0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(11):5510–5514

doi: 10.1210/jc.2003-030797

Differential Responses of Circulating Ghrelin to High-Fat or High-Carbohydrate Meal in Healthy Women

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The effects of specific nutritional factors on ghrelin secretion have not been investigated in humans. Therefore, we assessed ghrelin responses to a high-carbohydrate meal (1217 kcal with 77% carbohydrates, 10% protein, and 13% lipids) and to an isocaloric high-fat meal (15% carbohydrates, 10% proteins, and 75% lipids) in 14 nonobese healthy women. Eleven subjects also rated their hunger feelings on visual analog scales. Circulating ghrelin abruptly fell after both meals, but, after the carbohydrate meal, its maximum percent decrease was significantly greater than after the fat meal ($P = 0.02$). Plasma insulin and glucose levels rose after the meals, but their increases were significantly higher after the carbohydrate meal than after the fat meal. No significant change was observed in circulating leptin after both meals. Moreover, compared with the fat meal, the carbohydrate meal had a significantly greater suppressant effect on hunger feelings. Plasma ghrelin changes were significantly associated with hunger changes ($P < 0.007$). These findings show that circulating ghrelin is differently suppressed by diet manipulations. The mechanisms responsible for such a phenomenon and its possible implication in the physiology of human satiety remain to be elucidated. (*J Clin Endocrinol Metab* 88: 5510–5514, 2003)

GHRELIN IS A recently identified peripheral peptide produced by enteroendocrine cells within the mucosal epithelial layer of the stomach. It was initially characterized as an endogenous ligand for the GH secretagogue receptor and, similarly to synthetic agonists of this receptor, stimulates pituitary GH release in both rats and humans (1–3). However, besides potent GH-releasing actions, ghrelin has other remarkable activities, including stimulation of lactotroph and corticotroph secretion, influence on sleep, control of gastric motility and acid secretion, cardiovascular effects, antiproliferative actions on neoplastic cell lines, and orectic activity coupled with control of energy balance (4).

Recent studies suggest that this peptide may provide a peripheral signal to the central nervous system to stimulate food intake and increase adiposity in both rats and humans. In rodents, intracerebroventricular or peripheral administration of ghrelin induces food intake, increases hypothalamic expression of neuropeptide Y (NPY) (a potent stimulator of feeding) and causes weight gain (5–8). Similarly, in humans, iv administration of ghrelin enhances appetite and increases food consumption (9). The ghrelin's effects on food intake are independent of GH secretion and appear to be mediated via either the agouti-related protein and the melanocortin receptor pathways and the activation of the hypothalamic NPY/NPY-1 receptor pathway. In particular, it has been shown that, in the rat, the satiety-reducing effect of ghrelin is related to the antagonism of the inhibitory effect of the adipocyte-derived hormone leptin on hypothalamic NPY production (6). This raises the possibility that ghrelin and leptin are part of a peripheral dynamic feedback system that regulates body weight (BW) and energy homeostasis by modulating satiety.

Abbreviations: BW, Body weight; CV, coefficients of variation; Δmax, maximum changes; NPY, neuropeptide Y.

Evidence has been provided that plasma ghrelin levels are influenced by acute and chronic changes in the energy balance. For instance, in rodents, plasma ghrelin levels increases upon fasting and return to baseline after intragastric administration of nutrients (8). Similarly, in humans, plasma ghrelin concentrations increase after fasting and decreases after habitual feeding (9–12), suggesting that this peptide reflects the acute nutritional state of the organism and may also serve as an indicator of short-term energy imbalance. Furthermore, it has been shown that plasma ghrelin rises nearly 2-fold shortly before each meal and falls to minimum levels within 1 h after eating, a profile that is consistent with a physiological role of ghrelin in both initiating individual meals and regulating their size (13).

Ingested nutrients are the most likely mediator of the rapid postprandial fall in circulating ghrelin, although it is not yet clear whether the presence of nutrients in the stomach and in the gut or the metabolic response to food ingestion is the major determinant of ghrelin fall after eating (14, 15). Moreover, no study has been performed to investigate whether the ghrelin response to oral feeding is influenced by specific nutritional factors. Therefore, the aim of the present study was to test whether the macronutrient content of the meals influence postprandial ghrelin secretion. To this purpose, we assessed in healthy subjects the ghrelin responses to two isoenergetic meals with different amounts of carbohydrates and lipids but similar amounts of proteins. Plasma concentrations of glucose, insulin, and leptin were also measured because these substances have been claimed to modulate ghrelin secretion in response to food intake (16–18).

Subjects and Methods

Subjects

Fourteen women, with a mean (±SD) age of 24.5 (±2.6) yr, were recruited for the study. They menstruated regularly, had normal exercise

and drinking habits, and a stable BW in the 6 months preceding the study, without food restriction or dieting or other abnormal eating behaviors, as ascertained by a clinical interview. Subjects with a body mass index above 27 kg/m^2 and/or antecedent of obesity were excluded from the study. Their mean (±SD) BW and body mass index were 60.0 (±6.1) kg and 21.5 (± 1.8) kg/m^2, respectively. All women were drug free for at least 6 wk prior to entering the study, had normal physical examinations, normal values of routine blood and urine tests, and a normal electrocardiogram. None of them was taking oral contraceptives or had a past history of alcohol or drug abuse.

Protocol

The experimental protocol was approved by the local ethical committee, and all subjects gave their written consent after being fully informed of the nature and procedures of the study.

The experiment used a within-subject repeated measure design in which each woman served as her own control. All subjects were tested two times, 1 month apart, in the follicular phases of two consecutive menstrual cycles (d 5–10 from menses). On the first occasion, they arrived at our Clinical Investigation Unit at 0800 h after a 12-h fast. At 0815 h, they received a breakfast of 200 kcal, with 77% carbohydrates, 10% proteins, and 13% fat. At 1145 h, an iv catheter was inserted into an antecubital vein and connected to a saline solution, which was slowly infused to keep the catheter patent. At 1200 h, a lunch of 1217 kcal (with 77% carbohydrates, 10% proteins, and 13% fat) was served. Blood samples were drawn immediately before (time zero) and 45, 60, 90, 120, and 180 min after the lunch. On the second occasion, subjects underwent the same experimental procedures already described but received a breakfast of 200 kcal with 15% carbohydrates, 10% proteins, and 75% fat and a lunch of 1217 kcal with 15% carbohydrates, 10% proteins, and 75% fat. The order of the high-carbohydrate and high-fat meals was randomized. Blood was collected in tubes with lithium heparin as anticoagulant. Plasma was separated by centrifugation and stored at −20 C.

Eleven subjects rated their hunger on visual analog scales that used a 10-cm line with labels at the extremities indicating the most negative (not at all hungry) and the most positive (extremely hungry) ratings. Subjects completed baseline ratings before the noon meal and then at the scheduled time points of blood withdrawals.

Biochemical analyses

Plasma ghrelin was measured by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA). The sensitivity of the method was 1 pg/tube; intra- and interassay coefficients of variation (CV) were less than 5.3 and 13.6%, respectively. Plasma insulin was determined by an immunoradiometric method, using a commercial kit purchased from Biochem Immunosystem (Milan, Italy). The lower detection limit was 2.1 pmol/liter; intra- and interassay CV were 2.6 and 4.7%, respectively. Plasma leptin values were determined by a sandwich ELISA, using a commercial kit purchased from Alexis Biochemicals (Laufelfingen, Switzerland). The sensitivity of the method was 0.2 ng/ml; the intra- and interassay CV were 6.1 and 8.5%, respectively.

Plasma glucose was determined by a commercial enzymatic UV method (Sigma Diagnostics, St. Louis, MO).

For each hormone, all of a single subject's samples were run in the same assay, and the order of tubes in the assay was randomized.

Statistics

The BMDP statistical software package (University of California, Berkeley, CA) (19) was used for data analysis.

Differences in both the hormone and hunger responses to the two isoenergetic meals were analyzed by two-way ANOVA with repeated measures, followed by the *post hoc* Tukey's test. One-way ANOVA and analysis of covariance were employed to analyze differences in the maximum decreases in plasma ghrelin after the two test meals. The Pearson's product moment correlation test was employed to analyze possible correlations among the variables. A stepwise multiple regression was used to test the effects of biochemical indices on the variability of hunger feelings after the two meals. A level of significance of $P < 0.05$ was used for all data analyses.

Results

Plasma ghrelin

The meal × time repeated measures ANOVA yielded a significant main effect for time ($F_{5,130} = 13.04$; $P < 0.0001$) and a significant meal × time interaction ($F_{5,130} = 2.88$; $P = 0.01$). These data indicate that circulating ghrelin changed significantly across sampling times and significantly differed over the samplings after the two test meals. Indeed, mean plasma ghrelin levels significantly dropped after both meals, but the drop after the carbohydrate meal was greater than that observed after the fat meal (Fig. 1, *left panel*). In each subject, when the lowest value of plasma ghrelin observed at any time point after meal consumption was expressed as a percentage of the preprandial (time zero) value (maximum post-

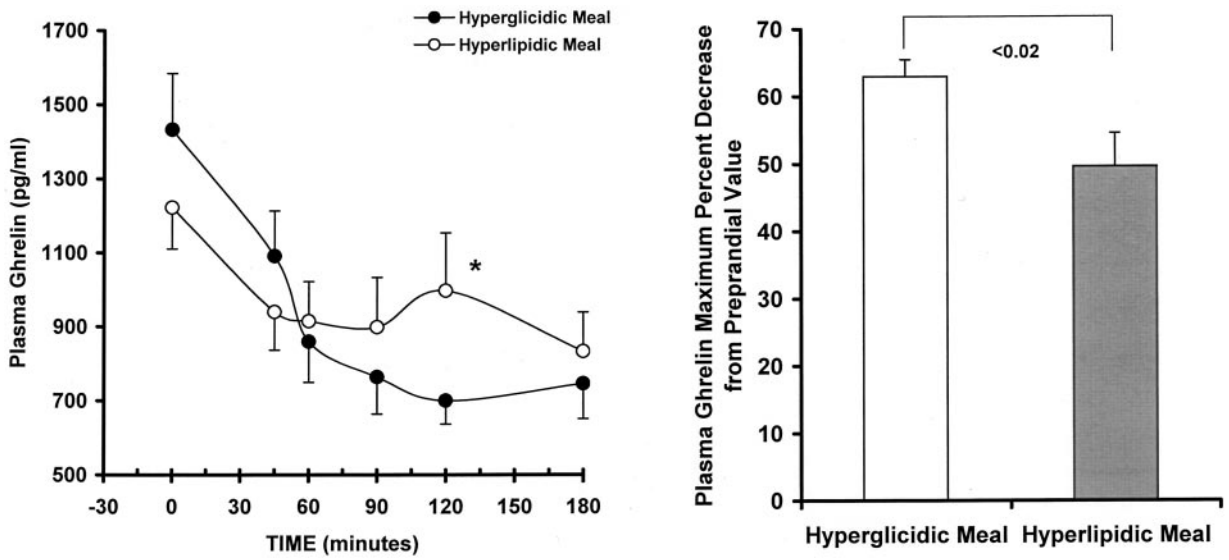

FIG. 1. Plasma ghrelin responses to a high-carbohydrate or a high-fat meal in healthy women. *Left panel*, Absolute values; *right panel*, maximum percentage of changes from preprandial values. Data are expressed as mean ± SEM. *, $P < 0.02$ *vs.* hyperlipidic meal (*post hoc* Tukey's test).

prandial decrease), one-way ANOVA showed that the mean maximum percent decrease in plasma ghrelin after a carbohydrate meal was significantly greater than after a fat meal (63.0 ± 9.3 *vs.* 49.7 ± 18.4%; $F_{1, 26} = 5.774$; $P = 0.02$) (Fig. 1, *right panel*). This difference was not affected by preprandial values of plasma ghrelin, as ascertained by the analysis of covariance ($F_{1,25} = 4.68$; $P = 0.04$).

Plasma leptin

The meal × time repeated measures ANOVA yielded a slight significant effect for time ($F_{5,130} = 2.38$; $P = 0.04$) but no significant meal × time interaction ($F_{5,130} = 0.69$; $P = 0.6$). These data indicate that circulating leptin did not change significantly across sampling times after the two different meals (Fig. 2, *upper panel*).

Plasma insulin

Data concerning circulating insulin in four subjects after the fat meal were not available because of technical drawbacks.

The meal × time repeated measures ANOVA yielded a significant effect for time ($F_{5,110} = 23.33$; $P < 0.0001$) for meal ($F_{1,22} = 12.66$; $P < 0.002$) and a significant meal × time interaction ($F_{5,110} = 3.80$; $P = 0.003$). These data indicate that circulating insulin changed significantly across sampling times and that, in the two test conditions, they displayed significant differences over the samplings. In fact, plasma insulin levels promptly increased after both types of meals and, at the end of the study period, were still higher than preprandial values. However, after the carbohydrate meal, circulating insulin was significantly higher than after the fat meal at all the time points (Fig. 2, *middle panel*).

Plasma glucose

The meal × time repeated measures ANOVA yielded a significant effect for time ($F_{5,130} = 14.65$; $P < 0.0001$) and for meal ($F_{1, 26} = 7.65$; $P = 0.01$) but no significant meal × time interaction ($F_{5, 130} = 1.53$; $P = 0.1$). These data indicate that circulating glucose showed similar changes across sampling times after the two different meals, but the magnitude of these changes statistically differed. In fact, in both conditions, plasma glucose levels promptly increased after the meal and then returned to preprandial values at the end of the study period. However, after the carbohydrate meal, circulating glucose was significantly higher than after the fat meal at 45-, 60-, and 90-min time points (Fig. 2, *bottom panel*).

Hunger

The meal × time repeated measures ANOVA yielded a significant main effect for time ($F_{5,100} = 22.83$; $P < 0.0001$) and a significant meal × time interaction ($F_{5,100} = 3.18$; $P = 0.01$). These data indicate that hunger changed significantly across the time and significantly differed over time in the two test conditions. Indeed, hunger ratings significantly decreased after both meals; however, 120 min after the fat meal, they already returned to baseline values, whereas after the carbohydrate meal, they were still significantly decreased at the end of the observation period (Fig 3).

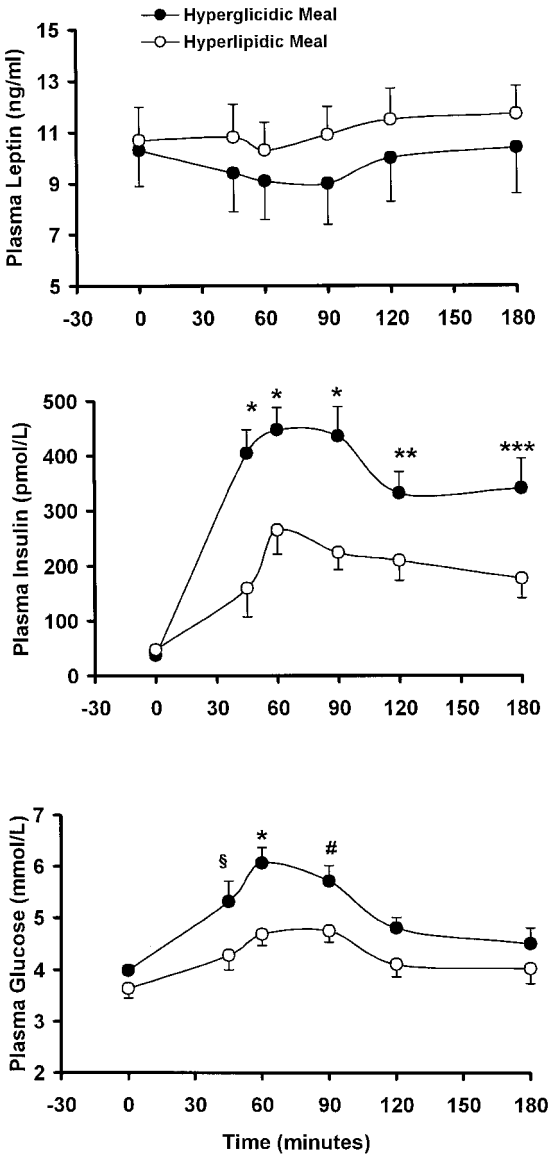

FIG. 2. Plasma leptin (*top panel*), insulin (*middle panel*) and glucose (*bottom panel*) responses to a high-carbohydrate or a high-fat meal in healthy women. Data are expressed as mean ± SEM. *, $P < 0.001$; **, $P < 0.05$; ***, $P < 0.005$; §, $P < 0.01$; #, $P < 0.02$ *vs.* hyperlipidic meal (*post hoc* Tukey's test).

Correlations

The areas under the curve from time zero to 180 min were calculated for leptin, glucose, and insulin. The Pearson's correlation test did not show any significant correlation between the area under the curve of leptin, glucose, or insulin and the maximum percent decreases in circulating ghrelin after both carbohydrate and fat meals.

To assess whether hunger was related to circulating ghrelin, glucose, or insulin, maximum changes (Δmax) from baseline values in both hunger scores, plasma ghrelin, insulin, and glucose levels were calculated for each subject in each experimental condition, and a stepwise multiple regression with Δmax hunger as the dependent variable and Δmax ghrelin, insulin, and glucose as independent variables

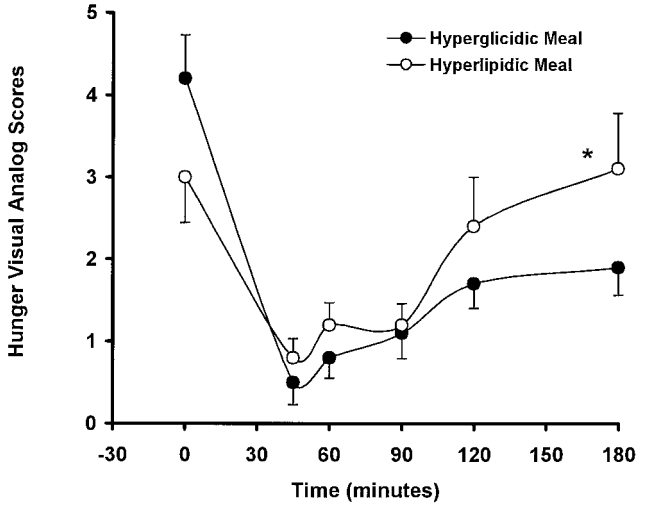

FIG. 3. Ratings of hunger according to time after the ingestion of a high-carbohydrate or a high-fat meal in healthy women. Data are expressed as mean ± SEM. *, $P < 0.02$ *vs.* hyperglicidic meal (*post hoc* Tukey's test).

was run. Plasma ghrelin changes were significantly associated with hunger changes ($F_{1,20} = 16.73$; $P < 0.007$; $R^2 = 0.45$). After removing this effect, no further significant associations emerged between hunger Δmax and both insulin and glucose Δmax.

Discussion

This study was designed to test whether meals differing in their macronutrient composition but with similar energy amounts differently affect postprandial ghrelin secretion in nonobese healthy women. The major findings were: 1) a carbohydrate meal induced greater postprandial suppressions in circulating ghrelin and hunger feelings than an isoenergetic fat meal; 2) the ghrelin response to both fat and carbohydrate meal was not correlated to physiological postprandial changes in circulating leptin, insulin, and glucose; and 3) a significant positive association occurred between postprandial changes in circulating ghrelin and hunger sensation.

In line with previous studies (10, 11, 20), present findings show that circulating ghrelin considerably falls after the ingestion of food, supporting a role for this peptide as a peripheral sensor of short-term positive energy imbalance. Moreover, they provide evidence that the postprandial fall of plasma ghrelin concentrations is higher after the consumption of a carbohydrate meal than after the ingestion of an isoenergetic fat meal. Consistent with our results, Weigle *et al.* (21) very recently reported that, in healthy individuals, absolute and percent meal-induced decreases of plasma ghrelin concentrations were higher during a 2-wk high-carbohydrate diet than during a 2-wk isocaloric moderate-fat diet. Therefore, these data altogether support the view that the nutrient composition of the diet influences circulating ghrelin levels in healthy subjects.

It has been suggested that the metabolic response to food ingestion, in terms of glucose and insulin changes, is the major determinant of the food-induced ghrelin suppression, because both glucose and insulin have a suppressant action on plasma ghrelin levels (13, 16, 17). Although in our study plasma glucose and insulin concentrations were higher after carbohydrate ingestion than after the fat meal, suggesting stronger suppressant signals for ghrelin in the first condition, we could not detect any significant relationship between the ghrelin responses (expressed as the maximum percent decreases from preprandial values) and both glucose and insulin modifications in the two test conditions. These findings are not in line with those of a previous study showing a positive correlation between postprandial changes of circulating ghrelin and the output of serum glucose (15). However, that investigation is not fully comparable to our study because of differences in the meal composition, the total amount of ingested calories, the gender of subjects, and the timing of blood sampling.

Moreover, it has been shown that the immediate suppressant effect of oral food on circulating ghrelin in humans cannot be reproduced by the combined iv and sc administration of glucose and insulin (15) or by iv administration of lipids (22), or by simple stomach expansion, as obtained by oral water load (14), whereas it can be induced by nonnutritive fiber ingestion (23) or by euglycemic hyperinsulinemia (22). All these data point to the uncertainty as to whether the true cause of meal-induced ghrelin suppression is the metabolic response, mechanical factors, the actual presence of nutrients in the gut, the food-induced taste stimulation, or the release of enteric hormones other than those investigated in this study. As a consequence, at present, it is not possible to define the precise mechanisms responsible for the reported different suppressant action of the high-carbohydrate and of the high-fat meal on circulating ghrelin in healthy women.

We also found that, as compared with a fat meal, the carbohydrate meal had a greater suppressing effect on the subjective hunger sensation. This is in line with the reported demonstration that oral carbohydrates have a greater satiating effect than lipids (24, 25). Moreover, in our subjects, we detected a significant association between meal-induced changes in plasma ghrelin and hunger feelings, with changes in plasma ghrelin being able to explain the 45% of the variance of hunger sensation. This finding seems to support the idea that circulating ghrelin, through its differential responses to diet manipulations, could be one of the factors involved in the mediation of the diverse satiating power of nutrients. However, in our experimental design, the subjects were not blind to the time of the day; therefore, we cannot exclude that appetite scores recorded on visual analog scales were determined as much by the passage of time as by plasma ghrelin levels. Further studies need to address this issue.

Some limitations of this study need to be discussed. First of all, we included only female subjects; hence, gender differences in the ghrelin responses to diet manipulations could have been missed. Secondly, we had no "sham-feeding" control condition, so we cannot exclude that the decrease of ghrelin after the meals may be due to the effects of a diurnal rhythm rather than the actual ingestion of nutrients. However, in our experimental design, each woman served as her own control, so it seems unlikely that the different decrease in circulating ghrelin observed after the two test meals could

be linked exclusively to diurnal oscillations of the hormone levels. Moreover, the validity of self-reporting visual analog scales in rating hunger sensation has been questioned. It cannot be automatically assumed that changes in self-reported hunger ratings actually reflect a decrease or increase in hunger, because factors other than the hunger sensation, such as the palatability of food or feelings of nausea, may influence this rating. Finally, we limited our observation to the third hour after the food ingestion, when circulating ghrelin still appears to be decreasing; therefore, we cannot exclude that ghrelin suppression after the high-fat meal could have been greater if the observation period had been longer.

In conclusion, we demonstrated that, in humans, circulating ghrelin is affected by the nutrient composition of the diet, because its decrease after a high-carbohydrate meal is greater than after a high-fat meal. The mechanisms responsible for such a phenomenon and its possible implication in the physiology of satiety remain to be determined.

Acknowledgments

Received May 5, 2003. Accepted July 21, 2003.

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References

1. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
2. **Hosoda H, Kojima M, Matsuo H, Kangawa K** 2000 Purification and characterization of rat des-Gln^{14}-ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. J Biol Chem 275:21995–22000
3. **Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K** 2000 Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 85:4908–4911
4. **Muccioli G, Tschop M, Papotti M, Deghenghi R, Heiman M, Ghigo E** 2002 Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. Eur J Pharmacol 440:235–254
5. **Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S** 2001 A role for ghrelin in the central regulation of feeding. Nature 409:194–198
6. **Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K** 2001 Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50:227–232
7. **Wren AM, Small CJ, Ward HL, Murphy KJ, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Moroan DGA, Ghatei MA, Bloom SR** 2000 The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141:4325–4329
8. **Tschöp M, Smiley DL, Heiman ML** 2000 Ghrelin induces adiposity in rodents. Nature 407:908–913
9. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KJ, Dhillo WS, Ghatei MA, Bloom SR** 2001 Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992–5995
10. **Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K** 2001 Stomach is a major source of circulating ghrelin and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 86:4573–4578
11. **Muller AF, Lamberts SWJ, Janssen JAMJL, Hofland L, Van Koetsveld P, Bidlingmaier M, Strasburger CJ, Ghigo E, Van der Lely AJ** 2002 Ghrelin drives GH secretion during fasting in man. Eur J Endocrinol 146:203–207
12. **English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JPH** 2002 Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab 87:2984–2987
13. **Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS** 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719
14. **Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe SI, Hosoda H, Kangawa K, Matsukura S** 2002 Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 87:240–244
15. **Caixàs A, Bashore C, Nash W, Pi-Sunyer F, Laferrère B** 2002 Insulin, unlike food intake, does not suppress ghrelin in human subjects. J Clin Endocrinol Metab 87:1902–1906
16. **Nakagawa E, Nagaya N, Okumura H, Enomoto M, Oya H, Ono F, Hosoda H, Kojima M, Kangawa K** 2002 Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: responses to the intravenous and oral administration of glucose. Clin Sci (Lond) 103:325–328
17. **Saad MF, Bernaba B, Hwu CM, Jinagouda S, Fahmi S, Kogosov E, Boyadjian R** 2002 Insulin regulates plasma ghrelin concentration. J Clin Endocrinol Metab 87:3997–4000
18. **Flanagan DE, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV, Sherwin RS** 2003 The influence of insulin on circulating ghrelin. Am J Physiol (Endocrinol Metab) 284:E313–E316
19. **Dixon J** 1985 BMDP Statistical software. Berkeley, CA: University of California Press
20. **Monteleone P, Martiadis V, Fabrazzo M, Serritella C, Maj M** 2003 Ghrelin and leptin responses to food ingestion in bulimia nervosa: implications for binge-eating and compensatory behaviours. Psychol Med 33:1–8
21. **Weigle DS, Cummings DE, Newby PD, Breen PA, Frayo RS, Matthys CC, Callahan HS, Purnell JQ** 2003 Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. J Clin Endocrinol Metab 88:1577–1586
22. **Mohlig M, Spranger J, Otto B, Ristow M, Tschöp M, Pfeiffer AF** 2002 Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. J Endocrinol Invest 25:RC36–RC38
23. **Nedvidkova J, Krykorkova I, Bartak V, Paperzova H, Gold PW, Alesci S, Pacak K** 2003 Loss of meal-induced decrease in plasma grhrelin levels in patients with anorexia nervosa. J Clin Endocrinol Metab 88:1678–1682
24. **Green SM, Delargy JH, Joanes D, Blundell JE** 1997 A satiety quotient: a formulation to assess the satiating effect of food. Appetite 29:291–304
25. **Reid M, Hetherington M** 1997 Relative effects of carbohydrates and protein on satiety—a review of methodology. Neurosci Biobehav Rev 21:295–308