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RAPID COMMUNICATION

Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa

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Abstract

Objective: Ghrelin is a new gastric hormone that has been identified as an endogenous ligand for the growth hormone (GH) secretagogue receptor subtype 1a (GHS-R1a). Ghrelin administration however not only stimulates GH secretion but also induces adiposity in rodents by increasing food intake and decreasing fat utilization. We hypothesized that impaired ghrelin secretion in anorexia nervosa may be involved in the pathogenesis of this eating disorder. To examine this hypothesis and to further investigate the role for ghrelin in regulating energy homeostasis, we analyzed circulating ghrelin levels in patients with anorexia nervosa and examined possible correlations with clinical parameters before and after weight gain.

Methods: Plasma ghrelin levels were measured in overnight fasting plasma samples from 36 female patients with anorexia nervosa (age: 25.0 ± 1.2 years, BMI: $15.2 \pm 0.2\,kg/m^2$) before and after weight gain following psychotherapeutic treatment intervention in a psychosomatic institution. Plasma ghrelin levels were also measured in fasting plasma samples from 24 age-matched female controls (31 ± 1.4 years, BMI: $22.9 \pm 0.45\,kg/m^2$). For quantification of ghrelin levels a commercially available radioimmunoassay (Phoenix Pharmaceuticals, USA) was used.

Results: Fasting plasma ghrelin levels in anorectic patients were significantly higher (1057 ± 95 pg/ml) than in normal age-matched female controls (514 ± 63 pg/ml $n = 24$, $P = 0.02$). Therapeutic intervention in a psychosomatic institution caused an BMI increase of 14% ($P < 0.001$) leading to a significant decrease in circulating ghrelin levels of 25%, ($P = 0.001$). A significant negative correlation between Δghrelin and ΔBMI was observed (correlation coefficient: -0.47, $P = 0.005$, $n = 36$).

Conclusion: We show for the first time that fasting plasma levels of the novel appetite-modulating hormone ghrelin are elevated in anorexia nervosa and return to normal levels after partial weight recovery. These observations suggest the possible existence of ghrelin resistance in cachectic states such as caused by eating disorders. Future studies are necessary to investigate putative mechanisms of ghrelin resistance such as a possible impairment of intracellular ghrelin receptor signaling in pathophysiological states presenting with cachexia.

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Introduction

Ghrelin, a recently discovered gastrointestinal peptide hormone (1–3), stimulates growth hormone secretion in rats and humans (1–10). Based on the observation of orexigenic and adipogenic effects in rodents (11–16), an additional role for ghrelin in the regulation of energy balance has been assumed (17, 18). In humans, ghrelin induces hunger (6, 9, 19) and ghrelin receptor agonists have been shown to acutely increase endogenous glucose levels (19) and to induce tissue specific insulin resistance (20). Ghrelin is mainly secreted by gastric endocrine cells into the systemic circulation, where plasma concentrations can be measured by immunoassay (1, 2). Plasma ghrelin levels are regulated by acute and chronic changes in energy balance: Fasting increases while feeding decreases circulating ghrelin concentrations (11, 21–23). Significantly decreased ghrelin levels have been observed in obese individuals (24). Circulating ghrelin levels are negatively correlated with leptin and insulin concentrations as well as with body mass index, body fat mass and adipocyte size (24, 25). To further investigate the participation of ghrelin in the neuroendocrine network regulating energy homeostasis (17, 18, 26–28) as well as a possible relevance of the novel 'hunger hormone'

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for eating disorders, we analyzed circulating ghrelin levels in anorectic patients before and after therapeutically induced weight gain. We hypothesized that ghrelin levels could possibly be decreased in patients with anorexia nervosa and that this could point to an involvement of ghrelin in the pathogenesis of anorexia nervosa. Results were compared with data from healthy controls and possible associations with changes in body mass were studied.

Methods

Subjects

We studied 36 female patients with anorexia nervosa (age 18 to 45 years, mean age: 25.0±1.2 years, body mass index (BMI): 15.2±0.2 kg/m^2) who had been enrolled in an inpatient intervention program at a psychosomatic treatment center (Klinik Roseneck, Prien, Germany). All patients had been examined and found in good health apart from their eating disorder. At the time point of admission to the hospital, blood samples were drawn after an overnight fast, then immediately chilled on ice and stored at −80 °C. The same procedure was performed when patients left the institution after successful treatment resulting in partial weight recovery (average duration of treatment: 66±25 days). For suitable controls, blood samples from 24 healthy, female volunteers (age 19 to 46 years, mean age 22.9±0.5 years, mean BMI: 21.9±0.6 kg/m^2) were drawn after an overnight fast and later analyzed for ghrelin concentrations. Written informed consent was obtained from each subject. The test procedure was reviewed and approved by the Ethics Committee of the Medical Faculty of the Ludwig-Maximilians-University, Munich, Germany.

Plasma samples and assay procedures: The samples were immediately centrifuged at 4 °C and the plasma was stored at −80 °C until being thawed for analysis. Human plasma ghrelin was measured by commercially available radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, CA, USA) that uses 125I-labeled bioactive ghrelin as a tracer and polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin (crossreactivity with human ghrelin fragment (AA's 23–28): 26.7%, Dr J Chang, Phoenixpeptide, personal communication). Generated data therefore most likely reflect total circulating ghrelin concentrations rather than levels of bioactive ghrelin. No crossreactivities with any relevant molecule have been found, including motilin-related-peptide (MTLRP) and motilin. Intra- and interassay-C.V.'s were below 5.3% and 13.6%, respectively. Dilution and spiking (synthetic Lilly ghrelin (7)) experiments performed in our laboratory yielded a linearity of 101–130% and a recovery of 89–106%.

Statistics

All values are given as means±S.E. Paired *t*-test (before and after weight gain) and student *t*-test (patients vs controls) were performed to make appropriate comparisons. Correlations between parameters were examined using Spearman's rank correlation coefficient.

Results

Average body weight of patients with anorexia nervosa was 41.4±0.8 kg and increased by 14.3% to 47.3±0.9 kg after therapy ($n = 36$, $P = 0.0001$). Mean body mass index of patients with anorexia nervosa was 15.2±0.2 kg/m^2 before treatment and increased to 17.4±0.3 kg/m^2 after therapy ($P < 0.001$, $n = 36$, Fig. 1a). Plasma ghrelin levels of patients with anorexia nervosa (1057±95 pg/ml) were 51.4% higher compared with plasma ghrelin levels in healthy controls (514.8±63 pg/ml, $n = 24$, $P < 0.001$, Fig. 1b). Furthermore, plasma ghrelin levels of patients with anorexia nervosa decreased after therapy-induced weight gain from 1057±95 pg/ml before therapy by 24.4% to 799±71 pg/ml ($n = 36$, $P = 0.04$, Fig. 1b). No significant difference was found between fasting plasma ghrelin levels in patients with anorexia nervosa after weight recovery and fasting plasma ghrelin levels of normal controls. A negative correlation between fasting plasma ghrelin levels and BMI was weak in patients with anorexia nervosa before therapy (correlation coefficient: −0.35, $P = 0.03$) and did improve after therapy-induced weight recovery (correlation coefficient: −0.49, $P = 0.03$). In addition, a significant correlation between changes of BMI (ΔBMI) and magnitude of plasma ghrelin decrease (Δghrelin) was found (correlation coefficient: −0.47, $P = 0.005$).

Discussion

Eating disorders are more prevalent in industrialized societies than in non-industrialized societies and affect an estimated 5 millions Americans every year (29). These disorders typically occur in adolescent females, although 5–15% of the cases of anorexia nervosa are diagnosed in boys and men (29). The mortality rate of patients with eating disorder is 12-times higher than in healthy young female adolescents, only 50% have a full recovery (29). Anorexia nervosa seems to be caused by a combination of genetic, neurochemical, psychodevelopmental and sociocultural factors (29, 30).

Recent progress in the field of energy homeostasis was triggered by the discovery of the adipocyte hormone leptin (31) and revealed a complex neuroendocrine network that appears to balance energy homeostasis and consecutively body weight by modulating appetite, energy expenditure and nutrition partitioning (26–28, 31). One of the latest additions to this model is the novel stomach hormone ghrelin, an

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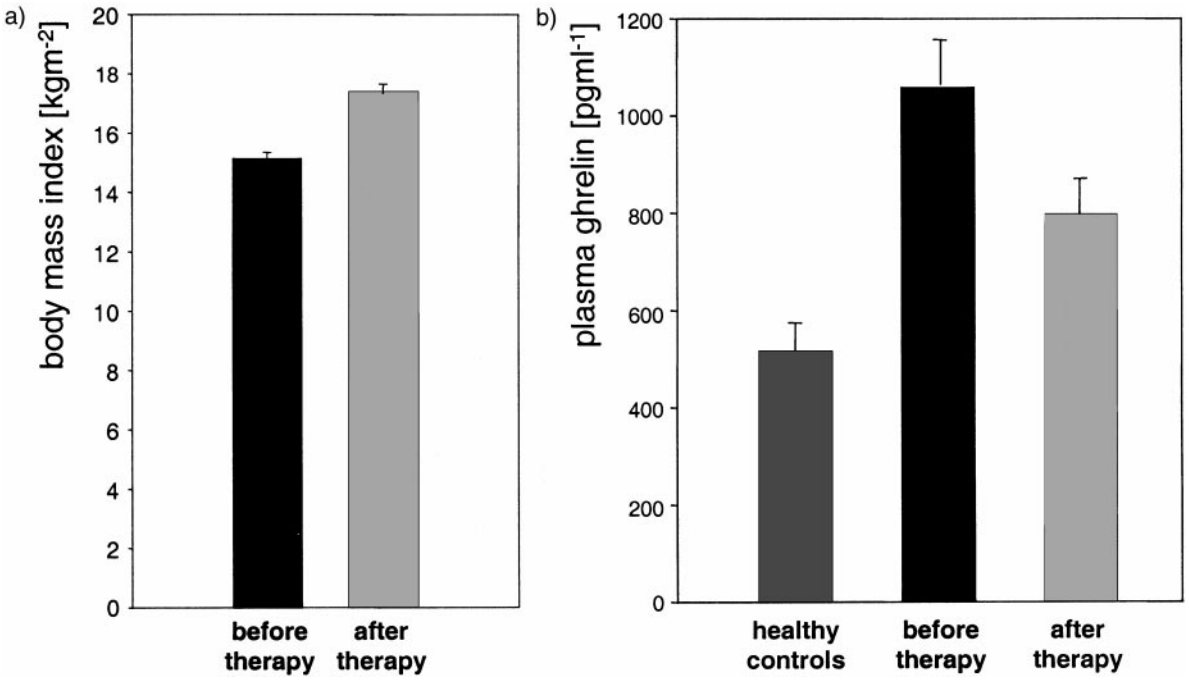

Figure 1 Plasma ghrelin in anorexia nervosa: Influence of therapy induced weight gain. a) Increase of body mass index due to therapeutic intervention during a stay in a psychosomatic hospital ($P < 0.001$, $n = 36$). b) Elevated human plasma ghrelin levels are significantly higher in patients with anorexia nervosa. Partial weight recovery (see Fig. 1a) decreases circulating ghrelin concentration ($n = 36$, $P = 0.04$).

endogenous agonist at the growth hormone secretagogue receptor (1–3, 17, 18). In addition to its ability to stimulate growth hormone secretion, ghrelin induces a positive energy balance resulting in adipogenesis (11). These effects are most likely mediated by hypothalamic neuropeptides (neuropeptide Y (NPY), Agouti-related protein (AGRP), proopiomelanocortin (POMC)) (13–18) and are ultimately a result of increased food intake (11–16) and reduced fat oxidation (11). Destruction of specific hypothalamic areas has been described to result in either extreme hyper- (32) or hypophagia (33, 34) in humans.

Based on these informations we originally hypothesized that impaired ligand binding to hypothalamic ghrelin receptors could have been of significance for the pathogenesis of eating disorders. In clinical studies, ghrelin and synthetic ghrelin receptor-agonists have been documented to induce hunger (6, 9, 19) and in the absence of growth hormone even generate an insulin resistant metabolic state (20). Circulating human ghrelin levels are decreased in obesity (24) and low after food intake (21–23). We proposed therefore earlier that ghrelin signals the CNS when it is necessary to switch to a more energy saving metabolism and to become hungry. The adipocyte-derived satiety hormone leptin induces weight loss in rodents and circulating leptin levels are increased in obesity but decreased in anorexia nervosa (35–39). Leptin and ghrelin may act as complementary peripheral signals reflecting acute and chronic changes in energy balance (18).

We show here for the first time that plasma ghrelin levels of patients with anorexia nervosa are significantly higher than plasma ghrelin levels of healthy controls. These data further support the hypothesis that the secretion of this new hormone that has effects opposite to leptin in rodents is regulated antipodal to leptin. Increased circulating human ghrelin levels in anorexia nervosa seem to make it unlikely that ghrelin is directly responsible for the pathogenesis of anorexia nervosa. While decreased leptin levels in anorectic patients might simply reflect reduced body fat mass (35–39), increased gastric ghrelin secretion in anorexia might however reflect a physiological effort to compensate lack of nutritional intake and stored energy. Rapid normalization of circulating human ghrelin levels after partial weight recovery, possibly support this concept. At this point we can not exclude the possibility that increased ghrelin levels in anorexia nervosa reflect a pathophysiological state of ghrelin resistance analogous to the model of leptin resistance in obesity. Further studies on conceivable changes in intracellular GHS-R-signaling in obesity are necessary to further address these questions. Since the existence of multiple ghrelin receptors or at least multiple ghrelin receptor subtypes is very likely (40), responsiveness of

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GH-secretion to ghrelin or its agonists does not exclude the possibility of ghrelin-resistant neuroendocrine appetite control centers.

Reduction of human plasma ghrelin levels after partial correction of a pathological BMI further support the hypothesis that ghrelin is a peripheral hunger hormone that physiologically reflects acute and chronic energy demand. We hypothesize that the teleological purpose of increased ghrelin levels in anorexia/cachexia consists in signalling the CNS that an increase in energy expenditure along with a more energy-saving metabolic state are necessary.

Relatively high plasma concentrations of growth hormone in patients with anorexia nervosa have been documented earlier (41–43). With respect to ghrelin's ability to induce GH-secretion at the pituitary, our observation of high ghrelin plasma levels in anorexia nervosa seems to be in accordance with published data on the regulation of the GH-IGF-I axis under negative energy balance conditions. Further clinical studies are necessary to investigate a possible causal connection between increased ghrelin levels and raised growth hormone concentrations under cachectic conditions. Decreased IGF-I levels in anorexia nervosa are often interpreted as an indicator of peripheral growth hormone resistance (44–46). In this context, a regulatory feedback loop between hepatic IGF-I production and gastric ghrelin secretion would be imaginable but no data are available until now, to proof such a concept.

In general, ghrelin correlates negatively with body mass index and body fat mass as shown in this and other studies. Existing orally active ghrelin receptor agonists might offer valuable treatment options, even though we show here relatively increased ghrelin levels in anorectic patients. Comparable to the use of insulin for the treatment of diabetes mellitus type 2 (where plasma insulin levels are often found to be increased), ghrelin receptor agonists might be a useful treatment option in anorectic patients with relatively high endogenous ghrelin levels. Small molecule ghrelin receptor agonists with potent activity after oral administration are already available. The fact that ghrelin injection induces hunger in healthy human volunteers seems to encourage the future investigation of these therapeutic options.

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References

1 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999 **402** 656–660.

2 Bowers CY. Unnatural growth hormone-releasing peptide begets natural ghrelin. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1464–1469.

3 Kojima M, Hosoda H, Matsuo H & Kangawa K. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. *Trends in Endocrinology and Metabolism* 2001 **12** 118–122.

4 Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF *et al.* Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *European Journal of Endocrinology* 2000 **143** 7–9.

5 Tolle V, Zizzari P, Tomasetto C, Rio MC, Epelbaum J & Bluet-Pajot MT. *In vivo* and *in vitro* effects of ghrelin/motilin-related peptide on growth hormone secretion in the rat. *Neuroendocrinology* 2001 **73** 54–61.

6 Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C *et al.* Preliminary evidence that ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *Journal of Endocrinological Investigation* 2000 **23** 493–495.

7 Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M *et al.* Ghrelin strongly stimulates growth hormone release in humans. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4908–4911.

8 Peino R, Baldelli R, Rodriguez-Garcia J, Rodriguez-Segade S, Kojima M, Kangawa K *et al.* Ghrelin-induced growth hormone secretion in humans. *European Journal of Endocrinology* 2000 **143** 11–14.

9 Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C *et al.* Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a non-natural peptidyl GHS, and GH-releasing hormone. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1169–1174.

10 Ghigo E, Arvat E, Giordano R, Broglio F, Gianotti L, Maccario M *et al.* Biologic activities of growth hormone secretagogues in humans. *Endocrine* 2001 **14** 87–93.

11 Tschöp M, Smiley DL & Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000 **407** 908–913.

12 Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S *et al.* The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000 **141** 4325–4328.

13 Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H & Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* 2000 **141** 4797–4800.

14 Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001 **409** 194–198.

15 Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N *et al.* Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001 **120** 337–345.

16 Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K *et al.* 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* 2001 **50** 227–232.

17 Inui A. Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nature Reviews Neuroscience* 2001 **2** 551–560.

18 Horvath TL, Diano S, Sotonyi P, Heiman ML & Tschöp M. Ghrelin and the regulation of energy balance: A hypothalamic perspective. *Endocrinology* (In Press).

19 Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M *et al.* Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *Journal of Clinical Endocrinology and Metabolism* 2001 (In Press).

20 Muller AF, Janssen JA, Hofland LJ, Lamberts SW, Bidlingmaier M, Strasburger CJ *et al.* Blockade of the growth hormone (GH)

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receptor unmasks rapid GH-releasing peptide-6-mediated tissue-specific insulin resistance. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 590–593.
21 Tschöp M, Wawarta R, Bidlingmaier M, Landgraf R, Riepl R & Folwaczny C. Postprandial decrease of human ghrelin levels. *Journal of Endocrinological Investigation* 2001 **24** 19–21.
22 Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, Kojima M *et al.* Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochemical and Biophysical Research Communications* 2001 **281** 1220–1225.
23 Cummings E, Purnell JQ, Frayo SR, Schmidova K, Wisse BE & Weigle DS. A pre-prandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001 **50** 1714–1719.
24 Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E & Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001 **50** 707–709.
25 Ravussin E, Tschöp M, Morales S, Bouchard C & Heiman ML. Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *Journal of Clinical Endocrinology and Metabolism* 2001 (In Press).
26 Ahima RS & Osei SY. Molecular regulation of eating behavior: new insights and prospects for therapeutic strategies. *Trends in Molecular Medicine* 2001 **7** 205–213.
27 Spiegelman BM & Flier JS. Obesity and the regulation of energy balance. *Cell* 2001 **104** 531–543.
28 Kalra SP, Dube MG, Pu S, Xu B, Horvath TL & Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocrine Reviews* 1999 **20** 68–100.
29 Becker AE, Grinspoon SK, Klibanski A & Herzog DB. Eating disorders. *New England Journal of Medicine* 1999 **340** 1092–1098.
30 Garner DM. Pathogenesis of anorexia nervosa. *Lancet* 1993 **341** 1631–1635.
31 Friedman JM. Leptin and the regulation of body weight. *Harvey Lectures* 1999–2000 **95** 107–136.
32 Reeves AG & Plum F. Hyperphagia, rage, and dementia accompanying a ventromedial hypothalamic neoplasm. *Archives of Neurology* 1969 **20** 616–624.
33 De Vile CJ, Sufraz R, Lask BD & Stanhope R. Occult intracranial tumours masquerading as early onset anorexia nervosa. *British Medical Journal* 1995 **311** 1359–1360.
34 Heron GB & Johnston DA. Hypothalamic tumor presenting as anorexia nervosa. *American Journal of Psychiatry* 1976 **133** 580–582.
35 Casanueva FF, Dieguez C, Popovic V, Peino R, Considine RV & Caro JF. Serum immunoreactive leptin concentrations in patients with anorexia nervosa before and after partial weight recovery. *Biochemical and Molecular Medicine* 1997 **60** 116–120.
36 Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E *et al.* Serum leptin levels in women with anorexia nervosa. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3861–3863.
37 Hebebrand J, van der Heyden J, Devos R, Kopp W, Herpertz S, Remschmidt H *et al.* Plasma concentrations of obese protein in anorexia nervosa. *Lancet* 1995 **346** 1624–1625.
38 Hebebrand J, Ballauff A, Hinney A, Herpertz S, Kopp W, Wewetzer C *et al.* Body weight regulation in anorexia nervosa with special attention to leptin secretion. *Nervenarzt* 1999 **70** 31–40.
39 Plata-Salaman CR. Leptin, anorexia nervosa, and anorexia of acute and chronic disease. *Nutrition* 1999 **15** 943–945.
40 Chen C. Growth hormone secretagogue actions on the pituitary gland: multiple receptors for multiple ligands? *Clinical and Experimental Pharmacology & Physiology* 2000 **27** 323–329.
41 Stoving RK, Hangaard J, Hansen-Nord M & Hagen C. A review of endocrine changes in anorexia nervosa. *Journal Psychiatric Research* 1999 **33** 139–152.
42 Ferrari E, Fraschini F & Brambilla F. Hormonal circadian rhythms in eating disorders. *Biological Psychiatry* 1990 **27** 1007–1020.
43 Masuda A, Shibasaki T, Hotta M, Suematsu H & Shizume K. Study on the mechanism of abnormal growth hormone (GH) secretion in anorexia nervosa: no evidence of involvement of a low somatomedin-C level in the abnormal GH secretion. *Journal of Endocrinological Investigation* 1988 **11** 297–302.
44 Rappaport R, Prevot C & Czernichow P. Somatomedin activity and growth hormone secretion. I. Changes related to body weight in anorexia nervosa. *Acta Paediatrica Scandinavia* 1980 **69** 37–41.
45 Argente J, Caballo N, Barrios V, Munoz MT, Pozo J, Chowen JA *et al.* Multiple endocrine abnormalities of the growth hormone and insulin-like growth factor axis in patients with anorexia nervosa: effect of short- and long-term weight recuperation. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2084–2092.
46 Counts DR, Gwirtsman H, Carlsson LM, Lesem M & Cutler GB Jr. The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 762–767.

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