Role of Cortisol in Menstrual Recovery in Adolescent Girls with Anorexia Nervosa

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ABSTRACT: Neuroendocrine abnormalities in anorexia nervosa (AN) include hypercortisolemia, hypogonadism, and hypoleptinemia, and neuroendocrine predictors of menstrual recovery are unclear. Preliminary data suggest that increases in fat mass may better predict menstrual recovery than leptin. High doses of cortisol decrease luteinizing hormone (LH) pulse frequency, and cortisol predicts regional fat distribution. We hypothesized that an increase in fat mass and decrease in cortisol would predict menstrual recovery in adolescents with AN. Thirty-three AN girls 12–18 y old and 33 controls were studied prospectively for 1 y. Body composition [dual energy x-ray absorptiometry (DXA)], leptin, and urinary cortisol (UFC) were measured at 0, 6, and 12 mo. Serum cortisol was measured overnight (every 30 min) in 18 AN subjects and 17 controls. AN subjects had higher UFC/cr·m² and cortisol area under curve (AUC), and lower leptin levels than controls. Leptin increased significantly with recovery. When menses-recovered AN subjects were compared with AN subjects not recovering menses and controls, menses-recovered AN subjects had higher baseline cortisol levels and greater increases in leptin than controls and greater increases in fat mass than AN subjects not recovering menses and controls (adjusted for multiple comparisons). In a logistic regression model, increasing fat mass, but not leptin, predicted menstrual recovery. Baseline cortisol level strongly predicted increases in the percentage of body fat. We demonstrate that 1) high baseline cortisol level predicts increases in body fat and 2) increases in body fat predict menses recovery in AN. (*Pediatr Res* 59: 598–603, 2006)

AN, a model of severe undernutrition, is associated with hypogonadotropic hypogonadism resulting in primary or secondary amenorrhea or delayed menarche. Weight recovery occurs in up to 50% of adolescents with AN and should result in recovery of the hypothalamo-pituitary-gonadal (H-P-G) axis (1). However, a temporal association between weight gain and menstrual recovery is not always observed (2,3). Not all adolescents with AN who resume menses are weight recovered, and not all weight-recovered adolescents with AN resume menstrual function. In addition, neuroendocrine predictors of menstrual recovery are unclear.

We have demonstrated higher cortisol (4) and lower leptin levels (3,5) in AN girls compared with healthy adolescents.

Leptin is an adipocytokine, and leptin-deficient or -resistant mice (6,7) and humans with leptin and leptin receptor mutations (8,9) are hypogonadal. In a recent study, leptin administration was associated with resumption of menses in five of eight women with hypothalamic amenorrhea (10). These data suggest that leptin is an important regulator of the H-P-G axis and that an increase in leptin along with an increase in fat mass may predict recovery of the H-P-G axis in AN. Conversely, Golden *et al.* (11) observed no differences in fat mass between AN girls who recovered menses and those who did not.

In addition, possible effects of cortisol on the H-P-G axis have been demonstrated. Cortisol in high doses decreases gonadotropin-releasing hormone (GnRH) secretion and LH pulse frequency in healthy women (12,13), and inverse associations have been noted between cortisol and LH pulse frequency (14) as well as between cortisol and menstrual frequency (15). Samuels *et al.* (16), conversely, did not observe a decrease in gonadotropin pulsatility following hydrocortisone administration. Girls with AN have hypercortisolemia (4), and it is unclear whether baseline cortisol levels or a reduction in cortisol may predict menstrual recovery. In addition, in a study of adult amenorrheic and eumenorrheic women of comparable low body mass index (BMI), our group reported greater fat mass, particularly trunk fat, in the eumenorrheic group, suggestive of effects of body composition on reproductive function (17). Our group has also demonstrated that cortisol is an important predictor of body composition in adults, with higher baseline cortisol levels predicting greater increases in trunk fat in adults with AN (18). It has not been determined, however, whether a higher baseline cortisol level, predicting greater increases in fat mass, also predicts greater chances of recovering menses. Relative contributions of hypercortisolemia, hypoleptinemia, and decreased fat mass to hypogonadism in adolescent AN are unclear, and it is uncertain whether recovery of any one of these factors better predicts menstrual recovery than others.

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Abbreviations: AN, anorexia nervosa; AUC, area under the curve; BMI, body mass index; DXA, dual energy x-ray absorptiometry; GnRH, gonadotropin-releasing hormone; H-P-G axis, hypothalamo-pituitary-gonadal axis; UFC/cr·m², urinary free cortisol normalized for creatinine and surface area

We hypothesized that, in AN girls, an increase in leptin associated with increases in fat mass contributes to and predicts menstrual recovery. In addition, we hypothesized that alterations in cortisol may predict menses recovery, either as a consequence of recovery of the H-P-G axis from decreasing cortisol levels or from greater increases in fat mass and leptin in AN girls with higher cortisol levels at baseline.

SUBJECTS AND METHODS

Subject selection. Data from two previously completed studies by Soyka *et al.* (19) and Misra *et al.* (3,4,20) were pooled to determine predictors of menstrual recovery in girls with AN. Of the 42 adolescent girls with AN (meeting the DSM-IV criteria) and 40 controls 12–18 y old enrolled in these studies, follow-up data at 6 or 12 mo was available for 37 AN subjects and 35 controls. Of these adolescents, 10 AN subjects and two controls were premenarchal. Among the premenarchal group, girls with delayed menarche [age older than 15.3 y (mean age at menarche + 2 SDs for American girls) (21)] were included in our study because delayed menarche was likely subsequent to low weight 3 and onset of menses expected following weight gain. Girls younger than 15.3 y old were not included because they were still in the normal age range for attaining menarche. Thus, four AN subjects and two controls were excluded from the final analysis, and 33 AN subjects and 33 healthy adolescents were evaluable for predictors of menstrual recovery (Fig. 1).

Baseline characteristics and methods of recruitment for 16 controls and 15 AN subjects have been reported in a previous article by Soyka *et al.* (19) and for 17 controls and 18 AN subjects in articles by Misra *et al.* (3,4,20). Characteristics of girls with AN did not differ in the two studies nor did characteristics for controls. The overall mean age was 16.1 ± 1.5 y in AN subjects and 15.4 ± 1.6 y in controls ($p = \text{not significant}$). Bone age was 15.7 ± 1.5 y in AN subjects and 15.8 ± 1.6 in healthy adolescents ($p = \text{not significant}$). Mean duration since diagnosis was 5.0 ± 9.4 mo, and mean duration of amenorrhea was 10.6 ± 10.2 mo. Mean age at menarche among postmenarchal girls was 12.8 ± 1.5 y in AN subjects *versus* 12.4 ± 1.0 y in controls ($p = \text{not significant}$). Postmenarchal AN subjects had been amenorrheic at least 3 mo at study initiation. Nineteen of 33 AN subjects (57.6%) recovered menstrual function during the 1-y follow-up. Recovery of menstrual function was defined as three or more menstrual periods over the preceding 6 mo with at least one menstrual period in the preceding 3 mo. Healthy controls did not have a history of eating disorders. Our institutional review board approved the study, and informed assent and consent was obtained from all.

Experimental protocol. Subjects were screened to rule out thyroid dysfunction, hypergonadotropic hypogonadism, and hyperprolactinemia and were evaluated at a baseline visit and at 6 and 12 mo. The baseline visit included a history and physical examination and fasting blood sample for leptin. Subjects from the study by Misra *et al.* (3,4,20) (17 controls and 18 AN

subjects) had frequent sampling for cortisol level performed every 30 min overnight (2000 h to 0800 h) at the General Clinical Research Center (GCRC) of Massachusetts General Hospital. Total AUC for cortisol was calculated using the computerized algorithm Cluster (1×2) (22). For all subjects, body composition was assessed by DXA. A 24-h urine collection was completed UFC and creatinine (cr), and baseline UFC/cr-m² was available in 29 AN subjects and 29 controls.

Subjects were followed over a year and leptin and body composition measurements repeated at 6 and 12 mo. Twelve-month data were available for 29 AN subjects and 29 controls, and 6-mo data in an additional four AN subjects and four controls. Among the four AN girls for whom only 6-mo data were available, two resumed menses by 6 mo and continued to maintain menstrual function, whereas the two AN girls who had not resumed menses at the 6-mo follow-up continued to be amenorrheic and of low weight at 12 mo (information from primary care provider and parent). These girls were included in the study given that their clinical and menstrual status at 12 mo was not different from that at 6 mo. Frequent sampling for cortisol was repeated at 10% increase in BMI in 11 of the 18 AN girls from the study by Misra *et al.* (4). Eight of these 11 had resumed menses. UFC/cr-m² data at follow-up were available in 16 AN and 13 controls [all from the study by Misra *et al.* (4) because the study by Soyka *et al.* (19) did not include urinary cortisol measurements after the baseline visit].

Anthropometric measurements. Height was measured at the GCRC in triplicate on a single stadiometer and averaged. Weight was measured on an electronic scale in a hospital gown. BMI was calculated as the ratio of weight (kg) to height (m²). Standards of Greulich and Pyle (23) were used to determine bone age. Body composition was determined using whole-body DXA (QDR 4500, Hologic Inc., Waltham, MA) (24,25). The percentage of trunk fat was calculated as follows: $[\text{trunk fat}/\text{total fat}] \times 100$ (26).

Biochemical assessment. We used radioimmunoassay (RIA) to measure serum cortisol (Diagnostic Products Corp., Los Angeles, CA) [limit of detection, 1 µg/dL; sensitivity, 0.21 µg/dL; coefficient of variation (CV), 2.5–4.1%] and leptin (Linco Diagnostics, St. Louis, MO) (sensitivity, 0.5 ng/mL; CV, 3.4–8.3%). Samples were stored at –80°C until analysis and were run in duplicate. UFC was measured by the hospital laboratory using the GammaCoat 125I RIA (Diasorin Inc., Stillwater, MN; detection limit 1 µg/dL; CV 7%).

Statistical methods. All data are described as mean $\pm$ SD. Data were analyzed using the JMP program (version 4, Cary, NC). A *t* test was used to calculate differences between means. When comparisons involved more than two groups, we used analysis of variance (ANOVA) followed by the Tukey-Kramer test for intergroup comparisons. A paired *t* test was used to compare endpoints at follow-up *versus* baseline. To compare proportions, we used Fisher's exact test. Correlational analysis was used to determine associations between continuous variables such as baseline serum or urinary cortisol, baseline body fat, and changes in body fat. Logistic regression was used to determine predictors of menstrual recovery and odds ratios calculated from parameter estimates of covariates. For the four subjects in whom only baseline and 6-mo data were available, 6-mo data were carried forward for analysis. For these girls, 12-mo values were imputed based on within-group average change between 6 and 12 mo, and we found similar differences between the groups as with carry forward analysis.

RESULTS

Comparison of AN girls who recovered menses versus those who did not recover menses versus healthy adolescents. Girls with AN recovering menses did not differ from AN girls not recovering menses for baseline BMI, fat mass, percentage of body fat, and leptin (Table 1). Both AN groups had lower BMI, fat mass, percentage of body fat, and leptin than controls. Baseline cortisol AUC [performed only in subjects from the study by Misra *et al.* (4)] was higher in AN recovering menses compared with the other groups ($p < 0.0001$, ANOVA) (Fig. 2). For girls who underwent frequent sampling for cortisol (4), when AN girls were dichotomized based on median cortisol AUC, eight of the nine girls with a cortisol level greater than the median resumed menses *versus* four of nine girls with a cortisol level below the median ($p = 0.07$). Baseline UFC/cr-m² trended higher in AN girls *versus* controls. Correlation between cortisol AUC and UFC/cr-m²

Figure 1. Flow diagram describing the process of selection of 33 girls with AN and 33 healthy adolescents from two studies (4,19) for determination of predictors of menstrual recovery.

Figure 2. Baseline cortisol AUC in AN girls who recovered menses ($n = 13$) (shaded column), AN girls who did not recover menses ($n = 5$) (solid column), and controls ($n = 17$) (open column). ANOVA demonstrated a significant difference between the groups ($p < 0.0001$). AN girls who recovered menses had higher baseline cortisol AUC than controls ($p < 0.05$).

Figure 3. Changes in fat mass in AN girls who recovered menses ($n = 19$) (shaded column), AN girls who did not recover menses ($n = 14$) (solid column), and controls ($n = 33$) (open column). ANOVA demonstrated a significant difference between the groups ($p < 0.0001$). AN girls who recovered menses had greater increases in fat mass than AN girls who did not resume menses and controls ($p < 0.05$ for both).

was fair ($r = 0.52$, $p = 0.002$ for all subjects; $r = 0.56$, $p = 0.01$ for AN girls).

Girls with AN recovering menses had greater increases in BMI, fat mass, percentage of body fat, percentage of trunk fat, and leptin than healthy adolescents, and greater increases in BMI, fat mass, and percentage of body fat than AN not recovering menses (Table 1). Change in fat mass in the three groups is illustrated in Figure 3. When AN girls were divided into two groups based on median change in BMI (Δ BMI) over follow-up, 12 of the 16 girls with Δ BMI greater than the median resumed menses *versus* seven of the 17 girls with Δ BMI less than the median ($p = 0.05$). Similarly, when AN girls were dichotomized based on median change in fat mass (Δ fat mass) over follow-up, 13 of 16 AN girls with Δ fat mass greater than the median resumed menses *versus* five of the 16 AN girls with Δ fat mass less than the median ($p = 0.006$). When AN girls were dichotomized based on median change in

leptin (Δ leptin), 11 of 16 girls with Δ leptin greater than the median resumed menses *versus* seven of 16 girls with Δ leptin less than the median ($p =$ not significant).

Final measurements of BMI, fat mass, percentage of body fat, percentage of trunk fat, and leptin and cortisol levels were compared (Table 1). Final BMI, fat mass, and percentage of body fat were higher in AN girls resuming menses *versus* those who did not but continued to be lower than in controls. All AN girls with a final percentage of body fat $>24.4\%$ regained menstrual function, whereas no AN girl with a final percentage of body fat $<18.1\%$ regained menstrual function (Fig. 4). Final percentage of trunk fat in AN girls resuming menses approached that in controls and was higher than in AN girls not resuming menses.

Paired t tests of baseline and 12-mo data in AN who resumed menses (Table 2) showed that girls recovering men-

Table 1. Differences between girls with anorexia nervosa who resumed menses versus those who did not resume menses and healthy adolescents

	Anorexia nervosa			p (ANOVA)
	Recovered menses ($n = 19$)	Not recovered menses ($n = 16$)	Controls ($n = 33$)	
Baseline BMI (kg/m^2)	$16.8 \pm 1.2^*$	$16.4 \pm 1.1^*$	22.3 ± 3.3	<0.0001
Baseline fat mass (kg)	$9.2 \pm 3.3^*$	$7.6 \pm 2.5^*$	18.7 ± 5.2	<0.0001
Baseline % body fat	$19.2 \pm 5.4^*$	$17.4 \pm 4.9^*$	31.1 ± 5.5	<0.0001
Baseline % trunk fat	34.1 ± 5.8	$31.3 \pm 4.6^*$	37.7 ± 5.4	0.001
Baseline leptin (ng/mL)	$4.1 \pm 3.0^*$	$3.5 \pm 2.4^*$	14.5 ± 6.7	<0.0001
Baseline UFC/cr- m^2 ($\mu\text{g}/\text{mg cr-m}^2$)§	$0.044 \pm 0.029^*$	0.040 ± 0.028	0.023 ± 0.011	0.006
Final BMI (kg/m^2)	$20.0 \pm 2.1^{*\dagger}$	$17.1 \pm 1.4^*$	22.6 ± 3.3	<0.0001
Final fat mass (kg)	$14.3 \pm 3.9^{*\dagger}$	$8.5 \pm 2.3^*$	18.5 ± 5.4	<0.0001
Final % body fat	$25.5 \pm 4.8^{*\dagger}$	$18.1 \pm 3.7^*$	30.3 ± 5.9	<0.0001
Final % trunk fat	36.5 ± 4.1	$32.3 \pm 4.2^*$	37.2 ± 5.6	0.01
Final leptin (ng/mL)	8.7 ± 5.3	$5.3 \pm 4.1^*$	13.6 ± 8.8	0.002
Final UFC/cr- m^2 ($\mu\text{g}/\text{mg cr-m}^2$)§	0.033 ± 0.031	0.030 ± 0.007	0.028 ± 0.011	ns
Δ BMI (kg/m^2)	$3.2 \pm 2.2^{*\dagger}$	0.8 ± 1.3	0.3 ± 1.2	<0.0001
Δ Fat mass (kg)	$5.1 \pm 3.9^{*\dagger}$	0.8 ± 2.7	0.1 ± 2.9	<0.0001
Δ % body fat	$6.3 \pm 5.5^{*\dagger}$	0.7 ± 5.1	-0.7 ± 3.5	<0.0001
Δ % trunk fat	$2.3 \pm 3.3^*$	1.1 ± 5.4	-0.4 ± 2.1	0.04
Δ Leptin (ng/mL)	$4.7 \pm 5.1^*$	1.9 ± 4.6	-0.9 ± 7.2	0.01
Δ UFC/cr- m^2 ($\mu\text{g}/\text{mg cr-m}^2$)§	-0.004 ± 0.035	0.000 ± 0.022	0.009 ± 0.016	ns

* Significantly different from controls (Tukey-Kramer test) ($p < 0.05$).

† Significantly different from AN girls who did not recover menses (Tukey-Kramer test) ($p < 0.05$).

§ Baseline UFC/cr- m^2 was available for 29 AN subjects and follow-up data for 16 AN subjects.

ns, not significant.

Figure 4. Final percentage of body fat in AN girls who resumed menses ($n = 19$) and those who did not ($n = 14$). All girls with a final percentage of body fat $>24.4\%$ resumed menses, whereas no girl with a final percentage of body fat $<18.1\%$ regained menstrual function.

Table 2. Paired t test analysis in AN girls who resumed menses ($n = 19$)

	Baseline	Final	p
BMI (kg/m^2)	16.6	18.7	<0.0001
Fat mass (kg)	8.5	11.7	<0.0001
% Body fat	18.4	22.2	0.001
% Trunk fat	32.2	38.2	0.09
Leptin (ng/mL)	3.7	7.2	0.0004
UFC/cr- m^2 ($\mu\text{g}/\text{mg cr-}\text{m}^2$)	0.036	0.033	ns
($n = 12$)			
Cortisol AUC ($\mu\text{g}/\text{dL}\cdot 12\text{ h}$)	6566	5902	ns
($n = 8$)			

SA, surface area; ns, not significant.

ses had significant increases in BMI, fat mass, percentage of body fat, percentage of trunk fat, and leptin, but not in UFC/cr- m^2 . Frequent sampling for cortisol was repeated in 11 AN girls who gained $>10\%$ of their BMI during follow-up. Eight of these girls also resumed menses. In this subset who underwent repeat frequent sampling, paired t tests in girls resuming menstrual function demonstrated significant increases in BMI (16.9 versus 19.4 kg/m^2 , $p = 0.0008$), fat mass (9.3 versus 13.4 kg, $p = 0.0002$), percentage of body fat (18.8 versus 24.1%, $p = 0.0008$), and leptin (4.6 versus 9.0 ng/mL , $p = 0.007$) similar to the group as a whole who resumed menses. Cortisol AUC decreased minimally from 6683 to 5940 $\mu\text{g}/\text{dL}\cdot 12\text{ h}$ in this subset ($p =$ not significant).

Cortisol as a predictor of changes in body composition over time in AN. Baseline cortisol AUC predicted Δ BMI ($r = 0.54$, $p = 0.02$) and was a strong predictor of Δ fat mass and percentage of body fat ($r = 0.70$, $p = 0.002$ and $r = 0.75$, $p = 0.0006$) (Fig. 5). Baseline fat mass and leptin did not predict Δ fat mass in AN. However, baseline percentage of body fat did predict Δ percentage of body fat ($r = -0.49$, $p = 0.005$). When baseline percentage of body fat, cortisol AUC, and leptin were entered into a regression model, the sole significant predictor of Δ percentage of body fat was baseline cortisol AUC, which contributed to 55.9% of the variability. UFC/cr- m^2 also predicted Δ percentage of body fat ($r = 0.40$, $p = 0.04$) (Fig. 5) and remained an independent predictor after adjusting for baseline percentage of body fat (8.3% of the variability). In the group as a whole, cortisol was again a

Figure 5. Relationship between baseline cortisol AUC and subsequent changes in percentage of body fat and between UFC/cr- m^2 and changes in percentage of body fat in AN. Strong positive associations were observed between cortisol AUC and changes in percentage of body fat ($r = 0.75$, $p = 0.0006$; $n = 18$) and between UFC/cr- m^2 and changes in percentage of body fat ($r = 0.40$, $p = 0.04$; $n = 29$).

significant and independent predictor of changes in percentage of body fat over time (data not reported).

Baseline characteristics in AN with baseline cortisol AUC above the median versus those with AUC below the median did not differ [BMI ($16.7 \pm 1.5\text{ kg}/\text{m}^2$ versus $16.7 \pm 1.1\text{ kg}/\text{m}^2$), fat mass ($9.2 \pm 3.5\text{ kg}$ versus $8.8 \pm 2.7\text{ kg}$), percentage of body fat ($18.7 \pm 4.5\%$ versus $18.2 \pm 4.5\%$), duration of illness ($8.4 \pm 2.5\text{ mo}$ versus $3.9 \pm 2.5\text{ mo}$), or duration of amenorrhea ($3.6 \pm 1.9\text{ mo}$ versus $5.3 \pm 1.9\text{ mo}$)].

Predictors of menstrual recovery (logistic regression).

When logistic regression was performed using Δ BMI, Δ body fat, and Δ leptin to determine which of these parameters predicted menstrual recovery, Δ body fat, but not Δ leptin or Δ BMI, was a significant predictor of menstrual recovery ($p = 0.01$). For every quartile increase in Δ fat mass, the odds ratio (OR) for recovering menses was 3.0 ($p = 0.009$), and for an increase in Δ fat mass from below the median to above the median, the OR of recovering menses was 9.5 ($p = 0.007$). Similarly, when final percentage of body fat, final BMI, and final leptin were entered into a logistic regression model, final percentage of body fat was a significant predictor of menstrual recovery ($p = 0.03$). The OR of resuming menses for final percentage of body fat above the median compared with below the median was 15.5 ($p = 0.003$). Trunk fat was not an independent predictor of menstrual recovery.

DISCUSSION

We demonstrate that a higher baseline cortisol level in AN predicts greater subsequent increases in body fat, and increase in body fat predicts menstrual recovery. Thus, AN girls with higher cortisol levels at baseline may gain more fat mass with weight gain, predicting greater chances of menstrual recovery. The role of cortisol in predicting menstrual recovery in AN may stem not from its effect on the H-P-G axis, but from the effect of prerecovery cortisol concentrations on subsequent changes in body composition.

In healthy women, high-dose cortisol causes either a decrease (12,13) or no change (16) in LH pulsatility, and Rickenlund *et al.* (15) have reported an inverse correlation between cortisol and menstrual frequency. AN is associated with hypercortisolemia in adults (27) and adolescents (4), and it is

tempting to postulate that hypercortisolemia may contribute to hypogonadotropic hypogonadism associated with AN. However, in this study, baseline serum cortisol was higher in AN girls who recovered menses compared with the group that did not recover menses and controls, and UFC/cr-m² was higher in AN recovering menses *versus* controls. No change in overnight serum cortisol occurred with weight gain in eight AN girls who also recovered menses. Given that marked increases in BMI, fat mass, and leptin did occur in this subset who underwent repeat frequent sampling following weight gain suggests that small sample size cannot explain the lack of change in cortisol with menses recovery. AN girls may have residual elevations in cortisol despite increased weight, and, indeed, adult AN studies do report that weight gain is not associated with changes in cortisol (18).

Baseline characteristics including BMI, fat mass, and duration of illness or of amenorrhea did not differ in AN subjects with higher *versus* lower cortisol values (based on median AUC). Thus, higher baseline cortisol was not indicative of severity of illness. Baseline cortisol was a strong and independent predictor of increase in fat mass after adjusting for baseline fat and leptin. Similarly, UFC/cr-m² independently predicted increase in body fat. AN girls with higher baseline cortisol are thus more likely to have greater increases in fat mass with weight gain, particularly because cortisol does not decrease with weight gain. Increase in fat mass, in turn, independently predicted menstrual recovery on logistic regression. This is in contrast to data reported by Golden *et al.* (11) in which fat mass did not differ in AN girls who recovered menses *versus* those who did not.

In our analyses, serum cortisol was a stronger predictor of changes in fat mass than was urinary cortisol. In a previous study (4), we reported that higher serum cortisol concentrations observed in girls with AN are a consequence of increased secretory frequency and half-life. We postulate that UFC/cr-m² is a less sensitive measure than serum cortisol of hypercortisolemia in AN due to increased cortisol half-life and reduced urinary clearance in AN. In addition, although detailed instructions were provided to all subjects regarding 24-h urine collection, we suspect lack of completeness of this collection in some adolescents noted to have very low 24-h urinary volumes. Although correction for creatinine would take into account some inaccuracies in collection, diurnal variation limits the accuracy of this correction method. Therefore, our serum frequent sampling cortisol data are likely more accurate than our urinary cortisol data. Studies have demonstrated that cortisol binding globulin (CBG) levels are not elevated in AN (28). Therefore, we do not believe that lack of a stronger correlation between serum and urinary cortisol is a consequence of high CBG levels in AN. In addition, baseline BMI and body composition did not differ between girls who did (4) and did not (19) have frequent sampling for serum cortisol performed (data not reported). Thus, it is not likely that innate differences between subjects from the two studies may have accounted for the stronger correlations of serum cortisol with body fat *versus* that of UFC/cr-m² with body fat. These data would, of course, be stronger if overnight serum cortisol estimations had been performed for all subjects.

Leptin is an adipocytokine that is low in AN (3,29–31), and leptin deficiency or resistance causes hypogonadism (6–9). Administration of Rh leptin to women with hypothalamic amenorrhea caused resumption of menstrual function in five of eight women (10). Given these data, we expected an association between change in leptin and resumption of menses. Indeed, AN girls who resumed menses had greater increases in leptin than controls. When compared with AN girls not recovering menses, change in leptin in the menses-recovered group was higher but did not reach statistical significance. A significant difference may have been observed with more subjects. However, with this sample size, we did observe significantly greater increases in fat mass in the group recovering menses *versus* the group not recovering menses. In addition, on regression modeling, increase in body fat (but not in leptin) predicted menstrual recovery. These data suggest that increase in fat mass may result in signals to the H-P-G axis that cause an awakening of this axis separate from those associated with increases in leptin. However, mediators of effects of increased fat mass on the reproductive axis are unclear, and more studies are necessary to confirm these findings.

In this study of adolescents with AN, we demonstrate that increases in fat mass are the most important predictor of menstrual recovery and that baseline cortisol most strongly predicts subsequent increases in fat mass. Although increases in leptin were greater in AN girls who recovered menses *versus* those not recovering menses, leptin did not independently predict menstrual recovery in this study. The mechanism whereby increases in fat mass cause recovery of the H-P-G axis independent of increases in leptin is unclear, but maybe related to signaling by fat-related factors yet to be characterized.

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