Biomarkers of Length of Stay On An Inpatient Eating Disorder Unit For Treatment of Anorexia Nervosa in Females

Courtney E. Breiner
University at Albany, State University of New York
Baiyu Qi
University of North Carolina at Chapel Hill
Laura M. Thornton
University of North Carolina at Chapel Hill
Kimberly A. Brownley
University of North Carolina at Chapel Hill
Tonya Foreman
University of North Carolina at Chapel Hill
Anna M. Bardone-Cone
University of North Carolina at Chapel Hill
Cynthia M. Bulik
Karolinska Institute
Jessica H. Baker (jessica_baker@med.unc.edu)
University of North Carolina at Chapel Hill

Research Article

Keywords: anorexia nervosa, magnesium, ghrelin, biomarkers, length of stay

Posted Date: January 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1228895/v1

Abstract

Background. Length of stay on an inpatient unit for treatment of anorexia nervosa (AN) is widely variable. Although previous research has used anthropometric and clinical variables and duration of illness to predict length of stay, there has been limited investigation of the predictive ability of biomarkers. Biomarkers, including those collected through a comprehensive metabolic panel (CMP) and appetite hormones, such as ghrelin and leptin, are impacted by disease presence and may play an etiological role in AN.

Methods. Using a series of regression models, we retrospectively evaluated the associations of these putative biomarkers at admission with length of inpatient stay in 59 females receiving treatment on an inpatient eating disorder unit for anorexia nervosa.

Results. Both lower levels of magnesium and higher active ghrelin levels at inpatient admission predicted length of stay.

Conclusions. This research provides further evidence supporting both biological and psychological components of AN, identifying potential biomarkers that could aid in prospective prediction of treatment needs. Ghrelin monitoring throughout inpatient stays may aid clinicians in better predicting physical recovery and renourishment from AN and prepare for stepdown from an inpatient setting. Further research is necessary to replicate and extend these findings across treatment settings.

Summary

Anorexia nervosa (AN) has one of the highest mortality rates of any mental disorder, due in part to the severe medical complications of the disorder. Inpatient medical stabilization is the highest level of care for the disorder. Currently, we have little information on how to predict how long an individual needs to remain in inpatient medical stabilization. This study examined which biological markers may be predictive of the length of treatment stay. In 59 women with AN, higher active ghrelin and lower magnesium levels at admission predicted a longer length of stay. Although inpatient programs typically track magnesium levels, ghrelin tracking is not currently part of the standard of care in most inpatient facilities. Our research suggests ghrelin monitoring throughout inpatient stays may be a marker of recovery and ability to step down to lower levels of care.

1. Background

Anorexia nervosa (AN) has one of the highest mortality rates of any mental illness and is more deadly than other eating disorders (Fichter & Quadflieg, 2016; Smink et al, 2012). AN involves severe psychological and medical sequelae (Westmoreland et al., 2016), and consequences of AN, such as extreme underweight status, malnutrition, nutrient deficiencies, and other medical complications, often require inpatient treatment for medical stabilization and psychological treatment. Directly conflicting with insurance-driven institutional efforts to shorten inpatient stays to decrease treatment costs (Weissman &

Rosselli, 2017; Wiseman et al., 2001), premature discharge from inpatient treatment for AN leads to patients discharging at lower weights than may be medically recommended (Sly et al., 2013; Vandereycken, 2003) and to adverse outcomes, such as requiring additional hospitalization(s) after discharge (Pike, 1998). Together, this may ultimately lead to higher rates of inpatient readmission and, paradoxically, additional treatment costs (Vandereycken, 2003). Reliable, prospective predictors of inpatient length of stay could improve inpatient clinical management, yet few established predictors exist. Biomarkers may be particularly useful for predicting length of stay due to their objective nature and established associations with AN-illness status in combination with the fact that many biological indices are already tracked as part of routine clinical care in inpatient treatment settings. Thus, the primary goal of this study was to identify potential biomarkers of length of stay for patients with AN on an inpatient eating disorder unit.

Significant genetic correlations have been observed between AN and body mass index (BMI), insulin resistance, fasting insulin, leptin, high density lipoprotein (HDL) cholesterol, and type 2 diabetes suggesting that some of the genetic factors that influence AN and certain metabolic and arthrometric phenotypes are shared (Watson et al., 2019). Additionally, AN is associated with alterations in several metabolic indices. For example, patients with AN have increased insulin sensitivity compared with women without an eating disorder, which persists into the weight-restoration period (Dostálová et al., 2007; Brown et al., 2003; Kim et al., 2019; Ilyas et al., 2019).

Appetitive hormone dysregulation is also observed in AN (Hebebrand et al., 2007). Patients with AN have significantly *lower* leptin and *higher* active and total ghrelin levels than healthy controls (Schalla & Stengel, 2018). Leptin levels increase with weight gain in AN (Hebebrand et al., 2007; Ruscica et al., 2016), giving possible insight regarding whether a patient is recovering (i.e., together, weight and leptin levels are two indicators of treatment progress; Misra & Klibanski, 2010). Conversely, both active and total ghrelin levels decrease with weight gain after refeeding without psychological intervention (Nakahara et al., 2007; Schalla & Stengel, 2018). The genetic and phenotypic associations observed between multiple biological indices and AN suggest potential avenues for the identification of inpatient length of stay biomarkers. Examining the utility of biomarkers in predicting treatment course is a natural extension of this research. It is important to note that although these biomarkers may be related to starvation rather than AN pathophysiology per se, they remain important to examine as low weight is often a primary reason for inpatient treatment.

The goal of this study was to examine whether peripheral biomarkers from blood samples of AN inpatient length of stay could be identified. Peripheral biomarkers from blood have the benefit of being able to index biological factors from a variety of systems (e.g., stomach, kidneys, endocrine system). Here, we included biological indices typically captured as part of a comprehensive metabolic panel (CMP)—given this is often routinely monitored during inpatient treatment—and the appetite hormones ghrelin and leptin. The only *a priori* hypotheses we had were in regard to appetite hormones: namely that lower levels of leptin and higher levels of ghrelin at admission would be significantly associated with a longer length of

stay. We considered the inclusion of the CMP as exploratory. Identifying biomarkers of inpatient length of stay for AN may provide additional information for AN treatment monitoring and stabilization.

2. Methods

2.1. Study population

Patients were recruited from an inpatient eating disorder unit at an academic medical center. Participants (N=73) were females at least 15 years of age who were referred and evaluated for inpatient treatment for AN. Patients were diagnosed with AN by the inpatient unit psychiatrist using standard semi-structured interviewing procedures. The inpatient program provides 24-hour in-hospital care for adolescents and young adults with eating disorders. The program focuses on nutritional rehabilitation, weight restoration, and medical monitoring. However, individual, group, and family therapy, along with nutritional counseling, are also provided. Patients undergo comprehensive lab monitoring on admission to the unit and throughout their hospitalization. They are monitored for signs and symptoms of refeeding syndrome. They are also evaluated daily by a psychiatrist. Psychotropic medications are prescribed if indicated.

Patients are typically hospitalized until they reach approximately 85% of target weight (if weight restoration is needed). When patients do not need weight restoration, they are hospitalized until they are medically stable and able to sustain their weight by mouth with food (rather than liquid supplement). Approximately 10% of patients require a nasogastric tube during their stay for nutritional rehabilitation. Patients are typically discharged to outpatient treatment or to a residential treatment center for individuals with eating disorders.

One patient withdrew consent. Of the 72 remaining patients, a total of 59 participants had complete data available for all of the biomarkers of interest and were included in the current analyses. Nineteen participants were considered “self-discharged,” defined as discharging prematurely or prior to the treatment team’s recommendation. This study was approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill. Along with providing informed consent to participate in the study, participants also signed a HIPAA consent form so that treatment information (e.g., chemistry labs, admission height/weight, discharge date) could be obtained from the medical records.

2.2. Patient characteristics

Age at inpatient admission, height, and weight were obtained for all participants. Inpatient staff measured height and weight at admission and discharge. BMI (kg/m^2) was calculated based on these values. Ideal body weight (IBW) was calculated as [100+5*(height in inches - 60)]; %IBW was calculated as (weight / IBW) *100.

2.3. Length of stay

Length of stay, the primary outcome variable, was defined as the number of days a participant received treatment on the inpatient unit. This was calculated as discharge date minus admission date. Self-discharge status was also coded as discharging prematurely or prior to the treatment team's recommendation (yes/no).

2.4. Blood samples

Blood samples were measured within 5 days of admission. Fasting blood draws occurred in the morning, prior to breakfast. Blood samples for the CMP, as well as magnesium and phosphorus, were delivered to and processed by UNC Healthcare McClendon Hospital Laboratory Services (CLIA-certified). The CMP included the following: sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, glucose, calcium, total protein, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels.

Additional blood samples were collected for leptin, total and active ghrelin, and estradiol assays. Blood samples for hormone assays were obtained during regularly scheduled blood draws at admission. Total ghrelin (pg/ml), active ghrelin (pg/ml), and leptin (ng/ml) plasma hormone levels were measured using double antibody Radioimmunoassay (RIA) reagents and protocols commercially available from EMD Millipore, Billerica, MA. Intra-assay coefficients of variability (CV's) are 7.9%, 6.7%, and 4.6% respectively, and inter-assay CV's are 14.7%, 9.6%, and 5.0%. The sensitivity of the total ghrelin assay is 93 pg/ml with a standard range of 120-7700 pg/ml. Active ghrelin assay has a sensitivity of 7.8 pg/ml with a standard range of 6.9-1770 pg/ml. The sensitivity of the leptin RIA is .437ng/ml, and the standard range is .78 to 100 ng/ml. Plasma 17B-estradiol levels were measured using a coated tube RIA kit from MPBiomedicals, Costa Mesa, CA. The intra- and inter- assay CV's are 3.5% and 7.6% respectively, and the sensitivity is 4 pg/ml with a standard range of 10-3000 pg/ml.

2.5. Statistical analysis

Statistical analyses were conducted in SAS 9.4 (SAS Inc.). Descriptive information was generated for patient characteristics and potential biomarkers at admission. We applied generalized linear model to predict length of stay on the inpatient unit from each biomarker. Due to a small sample size, we only included age and BMI as covariates in each model to reduce power lost.

Based on the results of the generalized linear models, we next used backward stepwise regression to obtain a more parsimonious prediction model, and to understand the importance of each biomarker in determining length of stay. This model included all significant biomarkers (p<0.05) from the linear regression models. Using stepwise regression allowed us to identify the best set of biomarkers to predict length of stay that we might evaluate further for clinical use. Age and BMI at admission were also included in the model as covariates but were allowed to leave the model in the elimination process. The variable with the least significant effect that did not meet the 0.05 significance threshold for staying in the model was removed from the model. The elimination process was repeated until no improvement of the model was achieved by removing new variables, and all remaining effects in the model met the

significance threshold (p<0.05) (Heinze et al., 2018). Adjusted R^2 were calculated to measure the proportion of the variance for length of stay that was explained by the final model. Standardized regression coefficients were calculated to estimate and compare the individual effects of each predictor.

Finally, based on the number of self-discharges (n=19) and the number of adolescents (n=10 aged 15-18), two post-hoc sensitivity analyses were conducted to assess the impact of self-discharge and age on the results. Specifically, generalized linear models and a backward stepwise regression model were conducted for 1) patients with approved discharge; 2) adults only to evaluate whether the same biomarkers that predicted length of stay in the full sample remained significant for the two sub-samples. Due to our limited sample size, in addition to statistical significance, we also used standardized regression coefficients to measure effect sizes.

Notably, because we considered this study exploratory, we decided *a priori* to not correct for multiple comparisons. Correction for multiple comparisons increases the risk for type-2 error (i.e., non-rejection of a false null hypothesis), which may prematurely discard useful observations (Rothman, 1990). Here, we judged potential clinical utility as more important than statistical significance. Future studies will need to confirm any observed associations.

3. Results

3.1. Demographics

Table 1 summarizes patient characteristics at admission. Our study sample consisted of 27 (45.76%) patients with restricting-type AN, 29 (49.15%) patients with binge-eating/purging-type AN, and 3 (5.08%) patients with atypical AN. The average age of the patients was 29.42 (*SD*=13.21) years, with a range of 15 to 77 years. The average BMI was 14.97 (SD=9.23) kg/m^2. The mean length of stay on the inpatient unit was 29.68 (*SD*=18.65) days. Of 19 patients who self-discharged, the mean length of inpatient stay was similar to the full sample mean: 27.26 (21.02) days, with a range of 3 to 78 days.

Baseline characteristics (i.e., age, AN subtype, BMI, %IBW, and all biomarkers at admission) did not differ between those who self-discharged and those who did not, indicating that those who self-discharged are a heterogeneous population in their own right, might be medically stable, and might not have a great impact on results. Thus, they were still included in our primary analysis.

Table 1. Patient Characteristics at Inpatient Admission for total sample and by discharge status.

Variables	*Total sample (N=59)*	*Self-discharge (n=19)*	*Approved discharge (n=40)*
	n (%)		
AN subtype at admission			
Restricting type	27 (45.76)	8 (42.11)	19 (47.50)
Binge-eating/purging type	29 (49.15)	10 (52.63)	19 (47.50)
Atypical AN	3 (5.08)	1 (5.26)	2 (5.00)
≥85% IBW	4 (6.78)	0 (0)	4 (10.00)
	Mean (SD)		
Age at admission (years)	29.42 (13.21)	31.47 (13.53)	28.45 (13.12)
BMI (kg/m^2)	14.97 (1.89)	14.63 (1.81)	15.14 (1.94)
%IBW	72.40 (9.23)	70.65 (8.56)	73.23 (9.52)
Biomarkers			
Sodium (mmol/L)	139.11 (2.73)	138.42 (2.57)	139.43 (2.77)
Potassium (mmol/L)	4.19 (0.53)	4.05 (0.55)	4.26 (0.51)
Chloride (mmol/L)	102.25 (4.27)	101.89 (5.18)	102.43 (3.82)
Urea Nitrogen (mg/dL)	13.75 (5.07)	12.42 (4.72)	14.38 (5.17)
Bicarbonate (mmol/L)	27.34 (3.64)	27.89 (3.71)	27.08 (3.62)
Creatinine (mg/dL)	0.78 (0.20)	0.79 (0.24)	0.77 (0.18)
Glucose (mg/dL)	79.05 (9.85)	79.58 (8.17)	78.80 (10.64)
Calcium (mg/dL)	9.32 (0.53)	9.25 (0.53)	9.36 (0.54)
Magnesium (mg/dL)	1.96 (0.19)	1.97 (0.22)	1.96 (0.18)
Phosphorus (mg/dL)	4.11 (0.69)	4.16 (0.65)	4.08 (0.72)
Total Protein (g/dL)	6.61 (0.89)	6.46 (0.90)	6.68 (0.89)
Albumin (g/dL)	3.93 (0.67)	3.85 (0.63)	3.97 (0.69)
Bilirubin (mg/dL)	0.55 (0.38)	0.48 (0.20)	0.58 (0.44)
Aspartate Aminotransferase (U/L)	31.86 (21.13)	32.05 (11.52)	31.78 (24.56)
Alanine Aminotransferase (U/L)	41.07 (50.81)	33.37 (17.32)	44.73 (60.48)

Alkaline Phosphatase (U/L)	55.85 (17.16)	53.74 (16.17)	56.85 (17.72)
Total ghrelin (pg/ml)	2115.36 (1135.26)	2055.29 (1038.10)	2143.89 (1190.26)
Active Ghrelin (pg/ml)	220.59 (108.37)	193.51 (85.44)	233.46 (116.48)
Leptin (ng/ml)	9.00 (6.53)	9.43 (8.79)	8.79 (5.26)
Estradiol (pg/mL)	75.96 (83.73)	89.86 (137.97)	69.37 (38.77)

AN: anorexia nervosa; IBW: ideal body weight; SD: standard deviation.

No variable differs significantly by discharge status.

3.2. Linear regression results

Table 2 shows the results from the generalized linear regression models predicting length of stay on the inpatient unit from each biomarker at admission. *Lower* magnesium and *higher* active ghrelin were significantly associated with a longer length of stay on the inpatient unit, after adjusting for admission age and BMI.

Table 2. Results from generalized linear regression analyses predicting length of stay on the inpatient unit from each biomarker at admission, adjusting for age and BMI at admission (*N*=59).

Variable	*DF*	*B*	*SE*	*β*	*t value*	*p-value*
Sodium	1	1.03	0.74	0.15	1.38	0.17
Potassium	1	3.18	3.73	0.09	0.85	0.40
Chloride	1	0.52	0.45	0.12	1.16	0.25
Urea Nitrogen	1	0.39	0.38	0.11	1.02	0.31
Bicarbonate	1	-0.22	0.54	-0.04	-0.41	0.69
Creatinine	1	-9.59	10.18	-0.10	-0.94	0.35
Glucose	1	-0.14	0.21	-0.07	-0.66	0.51
Calcium	1	2.22	4.23	0.06	0.52	0.60
Magnesium	1	-22.71	9.75	-0.23	-2.33	0.02
Phosphorus	1	0.54	3.02	0.02	0.18	0.86
Total Protein	1	-1.41	2.40	-0.07	-0.59	0.56
Albumin	1	-2.12	3.24	-0.08	-0.65	0.52
Bilirubin	1	-0.47	5.20	-0.01	-0.09	0.93
Aspartate Aminotransferase	1	0.08	0.09	0.09	0.82	0.41
Alanine Aminotransferase	1	0.07	0.04	0.19	1.81	0.08
Alkaline Phosphatase	1	0.02	0.12	0.02	0.18	0.86
Total Ghrelin	1	0.001	0.002	0.11	0.94	0.35
Active Ghrelin	1	0.04	0.02	0.23	2.03	0.05
Leptin	1	0.24	0.32	0.08	0.75	0.46
Estradiol	1	0.004	0.02	0.02	0.16	0.88

DF: degree of freedom; B: regression coefficient; SE: standard error; β: standardized regression coefficient.

Variables with significant association (p<0.05) with length of stay are bolded.

3.3. Stepwise regression results

Backward stepwise regression was used to identify which biomarkers played the most significant roles in determining length of stay on the inpatient unit (**Table 3**). Significant biomarkers from generalized linear regression models (magnesium and active ghrelin) were entered into the model along with age and BMI at admission. Magnesium, active ghrelin, and BMI were retained in the final model and jointly accounted

for 45% of the variance in length of stay. BMI (β=-0.59) had the largest effect on length of stay, followed by active ghrelin (β=0.21) and magnesium (β=-0.20). This analysis was fully powered (power=0.82).

Table 3. Biomarkers retained in the backward stepwise regression model predicting length of stay on the inpatient unit (*N*=59).

Parameter	*DF*	*B*	*SE*	*β*	*t value*	*p-value*	Adj R-Sq
							0.45
Magnesium	1	-19.70	9.65	-0.20	-2.04	0.05	
Active Ghrelin	1	0.04	0.02	0.21	2.10	0.04	
BMI	1	-5.79	1.00	-0.59	-5.81	<0.01	

DF: degree of freedom; B: regression coefficient; SE: standard error; β: standardized regression coefficient; Adj R-Sq: adjusted r-squared. *3.4. Post-hoc sensitivity analysis*

A sensitivity analysis was conducted to address the impact of participants who self-discharged on the results. Specifically, analyses were repeated including only those patients who did not self-discharge (*n*=40) (**Table 4)**. We did not identify any new significant biomarkers of length of stay in this subgroup. At admission, magnesium (β changed from -0.23 to -0.19) and active ghrelin (β changed from 0.23 to 0.22) were no longer significantly associated with length of stay. However, this sensitivity analysis was not fully powered (power=0.62).

A second sensitivity analysis showed that the effect of significant biomarkers on length of stay for the total sample was amplified for adults only (*n*=49), who accounted for the majority of the sample (83%; **Tables 4 and 5)**. At admission, *lower* magnesium (β changed from -0.23 to -0.30) and *higher* active ghrelin (β changed from 0.23 to 0.28) remained significantly associated with a longer length of stay. In the final stepwise model, magnesium (β=-0.26), active ghrelin (β=0.23), and BMI (β=-0.58) were retained and jointly accounted for 48% of the variance in length of stay. This sensitivity analysis was not fully powered (power=0.73).

Table 4. Post-hoc sensitivity analysis: generalized linear regression results predicting length of stay on the inpatient unit from each biomarker at admission for patients with approved discharge (*n*=40) and for adults only (*n*=49), adjusting for age and BMI at admission.

Variable	*DF*	*B*	*SE*	*β*	*t value*	*p-value*
Approved Discharge Only (*n*=40)						
Sodium	1	0.57	0.78	0.09	0.74	0.46
Potassium	1	-0.57	4.37	-0.02	-0.13	0.90
Chloride	1	0.42	0.56	0.09	0.75	0.46
Urea Nitrogen	1	0.01	0.41	0.003	0.03	0.98
Bicarbonate	1	-0.06	0.60	-0.01	-0.10	0.92
Creatinine	1	-9.41	11.81	-0.09	-0.80	0.43
Glucose	1	-0.04	0.20	-0.02	-0.17	0.86
Calcium	1	-1.59	4.95	-0.05	-0.32	0.75
Magnesium	1	-19.05	11.32	-0.19	-1.68	0.19
Phosphorus	1	-2.98	3.02	-0.12	-0.98	0.33
Total Protein	1	-2.40	2.62	-0.12	-0.92	0.37
Albumin	1	-2.37	3.39	-0.09	-0.70	0.49
Bilirubin	1	-0.10	4.88	-0.003	-0.02	0.98
Aspartate Aminotransferase	1	0.12	0.08	0.17	1.40	0.17
Alanine Aminotransferase	1	0.06	0.03	0.21	1.80	0.08
Alkaline Phosphatase	1	0.04	0.12	0.04	0.33	0.74
Total Ghrelin	1	0.002	0.002	0.14	1.04	0.30
Active Ghrelin	1	0.03	0.02	0.22	1.85	0.07
Leptin	1	0.004	0.42	0.001	0.01	0.99
Estradiol	1	-0.08	0.05	-0.17	-1.42	0.16
Adults Only (*n*=49)						
Sodium	1	1.03	0.83	0.15	1.24	0.22
Potassium	1	1.86	4.75	0.05	0.39	0.70
Chloride	1	0.47	0.49	0.11	0.95	0.35
Urea Nitrogen	1	0.36	0.42	0.10	0.86	0.39
Bicarbonate	1	-0.23	0.59	-0.05	-0.39	0.70
Creatinine	1	-11.38	11.11	-0.12	-1.02	0.31

Glucose	1	-0.09	0.27	-0.04	-0.34	0.73
Calcium	1	2.86	4.59	0.08	0.62	0.54
Magnesium	1	-29.60	10.81	-0.30	-2.74	0.01
Phosphorus	1	1.33	3.41	0.05	0.39	0.70
Total Protein	1	-0.65	2.83	-0.03	-0.23	0.82
Albumin	1	-1.09	3.76	-0.04	-0.29	0.77
Bilirubin	1	-0.85	5.67	-0.02	-0.15	0.88
Aspartate Aminotransferase	1	0.08	0.10	0.09	0.73	0.47
Alanine Aminotransferase	1	0.07	0.04	0.19	1.62	0.11
Alkaline Phosphatase	1	0.001	0.13	0.001	0.01	0.99
Total Ghrelin	1	0.001	0.002	0.11	0.85	0.40
Active Ghrelin	1	0.05	0.02	0.28	2.30	0.03
Leptin	1	0.36	0.37	0.12	0.97	0.34
Estradiol	1	0.002	0.02	0.01	0.10	0.92

DF: degree of freedom; B: regression coefficient; SE: standard error; β: standardized regression coefficient.

Variables with significant association (p<0.05) with length of stay are bolded.

Table 5. Post-hoc sensitivity analysis: biomarkers retained in the backward stepwise regression model predicting length of stay on the inpatient unit for adults only (n=49).

Parameter	*DF*	*B*	*SE*	*β*	*t value*	*p-value*	Adj R-Sq
							0.48
Magnesium	1	-25.55	10.57	-0.26	-2.42	0.02	
Active Ghrelin	1	0.04	0.02	0.23	2.13	0.04	
BMI	1	-5.77	1.08	-0.58	-5.36	<0.01	

DF: degree of freedom; B: regression coefficient; SE: standard error; β: standardized regression coefficient; Adj R-Sq: adjusted r-squared

4. Discussion

This study addresses an important gap in the literature, namely whether peripheral blood-based biomarkers associated with length of stay can be identified. In the initial analyses, two variables at

admission predicted longer length of stay on an inpatient unit: lower magnesium and higher active ghrelin. Our findings highlight the potential for using biological indices as additional indicators of length of stay and implicate metabolic traits as contributors to treatment course.

Low magnesium levels are common in individuals with severe AN, with hypomagnesemia occurring in up to 16% of patients at admission (Birmingham et al., 2004; Raj et al., 2012). Hypomagnesemia is a marker of refeeding syndrome (Skowrońska et al., 2019), which can ultimately lead to significant medical complications of AN, including cardiovascular disorders and sudden cardiac death (Di Cola et al., 2014). Therefore, low magnesium may predict a longer length of stay for two interrelated reasons: (1) it is indicative of more severe illness and thus (2) patients require longer refeeding periods in efforts to decrease risk of refeeding syndrome. Importantly, our results suggest that patients with lower magnesium, even if it is not at the level of clinical hypomagnesemia, may still have longer lengths of stay.

Secondly, our results indicate the potential utility of ghrelin monitoring, which is not routinely assessed during AN treatment. Active ghrelin represents the acetyl isoform of ghrelin and is an appetite stimulant, rising before food intake, along with involvement in a number of other physiological functions such as lipid storage, body weight, energy expenditure, learning and memory and mood and anxiety (Hillman et al., 2011; Müller et al., 2015). Active ghrelin has a short half-life and is subsequently converted to non-acetyl (i.e., inactive) ghrelin (Tong et al., 2013). Total ghrelin represents active plus inactive ghrelin.

Although our results are consistent with previous findings suggesting that patients with AN have increased plasma ghrelin levels compared with those without an eating disorder, our results extend this research by showing that patients with higher admission or persistent elevations in active ghrelin may need longer inpatient stay. Nutritional rehabilitation and initial weight gain do not necessarily equate with metabolic “recovery” as indexed by ghrelin, and thus higher active ghrelin may display a need for a longer stay to ensure medical stabilization and appropriateness for. Because ghrelin is involved in a number of indices likely relevant to AN treatment (e.g., appetite, mood, weight), this could suggest that persistently elevated active ghrelin levels may represent a biomarker of more severe, chronic, or metabolically unstable AN symptomatology requiring longer inpatient stays.

Collectively, our results indicate that biomarkers may be useful for predicting AN inpatient treatment length of stay. Although other disease-specific markers have been used previously [e.g., duration of illness, (Lievers at al., 2009), BMI and previous hospital admissions (Maguire et al., 2003), early treatment response (Wales et al., 2016), and autonomous control over treatment (Thaler et al., 2016)], their predictive abilities are limited and only marginally improve when combined with psychotherapist prognosis rating (Fichter & Quadflieg, 2020). Biomarkers have the benefit of being objective measures that do not rely on self-report or subjective clinical judgment. Additionally, peripheral blood-based biomarkers can be easily integrated into routine collection procedures. In particular, our findings suggest active ghrelin could be a useful addition for regular monitoring throughout routine clinical care given the prospective nature of active ghrelin at admission ultimately predicting inpatient length of stay. Definitions

of AN outcome and recovery may need to be reconceptualized to include hormone regulation and metabolic recovery.

There are limitations of this study that should be considered. First, although our total sample size (*n*=59) had enough power to detect statistically significant biomarkers of length of stay, we had limited statistical power when conducting sensitivity analyses. In our first sensitivity analysis, removing individuals who self-discharged, we did not replicate findings from the total sample. Theoretically, lack of replicability may also have been due to removing individuals who self-discharge, however we believe this explanation to be unlikely due to the heterogeneity of individuals who self-discharged in this sample. The range of days spent on the inpatient unit was wide (10 to 78 days), indicating that this was not simply a group of individuals initiating discharge soon after admission and therefore receiving limited treatment. Additionally, the average length of stay of those who did self-discharge was not significantly different from those who did not. Secondly, our effect sizes had minimal change in our sensitivity analysis excluding individuals who self-discharged, further implicating a lack of power as a reason for these findings. However, it is important to note that we do not have the specific details about the reasons for premature discharge (i.e., initiated by the patient, whether the stay was truncated by insurance coverage, other reasons), as reasons for such discharge may modify observed effects (e.g., group interaction).

In addition to limitations related to those who self-discharged, our sample included a small number of adolescent patients (*n*=10) which may have influenced results. Importantly, a sensitivity analysis excluding adolescents showed larger effect sizes for both magnesium and active ghrelin in the adult group. Biomarker predictors of inpatient length of stay may differ between adolescents and adults, and further research examining biomarkers solely in adolescents may be important. Studies of AN biomarkers including both adults and adolescents can lead to mixed findings (Peyser et al., 2021) and remains a limitation of research on biomarkers of AN. Although ghrelin is higher in both adolescent and adult females with AN compared to healthy controls (Misra et al., 2005; Tolle et al., 2003), to our knowledge, no research has examined the difference in this biomarker between age groups with AN. Therefore, research examining the predictive nature of both ghrelin and magnesium on length of stay in adolescents is vital. Importantly, this sensitivity analysis was also under-powered.

In addition to low power in sensitivity analyses and thus an inability to directly compare groups (e.g., self-discharge versus clinician-discharge, adolescents versus adults, AN-BP versus AN-R), our study was limited to solely females with AN. It is unclear if there are gender differences in biomarkers of AN, as most research has been conducted on females. Larger studies with appropriate power to conduct analyses by age group, subtype of AN, and other demographic characteristics must be conducted to replicate our findings across groups. Finally, our study did not include a comparison of controls without a lived experience with eating disorders.

Despite the limitations, the results of this study identified objective peripheral blood-based biomarkers associated with length of stay on an inpatient unit that may aid in identifying patients who require more time on an inpatient unit and, with continued research, could lead to prospective identifiers of treatment

needs and readiness for discharge. Particularly important, this study may be more representative of patients with AN given the research was conducted in a treatment setting and not in a strictly research setting (e.g., clinical trial) and thus, displays additional utility of biological indices already being collected throughout treatment. Finally, using these biomarkers may aid in our ability to argue for a longer length of inpatient stays for patients with AN; one concern for Americans with eating disorders is limited insurance coverage for inpatient treatment (Escobar-Koch et al., 2010). Future research should examine the predictive nature of the identified biomarkers across follow-up periods, and whether or not they remain associated with treatment outcomes beyond discharge.

5. Conclusions

The current study provided preliminary evidence of the utility of biomarkers in predicting the length of stay of female patients with anorexia nervosa on an inpatient eating disorder unit. Lower magnesium and higher ghrelin at intake were predictive of longer treatment stays; it may be clinically useful to include ghrelin monitoring throughout inpatient treatment for AN. Long-term, biomarkers represent another tool that could be used in the clinical management of AN and the determination for the appropriateness of inpatient discharge, ultimately improving the likelihood for positive outcomes and full recovery.

Abbreviations

AN: anorexia nervosa

BMI: body mass index

IBW: ideal body weight

Declarations

Ethics approval and consent to participate: This study was approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill.

Consent for publication: N/A

Availability of data and materials: The dataset analyzed during the current study are not publicly available due to the small number of participants and potential for data to be identified, however, data are available from the corresponding author on reasonable request.

Competing interests: The authors declare no competing interests.

Funding: This work was supported by the National Institutes of Health [R01 MH095860; K01 MH106675; R01 MH120170; R01 MH119084; R01 MH118278; U01 MH109528], the Brain and Behavior Research Foundation, the Swedish Research Council (Vetenskapsrådet. [538-2013-8864]), and the Lundbeck Foundation [R276-2018-4581].

Authors' contributions: Data was collected by LMT, KAB, TF, AMB, CMB, and JHB. JHB, LMT, BQ, and CEB conceptualized the current study. BQ and LMT analyzed data and interpreted analyses. CEB and BQ co-wrote this article. LMT, KAB, TF, AMB, CMB, and JHB edited and prepared the article for submission.

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