Insulin, glucose, and pancreatic polypeptide responses to a test meal in restricting type anorexia nervosa before and after weight restoration

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ANOREXIA NERVOSA (AN) is a highly morbid pathological condition with the highest mortality rate among psychiatric disorders (35, 46). This disorder is characterized by multiple metabolic and endocrine abnormalities, including failure of the homeostatic responses to malnutrition and semistarvation. Although most of these alterations are believed to be state related and to reverse with weight restoration, some may be trait-related vulnerability factors that differentiate at-risk individuals. Whether trait or state related, some of these alterations may play a role in sustaining the rewarding nature of restricting behavior, thereby acting as mediators for AN.

Unlike starvation, AN is characterized by an excess of both orexigenic and anorexigenic signaling, indicating dysregulation of hunger and satiety (25). Reduced hunger and desire to eat, coupled with increased fullness and satiety after a test meal, have been reported in AN (21, 31), although the capacity to respond to physiological hunger and satiety cues may not be entirely absent (11, 32). Delayed gastric emptying and intestinal transit times are likely contributors to the reports of increased fullness and satiety after meals (8, 13, 22, 24, 26).

Some investigators argue that these findings reflect tight cognitive control of normal appetite. However, relative to healthy comparison women, those with AN show reduced salivation, a heightened autonomic response to food, and fear and disgust in response to images of food (27, 43), suggesting conditioned responses similar to those seen in simple phobias.

Knowledge regarding signals and systems involved in meal-to-meal regulation of food intake is rapidly expanding. Although newly found systems are studied exhaustively as they relate to obesity, their roles and responses in AN are left largely unexamined. As it stands, it is difficult to determine whether disturbances in meal-related physiological responses are cause or consequence of the disease and whether altered responses are maintained following recovery. Although some of the physiological and endocrine factors involved in long-term body weight maintenance have been examined in cases of AN (6, 12, 20, 23, 29), short-term peptide responses to a standardized solid, mixed-nutrient meal have been less well described. Studies have often yielded conflicting or inconsistent results due to methodological differences and problems with research design. In the case of glucose levels and insulin secretion in AN, for example, mixed results may be attributed to the use of oral glucose tolerance tests (OGTT) or liquid meals (10, 39, 47) rather than a mixed macronutrient solid meal representative of what patients are fed in a standard behavioral treatment program for AN. Furthermore, most studies have administered a test meal or OGTT only at the start of treatment in the starved state (39, 47) or have failed to specify at what point in refeeding subjects were tested (42). When studies have examined responses to a solid meal they have often been limited to sample sizes of less than 10 subjects per group (see Ref. 10 for a review). Finally, studies have failed to consistently analyze results for restricting AN separately from those for binge/purge AN. As noted by Casper (10), the metabolic disturbances arising from these two subtypes of AN are likely to differ, since the former is characterized by sustained restriction and the latter by a pattern of restriction alternating with binge and/or purge behaviors (10).

We hypothesized that in AN there are reversible abnormalities in the endocrine responses to ingestion of a meal that are dependent on weight gain for restoration. The primary aim of this study was to avoid methodological flaws common in prior studies and to characterize physiological responses to food intake in anorexics at three time points: admission to the Eating Disorders Program at Johns Hopkins University Hos-
pital, 2 wk after admission and adherence to a refeeding protocol, and when individuals reached a body mass index (BMI) of 19 kg/m². These data include measures of glucose, insulin, and pancreatic polypeptide (PP), as there are currently either contradictory or incomplete characterizations of how ingestion of a solid meal influences these important physiological parameters. Our data provide insight into the physiological effects of AN and how weight restoration influences endocrine responses to ingestion of a solid, mixed-nutrient test meal.

SUBJECTS AND METHODS

Subjects

Thirteen AN subjects (12 female, 1 male) and 13 healthy controls (12 female, 1 male) participated in the study. AN subjects were inpatients in the Johns Hopkins University Eating Disorders Program. As chronic restriction is likely to result in different metabolic consequences than is starvation alternating with binge-purge behaviors, we limited our study sample to the restricting subtype of AN. The average age was 27.8 ± 2.3 yr, and diagnosis was confirmed by administration of the Structured Clinical Interview for DSM-IV-TR (16, 17). Control subjects were of normal weight (mean BMI 23.6 ± 1.3 kg/m²; Fig. 1) and were age matched to the AN subjects with a mean age of 26 ± 2.5 yr. Control subjects were recruited via advertisements placed throughout the Johns Hopkins Hospital and screened with the SCOFF questionnaire (28) to exclude individuals with a possible eating disorder. All subjects provided written informed consent to participate, and the study protocol was approved by the Johns Hopkins Institutional Review Board.

Methods

On test days, anorexic subjects were escorted to the Johns Hopkins University General Clinical Research Center (GCRC, outpatient) at 7:30 AM. Height and weight were measured, and subjects were then asked to sit quietly for 15 min. After 15 min, a GCRC nurse placed a heparlock in the forearm of the subject and blood was drawn (r = 0) followed by a second blood draw 15 min later (r = 15). After another 10 min, the test meal was placed in front of the subject (r = 25). The subject was instructed to refrain from eating until a blood draw was made at 30 min. Immediately after the 30-min draw, the subject was instructed to take the first bite and informed that the meal must be completed within 50 min (by r = 80). The test meal consisted of one banana, one English muffin, two tablespoons butter, two hard-boiled eggs, one cup of skim milk, and three-quarters of a cup of dry flake cereal for a total of ~650 kcal (5). Within each testing session, blood samples were taken 15, 30, 45, 60, 90, and 120 min after the subject began eating. A volume of 9 ml was drawn at each time point. Subjects were monitored throughout the testing session.

Blood was collected into K+-EDTA tubes and placed on ice. Assays included nine time points for glucose, insulin, and PP. Glucose was measured on a Roche/Hitachi MODULAR (P and D) instrument using the Glucose H/K enzymatic kit from Roche Diagnostics. Insulin was measured on a Tosoh AIA instrument using the ST AIA-Pack IRI kit from Tosoh designed for the quantitative determination of insulin (IRI) in human serum or heparinized plasma. PP levels were determined by radioimmunoassay (Quest Diagnostics, San Juan Capistrano, CA).

Anorexic subjects were tested on three separate days, within 72 h of hospitalization, 2 wk after the first test, and when their BMI reached 19 kg/m². All were fasting from 6 PM on the day prior to testing. Control subjects were tested on only one occasion and were admitted to the inpatient GCRC at 4:00 PM on the day prior to the test breakfast. They were fed a standard meal at 5:00 PM the evening prior to testing and not allowed to consume anything other than water from 6:00 PM until the morning test session. The breakfast test session was identical to that described for the anorexic subjects.

Statistical Analyses

One-way analysis of variance (ANOVA) was employed to test for differences between anorexic and control subjects in preprandial hormone levels and BMI. Repeated-measures ANOVAs were conducted to determine differences in postprandial responses for subjects with AN at the three test sessions. Post hoc analyses of statistical differences were made by Tukey’s HSD tests. Values for control subjects were compared with those of anorexic subjects at each testing session (anorexic subjects at the first test session: AN1; anorexic subjects at the second test session: AN2; anorexic subjects at the third test session: AN3) by individual Student’s t-tests. Values are reported as means ± SE. Data on glucose, insulin, and PP serum levels in response to the meal were analyzed two ways, first by comparing baseline and peak values and second by comparing area under the curve (AUC).

RESULTS

Preprandial Baseline Measurements of Glucose, Insulin, and PP

The average length of time from the first test session to the third test session in anorexics was 43 ± 6 days. As shown in Table 1, the body mass index (BMI) of anorexics at the
The first testing session was significantly lower than that of controls (16.84 ± 0.26 and 23.8 ± 0.62 kg/m², respectively). This difference persisted for the second and third testing sessions, such that, at all three test sessions, the BMI of anorexic subjects was significantly less than that of controls.

Baseline levels of glucose, insulin, and PP were calculated as the means ± SE of the levels measured in the first three blood draws, which took place prior to consumption of the test meal. Data are shown in Table 1. Baseline glucose levels for AN1 and AN2 were significantly lower than those of controls (P < 0.01), with baseline glucose at AN1 significantly lower than at AN2 and AN3 and baseline glucose at AN2 significantly lower than at AN3 (P < 0.05 in all cases). There were no significant differences in baseline insulin levels between control subjects and anorexics at any of the three testing sessions. By contrast, baseline PP levels were significantly elevated in the anorexic group at the first test session (AN1) compared with the second (AN2) and third (AN3) sessions and to those of controls (P < 0.05 in all cases).

### Glucose, Insulin, and PP Responses to the Test Meal

**Glucose response to the test meal.** Changes in serum glucose levels in response to ingestion of the test meal are depicted in Fig. 1. Values are mean ± SE changes from baseline. Fifteen and thirty minutes after the start of the test meal, serum glucose levels were significantly lower in AN1, AN2, and AN3 compared with controls (P < 0.01 in all cases). Forty-five minutes after the start of the meal, glucose levels were significantly lower in AN1 and AN2 but not in AN3 compared with controls (P < 0.05 in both cases). AN2 levels remained significantly depressed compared with controls for the entirety of the study. Comparison of serum glucose levels in subjects at AN1, AN2, and AN3 revealed significant differences between the first (AN1) and second (AN2) test sessions such that glucose levels in subjects at AN1 30, 60, 90, and 120 min after the start of the meal were significantly elevated compared with those for AN2. Differences between AN1 and AN3 occurred 30 and 90 min after the start of the meal, at which time glucose levels were increased in AN1 compared with AN3 (P < 0.05) and at 60 min, at which time glucose levels were significantly elevated in AN3 compared with AN1. Figure 1B shows the AUC above baseline for serum glucose. The AUC was significantly greater for control subjects than for anorexics at AN1, AN2, and AN3 (P < 0.01 at all points). Comparison of AUC among anorexic trials reveals a significant increase in AUC at AN3 compared with AN1 (P < 0.05). As shown in Table 1, the peak level for serum glucose was significantly greater in control subjects than in anorexics at the first and second test sessions (AN1 and AN2, P < 0.01) but not at the third (AN3). However, there was more time between onset of the meal and peak glucose value in AN3 (60 min) compared with controls (30 min).

**Insulin response to the test meal.** As depicted in Fig. 2A, the insulin response to ingestion of the test meal was delayed in anorexic subjects compared with controls, and this delay persisted with refeeding. Peak insulin levels occurred in controls 30 min after the start of the meal, whereas AN1 peaked at 60 min and AN2 and AN3 did not peak until 90 min after the meal.

### Table 1. Baseline and peak values for control and anorexic subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AN1</th>
<th>AN2</th>
<th>AN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.8±2.5</td>
<td>27.8±2.3</td>
<td>18.0±0.2†</td>
<td>19.7±0.62‡</td>
</tr>
<tr>
<td>BMI</td>
<td>23.8±0.6</td>
<td>16.8±0.3*</td>
<td>78.9±1.4**</td>
<td>83.7±1.5‡</td>
</tr>
<tr>
<td>Baseline glucose, mg/dl</td>
<td>85.1±2.0</td>
<td>75.56±1.6*</td>
<td>4.2±0.8</td>
<td>4.0±0.6</td>
</tr>
<tr>
<td>Baseline insulin, μU/ml</td>
<td>4.5±0.6</td>
<td>4.7±0.8</td>
<td>105.0±2.20</td>
<td>19.9±1.6</td>
</tr>
<tr>
<td>Baseline PP, ng/ml</td>
<td>177.2±25.2</td>
<td>276.2±66.0*</td>
<td>137.3±2.6*</td>
<td>19.9±1.6</td>
</tr>
<tr>
<td>Peak glucose, mg/dl</td>
<td>36.1±3.2</td>
<td>16.9±4.8*</td>
<td>48.5±7.4*</td>
<td>56.7±7.3</td>
</tr>
<tr>
<td>Peak insulin, μU/ml</td>
<td>64.4±4.6</td>
<td>68.2±11.4</td>
<td>4.5±0.8</td>
<td>4.0±0.6</td>
</tr>
<tr>
<td>Peak PP, ng/ml</td>
<td>583.4±30.9</td>
<td>765.8±61.8*</td>
<td>105.0±2.20</td>
<td>19.9±1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. AN1, AN2, and AN3, 1st, 2nd, and 3rd anorexia nervosa subjects’ test sessions; PP, pancreatic polypeptide. *Significantly different from control subjects; †significantly different from AN1; ‡significantly different from AN2.
began. Although there were no differences in baseline insulin levels, the peak insulin value for control subjects was significantly greater than that for AN2 (P < 0.05). There were no significant differences in maximum insulin levels between control subjects and anorexics at AN1 or AN3. Interestingly, AUC was not different between AN1 and controls; however, it was significantly decreased in AN2 and AN3 compared with AN1 (Fig. 2B).

**PP response to the test meal.** Changes from baseline PP levels are depicted in Fig. 3A. Thirty minutes after the start of the meal, PP levels were significantly elevated in anorexic subjects, regardless of test session, compared with controls. This difference persisted at the 45-min time point in AN2 and AN3. At the final time point, 2 h after the meal began, PP levels were significantly elevated in anorexics at all three test sessions (AN1: P < 0.05; AN2 and AN3: P < 0.001) compared with controls. There were no differences in PP values between AN1, AN2, and AN3 at any time point, nor were there differences in peak values for the three testing sessions. The peak level of PP in control subjects, as shown in Table 1, was significantly lower than that of anorexic peak levels regardless of test session (P < 0.05 in all cases). Similarly, the AUC for PP, as depicted in Fig. 3B, was significantly lower for control subjects than for AN1, AN2, or AN3 (P < 0.05 for all cases).

![Figure 3A](image1.png)  
**Figure 3A.** Changes in plasma pancreatic polypeptide (PP) levels from baseline during and after the test meal. Values are means ± SE, and plasma PP levels (ng/ml) in subjects are represented as change from baseline. *Time points at which control levels were significantly different from AN1, AN2, and AN3 (P < 0.05). The time during which subjects had access to the meal is denoted by the solid line above the x-axis (50 min total). Differences in the area under the curve are represented in B. *Values significantly different from those of control subjects (P < 0.05).

![Figure 3B](image2.png)  
**Figure 3B.** Changes in area under the curve (AUC) during and after the test meal. Values are means ± SE, and areas under the curve are represented as change from baseline. *Values significantly different from controls (P < 0.05). AN refers to anorexia nervosa stimulation condition.

**DISCUSSION**

Our results demonstrate that disturbances in PP and insulin response curves to ingestion of a solid test meal in untreated, undernourished individuals with AN compared with normal-weight controls persist with weight restoration. By contrast, changes in the glucose response curve to a meal begin to resemble those of control subjects as anorexic individuals undergo weight restoration. Both peak and baseline levels of serum glucose at AN3 compared with AN1 indicate partial normalization with weight restoration. Low fasting glucose levels in the starved state that normalize with weight restoration are consistent with prior studies of ill (10, 36) and of weight-restored AN individuals (9, 10).

With respect to insulin, previous studies have shown variable baseline fasting insulin levels in AN compared with controls, including depressed levels (39), elevated levels (42), and, as in this study, normal levels (10, 48). Peak insulin response values in AN did not differ from those in controls, although there was a consistent delay in both the glucose and insulin response curve to a test meal across AN trials. The right shift of these curves in AN subjects may be due to the slower rate of meal consumption in this group compared with controls. Most AN subjects took the full 50 min to complete the meal, whereas controls were typically done within ~30 min. Future studies should control for the rate of food intake or limit meal time to a short interval of 10–20 min to control for this variable. Although differences in the speed of meal consumption across groups likely affected our data, the fact that the AUC analysis also yielded significant group differences argues that these differences cannot be attributed solely to differential rates of food consumption between groups. Interestingly, studies using an OGTT have also found a delay in the insulin response curve in both ill (39) and weight-restored AN subjects (10). Gastric and gut dysmotility associated with AN (26, 34) may be an additional factor contributing to the right shift in glucose and insulin response curves observed in AN compared with control subjects, and the lower AUC for glucose and insulin in starved AN may reflect malabsorption. We cannot, however, explain why the AUC for insulin at AN1 is comparable to that of controls whereas it is depressed at AN2 and AN3 as though there is increased sensitivity to insulin with refeeding.

Previous work by Uhe et al. (45) described basal and postprandial levels of glucose and insulin in AN after a standard mixed-nutrient meal before and after refeeding. The data, however, are confounded by methodological concerns, since subjects appear to have been only partially weight restored and were retested at an ill-defined point specified only as a BMI above 17. Furthermore, subtype of AN was not specified (45). Although Uhe et al. found that glucose and insulin levels did not differ at baseline or postprandially in anorexics compared with controls, we found that both baseline and postprandial glucose levels were significantly lower in anorexics regardless of test session (AN1, AN2, or AN3) compared with controls.

Baseline levels of PP in anorexic subjects at the first test session (AN1) and the PP response curve to a mixed-nutrient test meal at all three test sessions was elevated, and alterations persisted with weight restoration. PP is produced in the pancreatic islets of Langerhans and released into circulation after...
ingestion of a meal during both cephalic and gastric phases of release. The increase in PP levels during sham feeding is mediated through the vagus nerve and has been used as a measure of vagal activity (14, 15, 40). The amount of PP released is dependent on the digestive state (1–4, 18, 33), and infusion of PP in healthy controls has been shown to reduce appetite and decrease food intake in human subjects, reducing cumulative energy intake over a 24-h period by 25% (7). In normal subjects, release of PP occurs at a low rate in the fasted state, is markedly increased throughout all phases of digestion, and remains elevated for up to 6 h postprandially (1). Our findings are consistent with and expand on those of Fujimoto et al. (19), in which subjects with AN or controls were given a fat-rich meal. Restricting but not bulimic subtype AN patients had elevated PP release in response to the test meal (19). We further demonstrate the persistence of alterations in PP responses to a mixed meal before, during, and after weight restoration in restricting AN compared with controls. Persistently elevated levels of PP in response to food intake in AN may suggest alterations in vagal responsivity to food ingestion in this patient population and are consistent with a possible role for PP as a contributor to the pathophysiology and maintenance of AN. Further evidence for alterations in meal-related PP release in AN comes from two studies, by Tomasik et al. (42) and Uhe et al. (45), in which elevated PP release was observed in response to a solid meal in underweight AN subjects. Both of these studies, however, failed to specify the subtype of AN subjects tested.

Recently, Sysko et al. (37) examined behavioral and psychological responses in subjects with AN before and immediately after weight restoration. This group found that weight-restored AN subjects still consumed significantly less food than control subjects despite changes in weight and decreases in psychological and eating-disordered symptoms. It is possible that persistent overproduction of PP in weight-restored AN contributes to this continued tendency to consume less food after treatment. Although weight is normalized, more time in the weight-restored state is likely to be required to fully reverse endocrine changes that occurred in response to prolonged underfeeding. Alternately, elevated PP levels in AN may be trait related and represent a susceptibility factor for this disorder. Studies of long-term-recovered patients and of first-degree relatives of AN probands may help to clarify these state- vs. trait-related questions.

It is also possible that elevated PP in subjects with AN is related to delayed gastric emptying. PP transgenic mice exhibit a reduced rate of gastric emptying in addition to decreased food intake (44). Patients with AN experience substantial delays in gastric emptying compared with healthy controls (13, 22, 24). This delay has been hypothesized to perpetuate eating disorders by exacerbating fear of fatness due to bloating or by causing rectal distension, which may reflexively inhibit gastric emptying. Long-term rehabilitation improves gastrointestinal symptoms in AN, including gastric emptying (30), whereas short-term refeeding does not (8), although the time course for this improvement is not currently known. Future studies should assess whether the length of time in the weight-restored state results in normalization of the glucose and insulin response following ingestion of a meal and whether PP levels normalize in the recovered state.

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