Impaired glucagon secretion to insulin-induced hypoglycemia in anorexia nervosa

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Abstract. In order to clarify the role played by pancreatic α-cell dysfunction in the impaired glucose recovery from hypoglycemia in patients with anorexia nervosa, the response of pancreatic α-cells to insulin-induced hypoglycemia was investigated in 16 patients with anorexia nervosa before and after treatment. The results were compared with those obtained after loading with arginine. Before treatment, despite comparable falls in plasma glucose levels, glucagon secretion was significantly reduced in the anorectic patients compared with control subjects. In addition, glucose recovery from hypoglycemia in the patients was attenuated. However, after treatment, both glucagon secretory activity and plasma glucose recovery following insulin-induced hypoglycemia were restored to normal. Plasma glucagon responses to arginine infusion were not significantly different in the untreated anorectic patients and control subjects. However, the plasma insulin response in the patients was significantly lower than in the control group. These results suggest that the impaired recovery of plasma glucose levels from insulin-induced hypoglycemia in patients with anorexia nervosa is primarily attributable to impaired pancreatic α-secretory capability. In addition, this abnormality in pancreatic α-cell function is reversible with treatment leading to improved nutrition and weight gain.

In 1974, Mecklenburg et al. reported that patients with anorexia nervosa, who showed normal increments in plasma cortisol and GH concentrations after stimulation, exhibited an unexplainable delay in plasma glucose recovery from insulin-induced hypoglycemia. Hypoglycemic coma (Zalin & Lant 1984; Ratcliffe & Bevan 1985) has recently been implicated as a potential cause of sudden unexplained death (Theander 1970; Bruch 1971; Warren & Vande Wiele 1973) in these patients. However, the mechanism by which severe hypoglycemia occurs and glucose recovery from hypoglycemia is impaired in anorectic patients is not known.

While glucagon, GH, cortisol and epinephrine are known to increase acutely following insulin-induced hypoglycemia, glucagon secretion has emerged as the primary supporter of plasma glucose recovery in a number of reports (Gerich et al. 1979; Clarke et al. 1979; Rizza et al. 1979; Cryer 1985). In anorectic patients, responses of GH, cortisol and epinephrine to hypoglycemia have been studied (Mecklenburg et al. 1974; Brauman & Gregoire 1975; Frankel & Jenkins 1975; Aro et al. 1977; Sato et al. 1988), whereas there has been no study of glucagon response to insulin-induced hypoglycemia. Recently, we (Kumai et al. 1988) reported that glucagon secretion was not suppressed by hyperglycemia after a glucose load in anorectic patients. Thus it seemed reasonable to suspect that impaired pancreatic α-cell function was involved in the delayed recovery of plasma glucose levels from insulin-induced hypoglycemia seen in patients with anorexia nervosa.

In order to clarify the role played by pancreatic α-cell dysfunction in the impaired glucose recovery from hypoglycemia in anorectic patients, plasma glucagon concentrations were measured in these patients, before and after 5 months of intens-
ive dietary therapy, and in control subjects during insulin-induced hypoglycemia. In addition, these results were compared with those obtained from arginine loading.

Subjects and Methods

Sixteen female patients (mean age: 21.9 ± 1.5 (SEM) years, median age 22 years, range 13–35), diagnosed as having anorexia nervosa according to the criteria of Feighner et al. (1972) were asked to participate in the study. Written informed consent was obtained from all participants, and they were then admitted to the Kyushu University Hospital. The control group consisted of 8 age-matched (mean age: 20.3 ± 0.7 years, median age 20 years, range 18–24) healthy females who were within 10% of ideal body weight and who had regular menstrual cycles and no family history of diabetes mellitus. The study was conducted in accordance with the principles of the Helsinki II Declaration. All patients underwent the insulin tolerance test within one week after admission and again shortly before discharge, approximately 5 months later. An arginine infusion test was performed within five days, but prior to initiation of dietary therapy after the initial insulin tolerance test. At the time of the initial test, careful dietary histories obtained from the anorectic patients revealed a total caloric intake ranging between 800–1200 Cal per day which contained approximately 120–180 g of carbohydrate. After an overnight fast, all tests were performed between 08.00–09.00 h. Following the completion of the initial insulin tolerance test and the arginine infusion test, the dietary intake of the patients was gradually increased so that by the time of the second insulin tolerance test, the participants were each ingesting 2570 Cal per day containing 400 g of carbohydrate. None of the patients or controls were taking any medications at the time of the studies.

An indwelling cannula was inserted into an antecubital vein of the supine subject and used for collection of blood samples. Thirty minutes later, an iv bolus of 0.1 IU/kg bovine regular insulin (Iszilin*, Shimizu Pharmaceutical Co, Shimizu, Japan) was given. Blood samples were collected for plasma glucose, pancreatic glucagon, GH and cortisol determinations, immediately before and at 30, 60, 90 and 120 min after the insulin injection. An arginine infusion test was performed by infusing 300 ml of a 10% arginine monohydrochloride solution (Morishita Pharmaceutical Co, Osaka, Japan) over 30 min. Blood samples were taken from an antecubital vein of the opposite arm before and at 15, 30, 60, 90 and 120 min for the determination of plasma glucose, insulin and pancreatic glucagon. Plasma glucose was measured with the autoanalyzer glucose oxidase method. The blood samples for plasma insulin estimation were collected in chilled tubes containing 2 mg EDTA, gently mixed and then centrifuged. The plasma was removed and stored at −20°C until assay. Insulin was determined using an insulin RIA kit obtained from Dainabot Co, Ltd (Tokyo, Japan). The inter- and intra-assay coefficients of variation of this insulin radioimmunoassay were 6.6% and 5.9%, respectively. The blood samples for plasma glucagon estimation were placed in chilled tubes containing 2 mg EDTA and 1000 units of Aprotinin (Trasylo*, Bayer, Leverkusen, West Germany), gently mixed, and immediately centrifuged at 4°C. The plasma was removed and stored at −20°C until assay. Glucagon was determined using a glucagon RIA kit obtained from Dainabot Co, Ltd (Yanaihara et al. 1979a,b). This antisem, OAL-123, does not significantly cross react with gut glucagon-like immunoreactivity (Yanaihara et al. 1979b). The inter- and intra-assay coefficients of variation of this assay were 7.6% and 8.1%, respectively. Plasma GH was determined using a GH RIA kit obtained from Dainabot Co, Ltd. Plasma cortisol was determined using a cortisol RIA kit obtained from Eiken Immunochimical Laboratories (Tokyo, Japan). The inter- and intra-assay coefficients of variation for GH were 3.9% and 6.1%, respectively. Those for cortisol were 6.2% and 7.3%, respectively.

The results are shown as the mean ± SEM. The glucagon area under the curve above the baseline (AUC) was calculated by the trapezoidal rule. Continuous variables were compared by one-way or repeated measures two-way analysis of variance (ANOVA), and paired and unpaired t-tests (two-tailed). Differences, where appropriate, on the one-way or two-way ANOVA were evaluated by Bonferroni post hoc tests.

Results

Relevant clinical data of the anorectic patients and controls are summarized in Table 1. The mean weight of the patients before treatment was significantly lower than that of the controls (34.1 ± 1.5 vs 48.6 ± 1.0 kg, P < 0.001). Following therapy, all 16 patients exhibited an increase in weight to a group average of 43.4 ± 0.9 kg.

As shown in Fig. 1, fasting plasma glucose levels in the patients with anorexia nervosa prior to treatment were lower than those of the control group (4.11 ± 0.12 vs 4.68 ± 0.09 mmol/l, P < 0.01). However, there was no significant difference in nadir glucose concentrations or the rate of fall in glycemia between the control group and the patients with anorexia nervosa. In the control group, plasma glucose levels rose after the nadir and returned to basal values. Plasma glucose recovery from hypoglycemia in the patients with anorexia nervosa prior to treatment was delayed and attenuated, and plasma glucose levels at 90 and 120 min were significantly lower than those of the con-
control group. Following therapy, fasting plasma glucose levels returned to normal (Fig. 1). The abnormal recovery of plasma glucose from hypoglycemia observed in the initial test was improved after treatment. Indeed, plasma glucose values at 90 and 120 min were significantly higher in patients with anorexia nervosa after treatment than before treatment. There was no significant difference in plasma glucose concentrations between the control group and the patients after treatment.

Base-line fasting plasma glucagon levels in the patients with anorexia nervosa prior to treatment were lower than those of the control group, (18.7 ± 1.5 vs 25.5 ± 1.4 pmol/l, P < 0.05), as shown in Fig. 2. With the administration of insulin, a significant and prompt increase in plasma glucagon was found in the control group. In contrast, a poor glucagon response to hypoglycemia was found in the untreated anorectic patients. After treatment, the glucagon responses were normalized (Fig. 2). All plasma glucagon levels were significantly higher than those in the initial insulin tolerance test (P < 0.01). When plasma glucagon response to insulin-induced hypoglycemia was expressed as area between the individual fasting levels and curves plotted over a 2-h period, the glucagon area (AUC) in the patients before treatment was 784.9 ± 155.8 pmol·1⁻¹·(2 h)⁻¹, which was signifi-

<table>
<thead>
<tr>
<th>Group and number</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Weight (kg) after treatment</th>
<th>Duration of illness (years)</th>
<th>Interval between tests (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (N = 8)</td>
<td>20.3 ± 0.7</td>
<td>156.4 ± 1.2</td>
<td>48.6 ± 1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anorexia nervosa (N = 16)</td>
<td>21.9 ± 1.5</td>
<td>155.6 ± 0.9</td>
<td>34.1 ± 1.5</td>
<td>43.4 ± 0.9²</td>
<td>5.4 ± 1.2</td>
<td>145.0 ± 14.4</td>
</tr>
</tbody>
</table>

All values are the mean ± SEM; the median and the range are in parentheses.

¹: P < 0.001 vs controls; ²: P < 0.001 vs before treatment.
Comparison of glucagon response expressed as area under curve above baseline (AUC) during insulin tolerance test between control subjects and patients with anorexia nervosa before and after treatment. The vertical bars show mean ± SEM. AUC: Glucagon: Glucagon area between the fasting levels and curve plotted over a period of 2 h. *: P < 0.05.

Fig. 3.

Plasma glucose, insulin and glucagon concentrations (mean ± SEM) during arginine infusion test in control subjects (○ — ○) and patients with anorexia nervosa before treatment (● — ●). *: P < 0.05 vs controls; **: P < 0.01 vs controls.

Fig. 4.

significantly less than the 1737.0 ± 310.3 in the patients after treatment and the 1829.4 ± 271.9 in the control group (Fig. 3).

Before treatment GH concentrations after insulin administration increased from 4.3 ± 0.8 to 8.6 ± 0.8 μg/l in anorectic patients at 60 min. The peak GH level tended to decrease in the untreated patients but did not differ significantly from the values seen in the patients after treatment (12.7 ± 2.3 μg/l) and in the control group (14.8 ± 2.6 μg/l). The plasma cortisol responses to insulin-induced hypoglycemia in the patients before treatment (peak: 90 min, 646 ± 45 nmol/l) were similar to those in the patients after treatment (90 min, 574 ± 43 nmol/l) and the control group (60 min, 560 ± 37 nmol/l).

In contrast to responses to insulin-induced hypoglycemia, the plasma glucagon responses to the stimulus of arginine infusion in the patients with anorexia nervosa prior to treatment were similar to those in the control group (Fig. 4). Plasma insulin levels in the patients were significantly lower than those in the control group from 30 min through to the 120 min mark.

Discussion

This report is the first to document impaired pancreatic α-cell secretion of glucagon to insulin-in-
duced hypoglycemia in patients with anorexia nervosa. Despite a reduction in circulating glucose levels comparable to those of the control group after insulin administration, the glucagon response of the anorectic patients to hypoglycemia was significantly lower than that of the control subjects. Concomitantly, following hypoglycemia, the restoration of glucose levels to normal occurred rapidly in the control group, but was significantly delayed in anorectic patients. In agreement with previous reports (Brauman & Gregoire 1975; Frankel & Jenkins 1975; Aro et al. 1977), the GH response of the anorectic patients before treatment to insulin-induced hypoglycemia tended to decrease in comparison with that of the control group, but the difference did not reach statistical significance. The plasma cortisol response of the patients before treatment to hypoglycemia were similar to that of the control subjects and almost corresponded to previous reports (Mecklenburg et al. 1974; Aro et al. 1977).

Investigations concerned with the relative importance of a number of glucose counterregulatory hormones (Gerich et al. 1979; Clarke et al. 1979; Rizza et al. 1979; Cryer 1985) have shown that glucagon is the dominant hormone in glucose recovery from insulin-induced hypoglycemia and secretion of GH and cortisol is not critical to recovery from insulin-induced hypoglycemia. The present inquiry is in accord with those findings and specifically suggests that impaired recovery from hypoglycemia in patients with anorexia nervosa is mainly due to an insufficient secretion of glucagon. In addition, after treatment, the abnormal pancreatic α-cell response is reversed, together with restoration to normal of the recovery of plasma glucose from insulin-induced hypoglycemia.

Previous reports (Gerich et al. 1979; Rizza et al. 1979) have also demonstrated that epinephrine becomes critical to recovery from insulin-induced hypoglycemia when glucagon secretion is deficient. Sato et al. (1988) reported that epinephrine did not increase in response to insulin and that responsiveness to insulin was partially recovered according to weight gain in a patient with anorexia nervosa. Thus, the possibility may exist that epinephrine partly contributes to improved glucose recovery from hypoglycemia in anorectic patients.

Besides counterregulatory hormones, insulin sensitivity and insulin clearance may affect glucose recovery from insulin-induced hypoglycemia. The peripheral effects of insulin have been reported increased in anorectic patients (Mecklenburg et al. 1974) and the present investigation may be in accord with this report. Insulin sensitivity and insulin clearance in patients with anorexia nervosa have been imported to be both normal (Castillo et al. 1985) and increased (Zuniga-Guajardo et al. 1986). This discrepancy, as yet unclarified, is probably due to differences of examination time and caloric intake. It remains to be elucidated whether insulin sensitivity and clearance affect glucose recovery from insulin-induced hypoglycemia in anorectic patients.

Cox et al. (1983) reported biochemical and ultrasonic abnormalities of the pancreas in 7 out of 10 patients with anorexia nervosa. They also reported the normalization of these abnormalities following dietary therapy. In addition, since another patient with malnutrition, who was diagnosed as having an astrocytoma of the medulla, showed similar pancreatic ultrasound changes, they suggested that malnutrition was the causative factor of these abnormal findings.

In this study, following dietary intervention, the abnormal pancreatic α-cell response to insulin-induced hypoglycemia was restored to normal. These results suggest that reduction in body weight and malnutrition appear to be major causative factors of the observed pancreatic α-cell dysfunction. On the other hand, the glucagon response to arginine loading in patients with anorexia nervosa was not significantly different from that in the control group and is consistent with previous reports (Sizonenko et al. 1975; Blickle et al. 1984). The difference in glucagon responses to the two different stimuli suggests that the α-cell in anorectic patients is selectively unresponsive to a reduction in glucose concentrations, whereas the response to amino acid stimuli appears to be intact.

In summary, the present study suggests that the impaired recovery of plasma glucose levels from insulin-induced hypoglycemia in patients with anorexia nervosa before treatment may be largely caused by an abnormality in glucagon secretion from pancreatic α-cells. This impaired glycemic recovery in anorectic patients is improved following dietary intervention concomitant with normalization of glucagon secretion.
Acknowledgments

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