

Eating pattern and the effect of oral glucose on ghrelin and insulin secretion in patients with anorexia nervosa

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(Received 29 November 2002; returned for revision 1 January 2003; finally revised 9 May 2003; accepted 10 June 2003)

Summary

OBJECTIVE Ghrelin is thought to be involved in the regulation of eating behaviour and energy metabolism in acute and chronic feeding states. Circulating plasma ghrelin levels in healthy humans have been found to decrease significantly after oral glucose administration. Because it is suggested that eating behaviour may influence the secretion of ghrelin and insulin in anorexia nervosa (AN), we examined the effect of oral glucose on ghrelin and insulin secretion in subtypes of AN patients.

DESIGN AND PATIENTS Twenty female AN patients and 10 age-matched female controls were subjects. The patients were subdivided into two subtypes based on eating behaviour as follows: 11 restricting type (AN-R), nine binge-eating and purging type (AN-BP). Subjects underwent an oral glucose tolerance test at 08:00 h. Blood was collected 0, 30, 60, 120 and 180 min after the glucose load.

RESULTS Both AN-R and AN-BP had a significant increased basal ghrelin level ($P < 0.01$) and a significantly decreased basal insulin level ($P < 0.05$) as compared to controls. The time of the nadir of mean ghrelin in AN-BP (120 min, 58.1% of basal level, 204.9 ± 34.3 pmol/l, mean \pm SEM) was delayed compared to controls (60 min, 60.2%, 74.3 ± 7.9 pmol/l), and in the AN-R group it kept decreasing for 180 min (80.0%, 182.4 ± 31.5 pmol/l). The peaks insulin levels in AN-BP (120 min, 319.3 ± 88.8

pmol/l) and AN-R (180 min, 418.9 ± 68.4 pmol/l) were also delayed as compared to controls (60 min, 509.2 ± 88.8 pmol/l). The glucose level at 180 min in AN-R was significantly ($P < 0.05$) higher than in controls.

CONCLUSIONS These findings suggest that differences in eating behaviour in AN may induce alterations in both ghrelin and insulin metabolism in the acute feeding state. Furthermore, metabolic changes in the restrictive eating pattern may be related to the pathophysiology of small quantitative meal intake in AN-R patients.

Ghrelin is involved in the regulation of GH release (Kojima *et al.*, 1999; Takaya *et al.*, 2000) and recently has been found to have other actions, including effects on appetite (Tschöp *et al.*, 2000; Cummings *et al.*, 2001; Wren *et al.*, 2001; Lawrence *et al.*, 2002), one heart (Nagaya *et al.*, 2001), pancreas (Wierup *et al.*, 2002) and carbohydrate metabolism (Shiyya *et al.*, 2002). This peptide is an orexigenic peptide that has effects similar to hypothalamic neuropeptides such as neuropeptide Y (NPY; Shintani *et al.*, 2001) and agouti-related protein (AGRP; Nakazato *et al.*, 2001). Plasma ghrelin concentrations in the gastric and truncal veins of normal rats increase in response to fasting and decrease upon refeeding (Tschöp *et al.*, 2000; Dornonville *et al.*, 2001; Toshinai *et al.*, 2001). Circulating plasma ghrelin in healthy humans is found to decrease significantly after oral and intravenous (i.v.) glucose administration (Shiyya *et al.*, 2002). On the other hand, intravenous administration of ghrelin stimulates circulating gastrin and insulin levels in rats (Lee *et al.*, 2002). These findings suggest the involvement of ghrelin in the regulation of eating behaviour and energy homeostasis in both acute and chronic feeding states.

The eating disorder anorexia nervosa (AN) is characterized by chronic food restriction and has been found to cause alterations in the release of gastrointestinal peptides (Baranowska *et al.*, 2000), including increased circulating ghrelin (Ariyasu *et al.*, 2001; Otto *et al.*, 2001), and causes various metabolic changes such as glucose metabolism (Drossman *et al.*, 1979), insulin sensitivity (Zuniga-Guajardo *et al.*, 1986; Kirriike *et al.*, 1990; Fukushima *et al.*, 1993) and insulin resistance (Scheen *et al.*, 1988). However, we previously found that after subdividing AN into two subtypes based on guidelines for the differences in eating behaviour as published in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV; American Psychiatric Association, 1994) there were increased plasma

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ghrelin levels in AN patients with habitual binge/purge behaviour (AN-BP) when compared to restricting behaviour (AN-R) despite similar body mass indices (Tanaka *et al.*, 2003b). The normal weight of a patient with bulimia nervosa with habitual binge-eating and purging has also been found to increase the ghrelin level (Tanaka *et al.*, 2002). Moreover, carbohydrate metabolism and insulin secretion in eating disorders are considered to be related to nutritional status and eating patterns (Casper *et al.*, 1988; Johnson *et al.*, 1994). These findings suggest that differences in eating behaviour may influence secretion of ghrelin and insulin in AN. Therefore in this study, we measured ghrelin and insulin responses to oral glucose loads in all subjects in order to examine the effect of acute feeding states on ghrelin and insulin secretion, and compared the states between the subtypes of AN patients and healthy controls for the purpose of clarifying the pathophysiology of eating behaviour.

Research design and methods

Subjects

Eleven female AN-R patients, nine female AN-BP patients who met DSM-IV guidelines and 10 age-matched apparently healthy female volunteers (controls) were the subjects in this study. Patients were admitted to our hospitals for inpatient treatment, and were excluded if they had a history of alcohol or substance abuse, or gastrointestinal disease. AN-BP patients had habitual binge-eating and vomiting at least twice a week over the preceding 3 months. Written informed consent was obtained from all participants before starting the study, which proceeded in accordance with the principles of the Declaration of Helsinki. Controls were recruited by advertisement in the local community and were paid for their participation. They had no history of psychiatric illness and metabolic diseases, ate normal diets and were within 10% of ideal body weight.

Protocol

Subjects were given a 75-g/225-ml glucose solution orally at 08:00 h after an overnight fast. A butterfly needle was inserted into a forearm vein and the catheter was kept patent by a saline infusion in order to collect blood samples efficiently. Blood was collected 0, 30, 60, 120 and 180 min after oral administration. During testing, all subjects remained in a recumbent position and no activity or eating was permitted. We measured body weight at the time blood samples were obtained. The AN patients were assayed within 1 week after admission and before the initiation of active treatment including medications such as psychotropics. The Institutional Committee of Kagoshima University approved the protocol.

Measurements

Plasma glucose was measured by the glucose oxidase method. Serum insulin was determined by an EIA kit (SRL, Inc., Tokyo, Japan). Blood was drawn into chilled tubes containing EDTA2Na (1 mg/ml) and aprotinin (500 U/ml). Plasma ghrelin was measured using radioimmunoassay (RIA) as described elsewhere (Shiia *et al.*, 2002). In brief, antiserum against the C-terminal region of human ghrelin was raised in New Zealand white rabbits immunized against synthetic human ghrelin[13–28] that had been coupled with maleimide-activated mariculture keyhole limpet haemocyanin. The antiserum recognized acylated ghrelin and nonacylated ghrelin equally on a molar basis. Human Tyr⁰-ghrelin[13–28] was radioiodinated by the lactoperoxidase method for use in the assay. Inter- and intra-assay variation was < 8 and < 6%, respectively. The limit of detection of this assay is 12 fmol/tube of human ghrelin. Two milliliters of plasma was diluted with 2 ml of 0.9% saline and applied to a Sep-Pak C-18 cartridge (Waters, Milford, MA, USA) pre-equilibrated with 0.9% saline. The cartridge was washed first with saline and then with a 0.1% trifluoroacetic acid (TFA) solution and peptides were eluted with a 60% acetonitrile (CH₃CN) solution containing 0.1% TFA. The eluate was evaporated, reconstituted with RIA buffer and subjected to RIA analysis. A diluted sample or a standard peptide solution (100 µl) was incubated for 24 h with 100 µl of the antiserum diluent (final dilution 1/20 000). The tracer solution (16 000 cpm/100 µl) was added, and the mixture incubated for 24 h. Bound and free ligands were separated by the second antibody method. All procedures were done at 4 °C. Recovery of human ghrelin added to the plasma was 90.7 ± 4.0% (*n* = 6).

Statistics

The subject groups (mean ± SEM) were compared using analysis of variance (ANOVA) and a posthoc Scheffé test, when data were normally distributed. The Kruskal–Wallis one-way ANOVA with a chi-square statistic was used to test group differences for the subject characteristic variables because the data distributions were skewed. A *P*-value of < 0.05 was considered statistically significant.

Results

Physiological characteristics for the subject groups are shown in Table 1. The mean body mass index (*P* < 0.01) and basal serum insulin level (*P* < 0.05) in both AN-R and AN-BP were significantly lower than those in controls. Basal plasma glucose levels in AN-R were significantly lower (*P* < 0.05) as compared to the control group. Basal plasma ghrelin in both AN-R and AN-BP were significantly higher (*P* < 0.01) as compared to controls (Table 1).

Table 1 Physiological characteristics (mean \pm SEM) of subject groups

	AN-R <i>n</i> = 11	AN-BP <i>n</i> = 9	control <i>n</i> = 10	Kruskal–Wallis*	<i>P</i>
Age (years)	18.5 \pm 1.4	20.9 \pm 1.4	21.0 \pm 0.6	4.2	0.13
Duration of illness (years)	2.2 \pm 0.5	5.2 \pm 1.3	–	–	–
Body mass index (kg/m ²)	13.3 \pm 0.4†	13.8 \pm 0.5†	21.4 \pm 0.4	–	–
Basal plasma ghrelin (pmol/l)	233.8 \pm 39.3	347.4 \pm 49.2	123.4 \pm 6.6	15.5	< 0.01
Basal plasma glucose (pmol/l)	4.2 \pm 0.1†	4.5 \pm 0.1	4.7 \pm 0.1	–	–
Basal serum insulin (pmol/l)	27.2 \pm 3.8	28.7 \pm 4.6	50.6 \pm 6.6	7.4	0.03

*The Kruskal–Wallis one-way ANOVA was used to test because the data distributions were skewed. †*P* < 0.05 v.s. control, using ANOVA and a posthoc Scheffé test.

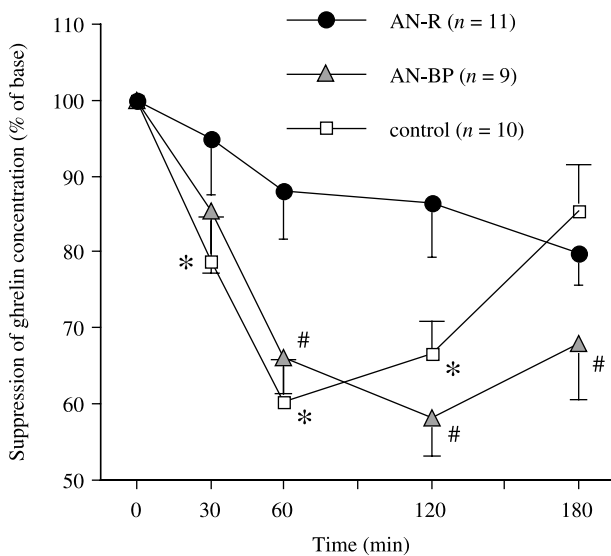


Fig. 1 Plasma ghrelin responses to an oral glucose tolerance test in female anorexia nervosa patients with restricting type (●, AN-R) and binge-eating/purging type (△, AN-BP), and age-matched controls (□). **P* < 0.05 in control vs. 0 min, #*P* < 0.05 in AN-BP vs. 0 min.

The mean plasma ghrelin concentrations in both controls and AN-BP patients decreased after administration of an oral glucose load, reaching nadirs of 60.2% (74.3 \pm 7.9 pmol/l, mean \pm SEM) and 58.1% (204.9 \pm 34.3 pmol/l) of basal levels, respectively, 60 min and 120 min after the glucose load, and increasing thereafter. However, the plasma ghrelin level in AN-R patients constantly decreased without reaching the nadir level for 180 minutes (80.0%, 182.4 \pm 31.5 pmol/l; Fig. 1).

The plasma glucose level 180 min after the glucose load in AN-R was higher than that in controls (*P* < 0.05; Fig. 2a). Though the peak glucose level occurred 60 min after the glucose load in both AN-BP and controls, the peak in AN-R occurred 120 min after the glucose load (Fig. 2a). After glucose loads in controls, the peak insulin level was found to occur at 60 min (509.2 \pm 88.8 pmol/l) while the peaks in AN-BP and AN-R

were, respectively, 120 min (319.3 \pm 88.8 pmol/l) and 180 min (418.9 \pm 68.4 pmol/l) after the glucose load (Fig. 2b).

Discussion

AN-R patients exhibited a constant decreased ghrelin level without reaching a nadir during the test and peaks were delayed for both glucose and insulin as compared to AN-BP and controls. Plasma ghrelin concentrations in healthy humans have been found to decrease significantly after oral and intravenous glucose administration (Shiyya *et al.*, 2002), and it has been suggested that there is delayed glucose absorption due to gastric and duodenal dysmotility in AN-R patients (Stacher *et al.*, 1986; Buchman *et al.*, 1994). However, these results might be caused by impaired regulation of ghrelin secretion as recent studies have shown that plasma ghrelin is not regulated by i.v. administration of glucose, or the combination of glucose and insulin (Caixás *et al.*, 2002; Schaller *et al.*, 2003). A previous study of carbohydrate metabolism (Nozaki *et al.*, 1994) also showed that initial insulin secretion was decreased in AN patients that had glucose level peaking 90 min or later in response to both oral and intravenous glucose. Because our study found that the glucose peak level in AN-R was 120 min, these findings suggest that AN-R patients might also have β -cell dysfunction in acute feeding states.

AN-BP patients had increased basal ghrelin, decreased basal insulin and a delayed nadir for ghrelin and the peak insulin as compared to controls, although the times for peak glucose were similar to controls. Our recent research (Tanaka *et al.*, 2003a) suggests that binge-eating with vomiting rather than binge-eating without vomiting may influence ghrelin levels in eating disorders. In bulimia nervosa patients with unstable weight and with binge-eating and frequent vomiting, a blunted insulin response to a glucose load was found. On the other hand, in these patients who were treated successfully by abstaining from binge-eating and vomiting for 4 weeks, there was a similar insulin response to that seen in normal controls (Russell *et al.*, 1996). These findings suggest that both abnormal eating behaviour and nutritional

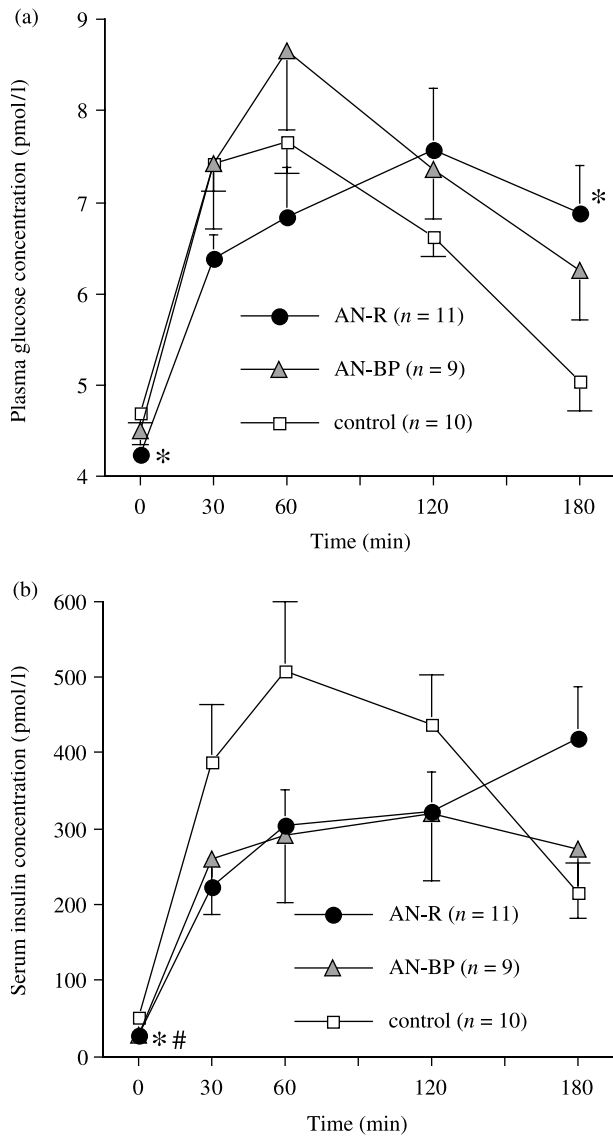


Fig. 2 (a) Comparison of plasma glucose concentration responses to an oral glucose tolerance test among AN-R (●), AN-BP (△) and controls (□). (b) Comparison of serum insulin concentration responses to an oral glucose tolerance test among AN-R, AN-BP and controls. * $P < 0.05$ in AN-R vs. control, # $P < 0.05$ in AN-BP vs. control.

depletion may be the cause of the results found in AN-BP, and that abstaining from binge-eating and vomiting may be important for inpatient treatment of AN-BP patients.

The present study suggests that differences in eating behaviour may influence the effect of oral glucose on both ghrelin and insulin secretion in AN patients. A few studies (Stordy *et al.*, 1977; Neuberger *et al.*, 1995) have shown that oral energy intake with nutritional rehabilitation required for weight gain is significantly different between AN subtypes. In particular, AN-R

patients required 30–50% more energy intake than AN-BP (Kaye *et al.*, 1986). These findings suggest that impaired regulations of both ghrelin and insulin seen with restrictive eating patterns may be the cause of the higher oral energy intake required for weight gain rather than the actual AN-BP behaviour.

Ghrelin, one of the gastric and orexigenic peptides, is found to inhibit the insulin response to the secretagogues glucose, arginine and carbachol (Egido *et al.*, 2002). In contrast, insulin is one of the anorexigenic peptides and has been shown to decrease plasma ghrelin in humans (Saad *et al.*, 2002). Ghrelin has also been documented to be present in α -cells, and increase the cytosolic free Ca^{2+} concentration in β -cells and stimulate insulin secretion (Date *et al.*, 2002b). These peptides, which are related to energy metabolism, have been suggested to be regulated through the vagal system (Herath *et al.*, 1999; Masuda *et al.*, 2000; Blat & Malbert, 2001; Date *et al.*, 2002a). Therefore, we consider the abnormal eating behaviour and nutritional change in AN to have some influence on the relationship between ghrelin and insulin secretion through the vagal system.

Finally, our study documents that both the time at the nadir of the plasma ghrelin level and the peak serum insulin level are delayed in AN patients, especially in AN-R as compared to controls. The present study suggests that differences in eating behaviour may influence the effect of oral glucose on both ghrelin and insulin secretion in AN patients before active treatment. Thus, alterations in both nutritional status and eating pattern may induce ghrelin and insulin metabolic changes in both the acute and chronic feeding state. Furthermore, these metabolic changes in the restrictive eating patterns may be related to the pathophysiology of small quantitative meal intake in AN-R patients.

Acknowledgements

This study was supported by a research grant from the Japanese Ministry of Health, Labor and Welfare.

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