From endocrine to rheumatism: do gut hormones play roles in rheumatoid arthritis?

Chih-Yen Chen¹,² and Chang-Youh Tsai¹,³

Abstract

RA is characterized by chronic inflammation in the musculoskeletal system, in which TNF-α is the key cytokine trigger. TNF-α, previously known as cachectin, is implicated in the modulation of body composition and energy expenditure. Gut hormones, including acyl ghrelin, des-acyl ghrelin, GIP, GLP-1 and PYY, have been known to be the major regulators of appetite, nutrition, energy expenditure and body mass formation. Emerging evidence indicates that blockade of TNF-α by biologics not only ameliorates rheumatoid inflammation, but can affect the secretion and action of gut hormones on appetite, body composition, energy expenditure, muscle catabolism and bone remodelling. A link between the gastrointestinal endocrine axis and the immune system may be established through the interaction of proinflammatory cytokines, including TNF-α and these gut hormones. With the ever-increasing understanding of rheumatoid inflammation and the invention of more biologics to modulate the cytokine network, more attention should be given to the possible immunomodulatory roles of gut hormones in autoimmune inflammatory reactions.

Key words: rheumatoid arthritis, gut hormones, energy balance, inflammation.

Introduction

RA is a chronic systemic inflammatory disease with predominantly articular involvement that is mediated by various cytokines, such as IL-1β and TNF-α [1]. Aside from joint destruction, RA is characterized by systemic constitutional symptoms including muscle atrophy, prostration, wasting and cachexia, which are closely associated with clinical morbidity and mortality [2, 3]. The culprit cytokine, TNF-α, not only plays a proinflammatory role in joint destruction, but also contributes to these constitutional symptoms. Formerly known as cachectin, TNF-α has a direct effect on the loss of muscle mass through mediating muscle protein breakdown [4] and inducing anorexia as well as physical inactivity [5].

Clinical experience with biologics for the treatment of RA has shown the effectiveness of anti-TNF agents in relieving pain and reducing joint destruction, with negligible side effects [6, 7]. Along with these improvements, the survival rate of RA patients has increased significantly.

Reduced appetite and impaired energy homeostasis are critical factors that negatively impact human survival [2]. Aside from directly suppressing the inflammatory destruction of various organs, the blockade of TNF-α may alter the circulating hormone milieu, leading to changes in food intake and energy balance, and thus an improvement of survival.

Accumulating evidence reveals that gut hormones actively participate in the regulation of inflammation, appetite and energy homeostasis [8]. Whether or not the alteration of gut hormones induced by anti-TNF-α therapy improves appetite and body weight balance is another interesting issue. Therefore an understanding of the immunophysiologic mechanisms of gut hormones may facilitate better clinical treatment for RA, decreasing its comorbidities and improving well-being.

Acyll ghrelin

Acyll ghrelin, a 28-amino-acid peptide, is secreted from the X/A-like cells of the stomach and acts as the natural cognate ligand for growth hormone secretagogue receptor (GHS-R, previously known as an orphan receptor) in the hypothalamus [9]. The second endogenous cognate ligand for GHS-R, des-Gln¹⁴-ghrelin, a 27-amino-acid peptide, created by alternative splicing of the ghrelin gene, constitutes one-fifth of ghrelin immunoreactivity in the rat stomach [10]. The two endogenous ligands are...
equally potent in exhibiting orexigenic actions in the satiated status [11]. Acyl ghrelin features a unique post-translational modification of O-n-octanoylation at serine 3 and is the only gastrointestinal signal that increases meal size. The acyl form of ghrelin acts on GHS-R1a and signal-423991175059_53959159z/subunit, resulting in the release of inositol triphosphate and Ca2+ [12]. Ghrelin O-acetyltransferase, a polytopic membrane-bound enzyme that attaches octanoate to serine-3 of ghrelin, has been identified and characterized [13]; it converts des-acyl ghrelin to acyl ghrelin. Acyl ghrelin and GHS-R are detectable in T cells, B cells, monocytes and neutrophils [14]. Furthermore, acyl ghrelin is strongly co-localized in the lipid raft of GM1+ cells, while preproghrelin is co-localized in Golgi bodies in activated T cells [15]. Acyl ghrelin has shown to inhibit IL-1β, IL-6 and TNF-α excreted from circulating immune cells in humans. In addition, acyl ghrelin inhibits leptin-induced increases in IL-1β, IL-6 and TNF-α from human T lymphocytes [15]. Acyl ghrelin also reduced basal expression of the autophagy-related genes ATG5 and ATG7 [16]. These results indicate that acyl ghrelin reduces TNF-α-driven apoptosis and autophagy in human visceral adipocytes and inhibits the proinflammatory reaction. Therefore acyl ghrelin functions as a key signal to link the metabolic axis with the immune system. The preprandial rise and postprandial fall of total ghrelin concentrations in healthy subjects and patients with type II diabetes mellitus (DM) after gastric bypass surgery suggest its role in human meal initiation and termination [17, 18]. Acyl ghrelin induces rodent adiposity in a growth hormone-releasing and feeding/stimu-423991175059_539591591ating independent manner [19]. In addition, a negative correlation was detected between circulating total ghrelin levels and BMI in humans [20]. Acyl ghrelin has been shown to increase the respiratory quotient (i.e. decrease energy expenditure) in obese subjects [21]. Furthermore, an immunological approach using oligoclonal antibodies against acyl ghrelin demonstrated decreased food intake and higher heat dispersion, as well as an increased rate of respiration [22]. Thus acyl ghrelin promotes positive energy balance.

Acyl ghrelin has been shown to stimulate bovine myo-423991175059_53959159blast differentiation in vitro [23]. Application of acyl ghrelin alleviated muscle atrophy and facilitated recovery from muscle atrophy via stimulation of the GH-STAT5-IGF-1 axis in the locally atrophied plantaris muscle in mice [24]. Injection of acyl ghrelin also improved body weight loss and ameliorated skeletal muscle catabolism induced by angiotensin II through the early restoration of decreasing IGF-1 mRNA in the skeletal muscle in mice [25]. On the other hand, the serum ghrelin level was reported to be positively correlated with trabecular bone mass density in women [26]. A recent study demonstrated clearly that acyl ghrelin improved bone structure via an age-dependent mechanism [27]. Furthermore, ghrelin O-acetyltransferase was proven to regulate intestinal bile acid reabsorption [28]. Thus the pleiotropic anabolic effects on eliciting feeding, promoting adipose tissue, improving muscle and bone protective functions underscore acyl ghrelin as an ideal molecule to combat human anorexia/cachexia or wasting disorders [29].

Table 1 shows plasma ghrelin levels at the baseline and after anti-TNF-α therapy in patients with RA from various studies. Plasma fasting acyl ghrelin levels were reported either lower [30] or similar [31] in RA compared with healthy controls at baseline. Elevation of plasma total ghrelin with decreased endothelial activation was noted after 120 min of acute infliximab injection [32]. One-year treatment with anti-TNF-α neither improved insulin resistance nor did it alter plasma acyl ghrelin in a Spanish population [33]; however, similar treatment resulted in decreased total ghrelin concentrations in a Polish population [34]. Our recently published article demonstrated that a transient decrease in fasting plasma acyl ghrelin and loss of the post-oral glucose challenge suppression of plasma acyl ghrelin occurred at 3 months during etanercept treatment [35]. These discrepancies among various studies, including ours, indicate that between the TNF-α and acyl ghrelin there must be additional factors that modify the magnitude of secretion of this gut hormone. Or the differences in the methodologies of measurement may have lead to this inconsistency.

### Des-acyl ghrelin

Des-acyl ghrelin, lacking O-n-octanoylation at serine 3, is produced in the stomach and is a major circulating molecule of ghrelin [36]. In different studies the plasma des-acyl ghrelin:acyl ghrelin ratio has been reported to range from 2.5:1 [37], 3:1 [38] and 9:1 [11, 39]. Compared with acyl ghrelin, the role of des-acyl ghrelin in inflammation has been overlooked [40].

Des-acyl ghrelin and acyl ghrelin stimulate lipid accumulation in human visceral adipocytes [41]. Co-administration of des-acyl ghrelin and acyl ghrelin counteracted the pancreatic polypeptide-releasing effect exerted by acyl ghrelin in a model of isolated mouse pancreatic islets [42]. Distinct from acyl ghrelin, des-acyl ghrelin can bind to specific receptors on the cardiomyocytes and enhance the insulin-induced translocation of glucose transporter-4 from nuclear to cytoplasmic compartments, ultimately resulting in the uptake of medium-chain fatty acids [43]. Taken together, des-acyl ghrelin behaves like a distinct gastric hormone, acting in conjunction with acyl ghrelin to antagonize acyl ghrelin reciprocally. In other words, it appears to have acyl ghrelin-independent effects.

In differentiating human omental adipocytes, des-acyl ghrelin reduced the activity of caspase-8 and caspase-3, as well as apoptosis induced by TNF-α. In addition, des-acyl ghrelin inhibited expression of the autophagy-related genes—ATG5, BECN1 and ATG7 [16]. A 2-fold increase in des-acyl ghrelin concentrations was found in patients with non-alcoholic steatohepatitis compared with morbidly obese controls [44]. These studies have provided compelling evidence for the critical role of des-acyl ghrelin in the inflammatory process.

Des-acyl ghrelin modulates the gene expression of the lipogenic and insulin signalling pathway [45], and also
which are found in the mucosa of the duodenum and the secretin family [51]. GIP is synthesized by K cells, formerly called gastric inhibitory polypeptide, is a member of the secretin family [51]. GIP is synthesized by K cells, which are found in the mucosa of the duodenum and jejenum, and circulates as a biologically active 42-amino-acid peptide [52]. Circulating GIP acts on its 7-transmembrane G-protein coupled receptors on β cells in the pancreas. The main function of GIP is to induce insulin secretion. GIP, together with glucagon-like peptide-1 (GLP-1), belongs to a class of molecules referred to as incretins. When glucose enters the duodenum, it stimulates the secretion of incretins. Worthy of note is that the amount of insulin secreted is greater when glucose is administered orally than when administered intravenously [53, 54].

GIP has an effect on lipogenesis through stimulation of lipoprotein lipase in adipocytes [55, 56]. GIP was shown recently to increase human adipose tissue blood flow under a condition of hyperinsulinemia and slight hyperglycaemia, resulting in re-esterification of free fatty acid and deposition of triacylglycerol in the abdominal subcutaneous adipose tissue [57]. In addition, GIP serves as an important actor in linking nutrient ingestion and bone formation. GIP induces bone formation and prevents age-related loss of bone mass and bone strength in murine models [58, 59].

In aggregate, these findings favour GIP as an important player in maintaining positive energy homeostasis. The data regarding GIP in patients with RA and the dynamic changes in GIP after anti-TNF-α therapy are essential for our understanding of rheumatoid inflammation and its consequences with regard to energy homeostasis as well as bone destruction. We reported recently that 12-month therapy with etanercept resulted in a decrease in fasting plasma GIP levels in patients with active RA [35]. However, after an oral glucose challenge, plasma GIP remained at elevated levels in active RA patients who were under constant therapy with etanercept. This suggests that plasma GIP may contribute to the energy homeostasis in RA patients during anti-TNF therapy. Several studies have failed to demonstrate the effect of GIP on appetite parameters, including hunger, desire to eat, satiety or prospective consumption, in healthy volunteers [61]. On the other hand, GIP has been demonstrated

TABLE 1 Summary of plasma total, acyl and des-acyl ghrelin concentrations in patients with RA

<table>
<thead>
<tr>
<th>Publication</th>
<th>Plasma total, acyl and des-acyl ghrelin concentrations</th>
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<tbody>
<tr>
<td>Koca et al. (2008) [31]</td>
<td>Baseline acyl and total ghrelin ↔ (compared with healthy subjects)</td>
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<tr>
<td>Otero et al. (2004) [30]</td>
<td>Baseline acyl ghrelin ↓ (compared with healthy subjects)</td>
</tr>
<tr>
<td>Gonzalez-Gay et al. (2008) [32]</td>
<td>Total ghrelin ↑ after acute infliximab injection</td>
</tr>
<tr>
<td>Ferraz-Amaro et al. (2011) [33]</td>
<td>Acyl ghrelin ↑ after a 12-month course of anti-TNF-α therapy (including adalimumab, etanercept and infliximab)</td>
</tr>
<tr>
<td>Magiera et al. (2011) [34]</td>
<td>Total ghrelin ↓ after a 12-month course of infliximab therapy</td>
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<tr>
<td>Chen et al. (2013) [35]</td>
<td>Acyl ghrelin ↓ and loss of the post-oral glucose challenge suppression of acyl ghrelin at 3 months during etanercept treatment (transiently). Des-acyl ghrelin ↑ after a 12-month etanercept therapy, whereas the post-oral glucose challenge suppression of des-acyl ghrelin remained constant throughout the course of etanercept treatment.</td>
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↔, unaltered; ↓, decreased; ↑, increased.

exhibits pro-anabolic as well as anti-catabolic effects on myotubes exposed to cytokines and decreased burn-induced muscle proteolysis in rats [46]. Des-acyl ghrelin alone has been shown to exhibit anorectic action on feeding behaviour in the hungry condition [47] and to oppose acyl ghrelin-induced hyperphagic effects in the satiated condition [48]. In addition, improvement of body weight and body composition was found to be associated with an increase in plasma des-acyl ghrelin levels in young men undergoing military service after 6 months of intensive long-term physical exercise [49]. Hence des-acyl ghrelin plays a role in muscle healing and acts as an active modulator in inflammation, appetite, gastric motility and energy homeostasis. In the recovery stage of rheumatoid inflammation resulting from anti-TNF therapy, des-acyl ghrelin may also contribute to the healing of muscle diseases secondary to joint destruction.

Emerging evidence indicates that total ghrelin is not the surrogate biomarker of either acyl ghrelin or des-acyl ghrelin [50], and it is recommended that both active forms of ghrelin be measured in the blood separately. However, there are no data available regarding des-acyl ghrelin levels in RA patients with or without anti-TNF therapy. Our recent study demonstrated that fasting plasma des-acyl ghrelin concentrations increased at 12 months of etanercept therapy, but they were not elevated at 3 months of therapy in patients with active RA (Table 1). Besides, the after-effect of oral glucose challenging on the suppression of plasma des-acyl ghrelin remained constant throughout the course of etanercept therapy in these patients [35]. Thus des-acyl ghrelin may yet be an unidentified player in mediating energy homeostasis in the anti-inflammatory process during RA treatment with biologics.

Glucose-dependent insulinoctropic polypeptide

Glucose-dependent insulinoctropic polypeptide (GIP), formerly called gastric inhibitory polypeptide, is a member of the secretin family [51]. GIP is synthesized by K cells, which are found in the mucosa of the duodenum and jejenum, and circulates as a biologically active 42-amino-acid peptide [52]. Circulating GIP acts on its 7-transmembrane G-protein coupled receptors on β cells in the pancreas. The main function of GIP is to induce insulin secretion. GIP, together with glucagon-like peptide-1 (GLP-1), belongs to a class of molecules referred to as incretins. When glucose enters the duodenum, it stimulates the secretion of incretins. Worthy of note is that the amount of insulin secreted is greater when glucose is administered orally than when administered intravenously [53, 54].

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to exhibit a proinflammatory property in vitro [62, 63]. Therefore the exact role of GIP in inflammatory diseases such as RA requires further investigation.

**Glucagon-like peptide-1**

Glucagon-like peptide-1 (GLP-1) is derived from the transcription product of the proglucagon gene, which also encodes GLP-2, glicentin (a gastric acid inhibitor) and oxyntomodulin [8]. GLP-1 is produced primarily by L cells in the distal small intestine and colon, where it co-localizes with oxyntomodulin and peptide YY (PYY). GLP-1, GLP-2, glicentin and oxyntomodulin are rapidly inactivated in the circulation by dipeptidyl peptidase-4. GLP-1 is known as the most powerful incretin in humans, and also engages in the ileal brake, accentuates glucose-dependent insulin release, inhibits glucagon secretion and increases pancreatic β cell growth. Manipulation of the GLP-1 system constitutes the basis of several major novel treatments for type 2 DM. In addition, overstimulation of the GLP-1 receptor offers an attractive pharmacologic anti-obesity strategy [8].

GLP-1 has been demonstrated to exhibit anti-inflammatory properties in vivo and in vitro. So we may presume its increase in secretion is parallel to the anti-TNF therapy in RA patients. An in vitro study revealed that the GLP-1 analogue liraglutide exhibited anti-oxidative and anti-inflammatory effects on endothelial cells with inhibition of protein kinase C-α, nicotinamide adenine dinucleotide phosphate oxidase, and nuclear factor kappa B (NF-κB) signalling and up-regulation of protective anti-oxidative enzymes [64]. GLP-1 was also shown to reduce macrophage infiltration and directly inhibit inflammatory pathways in adipocytes and adipose tissue macrophages in an obese mouse model of diabetes [65]. Therefore, whether or not GLP-1 exerts its potential anti-inflammatory effects in patients with RA during anti-TNF therapy is an open question.

GLP-1 has been shown to regulate appetite, including decreasing hunger, desire to eat and prospective consumption, and to delay gastric emptying in healthy volunteers [61]. These anorectic and gastric motility-inhibitory properties support the role of GLP-1 as a negative regulator of food intake and energy homeostasis. Another animal study provided direct evidence of the effect of GLP-1 on neuronal activation in the central nervous system. Peripheral injection of GLP-1 differentially induced neuronal activation in the brainstem and hypothalamus of mice using a manganese-enhanced MRI technique [66]. In addition, higher fasting plasma concentrations of GLP-1 are associated with higher resting energy expenditure and fat oxidation rates in humans [67]. Injection of the GLP-1 analogue exenatide reduced body weight remarkably in Caucasians and Asians with type 2 DM [68, 69]. Our recently published data indicate that long-term therapy with etanercept results in weight gain but failed to alter the fasting plasma GLP-1 levels in active RA patients [35]. On the other hand, we could not find any change in plasma GLP-1 with an oral glucose challenge before and after anti-TNF-α treatment in these patients. Therefore, despite its potential anti-inflammatory property, plasma GLP-1 may not necessarily be implicated in the regulation of energy balance in patients with RA during anti-TNF-α therapy.

**Peptide YY**

PYY is a 36-amino-acid peptide secreted by L cells in the ileum and colon in response to feeding. Intravenous infusion of PYY reduces appetite in humans [70, 71]. PYY belongs to the PP-fold family and shares structural homology with neuropeptide Y and pancreatic polypeptide. PYY1-36 and PYY3-36 are two forms of PYY, whereas the major circulating form is a truncated 34-amino-acid form, PYY3-36, created by cleavage of the N-terminal Tyr-Pro residues by dipeptidyl peptidase-4 [8]. PYY3-36 shows selectivity for the Y2 receptor with high affinity, although it has some affinity for Y1 and Y5 receptors. The Y2 receptor is thought to function as an auto-inhibitory presynaptic receptor, expressed on NPY neurons [8]. In addition to its anorectic action when administered alone, PYY3-36 acts synergistically with GLP-1 receptor agonist, such as GLP-1 and oxyntomodulin, to reduce food intake in mice and humans [70, 72].

PYY has been reported to exhibit an anti-inflammatory property. An in vitro study demonstrated that PYY attenuates transcription factor activities, including NF-κB, Smad3/4 and peroxisome proliferator-activated receptors [α/γ/β] (PRAR-α/γ/β), in acinar cells of a TNF-α-induced rodent pancreatitis [73]. Intravenous infusion of PYY delayed gastric emptying in healthy adults [74], while plasma PYY concentrations correlated with impaired gastric emptying in critically ill patients [75]. In addition, plasma PYY concentrations were shown to be positively correlated with postprandial energy expenditure [76]. Due to its pleiotropic effects on modulating appetite, inflammation, gastric motility and energy expenditure, PYY might be a candidate for regulating energy balance during inflammation in patients with RA. The results from our published report indicate that long-term therapy with etanercept cannot affect fasting plasma PYY levels, despite weight gain [35]. No change in plasma PYY after the oral glucose challenge was observed before and after anti-TNF treatment in these patients. Hence plasma PYY may contribute minimally to the regulation of energy balance in patients with RA during anti-TNF therapy.

**Conclusion**

As anti-TNF treatment can achieve a high remission rate in patients with RA, whether this therapy can also change energy handling, leading to better body composition and physical function in these subjects, deserves attention. A growing body of evidence indicates that gut hormones may serve as key signals in coupling the metabolic axis with the immune system (Fig. 1) and may be new players during immunomodulatory therapy for RA patients. Gut hormones and leptin antagonize each other in regulating appetite and energy balance [27]. In addition, TNF-α receptor may derange the hypothalamic–pituitary–adrenal
axis [77] (decrease the hypothalamic-pituitary-adrenal axis tone with or without increased sympathetic outflow) as well as the synthesis of non-adrenal gonadal hormones [78]. Anecdotal reports have shown an increased incidence of DM in patients with RA treated or not with anti-TNF biologics. Whether or not this is relevant to the gut hormone imbalance caused by rheumatoid inflammation per se or by anti-TNF therapy is an intriguing issue to be investigated in depth. In addition, currently available data suggest that the interplay between gut hormones exists during rheumatoid inflammation. Manipulation of TNF-α may alter the circulating gut hormonal milieu and further regulate substrate utilization and promote metabolic flexibility. A great many novel biologics, including anti-B cell therapy, anti-IL-6, anti-IL-23/IL-12 p40 receptor and anti-IL-17, as well as other cytokine therapies, have come and will come into the large arena of RA treatment, both now and in the future. The interplay of gut hormones with these immunocompetent cells or cytokines/cytokine receptors is largely unknown and will require vigorous study. Finally, because a significant variation in visceral fat content exists between Asians and Caucasians, namely the Y-Y paradox [79], the investigation of gut hormones in RA patients who are treated with various biologics should be conducted among different ethnicities in a prospective, longitudinal and systematic manner.

**Rheumatology key messages**

- Gut hormones modulate inflammation, appetite and energy balance in RA patients.
- Anti-TNF alters circulating gut hormones, energy handling, physical function and well-being in RA patients.

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