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Review

Capromorelin: a ghrelin receptor agonist and novel therapy for stimulation of appetite in dogs

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Abstract

Ghrelin is a hormone, secreted from cells in the stomach, which is important in the regulation of appetite and food intake in mammals. It exerts its action by binding to a specific G-protein-coupled receptor, the growth hormone secretagogue receptor 1a (GHS-R1a) which is found in areas of the brain associated with the regulation of food intake. Ghrelin causes a release of growth hormone (GH) through binding to GHS-R1a in the hypothalamus and pituitary gland. A class of compounds known as growth hormone secretagogues, or ghrelin receptor agonists, were developed for therapeutic use in humans for the stimulation of GH in the frail elderly, and have subsequently been studied for their effects on increasing appetite and food intake, increasing body weight, building lean muscle mass, and treating cachexia. Subsequent research has shown that ghrelin has anti-inflammatory and immunomodulatory effects. This article reviews the basic physiology of ghrelin and the ghrelin receptor agonists, including the available evidence of these effects *in vitro* and *in vivo* in rodent models, humans, dogs and cats. One of these compounds, capromorelin, has been FDA-approved for the stimulation of appetite in dogs (ENTYCE®). The data available on the safety and effectiveness of capromorelin is reviewed, along with a discussion of the potential clinical applications for ghrelin receptor agonists in both human and veterinary medicine.

Keywords: Anorexia, Appetite stimulation, Cachexia, Growth hormone secretagogue, Insulin-like growth factor 1.

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Introduction

Ghrelin is a hormone produced by specific cells in the stomach. It exerts its action by binding to receptors in the hypothalamus involved in the regulation of food intake. Ghrelin causes an increase in the pituitary secretion of growth hormone (GH). Drugs that bind and activate the ghrelin receptor have been explored for their effect on appetite, weight gain, frailty, cachexia, inflammation and gut motility. This manuscript reviews the mechanisms of action of ghrelin and this class of drugs for use in animals, and introduces a novel drug for the stimulation of appetite in dogs: capromorelin, a small molecule ghrelin receptor agonist.

Ghrelin

This unique hormone is a 28 amino acid peptide, in which the serine 3 residue is n-octanoylated (Kojima *et al.* 1999). Octanoylation is essential for the hormone's activity, and is achieved by the ghrelin O-acyltransferase enzyme (Yang *et al.* 2008). Ghrelin is produced in the oxyntic glands of the gastric fundus, specifically in a distinct endocrine cell type found in the mucosal epithelium (Kojima *et al.* 1999; Date *et al.* 2000; Kojima & Kangawa 2002). The amino acid sequence of ghrelin is well conserved across species, with the 10 amino acids at the N-terminus identical in humans, pigs, cows, sheep, cats and dogs (Tomasetto *et al.* 2001; Kojima & Kangawa 2002,

2005; Ida *et al.* 2007). Ghrelin is not secreted into the lumen of the GI tract, but rather into blood vessels for systemic distribution.

Food intake regulates ghrelin secretion, which is increased during fasting and decreased after food consumption in humans and other mammalian species (Cummings *et al.* 2004; Sun *et al.* 2004). The role of ghrelin on the control of feeding behaviour was shown in dogs (Bhatti *et al.* 2006) and cats (Ida *et al.* 2007).

Ghrelin receptors

Ghrelin exerts its effects through a specific G-protein-coupled receptor called the growth hormone secretagogue receptor 1a (GHS-R1a). Another GHS-R cDNA is produced by alternative splicing which produces a COOH-terminal truncated form (GHS-R1b) that is pharmacologically inactive (Kojima & Kangawa 2005). The GHS-R1a is expressed in several areas of the hypothalamus associated with feeding behaviour, including the arcuate and ventromedial nuclei (Kojima & Kangawa 2005). GHS-R1a is also found in the pituitary gland, and other organs such as bone, heart, lung, liver, kidney, pancreas and immune cells (Papotti *et al.* 2000; Smith *et al.* 2005).

Research shows that the GHS-R1a can amplify dopamine signalling *in vitro*, by a mechanism consistent with formation of heterodimers of the GHS-R and dopamine receptors in neurons that co-express these receptors. This dimerization is also detected *in vivo* in rodents indicating that dopamine signalling may be affected by these receptor interactions, particularly dopamine reward signalling (Papotti *et al.* 2000; Smith *et al.* 2005). Other research has shown that the serotonin 2C receptor can form heterodimers with GHS-R1a, and this dimerization may also be involved in regulation of appetite and food reward (Schellekens *et al.* 2013).

Ghrelin receptor agonists

In the 1980s, Bowers *et al.* (1984) identified a small peptide, GHRP-6, that stimulated GH release both *in vitro* and *in vivo* by an unknown mechanism, which subsequently was found to act through a novel receptor. GHRP-6 was used as a structural template to develop small molecule compounds that could stimulate GH, potentially to be used as a treatment for declining GH in the frail elderly (Smith 2005). This work led to the cloning of GHS-R1a, and development of a class of small molecules called growth hormone secretagogues (GHS); compounds that when administered orally, caused the pituitary to release GH. Subsequently, ghrelin was identified as the endogenous ligand of the GHS-R1a, and ghrelin's role in regulating food intake, as well as stimulating GH, was elucidated.

A number of GH secretagogue compounds, also known as ghrelin receptor agonists, have been identified (Table 1). Some, such as relamorelin (also known as BIM-28131, under development for diabetic gastroparesis) and ulimorelin (for gastrointestinal functions) are peptide compounds designed to be given by injection, and others are small molecules that can be administered orally such as anamorelin (under development for cancer cachexia), macimorelin (for GH deficiency) and capromorelin (Muller *et al.* 2015). All of these compounds act by mimicking ghrelin and binding the GHS-R1a.

Capromorelin, also referred to as AT-002, RQ-5, and CP-424,391 was developed originally for the treatment of human frailty (Carpino *et al.* 2002). Capromorelin binds the human GHS-R1a *in vitro* and caused GH release both in pituitary cell cultures and *in vivo* after oral dosing in rats and dogs (Carpino *et al.* 2002, 2003). GHSs, including capromorelin, were initially investigated for their ability to stimulate GH and it was not until after the discovery of ghrelin that their therapeutic potential for appetite stimulation was recognized. Capromorelin is the first ghrelin receptor agonist to achieve FDA approval, and it is indicated for the stimulation of appetite in dogs (ENTYCE®, capromorelin oral solution, Aratana Therapeutics, Inc., Leawood, KS).

Biological effects of ghrelin and ghrelin receptor agonists

As a peptide, ghrelin has a relatively short serum half-life, while the ghrelin receptor agonists have been optimized for therapeutic use, with better oral

Table 1. Ghrelin receptor agonists (GH secretagogues) for human health

Compound	Synonyms	Company	Peptide/Chemical	Phase	Indication
Anamorelin	ONO-7643 ST-1291 RC-1291	Helsinn Ono (JP) (Sapphire, Rejuvenon)	Chemical	Phase 3	Cancer cachexia
Capromorelin	CP-424,391 RQ-00000005	Pfizer Raqualia	Chemical	Phase 2, discontinued	Frailty in elderly
Examorelin	Hexarelin EP-23905 MF-6003	(Europeptides, Mediolanum Farmaceutici)	Peptide	Phase 2, discontinued	GH deficiency
Ibutamoren	MK-0677	Merck	Chemical	Phase 2, discontinued	Fibromyalgia Alzheimer's disease Frailty in elderly
Ipamorelin	NNC 26-0161	Helsinn (Novo Nordisk)	Peptide	Phase 2, discontinued	Postoperative ileus
Macimorelin	AEZS-130 EP1572 JMV-1843	Aeterna Zentaris	Chemical	Phase 3	Evaluation of adult GH deficiency
Pralmorelin	GHRP 2 GPA 748 KP-102	Kaken	Peptide	Approved in Japan	Evaluation of GH deficiency
Relamorelin	BIM-28131 RM 131	Allergan (Motus, Rhythm, Ipsen)	Peptide	Phase 2	Diabetic gastroparesis
Sermorelin	GRF 1-29	Serono	Peptide	Approved US, discontinued	GH deficiency
Tabimorelin	NN-703 NNC 26-0703	Novo Nordisk	Chemical	Phase 2, discontinued	GH deficiency
Ulimorelin	LP101 TZP-101	Lyric (US) (Tranzyme) Norgine (ex US)	Peptide	Phase 2	Nutrition disorders

bioavailability and longer half-lives, as compared to ghrelin (Carpino *et al.* 2002; Smith 2005). For example, capromorelin has a Tmax (mean 0.83 h) and serum half-life (mean 1.19 h) which indicate that absorption of capromorelin occurs in the proximal gastrointestinal tract. *In vitro* (human liver microsomes) and *in vivo* (rats) metabolism studies suggest that capromorelin is metabolized by hepatic enzymes. Following oral administration of radiolabelled capromorelin to dogs, the radio-label was excreted in urine (37%) and in faeces (62%) within 72 h. Based on metabolite analysis, elimination of capromorelin occurs primarily through phase 1 hepatic metabolism and the metabolites are excreted primarily in the faeces with the remainder in the urine (Aratana Therapeutics, Inc., data on file).

The main actions of this class of drugs include the stimulation of appetite, increasing food intake and the stimulation of growth hormone, which subsequently increases insulin-like growth factor 1 (IGF-1). In addition to these actions, ghrelin and ghrelin receptor agonists have been shown to increase muscle mass, stimulate bone formation, have effects on gut motility and have anti-inflammatory activity. These biological effects are reviewed below.

Increased appetite and food intake

Studies have demonstrated that fasting increases and food intake suppresses systemic ghrelin levels in dogs (Yokoyama *et al.* 2005; Bhatti *et al.* 2006) and cats (Ida *et al.* 2007). This was also seen in human studies, where administration of ghrelin stimulated appetite and weight gain (Wren *et al.* 2001; Cummings *et al.* 2004; Klok *et al.* 2007). Ghrelin administration directly activates neurons in the arcuate nucleus of

the hypothalamus, and works indirectly through the vagus nerve to trigger the sensation of hunger and enhance feeding (Date *et al.* 2000).

Capromorelin has been studied to determine its effects on food intake and weight gain in the dog. In a 7-day study in Beagle dogs, treatment with doses of 3.0 mg/kg once or twice a day, or 4.5 mg/kg once a day were compared to treatment with placebo. Capromorelin treatment resulted in significant mean increases in food intake ranging from 36 to 58%, and significant increases in body weight ranging from a mean of 3.8 to 4.5% over the 7 days of treatment (Zollers *et al.* 2017b). Food intake and body weight increases were also seen in a 4-day study in laboratory Beagles, where the commercial formulation of capromorelin (ENTYCE®) was studied (Zollers *et al.* 2017a).

Capromorelin was shown to have a similar effect in laboratory cats where cats were dosed with placebo or capromorelin at doses ranging from 1 to 3 mg/kg for 21 days. Food intake increased in placebo-treated cats by about 11% over baseline, while capromorelin-treated groups showed mean increases ranging from 25 to 46% over baseline. Body weights of capromorelin-treated cats increased between approximately 3.9-5.4%, while the placebo group lost weight (approximately 1.1%) (Zollers *et al.* 2015). Statistically significant increases in food intake and body weight were seen in a 91-day study of laboratory cats treated with 6 mg/kg capromorelin, at day 91, mean weight increases were 0.8 kg and 0.16 kg for the capromorelin and placebo-treated groups respectively (Wofford *et al.* 2017).

Stimulation of GH and IGF-1

Ghrelin and ghrelin receptor agonists have activity at both the hypothalamic and pituitary level to stimulate the secretion of GH (Smith *et al.* 1997). Activation of GHS-R1a in the hypothalamus stimulates GH-releasing hormone secretion from arcuate nucleus neurons (Dickson & Luckman 1997) and in the anterior pituitary promotes exocytosis of vesicles containing GH (Herrington & Hille 1994; Muller *et al.* 2015). In healthy humans, intravenous ghrelin caused the release of GH in a dose-dependent manner, with peak GH values measured at 30 min after administration (Takaya *et al.* 2000). GH then stimulated IGF-1 production in the liver. IGF-1 acts as negative feedback to GH secretion, which serves to avoid GH overstimulation in response to subsequent doses of ghrelin and ghrelin receptor agonists.

MK-0677 was shown to increase serum GH and IGF-1 in Beagle dogs after daily oral dosing for up to 14 days. The initial GH stimulation was attenuated on subsequent days, likely due to the negative feedback of IGF-1 on GH secretion, preventing hyperstimulation of the GH axis (Hickey *et al.* 1997).

A recent laboratory study in Beagle dogs evaluated daily oral dosing of capromorelin for 7 days, either once a day at 3.0 or 4.5 mg/kg body weight, or twice a day at 3.0 mg/kg. A single dose of capromorelin treatment resulted in an increase in serum GH, which returned to baseline 8 h after dosing, likely because of the negative feedback of IGF-1. After 7 days of treatment, capromorelin administration still stimulated serum GH but the effect was attenuated. Serum IGF-1 concentrations increased after capromorelin dosing, remained elevated during the 7-day study and returned to pre-study levels 2 days after treatment finished (Zollers *et al.* 2017b).

In laboratory cats treated for 21 days with 1–3 mg/kg capromorelin, a sustained increase in IGF-1 was measured (Zollers *et al.* 2015) and in a 91-day safety study in laboratory cats, treatment with 6 mg/kg capromorelin increased GH and IGF-1 levels as compared to placebo treatment (Wofford *et al.* 2017).

Both MK-0677 (Hickey *et al.* 1997) and capromorelin (Zollers *et al.* 2016) have been shown to cause an increase in serum cortisol levels after one dose in dogs. Similar to GHS effects on GH, cortisol returned to pre-treatment values by 6–8 h post treatment and the magnitude of cortisol stimulation was attenuated following subsequent doses. This is consistent with observations wherein intravenous administration of ghrelin in humans resulted in increased adrenocorticotropic hormone (ACTH) and, subsequently, increased cortisol serum levels (Arvat *et al.* 2001).

GH and IGF-1 and their receptors have been implicated in possible promotion of tumour growth (Perry

& Wang 2012). IGF-1 receptors have been shown to be present on canine malignant melanoma cell cultures *in vitro* (Thamm *et al.* 2010) and elevated serum and tumour GH and IGF-1 have been associated with the malignant phenotype of canine mammary cancer (Queiroga *et al.* 2010). In a 1-year safety study in normal Beagles, there was no evidence of tumour initiation from high daily doses of capromorelin (Zollers *et al.* 2016a). There are no studies elucidating the effects of ghrelin receptor agonists in tumour-bearing animals. In humans, epidemiological studies of a possible association between serum IGF-1 levels and cancer risk did not establish causality (Cohen *et al.* 2000). Increased GH also stimulates an increase in IGF-binding protein-3 levels, and increased IGF-binding protein 3 has been shown to be negatively correlated with the risk of cancer in humans (Cohen *et al.* 2000). Human cancer patients in two large-scale Phase III clinical studies of anamorelin did not show that treatment with this ghrelin receptor agonist promoted tumour growth (Temel *et al.* 2016).

Effect on muscle

Treatment with exogenous GH has been shown to increase IGF-1 levels and to induce hypertrophy of muscle fibres in laboratory dogs (Molon-Noblot *et al.* 1998). This suggests that treatment with a ghrelin receptor agonist, which causes increases in GH and IGF-1, may result in positive effects on lean muscle mass. In a long-term laboratory study using limb immobilization in Beagle dogs, a ghrelin receptor agonist (an analogue of MK-0677) was studied for its effect on muscle. GH and IGF-1 were increased in treated dogs and the size and strength of the quadriceps muscle was shown to be increased after remobilization, as compared to placebo treatment (Lieber *et al.* 1997).

Ghrelin may have other effects on muscle, not mediated by GH release. Chen *et al.* characterized molecular pathways involved in muscle atrophy induced by tumour implantation and cisplatin treatment in mice, and studied the effect of ghrelin treatment in this model. They concluded that ghrelin prevented muscle atrophy by down-regulating inflammation and myostatin gene expression, and activating genes involved in muscle fibre synthesis. Ghrelin appeared to target muscle cells directly, resulting in improved muscle strength and survival (Chen *et al.* 2015).

Effect on bone

Using transgenic mice deficient in GHS-R1a compared to wild-type mice, ghrelin was shown to have dual effects on osteoclastogenesis, inhibiting osteoclast progenitors directly and stimulating osteoclastogenesis (van der Velde *et al.* 2012). *In vitro*, ghrelin has been shown to stimulate human osteoblast growth (Delhanty *et al.* 2006), stimulate proliferation and differentiation and inhibit apoptosis in osteoblastic cells (Kim *et al.* 2005). Treatment with ghrelin was shown to increase bone mineral density in rats, demonstrating that ghrelin can stimulate bone formation (Fukushima *et al.* 2005).

Effect on gut motility

Ghrelin has been shown to stimulate gut motility in rats (Masuda *et al.* 2000; Fukushima *et al.* 2005). In human volunteers, relamorelin increased the frequency of distal antral stomach motility contractions, without significant effects on amplitude (Nelson *et al.* 2016). Relamorelin is under development in humans for treatment of diabetic gastroparesis.

In dogs, however, an intravenous injection of canine ghrelin did not stimulate the motor activity of the digestive tract in either the fasted or fed state, and did not accelerate gastric emptying. The biological activity of the canine ghrelin used in the study was confirmed by measuring its stimulatory effect on GH release (Ohno *et al.* 2006). Further, in a 12-month safety study of capromorelin at doses up to 40 mg/kg daily in laboratory Beagle dogs, no clinical signs associated with increased gut motility were observed (Zollers *et al.* 2016a).

Anti-inflammatory and immunomodulatory effects

There is evidence for ghrelin as an anti-inflammatory agent, and it may play a role in immunoregulation of

cytokines (Baatar *et al.* 2011). A number of investigations over the past 15 years have demonstrated ghrelin to be a potent anti-inflammatory mediator, to promote lymphocyte development in the bone marrow and thymus, and to reverse age-associated thymic involution.

Many *in vitro* studies have shown that ghrelin modulates release of a variety of cytokines. For example, in lipopolysaccharide (LPS)-stimulated murine macrophages treated with ghrelin, the production of pro-inflammatory cytokines interleukin-1 β (IL-1β) and tumour necrosis factor α (TNFα) was inhibited. Exogenous ghrelin pre-treatment resulted in a decrease in LPS-induced nuclear factor-kappa B (NFκB) activation (Waseem *et al.* 2008). In a study of human monocytes and T cells, ghrelin was shown to inhibit pro-inflammatory cytokine expression, specifically IL-1β, IL-6 and TNFα, and biologically active GHS-R was shown to be expressed in human T lymphocytes and monocytes. In addition, ghrelin was shown to be produced by T lymphocytes (Dixit *et al.* 2004). Ghrelin and the agonist pralmorelin (also known as GHRP-2) prevented endotoxin-induced IL-6 release from rat peritoneal macrophages *in vitro*, suggesting that this effect is mediated by GHS-R1a directly on immune cells (Granado *et al.* 2005).

In mice treated with ghrelin prior to and after LPS administration, ghrelin had a potent anti-inflammatory effect on the induced endotoxaemia, inhibiting the production of IL-1β, IL-6 and TNFα expression in liver and spleen tissue (Dixit *et al.* 2004).

A number of studies have demonstrated positive anti-inflammatory effects in rodents in models of sepsis, endotoxaemia, intestinal ischaemia and cardiovascular disease (Baatar *et al.* 2011). In a rat model of chronic kidney disease, which resulted in uraemia and decreased lean muscle mass, treatment with ghrelin resulted in the expected increase in food intake and body weight, but additionally, there was a decrease in circulating inflammatory cytokines relative to saline-treated rats (DeBoer *et al.* 2007).

In a rat model of chronic heart failure, it was demonstrated that ghrelin and the ghrelin receptor agonists, BIM-28131 (relamorelin) and BIM-28125, decreased the elevated TNFα plasma concentrations (Lenk *et al.* 2013). Granado *et al.* (2005) reported that a ghrelin receptor agonist peptide, GHRP-2, given for 8 days in a rat model of acute arthritis, decreased the arthritis score, paw volume and serum IL-6.

Chronic administration of a ghrelin receptor agonist (an analogue of MK-0677) to old mice restored GH and IGF-1 levels, and also stimulated growth, differentiation and cellularity of the thymus, which normally involutes with age. Spleens from treated mice showed that the treatment promoted lymphoid cell division. Additionally, treated aged mice were resistant to developing tumours when administered a lymphoma cell line (Koo *et al.* 2001).

As yet, potential anti-inflammatory and immunomodulatory effects of ghrelin and ghrelin receptor agonists in dogs and cats have not been studied.

Clinical applications

Ghrelin receptor agonists were developed as potential treatment for frailty in elderly humans, because of their action to increase GH, which is known to decline with age, and for their potent impact on appetite, which is often reduced in the frail elderly (Smith 2005; Landi *et al.* 2016). Given new understanding of direct effects of ghrelin on muscle, gastrointestinal motility, inflammation and immune regulation, new clinical applications are being explored (Smith *et al.* 2005).

Decreased food intake and loss of appetite

In dogs and cats, loss of appetite (anorexia) or reduced appetite (hyporexia) can be caused by a variety of clinical conditions (Delaney 2006; Agnew & Korman 2014). Metabolic changes associated with disease or injury can result in loss of lean body mass, negative protein balance and other undesirable effects such as reduced wound healing, weakness and poorer overall prognosis (Chan 2004; Chan & Freeman 2006; Freeman 2012). In humans, malnutrition has been shown to increase morbidity and mortality related to infection, with poor nutritional status impacting immunity, and infection causing appetite suppression, while increasing energy

expenditure when fever is present (Bresnahan & Tanumihardjo 2014). In dogs and cats, it has been shown that increased energy intake was correlated with earlier hospital discharge, and animals with low body condition scores had greater mortality (Brunetto *et al.* 2010). In a study of dogs with septic peritonitis, earlier nutritional support was associated with a shorter hospitalization (Liu *et al.* 2012).

Assessment of quality of life of veterinary cancer patients concludes that better appetite, weight and body condition scores are an important part of a better quality of life (Hershey *et al.* 2016). Nutritional assessment has been included in the global initiative to standardize the physical examination for dogs and cats, and evaluations of body weight, body condition score and muscle condition scores are considered a critical component of the physical examination (Freeman *et al.* 2011). In cats, inappetence can result in hepatic lipidosis and it has been reported that 'prolonged inadequate nutrition may be more detrimental to the patient than the primary underlying disorder' (Agnew & Korman 2014).

These results highlight the fact that stimulation of appetite and increasing food intake in sick animals will have significant beneficial effects. Ghrelin receptor agonists can be useful in increasing appetite and food intake in anorexic or hyporexic dogs and cats, regardless of the underlying cause.

A prospective, placebo controlled, masked clinical study of capromorelin (ENTYCE®) was conducted in client-owned dogs that presented to veterinary clinics with at least 2 days of reduced appetite, as reported by the owners, due to a variety of causes (Zollers *et al.* 2016b).

Dogs were randomized to either placebo or capromorelin (3 mg/kg/day) treatment groups. Owners were required to agree not to feed the dogs anything different than their normal diet, and after 4 days, owners scored their dog's appetite, using a questionnaire. Capromorelin treatment improved appetite compared to placebo ($P = 0.008$). As expected for a subjective end point, there was a high placebo effect, with 44.6% of placebo-treated dog's showing an increased appetite, compared to 68.6% of capromorelin-treated dogs, as reported by their owners. Although this study included dogs of various breeds, with underlying pathologies, and on various concomitant medications, capromorelin was demonstrated to significantly stimulate appetite.

Frailty, loss of muscle and weight loss

Ghrelin receptor agonists may be useful in frail ageing animals that have lost lean body mass, either due to anorexia or hyporexia, or atrophy secondary to surgery or restraint.

In a laboratory study, Beagles that had hind legs artificially immobilized for 10 weeks, resulting in muscle atrophy, showed faster muscle recovery if treated with an analogue of MK-0677 (Lieber *et al.* 1997). In humans, studies have shown that perioperative nutritional support improves clinical outcomes in malnourished patients (Zhong *et al.* 2015).

In 395 older humans with mild functional limitations, capromorelin was studied for its effects on body composition and physical performance effects. As expected, a sustained dose-related increase in GH and IGF-1 was seen. At 6 months, mean body weight increased 1.4 kg in capromorelin-treated subjects, while placebo-treated subjects lost a mean of 0.2 kg ($P = 0.006$). Lean body mass increase in capromorelin-treated patients was statistically significant, and various measures of physical function such as stair climbing and tandem walk improved (White *et al.* 2009). Similar long-term evaluations of the effects of capromorelin on body weight, composition and function in dogs have not been conducted.

In chronic kidney disease (CKD), weight loss resulting in frailty is common. An evaluation of weight loss in relation to diagnosis and progression of CKD in cats showed that weight loss can be detected in CKD prior to diagnosis, accelerated after diagnosis and was associated with shorter survival (Freeman *et al.* 2015). Ghrelin receptor agonists may prove useful in CKD, to slow or reverse the weight loss associated with this condition.

Cachexia

Cachexia is a wasting syndrome, which results in a loss of lean body mass, muscle atrophy, weakness and loss of appetite and cannot be reversed simply

by increased nutrition. It is seen in patients with a variety of conditions – most common in veterinary medicine are cardiac cachexia in dogs and cachexia associated with chronic kidney disease in cats (Freeman 2012). Cancer cachexia appears to be common in humans, and can be seen in both dogs and cats with various tumours. In a retrospective study of 100 dogs with cancer, cachexia was defined as a body condition score of ≤3 on a 9-point scale. Using this definition, 4% of patients were cachexic. However, 23% of dogs had lost more than 10% of body weight, and another 14% had lost between 5 and 10% (Michel *et al.* 2004).

In a study of feline cancer patients, muscle mass was reduced in 91% of the 57 cats in the study, and cats that had a body condition score of <5/9 had a mean survival time of 3.3 months compared to 16.7 months for cats with a score of >5/9. This difference was statistically significant (Baez *et al.* 2007). More research is needed to define the prevalence of cancer cachexia in veterinary patients.

Cachexia secondary to cardiac disease has been reported in dogs. In dogs with heart failure, survival was greatest in dogs that gained weight, although body condition scores were not predictive, perhaps because they are a subjective measurement. Given that ghrelin causes an increase in appetite, body weight, GH and IGF-1, and has positive effects on lean muscle and anti-inflammatory effects, it is likely that ghrelin receptor agonists may be proven to be effective in the treatment of cachexia in dogs and cats. As yet, there is no direct evidence of this in these species, but research in animal models and human clinical trials suggests that administration of ghrelin receptor agonists may be useful in the treatment of cachexia.

To study if ghrelin treatment could positively impact cachexia related to CKD, a rat model was used, in which rats develop uraemia and increases in BUN and creatinine after partial nephrectomy. Ghrelin treatment demonstrated an improvement in the accrual of lean body mass (DeBoer *et al.* 2007).

In a study of human cachexia secondary to chronic heart failure, Nagaya *et al.* administered synthetic ghrelin intravenously twice a day to 10 patients with chronic heart failure. Three weeks of ghrelin therapy increased left ventricular ejection fraction in association with left ventricular mass, increased peak workload and oxygen consumption during exercise and improved muscle wasting as indicated by increases in muscle strength and lean body mass. In eight control patients, these positive effects were not observed (Nagaya *et al.* 2004).

Anamorelin is under development in human medicine to treat cancer cachexia. In a randomized, double-blind Phase II study, patients with non-small cell lung cancer with cachexia characterized by more than 5% weight loss over 6 months were treated with placebo or 50 or 100 mg oral anamorelin daily for 12 weeks. Lean body mass and total body weight increased in the anamorelin groups compared to the placebo group (Takayama *et al.* 2016). Subsequently anamorelin (100 mg/day for 12 weeks) was tested in two randomized, double-blind Phase III trials in lung cancer patients with cachexia; over 900 patients were enrolled. Anamorelin significantly increased lean body mass and body weight compared to placebo (Temel *et al.* 2016).

Safety

Because ghrelin receptors are found in a variety of tissues in the body, and the hormone has a wide range of effects on GH, IGF-1, appetite, food intake, immune function and inflammation, it is important to be aware of potential adverse effects of this class of compounds, and specifically, capromorelin.

Capromorelin has been evaluated in a 12-month safety study in laboratory Beagles. It was administered orally as a solution, by daily gavage, at doses of 0.0 (placebo), 0.3, 7 or 40 mg/kg, with eight dogs in each group (four males and four females). Safety was evaluated by physical examinations, including electrocardiograms and ophthalmic examinations, serum chemistry and haematology evaluations. At the end of the study, dogs were humanly euthanized and full necropsies were performed with histology completed on multiple tissues. When corrected for differences in formulation between that used in this study and the marketed formulation, the 40 mg/kg dose is equivalent to approximately 17.5 times the clinical dose of ENTYCE® (3 mg/kg), indicating a wide safety margin (Zollers *et al.* 2016a).

Of interest are the parameters in this study related to the cardiovascular system, as ghrelin receptors have been shown to be expressed in the heart. Dogs in the study described above in the 7 and 40 mg/kg capromorelin dose groups showed a minor lengthening of the PR interval, but no evidence on physical examination of cardiotoxicity, and no altered cardiac histopathology. This finding is consistent with first-degree AV block that occurs spontaneously in Beagle dogs. In a study of ghrelin given intravenously twice a day for 3 weeks in human patients with chronic heart failure, the treatment was seen to improve left ventricular function (Nagaya *et al.* 2004).

The field study (Zollers *et al.* 2016b) was conducted in client-owned dogs (171 dogs treated with capromorelin and 73 dogs treated with placebo in the intention to treat population) treated for 4 days after being presented with a variety of underlying conditions resulting in at least 2 days of inappetence; there was no report of any cardiovascular adverse event potentially related to treatment.

A safety study was conducted in laboratory cats treated with either placebo or capromorelin given at 6 mg/kg for 91 days. Cats had physical examinations, and serum chemistry and haematology evaluations. Other than some salivation and head shaking after dosing, no adverse effects were seen (Wofford *et al.* 2017).

Independent of its effects on GH and IGF-1, ghrelin plays a role in energy homoeostasis (Muller *et al.* 2015). Ghrelin treatment results in decreased glucose-stimulated insulin secretion (GSIS) with subsequent increased serum glucose in humans and rodents (Broglio *et al.* 2001; Reimer *et al.* 2003), an effect which depends on ghrelin/GHS-R1a activity in the pancreas (Kurashina *et al.* 2015) and is independent of GH signalling (Vestergaard *et al.* 2008). Genetic or pharmacological blockade of ghrelin activity specifically in the pancreas results in increased GSIS (Dezaki *et al.* 2004). In addition to effects on insulin, studies have indicated a role for ghrelin/GHS-R1a in stimulating glucagon secretion (Chuang *et al.* 2011) and glucagon-like peptide 1 (GLP-1) (Gagnon *et al.* 2015). In healthy humans, exogenous administration of ghrelin resulting in supraphysiological ghrelin concentrations has been reported to result in reduced insulin sensitivity (Tong *et al.* 2010). However, when ghrelin was infused such that serum ghrelin concentrations were consistent with prolonged fasting, GSIS was reduced but insulin sensitivity was not affected (Tong *et al.* 2013). In two human Phase III clinical trial of anamorelin, the most common treatment-related adverse events were diabetes and hyperglycaemia, but the incidence was low. Combining both trials, diabetes was seen in 1.54% of anamorelin-treated patients and 1.24% of the placebo-treated patients, while hyperglycaemia was seen in 4.77% and 1.24% of the capromorelin and placebo-treated patients respectively (Temel *et al.* 2016). Similarly, in a study in 395 older patients, capromorelin caused small increases in fasting glucose and glycosylated haemoglobin, which were considered of 'minimal clinical consequence' (White *et al.* 2009).

Dogs treated with high-dose capromorelin (17.5× the clinical dose) for 12 months did not show changes in serum or urinary glucose concentrations (Zollers *et al.* 2016a). In a 91-day cat safety study, serum and urinary glucose, and serum fructosamine were measured. Capromorelin treatment was associated with an increase in serum glucose but values for individual cats did not exceed the reference range. The treatment effect overall was found to be statistically significant for fructosamine, but the mean fructosamine concentrations were higher in the placebo than in the capromorelin-treated group at days 30 and 91, and no fructosamine value was outside of the reference range (Wofford *et al.* 2017).

Conclusions

Ghrelin is a key mediator of appetite, food intake and GH and IGF-1 release. Capromorelin is a ghrelin receptor agonist that binds GHS-R1a, thereby stimulating appetite and causing increases in body weight. In addition, capromorelin treatment results in an increase in GH and IGF-1 which may be beneficial in building lean muscle mass. These actions have been shown to be beneficial in a variety of clinical conditions, including inappetence and cachexia secondary to chronic kidney disease, heart failure and cancer.

Ghrelin has also been shown to have specific anti-inflammatory effects and holds promise for use in therapeutic applications related to both acute and chronic inflammation. Further research is needed to fully explore the potential of ghrelin receptor agonists such as capromorelin in common chronic conditions leading to weight loss and wasting in cats and dogs.

Capromorelin is the first ghrelin receptor agonist, also known as a GH secretagogue, to be FDA-approved for veterinary use, specifically for the stimulation of appetite in dogs. It represents a new tool for veterinarians to use in dogs with a variety of conditions in which anorexia or hyporexia play a part.

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Conflict of interest statement

All authors are stockholders in Aratana Therapeutics, Inc. Drs. Heinen and Wofford are current employees and Drs. Rhodes and Zollers are previous employees of Aratana Therapeutics, Inc.

Ethics statement

No ethical approval was required as this is a review article with no original research data.

Contribution

LR led the literature review and drafted the manuscript. EH searched available sources for the information which resulted in Table 1. All authors contributed information and participated in review of the final manuscript.

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