Chapter 7

Effects of food intake and fasting on the plasma ghrelin concentration in healthy dogs

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Abstract

The growth hormone (GH)-releasing peptide ghrelin, primarily secreted by the stomach, causes weight gain by increasing food intake and reducing fat utilization and seems to play a role in meal initiation in several mammalian species. In this study, blood samples were collected from 8.30 a.m. to 5 p.m. in nine healthy Beagle dogs when food was administered as usual at 10 a.m., after a 1-day fast, after a 3-day fast, and after re-feeding at 10 a.m. the next day. Circulating concentrations of acylated ghrelin, GH, insulin-like growth factor-I (IGF-I), glucose, and insulin were determined.

The overall mean plasma ghrelin concentrations were significantly lower when food was administered than after fasting. Shortly after feeding, the time-specific mean plasma ghrelin concentrations decreased, but this decline did not reach statistical significance. The overall mean plasma GH and IGF-I concentrations did not differ significantly between the 4 different periods. The time-specific mean plasma GH concentration differed significantly over time with a maximum concentration at 0 min. This concentration did not change differently over time in the 4 different periods. Circulating overall mean glucose and insulin concentrations were significantly higher after re-feeding the day after the 3-day fast compared with the 3 other periods. The time-specific mean plasma glucose and serum insulin concentrations differed significantly over time. In addition, the course of these concentrations over time differed significantly in the 4 sampling periods.

In conclusion, in dogs fasting and food intake are associated with higher and lower circulating ghrelin concentrations, respectively, suggesting that also in this species ghrelin participates in the control of feeding behavior and energy homeostasis. The changes in plasma ghrelin concentrations are not associated with similar changes in plasma GH concentrations, whereas insulin and glucose concentrations appear to change reciprocally with the ghrelin concentrations.

Introduction

In 1999, Kojima and co-workers reported the isolation of ghrelin from the rat stomach (Kojima et al., 1999). The presence of ghrelin in the stomach has been confirmed in humans, cows, pigs, dogs, and horses (Date et al., 2000; Hayashida et al., 2001; Tomasetto et al., 2001). Ghrelin acts as the ligand for the growth hormone secretagogue receptor type 1a (GHS-R 1a) and is able to stimulate growth hormone (GH) secretion from pituitary cells in many species such as rats, humans, goats, dogs, and fish (Kojima et al., 1999, Date et al., 2000; Hayashida et al., 2001, Bhatti et al., 2002; Kaiya et al., 2003; Bhatti et al., 2006 in press). The mature ghrelin molecule contains 28 amino acids and its third Serine residue is acylated by *n*-octanoic acid which is essential for binding to the GHS-R 1a, for the GH-releasing capacity of ghrelin, and most likely for its other endocrine actions (Kojima et al., 1999; Bednarek et al., 2000; Muccioli et al., 2001).

Through activation of pathways distinct from those leading to GH secretion, ghrelin also acts as a potent orexigenic peptide (Kamegai et al., 2000; Tschop et al., 2000; Wren et al., 2000; Wren et al., 2001). In rodents, ghrelin causes weight gain by increasing food intake and reducing fat utilization (Kamegai et al., 2000; Wren et al., 2000; Nakazato et al., 2001). Ghrelin is the only known circulating orexigen, and has a potency similar to that of neuropeptide Y (NPY). This appetite-stimulating effect appears to be mediated, at least in part, through activation of NPY/agouti gene-related peptide (AGRP) neurons in the hypothalamic arcuate nucleus, 94 % of which express the GHS-R (Willesen et al., 1999). NPY and AGRP are thought to be mediators of the ghrelin-induced increased food intake because antagonism of either NPY or AGRP signalling in the brain attenuates the orexigenic potency of injected ghrelin (Dickson et al., 1997; Kamegai et al., 2000; Asakawa et al., 2001; Shintani et al., 2001).

Ghrelin may also play a role in meal initiation in humans, since the concentration of this peptide increases immediately prior to a meal and decreases after eating (Cummings et al., 2001). Also in sheep and cows it has been demonstrated that preprandial ghrelin surges occur as many times per day as meals are provided (Sugino et al., 2002a,b; Miura et al., 2004). These results indicate that ghrelin secretion may be a trigger for endogenous appetite signals.

Consistent with this, circulating ghrelin concentrations are increased in anorexia and cachexia and reduced in obesity (Shiiya et al., 2002; Cummings and Shannon, 2003; Tolle et al., 2003). These changes are opposite to those induced by leptin, an adipocyte-derived

hormone that reduces appetite and increases energy expenditure in animal models. Thus both ghrelin and leptin reflect the metabolic balance and may drive neuroendocrine and metabolic responses to changes in nutritional status (Inui et al., 2001; Yoshihara et al., 2002; Cummings and Foster, 2003).

Ghrelin production in the stomach is not only stimulated by fasting but is also related to glucose and insulin metabolism. In humans, oral and intravenous glucose administration decreases plasma ghrelin concentrations, whereas lipids or high-fat diets suppress the postprandial ghrelin concentrations less effectively (Mohlig et al, 2002; Monteleone et al., 2003; Weigle et al., 2003). Moreover, Blom et al. (2005) have shown in humans that the ghrelin response to a high-carbohydrate meal is related to insulin secretion.

So far there are only a few reports (Jeusette et al., 2005a,b; Yokoyama et al., 2005) on the role of ghrelin in dogs, a carnivorous animal with an evolutionary background of being able to cope with long periods of starvation. However, nowadays the domestic dog does not need to endure starvation but rather food surplus, that often leads to obesity.

In this study we investigated the physiological endocrine effects of food intake and fasting in healthy Beagle dogs. Therefore, the circulating concentrations of ghrelin, GH, insulin-like growth factor-I (IGF-I), glucose, and insulin were measured when food was administered at the usual time, after a 1-day fast, after a 3-day fast, and after re-feeding the next day.

Materials and methods

Dogs

Nine normal-weight ovariohysterectomized Beagle dogs, 5 years of age, were used. They received a commercial dog food (Hill's Science Plan - Adult - Canine Maintenance®) at 10 a.m. each day and were given water *ad libitum*. The mean body weight of the dogs was 12 kg (range 9.5-14.0 kg). The dogs were accustomed to the laboratory environment and procedures such as collection of blood samples.

Study design and blood sample collection

The dogs were randomly assigned to three groups. For the second and third group the study started one and two days, respectively, after that of the first group. All dogs were put on a food-fast regimen for five consecutive days: on day 1, food was given as usual at 10 a.m.; on days 2, 3, and 4, food was withheld; and on day 5, the dogs were re-fed at 10 a.m.

Blood samples were collected by jugular venipuncture on days 1 (= period 1), 2 (= period 2), 4 (= period 3), and 5 (= period 4) at 8.30 a.m., 10 a.m., 10.30 a.m., 11 a.m., 11.30 a.m., 12.30 p.m., 1.30 p.m., 3 p.m., and 5 p.m. In these blood samples the concentrations of acylated ghrelin, GH, glucose, and insulin were determined. Plasma IGF-I concentrations were determined only in the blood samples collected at 8.30 a.m. and 10 a.m.

For the determination of plasma ghrelin concentrations, the collection procedures and storage were carried out in accordance with the protocol supplied by the manufacturer (Ghrelin (active) radioimmunoassay (RIA) kit, Linco Research, St Louis, MO, USA). Blood samples were immediately transferred to ice-chilled EDTA-coated tubes and centrifuged at 4° C for 10 min. Per one ml plasma, 50 µl of 1 N HCl and 10 µl phenylmethylsulfonyl fluoride was added. Plasma was immediately stored at -25° C until analysis.

For the determination of plasma GH and IGF-I concentrations, the blood samples were immediately transferred to ice-chilled EDTA-coated tubes and centrifuged at 4^{o} C for 10 min. Plasma was stored at -25^{o} C until assayed.

Blood samples for the measurement of plasma glucose concentrations were collected in sodium-fluoride tubes, and those for the measurement of serum insulin concentrations were collected in tubes with cloth activator.

Hormone determination

Plasma ghrelin concentrations were measured with a (human) ghrelin RIA kit (Linco Research, St Louis, MO, USA). This assay specifically measures biologically active ghrelin (Janssen et al., 2001). The RIA technique detects human ghrelin with equal accuracy in the dog (Yokoyama et al., 2005). Values of ghrelin measured with the human ghrelin RIA kit after serial dilutions of various dog samples showed good linearity, indicating that in this assay canine ghrelin behaves immunochemically similar to that of the human ghrelin standards. The sensitivity of the assay was 10 ng/l.

Plasma GH concentrations were measured using a commercially available RIA for porcine and canine GH (PGH-46HK; Linco Research, St. Charles MS). The intra-assay coefficient of variation value was 7.6 % at a plasma concentration of 4.4 µg/l. The sensitivity of the assay was 1 µg/l.

Total plasma IGF-I concentrations were measured after acid-ethanol extraction to remove interfering IGF binding proteins. Plasma IGF was extracted using a mixture of 87.5 % (v/v) ethanol and 12.5 % 2 M formic acid. Tubes containing 100 µl plasma and 400 µl of the ethanol-formic acid mixture were mixed thoroughly and incubated for 30 min at room

temperature. After centrifugation for 30 min at 5500 g at 4° C, a 50 µl aliquot of the supernatant was diluted 1:50 with assay buffer containing 63 mM Na_2HPO_4 (pH 7.4), 13 mM Na_2EDTA, and 0.25 % (w/v) BSA. The extraction efficiency amounted to 92.5 ± 5.7 %. Plasma IGF-I concentrations were measured with a heterologous RIA validated for the dog (Favier et al., 2001). The intra-assay coefficient of variation was 8.6 % at a plasma concentration of 100 µg/l. The sensitivity of the assay was 10 µg/l. IGF-I antiserum AFP4892898 and human IGF-I for iodination were obtained from the National Hormone and Peptide Programme (Harbor-UCLA Medical Center, Torrance CA).

Serum immunoreactive insulin concentrations were measured by immunoradiometric assay (IRMA). The two-site IRMA method (INS-Irma®: BioSource Europe S.A., Nivelles, Belgium) had an intra-assay and interassay coefficient of variation of 4.5 % and 4.7 %, respectively. According to information supplied by the manufacturer, there is 0 % cross-reactivity with human pro-insulin. Serial dilutions of canine serum were parallel to the standard curve of human insulin. The sensitivity of the assay was 7 pmol/l.

Statistical analysis

Changes in circulating concentrations of ghrelin, GH, glucose and insulin were analyzed based on a mixed model with dog as random effect (repeated measures ANOVA with compound symmetry structure) and period (1 to 4), blood sampling times (8.30 a.m., 10 a.m., 10.30 a.m., 11 a.m., 11.30 a.m., 12.30 p.m., 1.30 p.m., 3 p.m., and 5 p.m.), and their interaction as categorical fixed effects. In the analysis both mean concentrations over the different sampling points (denoted by overall mean concentration) and concentrations at different sampling points (denoted by time-specific mean concentration) were compared, with mean referring to the mean over the different dogs. The four periods were compared pair wise using Tukey's multiple comparisons technique at a global significance level of 5 %. Plasma IGF-I concentrations between the four periods were compared with one-way ANOVA. All values are expressed as mean ± SEM.

Analyses were performed with SAS version 9.1 for Windows (Insightful Corp., Seattle, US).

Ethics of the study

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Ghent University.

Results

The overall mean plasma ghrelin concentration when food was given at day 1 (152 ± 34 ng/l) was significantly (P = 0.005) lower than after a 1-day fast (181 ± 42 ng/l). The overall mean plasma ghrelin concentration after re-feeding the day after the 3-day fast (143 ± 32 ng/l) was significantly lower than after a 1-day fast (181 ± 42 ng/l, P = 0.001) and after a 3-day fast (183 ± 53 ng/l, P = 0.009) (Figure 1a and Figure 1b).

The time-specific mean plasma ghrelin concentrations decreased shortly after feeding, but this decline did not reach statistical significance. Additionally, the time-specific mean plasma ghrelin response did not change differently over time when the 4 periods were compared (Figure 1a). There was a considerable interindividual variation in the pre-food and post-food changes of the time-specific plasma ghrelin concentration (Figure 1c).

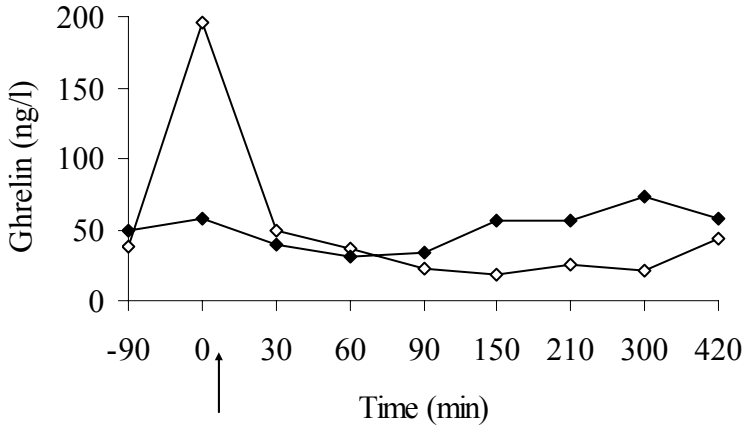

Figure 1c. Plasma ghrelin profiles in two Beagle dogs before (-90 and 0 min) and after (30, 60, 90, 150, 210, 300, 420 min) ingestion of a meal at 10 a.m (arrow). Note the heterogeneity in the rise before and the decline after food intake.

The overall mean plasma GH concentrations in the dogs when food was given at day 1, after a 1-day fast, after a 3-day fast, and after re-feeding the next day (1.7 ± 0.3 µg/l, 1.5 ± 0.2 µg/l, 1.4 ± 0.3 µg/l, and 1.5 ± 0.3, respectively) did not differ significantly (Figure 2). The time-specific mean plasma GH concentration differed significantly over time (P<0.0001) with a maximum concentration at 0 min. The time-specific mean plasma GH concentration did not change differently over time in the 4 different periods (Figure 2). No significant differences were found in the overall mean plasma IGF-I concentrations when the dogs were given food at day 1 (63 ± 9 µg/l), after a 1-day fast (60 ± 8 µg/l), after a 3-day fast (53 ± 7 µg/l), and after re-feeding the next day (47 ± 6 µg/l).

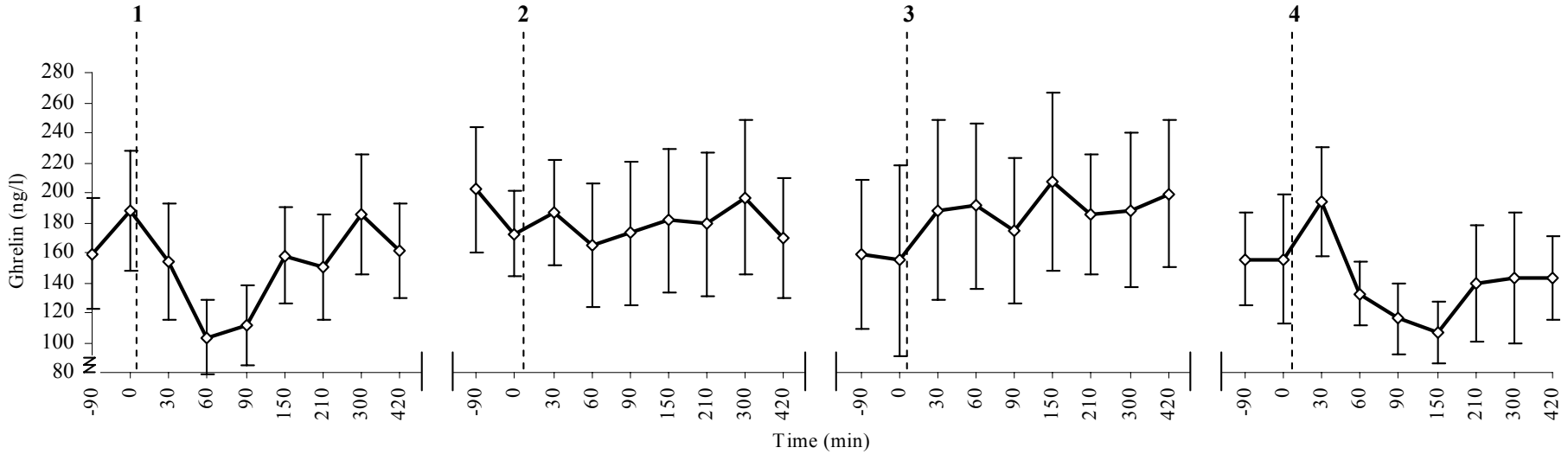

Figure 1a. Mean (± SEM) plasma ghrelin concentrations in 9 Beagle dogs before (-90 and 0 min) and after (30, 60, 90, 150, 210, 300, 420 min) ingestion of a meal at 10 a.m. **(1)**, after a consecutive 1-day fast **(2)**, after a 3-day fast **(3)**, and before and after ingestion of a meal at 10 a.m. the day after the 3-day fasting period **(4)**.

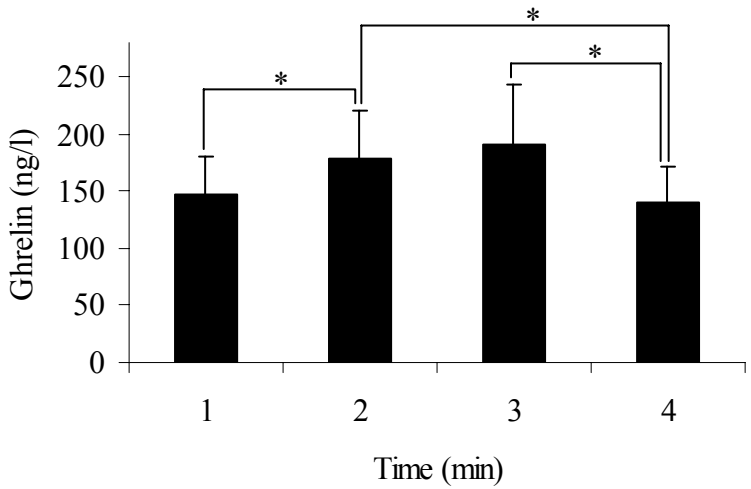

Figure 1b. Mean (+ SEM) plasma ghrelin concentration when a meal was given at 10 a.m. **(1)**, after a 1-day fast **(2)**, after a 3-day fast **(3)**, and when a meal was given again at 10 a.m. the day after the 3-day fasting period **(4)**. Significant differences between periods are indicated with an asterisk.

The overall mean plasma glucose concentrations when food was given at day 1 (4.5 ± 0.1 mmol/l), after a 1-day fast (4.6 ± 0.1 mmol/l), and after a 3-day fast (4.5 ± 0.1 mmol/l) were significantly lower ($P < 0.0001$) compared with this concentration after re-feeding the day after the 3-day fast (5.0 ± 0.2 mmol/l) (Figure 3).

The overall mean serum insulin concentrations when food was given at day 1 (28 ± 5 IU/l) and after re-feeding the day after the 3-day fast (27 ± 4 IU/l) were significantly higher ($P < 0.0001$) than after a 1-day fast (14 ± 2 IU/l) and after a 3-day fast (12 ± 2 IU/l) (Figure 4).

The time-specific mean plasma glucose and serum insulin concentrations differed significantly ($P<0.0001$) over time (Figures 3 and 4, respectively). In addition, the course of these concentrations over time differed significantly ($P<0.0001$) in the 4 sampling periods (Figures 3 and 4, respectively).

Discussion

The results of the present study demonstrate a significant difference in overall mean plasma ghrelin concentrations between food intake and fasting, characterized by lower plasma ghrelin concentrations when a meal was administered and higher plasma ghrelin concentrations during fasting. These findings are consistent with those for other species. For example, in rodents, circulating ghrelin concentrations increase with fasting and are suppressed within min by re-feeding or enteral infusions of nutrients but not water (Tschop et al., 2000; Asakawa et al., 2001). These observations suggest that the gastric peptide ghrelin may play an important role in controlling feeding behaviour and energy homeostasis.

The higher plasma ghrelin concentrations during fasting may be consistent with a physiological role for this hormone in increasing appetite and the initiation of food intake. Similar to the situation in rodents, circulating ghrelin concentrations in humans are rapidly suppressed by food intake, and 24-h plasma ghrelin profiles reveal marked preprandial increases and postprandial decreases associated with every meal (Cummings et al., 2001). Although meal-time hunger is a common, daily experience in humans and animals, the molecular determinants of this sensation remain incompletely understood. Several observations, mostly from animal studies, indicate that ghrelin contributes to the sensation of hunger and participates in meal initiation.

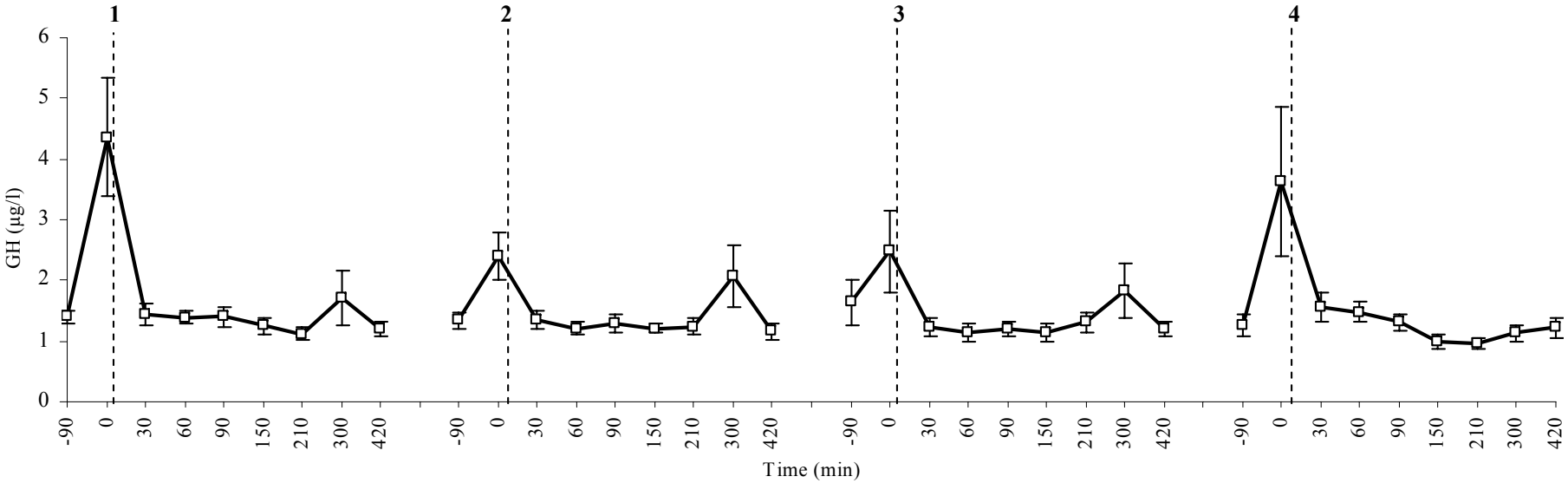

Figure 2. Mean (± SEM) plasma GH concentrations in 9 Beagle dogs before (-90 and 0 min) and after (30, 60, 90, 150, 210, 300, 420 min) ingestion of a meal at 10 a.m. **(1)**, after a consecutive 1-day fast **(2)**, after a 3-day fast **(3)**, and before and after ingestion of a meal at 10 a.m. the day after the 3-day fasting period **(4)**.

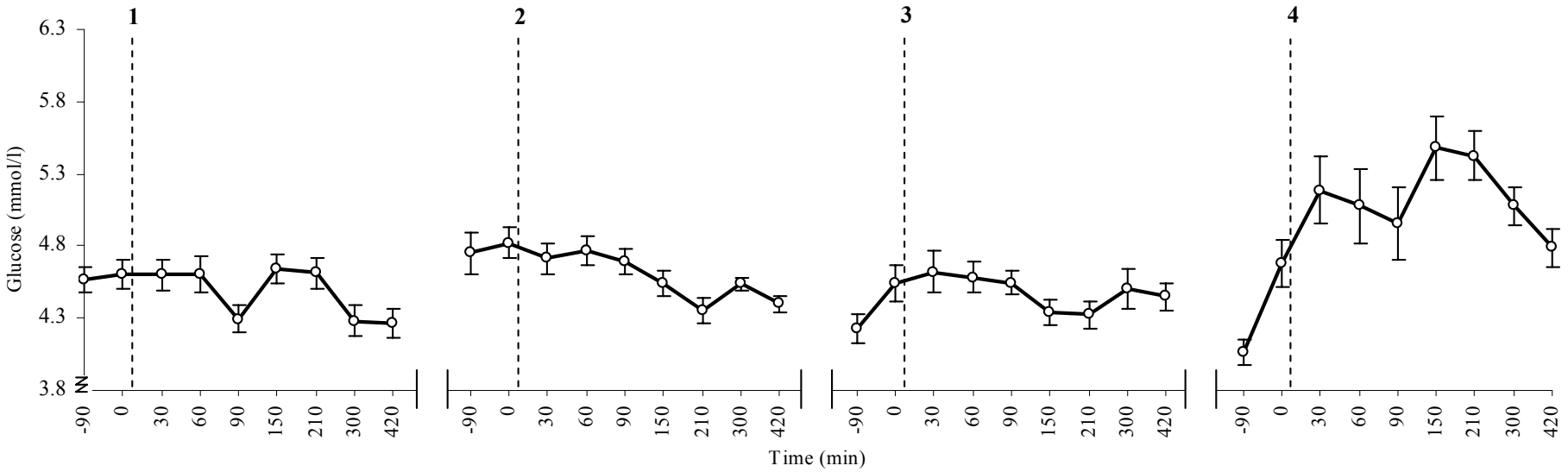

Figure 3. Mean (± SEM) plasma concentrations of glucose in 9 Beagle dogs before (-90 and 0 min) and after (30, 60, 90, 150, 210, 300, 420 min) ingestion of a meal at 10 a.m. **(1)**, after a consecutive 1-day fast **(2)**, after a 3-day fast **(3)**, and before and after ingestion of a meal at 10 a.m. the day after the 3-day fasting period **(4)**.

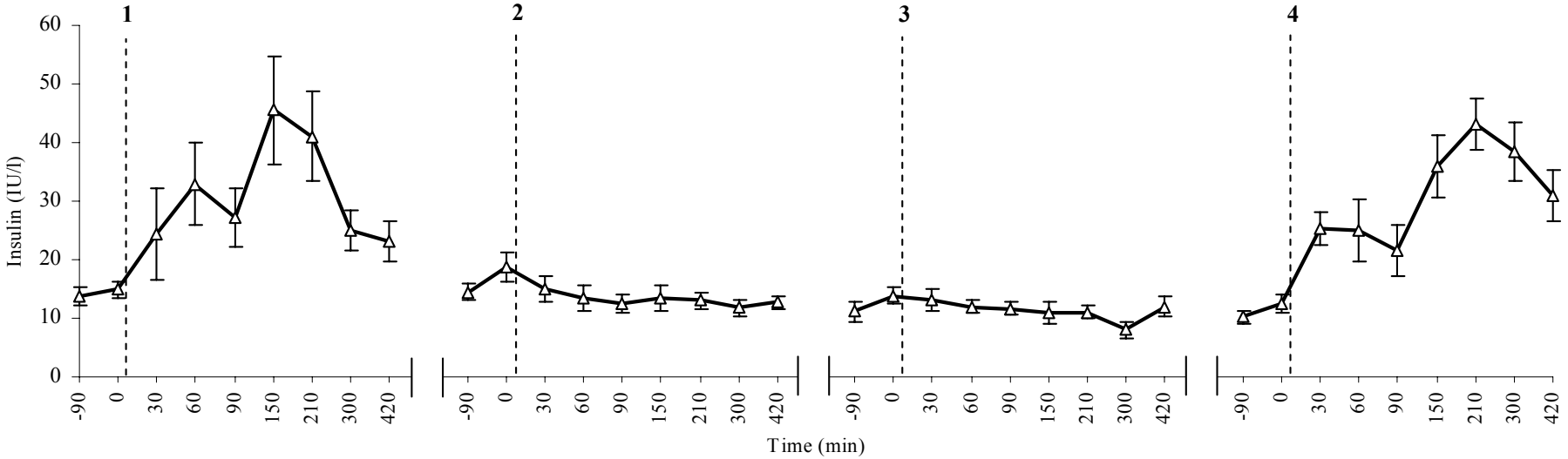

Figure 4. Mean (± SEM) serum concentrations of insulin in 9 Beagle dogs before (-90 and 0 min) and after (30, 60, 90, 150, 210, 300, 420 min) ingestion of a meal at 10 a.m. **(1)**, after a consecutive 1-day fast **(2)**, after a 3-day fast **(3)**, and before and after ingestion of a meal at 10 a.m. the day after the 3-day fasting period **(4)**.

Firstly, the majority of circulating ghrelin is produced by the stomach and duodenum, organs well positioned to detect recently ingested food (Kojima et al., 1999; Gnanapavan et al., 2002; Date et al., 2000). Secondly, despite being produced peripherally, ghrelin acts centrally to stimulate food intake (Tschop et al., 2000; Nakazato et al., 2001; Wren et al., 2000). Thirdly, the orexigenic actions of ghrelin are rapid and short-lived, increasing both food intake (Asakawa et al., 2001) and gastric acid secretion (Masuda et al., 2000) within 20 min after intraperitoneal injection, a time course that is consistent with a role in meal initiation. Fourthly, exogenous ghrelin triggers eating when administered at times of minimal spontaneous food intake (Nakazato et al., 2001; Wren et al., 2000). Finally, the most clearly documented targets of ghrelin action in the brain are the hypothalamic neurons that co-secrete the well-known orexigens, NPY and AGRP (Cummings and Shannon, 2003). These neuropeptides are implicated in the central regulation of meal initiation as their expression increases at times of maximal spontaneous food intake in rodents, whereas that of other neuropeptides involved in energy balance remain relatively constant throughout the day (Lu et al., 2002).

With regard to the plasma ghrelin concentration in the present study, neither a significant time effect, nor a significant interaction between time and the four different periods was observed. Nevertheless, at day 1 the highest time-specific plasma ghrelin concentrations were observed immediately before feeding. It is possible that this insignificant preprandial rise occurred as an anticipatory response to feeding because the dogs had been fed at the same time of the day for several years. That a psychological factor might have played a role in the present study is further supported by the observation that, after a few days of fasting, the highest time-specific plasma ghrelin level was observed not before food administration but immediately after the "unexpected" administration of food on day 5. In line with this assumption, Sugino et al. (2002a) demonstrated that psychological factors, i.e. an expectation of food administration, may stimulate ghrelin secretion just before feeding in sheep. This transient increase in ghrelin secretion just before feeding may be part of a conditioned emotional response. It is well known that secretion of saliva and gastric acid preceding food intake is induced by a conditioned emotional response through the stimulation of the vagal nerve (Harding and Leek, 1973). In this respect, ghrelin secretion may be induced by the vagal neural system in the same manner as the secretion of saliva and gastric acid.

Like in humans and rodents, also in our dogs the time-specific mean plasma ghrelin concentrations decreased shortly after food intake, but this decline did not reach statistical significance. However, in another study it was demonstrated that also in dogs circulating

plasma ghrelin concentrations decrease significantly after eating (Yokoyama et al., 2005). The results of the present study do show that feeding results in a significantly lower overall mean plasma ghrelin concentration compared to fasting in dogs. The mechanism by which nutrients suppress ghrelin concentrations are beginning to be elucidated. Absorbed nutrients are thought to be the most likely mediators of the postprandial decrease in plasma ghrelin concentrations in rodents (Tschop et al., 2000). Ingested nutrients suppress ghrelin release in rats and humans with an efficacy of carbohydrates > proteins > lipids (Overduin et al., 2005). Surprisingly, food-related ghrelin suppression does not require luminal nutrient exposure in the stomach or duodenum, the principal sites of ghrelin production (Williams et al., 2003a; Overduin et al., 2005). Instead, signals mediating this response originate further downstream in the intestines and from post-absorptive events. In addition to nutrients, also changes in plasma insulin concentrations, intestinal osmolarity, and enteric neural signalling probably play a role, whereas gastric distension, vagal nerve activity, and glucagon-like peptide-1 are not required (Williams et al., 2003b; Gelling et al., 2004).

There was considerable interindividual variation in time-specific mean plasma ghrelin concentrations in our dogs, as has been reported previously in humans (Cummings et al., 2001; Janssen et al., 2001). Interestingly, a strong correlation in plasma ghrelin concentrations has been found within one individual when samples are collected with an interval of 1 year (Janssen et al., 2001). This suggests that the variation in plasma ghrelin concentrations is mainly determined by the variation between individuals and less by intra-individual factors. Also, a large heterogeneity was found in the pre-food surge and post-food decline between dogs, indicating that not only basal ghrelin concentrations but also the ghrelin responses have a strong individual variation.

Time-specific mean plasma GH concentrations increased just before the time when feeding normally occurred. Also in cows a single GH surge during feeding has been demonstrated (Gaynor et al., 1995). The two principal hypothalamic regulators of GH secretion, GHRH and somatostatin, do not seem to be responsible for the rise in circulating GH concentrations around feeding (McMahon et al., 2000). Because ghrelin is also a potent GH-releasing peptide, it can be hypothesized that a preprandial rise in circulating ghrelin concentrations may be responsible for the preprandially increased GH secretion. Indeed, some studies in humans (Cummings et al., 2001), goats, and sheep (Sugino et al., 2002a,b) have demonstrated that a preprandial rise in plasma ghrelin concentration is associated with a GH surge. The results of the present study, however, do not provide evidence for such a relationship in dogs, because the significant preprandial GH surge was not associated with a

significant preprandial ghrelin surge. Moreover, in contrast to the overall mean plasma ghrelin profiles the overall mean plasma GH profiles did not differ significantly in the fed compared to the fasted state. In addition, the overall mean plasma IGF-I concentrations did not differ between the several food-fast regimens. Similar to the situation in our dogs, a link between plasma ghrelin concentrations and plasma GH concentrations could not be demonstrated in cows (Miura et al., 2004).

The overall mean plasma profiles of ghrelin on the one hand and the overall mean profiles of insulin and glucose on the other hand changed reciprocally after feeding and fasting in our dogs. While the overall mean plasma ghrelin concentrations were significantly lower after feeding than after fasting, the opposite was true for the overall mean serum insulin concentrations. These findings are in agreement with a study in humans, in which plasma ghrelin concentrations changed oppositely to circulating insulin concentrations (Cummings et al., 2001). The observation that both ghrelin and insulin are involved in the physiological response to food intake as well as in body weight regulation and that they display reciprocal 24-h profiles (Cummings et al., 2001), raises the question whether insulin negatively regulates ghrelin or *vice versa*. The former hypothesis has been investigated by many groups (Saad et al., 2002; Flanagan et al., 2003; Kamegai et al., 2004). Taken together, these studies demonstrated that while insulin can suppress ghrelin release when administered in supraphysiologic doses or at high-normal concentrations for prolonged periods of time, physiological concentrations of insulin do not appear to regulate ghrelin release (Caixas et al., 2002; Schaller, et al., 2003; Soriano-Guillen et al., 2004). It has also been suggested that ghrelin may act as a counter-regulatory hormone blocking insulin secretion and insulin action to maintain blood glucose concentrations (Broglio et al., 2001; Cummings et al., 2005). Indeed, several studies have shown that ghrelin can inhibit glucose-mediated insulin secretion, both *in vitro* and *in vivo* (Egido, et al., 2002; Reimer, et al., 2003; Colombo, et al., 2003). Similarly, exogenous ghrelin administration decreases circulating insulin concentrations in mice (Reimer, et al., 2003) and humans (Broglio et al., 2003). Recently, a novel ghrelin-producing pancreatic islet cell type has been identified, the ε-cell (Wierup et al., 2004). These cells are derived from the same progenitors as are the four classical islet cell types (producing insulin, glucagon, somatostatin, and pancreatic polypeptide), and can replace the other islet cells when the latter are eliminated by for example genetic deletion of vital transcription factors. Because ghrelin is highly expressed in the fetal pancreas (6-7 times more than in the stomach) (Chanoine and Wong, 2004), it may participate in pancreatic islet development. Preliminary evidence also suggests that ghrelin has paracrine effects on insulin

secretion in adults. If ghrelin derived from the pancreatic islets, rather than from the gastrointestinal tract, is the more critical regulator of insulin release, this raises the interesting possibility of an intra-islet ghrelin/insulin glucoregulatory axis.

In conclusion, in dogs, fasting and food intake are associated with higher and lower circulating ghrelin concentrations, respectively, suggesting that also in this species ghrelin participates in the control of feeding behaviour and energy homeostasis. The changes in plasma ghrelin concentrations are not associated with similar changes in plasma GH concentrations, whereas circulating insulin and glucose concentrations appear to change reciprocally with the ghrelin concentration.

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