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# Symptoms of depression and anxiety in anorexia nervosa: Links with plasma tryptophan and serotonin metabolism.

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Tryptophan

**Summary** Depressive, anxiety and obsessive symptoms frequently co-occur with anorexia nervosa (AN). The relationship between these clinical manifestations and the biological changes caused by starvation is not well understood. It has been hypothesised that reduced availability of tryptophan (TRP) could reduce serotonin activity and thus trigger these comorbid symptoms. The aim of this study, during re-feeding in individuals with AN, was to analyse covariations across measures of nutritional status, depressive and anxiety symptoms, and peripheral serotonin markers.

Depressive and anxiety symptoms, nutritional status and serotonin markers — whole blood serotonin content, plasma TRP and the ratio between TRP and large neutral amino acids — were assessed for 42 AN participants at admission to inpatient treatment and after re-feeding. Biological measures were compared to those obtained in 42 non-eating disordered subjects. For those with AN, psychological, nutritional and biological parameters improved significantly during hospitalisation. Levels of serotonin markers were significantly lower in the AN group compared to the control group, at admission and at discharge. Increase in the TRP/LNAA ratio was correlated with a decrease in depressive symptoms. In addition, there was a positive correlation between serotonin levels and symptoms of both anxiety and depression at discharge. We speculate that enhanced TRP availability during re-feeding, as a result of the increase in the TRP/LNAA ratio, could restore serotonin neurotransmission and lead to a decrease in depressive symptoms. The association between serotonin and anxiety and depressive symptoms would be consistent with numerous observations indicating abnormal functioning of the serotoninergic system in AN.


## 1. Introduction

Anorexia nervosa (AN), a disorder of unknown aetiology, is frequently associated with symptoms of anxiety, obsessive-compulsiveness and depression (Godart et al., 2002, 2007). The presence of the latter symptoms could be partly attributable to effects of a state of starvation (Keys et al., 1950; Pollice et al., 1997). Available studies in AN that have explored the link between nutritional status on the one hand, and anxiety and depressive symptoms on the other, yield inconsistent results (Mattar et al., 2011c). In addition, the pathophysiological mechanisms that might underlie these symptoms are not well understood.

Malnutrition has an impact on peripheral and central serotoninergic pathways, probably by way of tryptophan (TRP) deficiency (an essential amino acid and precursor of serotonin) (Kaye et al., 2009). Furthermore, the serotonin (5-HT) system is involved in various psychiatric disorders (depression, anxiety, obsessive symptoms and impulsivity) and in the regulation of the feeling of satiety. Consequently, it has been suggested that 5-HT activity might be important in the physiopathology of AN (Brewerton, 1995; Kaye et al., 2009). In animal models, hypothalamic 5-HT levels in rats are lowered by fasting (Haleem and Haider, 1996). Likewise, in patients with AN, there is a correlation between peripheral and central levels of serotoninergic markers (Nakatani et al., 2008; Liu et al., 2011). In addition, an increase in TRP intake leads to an increase in cerebral serotonin and/or 5-hydroxyindoleacetic acid (5HIAA — the main catabolite of serotonin), while conversely TRP depletion leads to a decrease in brain serotonin synthesis (Russo et al., 2009). However, reviews of the literature on studies measuring these peripheral serotoninergic markers in AN yield conflicting results (Attia et al., 2005). No study has evidenced differences between underweight AN patients and non-eating disordered controls on indices of blood serotonin levels (Askenazy et al., 1998; Ehrlich et al., 2008, 2009b; Comai et al., 2010). Certain studies report a decrease in plasma TRP and a decrease in the TRP/LNAA ratio (LNAA for large neutral amino acids) in acutely underweight anorexic patients (Schreiber et al., 1991; Askenazy et al., 1998; Ehrlich et al., 2009a; Comai et al., 2010). In fact, TRP competes with other LNAAs for the same transport system across the blood—brain barrier (Fernstrom and Wurtman, 1997). The TRP/LNAA ratio is thus predictive of transfer of plasma TRP to the CSF. Other studies have evidenced alteration in the serotoninergic pathway in underweight anorexic patients — a decrease in CSF 5-HIAA (Kaye et al., 1988), a decrease in monoamine oxidase activity (Díaz-Marsá et al., 2000), and reduced platelet paroxetine binding, indicating lower levels of peripheral serotonin transporter (Bruce et al., 2006) and a drop in the secretion of prolactin after administration of serotoninergic agonists such as m-CPP (Hadigan et al., 1995) and D-fenfluramine (Monteleone et al., 1998). After re-feeding, Kaye et al. (1988, 1984) showed rapid normalisation of 5HIAA levels in the CSF as soon as there was weight gain. One study noted a normalisation of plasma TRP and the TRP/LNAA ratio (Attia et al., 2005), but this result was not found by another team (Ehrlich et al., 2009a). Many issues remain unanswered: in AN, are serotonin anomalies constitutive factors, caused by restrictive eating during the acute phase, the scars of chronic malnutrition persisting after re-feeding, or a combination of some of these parameters?

Some studies have reported a decrease in anxiety and depressive symptoms in AN with weight gain (Pollice et al., 1997), and others, the normalisation of serotoninergic biological parameters (Attia et al., 2005). However, no study to date has addressed these two elements conjointly. Furthermore, no study has accurately detailed the nutritional state of the patients (Mattar et al., 2011b), all studies using either body mass index (BMI) or solely body weight to assess the nutritional status. As body weight alone is not a sensitive tool, it has recently been recommended that at least two methods should be used, such as Bioelectrical Impedance

Analysis (BIA) for body composition, and BMI in order to assess nutritional status in AN more comprehensively (Mattar et al., 2011b). The simultaneous exploration of anxiety and depressive symptoms, nutritional status and the peripheral metabolism of tryptophan and serotonin could determine whether there are links among these different parameters in the acute phase and after re-feeding and weight gain. New insights into these mechanisms could help to understand AN physiopathology and to develop better therapies.

## 2. Materials and methods

This project was part of a multi-centre prospective study called EVHAN (Evaluation of Hospitalisation for AN, registered in Eudract n° 2007-A01110-53, Clinical trials), and was approved by the Ile de France III ethics committee and CNIL (Commission National Informatique et Libertés). Written informed consent was obtained from all patients, in accordance with the Helsinki criteria.

### 2.1. Clinical sample

Forty-two AN subjects hospitalised consecutively between November 2009 and July 2010 for AN in 5 specialised psychiatry departments in France were included in the study (see Appendix 1 for list). The inclusion criteria were as follows: patients hospitalised for AN, aged between 13 and 65, and agreeing to take part in the study (with parental authorisation for subjects under 18 years of age). Current AN diagnosis was based on the DSM-IV-TR criteria (American Psychiatric Association, 2000) obtained using the CIDI 3.0 and the EDE-Q (Fairburn and Beglin, 1994) with the following BMI criteria: BMI $<$ 10th percentile up to 17 years of age, and BMI $<$ 17.5 for 17 years of age and above. Patients were excluded if they had a potentially confounding somatic pathology (such as diabetes, Crohn's disease, or metabolic disease). A control group, forty-two non-eating disordered subjects, was recruited in the same geographic area as the AN subjects, during a post-hospitalisation outpatient follow-up (at least 2 months after hospitalisation) in orthopaedic departments in Lariboisière and Robert Debré hospitals in Paris, France. They were screened with the hospital anxiety and depression scale (HADS) (Zigmond and Snaith, 1983; Lépine et al., 1985) in order to ensure they were below threshold scores for depression ($<$6) and anxiety ($<$12), so that none of them was presenting with any current anxiety or depressive disorder. These non-eating disordered subjects were also not taking any medication, thus being assessed by toxicology screening.

They were matched to clinical participants for gender and age.

### 2.2. Clinical evaluation

#### 2.2.1. Clinical questionnaires

Clinical participants were evaluated in the first two weeks of hospitalisation, and then in the two weeks preceding discharge.

Eating disorder symptoms were assessed using the eating attitudes test 26 (EAT-26) and the eating disorder examination questionnaire (EDE-Q). The EAT explores three domains: dieting, bulimia and food preoccupation, and oral control (Garner et al., 1982; Leichner et al., 1994). The EDE-Q evaluates frequency of symptoms in the previous 28 days, and explores four domains: restraint, eating concerns, shape concerns and weight concerns (Fairburn and Beglin, 1994). Depressive symptoms were assessed using two self-report questionnaires: the Beck depression inventory II (BDI II) (Beck et al., 1961; Bourque and Beaudette, 1982) and the hospital anxiety and depression scale (HADS) which evaluates anxiety and depressive symptoms (Zigmond and Snaith, 1983; Lépine et al., 1985). Obsessive-compulsive symptoms were assessed using the Maudsley obsessive-compulsive inventory (MOCI) (Hodgson and Rachman, 1977; Hantouche and Guelfi, 1993) a self-report questionnaire. Finally, social phobia symptoms were assessed using the Liebowitz social phobia scale (LSAS) (Yao et al., 1999), a clinical interview.

#### 2.2.2. Nutritional evaluation

Body weight (standard scales, SECA, Germany) and height (SECA gauge, Germany) were recorded for the calculation of body mass index (BMI) (weight divided by height in metres squared).

Body composition was measured using the Bioelectrical Analyzer (FORANA, Helios, Frankfurt, Germany) with an alternating electric current at 50 kHz and 800 mAmp and 4 skin electrodes (BIANOSTIC, DataInput, Darmstadt, Germany). The principles for measuring body composition using BIA have been previously described. Body components (fat and fat-free mass) were estimated using the Deurenberg equation (Mattar et al., 2011a). Subsequently, these measures were converted into indexes (fat mass index — FMI-and-fat-free mass index — FFMI) by dividing fat and fat-free mass by height in metres squared. This enables more reliable comparisons between the present subjects and those in other studies (Mattar et al., 2011a).

#### 2.2.3. Biological explorations

The following titrations were performed after admission and at discharge for the AN patients, and once for the control subjects:

- serotoninergic pathway: total blood serotonin, plasma tryptophan and large neutral amino acids (LNAA). Titration of serotonin, tryptophan and LNAA were performed using HPLC and fluorimetric detection (Kema et al., 1993).
- albuminaemia and pre-albumineamia.
- plasma leptin (ELISA sandwich technique, R&D DLP00 kit) used as a biological marker for fat mass, since leptin is produced by adipocytes (Mantzoros et al., 1997).

All samples were obtained between 7:30 and 8:30 a.m. after an overnight fast. In the 48 h preceding the sampling, subjects had a specific diet without foods rich in tryptophan (banana, citrus fruit, chocolate, dried fruit, nuts, tomato and avocado) so as to restrict the influence of dietary factors on titration results (Fernstrom and Wurtman, 1997).

#### 2.2.4. Other clinical data

Since selective serotonin reuptake inhibitors (SSRIs) can lead to a depletion in platelet serotonin (Alvarez et al., 1999), antidepressant treatments administered during hospitalisation were noted. Clinical criteria liable to affect the results

of titrations were obtained from structured research interviews, for instance past or present smoking (Rendu et al., 2011), oral contraceptive use (Ehrlich et al., 2008) and the number of hours of physical activity per week (walking, running, cycling, swimming, housework and other) (Favaro et al., 2000).

### 2.3. Statistical analyses

Statistical analyses were performed on SPSS 17.0. Data are reported as mean (*M*) and standard deviation (SD) for quantitative variables, and ratios (*n*/*N*) and percentages for qualitative variables. Prior to analysis, we verified that all variables were normally distributed (Kolmogorov—Smirnov test). Group differences (between AN participants at admission and at discharge, and between AN and non-eating disordered subjects) were tested by paired sample *t*-tests. Multivariate analysis was used for adjustment on BMI, with linear regression. Indeed, BMI can be a confounding factor (Mattar et al., 2011c; Rendu et al., 2011). Because of the small size of subgroups, non-parametric Mann—Whitney *U* tests were used for comparisons of AN participants according to the AN subtype, presence of antidepressant treatment, oral contraceptive or tobacco use. Pearson correlation coefficients were calculated to assess relationships between clinical, biochemical and nutritional variables. We did not find any difference between AN-R and AN-BP subjects in terms of clinical parameters or biological titrations, at admission or at discharge, so the AN group was considered as a whole in this study.

As recommended for exploratory studies, we did not perform multiple test adjustments (Bender and Lange, 2001).

## 3. Results

### 3.1. Description of clinical participants and non-eating disordered samples

Forty women and two men were included consecutively in the study. The mean age of the AN participants was 17.0 years (3.25) (range = 13.2—32.8) and their mean BMI 14.1 (1.41) (Table 1). Forty-two non-eating disordered subjects were matched for age and gender. Their mean age was 17.1 (3.27) (range = 13.6—33.4). Their mean BMI was 19.4 (2.24), significantly higher than in the AN group ($t = 13.15$, $p < 0.001$).

### 3.2. Evolution of clinical and biological variables during re-feeding

At admission, AN participants had high levels of depressive, anxiety and eating symptoms. All the symptoms decreased significantly between admission and discharge (Table 2). Concerning nutritional status, the FMI, FFMI and BMI of AN participants increased significantly in the course of hospitalisation (Table 2). BMI at discharge was however still below that of non-eating disordered subjects ($t = 3.51$, $p = 0.001$). For biological variables (serotonin, TRP, LNAA and TRP/LNAA), all the AN group measures were significantly below the non-eating disordered group values (Table 3). All of them increased significantly between admission and discharge.

**Table 1** Patient characteristics at admission.

| Characteristics | Mean (SD) |
|---|---|
| Age (years) | 17.0 (3.25) |
| BMI at the time of hospitalisation | 14.1 (1.41) |
| Lifetime minimum BMI | 13.3 (1.61) |
| Lifetime maximum BMI | 19.7 (3.1) |
| AN duration (months) | 29.8 (25.32) |
| Length of hospitalisation (months) | 4.90 (4.37) |
| Physical activity (hours per week) | 9.70 (8.27) |
| **Characteristics** | **(*N*)%** |
| AN-R | (19) 45% |
| AN-BP | (23) 55% |
| Antidepressants (admission) | (11) 26.2% |
| Antidepressants (discharge) | (18) 43% |
| Current smoker | (6) 14.3% |
| Past smoker | (3) 7.1% |
| Oral contraception | (3) 7.1% |

AN-BP, anorexia nervosa bingeing—purging type; AN-R, anorexia nervosa restricting type; BMI, body mass index; SD, standard deviation

Nevertheless at the end of hospitalisation the levels remained below those of the non-eating disordered subjects. These results were still significantly different after adjustment for BMI. At admission all AN participants had albumin levels in the normal range. Only 3 participants had prealbumin levels below normal (results not shown).

**Table 2** Description and comparison of eating disorders, anxiety, depressive symptoms and nutritional status at admission and discharge.

| | Admission *M* (SD) | Discharge *M* (SD) | *p* |
|---|---|---|---|
| BDI | 27.4 (13.7) | 11.8 (10.5) | <0.001 |
| HADS depression | 8.86 (4.61) | 4.83 (3.63) | <0.001 |
| HADS anxiety | 12.0 (4.23) | 8.20 (4.41) | <0.001 |
| MOCI | 12.3 (5.6) | 9.11 (5.66) | <0.001 |
| LSAS fear | 24.6 (15.9) | 16.9 (15.4) | <0.001 |
| EAT global score | 32.6 (17.6) | 14.1 (15.2) | <0.001 |
| EAT dieting | 17.5 (10.8) | 8.98 (9.45) | <0.001 |
| EAT bulimia | 5.80 (4.22) | 2.53 (3.46) | <0.001 |
| EAT control | 9.33 (4.70) | 2.80 (3.89) | <0.001 |
| EDE-Q global | 3.65 (1.49) | 2.50 (1.38) | <0.001 |
| EDE-Q restraint | 3.66 (1.96) | 2.20 (1.31) | <0.001 |
| EDE-Q eating | 3.27 (1.57) | 2.25 (1.44) | <0.001 |
| EDE-Q shape | 4.14 (1.60) | 2.97 (1.70) | <0.001 |
| EDE-Q weight | 3.47 (1.50) | 2.51 (1.59) | 0.002 |
| BMI | 14.1 (1.25) | 17.5 (1.56) | <0.001 |
| FFMI | 12.5 (0.94) | 14.0 (1.51) | <0.001 |
| FMI | 1.86 (1.14) | 3.84 (1.70) | <0.001 |

BDI, Beck depression inventory; BMI, body mass index; EAT, eating attitudes test; EDE-Q, eating disorder examination questionnaire; FFMI, fat-free mass index; FMI, fat mass index; HADS, hospital anxiety and depression scale; LSAS, Liebowitz social anxiety scale; SD, standard deviation; MOCI, Maudsley obsessive-compulsive inventory.

**Table 3** Description and comparison of serotonin, tryptophan, LNAA titration values for patients (at admission and discharge) and non-eating disordered subjects.

| | Admission *M* (SD) [*n*] | Discharge *M* (SD) [*n*] | Non-eating disordered subjects *M* (SD) [*n*] | AN admission vs. discharge *p* | AN admission vs. non-eating disordered subjects *p* | AN discharge vs. non-eating disordered subjects *p* |
|---|---|---|---|---|---|---|
| Serotonin (nmol/L) | 73.8 (44.8) [40] | 178.5 (80.3) [29] | 405.8 (155.6) [42] | <0.001 | <0.001 | <0.001 |
| TRP (μmol/L) | 13.5 (3.35) [39] | 18.8 (4.81) [28] | 49.9 (6.92) [42] | <0.001 | <0.001 | <0.001 |
| LNAA (μmol/L) | 144.2 (34.9) [37] | 172.5 (37.5) [27] | 423.0 (128.3) [42] | <0.001 | <0.001 | <0.001 |
| TRP/LNAA | 0.096 (0.011) [37] | 0.110 (0.011) [27] | 0.131 (0.048) [42] | 0.001 | <0.001 | = 0.032 |

LNAA, large neutral amino acids; *n*, number of blood samples collected for each biological titration; SD, standard deviation; TRP, tryptophan.

### 3.3. Links between clinical symptoms, biological variables and nutritional status

The change in depression scores measured using the BDI was negatively correlated with change in the TRP/LNAA ratio ($r = -0.442$, $p = 0.035$) — the lower the level of depression, the higher the TRP/LNAA ratio. These results were not linked to non-specific somatic factors assessed by the BDI: there was for instance no correlation between the TRP/LNAA ratio and items evaluating appetite or fatigue in the BDI.

At discharge, a higher score on the HADS anxiety scale was associated with higher serotonin levels ($r = 0.414$, $p = 0.032$): the higher the serotonin levels at discharge, the more marked were anxiety symptoms. Likewise for depressive symptoms, the higher the serotonin levels at discharge, the higher the score on the BDI ($r = 0.415$, $p = 0.031$).

### 3.4. Factors liable to influence biological titrations

At admission and at discharge, neither the presence of antidepressant treatment, nor that of oral contraception or past or present tobacco use was linked to titration values for serotonin, tryptophan, LNAA or TRP/LNAA. Similarly, titration results did not differ according to the number of hours of physical activity, or the AN subtype (restricting type or bingeing—purging type).

After multivariate analysis and adjustment on BMI, the link between variations in symptoms on the BDI and the TRP/LNAA ratio, and between the variations in the BDI and BMI, was significant (respectively $p = 0.053$ and $p = 0.021$). It means that both variables together significantly predict the change in the depression score ($F = 5.56$, $p = 0.013$) and are independent determinants of the reduction of depressive symptoms (Table 4). Finally, the link between the BDI score and serotonin at discharge reached threshold significance ($p = 0.056$) and the link between anxiety and serotonin was still significant ($p = 0.032$) (Table 4).

**Table 4** Multivariate analysis after adjustment on BMI.

| Variable to be explained | Explicative variables | $\beta$ | *p* |
|---|---|---|---|
| Δ BDI | Δ TRP/LNAA | −0.377 | 0.053 |
| | Δ BMI | −0.461 | **0.021** |
| Discharge BDI | Discharge serotonin | 0.376 | 0.056 |
| | Discharge BMI | −0.224 | 0.244 |
| Discharge HADS | Discharge serotonin | 0.429 | **0.032** |
| | Discharge BMI | −0.121 | 0.524 |

BDI, Beck depression inventory; BMI, body mass index; HADS, hospital anxiety and depression scale; LNAA, large neutral amino acids; TRP, tryptophan.

## 4. Discussion

This work is original because it studies the link between psychological state (anxiety and depressive symptoms), nutritional status and serotonin markers in AN conjointly. To our knowledge, this study is the first to have highlighted low blood serotonin, plasma tryptophan, LNAA and the TRP/LNAA ratio concomitantly with malnutrition. While there was an improvement in these parameters following re-feeding and weight gain, after re-feeding, levels nevertheless remained below those of non-eating disordered subjects. To date, studies that have measured peripheral markers of the serotoninergic pathway in AN have reported heterogeneous results for plasma tryptophan and the TRP/LNAA ratio, and none has shown low levels of serotonin (platelet, total blood or plasma) compared to non-eating disordered subjects.

At admission, AN participants presented anxiety and depressive symptoms with biological disturbances of the serotoninergic pathway: severely decreased values for tryptophan, serotonin and the TRP/LNAA ratio compared to non-eating disordered subjects. This result is in line with data from the literature concerning high levels of anxiety and depressive symptoms in AN, especially during the acute phase (Kaye, 2008; Mattar et al., 2011c). The decrease in serotonin and tryptophan levels can be interpreted as the consequence of the major nutritional deficiencies observed in these AN participants (Attia et al., 2005). Anomalies in the serotoninergic pathway are thought to play a role in various behavioural changes observed in AN subjects: restricted eating, depressed mood, anxiety, hyperactivity and impaired impulse control (Haleem, 2012). TRP, an essential amino acid, is the exclusive precursor of serotonin. Therefore, a TRP deficit could alter serotoninergic neurotransmission. The fact that the increase in the TRP/LNAA ratio in the course of

re-feeding is linked to a decrease in depressive symptoms provides arguments in favour of a possible impact of the normalisation of the biological markers of the serotoninergic pathway on AN mood symptoms. Indeed, intake of essential amino acids during re-feeding and consecutive weight gain seem to lead to an increase in the TRP/LNAA ratio. This ratio is predictive of the transport of tryptophan through the blood—brain barrier towards the CSF (van Donkelaar et al., 2011). Tryptophan is then used for the synthesis of cerebral serotonin which restores serotoninergic transfer and leads to a decrease in depressive symptoms. In the general population, the repercussions of fasting on the serotoninergic pathway have likewise been evidenced. Among women, three weeks' low calorie diet can lead to a decrease in tryptophan and in the TRP/LNAA ratio. It also increases the prolactin response to serotoninergic agonist administration, suggesting a disturbance of the brain serotoninergic pathway during fasting. Indeed, prolactin secretion is regulated by serotonin. These effects are however not observed among men (Anderson et al., 1990; Walsh et al., 1995). Women could therefore be more vulnerable to the consequences of dietary restrictions, in particular for the serotoninergic pathway (Steiger et al., 2011). Given that AN mainly affects females, this could be an interesting line of research. Among young women with AN, prolonged fasting could lead to disturbances of the serotoninergic pathway, and contribute to the triggering of anxiety and depressive symptoms.

Furthermore, at discharge, positive correlations between serotonin levels and anxiety score on the one hand, and between serotonin levels and depression score on the other were found in this study. Before discussing this point, we must mention that although we found a link between biological parameters and clinical symptoms, it cannot be concluded that they are causally related. But these results are in line with the results reported by other teams, who also found a positive correlation between total blood serotonin levels and the level of anxiety measured on the HARS in a group of malnourished AN participants classified as impulsive (Askenazy et al., 1998). In our study however, the anxiety symptoms receded during hospitalisation. We hypothesise that anxiety symptoms linked to malnutrition disappear, but that symptoms that are independent from the nutritional state persist. These symptoms could be linked to anxiety disorders often associated in AN, the onset of which often precedes the eating disorder (Godart et al., 2002). This link could indicate basal hyperfunctioning of the serotoninergic pathway in AN, which has been suggested to play a role in anxiety in AN (Kaye et al., 2009). Indeed, genetic anomalies observed in the serotoninergic pathway provide arguments in favour of constitutional anomalies in AN (Steiger et al., 2009; Rask-Andersen et al., 2010). In addition, imaging studies suggest an anomaly in the balance between the activity of 5HT1A and 5HT2A receptors. Indeed, in both malnourished and recovered AN patients, some studies reported elevation of postsynaptic 5HT1A potential and diminished 5HT2A receptor binding potential (Kaye et al., 2009). The fact that these anomalies persist beyond the starvation period could indicate that they are trait-anomalies, although they could also be a long-term consequence of prolonged dietary deficiencies. Our results are compatible with the fact that, in AN participants, acute tryptophan depletion leads to a decrease in anxiety (Kaye et al., 2003). Thus, via restrictive dietary behaviours, subjects with a genetic predisposition could reduce their tryptophan intake in order to decrease cerebral serotonin synthesis and compensate for the serotoninergic hyperfunctioning that underpins their chronic state of anxiety. However, in a second phase, chronic malnutrition itself and tryptophan deficiency could generate anxiety symptoms, thus creating a vicious circle process in AN (Kaye et al., 2009).

In addition, the activation of 5HT1B and 5HT2C postsynaptic receptors have an important role in the inhibition of appetite. If over-activated, these receptors could play a part in the triggering and maintenance of restrictive eating behaviours. First, subjects suffering from AN could restrict their food intake to alleviate anxiety. 5HT1B and 5HT2C receptor anomalies could increase satiety and drive further restriction. Then dietary restriction could trigger new symptoms. For example, a decrease in hypothalamic 5HT concentrations is known to elicit hyperactivity (Haleem, 2009), which aggravates weight loss. Low 5HT levels could reinforce impulsivity and lead to the emergence of bulimic symptoms. This might explain the frequent overlap between the bulimic and the restrictive forms of AN and the alternating between these two eating behaviours.

Certain authors have hypothesised that there could be links between increased serotoninergic activity and anxiety, dysphoric mood, inhibition, inflexibility and preference for order (Kaye et al., 2009; Steiger et al., 2011). These results are compatible with personality traits and comorbidities often observed in AN participants. The positive correlation found between blood serotonin and depressive symptoms at discharge in this study could seem surprising. Concerning serotonin levels in depression, results are conflicting: some studies report a decrease and others an increase in serotonin levels in depressed subjects compared to non-depressed controls (Schins et al., 2004). If we consider the link between an increase in the TRP/LNAA ratio and the decrease in depressive symptoms, and the link between blood serotonin and anxiety or depression at discharge, it is possible that the TRP/LNAA ratio and blood serotonin, although both indicative of CSF serotonin levels (Liu et al., 2011; van Donkelaar et al., 2011), are not in fact associated with the same aspects of clinical symptom patterns. The serotonin system involves 14 or more receptors and interacts with many other molecules in the brain (Kaye et al., 2013). It is also possible that other neuro-transmission pathways not explored here play a part in the physiopathology of these symptoms. For instance some authors have underlined the hedonic nature of the self-discipline exhibited by these participants. They have hypothesised an implication of the dopaminergic pathway and reward-processing in starvation and hyperactivity for instance, encountered in AN (Kaye, 2008).

It can be noted that regarding depressive symptoms, only correlations with the BDI were evidenced. Compared to the HADS, the BDI assesses more numerous aspects of depression, in particular cognitive elements. These results do not appear to derive solely from the specific BDI items assessing appetite and fatigue, since no correlation was specifically found for these two items.

Concerning factors liable to alter blood levels of the markers measured, no difference was found in relation to the various elements considered. Despite the fact that selective serotonin reuptake inhibitors (SSRIs) lead to a depletion in platelet serotonin (Alvarez et al., 1999) we did not find this result in our sample, whether on entry or at discharge. It is possible that levels were already so low on account of malnutrition that the depletion caused by the drug was no longer observable. The frequent lack of efficacy of antidepressants

observed in the acute phase of AN could in fact be linked to repercussions of tryptophan deficiency on the synthesis of brain serotonin (Barbarich et al., 2004). Only one study investigated the use of a nutritional supplement containing TRP and it did not find an increase in the efficacy of fluoxetine in individuals with AN who were underweight. Nevertheless, only 9 subjects completed the 26-week study, and only weight gain and mood change were investigated (Barbarich et al., 2004). Other studies are needed to test the efficacy of nutritional supplements, which are simple, inexpensive and have few side effects.

Our study presents several limitations. The small sample size decreases the statistical power, in particular for the comparison of sub-groups and multivariate analyses. Further to this, an evaluation at the end of hospitalisation only provides data subsequent to partial re-feeding. BMI is still low, and although biological titration results are improved, the AN group values are still below those of the non-eating disordered subjects. Unfortunately, non-eating disordered subjects did not complete all the clinical questionnaires and we could not compare the AN subjects to a control group for the clinical parameters.

Although the titrations were preceded by 48 h of a diet poor in serotonin and tryptophan, the exact composition of meals was not recorded, so that any impact on titration results was not assessed. In addition, some patients received SSRI treatment during hospitalisation. The correlations observed could on the other hand be linked to a non-specific global effect of the clinical improvement. Finally we chose a dimensional approach instead of a categorical model. In AN, disentangling depression comorbidity from depressive symptoms caused by malnutrition is a complex question. The dimensional approach is usually used to evaluate the evolution of depressive symptoms during re-feeding. Due to these various limitations, our results should be considered as exploratory.

To conclude, anxiety and depressive symptoms decrease in the course of re-feeding in AN. Our study underlines the fact that the bio-availability of tryptophan could be one of the factors contributing to the decrease in anxiety and depressive symptoms encountered in the course of re-feeding in AN. Longitudinal studies on larger samples and over long-term follow-up to complete weight restoration are needed to confirm these results.

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## Conflict of interest

All authors declare that they have no conflict of interest.

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## Appendix 1

Five clinical psychiatric departments were involved in this study: in Institut Mutualiste Montsouris, Paris, Pr. Corcos; in CHU Bellevue St Etienne, Pr. Lang: in CHU Charles Perrens, Bordeaux, department headed by Pr. Bouvard; in CHU Bordeaux, Pr. Pommereau; and in CHU Rouen, Pr. Gerardin.

## Appendix 2

Evhan Group: Nathalie Godart; Sylvie Berthoz; Jeanne Duclos; Lama Mattar; Hélène Roux; Marie Raphaële Thiébaud; Christophe Lalanne; Sarah Vibert; Tamara Hubert; Annaig Courty; Damien Ringuenet; Jean-pierre Benoit; Corinne Blanchet; Marie-Rose Moro; Laura Bignami; Clémentine Nordon; Frédéric Rouillon; Solange Cook; Catherine Doyen; Marie-Christine Mouren Siméoni; Priscille Gerardin; Sylvie Lebecq; Marc-Antoine Podlipski; Claire Gayet; Malaika Lasfar; Marc Delorme; Xavier Pommereau; Stéphanie Bioulac; Bouvard; Jennifer Carrere; Karine Doncieux; Sophie Faucher; Catherine Fayollet; Amélie Prexl; Stéphane Billard; François Lang; Virginie Mourier-Soleillant; Régine Greiner; Aurélia Gay; Guy Carrot; Sylvain Lambert; Morgane Rousselet; Ludovic Placé; Jean-luc Venisse; Marie Bronnec; Bruno Falissard; Christophe Genolini; Christine Hassler; Jean-Marc Tréluyer; Olivier Chacornac; Maryline Delattre; Nellie Moulopo; Christelle Turuban; Christelle Auger

## Appendix 3. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.09.009.

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