

Purging behavior modulates the relationships of hormonal and behavioral parameters in women with eating disorder

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Running title: ED: metabolic correlates of purging behavior

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Abstract

Background/aims: There is ample consensus that there is a neurophysiological basis for the eating disorders (ED). Traits of personality translate into behavioural traits, purging being a well defined transversal example. Direct implication of steroid hormones on ED has been seldom studied, despite their effects on behaviour.

Methods: After psychological interview analysis 57 ED female patients (31 purgative and 26 non-purgative), and 17 female controls were studied. Metabolic parameters and analysis of androgen, estrogen and glucocorticoid hormones were determined in parallel to the psychopathological profile (EDI-2 and SCL-90-R) and anthropometric measurements.

Results: Psychometric tests showed clear differences between ED and controls, but there were few hormonal-metabolic significant differences. In purgative ED there were repeated (significant) positive correlations with CBG, and negative with SHBG, versus eating and general psychopathology. In non-purging, there were positive correlations for deoxycortisol, free fatty acids, albumin and negative for aspartate aminotransferase and psychopathological traits.

Conclusion: The data hint at CBG/corticosteroids and sexual hormones/SHBG to be involved in purging behaviour and its psychopathology and severity scores. Correlations of selected psychometric data and the CBG/SHBG levels in purging may eventually result in clinical markers. This approach may provide additional clues for understanding the pathogenesis of the ED.

Homonal and metabolic changes in eating disorders (ED) have widely been described and studied in the literature (1,2). Their metabolic causes, however, have been seldom analyzed, mainly because of the limited availability of data, and the wide complexity of the populations studied (3). This is in part a consequence of diagnostic of the ED is, in itself, a fairly complex issue (4) and the intrinsic variability of most metabolic and hormonal parameters in human populations that are otherwise “normal”. Nevertheless, this relationship may run deeper than usually assumed: the common occurrence of amenorrhea in deeply affected anorectic individuals suggests that at least the hypothalamus-pituitary-gonadal axis is affected and their biological implications known.

Biological vulnerability in ED

Although genetic predisposition to ED has received considerable attention (5,6), is difficult to arrive at clear conclusions because of the variability on the manifestations, intensity and symptom-focusing of these disorders (7). Most of these studies only hint at increased frequency (or predisposition) because of the presence of certain alleles in a number of genes related with control of energy, appetite, behavior or other pathways (8,9), but there is not a clear-cut direct relationship with the ED (10,11). The intrinsic variability that characterizes any group of humans (height, weight, metabolic parameters, hormonal cycles), even if limited to one sex and a fairly narrow limit of age, makes very difficult to find well-defined group differences in parameters that otherwise are subjected to homeostatic control and tend to remain stable in spite of powerful manipulation. This problem is compounded by the intrinsic variability added by the analytical methods (in fact of any analytical method).

The markedly individual or personal nature of human psychological makeup is a key factor adding variability to any group-based analysis of metabolic-psychological relationships. The complexity of the etiology and even manifestations of eating diseases can be simplified by ascribing each patient to a particular category of the ATP-III, but the continuum of individual patient characteristics will no doubt affect not only psychological reactions but also hormonal correlates inducing metabolic consequences, thus increasing the “gray zone” between specific diagnostic groups. The obvious consequence is the relative lack of studies showing clear metabolic changes attributable to specific manifestations of eating disorders (12,13). There are a few studies showing the tip of the iceberg (14), and it is generally

acknowledged that metabolic correlates of eating diseases can be first-order elements both for their diagnostic and as key elements for understanding their pathogenic mechanisms (15).

Hormonal correlates and ED

There are a number of studies linking metabolic and hormonal changes with ED, both in humans and animal models (16), but changes induced by ED on the main hormonal and metabolic parameters have been only sparsely studied. Steroidal hormones, a case in point, are seldom analyzed in blocs including a number of different molecular species as a way to better understand the changes of their patterns in disease. The rapid inter-conversion of a number of corticosteroids (17), and, especially androgen and estrogen hormones and their precursors are a key characteristic of their function and a powerful means for their control. Although, hormonal profiles and influence in ED have widely been studied, such as estrogens (3,18,19), androgens (20), altered hypothalamic-pituitary adrenal (21) and hypothalamic-pituitary-gonadal axes functions (15) their relationships with specific phenotypical characteristics of ED have been seldom described. Although specific clinical traits such as purging behaviour have frequently been associated to higher psychopathology and poorer prognosis (21-24), their potential association with hormonal functioning has rarely been investigated.

The division of ED along the purging/non-purging line is based on its clear association with medical risk (25) and follows previous studies in which this subtyping has been widely used (26), on the basis of medical consequences and empirical phenotyping (27)

Aims of the study

Given the current gaps in the literature, the goal of the present study was to overcome the limitations of the previous studies by comparing ED individuals and healthy eating controls, across a broad range of hormonal components (Supplemental Figure 1): i.e. the key steroidal hormones and the transporting globulins: SHBG (28) and CBG (29), together with phenotypical characteristics (namely purging behaviour, clinical symptoms and general psychopathology).

Our specific goals were threefold and included: 1) to explore the distribution of hormonal parameters in a sample of EDs individuals and to analyze whether they are different when compared with healthy-eating controls; 2) to compare whether hormonal parameters differ between purging and non-purging ED, as a way to determine the eventual implication of steroid hormones in the manifestation of this

marker symptom; 3) to assess the relationship of differential hormonal features with other phenotypical traits in ED (clinical and psychopathological variables);

We hypothesized that hormonal function may be associated not only to ED categories but to some specific ED clinical features associated to severity (namely to ED severity and general psychopathology). This approach opens a new avenue for the study of the pathogenicity of eating disorders and their metabolic correlates in populations otherwise diverse, and would allow us to distinguish a specific metabolic-hormonal pattern in ED.

Materials and Methods

Participants

The final sample included 57 female ED patients (12 AN and subthreshold AN (21.0%), 31 BN and subthreshold BN (54.4%), and 14 with Eating Disorders Non Otherwise Specified-EDNOS (24.6%), [including 5 Binge Eating Disorder (8.8%)]. In total there were 35 purgative and 22 non purgative ED patients, consecutively admitted to the Eating Disorders Unit in the Department of Psychiatry at the University Hospital at Bellvitge (Barcelona, Spain). All participants were diagnosed according to DSM-IV criteria, using a semi-structured clinical interview [SCID-I] (30), conducted by experienced psychologists or psychiatrists. A total of 17 health-eating women, the comparison group (CG), were volunteers recruited from the staff of our Centers. The exclusion criteria were: current treatment with hormones (including anovulatory combinations), diabetes and other metabolic diseases (including polycystic ovary syndrome), drug consumption (including tobacco and alcohol), as well as other infectious, hormonal or metabolic diseases and lifetime ED in the case of CG individuals. The Ethics Committee of the University Hospital at Bellvitge approved this study, and informed consent was obtained from all participants.

Psychometrical measures

--Eating Disorders Inventory 2 (EDI-2)

The EDI-2 (31) is a reliable and validated 91-item multidimensional self-report questionnaire that assesses different cognitive and behavioral characteristics, which are typical for eating disorders. The questions are answered on a 6-point Likert scale. This instrument was validated in a population of

Spaniards (32) and had a mean internal consistency of 0.63 (coefficient alpha). Inter-rates reliability ranged between good to excellent (values were 0.77 to 0.96).

--Symptom Checklist- Revised (SCL-90-R)

We used the SCL-90-R (33) to assess a broad range of psychopathological symptoms. This test contains 90 items and helps to measure 9 primary symptom dimensions. In addition, it includes three global indices: 1) a global severity index (GSI), designed to measure overall psychological distress; 2) a positive symptom distress index (PSDI), designed to measure the intensity of symptoms; and 3) a positive symptom total (PST). The global severity index can be used as a summary of the test..This scale has been validated in a population of Spaniards (34), and had a mean internal consistency of 0.75 (coefficient alpha). Inter-rates reliability was between good to excellent, with values comprised between 0.70 and 0.90.

Procedure

Experienced psychologists and psychiatrists with master's or doctoral degrees (all them extensively trained in the use of the instruments) completed the clinical assessment during two structured face-to face interviews, before any psychological or pharmacological treatment was initiated. The first interview included questions about regularity of their menses and the mean duration and time of their ovulation cycle at the moment of the analyses. In addition to the clinical interview, demographic information was obtained through self report questionnaires.

In this session, basic anthropological measurements were carried out. Body weight and height were measured using standard procedures, and were used to calculate the body mass index (BMI) in kg/m². The percentage of body fat was estimated with a bioimpedance system (Body Mass Analyzer BC-418MA, Tanita Corp. Tokyo, Japan).

Hormonal and metabolic measurements

Venous blood samples were taken in the morning, after an overnight fast; serum was separated and stored at -80°C; later it was used for the analysis of glucose, total cholesterol, triacylglycerols, bilirubin, albumin, total serum protein, urea, alanine and aspartate aminotransferases, lactate dehydrogenase and ketone bodies using dry chemistry strips (Spotchem Strips, Menarini, Firenze. Italy); free fatty acids

were measured with a kit (NEFA kit, Wako, Osaka Japan). Steroid hormones (except hormone sulfates) were measured by HPLC-MS/MS following the procedure described in the Supplemental Methods.

Specific radioimmunoanalysis / ELISA kits were used for the measurement of: insulin (RIA HI-14K, Millipore, Billerica MA USA). sex hormone-binding globulin (SHBG) (IRMA KP32CT, RADIM, Roma Italy). corticosteroid-binding globulin (CBG) (RIA KIP1809, BioSource, Nivelles Belgium), estrone sulfate (RIA DSL-5400 Diagnostic Systems Laboratories, Webster Texas USA) and dehydroepiandrosterone (DHEA) sulfate (RIA DSL-3500 Diagnostic Systems Laboratories). The HOMA value was calculated from insulin and glucose data (35).

Statistical analyses

The statistical analysis was carried out with PASW17 statistics (SPSS system). T-TEST procedures (and the non-parametrical Mann-Whitney test for data with high asymmetries) explored differences in clinical and psychological measures between groups (purging vs non-purging and ED vs CG).

Heterotypical association between hormones and metabolic measures (considered as predictors) with psychometrical scores (considered as outcomes) was valued through multiple lineal regressions. One independent model was defined for each psychometrical outcome, entering simultaneously the set of hormones or metabolic incomes (ENTER procedure). Beta-coefficients obtained with these models valued the specific contribution of each predictor on the criteria, adjusted to the presence of the other variables considered of the same set; the R^2 coefficient valued the total predictive accuracy of the model.

Homotypical association between variables pertaining to the same group (hormones. metabolic and psychometrical) was valued with partial-correlations. These coefficients measures the degree of association between pairs of variables controlling (adjusting) the effect of other covariates. In this study, one independent partial-r-coefficient was obtained for each pair of variables considering as covariates the rest of variables of the same group different from zero. Paired correlations were considered relevant only when statistical significance emerged ($p < 0.05$) and effect sizes of R-coefficients were good ($|r| > 0.35$).

Results

Clinical, general psychopathology, anthropometric and metabolic description among the groups

The mean values obtained among the ED groups (purging vs. non-purging) for all anthropometric values are shown in Supplemental Table 1. There were no significant differences between groups except for higher body fat in non-purging than in CG. Table 1 presents the comparison of the EDI2 and SCLR90R test scores between the three experimental groups. There were significant differences for all items between ED as a whole and for both purging and non-purging women. However, no differences were observed between both ED groups. The results for SCLR90R test were fully comparable to those obtained with the EDI2 test: there were significant differences versus CG for all ED and the subgroups purging and non-purging, but there were no differences between these two subgroups.

Table 2 shows the serum steroid.hormone related parameters in CG, ED and the two subgroups of purging and non-purging ED women. The differences observed were sparse. When considered the whole of ED versus CG estrone sulfate and cortisone levels were lower in ED, and androstenedione, higher. Comparison of CG and non-purging was coincident with all ED in estrone sulfate and androstenedione, but not in cortisone: however the differences versus CG were significant for β -estradiol. SHBG levels were higher in purging women than in CG. There were significant differences between purging and non-purging groups for androstenedione and DHEA-sulfate, with levels higher, in both cases, in non-purging women.

Supplemental Table 2 depicts the serum metabolite data for ED and CG. There were no statistically significant differences between groups for any of the parameters studied.

Interaction between hormonal-metabolic parameters and ED and general psychopathology between purging and non-purging ED

The analysis of paired correlation data applied to the EDI2 psychometric aspects showed a considerable number of significantly interrelated parameters (Table 3). Thus in ED non-purging women, BMI, body weight and body fat were positively correlated to body dissatisfaction and bulimia. Deoxycortisol was correlated to interpersonal distrust, ineffectiveness, impulse regulation, ascetism and social insecurity. Free fatty acids were correlated to ineffectiveness, impulse regulation, ascetism, and social insecurity. Other correlations were limited to one or two factors: pregnelone to interpersonal distrust, progesterone to impulse regulation, androstenedione to ascetism, alanine aminotransferase to bulimia, albumin to perfectionism and both urea and plasma proteins to eating disorder inventory total.

The pattern of negative correlations for ED non-purging women were more limited: aspartate aminotransferase to interoceptive awareness, interpersonal distrust, ineffectiveness, impulse regulation, social insecurity, and eating disorder inventory total, cortisol to interpersonal distrust.

ED Purging women showed positive correlations between CBG and interoceptive awareness, bulimia, ineffectiveness, ascetism, and eating disorder inventory total; aspartate aminotransferase to interpersonal distrust, ineffectiveness and perfectionism; urea to interoceptive awareness, lactic dehydrogenase to perfectionism, , uric acid to body dissatisfaction, glucose and alanine aminotransferase to ineffectiveness, deoxycortisol to interpersonal distrust and social insecurity.

Negative correlations for purging ED women were centered on SHBG, which correlated with interoceptive awareness, bulimia, ineffectiveness, maturity fears, impulse regulation, ascetism, social insecurity, and eating disorder inventory total. Other negative correlations were: pregnelone to drive for thinness, aldosterone to body dissatisfaction, height to maturity fears, albumin to ascetism.

CG showed negative correlations for deoxycortisol to drive for thinness, body dissatisfaction, bulimia, and impulse regulation; BMI to drive for thinness, body dissatisfaction and perfectionism, body fat to drive for thinness and perfectionism, HOMA to ineffectiveness, maturity fears and social insecurity, glucose to maturity fears, social insecurity and eating disorder inventory total, plasma proteins to maturity fears, CBG to impulse regulation, bilirubin to eating disorder inventory total,

Negative correlations for control women were cortisol and cortisone to body dissatisfaction, estrone sulfate to drive for thinness,

On Table 3 only the global severity index of the SCL90R test is shown. ED non-purging women showed correlations for total protein and albumin, and negative correlation for alanine aminotransferase. In purging women, the correlation was positive for CBG and negative for SHBG. The CG showed only positive correlations for both glucose and bilirubin.

Discussion

The present study aimed to explore whether specific ED cluster of patients (purging vs. non-purging) are differentiated on hormonal-metabolic parameters, when compared with healthy-eating controls, and to analyze whether there is an interaction between the hormonal-metabolic functioning and phenotypical

features (purging behaviour, eating and general psychopathology) in ED patients. Our observations indicate that potentially meaningful correlations do exist between eating severity and general psychopathology and specific hormonal and metabolic functioning, and that purging and non-purging subjects respond in a different hormonal-metabolic way.

When comparing the absolute mean values of the items analyzed between the three experimental groups we observed only small differences if any. The limited number of significant differences between purgative and non-purgative groups (and from these and the CG), suggest that most of the psychometric hormonal and metabolic data presented fall well within the limits of “normalcy”. There were no marked differences due to purging behavior, and neither were (considering only the metabolic and hormonal parameters) between controls and patients with a clinically defined eating disease. This was also expected, since most of the subjects’ weight was normal and there were no deviations from the range of normalcy as those observed in deeply altered hormonal states. These results are comparable to a number of similar studies in which there were no marked effects of ED on most metabolic indicators other than those expected by altered food intake (36). Nevertheless, genetic studies have shown a number of allele distributions closely related with eating disorders (37,38), and, evidently, gene expression ultimately translates into metabolic effects.

The application of a deeper-layer analysis of possible correlations between the data, i.e. determining the possible differences in relationships between different parameters showed distinct patterns between both psychometric and metabolic factors, in a way that clearly differentiated the three groups. The statistically defined association of parameters helps explain the basic patterns of change, often obliterated in direct comparisons by the “noise” of human variability, individual specificity of the eating disorder and the variability inherent to the analytical techniques.

Purging behavior is a key diagnostic element for the classification of the eating disorders and is a distinct qualitative and qualifying trait (22,24). The application of the statistical correlation analysis to a sample of eating-disordered women on the basis of purging behavior resulted in two well marked behavioral patterns that transcend the simple sharing of ED diagnostic categories. However, the most important finding is that these different patterns associated with purging behavior are also closely correlated with a number of hormonal and metabolic parameters in a well differentiated pattern.

We centered a large part of the efforts for this study on the analysis of a large number of steroid hormones because of their relationship with behavior (39,40) and their alterations under stress (41) depression-like conditions (42,43), sexual drive, cycles (44) and role-related behavior (45). In the obese, estrone levels are a correlate of body fat (46). Estradiol facilitates fat mobilization (47) and controls gonadotropin synthesis in the brain (48). Testosterone is an important controller of sexual behavior in women (49), and competes with estradiol for binding to SHBG (50). Unfortunately, most values for testosterone were below the limit of detection of the methodology used, which at least allows us to prove that the analyzed women had normal or lower than normal levels of testosterone; whereas those of estradiol were within the normal range (51). Glucocorticoids are affected by rhythms (52), control energy metabolism (53) and the immune response to inflammation (54). Cortisol is carried in blood mainly by CBG (55), which also helps modulate the corticosteroid response (56,57).

Purging is clearly associated with hormone function, as shown by the positive correlation of ED psychopathology and general psychopathology with CBG levels and their negative relationship with SHBG, traits not observed in CG of ED non-purging. The levels of estradiol were lower in non-purging than in CG, but unaffected in ED purging, which also showed higher levels of SHBG than either group. A trend to increase SHBG levels as that observed in purging women may result in lower availability of free sex hormones and is in line with the SHBG increase under conditions of malnutrition and anorexia (58,59). Estradiol is probably SHBG main binding hormone, since testosterone levels were very low in all cases, but its levels did not show significant differences between groups. These findings confirm a marked reversed interrelationship between purging and SHBG. However, it is unclear how SHBG may help define the metabolic profile of purging women.

The case for CBG mirrors that of SHBG, but now the correlations of CBG with purging and of deoxycortisol in non-purging ED patients were positive.

The inactive/active corticosteroid (cortisone/cortisol) concentrations ratio was 17.5 for non-purging, 19.9 for purging and 24.1 for CG ($p < 0.01$ for ED and non-purging, and $p < 0.05$ for purging). This difference points to an overall increased reconversion of inactive cortisone into active cortisol via 11β -hydroxysteroid dehydrogenase (17,41) in the ED patients, irrespective of the purgative factor analyzed here. This may represent a mechanism of homeostatic preservation or increased hormone turnover rather than a direct increase in hormone activity, since cortisol levels were normal in all three groups

The high correlations of purging ED with CBG and the unchanged ratio of CBG versus cortisol in all three groups suggests that in purging women there may be relatively maintained (but tighter) activity of glucocorticoids when compared with CG. The correlations of corticosteroid activity, including CBG hint at them to play a role in the particular behavioral act of purging, in parallel to their implication in stress, and on most reactions to harm, external interaction and depression (60,61).

The positive correlations of BMI, body weight, body fat, albumin and other indicators of possible tendency to excess of nutrients were in part shared by CG, and clearly reflect both the trend to increase food intake (repeated correlations with BMI, free fatty acids and albumin in non-purging and BMI, glucose, uric acid in CG), and concerns with eating and weight. However, the remarkable and repeated negative correlation with aspartate aminotransferase, an index of liver failure (62) may suggest that there is not an excessive "excess" of nutrients driving to a complicated situation such as liver steatosis, obesity or other metabolic syndrome components. In non purging women, metabolic control seems tighter than in CG, and the negative correlations with aminotransferases contrast with the positive correlations found in purging women.

Positive correlations in the purging women show a disturbing assortment of markers of liver dysfunction (serum aminotransferases, urea, uric acid, lactic dehydrogenase). None of these parameters is out of the normalcy range, but their association suggests that the purging behavior is a harbinger of possible future hepatic alterations. Evidently, the loss of electrolyte and the upper GI tract damage induced by forced vomiting (63) can elicit protracted negative consequences, but the possible implication of the liver has not yet been established. The data presented here suggest a need for the control of liver well being in the EDs associated with purging.

The ED components of the purging women may be translated in a deep preoccupation by their image, bulimia and the ability to act against its consequences, i.e. the decision to vomit. Probably in this second active part is where the relative lowering of estrogenic function may help favor a more action-prone brain setting (64). Bulimic behavior is again negatively associated to SHBG levels (unpublished results), reinforcing the notion that there is an inverse relationship between the impulse to act and SHBG levels, i.e. higher free circulating sex hormones. The lack of correlation with parameters more directly related with body weight and shape suggests that purging behavior is not a simple component of the drive eliminate excess energy intake, but is rooted in sex hormone/ glucocorticoid functional relationships.

In the non-purging group the ED substrate is the same (body dissatisfaction, bulimia), but the fear for the consequences was greater than the drive for action. There was no correlation with sex hormones or their transporting globulin, no metabolic correlations with liver function indexes, but a number of positive correlations with BMI, body fat and free fatty acids. This reflects a more “normal” approach to the problem of bulimia, and a lot more of passiveness on the part of the patient with respect to the eventual consequences. The image problem, however, persists because no “actions” are taken, and as a consequence the metabolic derangements associated with these actions did not appear; or at least not more than in CG, which EDI2 responses were also fairly well correlated with BMI, HOMA and body fat. Since the BMI of non-purging women was higher than that of CG (probably, at least in part, consequence of the combination of bulimia and not purging), but in the limit of normalcy and overweight, no significant alteration in the insulin-glucose handling (HOMA) can be appreciated. Controls, however, showed deeper correlations for these parameters in the absence of both overweight and bulimia, which suggests that other factors may probably help protect the non-purging women from the unbalancing of glycemia / insulinemia.

The differential degrees of interaction between psychopathology, (both eating and general), and the hormonal functioning, when considered purging and non-purging women, suggest that both disorders have not only phenotypical and symptomatological differences, but also may have some differential underlying biological functioning.

Psychopathology, and indirectly degree of severity in ED, and reproductive steroid hormones may interact and reinforce each other. This finding is in concordance with previous studies where sex hormones, not only played a role in brain development, but also were associated to general psychopathology and mental disorders (65,66).

In sum, the data presented show that correlations of selected eating and general psychopathological symptoms with BMI in non-purging and the CBG and SHBG correlations in purging may eventually result in possible markers of this aspect of the disorder. The application of this approach may provide additional clues for understanding the pathogenesis and development of the eating diseases. The recommendation for long-term control of liver function in purging women is also a conclusion that can be drawn from this study.

Metabolic correlates, thus, provide a physiological substrate to the psychological findings and may help unravel the mechanisms that define these behavioral traits despite the interference of variability, personality factors and complexity of the task.

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Table 1 – Mean values for the EDI2 and SCLR90R test items of controls, and ED purging and non-purging women.

parameter	ED non-purging	ED purging	controls	statistical significance			
				C-ED	C-NP	C-P	P-NP
EDI2 test							
drive for thinness	15.4 ± 1.2 (18)	16.06 ± 1.02 (33)	2.70 ± 1.31 (10)	***	***	***	
body dissatisfaction	18.2 ± 1.8 (18)	17.82 ± 1.43 (33)	7.7 ± 3.5 (10)	***	**	**	
interoceptive awareness	13.9 ± 1.5 (18)	13.12 ± 1.19 (33)	2.10 ± 0.64 (10)	***	***	***	
bulimia	5.39 ± 1.36 (18)	8.70 ± 0.99 (33)	0.50 ± 0.34 (10)	***	*	***	
interpersonal distrust	7.78 ± 1.48 (18)	5.06 ± 0.96 (33)	2.00 ± 0.75 (10)	*	**		
ineffectiveness	12.72 ± 1.95 (18)	11.33 ± 1.13 (33)	3.50 ± 1.05 (10)	***	**	***	
maturity fears	9.56 ± 1.27 (18)	7.76 ± 0.93 (33)	3.70 ± 1.36 (10)	**	**	*	
perfectionism	7.22 ± 1.16 (18)	5.70 ± 0.74 (33)	2.00 ± 0.92 (10)	**	**	*	
impulse regulation	8.33 ± 1.76 (18)	7.91 ± 1.34 (33)	0.30 ± 0.21 (10)	**	**	**	
ascetism	8.72 ± 0.98 (18)	8.18 ± 0.76 (33)	3.10 ± 0.59 (10)	***	***	***	
social insecurity	8.78 ± 1.46 (18)	7.48 ± 0.79 (33)	3.40 ± 0.95 (10)	**	*	*	
eating disorder inventory total	116 ± 11 (18)	109 ± 8 (33)	31.0 ± 6.9 (10)	***	***	***	
SCLR90R test							
global severity index	1.96 ± 0.20 (18)	2.06 ± 0.12 (31)	0.56 ± 0.12 (10)	***	***	***	
positive symptom index	69 ± 4 (18)	72 ± 2 (31)	33.4 ± 5.69 (10)	***	***	***	
positive symptom distress index	2.48 ± 0.16 (18)	2.54 ± 0.09 (31)	1.46 ± 0.10 (10)	***	***	***	

The data are mean ± sem; the number of values (N) for each parameter is given between parentheses. Statistical significance of the differences between groups (Student's *t* test): * = P<0.05; ** = P<0.01; ***=P<0.001. C-ED = comparison of control and all ED patients; C-NP = comparison between CG and non-purging; C-P = comparisons between CG and purging; P-NP = comparisons between purging and non-purging groups

Table 2– Mean values for the serum steroid hormone-related data of controls, and ED purging and non-purging women.

parameter	units	ED non-purging	ED purging	controls	statistical significance			
					C-ED	C-NP	C-P	P-NP
estrone	pM	0.24 ± 0.02 (19)	0.25 ± 0.03 (34)	0.35 ± 0.07 (18)				
estrone sulfate	nM	6.02 ± 0.63 (18)	6.74 ± 0.78 (33)	8.80 ± 0.90 (17)	*	*		
β-estradiol	pM	0.27 ± 0.04 (20)	0.47 ± 0.10 (34)	0.45 ± 0.06 (18)		*		
estriol [A]	pM	0.06 ± 0.03 (3/16)	0.01 ± 0.01 (4/30)	0.01 ± 0.00 (2/16)				
cortisone	nM	56.8 ± 4.4 (20)	56.1 ± 2.6 (33)	66.3 ± 5.0 (17)	*			
cortisol	nM	325 ± 30 (20)	282 ± 19 (33)	275 ± 32 (17)				
deoxycortisol	pM	5.41 ± 2.92 (16/3)	2.84 ± 0.65 (23/11)	3.90 ± 1.30 (8/8)				
androstenedione	pM	9.62 ± 1.51 (11/8)	4.88 ± 0.43 (19/15)	3.69 ± 0.69 (10/7)	*	**		***
DHEA-sulfate	μM	6.37 ± 0.66 (21)	4.80 ± 0.44 (35)	5.56 ± 0.37 (17)				*
testosterone [A]	pM	<0.6 (0/21)	<0.6 (0/35)	1.82 (1/17)				
17OH-progesterone [A]	pM	<6.0 (0/21)	11.3 ± 2.7 (5/29)	19.5 ± 7.6 (2/16)				
progesterone	pM	17.7 ± 6.2 (4/15)	23.0 ± 5.5 (15/19)	25.9 ± 5.5 (8/10)				
pregnelonone [A]	pM	26.4 ± 7.0 (6/13)	62.2 ± 26.8 (7/27)	14.6 (1/17)				
SHBG	nM	46.2 ± 5.8 (21)	56.0 ± 5.2 (35)	39.0 ± 4.1 (18)			*	
CBG	nM	1202 ± 66 (21)	1181 ± 49 (35)	1083 ± 39 (18)				

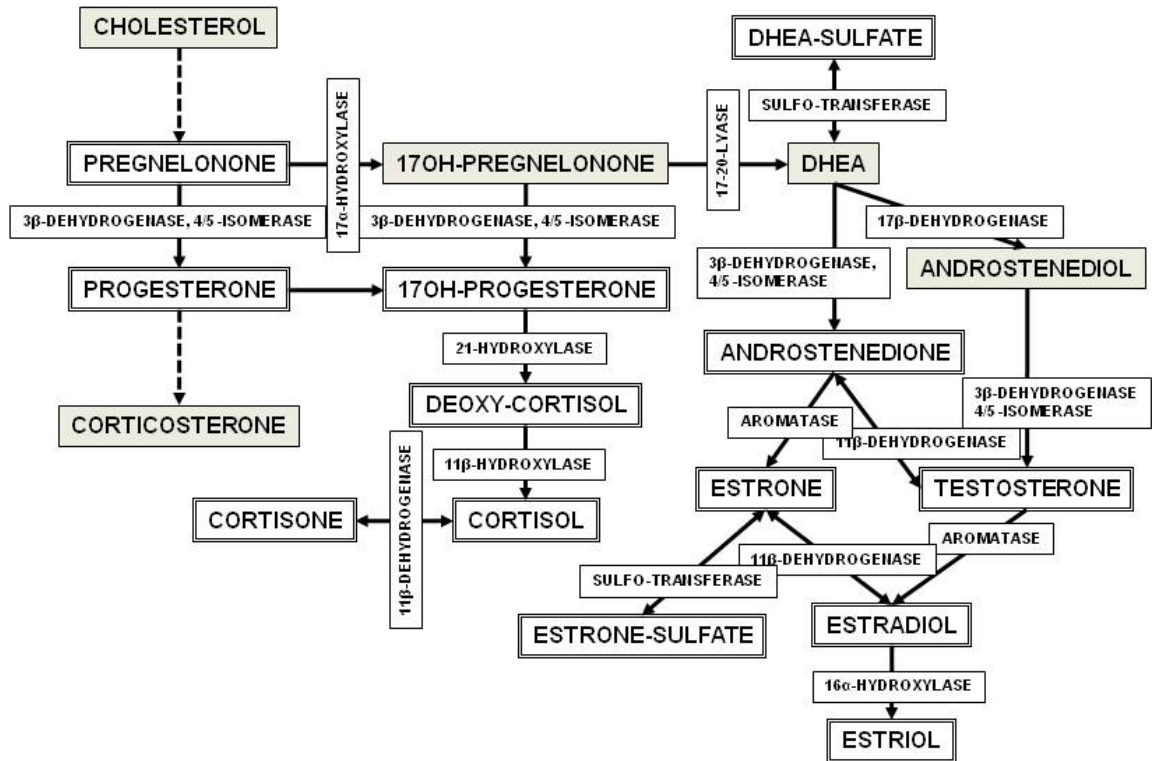
The data are mean ± sem; the number of values (N) for each parameter is given between parentheses. Statistical significance of the differences between groups (Student's *t* test): * = P<0.05; ** = P<0.01; ***=P<0.001. C-ED = comparison of control and all ED patients; C-NP = comparison between CG and non-purging; C-P = comparisons between CG and purging; P-NP = comparisons between purging and non-purging groups

NOTES: [A] The data in these rows show only the mean of the values measured that were actually over the limit of detection of the hormone; in consequence they are not representative of the actual values, largely closer to that limit of detection, i.e. “not detected”. We allowed these figures only as an indication of the differences (or lack of) between groups; in these cases, a second **N** value, in *italics*, is included to indicate how many samples were below the limit of detection of the test.

Table 3 - Correlations between components of the EDI2 and SCL90R tests with hormonal, anthropometric and metabolic parameters in a group of controls and women with eating diseases with respect to the purging or non-purging nature of their disease

	acronym	ED- non-purging (N=24-26)		ED-purging (N=33-36)		control group (N=17-18)	
		+ correlation	- correlation	+ correlation	- correlation	+ correlation	- correlation
drive for thinness	DT				pregnenolone ⁹	BMI · deoxycortisol ⁸ body-fat ·	estrone-sulfate
body dissatisfaction	BD	BMI · body weight · body fat		uric acid	aldosterone ¹⁹	BMI · deoxycortisol ⁸	cortisol · cortisone
interoceptive awareness	IA		AspT	CBG · urea	SHBG		
bulimia	B	BMI · body weight · AlaT · body fat · insulin · HOMA		CBG	SHBG	deoxycortisol ⁸	
interpersonal distrust	ID	deoxycortisol · pregnenolone	AspT · cortisol	deoxycortisol · AspT			
ineffectiveness	I	free-fatty-acids · deoxycortisol	AspT	CBG · glucose · HOMA · AspT AlaT	SHBG	HOMA	
maturity fears	MF				SHBG · height	glucose · HOMA · plasma-proteins	
perfectionism	P	albumin		AspT · LDH		BMI · body-fat	
impulse regulation	IR	deoxycortisol progesterone ⁸ free-fatty-acids	AspT		SHBG	uric acid · CBG · deoxycortisol ⁸	
ascetism	A	deoxycortisol androstenedione free-fatty-acids		CBG	SHBG · albumin		
social insecurity	SI	deoxycortisol · free-fatty-acids	AspT	deoxycortisol	SHBG	glucose · HOMA	
EDI2 total scores	EDI	urea · plasma-proteins · albumin	AspT	CBG	SHBG	glucose · bilirubin	
SCL90R Global severity index	GSI	total protein · albumin urea	AspT	CBG	SHBG	glucose · bilirrubin -	

Only significant correlations ($p < 0.05$) have been included; data **in bold** correspond to $p < 0.01$. Data for most hormones values were calculated from a lower number of individuals because of lower-than-threshold measurements obtained in a number of subjects; superscript figures indicate a low number (N) of individuals with valid measurements in the group.



SUPPLEMENTARY ONLINE MATERIAL

Figure 1

Steroid hormones that have been analyzed in the present study and their overall metabolic relationships.

The hormones measured in this study are presented on white rectangles.

Supplementary methods

Analysis of hormones by HPLC-MS/MS

Hormone standards were obtained from Steraloids (Newport RI USA) and Sigma (St Louis MO USA), and the deuterated internal standards from CDN Isotopes (Pointe Claire Ontario Canada). Chromatographic-grade solvents were from Riedel de Haën (Seelze Germany). Calibration curves were prepared for all steroid hormones in the range 1 to 4000 nM. The ratio between the peak area of each endogenous steroid and the deuterated steroid standard was correlated with the concentration using linear regression analysis to quantify serum steroid levels.

All samples were measured by HPLC-MS/MS in single continuous batches for each group of hormones, with a number of blanks and standards interspersed between the samples in order to obtain a good degree of homogeneity in the measurements. Three sets of serum samples (0.400 mL each, in duplicate) were used. The first was duped with 0.010 mL of 50 nM d4-estrone and 25 nM d4-estradiol as internal standards. Samples were extracted with 2 mL dichloromethane. The organic phase was dried under a gentle nitrogen stream; dry residues were resuspended in 0.075 mL of 100 mM bicarbonate buffer. pH 10.5. and 0.075 mL of 1g/L dansyl-chloride (Sigma) in acetone (1) were added, then vortexed and incubated 5 min at 60 °C. This suspension was directly injected to the HPLC-MS/MS system.

The second set of samples (0.400 mL) was loaded with 0.025 mL of 1 µM d7-androstenedione and 1 µM d2-testosterone as internal standards. Samples were extracted with 1.5 mL acetonitrile; the organic phase was dried with nitrogen and re-suspended in 0.120 mL of 5 mM ammonium acetate buffer containing 1g/L formic acid. pH 4.0. This suspension was directly injected to the HPLC-MS/MS system.

The third set of serum samples (0.400 mL) were mixed with 0.025 mL of a working stock mixture of d8-cortisol (1×10^{-5} M) added as an internal standard, and the tubes were incubated on ice for 20 min. Acetonitrile (1.5 mL) was then added and the tubes were vortexed and centrifuged to remove proteins. Supernatants were transferred into glass tubes and evaporated to dryness under nitrogen; dried residues were re-dissolved in 0.120 mL of the mobile phase, which was injected to the HPLC-MS/MS system.

Sample analyses were performed by LC-MS/MS (Varian. Palo Alto. CA. USA) in an instrumental setup consisting of: 210 pump with an on-line degassifier, 410 autosampler, and 1200L triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operated in positive ion mode.

Chromatographic separation was carried out using an Atlantis column 50 x 2.1 mm 3 μ m (Waters, Milford USA) at 0.3 mL/min for estrogens, and an Inertsil ODS-3 column 150 x 3.0 mm 3 μ m (Varian) at 0.2 mL/min for androgens. The eluent for estrogens was: a) 95% water-5% acetonitrile (with 0.1% formic acid). b) 95% acetonitrile-5% water (with 0.1% formic acid). The gradient (for b) was: min 0: 70 %. min 0.24-2: 85%. min 2-3.3: 85-95%. min 3.3-4: 95-70%. min 4-7: 70 %.

The eluent for androgens was: a) 5 mM ammonium acetate (with 0.1% formic acid). b) acetonitrile. The gradient (for b) was: min 0-28: 30 55 %. min 28-32: 55 90 %. min 32-48: 90 %. min 48-50: 90 30 %. min 50-55: 30 %.

The limit of sensitivity for estrogens (referred to serum) was established at 25 pM. The limit of sensitivity for both androgens was set at 0.6 nM. Interassay precision for hormone analyses within the present range of measurement were: 20% estrone, 21 % estradiol, 16% androstenedione, and 17% testosterone.

The chromatographic separation of corticosteroids was performed in a 150 x 3.0 (i.d.) mm Intersil 3 ODS-3 column (Varian, Palo Alto CA USA). The mobile phase consisted of two eluents, solvent a (5mM ammonium acetate. 0.1% formic acid) and solvent b (acetonitrile), at a flow rate of 0.2 mL/min. Samples of 0.050 mL were injected into the LC-MS/MS, after which the injector was washed five times with mobile phase. The total run time was 55 min. A linear gradient from 30% to 55% b was programmed for the first 28 min, followed by a second linear gradient to 90% b for 4 min. and isocratic mode for the next 16 min. Then, the system was returned to the initial proportion of 30% solvent b over the following 2 min and maintained for the final 5 min of each run. Detection limits of the method (referred to serum) were 2 nM for cortisol and 10 nM for cortisone.

Reference

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Supplementary Table 1. Mean values for the anthropometric parameters of controls, and ED purging and non-purging women.

parameter	units	ED non-purging	ED purging	controls
Age	years	31.3 ± 1.9 (21)	28.5 ± 1.5 (35)	33.4 ± 2.1 (17)
Weight	kg	69.0 ± 6.2 (18)	61.1 ± 3.3 (30)	55.8 ± 1.9 (15)
Height	m	1.63 ± 0.01 (18)	1.61 ± 0.01 (30)	1.61 ± 0.01 (15)
BMI	kg/m ²	25.8 ± 2.2 (18)	23.5 ± 1.2 (30)	21.5 ± 0.7 (15)
Body fat	%	29.8 ± 3.1 (13)	21.0 ± 3.3 (16)	22.1 ± 1.9 (15)

The data are mean ± sem; the number of values (N) for each parameter is given between parentheses.. The only significant difference between groups ($p < 0.05$, Student's t test) was between non-purging and controls for body fat. The rest of comparisons yielded non statistically significant differences

Supplementary Table 2. Mean values for other serum metabolic data of controls, and ED purging and non-purging women.

parameter	units	ED non-purging	ED purging	ED controls
glucose	mM	6.41 ± 0.93 (21)	5.23 ± 0.18 (35)	5.73 ± 0,27 (17)
insulin	pM	66.2 ± 7.9 (21)	55.7 ± 3.2 (35)	64.3 ± 5.4 (16)
HOMA index		3.65 ± 0.96 (21)	2.16 ± 0.14 (35)	2.74 ± 0.28 (16)
total cholesterol	mM	4.63 ± 0.26 (21)	4.52 ± 0.16 (35)	4.81 ± 0.22 (18)
urea	mM	2.03 ± 0.11 (21)	1.91 ± 0.10 (35)	2.10 ± 0.12 (18)
total bilirubin	µM	15.8 ± 1.4 (21)	13.9 ± 0.8 (35)	16.9 ± 1.5 (18)
aspartate aminotransferase	nkat/l	348 ± 20 (21)	334 ± 19 (35)	317 ± 30 (18)
alanine aminotransferase	nkat/l	373 ± 93 (21)	232 ± 16 (35)	282 ± 42 (17)
lactate dehydrogenase	µkat/l	6.01 ± 0.36 (21)	5.84 ± 0.32 (35)	5.37 ± 0.34 (17)
total protein	g/l	68.9 ± 3.0 (21)	69.8 ± 1.5 (35)	72.6 ± 2.3 (18)
albumin	g/l	47.9 ± 2.4 (21)	49.5 ± 1.0 (35)	48.0 ± 2.4 (18)
triacylglycerols	mM	1.00 ± 0.23 (21)	0.824 ± 0.07 (35)	0.69 ± 0.06 (18)
uric acid	µM	261 ± 16 (21)	270 ± 10 (35)	271 ± 14 (18)
free fatty acids	mM	0.60 ± 0.07 (21)	0.60 ± 0.07 (35)	0.58 ± 0.06 (18)

The data are mean ± sem; the number of values (N) for each parameter is given between parentheses. There were no statistically significant differences between groups (Student's *t* test).