Myo-inositol Levels in the Dorsal Anterior Cingulate Cortex Predicts Anxiety-to-Eat in Anorexia Nervosa

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Abstract

Background. Anorexia nervosa (AN) is a mental and behavioral health condition characterized by an intense fear of weight or fat gain, severe restriction of food intake resulting in low body weight, and distorted self-perception of body shape or weight. While substantial research has focused on general anxiety in AN, less is known about eating-related anxiety and its underlying neural mechanisms. Therefore, we sought to characterize anxiety-to-eat in AN and examine the neurometabolic profile within the dorsal anterior cingulate cortex (dACC), a brain region putatively involved in magnifying the threat response.

Methods. Women seeking inpatient treatment for AN and women of healthy weight without a lifetime history of an eating disorder (healthy controls; HC) completed a computer-based behavioral task assessing anxiety-to-eat in response to images of higher (HED) and lower (LED) energy density foods. Participants also underwent magnetic resonance spectroscopy of the dACC in a 3 Tesla scanner.

Results: The AN group reported greater anxiety to eat HED and LED foods relative to the HC group. Both groups reported greater anxiety to eat HED foods relative to LED foods. The neurometabolite myo-inositol (mI) was lower in the dACC in AN relative to HC, and mI levels negatively predicted anxiety to eat HED but not LED foods in the AN group only. mI levels in the dACC were independent of body weight, body mass, and general anxiety.

Conclusions: These findings provide critical new insight into the clinically challenging feature and underlying neural mechanisms of eating-related anxiety and indicate mI levels in the dACC could serve as a novel biomarker of illness severity that is independent of body weight to identify individuals vulnerable to disordered eating or eating pathology as well as a potential therapeutic target.

Introduction

Anorexia nervosa (AN) is a mental and behavioral health condition characterized by an intense fear of weight or fat gain, severe restriction of food intake resulting in low body weight, and distorted self-perception of body shape or weight [1]. AN has the second highest mortality rate of all psychiatric illnesses [2] and affects individuals of all ages, sexes, and genders [3,4]. Biological women are particularly vulnerable, with the age of onset occurring around puberty [5]. Weight restoration to a Body Mass Index (BMI) of 19-21 remains the mainstay of treatment for severe AN, but relapse rates in the first year following weight restoration range from 30-50% [6,7]. The mechanisms that maintain the disorder and underlie these high relapse rates remain unknown.

Anxiety is a risk factor shown to exacerbate disease severity and promote relapse in AN [8]. Meal-associated anxiety is a striking clinical feature in patients with AN, especially when confronted with pressure to consume higher energy density (HED) foods[9]. Consequently, individuals with AN consume largely, if not exclusively, low calorie foods and severely limit consumption of calorically dense foods [10-16]. Despite this clinical feature, most treatments do not directly target food-related anxiety and focus instead on cognitive symptoms such as disturbances in body image [17]. Furthermore, while substantial research has focused on general anxiety in AN, less is known about eating-related anxiety and its underlying neural mechanisms.

In general, brain regions implicated in the development and expression of anxiety responses in humans include the amygdala, insula, bed nucleus of the stria terminalis (BNST), and the

prefrontal cortex (PFC) [18-21]. Neuroimaging techniques, including structural and functional magnetic resonance imaging (MRI) can provide insights into human brain structure and function. Functional magnetic resonance imaging (fMRI) studies indicate that individuals with generalized anxiety disorder show greater activation in the amygdala, ventral PFC (vPFC), and anterior cingulate cortex (ACC) to fearful stimuli [22,23] as well as increased functional connectivity among the amygdala, vPFC, and ACC [24,25]. In individuals with AN, relative to HC, neuroimaging studies have shown reductions in gray matter volume[26], decreased blood perfusion (at least in the restricting subtype of AN) [27-29], and increased activation to high calorie food images in the dorsal ACC (dACC) or ACC more broadly [30,31]. Given the increased activation in the dACC in response to food cues in AN and its putative involvement in magnifying the threat response in both animals and humans, we sought to examine the neurometabolic profile within the dACC to understand the relationship between brain chemistry within the dACC and anxiety-to-eat in women with AN and in women with healthy weight and without a lifetime history of an eating disorder (healthy controls; HC).

Proton magnetic resonance spectroscopy (MRS) is a widely used noninvasive neuroimaging technique for measuring brain metabolism *in vivo*. While measurement of low-concentration brain metabolites (e.g., gamma-aminobutyric acid (GABA), glutathione (GSH)) has been challenging with conventional MRS, recent methodological advances in our laboratory have enabled edited MRS to simultaneously measure levels of multiple low-concentration, coupled metabolites[32,33]. Briefly, the Hadamard Editing Resolves Chemicals Using Linear-combination Estimation of Spectra (HERCULES)[34] editing scheme enables the extraction of distinct edited spectra from different Hadamard combinations. To date, no study has investigated metabolite levels in individuals with AN using HERCULES.

This study was designed to characterize anxiety-to-eat in AN and identify key neurometabolic features in the dACC that may sustain AN. We hypothesized that women with AN would show greater anxiety to eat HED foods relative to lower energy density (LED) foods and greater anxiety-to-eat relative to HC. Consistent with previous MRS research in the dACC, we hypothesized that the AN group would show lower myo-inositol (mI) [35], N-acetyl aspartate (NAA) [35,36], and glutamine/glutamate (Glx) [35] relative to levels in HC. Exploratory analyses would then be conducted to determine relationships between anxiety-to-eat and levels of neurometabolites in the dACC that significantly differed between groups.

Methods and Materials

Participants

Participants were recruited for an ongoing prospective observational study investigating anxiety-to-eat (K01MH127178). Data collection occurred between September 22, 2022 and November 30, 2023. The protocol was approved by the institutional review board at The Johns Hopkins School of Medicine (IRB00214417). Women with AN were recruited from the Johns Hopkins Hospital during the initial phase of inpatient treatment, and HCs were recruited from the local community. For women with AN, eligibility criteria included the following: met DSM-V criteria for AN; a current patient in the Johns Hopkins Inpatient Eating Disorders Program; 13-70 years of age; able to read, speak, and comprehend English fluently; and having no contraindications for magnetic resonance imaging (MRI; including pregnancy, claustrophobia, metal implants, history of brain injury). For HCs, eligibility criteria included: a body mass index (BMI) between 19-24.9 kg/m^2; 13-70 years of age, no lifetime history of an eating disorder or axis I disorder; able to read, speak, and comprehend English fluently; and no MRI contraindications as listed above. All participants provided written informed consent prior to study assessments.

Measures

Overview. Participants completed two study visits (average interval of 5.03 days ± 0.79 days between visits) and were instructed not to consume any food or beverages apart from water after midnight the day prior to each visit. A rapid finger prick blood test was conducted using a lancet and glucometer to confirm that participants arrived fasted (<100 mg/dL). During the first visit, participants completed a computer-based assessment of anxiety-to-eat and reported interoceptive measures on a 13-inch Macbook Air computer. Body weight and height were collected using a Tanita MC-980U plus body composition scale in the HC group and from electronic medical records in the AN group. A link to complete psychometric inventories was emailed to participants following completion of the first study visit. During the second visit, participants completed the scanning protocol. Assessments are described in detail below.

Psychophysical Scaling Assessments. Participants reported their levels of hunger, thirst, fullness, desire to eat, anxiety, and stress on a 145 mm visual analog scale (VAS), where "0" (left scale anchor) represented the least degree ever experienced and "+100" (right scale anchor) represented the greatest degree ever experienced of the respective variable of interest. Participants then completed a computer-based behavioral task that included 20 images of foods common to a Western diet and presented at one standard serving size on a 10" white plate. Participants received the prompt "How much anxiety would you feel to eat this portion of [food name]" and then rated their level of anxiety to eat the portion of food on a 145 mm VAS scored from "0" ("the least anxiety ever experienced"; left scale anchor) and "+100" ("the most anxiety ever experienced"; right scale anchor). Ten of the 20 food images were HED foods (1.9-4.9 kcal/gram; e.g., cookies, cake, stirfry, French toast) and 10 were LED foods (0.2-1.4 kcal/gram; e.g. broccoli, sushi, banana, yogurt).

Psychometrics. Participants completed the State-Trait Anxiety Inventory (STAI)[37] and the Eating Disorder Examination Questionnaire (EDEQ)[38]. The STAI is a self-report measure of the presence and severity of generalized state (STAI-S) and trait (STAI-T) anxiety. The EDEQ is a self-report measure that assesses the range, frequency, and severity of eating disorder behaviors and is comprised of four subscales (eating restraint, eating concern, shape concern, weight concern). Both the STAI and EDEQ have good psychometric properties [39-42].

Neuroimaging Protocol. Data were collected on a Philips 3T Ingenia Elition RX scanner (Philips Healthcare, Best, the Netherlands) equipped with a 32-channel receive-only head coil. The scanning protocol included a 1 mm^3 isotropic T1 MPRAGE (TR/TE=2.9/6.5ms, Flip angle=8°; 211 slices; voxel size = 1 mm^3 (isotropic voxels) for MRS voxel placement and segmentation. The MRS voxel size was 30 (AP) x 30 (CC) x 30 (RL) mm^3 in dACC, as shown in Figure 1. MR spectra were acquired using the following parameters: PRESS localization; TR/TE=2000/80 ms; 256 transients; HERCULES editing with 20-ms editing pulses are applied at: (A) 4.58 and 1.9 ppm; (B) 4.18 and 1.9 ppm; (C) 4.58 ppm; and (D) 4.18 ppm. Edited signals from γ-aminobutyric acid (GABA), glutathione (GSH), NAA, N-acetylaspartylglutamate (NAAG), and lactate (Lac) are separated with reduced signal overlap into distinct Hadamard combinations: (A+B+C+D); (A+B-C-D); and (A-B+C-D); 2048 datapoints; 2 kHz spectral width; and VAPOR water suppression and interleaved water reference correction [34]. The dACC voxel was placed medially, and with the bottom aligning with top of the body of the corpus callosum (as shown in Figure 1).

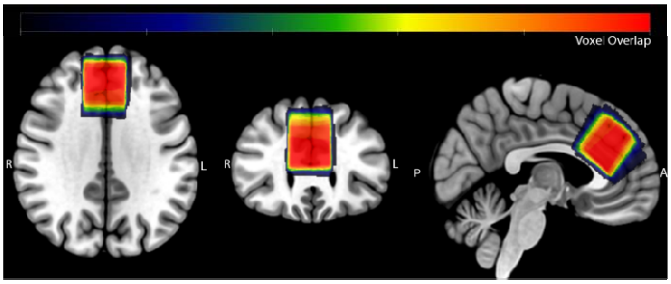

Figure 1 Location of the dACC voxels. The native space binary voxel mask for each participant was normalized to MNI space and overlaid onto the spm152 template to display anatomical overlap in voxel positioning between participants. Warmer (red) colors denote areas of greater overlap across participants.

Data Analysis

Three HCs had BMI values outside the defined lean range of 19-24.9 (i.e., 18, 25, and 25) and were subsequently excluded from the dataset. From the remaining 27 HCs, 16 were selected to closely match the age distribution of the test group; birth month and year were matched as close as possible between groups. This approach was taken to control for observed age-related effects on neurometabolite levels as measured by MRS [43] and to equalize the sample size between groups. Notably, analyses including the full sample of 27 HCs were conducted and yielded similar results to the age-matched groups discussed below. One participant in each group did not complete the psychometric instruments (STAI, EDEQ).

IBM SPSS Statistics version 28 and GraphPad Prism version 10 were used to analyze and plot the demographic, psychometric, and psychophysical data. An alpha level of 0.05 was predetermined for all analyses. A priori analyses of variance (ANOVAs), t-tests, and chi-square tests were conducted to examine differences in demographics, anxiety-to-eat ratings, and neurometabolite levels in the dACC between groups. Multiple linear regression was conducted to obtain predictive models for the main variable of anxiety-to-eat using a stepwise procedure to automatically select the significant predictors among the independent variables body weight, BMI, general anxiety, and mI for each participant group. Subsequent post hoc regression analyses and bivariate correlations were performed to explore the contribution and relationship of other factors (i.e., general anxiety as measured by VAS ratings, STAI metrics, body weight, BMI, blood glucose levels) with anxiety-to-eat ratings and mI levels in the dACC.

MRS data were analyzed using the open-source analysis toolbox Osprey (version 2.4.0) [44] within MATLAB R2022a. The analysis procedures followed consensus-recommended processing guidelines for MRS data [45,46]. Briefly, standardized analysis steps in Osprey included eddy-current correction based on the unsuppressed water reference [47], frequency- and phase-correction using probabilistic spectral alignment [48], removal of residual water signals using a Hankel singular value decomposition (HSVD) filter [49], and modeling of the metabolite peaks [44,50]. An appropriate custom basis set was generated using MRSCloud [51] with simulated profiles for ascorbate (Asc), aspartate (Asp), creatine (Cr), GABA, glycerophosphocholine (GPC), GSH, glutamine (Gln), glutamate (Glu), mI, Lac, NAA, NAAG, phosphocholine (PCh), phosphorcreatine (PCr), phosphoryl ethanolamine (PE), scyllo-inositol (sI), and taurine (Tau). The modeled spectral range was from 0.2 to 4.2 ppm and the default Osprey baseline knot spacing of 0.4 ppm was used. Amplitude-ratio soft constraints were applied to the amplitudes of the macromolecules (MM), lipids, and NAAG/NAA peaks as defined in the LCModel manual (http://s-provencher.com/pub/LCModel/manual/manual.pdf). The amplitudes of GABA and the co-edited MM at 3.0 ppm were soft-constrained in a one-to-one ratio, as suggested by previous work [52]. The water-reference data were modeled in the frequency domain with a dedicated

water basis function and a six-parameter model (amplitude, zero- and first-order phase, Gaussian and Lorentzian line broadening, and frequency shift). A summary of the experimental methods and data quality—following the minimum reporting standards in MRS [52]—is presented in Supplemental Table 1. For each participant, NAA, tCho, mI, Glu, Gln, and Glx were estimated from sum spectra, and Asc, Asp, GABA, GSH, NAAG, Lac, and PE levels were estimated after correcting for partial volume effects in each voxel as described in Gasparovic et al. [53]. Because GABA levels are reported to differ between brain gray and white matter [54], GABA levels were alpha-corrected [55] and final GABA estimates are reported as GABAplus, including the contribution of the modeled co-edited macromolecules[56]. GSH or GABA concentrations equal to 0 were interpreted as evidence of failure to fit and those data points were excluded from further analysis. The normality distribution and homogeneity of variance of metabolites were assessed using the Shapiro-Wilk normality test and F-test, respectively.

Results

Participant Characteristics. The AN group was comprised of 16 women with AN (13 restricting subtype, 3 binge/purge subtype). The HC and AN groups were similar in age ($F_{(1,31)}$=0.010, p=0.919), ethnicity (x^2=2.133, p=0.144), and education (x^2=8.291, p=0.081), and the HC group was more racially diverse than the AN group (x^2=10.863, p=0.028). The AN group weighed less ($F_{(1,31)}$=18.213, p<0.001), had a lower BMI ($F_{(1,31)}$=37.179, p<0.001), and had higher EDEQ global and subscale scores (see Supplemental Table 2) relative to the HC group. The mean and standard error of the mean (S.E.M.) or frequency of values of the participant characteristics are displayed in Table 1.

Table 1. Characteristics [Mean ± S.E.M. or Frequency (N)] of the women comprising the healthy control (HC) group and anorexia nervosa (AN) group.

Variable	**HC Group Frequency (N) or Mean ± SEM**	**AN Group Frequency (N) or Mean ± SEM**
Age (years)	24.59 ± 2.26	24.22 ± 2.89
Race	Asian (4), Black (1), Multiple (4), Other (2) White (5)	Asian (1), White (14), Multiple (1)
Ethnicity	Hispanic (2), Not Hispanic (14)	Hispanic (0), Not Hispanic (16)
Education	High School/GED (6), BS/A (6), MS/A (4)	No degree (5), High School/GED (5), AA (1), BS/A (4), MS/A(1)
Body Weight (lbs)	129.86 ± 4.55	104.02 ± 3.99
BMI	21.95 ± 0.44	17.58 ± 0.57

SEM=Standard Error of Mean; HC=healthy control; AN=anorexia nervosa; N=sample size; GED=General Education Development; AA=Associate in Arts; BS/BA=Bachelor of Science/Arts; Master of Science/Arts; lbs=pounds; BMI=body mass index

Interoception Measures. The AN group reported less hunger, less desire to eat, and more anxiety relative to the HC group. No differences were observed between groups in reported thirst, fullness, stress, and boredom. The mean and S.E.M. response and statistical outcome for each interoceptive factor is displayed in Supplemental Table 3.

Anxiety-to-Eat. Main effects of energy density (F(1,30)=37.671, p<0.001) and group (F(1,30)=27.338, p<0.001) as well as a significant energy density by group interaction (F(1,30)=10.602, p<0.003) on anxiety-to-eat ratings were observed. The AN group reported

greater anxiety to eat a standard serving size of HED foods (p<0.001) and LED foods (p=0.012) relative to responses by the HC group (Figure 2). Both groups reported greater anxiety to eat HED foods relative to LED foods (HC: p=0.050; AN: p=0.001).

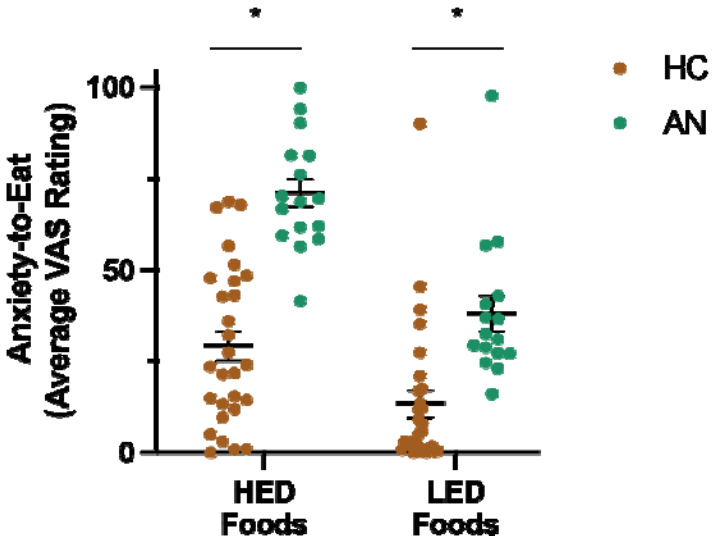

Figure 2. Mean ± S.E.M. anxiety-to-eat ratings for a standard serving size of higher energy dense (HED) and lower energy dense (LED) foods in the healthy control (HC; gold) and anorexia nervosa (AN; cyan) groups. The AN group reported greater anxiety to eat both HED and LED foods relative to the HC group. * represents significant p-value at alpha level of 0.05.

MRS. MRS data quality was generally high (see Supplemental Table 1). The following neurometabolites in the dACC were measured: mI, GABA with co-edited macromolecules (GABAplus), total choline (tCho), GSH, Glx, NAA, NAAG, Lac, and tCr. Metabolite levels in the dACC region are summarized in Table 2, and their sum spectra overlaid for all participants in each group can be found in Figure 3. Only mI levels in the dACC differed between groups (Figure 4, Table 2).

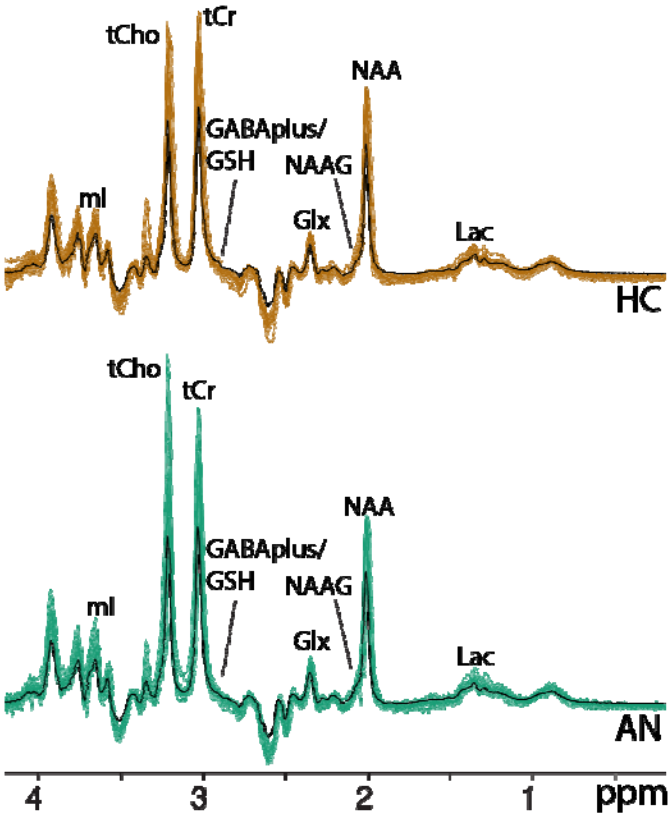

Figure 3. In vivo HERCULES data showing the overlaid sum spectra acquired from the dorsal anterior cingulate cortex (dACC) region. The key spectral features of the nine edited metabolites are visible. The black line within each spectrum represents the mean spectrum of each group. The gold spectra are those acquired from the healthy control (HC) group, while the cyan spectra are those acquired from the anorexia nervosa (AN) group.

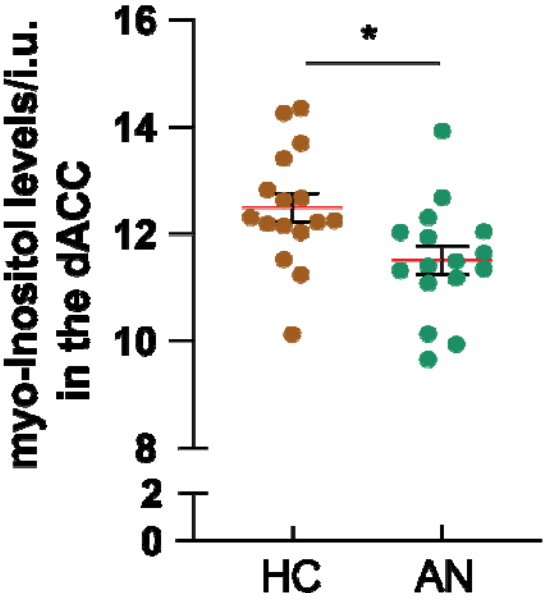

Figure 4. Myo-inositol (mI) levels in the dorsal anterior cingulate cortex (dACC) as measured by MRS in the healthy control (HC; gold) and anorexia nervosa (AN; cyan) groups. The AN group had lower mI levels in the dACC compared to HC. * represents significant p-value at alpha level of 0.05. The red line represents the mean values.

Table 2. Metabolites levels in institutional units in the dorsal anterior cingulate cortex (dACC) of women comprising the healthy control (HC) group and the anorexia nervosa (AN) group.

Neurometabolite	HC Group Mean ± S.E.M.	AN Group Mean ± S.E.M.	Statistic
mI	12.50 ± 0.27	11.51 ± 0.26	$F_{(1,30)}$=6.783, p=0.014*
GABAplus	7.74 ± 0.43	8.42 ± 0.19	$F_{(1,30)}$=2.097, p=0.158
tCho	3.10 ± 0.098	3.27 ± 0.09	$F_{(1,30)}$=1.770, p=0.193
GSH	3.63 ± 0.19	3.91 ± 0.16	$F_{(1,30)}$=1.203, p=0.281
Glx	15.59 ± 0.61	14.76 ± 0.48	$F_{(1,30)}$=1.128, p=0.297
NAA	16.17 ± 0.27	15.80 ± 0.28	$F_{(1,30)}$=0.921, p=0.345
NAAG	0.62 ± 0.11	0.73 ± 0.12	$F_{(1,30)}$=0.449, p=0.508
Lac	1.01 ± 0.06	1.10 ± 0.08	$F_{(1,30)}$=0.335, p=0.567
tCr	12.78 ± 0.25	12.90 ± 0.19	$F_{(1,30)}$=0.128, p=0.723

mI: myo-inositol; GABAplus: γ-aminobutyric acid + co-edited macromolecules; tCho, total Choline; GSH, glutathione; Glx, Glu + Gln; NAA, N-acetyl aspartate; NAAG, N-acetyl aspartyl glutamate; Lac, lactate; tCr, total Creatine; * represents significant p-value at alpha level of 0.05.

*Relationship Between mI Levels in the dACC and Anxiety-to-Eat***.** mI levels in the dACC significantly predicted anxiety to eat HED foods ($F_{(1,14)}$=8.342, p=0.012) in individuals with AN. Specifically, for every unit decrease in mI in the dACC, there was an estimated nine-unit increase in anxiety to eat HED foods (β=-8.881, SE(β)=3.075; R^2=0.373; Figure 5). General anxiety (β=0.012, p=0.957), BMI (β=0.289, p=0.194), and body weight (β=0.171, p=0.441) were not significant predictors and therefore were not retained in the model. Models could not be fit for anxiety to eat LED foods in AN or anxiety to eat HED or LED foods in HC.

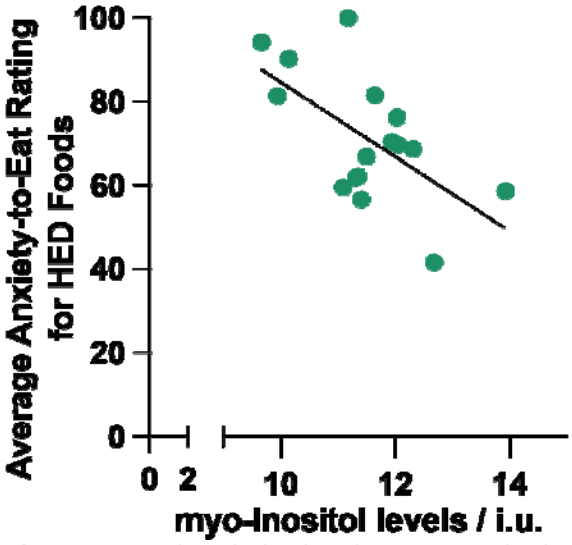

Figure 5. mI levels in the dACC negatively predicted anxiety to eat a standard portion of higher energy dense (HED) foods in the anorexia nervosa (AN) group only.

Post-hoc simple linear regression analyses showed no relationship between mI levels in the dACC and body weight, BMI, state anxiety, trait anxiety, anxiety as measured by the VAS, or fasting blood glucose levels in either group. Statistical outcomes in Supplemental Table 4.

Discussion

The goal of this project was to characterize anxiety-to-eat, which is a central yet understudied feature of AN psychopathology, and to identify its underlying neurobiological mechanisms. Our primary findings were that anxiety-to-eat was higher for HED relative to LED foods in both AN and HC, and anxiety to eat both HED and LED foods was greater in AN relative to HC. Only the level of mI in the dACC emerged as a significant predictor of anxiety to eat HED foods in individuals with AN but not in HC. These data have four significant clinical and scientific implications.

First, these findings provide new insight into the clinically challenging feature of eating-related anxiety in AN. Our data complement previous research showing that women with eating disorders report greater anxiety to consume high versus low calorie foods and beverages [31,57], and extend these findings to demonstrate that anxiety to eat both HED *and* LED foods is greater in women with AN relative to HC. Moreover, our data demonstrate that HC report greater anxiety to eat HED relative to LED foods as observed in AN, suggesting that the *degree* as opposed to the *presence* of anxiety-to-eat is central to AN psychopathology. These data in HC are inconsistent with the report by Ellison et al., [31] demonstrating that women without a history of an eating disorder show no such distinction in anxiety to consume high and low-calorie beverages. Differences in methods may underly the inconsistency between the two studies. Ellison et al., [31] used images of beverages labeled as high or low calorie and collected anxiety ratings using a Likert scale, whereas we presented images of one standard portion size of 10 HED and 10 LED foods and collected ratings using a VAS that allows for valid across-group comparisons [58,59]. Further, the variety of the foods presented in our study may have elicited greater variability in ratings of anxiety-to-eat, reflective of differences in food liking, healthiness, or other factors in HC and AN that may not have been captured solely by images of beverages.

Second, we found lower mI levels in the dACC of individuals with AN relative to HC, consistent with prior MRS findings in AN[35]. mI is a carbocyclic sugar and a precursor molecule to numerous phosphatidyl-inositol species in the second-messenger phosphatidyl-inositol systems including adrenergic, serotonergic, and glutamatergic pathways[60,61] shown to be dysregulated in AN [62-67]. Lower mI in the dACC may reflect heightened cellular activity or signaling within phosphatidyl-inositol circuits, leading to a depletion of phosphatidyl-inositol and the mI precursor. This would be consistent with prior research showing greater activity in the dACC, or ACC more broadly, to high calorie food images in AN relative to HC[30,31]. Conversely, lower mI in the dACC may be attributable to lower numbers of astrocytes, as demonstrated in rodent models of AN [68,69], or reduced dACC volume[26,68] in AN relative to HC. A limitation in MRS technology is the inability to distinguish cell-specificity (i.e., neuron, glia) of measured neurometabolites. Because mI is primarily derived from de novo synthesis from D-glucose-6-phosphate[70] but also from diet [71], the lower levels of mI in AN may alternatively stem from reduced caloric intake or inadequate nutrition for mI synthesis. This is unlikely given that levels of mI were independent of body weight, body mass, and fasting glucose (in both groups). Other studies have also shown no association between BMI and dACC metrics during the acute illness as well as following weight restoration[26,29,72]. The lack of a relationship between mI in the dACC and body mass or body weight may explain why persistent disturbances in eating behaviors are observed despite improvements in psychological characteristics and weight restoration in AN [14,73]. No differences in other metabolite levels in the dACC were observed between groups. The novel editing scheme HERCULES used here simultaneously quantifies multiple low-concentration coupled metabolite levels which allows for substantially reduced scan time. This editing scheme ensures that each target signal is treated independently, enabling the extraction of distinct edited spectra from the dataset in the different Hadamard combinations. Inconsistencies in the literature may derive from the increased sensitivity and specificity of the approach used here [35,36].

Third, mI levels in the dACC negatively predicted the degree of anxiety to eat HED foods irrespective of body mass, body weight, or general anxiety in AN. Furthermore, mI in the dACC was not associated with other eating disorder pathology as measured by the EDEQ in either group (AN: $p \geq 0.138$; HC: $p \geq 0.245$). Collectively, these data suggest that mI in the dACC is specific to and may underlie anxiety-to-eat in AN. The dACC plays a critical role in affective decision-making by linking behaviors with positive and negative consequences[74-77]. In AN, HED foods acquire the capacity to elicit negative anticipatory responses such as anxiety-to-eat due to the associated fear of weight gain if consumed. This *perceived* immediate causal consequence of consuming HED foods (increased body fat or body weight gain) and resulting motivational state (anxiety to eat) may engage the dACC and activate avoidance behavior. This dynamic is not typically observed in individuals without an eating disorder and may explain the absence of a relationship between mI levels in the dACC and anxiety-to-eat in HC. These findings indicate that mI levels in the dACC could serve as a novel biomarker of illness severity that is independent of body weight to identify individuals vulnerable to disordered eating or eating pathology as well as representing a potential therapeutic target. Indeed, randomized clinical trials of mI supplementation have demonstrated promising results in other psychiatric conditions [78-80].

Fourth, our data indicate that anxiety-to-eat is a distinct construct from general anxiety in AN. Only anxiety toward eating HED foods was linked to mI levels in the dACC in AN; anxiety as measured by the STAI-T, STAI-S, and anxiety in the moment (VAS metric) was not associated with mI levels in the dACC. Moreover, there was no relationship between anxiety to eat HED foods and state anxiety ($r=-0.13$, $p=0.645$), trait anxiety ($r=0.463$, $p=0.082$) or anxiety in the moment as measured via VAS ($r=0.16$, $p=0.553$) in the AN group. The distinctness of anxiety-to-eat from general anxiety may explain why anxiolytics have had limited effectiveness in the

treatment of AN[81,82]. Specifically addressing eating-related anxiety during treatment may be an effective treatment strategy, especially considering that maladaptive restrictive eating behaviors typically persist despite improvements in psychological characteristics and weight restoration [14,73]. Here, anxiety to eat HED foods was positively correlated with eating restraint ($r=0.77$, $p<0.001$), eating concern ($r=0.81$, $p<0.001$), weight concern ($r=0.76$, $p<0.001$), and shape concern ($r=0.81$, $p<0.001$) as measured by the EDEQ in the AN but not HC group (p-values ≥ 0.241), underscoring the potential significance and contribution of anxiety-to-eat in the maintenance of AN.

Limitations. While this sample size is comparable to if not larger than similar observational or neuroimaging studies in the extant AN literature and sufficient to identify significant relationships between AN psychopathology and brain chemistry, the sample size is arguably small. Larger longitudinal studies are needed to replicate these results and link changes in brain chemistry (i.e. mI in the dACC) with improvements in anxiety-to-eat in AN. The MRS method used here corrected for water and metabolite relaxation constants in tissues based on the assumption that T1/T2 values are uniform as indicated in Wansapura et al.,[83]. Changes in water and metabolite relaxation rates have been observed with age [43,84,85] and were the justification for age-matching the groups.

Conclusion. Anxiety-to-eat is a clinically relevant and challenging feature of AN. It is a distinct and separate construct from general anxiety and therefore should be addressed separately in the treatment of AN. The presence of anxiety-to-eat in individuals of healthy weight and without an eating disorder indicates that the *degree* of anxiety-to-eat rather than the presence of anxiety-to-eat is pathological in women with AN. Lower mI in the dACC in individuals with AN appears to underlie anxiety-to-eat and may serve as a potential therapeutic target. mI levels in the dACC were unrelated to body weight or BMI but positively associated with eating disorder metrics, warranting reconsideration of the degree of underweight BMI as the defining factor of severity of illness in the DSM-5. Identifying and understanding the potential roles of mI in the maintenance of AN is warranted. Further longitudinal studies are needed to investigate whether this brain: behavior relationship is rectified or persists following weight restoration and to elucidate its role in recovery, remission, and relapse from AN.

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Supplemental Material

Supplemental Table 1: Summary following minimum reporting standards in MRS generated in Osprey See Lin et al. 'Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations. NMR in Biomedicine. 2021;e4484. doi.org/10.1002/nbm.4448

1.Hardware	
a. Field strength [T]	3 T
b. Manufacturer	Philips
c. Model (software version if available)	R 5.7.1
d. RF coils: nuclei (transmit/receive), number of channels, type, body part	^{1}H, 32 channel, head
e. Additional hardware	-
2. Acquisition	
a. Pulse sequence	HERCULES (Johns Hopkins University Patch)
b. Volume of interest (VOI) locations	dACC
c. Nominal VOI size [mm^3]	dACC:30 x 30 x 30 mm^3
d. Repetition time (TR), echo time (TE)[ms]	TR 2000 ms, TE 80 ms
e. Total number of averages per spectrum i. Number of averaged spectra per subspectrum	320 total averages with 80 averages per subspectrum
f. Additional sequence parameters i. Editing pulse parameters	F1: 2000 Hz, 2048 points GABA at 1.9 ppm, GSH at 4.56 ppm
g. Water suppression method	VAPOR
h. Shimming method, reference peak, and threshold of acceptance of shim chosen	1st and 2nd shimming pencil beam, water
i. Trigger or motion correction	No trigger or active motion correction
3. Data analysis methods and outputs	
a. Analysis software	Osprey 2.4.0
b. Processing steps deviating from Osprey	Final alignment of the averaged sub-spectra by minimizing the choline (not water) peak; co-edited MMs at 3 ppm were modelled using the “1to1GABAsoft” model
c. Output measure	tCr, rawWaterScaled, CSFWaterScaled, TissCorrWaterScaled
d. Quantification references and assumptions, fitting model assumptions	Asc, Asp, Cr, GABA, GPC, GSH, Gln, Glu, mI, Lac, NAA, NAAG, PCh, PCr, PE, scyllo-inositol, Tau, 8 MM basis functions in the sum spectrum ($MM_{0.94}$, $MM_{1.22}$, $MM_{1.43}$, $MM_{1.70}$, $MM_{2.05}$, Lip09, Lip13, Lip20) Fitting method: Osprey baseline knot spacing 0.4 ppm
4. Data quality	
HERCULES, dACC	
a. SNR (Cr), linewidth (Cr) [Hz, OFF	SNR 233 ± 40, linewidth 5.05 ± 0.66 Hz

spectra]	
c. Quality measures of postprocessing model fitting (Mean Relative Amplitude Residual (Residual/Noise)) sum diff1 diff2	 22.35 ± 22.18 5.20 ± 1.42 5.35 ± 1.76
d. Mean spectrum created with OspreyOverview	Figure 3

T: Tesla, RF: radiofrequency, HERCULES: Hadamard Editing Resolves Chemicals Using Linear-combination Estimation of Spectra, mm: millimeter, ms: millisecond, Hz: frequency, ppm: parts per million, VAPOR: Variable Power radiofrequency pulses with Optimized Relaxation delays, dACC: dorsal anterior cingulate cortex, SNR: signal-to-noise ratio, GABA: γ-aminobutyric acid, GSH: glutathione, Asc: ascorbate, Asp: aspartate, Cr: Creatine, GPC: glycerophosphocholine, Gln: glutamine, Glu: glutamate, mI: myo-inositol, Lac: lactate, NAA: N-acetyl aspartate, NAAG: N-acetyl aspartyl glutamate, PCh: phosphocholine, PCr: phosphorcreatine, PE: phosphoryl ethanolamine, Tau: taurine, MM: macromolecules, Lip: lipid.

Supplemental Table 2. Mean ± S.E.M. global and subscale scores on the EDEQ in women comprising the healthy control (HC) group (N=15) and anorexia nervosa (AN) group (N=15).

EDEQ Score	**HC Group**	**AN Group**	**Statistic**
Global	0.95 ± 0.26	3.13 ± 0.43	$t_{(28)}$=-4.338, p<0.001*
Eating Restraint	0.83 ± 0.23	2.81 ± 0.50	$t_{(28)}$=-3.607, p<0.001*
Eating Concern	0.52 ± 0.27	2.45 ± 0.40	$t_{(28)}$=-3.993, p<0.001*
Shape Concern	1.34 ± 0.34	3.87 ± 0.46	$t_{(25.8)}$=-4.417, p<0.001*
Weight Concern	1.09 ± 0.36	3.37 ± 0.47	$t_{(26.2)}$=-3.841, p<0.001*

SEM=Standard Error of Mean; HC=healthy control; AN=anorexia nervosa; N=sample size; * represents significant p-value at alpha level of 0.05.

Supplemental Table 3. Mean ± S.E.M. response for interoceptive factors in women comprising the healthy control (HC) group and anorexia nervosa (AN) group.

Variable	**HC Group**	**AN Group**	**Statistic**
Hunger	48.37 ± 4.26	31.75 ± 6.06	$F_{(1,30)}$=5.033, p=0.032*
Thirst	49.75 ± 5.23	51.88 ± 5.07	$F_{(1,30)}$=1.169, p=0.288
Fullness	37.50 ± 3.56	48.81 ± 6.72	$F_{(1,30)}$=2.214, p=0.147
Desire to Eat	55.69 ± 5.69	32.00 ± 5.63	$F_{(1,30)}$=8.749, p=0.006*
Anxiety	25.88 ± 6.44	49.94 ± 5.91	$F_{(1,30)}$=7.589, p=0.010*
Stress	31.44 ± 7.84	49.44 ± 5.77	$F_{(1,30)}$=3.420, p=0.074
Boredom	41.19 ± 5.05	42.50 ± 4.79	$F_{(1,30)}$=0.036, p=0.852

SEM=Standard Error of Mean; HC=healthy control; AN=anorexia nervosa; * represents significant p-value at alpha level of 0.05.

Supplemental Table 4. Statistical outcomes of post-hoc simple linear regression analyses conducted on levels of myo-inositol (mI) in the dACC with variables of interest in women comprising the healthy control (HC) group and anorexia nervosa (AN) group.

Variable	**HC Group**	**AN Group**
Body weight	$F_{(1,14)}$=1.379, p=0.260	$F_{(1,14)}$=0.206, p=0.657
BMI	$F_{(1,14)}$=3.486, p=0.083	$F_{(1,14)}$=1.279, p=0.277
STAI-S	$F_{(1,13)}$=1.408, p=0.257	$F_{(1,13)}$=0.027, p=0.873
STAI-T	$F_{(1,13)}$=3.472, p=0.085	$F_{(1,13)}$=1.686, p=0.217
VAS Anxiety	$F_{(1,14)}$=0.296, p=0.595	$F_{(1,14)}$=0.881, p=0.364
Fasting blood glucose	$F_{(1,14)}$=0.892, p=0.361	$F_{(1,14)}$=0.368, p=0.554

BMI: body mass index, STAI-S: State-Trait Anxiety Inventory-state; STAI-S: State-Trait Anxiety Inventory-Trait, VAS: visual analog scale

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