Evidence for Metabolic and Endocrine Abnormalities in Subjects Recovered From Anorexia Nervosa

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Subjects with anorexia nervosa (AN) at low weight display metabolic, endocrine, and behavioral abnormalities. Whether these various differences are a consequence of the condition and persist after recovery is unclear. We tested the hypothesis that abnormalities in the insulin and leptin axes and in the desire to eat persisted in subjects who had recovered from AN in terms of body mass index (BMI) and menstrual function. Endocrine, metabolic, and psychological parameters were assessed by sampling under fasting conditions and serially in response to a standard meal. Subjects included 18 females recovered from AN and 18 female controls and measures included plasma insulin, leptin, glucose and β-hydroxybutyrate (β-HBA) concentrations together with desire to eat. Fasting glucose concentrations were normal in both groups, but fasting insulin concentrations were significantly lower and the fasting glucose/insulin ratio significantly higher in the recovered subjects. The glucose concentration was significantly higher at the end of the meal period in the recovered group. The peak increase of insulin during the meal was significantly less in the recovered group and in response to the meal, glucose/insulin ratios were significantly higher for the first 45 minutes indicating a delayed insulin response. Fasting β-HBA concentrations were not significantly different between groups, but postmeal decreases were significant and larger in the recovered AN group. Fasting and meal-related leptin concentrations were not significantly different between the groups and in both groups were correlated with BMI. In controls, but not in recovered subjects, the reported desire to eat was correlated with plasma glucose and leptin concentrations. The insulin, glucose and β-HBA data indicated the presence of insulin hypersensitivity in the recovered subjects. As the insulin response to the meal was blunted and apparently delayed, there may be a persistent alteration in pancreatic function as a long-term pathological consequence of the anorexia. Alternatively, these data indicate a possible trait marker for AN.

IN ANOREXIA NERVOSA (AN), there is a failure in the normal homeostatic response to weight loss.[1] The cellular and/or systemic mechanisms underlying the problem are unclear. One possibility is that the insulin and leptin axes were dysfunctional: both these hormones circulate at concentrations proportional to body fat content[2,3] and have key roles in the feedback from peripheral metabolic events to the central nervous system (CNS).[4] Insulin has a complex role in controlling carbohydrate and lipid metabolism,[5,6] and leptin is involved in long-term regulation of body fat stores.[7] A broader metabolic role has been proposed for leptin[8] in which insulin and leptin interact. For example, insulin is involved in controlling glucose utilization and fat deposition in adipocytes, ie, parameters involved in leptin synthesis and release.[9-11] This enables an interaction between the acute regulation of energy intake[12] and the more long-term "lipostatic" effects of leptin. In addition, leptin expression has a diurnal variation, which is modulated by insulin.[13] In the CNS, leptin and insulin moderate the activity of neurones involved in the control of energy homeostasis.[14,15]

In terms of weight regulation, there may be a greater biologic input at the stage of satiation than at the initiation of eating.[16] Thus, intake may be substantially controlled by mechanisms that adjust meal size. In AN, biologic mechanisms involved in terminating a meal may be overactive, contributing to weight loss and the escape from normal homeostasis. During a meal, feedback related to satiety is conveyed from the upper gastrointestinal tract (via the vagus) to the CNS: in addition, blood glucose is monitored by hypothalamic and brainstem nuclei,[17] a possible satiety factor.[18] Insulin may also have a direct role in satiety,[4,5,14,19] but this is not supported by other studies.[20]

Increased and decreased glucose and insulin responses to standard meals have been reported in AN at a low weight or upon recent regaining of a relatively normal body weight.[21] Thus, it is unclear if abnormalities were a consequence of low body weight or altered food intake.[22] Leptin levels have been reported to be reduced in underweight AN subjects,[23] but in proportion to body mass index (BMI).[24] A human leptin-deficient phenotype has been described, the affected family members being very obese.[25] Thus, an overactive leptin system can be hypothesized to cause extremes of weight loss. In humans, plasma leptin concentrations decrease substantially after 1 to 2 days of fasting and rapidly return to normal on refeeding[26]: under normal circumstances however, there is no apparent acute change in leptin in response to food,[27] although in rodents, there is an acute increase.[28]

The broad hypothesis tested was that in recovered AN subjects there are persisting endocrine, metabolic, and psychometric changes, which will be evident under fasting conditions and in response to a standard meal. Specifically, these were a normal glucose and a decreased insulin response to the standard meal, with decreased levels of both factors in the fasting state, a significant increase in leptin during the meal and abnormalities in the responses on a visual analogue scale (VAS) measuring hunger.

SUBJECTS AND METHODS

The study examined insulin and leptin responses to a standard meal in subjects recovered from an episode of AN. These subjects were

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Table 1. β-Hydroxybutyrate-Insulin Ratios

Time	Controls	Recovered Subjects
11:15 (baseline)	0.35 (0.01-1.12)	0.28 (0.04-1.75)
11:30	0.22 (0.04-1.10)	0.25 (0.02-2.14)
11:45	0.08 (0.00-0.39)	0.11 (0.00-2.68)
12:00	0.06 (0.01-0.24)	0.04 (0.00-1.08)
12:15	0.05 (0.00-0.36)	0.02 (0.00-0.27)
12:45	0.07 (0.01-0.71)	0.02 (0.00-0.25)
13:45	0.09 (0.01-0.62)	0.04 (0.00-0.26)
11:45	0.18 (0.02-1.30)	0.08 (0.01-0.33)

NOTE. Data presented as median (range).

chosen so that any observed abnormalities could be considered as candidates for a physiological predisposition to the disorder. To facilitate interpretation of these endocrine responses, parallel samples of plasma glucose and β-hydroxybutyrate (β-HBA) were also assayed.

Eighteen female subjects who had recovered from an episode of AN (according to Diagnostic and Statistical Manual [DSM]-IIIR) were recruited: recovery was defined as a BMI >18.5 kg/m^2, a resumption of menses for at least 3 months, and 'relatively normal' eating habits (ie, the criteria were largely biological). Thus, bulimic subjects were excluded and with the acknowledgment that eating habits of normal women vary widely. The range of eating habits in the normal population is demonstrated by the responses of 2 populations to the Eating Disorders Inventory (Table 1). They were compared with a group of female controls with no history of an eating disorder. Eating disorder symptoms were assessed in both groups using a number of standard methods, including the Eating Disorders Examination,[29] the Eating Disorders Inventory,[30] and the Bulimic Inventory Test (Edinburgh) (BITE).[31]

The standard meal consisted of 6 quarters of cottage cheese sandwiches, prepared from wholemeal bread, cottage cheese, low-fat spread, and a slice of cucumber. It contained the following macronutrients 24.6 g protein, 46.8 g carbohydrate, 10.68 g fat, and 9.36 g fiber, giving a total caloric value of 310.2 kcal. This meal was chosen as it was (1) considered to be 'non-threatening' to a recovered subject, (2) reasonably well liked by the subjects, and (3) easily reproduced.

Subjects arrived at 10:45 AM after fasting from midnight the previous evening, and the indwelling cannula was inserted. Blood was collected at 11:00, 11:15, 11:30, 11:45, 12:00, 12:15, 12:45, 1:45, and 2:45. The meal, which was presented at 11:15, had to be eaten by 12:15. Subjects were allowed diet cola or black coffee with the meal and free access to mineral water throughout the day.

Blood samples were collected from an indwelling cannula in a forearm vein. The cannula was flushed with heparin after each sample to prevent clot formation. On each occasion, 15 mL was collected into sodium heparin tubes containing 200 μL aprotonin (Trasylol, Bayer, Germany). Blood was centrifuged (4°C, 10 minutes, 2,000 *g*), the plasma separated, and aliquots stored in 2-mL sample tubes at -80°C before analysis.

On each occasion that blood was drawn, subjects were asked to complete a VAS to determine feelings related to hunger (fE). The VAS scale used was: No desire to eat – craving for some food, the scale consisted of a 10-cm line between the 2 end points.

Leptin was analyzed by radioimmunoassay,[32] insulin by coated tube radioimmunoassay (DPC, Llanberis UK), glucose by a glucose oxidase method (Sigma, Poole, UK), and β-HBA by a β-hydroxybutyrate dehydrogenase method (Roche, Lewes, UK): the latter 2 analyses were performed on an automated analyzer (MIRA-S, Roche).

Statistical Analysis

Summary statistics were used to describe clinical and demographic data and to compare recovered subjects with controls. Endocrine measures were (1) baseline; (2) area under the curve (AUC, using the trapezoid rule); and (3) delta, defined as peak increase from baseline. The second time point (t2) (11:15 AM) rather than t1 was taken as the baseline (fasting level) to allow for the effects of venipuncture. Log-transformed data was used to achieve normality in the data sets. To compare AUC between the groups, analysis of variance (ANOVA) was performed on the log-transformed data, with (log) baseline as covariate. *T* tests were used to compare (log transformed) delta values between the groups. Significance was set at the 5% level, with the acknowledgment that this must be exploratory rather than definitive where several tests were performed.

RESULTS

There were no significant differences between the control and recovered subjects for BMI, with mean (standard deviation [SD]) values of 22.4 (3.1) and 22.2 (3.0) kg/m^2, respectively and neither was there a significant difference in age [mean (SD): 28.1 (8.5) and 30.6 (7.9) years], weight [mean (SD): 61.8 (10.2) and 59.6 (11.0) kg], or height [1.66 (0.08) and 1.64 (0.08) m], respectively. In the recovered group, 11 of the subjects had a history of other psychiatric complaints compared with 5 of the controls (χ^2 with continuity correction = 2.81, $P = .09$), and 11 of the recovered subjects had a family history of psychiatric disorders compared with 7 of the controls (χ^2 with continuity correction = 1.00, $P = .32$). The scores obtained by each group for the Eating Disorders Inventory (EDI), the BITE, and the Eating Disorders Examination (EDE), are shown in Tables 2, 3, and 4 together with published data for several different populations. For further demographic details of the 2 groups see Ward et al.[33]

Fasting glucose concentrations were not significantly differ-

Table 2. Results From the Study and Published Ranges for the EDI

Subscale	Recovered Subjects	Controls	Eating Disorder Subjects	College Females
Drive for thinness	7.3 (4.6)	2.8 (4.6)	14.5 (5.6)	5.5 (5.5)
Bulimia	1.7 (3.4)	0.4 (0.9)	10.5 (5.5)	1.2 (1.9)
Body dissatisfaction	13.6 (9.1)	9.2 (8.1)	16.6 (8.3)	12.2 (8.3)
Ineffectiveness	7.7 (5.1)	4.8 (5.0)	11.3 (7.8)	2.3 (3.6)
Perfectionism	7.1 (3.0)	3.1 (3.8)	8.9 (4.9)	6.2 (3.9)
Intrapersonal distrust	3.6 (4.0)	2.1 (2.9)	5.8 (4.7)	2.0 (3.1)
Interoceptiveawareness	5.2 (5.2)	2.1 (2.9)	11.0 (6.1)	3.0 (3.9)
Maturity fears	2.4 (3.4)	3.1 (4.2)	4.5 (4.7)	2.7 (2.9)

NOTE. The published data for college females and eating disorders subjects are from Williamson et al.[34] Difference between recovered subjects and controls significant for drive for thinness ($P = .001$) and perfectionism ($P = .0004$).

Table 3. Results From the Study and Published Ranges for the EDE

Subscale	Recovered Subjects	Controls	AN	BN	Restrained Eating Controls	Normal Controls
Restriction	1.21 (1.21)	0.67 (1.04)	3.17 (1.47)	3.45 (1.18)	3.15 (0.33)	0.79 (0.97)
Eating concern	0.98 (1.39)	0.12 (0.26)	2.17 (1.62)	2.63 (1.42)	1.25 (0.23)	0.20 (0.51)
Weight concern	1.42 (1.08)	0.64 (0.77)	2.40 (1.48)	3.73 (0.39)	2.12 (0.19)	1.00 (0.87)
Shape concern	3.41 (2.43)	1.23 (1.58)	2.85 (1.22)	3.90 (1.28)	2.55 (0.20)	1.14 (0.89)
Bulimia	2.94 (9.87)	0.56 (1.29)	1.58 (1.55)	2.17 (0.86)	0.14 (0.10)	—

NOTE. The published data for AN, BN, restrained control subjects, and normal control subjects in Fairburn and Copper.[29]
Abbreviations: AN, anorexia nervosa; BN, bulimia nervosa.

ent between the groups, (mean (SEM): 5.03 (0.09) mmol/L in the recovered group and 5.18 (0.11) mmol/L in controls). Figure 1A shows glucose responses to the meal: (log transformed) AUC (recovered, 0.60 [−2.58 to 3.58] versus controls −0.17 [−3.10 to 3.18], $P > .1$) or increases from baseline values (1.20 [0.10 to 3.10) versus 0.90 [0.10 to 2.90], respectively, $P > .1$) were not significantly different between the groups, and there was no apparent difference in response time to the meal. There were differences in the shape of the response curve: at t6, glucose concentrations were significantly higher (by approximately 15%) in the recovered AN group (5.81 (0.30) mmol/L versus 4.92 (0.30) mmol/L in controls (*t* test; $P = .04$).

Fasting (baseline) insulin concentrations were significantly different between groups (*t* test; $P < .001$). Median (range) levels were some 30% lower in the recovered group (4.65 [3.9 to 7.4] mIU/L) than in controls (6.8 [5.0 to 11.6] mIU/L). Figure 1B shows insulin responses to the meal: the increase of insulin was significantly lower ($P = .018$, *t* test on log-transformed data) in the recovered group (22.2 [8.7 to 61.0] mIU/L) than in controls (30.45 [18.3 to 82.8] mIU/L). The AUC value (corrected for baseline) was some 10% lower in the recovered group (37.9 [2.01 to 127.40] versus 41.58 [13.94 to 117.61]), but the difference was not significant.

To provide further information on the relationship between plasma glucose and insulin concentrations, glucose/insulin ratios were examined across the period of the study: these are shown in Fig 1C. Analysis of these data using repeated measures ANOVA showed a significant effect of time ($P < .001$) and group by time ($P = .03$). Post hoc analysis revealed a significant difference (*t* test: $P < .001$) in the fasting glucose/insulin ratios between the 2 groups, the values being 1.01 (0.05) and 0.74 (0.04) for the recovered and control groups, respectively. This is consistent with the fasting glucose and insulin data, which showed the presence of insulin hypersensitivity in the recovered group. In the recovered group, the increased glucose/insulin ratio persisted throughout the period of the study, but the difference between the groups was only significant at the 3 time points after food ingestion. The data shown in Figs 1B and C is consistent with a delayed insulin response to increasing glucose in the recovered subjects.

Table 4. Results From the Study for the BITE

	Recovered Subjects	Controls
Symptom	7.5 (0-24.0)	6.0 (1.0-14.)
Severity	0.5 (0-6.0)	0 (0-3.0)

Fasting β-HBA concentrations were not significantly different between the groups (1.85 [0.45] mg/dL in the recovered group and 3.03 [0.64] mg/dL in controls). However, the mean value in the recovered group was some 40% lower than in controls. Figure 1D shows the β-HBA response to the meal: (log transformed) AUC was significantly different between the groups (−1.63 [−13.65 to 0.38] in recovered subjects versus −1.68 [−17.89 to 10.13] in controls, ANOVA with [log transformed] baseline as covariate: $P < .001$). There was no significant difference between the decrease from baseline values (−1.08 [−6.61 to −0.13] versus −0.99 [−6.33 to 0.13], respectively). Because of insulin's role in the regulation of fat stores, the ratios of β-HBA to insulin were examined. Analysis by repeated measures ANOVA revealed a significant effect of time ($P < .001$), but no significant effect of group by time ($P = .1$). However, it can be seen in Table 1 that in the recovered group, the ratio was consistently lower throughout the study period.

Fasting leptin concentrations were approximately 10% lower in the recovered group, but there were no significant differences between groups (10.07 [1.17] ng/mL in the recovered group and 11.26 [1.40] ng/mL in controls). Using linear regression, BMI can be used to predict baseline leptin in both groups (recovered: adjusted $r^2 = .42$, $P = .002$; control: adjusted $r^2 = .28$, $P = .01$), ie, the relationship was slightly stronger in the recovered group. Figure 1E shows leptin responses to the standard meal: there was no significant difference in AUC between the 2 groups.

There was no significant difference between the groups in the Desire to Eat (fE) scale over the test period for either AUC or delta (Fig 1F). As can be seen, both groups showed similar scores in the premeal period, followed by identical and rapid decreases in the desire to eat, during the meal. In the postmeal period, both groups showed a virtually identical profile, which demonstrates a gradual reappearance of the desire to eat.

DISCUSSION

Subjects in this study appeared to have recovered from AN, as determined by DSM IIIR criteria. In addition, their group mean BMI was virtually identical to that of controls. However, comparison to a control group showed significant endocrine and metabolic differences in fasting states and in dynamic responses to a meal. There were no significant differences between the groups for scores from the BITE, but the recovered

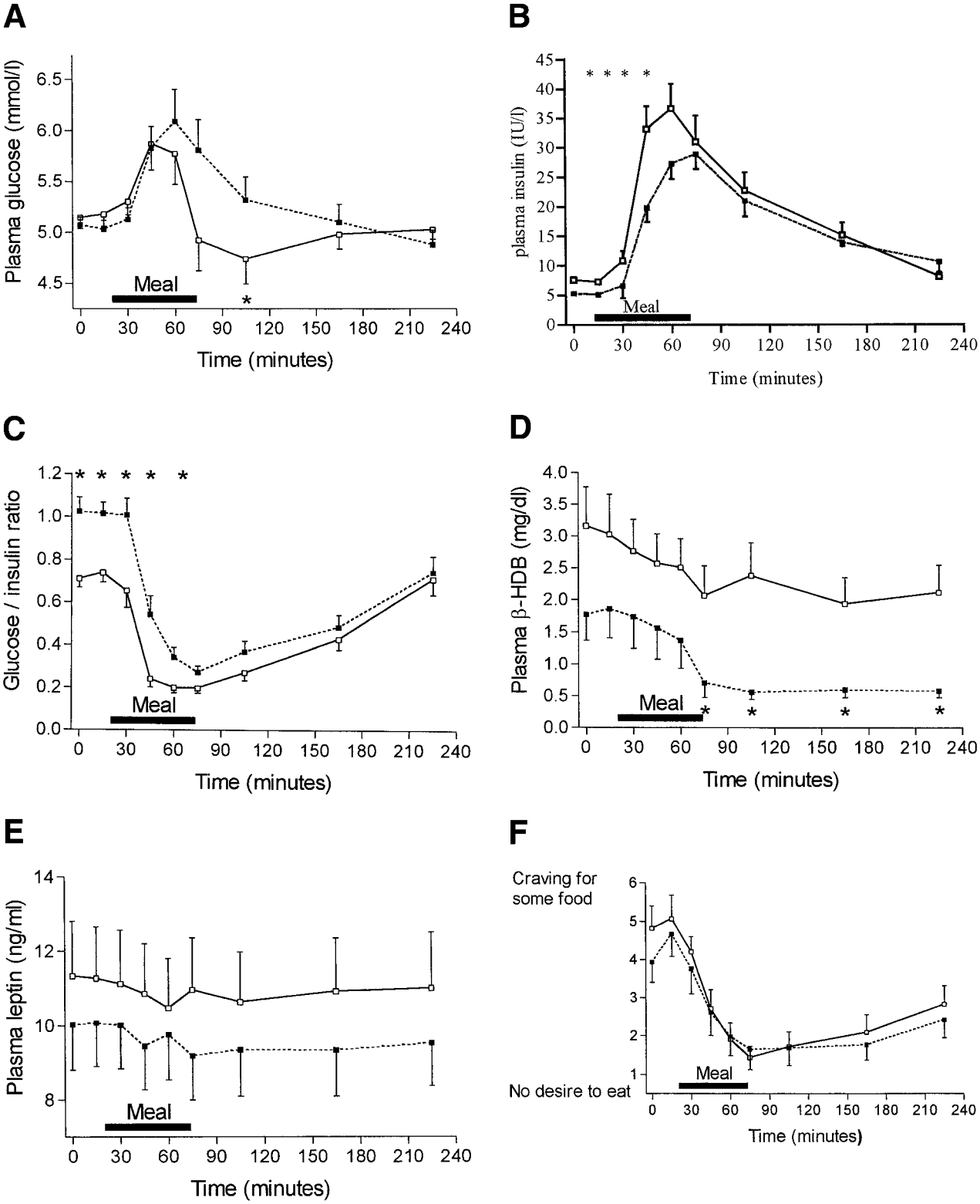

Fig 1. (A) Plasma glucose responses to the standard meal (mean ± SEM), *$P < .05$. (B) Plasma insulin responses to the standard meal (mean ± SEM), *$P < .05$. (C) Glucose/insulin ratios in response to the standard meal (mean ± SEM), *$P < .05$. (D) Plasma β-hydroxybutyrate responses to the standard meal (mean ± SEM), *$P < .05$. (E) Plasma leptin responses to the standard meal (mean ± SEM), *$P < .05$. (F) Desire to eat (fE) responses to the standard meal. (■) Recovered group; (□) control group.

group scored significantly higher on 2 of the EDI subscales (drive for thinness and perfectionism), but these values were not markedly higher than the published normal ranges based on scores obtained from college females (Table 2). In a similar fashion, the scores for the EDE were significantly higher in the recovered group (Table 3), but again similar to published ranges for restrained control subjects (for a full discussion of these results, see Ward et al[33]). There were no significant differences between the groups for the scores obtained in the BITE (Table 4).

The present data showed that in subjects recovered from AN, fasting plasma glucose concentrations were normal, but were sustained in the presence of significantly decreased fasting insulin concentrations. That fasting glucose concentrations were not different between the groups indicates that in the fasting state, the recovered AN group was relatively more sensitive to insulin, ie, they were displaying insulin hypersensitivity. The origins of this hypersensitivity are unclear, but for example, may be related to levels of exercise or body fat content as recovered subjects were reported to have reduced body fat and increased physical activity.[35] Increased physical activity and decreased body fat would allow these recovered subjects to have BMI values comparable to controls and to show apparent insulin hypersensitivity. It may be that these 'recovered' women remain below their set point that would be achieved if they were eating without constraint. This finding warrants further investigation with a more specific measure of insulin sensitivity, for example an intravenous glucose tolerance test. Future studies should include more detailed measurements of body fat distribution and estimates of the body weight set point derived from the body weights of close family members.

In response to the standard meal, the initial increases in plasma glucose were similar in both groups, and thus it seems that glucose absorption from the gut was normal. The glucose response to the meal is similar to that seen in recovered subjects in some, but not all investigations[21]: differences between studies, may be related to the fact that glucose and insulin responses to a meal are affected by meal composition[36] or because AN subjects (either currently underweight or recovered) respond differently to individual macronutrients or to subsatiating levels of calorie intake. Interstudy differences are also seen with insulin responses to a test meal: reduced responses have been reported in low weight individual subjects,[37] but others have shown normal or increased responses,[38] and in recovered subjects, the response has been reported to be normal (see Ploog and Pirke[22] for review). In this study, the insulin/glucose responses to the standard meal showed an apparently delayed and blunted insulin response with a resultant delay in the postmeal insulin-induced decrease in glucose concentration. This delayed and blunted insulin response to a meal in the recovered subjects is reflected in the significantly higher glucose/insulin ratios during the period of meal ingestion. It is proposed that there is some alteration in pancreatic function as a consequence of the anorexia, for example, as a result of chronic low weight, altered electrolyte balance, or chronic laxative abuse.[39] The blunted insulin response and the relatively elevated fasting plasma glucose/insulin ratio are consistent with that seen in prediabetic individuals and is consistent with a report, which suggests that subjects recovering from AN are insulin resistant.[40] Alternatively, as the data is derived from recovered subjects at normal weight, differences in the glucose/insulin axis may be trait markers for AN. Finally, the differences in insulin and glucose response may be related to differences in the speed at which the meal was eaten; this was not accurately recorded in this study. Whether the effects are trait or state markers remains to be established, but in either case, as insulin is anabolic and will tend to increase in starvation, it suggests that recovered AN subjects are less sensitive to low energy states, and this may be a way in which they can escape from normal appetitive mechanisms.

In the recovered AN group, the significantly lowered fasting concentration of insulin would be expected to be associated with a reciprocal increase in β-HBA, ie, with evidence of increased fat metabolism. However, their fasting β-HBA concentration was some 40% lower than in controls. Furthermore, in the recovered AN group, β-HBA concentrations were significantly decreased in response to the meal (by some 70% *v* some 30% in controls). Thus, both the fasting β-HBA concentration and the β-HBA response to the standard meal were consistent with a pattern of fat conservation in recovered subjects. This is in contrast to AN subjects at low weight when they have elevated plasma β-HBA concentrations, which are linked to dietary restraint,[38,41] low weight, and increased fat mobilization. The present data is also in contrast to the metabolic profile, which results from low insulin levels in diabetes mellitus. The data on β-HBA (fasting and in response to the meal) suggests that subjects recovered from AN were more sensitive than controls to the antilipolytic effects of insulin and supports the glucose/insulin data in that it indicates that in recovered subjects, processes involving insulin are altered, and that they may preferentially metabolise glucose. Interestingly, a study of low-weight subjects with AN has shown a decreased rate of lipid oxidation,[42] therefore, a study designed to examine the respiratory quotient of subjects recovered from AN is required to confirm this possibility.

In this study, plasma leptin concentrations and BMI were correlated in both groups. In fact, in the recovered group, the fasting plasma leptin concentration was some 10% lower than in the controls, and the correlation between it and BMI was stronger and more significant. It is possible, therefore, that the recovered subjects were slightly closer to their lipostatic "set point" than the controls. A correlation between plasma leptin concentrations and BMI has been reported in normal weight obese subjects and bulimic subjects.[24,32,41-45] There was no apparent immediate leptin response to the meal in either group, and this has been observed in studies of normal weight and obese subjects.[27]

The data from the fE scale showed almost identical responses in both groups. This may be due to (1) the recovered group had normal feelings of hunger or (2) the recovered group was responding to external cues, ie, they were directly relating the amount they ate to the marks they made on the VAS scale (ie, the knowledge that they had eaten a meal means that they no longer desired to eat). We have reported similar findings with respect to the response to the anorectic agent, d-fenfluramine. This agent had no effect on food intake in the recovered subjects.[33] AN subjects at a low weight do not have normal

feelings of hunger or desire to eat where it has been shown pre and postmeal hunger ratings were decreased compared with controls. Thus, feelings of hunger and the rapid decreases, which occur in response to eating, are apparently normal in the recovered group. However, the lack of a relationship between these feelings and the change plasma glucose (a recognized satiety agent[18]) suggests that the recovered subjects, unlike the controls, are not using this particular biological cue to determine satiety, as has been proposed by others.[45] While glucose may be such a factor, many investigators (see Schwartz et al[14] and Flier and Maratos-Flier[15] for example) do not consider glucose to be a significantly important independent satiety factor. It is unclear whether this is a predisposing factor for AN or one that has been learned during the course of the illness.

In summary, the present data suggest that there is an abnormality in the insulin/glucose axis in recovered AN subjects. It remains unclear whether it is a state or trait marker. It is manifest as a blunted and delayed insulin response to increasing plasma glucose and results in preferential metabolism of glucose and to conservation of fat stores. The lipostatic function of leptin, as manifest in the relationship between plasma leptin and BMI, is normal in recovered AN subjects. Finally, in this recovered AN group, there is no relationship between glucose and the development of hunger. This may indicate a deficiency in the link between the hypothalamic feeding centers and peripheral sensors or to the possibility that this group uses external cues to determine feelings of hunger or the desire to eat.

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