety of stimuli and governed by the endocrine cells of the stomach. Gastrin is released from the G cells of the antral mucosa and travels through the blood stream to the corpus where the enterochromaffin-like cells are stimulated to secrete histamine which, in turn, stimulates the parietal cells to secrete acid^[2]. In contrast, somatostatin and prostaglandins, inhibit acid secretion^[2].

The gut-brain axis and ghrelin

Gut-derived regulatory peptide hormones are typically considered to be important in the control of basic enteric physiology including gastric acid secretion, digestion, gut motility and blood flow. Additional gastric hormones have been identified which point to further critical roles for the stomach in controlling appetite and satiety. *Via* interaction with their cognate receptors in the brain, these hormones

form part of an important signalling network often referred to as the gut-brain axis which co-ordinates many of the functions of the gut, including appetite modulation. The most potent orexigenic hormone and the only circulating orexigen described thus far is ghrelin.

Ghrelin is a peptide hormone consisting of 28 amino acids with a hydrophobic octanoyl moiety (or to a lesser extent decanoic acid) esterified to the third residue, serine^[3]. Ghrelin was discovered by “reverse pharmacology” using the endogenous growth hormone secretagogue receptor (GHS-R) which had been considered an orphan G protein-coupled receptor prior to discovery of ghrelin, but is now accepted as the major ghrelin receptor^[4]. Initially characterised as a new component of the growth hormone (GH)/insulin-like growth factor axis, ghrelin was shown to be a potent stimulator of GH secretion from mammalian somatotroph cells after activation of the GHS-R. This activity is reliant upon a complex interaction of signalling pathways incorporating the phospholipase C, cAMP and nitric oxide/cGMP systems^[5].

Human ghrelin is produced predominantly by the P/D₁ enteroendocrine cells of the gastric fundus (known as X/A-like cells in rodents) and the octanoylated mature hormone is secreted into the general circulation *via* the capillary networks of the gastric lamina propria^[6]. Interestingly, whilst ghrelin expression and secretion is greatest in the gastric mucosa, the GHS-R is present at its highest level in the pituitary^[7] and hypothalamic nuclei^[4], leading to speculation that ghrelin and its receptor have evolved to provide a link between peripheral energy homeostasis and GH secretion^[8].

The mechanisms underlying the co- and post-translational modifications of ghrelin have only recently begun to be deciphered. Hydrophobicity conferred by acylation may allow for the bidirectional transport of ghrelin across the blood-brain barrier^[9]. This modification also facilitates the binding of ghrelin to the GHS-R and is essential for GHS-R-mediated ghrelin activity. GOAT is a lipid transferase from the membrane bound *o*-acyl transferase (MBOAT) family of acyl transferases that specifically acylates proghrelin^[10]. Expression of murine GOAT is highest in the stomach, followed by pancreas, small intestine and colon^[11], although in human tissues the pancreas may be the major site of expression^[10]. As anticipated, GOAT expression is enriched in the ghrelin-producing gastric enteroendocrine cells of mice although a small subset of ghrelin immunopositive cells appear to be devoid of GOAT^[12], supporting the hypothesis that unacylated ghrelin has independent biological functions. Furthermore, it is tempting to speculate that unacylated ghrelin secreted from the stomach could be acylated by paracrine GOAT-expressing cells. Mice with genetic disruption of GOAT completely lack circulating acylated ghrelin but display supraphysiological levels of serum unacylated ghrelin, suggesting that GOAT may play a role in the translational control of ghrelin synthesis and/or secretion and activity^[13] or that ghrelin feedback mechanisms are disrupted in the absence of GOAT.

Plasma ghrelin secretion is episodic and concentrations

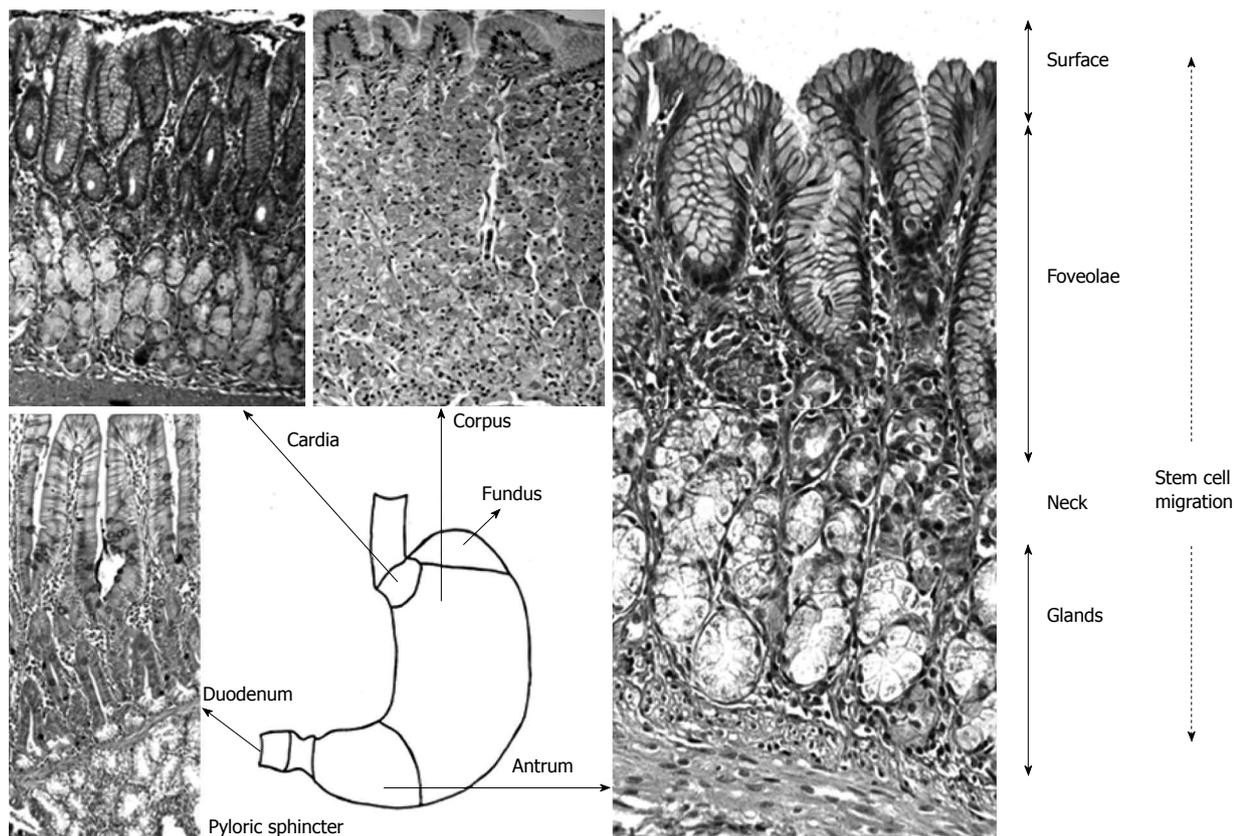


Figure 1 The regions of the stomach. In the stomach, undifferentiated stem cells located in the neck region migrate outwards or inwards specializing into mucous, parietal, chief and endocrine cells. In this figure, the antrum is shown at a three-fold larger magnification than the other images of the stomach. Ghrelin expression has been detected throughout the human gastric mucosa with strongest expression in the oxyntic mucosa.

in adults of healthy body mass index (BMI) are reported to range from 100-200 fmol/mL^[3] with healthy women having higher levels of acyl and total ghrelin than healthy men^[14]. Short-term fasting stimulates ghrelin secretion which may then initiate feeding in humans and rodents; post-prandial ghrelin serum levels are then reduced dramatically^[15]. In obesity, plasma ghrelin is decreased compared to normal counterparts^[16]. This phenomenon has previously been thought to be due to the reduced need for production and secretion of orexigenic ghrelin during periods of caloric excess. Ghrelin targets central nervous system appetite networks on multiple levels with a complex interplay occurring between it and other appetite-modulating peptides including neuropeptide Y, agouti-related protein, pro-opiomelanocortin and leptin^[17]. Whilst it is well established that pharmacological doses of ghrelin profoundly influence food intake, recent studies dissecting the mechanisms of the ghrelin acylation process have challenged the traditional dogma surrounding the endogenous ghrelin/GHS-R system and its role in appetite modulation that has existed since its discovery over ten years ago. Using GOAT transgenic knockout mouse models, Kirchner *et al.*^[13], have proposed that ghrelin and GOAT are primarily lipid-sensing molecules that are highly sensitive to changes in dietary fatty acid intake, specifically medium chain fatty acids and that acyl ghrelin signals to the brain that energy-dense food is available. In this regard, diets rich in octanoate (found in breast milk, coconut milk and

commercial infant formula) will result in an increase in the fatty acid substrate for GOAT and, therefore, a theoretical increase in circulating, acylated ghrelin. Experimental studies have shown that ghrelin influences metabolic substrate utilization in rodents^[18]. Additionally, it appears that GOAT plays a crucial role in this facet of ghrelin activity because the GOAT knockout mouse displays increased fat oxidation and is leaner than wild-type mice when given a high fat diet^[13]. Taken together, these studies advocate a role for ghrelin/GOAT and the GHS-R in sensing and reversing states of energy deficiency and reducing fat utilisation, suggesting that during times of nutrient excess, inhibition of ghrelin activity may reduce hyperphagia, obesity and type 2 diabetes. During prolonged caloric restriction, however, ghrelin activity is essential for maintaining blood glucose levels and preventing death in mice and this protective effect is mediated *via* increased GH secretion^[19].

ACYLATED GHRELIN IS NOT THE ONLY GHRELIN

The ghrelin/GHS-R axis may be expanded to incorporate several ghrelin isoforms that have been identified, including the splice variant des-Gln14-ghrelin and the non-modified des-octanoyl or unacylated ghrelin^[20]. Despite being the most predominant species of ghrelin in serum, unacylated ghrelin cannot bind to and, therefore,

does not activate the GHS-R. Initially thought to be a by-product of bioactive ghrelin degradation, unacylated ghrelin now appears to be an important hormone with an array of biological activities, particularly in the cardiovascular system, bone physiology, reproductive axes and prenatal growth^[20]. Many actions of unacylated ghrelin oppose those of acylated ghrelin. For example, the GH/IGF-1 axis is downregulated in transgenic mice overexpressing the non-modified isoform of ghrelin, resulting in a smaller than normal phenotype^[21], and food intake and gastric emptying are suppressed in mice administered unacylated ghrelin^[22]. It seems likely that these apparently inverse biological properties are the result of alternative receptor activation, and support the notion of the existence of undiscovered non-GHS-R ghrelin receptor(s). The differences and similarities between the isoforms in regards to function in gastrointestinal and selected peripheral tissues is summarised in Figure 2.

Interactions of ghrelin with the anorectic, pro-inflammatory adipokine leptin

It is well documented that ghrelin and the adipokine leptin exert mutually antagonistic regulatory effects on energy balance and appetite at the hypothalamic level. Whereas ghrelin stimulates NPY neurons in the arcuate nucleus, leptin inhibits these neurons and stimulates proopiomelanocortin (POMC) neurons, thereby suppressing food intake. The stomach contributes, in part, to plasma leptin levels, although the majority of leptin production occurs in adipose tissue. Stomach-derived leptin is expressed in the lower half of the gastric fundic glands in an endocrine cell type distinct from ghrelin-producing cells^[23]. The gastric co-expression of these two antagonistic hormones involved in appetite modulation, generation of satiety signals and energy homeostasis again highlights the importance of the stomach as an endocrine organ.

Ghrelin suppresses inflammation in rodent models of disease and in humans

Ghrelin and GHS-R are expressed in immune cells, and manipulation of ghrelin/GHS-R expression and activity affects T cell function. In immune cells, as in the hypothalamus, ghrelin again antagonises leptin. Human T cells stimulated by leptin increase their production of the pro-inflammatory, anorectic cytokines IL-1 β , IL-6 and TNF α as well as displaying increased GHS-R1a expression. Co-treatment with ghrelin inhibits leptin-induced cytokine levels in a dose-dependent manner^[24]. Knockdown of ghrelin in primary human T cells increases Th1 cytokine production and IL-17 secretion suggesting a role for autocrine/paracrine ghrelin in the endogenous restraint of pro-inflammatory cytokine production and release^[25]. Studies demonstrating an *in vivo* anti-inflammatory role for ghrelin are rapidly increasing in number and include mouse models of pancreatitis and colitis^[26]. The therapeutic effect of ghrelin has been attributed to the down-regulation of pro-inflammatory cytokines (including IL-12, IFN- γ , and TNF- α), recruitment of inflammation-suppressing regulatory T cells and increased levels of the

anti-inflammatory cytokine IL-10^[26]. Recently, ghrelin has also been shown to inhibit secretion of pro-inflammatory high-mobility group box 1 (HMGB1; a DNA-binding cytokine that is a critical late mediator of inflammation) from activated macrophages, and treatment with ghrelin reduces serum HMGB1 levels in rodent models of sepsis^[27]. Pro-inflammatory cytokine production in the resident macrophages of the brain and spinal cord (microglia) is also reduced by ghrelin treatment, thereby reducing the severity of experimental autoimmune encephalomyelitis, a model of multiple sclerosis^[28]. In clinical trials, ghrelin has been used with success as an anti-inflammatory agent in cachexic patients with chronic respiratory infection and inflammation. In these patients, ghrelin treatment increased body weight and significantly suppressed inflammation in the lungs by decreasing neutrophil infiltration/accumulation and reducing serum TNF- α ^[29].

Exogenous ghrelin is gastroprotective and prokinetic

Gastric mucosal injury is attenuated by central and peripheral administration of ghrelin in rodent models of gastric and duodenal disease. Ghrelin also accelerates healing in these ulcerogen models by increasing mucosal healing, proliferation and blood flow,^[30] and these factors have been shown to be dependent on an intact GH/IGF axis, endogenous nitric oxide activity and vagal and sensory nerve integrity^[31,32]. Ghrelin protects gastric mucosal cells from apoptosis induced by *H. pylori* LPS by increasing constitutive nitric oxide synthase activity, reducing caspase-3 and inducible nitric oxide synthase^[33]. Unsurprisingly, given its structural similarity to the classical prokinetic peptide motilin, ghrelin also has a prokinetic effect on gut motility in many species including humans^[34] and the benefits of this are being evaluated in clinical trials in patients with gastrointestinal motility disorders.

Non-infectious gastric disorders disturb ghrelin expression

Non-infectious gastric disorders are known to impact ghrelin secretion from the stomach and subsequent circulating ghrelin levels. Ghrelin-producing endocrine tumours of the stomach induce supra-physiological ghrelin levels^[35] which may result in desensitization of the GHS-R. Recently, methyl donor deficiency (MDD) in pregnant rats was shown to result in reduced circulating ghrelin but not ghrelin gene transcription in the offspring^[36]. The altered polarity of the gastric fundic glands due to the nutritional deficiency in these rats may be responsible for a reversal in normal ghrelin secretion; that is, ghrelin may be secreted into the gastric lumen instead of the normal secretion pattern into the blood. The authors speculated that the reduction in ghrelin could be the cause of the significant intrauterine and post-natal growth restriction associated with MDD.

Autoimmune atrophic gastritis has also been shown to decrease ghrelin production and secretion^[37] and acylation of proghrelin may be disrupted - it is tempting to speculate that this may be due to the effects of inflammation on the activity and or expression of the more recently discovered GOAT.

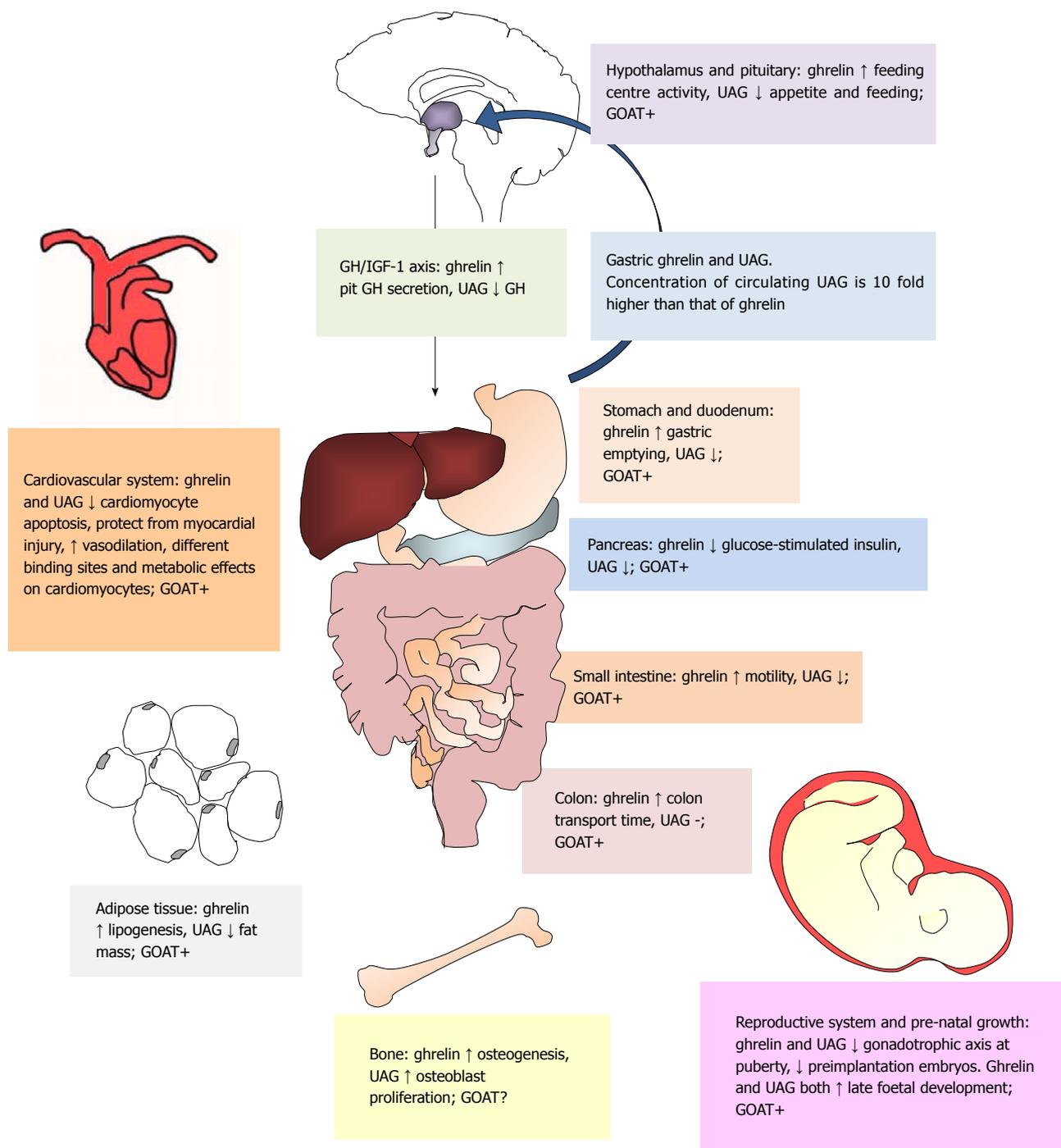


Figure 2 Diagram summarising the similarities and diversities of ghrelin and unacylated ghrelin activities in gastrointestinal and selected peripheral tissues/systems. The expression of ghrelin *o*-acyltransferase (GOAT) in most peripheral tissues suggests that acylation of locally-produced unacylated ghrelin (UAG) is possible. GOAT + indicates tissues that have been found to express mRNA for GOAT. GOAT? indicates that GOAT expression status is unknown. Pit: Pituitary; ↑: Increases or stimulates; ↓: Decreases; -: Effect unknown.

Atrophic gastritis is also associated with chronic *H. pylori* infection in a subset of patients. Of the infectious agents causing gastric pathogenicity, *H. pylori* is the most common one, however, other *Helicobacter* species can cause milder forms of gastritis^[38].

H. PYLORI INFECTION

H. pylori is a Gram-negative, rod/spiral-shaped micro-aer-

ophilic bacterium that causes life long infection. *H. pylori* has strategies that allows its survival and persistence in the inhospitable environment of the stomach. Adherence of *H. pylori* to the mucosal surface supports gain of nutrients and also facilitates entry into epithelial cells and the underlying mucosal tissue as shown *in vivo*^[39,40] and *in vitro*^[41]. Adherence of *H. pylori* is dependent on expression of bacterial attachment proteins (adhesins) and the cognate host oligosaccharides (glycans), displayed by glycoproteins

and glycosphingolipids in gastric epithelium and also by mucins in the gastric mucus layer^[42-44]. The microbe is well adapted to the gastric mucus niche, having long whip-like flagella facilitating locomotion through the mucus layer. In addition, surface-bound urease that catalyzes the hydrolysis of urea to ammonia and carbon dioxide allows the microbe to “neutralize” its microenvironment. Flagella, urease, adhesins, and genes encoding proteins with a predicted function in chemotaxis are essential for *H. pylori* colonization of laboratory animals^[45].

The majority of *H. pylori* infected individuals remain asymptomatic, but 10%-15% develop peptic ulcers, and 3% gastric non-cardia adenocarcinoma^[46]. Since most infected individuals show no clinical symptoms, the pathological process must be influenced by host/environmental factors in addition to the genotype of the infecting strain. Inter-individual factors, such as the type of host structures that *H. pylori* can bind to (blood group dependent), account for some of the variance of pathology as a consequence of infection^[47]. Stress is another factor that may work in conjunction with *H. pylori* or cause ulcers through alternative pathways, as people exposed to disasters as well as prisoners of war have increased occurrence of ulcers, and wound healing is slower in stressed individuals^[48]. The intense humoral and cellular immune responses associated with gastric colonization are usually unable to eradicate *H. pylori* and may play a major role in the morbidity of the disease.

H. pylori infection is one of the most common bacterial infections in the world, colonizing approximately half the population^[49]. Colonization usually occurs before the age of ten^[49], and once established within the gastric mucosa, the bacterium can persist for life, although transient infection occurs in a few individuals^[50].

The classification of *H. pylori* as a human carcinogen is mainly based on results from prospective epidemiological studies showing a relationship between infection with *H. pylori* and subsequent development of gastric carcinoma. Eradication of *H. pylori* has also resulted in reversal of pre-neoplastic conditions^[51], and *H. pylori* eradication in gastric ulcer patients reduces the risk of developing gastric cancer^[52], further supporting an association between *H. pylori* infection and cancer development.

Effect of *H. pylori* infection on the gastric endocrine system

In the majority of infected individuals, *H. pylori* infection is antrum predominant, and acid production by the largely unaffected corpus is enhanced leading to an increased risk of duodenal ulcers. In a minority, infection is corpus predominant, and infection is associated with progressive gastric atrophy and intestinal metaplasia, subsequent hypochlorhydria and an increased risk of gastric cancer^[53,54]. Possibly, the initial infection starts in the antrum, and in individuals with a lower basic acid production, the infection spreads to the corpus, as supported by the observations that relatives of gastric cancer patients have a lower basic acid production than the general population^[55], and that patients with antral predominant *H. pylori* infection

and gastritis developed corpus predominant infection and gastritis after treatment with acid suppressive therapy^[56]. Hypochlorhydria permits infection with other bacteria that may enhance the production of carcinogenic (e.g. *N*-nitroso) compounds. Thus, impaired control of gastric juice secretion is observed in chronic gastritis due to *H. pylori* infection, but the site/type of infection determines what effect *H. pylori* has on the endocrine system, or the downstream results of the endocrine system such as acid production. Further differences can be attributed to the development of intestinal metaplasia in a subset of individuals with chronic gastritis, and the endocrine-cell population present in the intestinal metaplasia resembles that found in the cryptal region of the normal small intestine^[57]. The literature is biased towards the antrum predominant infection, as these represent the majority of infections, and the effects of corpus predominant infection may therefore be disguised in many studies.

Effects of *H. pylori* on endocrine cells

Colonization of *H. pylori* in the human stomach results in release of chemoattractants such as IL-8, IL1 β and TNF α , which stimulate G cells, and the gastrin cell count is increased in *H. pylori* infected gastric mucosa^[58]. The number of D cells producing somatostatin decreases simultaneously^[59]. Similarly, some species of swine *Helicobacter* alter the number of endocrine cells in gastric mucosa. Some of these alterations, for example an increase in the number of G cells, decrease in the number of D cells and especially an increase in the ratio of G to D cells can be responsible for the development of gastroesophageal ulcers in swine^[60].

The impact of *H. pylori* on circulating plasma ghrelin levels highlights the importance of GOAT

Due to the gastric location of ghrelin-producing cells it is intuitive that ghrelin production, acylation and/or secretion might be compromised by chronic gastritis and atrophy and that this in turn will affect appetite, weight and BMI.

There have been contradictory reports in the literature in regards to the effect of *H. pylori* infection on circulating ghrelin levels and some of these discrepancies may be due to differences in populations (age, race, geography, gender, diet and overall health), extent of disease (for example whether atrophy is present or not) and *H. pylori* strain differences. This is also complicated by the use of different immunoassays to measure ghrelin and by the fact that acylated ghrelin is highly unstable and degrades rapidly to unacylated ghrelin^[61]. The optimum method of plasma ghrelin measurement is a contentious issue - a number of researchers maintain that measurement of total ghrelin is reflective of active ghrelin levels and is an adequate approach. Most studies examining the impact of *H. pylori* have examined total ghrelin levels only and the majority of these studies determined that infection decreases plasma ghrelin levels (Table 1). However, an important theme that emerges from these studies is that plasma total

Table 1 Original studies measuring total ghrelin only (in chronological order)

Subjects	Nationality	Tissue	Total ghrelin in <i>H. pylori</i> infection (or cure)	Ref.
39 Adults F	Turkish	G	-	[78]
10 Adults F + M	UK	P	↑ after <i>H. pylori</i> cure	[79]
68 Adults F + M	Japanese	P	↓	[80]
160 Adults F + M	Japanese	G + P	↓ more so as atrophy increases	[81]
89 Adults F + M	Japanese	P	↓ but did not recover after cure	[82]
225 Adults F + M	Japanese	P	↓ in chronic gastritis	[83]
61 Adults F + M	Japanese	G + P	↓ and associated with virulence strain	[84]
132 Adults F + M	Japanese	P	↓	[85]
100 Adults F + M	Polish	P	↓	[86]
56 Adults F + M	Japanese	P	↓	[87]
146 Children F + M	Polish	P	↓	[88]
134 Adults F + M	Japanese	G + P	↑ or ↓ after cure, depending on BMI. Ghrl mRNA ↑	[89]
62 Adults (> 75 yr) F + M	French	G + P	↓	[90]
120 Adults, 60 children F + M	Polish	P	↓	[91]
63 Adults F + M	Korean	G + P	- no healthy control group	[92]
50 Adults F + M	Turkish	P	- in absence of atrophic gastritis	[93]
85 Prepubertal children F + M	Italian	P	↓ related to severity of gastritis	[69]
256 Adults M	American	G + P	-	[94]
100 Adults F + M	Chinese	P	↓	[95]
22 Adults F + M	Korean	G + P	↑ ghrl mRNA after cure	[96]
341 Adults F + M	Chinese	P	↓ in males only	[97]

F: Female; M: Male; G: Gastric tissue; P: Plasma; Ghrl: Ghrelin; ↑: Increased; ↓: Decreased.

Table 2 Original studies measuring the effect of *H. pylori* infection on plasma acyl and unacylated ghrelin levels

Subjects	Nationality	Tissue	Acyl and total ghrelin in <i>H. pylori</i> infection (or cure)	Ref.
69 Adults F + M	Japanese	P	↓ acyl ghrl in atrophy only	[98]
50 Adults F + M	Italian	P	↑ acyl ghrl and acyl/total ratio in atrophy cf. healthy controls	[63]
220 Adults F + M	Japanese	P	↓ acyl ghrl associated with atrophy and ↑ after cure	[62]

F: Female; M: Male; P: Plasma; Ghrl: Ghrelin; ↑: Increased; ↓: Decreased.

ghrelin is reduced only in the presence of gastric atrophy and that in a subset of these studies, plasma ghrelin levels are negatively correlated with the severity of atrophy^[62,63].

The potentially different, even perhaps inverse, biological roles of acyl- and unacylated ghrelin suggest that the ratio of modified to unmodified ghrelin is highly important. In the context of *H. pylori* infection, few studies have taken this into account and in those that have, the findings are again contradictory (Table 2). In Japanese adults^[62], the acylated ghrelin/total ghrelin ratio as well as plasma acyl ghrelin levels are reduced, whereas in a study conducted on Western males^[63], there was a significant increase in acyl ghrelin and indeed the ratio of acylated ghrelin/total ghrelin which the authors speculate may be due to an endogenous, compensatory increase in the acylation process in response to a loss of total ghrelin secretion. A logical extension of this work would be to assess the level of expression and activity of GOAT during *H. pylori* infection and chronic gastritis and gastric atrophy.

The substrates for GOAT are the prohormone proghrelin and dietary medium chain free fatty acids which are used directly by GOAT. GOAT preferentially acylates proghrelin with octanoic acid, however, other species of acyl ghrelin (including C10:0 ghrelin) are present in the circulation at low levels and even unnatural forms of ghrelin

(C7:0 ghrelin) can be synthesised in mice when they are fed a diet rich in n-heptanoic acid or glyceryl triheptanoate^[13]. This new research suggests another potential reason for the effect of *H. pylori* infection on acyl ghrelin levels in addition to ghrelin endocrine cell destruction by gastric atrophy and or inflammatory cell destruction. Given that infection has been associated with malabsorption due to hypochlorhydria, vomiting, dyspepsia and with increased susceptibility to other enteric pathogens, especially in children^[64], it is tempting to speculate that altered dietary intake or dysregulated absorption of fatty acid substrate for GOAT could alter the ratio of acyl to total ghrelin during *H. pylori* infection. In this regard, a proportion of medium chain triglycerides are absorbed directly in the stomach as well as the small intestine^[65], and chronic gastritis leading to gastric atrophy may impair this absorption and reduce the ability of GOAT to acylate ghrelin. GOAT expressed in the small intestine of humans most likely contributes to systemic acyl ghrelin levels, and duodenal ulceration due to *H. pylori* infection may disrupt acyl production and secretion in this region of the gut. The development of techniques to assess human GOAT expression and function and the use of GOAT transgenic and knockout animal models for *H. pylori* infection experiments will allow researchers to explore this empirically in the near future.

***H. pylori*, malnutrition and growth failure**

Children with chronic gastrointestinal disease, including Crohn's disease, are known to have a higher incidence of growth retardation and pubertal delay when compared to their healthy counterparts^[66]. In a significant proportion of *H. pylori*-positive children, growth impairment, as indicated by a reduced mean height to below the 25th percentile, is evident and may be linked to *H. pylori* associated factors including, but not limited to, dyspepsia, diarrhoea, malnutrition and iron deficiency anaemia^[67]. Due to its potent stimulation of GH release, ghrelin may contribute to postnatal growth in humans. Ghrelin is synthesised and acylated by the placenta and is detected in foetal circulation from week 20-23, indicating that it may also play an important role in pre-natal growth^[68]. In support of this is a study showing that ghrelin deficiency in rat pups due to MDD contributes to severe intrauterine growth retardation^[36]. Whether there is a causative relationship between reduced circulating ghrelin during *H. pylori* infection and growth retardation/delay in infected children has not been adequately tested. Successful early eradication of *H. pylori* in a small study of infected children was associated with increased BMI and lean and fat mass, but not ghrelin, which was actually decreased^[69]. This may suggest that changes in ghrelin levels after *H. pylori* cure are epiphenomenal, however, only total ghrelin was measured in this study and the determination of the ratio of acyl to total ghrelin in cured children would have been more informative.

In contrast to the Pacifico *et al* study in which paediatric subjects cured of *H. pylori* have decreased plasma ghrelin, most studies report an increase in gastric ghrelin expression and secretion into the circulation after *H. pylori* cure in adults^[70,71]. However, in agreement with Pacifico *et al*, a study by Choe *et al*^[70] showed no increase in plasma or tissue ghrelin levels in children post *H. pylori* eradication and indeed no difference in ghrelin expression between *H. pylori* positive and negative children before treatment. It may be that the gastropathology of infected children differs from infected adults resulting in no significant impact on ghrelin-producing cells. Neutrophilic activity is associated with decreased density of ghrelin-producing cells^[71] and the neutrophilic infiltration of the mucosa is less in infected children than it is in adults^[72]. Gastric atrophy, which typically takes more than 10 years of chronic infection to develop, is rarer in children from Western developed countries, although the incidence of *H. pylori* associated atrophy is significantly higher in the Japanese paediatric population^[73]. Even in adults, specific measurement of acyl ghrelin in patients with varying severity of atrophic gastritis showed that atrophy is the defining factor for changes in plasma acyl ghrelin, irrespective of *H. pylori* status^[62].

IS OBESITY A POTENTIAL CONSEQUENCE OF *H. PYLORI* ERADICATION AND DOES GHRELIN HAVE A ROLE?

Human populations have evolved with very high rates of

chronic *H. pylori* infection. Therefore, it is likely that our metabolic rheostats have evolved to work in the context of chronic gastric infection. Indirect evidence such as the lower BMI and adiposity in *H. pylori* infected compared to uninfected populations, coinciding with lower levels of circulating ghrelin in infected individuals that can be increased after eradication, hints at a role for *H. pylori* as an obesity preventing agent. Whether there is a causative relationship between the falling prevalence of *H. pylori* infection and rising obesity rates in developed countries, where fewer children are becoming colonized, is a highly controversial topic. In a large study with 6724 adult patients who comprised a probability sample of the USA population, *H. pylori* seropositivity (either CagA-positive or -negative strains) was not associated with BMI or serum leptin levels, and ghrelin levels were not measured^[74]. Serological methods used in this study may not be as accurate at measuring *H. pylori* activity as other techniques. Nonetheless, there is no strong data to support a protective role of *H. pylori* in the prevention of obesity in developed countries. However, this lack of evidence may be due to the fact that both dietary habits associated with obesity and *H. pylori* infection are more prevalent in groups with low socioeconomic status^[75-77]. Eradication can restore a normal BMI in adults and growth velocity in children, however, this occurs particularly in developing countries and in Japan, where severity of atrophic gastritis is more pronounced. Whether ghrelin and other appetite-regulating peptides have a physiological role in this process is yet to be determined.

CONCLUSION

H. pylori infection affects the gut-brain axis due to its direct effects on gastric mucosa and on the enteroendocrine cell population including ghrelin-producing cells. Ghrelin is anti-inflammatory, anti-apoptotic and wound healing in gastritis, therefore, loss of ghrelin acylation during gastritis may impair healing. The relationships between *H. pylori*, ghrelin and BMI, adiposity and appetite remain speculative, but a greater understanding may give important insights into the pathophysiological processes underlying obesity in Western populations (Figure 3). By measuring gastric GOAT during infection in the future, we can shed more light on the effect of inflammation and infection on acyl ghrelin levels as opposed to the less illuminating total ghrelin levels.

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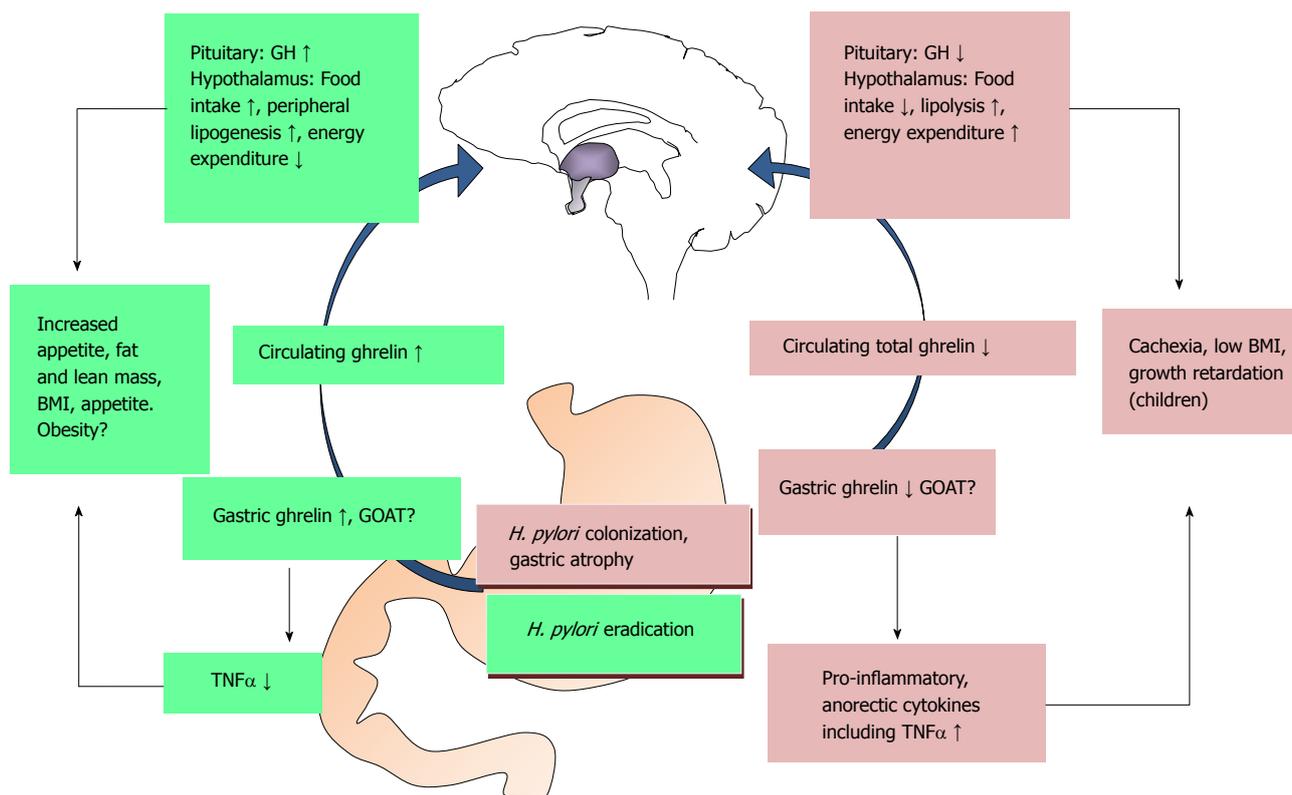


Figure 3 Potential pathways leading to reduced appetite, weight loss and pro-inflammatory state in chronic *Helicobacter pylori* infection, implicating the ghrelin axis. The majority of studies identify reduced gastric and circulating ghrelin in patients with chronic infection and gastric atrophy which may in turn lead to decreased growth hormone (GH) secretion and somatic growth in children. Reduction in ghrelin signalling in the hypothalamic feeding centres during chronic infection may be responsible for the observed reduced body mass index (BMI) in patients; *Helicobacter pylori* (*H. pylori*) eradication and subsequent restoration of ghrelin may reverse this situation. It is speculated that this in turn can contribute to obesity. GOAT? indicates that the effect of *H. pylori* status on GOAT expression is unknown. \uparrow : Increase; \downarrow : Decrease. TNF: Tumor necrosis factor.

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