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ORIGINAL ARTICLE

Oral glucose load inhibits circulating ghrelin levels to the same extent in normal and obese children

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Summary

Objective The presence of both the GH secretagogue (GHS) receptor and ghrelin in the pancreas indicates an involvement of this hormone in glucose metabolism. Ghrelin secretion is increased by fasting and energy restriction, decreased by food intake, glucose load, insulin and somatostatin in normal adults; however, food intake is not able to inhibit circulating ghrelin levels in children, suggesting that the profile of ghrelin secretion in children is different from that in adults. Moreover, how ghrelin secretion is regulated in childhood as a function of fat mass is still unclear.

Design and subjects We studied the effect of oral glucose load (75 g solution orally) on circulating total ghrelin levels in 14 obese children (group A, four boys and 10 girls, aged 9·3 ± 2·3 years) and 10 lean children (group B, five boys and five girls, aged 9·7 ± 3·8 years).

Measurements In all the sessions, blood samples were collected every 30 min from 0 up to +120 min. GH, insulin and glucose levels were assayed at each time point.

Results Glucose peaks following an oral glucose tolerance test (OGTT) in groups A and B were similar; however, both basal and OGTT-stimulated insulin levels in group A were higher than in group B ($P < 0{\cdot}05$). Basal total ghrelin levels in group A (281·3 ± 29·5 pg/ml) were lower ($P < 0{\cdot}0005$) than in group B (563·4 ± 81·5 pg/ml). In both groups A and B, the OGTT inhibited total ghrelin levels ($P < 0{\cdot}005$). In terms of absolute values, total ghrelin levels in group A were lower ($P < 0{\cdot}0005$) than those in group B at each time point after glucose load. The percentage nadir in total ghrelin levels recorded in group A (−25% at 90 min) was similar to that recorded in group B (−31% at 120 min). Total ghrelin levels were negatively associated with BMI ($r = 0{\cdot}5$, $P < 0{\cdot}005$) but not with glucose or insulin levels.

Conclusion Ghrelin secretion is reduced in obese children. It is, however, equally sensitive in both obese and lean children to the inhibitory effect of oral glucose load.

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Introduction

Ghrelin is a 28-amino-acid peptide predominantly produced by the stomach but also expressed in several other tissues including the endocrine pancreas.[1–7] In its acylated form, ghrelin displays strong GH-releasing action mediated by the activation of the GH secretagogue receptor type 1a (GHS-R1a).[1,3–5] GHS receptors are concentrated in the hypothalamus–pituitary unit but are also distributed in other central and peripheral tissues, including the endocrine pancreas.[2,3,6,8] In fact, ghrelin also exerts other endocrine and nonendocrine actions either at the central or the peripheral level. In particular, ghrelin has been demonstrated to exert a modulatory action on appetite and food intake; this action takes place at the central level and is likely to be mediated by the neuropeptide Y (NPY) and agouti-related protein (AGRP) system.[9,10] In addition, ghrelin exerts remarkable metabolic actions at the peripheral level, being able to modulate the pancreatic β-cell function as well as glucose and lipid metabolism.[5,6,11–13]

Circulating ghrelin levels are mainly represented by an unacylated form and this mostly reflects gastric secretion; in fact, they are reduced by 70% after gastrectomy as well as after gastric bypass in humans.[14,15] Ghrelin secretion occurs in a pulsatile manner without strict correlation with GH levels but in association with food intake episodes and sleep cycles in rats; furthermore, in humans ghrelin secretion undergoes remarkable variations throughout the day and ghrelin peaks anticipate food intake, suggesting that the latter is triggered by ghrelin discharge.[16]

Ghrelin secretion shows negative association with body mass index (BMI); circulating total ghrelin levels are increased in anorexia and cachexia but reduced in obesity both in adulthood and in childhood,[14,17–19] a notable exception being obese patients with Prader–Willi syndrome.[20,21] In agreement with the major link between nutrition and ghrelin, its circulating levels are increased by fasting and energy restriction but decreased by food intake and overfeeding.[14,17,18] These variations in ghrelin secretion predicted its clear negative association with insulin secretion.[4,12,16,22–26]

It has already been demonstrated that ghrelin secretion is reduced by both oral and intravenous glucose load, but also by insulin-induced hypoglycaemia.[22,27,28] An inhibitory input on ghrelin also comes from the activation of gastric somatostatin receptors as indicated, so that the most remarkable inhibition of circulating ghrelin levels is observed under exposure to somatostatin and its analogues.[29,30] Regarding the mechanisms underlying the influence of glucose and insulin on ghrelin secretion, there is still controversy; in particular, it is still a matter of debate whether they play a direct or an indirect inhibitory role.[26,31]

The inhibitory influence of both glucose and food intake has been reported to be a function of either age or fat mass. In fact, glucose load is reported to be unable to inhibit ghrelin secretion in anorexia nervosa while food intake does not modify it in childhood.[18]

Based on the above, we studied the ghrelin response to an oral glucose tolerance test (OGTT) in both obese and lean children to clarify the effects of glucose load on total ghrelin secretion as a function of age and fat mass.

Subjects and methods

Twenty-four prepubertal children were enrolled in this study; 14 obese children (group A, four boys and 10 girls, aged 9·3 ± 2·3 years) and 10 lean children (group B, five boys and five girls, age 9·7 ± 3·8 years). The BMI was above the 90th percentile for group A and from the fifth to the 90th percentile for group B; specifically, the mean BMIs in groups A and B were 26·0 ± 0·6 *vs.* 17·5 ± 0·6, $P < 0·0005$, respectively. Children in group A as well as those in group B had no endocrine disorder and were receiving no medication at the time of the study. All the children's parents were informed as to the purpose of the study and gave their consent as required by the local ethics committee. Control group subjects were enrolled during a programme of auxological and metabolic screening and their parents gave their consent to the evaluation of glucose tolerance with the OGTT.

The children's heights were measured by Harpenden's statiometer and their weight was measured by using an electronic scale. BMI was calculated as body weight divided by height squared (kg/m^2). All children in both groups were of normal stature and growing normally. As anticipated, all children were prepubertal.

All subjects underwent the OGTT (75 g glucose solution orally), which was performed in the morning after an overnight fast. Blood samples were taken by an intravenous catheter kept patent by slow infusion of isotonic saline at baseline and then 30, 60 and 120 min after the OGTT. At each time point, circulating ghrelin, glucose and insulin levels were measured.

Human ghrelin (pg/ml) was assayed, after extraction on reverse-phase C18 columns, by a radioimmunometric assay (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) using ^{125}I-labelled bioactive ghrelin as a tracer and a rabbit polyclonal antibody *vs.* octanoylated and des-octanoylated human ghrelin. The sensitivity was 30 pg/tube. Based on our data, the intra-assay coefficient of variation (CV) was 0·3–10·7%.

Serum insulin levels (1 μIU/ml = 7·175 pmol/l) were measured by a chemiluminescent enzyme-labelled immunometric assay (Diagnostic Products). The sensitivity was 2 μIU/ml. Intra- and interassay CVs were 2·5–8·3% and 4·4–8·6%, respectively.

Plasma glucose (mmol/l) was measured by the glucose oxidase method on a Beckman Glucose Analyser (Fullerton, CA, USA).

Data are presented as mean ± SEM of absolute and delta percentage values. The statistical analysis was performed by an analysis of variance (ANOVA) test (to evaluate the ghrelin, insulin and glucose responses to the OGTT) and an unpaired Student's *t*-test (to evaluate data at baseline in obese *vs.* lean children) by using Statview 5 software for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Basal insulin levels in group A were higher than in group B (252·4 ± 68·1 *vs.* 36·6 ± 4·8 pmol/l; $P < 0·05$) (Fig. 1b), whereas basal glucose levels were similar in groups A and B (4·4 ± 0·1 *vs.* 4·6 ± 0·1 mmol/l) (Fig. 1a). In addition, glucose peaks following an OGTT in group A (6·7 ± 0·3 mmol/l) and group B (7·3 ± 0·6 mmol/l) were similar (Fig. 1a), whereas OGTT-stimulated insulin levels in group A were higher than in group B (732·1 ± 106·6 *vs.* 268·3 ± 66·6 pmol/l; $P < 0·005$) (Fig. 1b). Basal total ghrelin levels in group A (281·3 ± 29·5 pg/ml) were lower ($P < 0·0005$) than in group B (563·4 ± 81·5 pg/ml) (Fig. 1c). In both groups A and B, OGTT inhibited total ghrelin levels ($P < 0·0005$) (Fig. 1c).

In terms of absolute values, after glucose load total ghrelin levels in group A were lower than those in group B at each time point ($P < 0·0005$) (Fig. 1c). However, the percentage nadir in total ghrelin levels recorded in group A (−25% at 90 min) was similar to that recorded in group B (−31% at 120 min) (Fig. 2).

Total ghrelin levels were negatively associated with BMI ($r = 0·5$, $P < 0·005$) (Fig. 3). Nevertheless, no correlation between ghrelin and insulin or glucose levels was recorded.

Discussion

The results of this study demonstrate that obese children show reduced secretion of total ghrelin, which, however, displays normal sensitivity to the inhibitory effect of oral glucose load. Total ghrelin levels in the obese are in fact inhibited by the OGTT to the same extent as they are in lean children. Total ghrelin levels are negatively associated with body mass but not with insulin or glucose levels.

It had been clearly demonstrated that ghrelin secretion is negatively associated with BMI and fat mass.[14,17,18,32] In fact, circulating total ghrelin levels are reduced in obesity both in adulthood and in childhood;[14,17,18] our present data confirm this negative correlation in childhood. A notable exception to this is shown by obese patients with Prader–Willi syndrome; the cause of this peculiarity remains unexplained.[20,21,33]

In agreement with the major link between nutrition, metabolism and ghrelin, the ghrelin circulating levels are increased by fasting and energy restriction but decreased by food intake and overfeeding, at least in adulthood.[14,16–18,21,34–36] Accordingly, these variations in ghrelin secretion under nutritional manipulations predict its clear negative association with insulin secretion.[16,17,22,27,28]

That ghrelin secretion is reduced by both oral and intravenous glucose load has been clearly demonstrated in adults, in whom it is also reduced by insulin-induced hypoglycaemia as well as under the euglycaemic–hyperinsulinaemic clamp.[22,27,28] Thus, either glucose or

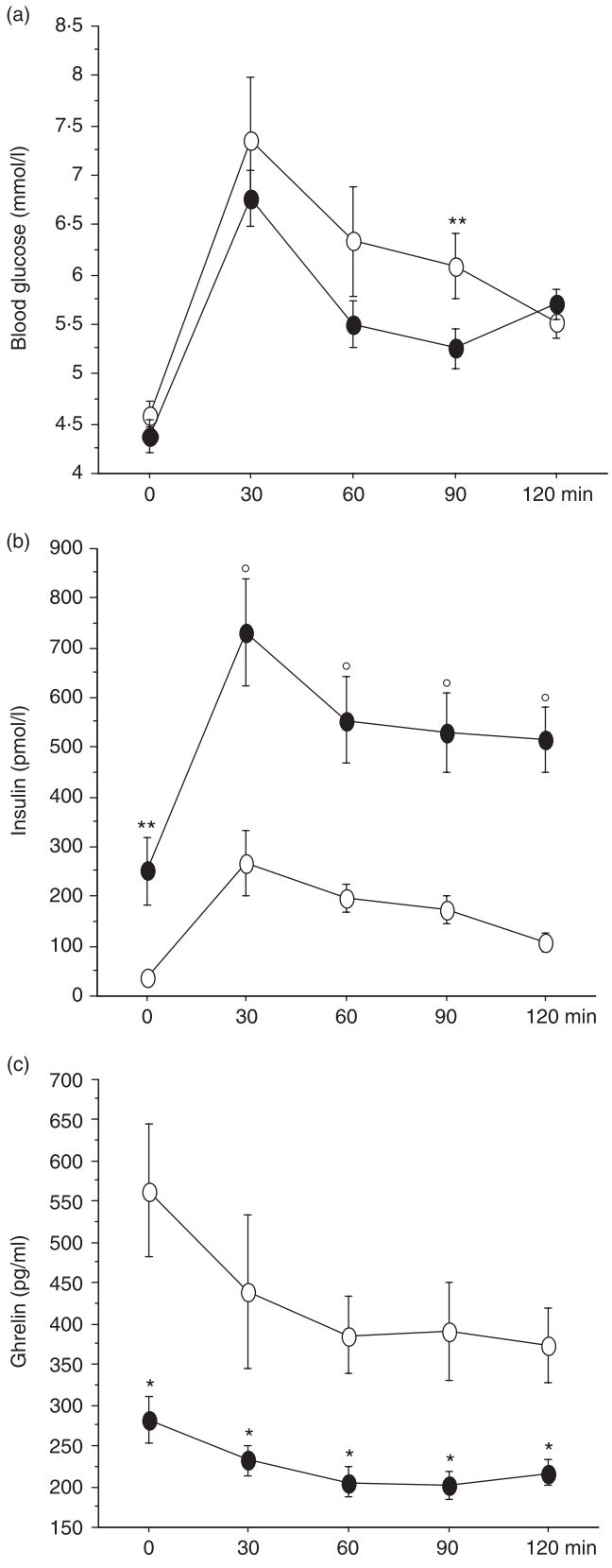

Fig. 1 Absolute values of (a) blood glucose (mmol/l), (b) insulin (pmol/l) and (c) total ghrelin (pg/ml) during OGTT in control (○) and obese (●) children (*$P < 0{\cdot}0005$, **$P < 0{\cdot}05$, °$P < 0{\cdot}005$ *vs.* normal children).

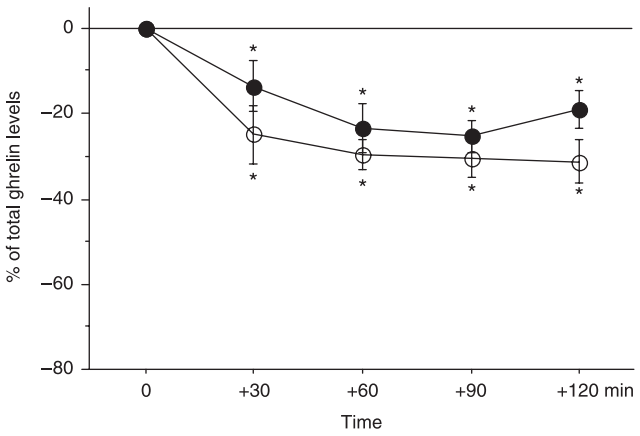

Fig. 2 Mean of delta percentage variation ± SEM of total ghrelin levels (○) normal children, (●) obese children (*$P < 0{\cdot}005$ *vs.* basal time).

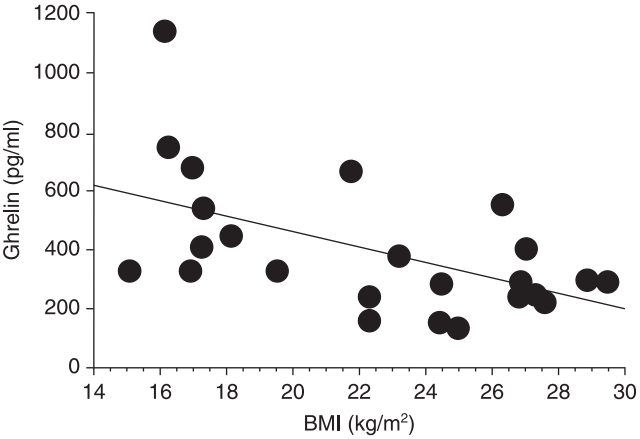

Fig. 3 Negative correlation between total ghrelin levels and BMI in all patients (groups A and B) ($r = 0{\cdot}5$, $P < 0{\cdot}005$).

insulin are inhibitory inputs on ghrelin secretion although whether they play a direct or an indirect inhibitory role is still a matter of debate.[26,31]

Previous studies demonstrated that fasting plasma ghrelin levels were high in anorectic patients, suggesting that ghrelin may play an important role in the pathophysiology of anorexia nervosa.[37] Hyperghrelinaemia in anorectic patients is caused at least partly by increased secretion of active ghrelin, and glucose ingestion suppresses active ghrelin release in these patients.[18,38] It has been reported that even the inhibitory effect of food intake on ghrelin secretion is not operative in childhood.[39]

Our present data demonstrate that ghrelin secretion is inhibited by oral glucose load in obese as well as in lean children. Obese children display a ghrelin hyposecretory state in basal conditions but, at least in terms of percentage decrease, hyperglycaemia inhibits circulating levels to the same extent as recorded in lean children. This occurs despite a clear-cut difference in both basal and OGTT-stimulated insulin secretion. Thus, our present data question previous studies showing the refractoriness of ghrelin secretion to the inhibitory effect of food intake in normal lean children[39] as well as to the inhibitory effect of standard light breakfast (SLB) and glucose load

in normal adults.[40,41] The inhibitory effect of oral glucose load on ghrelin secretion in lean and obese adolescents has been reported;[19] moreover, glucose load and food intake lead to a rapid fall in plasma ghrelin concentration, suggesting that plasma ghrelin reflects an acute feeding state and may also serve as an indicator of short-term energy balance.[18] Thus, the lack of significant reduction of ghrelin secretion following food intake in children reported by some authors could be related to the small number of subjects studied. Alternatively, a meal would be able to inhibit ghrelin only when sufficient amounts of adsorbed carbohydrates elevate glucose and insulin levels enough.[42] The low glucose content of the SLB would be another explanation for the lack of ghrelin inhibition in children, although the same SLB was able to inhibit ghrelin secretion in adults.[40]

The negative association between ghrelin secretion and fat mass, which, in turn, is positively correlated to insulin secretion, would imply that hyperinsulinism has a role in causing the well-known ghrelin hyposecretory state that connotes obesity.[14,16,17,43] This would question the major role of insulin per se in controlling ghrelin secretion, at least in childhood. Accordingly, despite hyperinsulinism, ghrelin levels are not reduced at all in obese patients with Prader–Willi syndrome.[20,21,33] Moreover, children with diabetes type 1 display reduced ghrelin secretion that is not modified by insulin replacement and reduction in glucose levels.[44] However, it should be emphasized that there is a major drawback of our studies, as well as in the majority of studies addressing ghrelin secretion in physiological and pathological states. In general, total ghrelin is measured, although its acylated form is assumed to be the only active form. However, it has been clearly shown that unacylated ghrelin is also an active peptide exerting remarkable metabolic actions.[5,11,13,45,46] Thus, a full understanding of the role played by ghrelin at the metabolic level in physiological and pathophysiological states will need an accurate distinction between its circulating acylated and unacylated forms. This knowledge could completely change our understanding of the relationships linking the ghrelin system with insulin secretion and sensitivity, glucose and lipid metabolism.

In conclusion, as in adulthood, obesity in childhood is associated with a significant reduction in total ghrelin secretion. The ghrelin hyposecretory state in obese children is normally sensitive to the inhibitory effect of glucose load.

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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 Smith, R.G., Leonard, R., Bailey, A.R., Palyha, O., Feighner, S., Tan, C., McKee, K.K., Pong, S.S., Griffin, P. & Howard, A. (2001) Growth hormone secretagogue receptor family members and ligands. *Endocrine*, **14**, 9–14.

3 Volante, M., Allia, E., Gugliotta, P., Funaro, A., Broglio, F., Deghenghi, R., Muccioli, G., Ghigo, E. & Papotti, M. (2002) Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1300–1308.

4 Broglio, F., Gottero, C., Benso, A., Prodam, F., Volante, M., Destefanis, S., Gauna, C., Muccioli, G., Papotti, M., van der Lely, A.J. & Ghigo, E. (2003) Ghrelin and the endocrine pancreas. *Endocrine*, **22**, 19–24.

5 van der Lely, A.J., Tschop, M., Heiman, M.L. & Ghigo, E. (2004) Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews*, **25**, 426–457.

6 Chanoine, J.P. & Wong, A.C. (2004) Ghrelin gene expression is markedly higher in fetal pancreas compared with fetal stomach: effect of maternal fasting. *Endocrinology*, **145**, 3813–3820.

7 Prado, C.L., Pugh-Bernard, A.E., Elghazi, L., Sosa-Pineda, B. & Sussel, L. (2004) Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 2924–2929.

8 Gnanapavan, S., Kola, B., Bustin, S.A., Morris, D.G., McGee, P., Fairclough, P., Bhattacharya, S., Carpenter, R., Grossman, A.B. & Korbonits, M. (2002) The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *Journal of Clinical Endocrinology and Metabolism*, **87**, 2988–2991.

9 Kalra, S.P. & Kalra, P.S. (2003) Neuropeptide Y: a physiological orexigen modulated by the feedback action of ghrelin and leptin. *Endocrine*, **22**, 49–56.

10 Chen, H.Y., Trumbauer, M.E., Chen, A.S., Weingarth, D.T., Adams, J.R., Frazier, E.G., Shen, Z., Marsh, D.J., Feighner, S.D., Guan, X.M., Ye Z., Nargund, R.P., Smith, R.G., Van der Ploeg, L.H., Howard, A.D., MacNeil, D.J. & Qian, S. (2004) Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology*, **145**, 2607–2612.

11 Muccioli, G., Tschop, M., Papotti, M., Deghenghi, R., Heiman, M. & Ghigo, E. (2002) Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. *European Journal of Pharmacology*, **440**, 235–254.

12 Ukkola, O. (2003) Ghrelin and insulin metabolism. *European Journal of Clinical Investigation*, **33**, 183–185.

13 Gauna, C., Meyler, F.M., Janssen, J.A., Delhanty, P.J., Abribat, T., van Koetsveld, P., Hofland, L.J., Broglio, F., Ghigo, E. & van der Lely, A.J. (2004) Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism*, **89**, 5035–5042.

14 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. *Journal of Clinical Endocrinology and Metabolism*, **86**, 4753–4758.

15 Cummings, D.E., Clement, K., Purnell, J.Q., Vaisse, C., Foster, K.E., Frayo, R.S., Schwartz, M.W., Basdevant, A. & Weigle, D.S. (2002) Elevated plasma ghrelin levels in Prader Willi syndrome. *Nature Medicine*, **8**, 643–644.

16 Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E. & Weigle, D.S. (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, **50**, 1714–1719.

17 Tschop, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E. & Heiman, M.L. (2001) Circulating ghrelin levels are decreased in human obesity. *Diabetes*, **50**, 707–709.

18 Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M.S., Tanaka, M., Nozoe, S., Hosoda, H., Kangawa, K. & Matsukura, S. (2002) Plasma

ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *Journal of Clinical Endocrinology and Metabolism*, **87**, 240–244.

19 Soriano-Guillen, L., Barrios, V., Martos, G., Chowen, J.A., Campos-Barros, A. & Argente, J. (2004) Effect of oral glucose administration on ghrelin levels in obese children. *European Journal of Endocrinology*, **151**, 119–121.

20 Cummings, D.E., Weigle, D.S., Frayo, R.S., Breen, P.A., Ma, M.K., Dellinger, E.P. & Purnell, J.Q. (2002) Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England Journal of Medicine*, **346**, 1623–1630.

21 DeIParigi, A., Tschop, M., Heiman, M.L., Salbe, A.D., Vozarova, B., Sell, S.M., Bunt, J.C. & Tataranni, P.A. (2002) High circulating ghrelin: a potential cause for hyperphagia and obesity in Prader–Willi syndrome. *Journal of Clinical Endocrinology and Metabolism*, **87**, 5461–5464.

22 Saad, M.F., Bernaba, B., Hwu, C.M., Jinagouda, S., Fahmi, S., Kogosov, E. & Boyadjian, R. (2002) Insulin regulates plasma ghrelin concentration. *Journal of Clinical Endocrinology and Metabolism*, **87**, 3997–4000.

23 Mohlig, M., Spranger, J., Otto, B., Ristow, M., Tschop, M. & Pfeiffer, A.F. (2002) Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. *Journal of Endocrinological Investigation*, **25**, RC36–RC38.

24 Flanagan, D.E., Evans, M.L., Monsod, T.P., Rife, F., Heptulla, R.A., Tamborlane, W.V. & Sherwin, R.S. (2003) The influence of insulin on circulating ghrelin. *American Journal of Physiology, Endocrinology and Metabolism*, **284**, E313–E316.

25 Reimer, M.K., Pacini, G. & Ahren, B. (2003) Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology*, **144**, 916–921.

26 Schaller, G., Schmidt, A., Pleiner, J., Woloszczuk, W., Wolzt, M. & Luger, A. (2003) Plasma ghrelin concentrations are not regulated by glucose or insulin: a double-blind, placebo-controlled crossover clamp study. *Diabetes*, **52**, 16–20.

27 McCowen, K.C., Maykel, J.A., Bistrian, B.R. & Ling, P.R. (2002) Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. *Journal of Endocrinology*, **175**, R7–R11.

28 Lucidi, P., Murdolo, G., Di Loreto, C., De Cicco, A., Parlanti, N., Fanelli, C., Santeusanio, F., Bolli, G.B. & De Feo, P. (2002) Ghrelin is not necessary for adequate hormonal counterregulation of insulin-induced hypoglycemia. *Diabetes*, **51**, 2911–2914.

29 Broglio, F., Koetsveld, P., Benso, A., Gottero, C., Prodam, F., Papotti, M., Muccioli, G., Gauna, C., Hofland, L., Deghenghi, R., Arvat, E., Van Der Lely, A.J. & Ghigo, E. (2002) Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. *Journal of Clinical Endocrinology and Metabolism*, **87**, 4829–4832.

30 Barkan, A.L., Dimarki, E.V., Jessup, S.K., Symons, K.V., Ermolenko, M. & Jaffe, C.A. (2003) Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. *Journal of Clinical Endocrinology and Metabolism*, **88**, 2180–2184.

31 Caixas, A., Bashore, C., Nash, W., Pi-Sunyer, F. & Laferrere, B. (2002) Insulin, unlike food intake, does not suppress ghrelin in human subjects. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1902–1906.

32 Bellone, S., Rapa, A., Vivenza, D., Vercellotti, A., Petri, A., Radetti, G., Bellone, J., Broglio, F., Ghigo, E. & Bona, G. (2003) Circulating ghrelin levels in newborns are not associated to gender, body weight and hormonal parameters but depend on the type of delivery. *Journal of Endocrinological Investigation*, **26**, RC9–11.

33 Goldstone, A.P., Patterson, M., Kalingag, N., Ghatei, A.M., Brynes, A.E., Bloom, S.R., Grossman, A. & Korbonits, M. (2005) Fasting and postprandial hyperghrelinemia in Prader–Willi syndrome is partially explained by hypoinsulinemia, and is not due to peptide YY3-36 deficiency or seen in hypothalamic obesity due to craniopharyngioma. *Journal of Clinical Endocrinology and Metabolism*, **90**, 2681–2690.

34 Hansen, T.K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J.S. & Jorgensen, J.O. (2002) Weight loss increases circulating levels of ghrelin in human obesity. *Clinical Endocrinology*, **56**, 203–206.

35 Altman, J. (2002) Weight in the balance. *Neuroendocrinology*, **76**, 131–136.

36 Callahan, H.S., Cummings, D.E., Pepe, M.S., Breen, P.A., Matthys, C.C. & Weigle, D.S. (2004) Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *Journal of Clinical Endocrinology and Metabolism*, **89**, 1319–1324.

37 Nedvidkova, J., Krykorkova, I., Bartak, V., Papezova, H., Gold, P.W., Alesci, S. & Pacak, K. (2003) Loss of meal-induced decrease in plasma ghrelin levels in patients with anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism*, **88**, 1678–1682.

38 Nakai, Y., Hosoda, H., Nin, K., Ooya, C., Hayashi, H., Akamizu, T. & Kangawa, K. (2003) Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. *European Journal of Endocrinology*, **149**, R1–R3.

39 Bellone, S., Castellino, N., Broglio, F., Rapa, A., Vivenza, D., Radetti, G., Bellone, J., Gottero, C., Ghigo, E. & Bona, G. (2004) Ghrelin secretion in childhood is refractory to the inhibitory effect of feeding. *Journal of Clinical Endocrinology and Metabolism*, **89**, 1662–1665.

40 Gottero, C., Bellone, S., Rapa, A., van Koetsveld, P., Vivenza, D., Prodam, F., Benso, A., Destefanis, S., Gauna, C., Bellone, J., Hofland, L., van der Lely, A.J., Bona, G., Ghigo, E. & Broglio, F. (2003) Standard light breakfast inhibits circulating ghrelin level to the same extent of oral glucose load in humans, despite different impact on glucose and insulin levels. *Journal of Endocrinological Investigation*, **26**, 1203–1207.

41 Broglio, F., Gottero, C., Prodam, F., Destefanis, S., Gauna, C., Me, E., Riganti, F., Vivenza, D., Rapa, A., Martina, V., Arvat, E., Bona, G., van der Lely, A.J. & Ghigo, E. (2004) Ghrelin secretion is inhibited by glucose load and insulin-induced hypoglycemia but unaffected by glucagon and arginine in humans. *Clinical Endocrinology*, **61**, 503–509.

42 Broglio, F., Benso, A., Gottero, C., Prodam, F., Grottoli, S., Tassone, F., Maccario, M., Casanueva, F.F., Dieguez, C., Deghenghi, R., Ghigo, E. & Arvat, E. (2002) Effects of glucose, free fatty acids or arginine load on the GH-releasing activity of ghrelin in humans. *Clinical Endocrinology*, **57**, 265–271.

43 Yoshihara, F., Kojima, M., Hosoda, H., Nakazato, M. & Kangawa, K. (2002) Ghrelin: a novel peptide for growth hormone release and feeding regulation. *Current Opinion in Clinical Nutrition and Metabolic Care*, **5**, 391–395.

44 Soriano-Guillen, L., Barrios, V., Lechuga-Sancho, A., Chowen, J.A. & Argente, J. (2004) Response of circulating ghrelin levels to insulin therapy in children with newly diagnosed type 1 diabetes mellitus. *Pediatric Research*, **55**, 830–835.

45 Broglio, F., Gottero, C., Prodam, F., Gauna, C., Muccioli, G., Papotti, M., Abribat, T., Van Der Lely, A.J. & Ghigo, E. (2004b) Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. *Journal of Clinical Endocrinology and Metabolism*, **89**, 3062–3065.

46 Asakawa, A., Inui, A., Sakamaki, R., Shinfuku, N., Ueta, Y., Meguid, M.M. & Kasuga, M. (2005) Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut*, **54**, 18–24.