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ORIGINAL PAPER

The 5-HTTLPR polymorphism, platelet serotonin transporter activity and platelet serotonin content in underweight and weight-recovered females with anorexia nervosa

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Abstract Serotonin (5-HT) pathways play an important role in the pathophysiology of anorexia nervosa (AN). In this study, we investigated functional characteristics of the platelet 5-HT transporter and platelet 5-HT content in AN patients at various stages of their illness in comparison to healthy control woman (HCW) controlling for the 5-HTTLPR deletion/insertion polymorphism and other confounding variables. Fasting blood samples of 58 acutely underweight AN patients (acAN, BMI = 15.2 ± 1.4), 26 AN patients of the initial acAN sample after short-term/partial weight restoration (BMI = 17.3 ± 0.9), 36 weight-recovered AN patients (recAN, BMI = 20.7 ± 2.2) and 58 HCW (BMI = 21.6 ± 2.0) were assessed for kinetic characteristics of platelet 5-HT uptake (V_{max}, K_m) and platelet 5-HT content. Plasma leptin served as an indicator of malnutrition. Mean V_{max} and K_m values were significantly higher in recAN subjects in comparison to HCW (2.05 ± 0.62 vs. 1.66 ± 0.40 nMol 5-HT/10^9 platelets min and 432 ± 215 vs. 315 ± 136 nMol, respectively) but there were no differences in platelet 5-HT content (464.8 ± 210.6 vs. 472.0 ± 162.2 ng 5-HT/10^9 platelets). 5-HT parameters in acAN patients and HCW were similar. 5-HTTLPR variants were not related to 5-HT platelet variables. In the longitudinal part of the study we found significantly increased 5-HT content but unchanged 5-HT uptake in AN patients after short-term/partial weight restoration. Our results highlight the importance of malnutrition for the interpretation of abnormalities in neurotransmitter systems in AN. Changes in platelet 5-HT transporter activity were related to the stage of the illness but not to 5-HTTLPR genotype. Increased V_{max} and K_m in recovered AN patients might mirror adaptive modulations of the 5-HT system.

Keywords Anorexia nervosa · Serotonin transporter · Platelet · 5-HTTLPR deletion/insertion polymorphism · Leptin

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Introduction

Serotonin (5-hydroxytryptamine, or 5-HT) pathways modulate the regulation of hunger and satiety [6]. Vice versa the 5-HT system is readily affected by nutritional intake [15]. Therefore, it has been hypothesised that pathologic eating behaviour in anorexia nervosa (AN) could be related to alterations in 5-HT activity [35]. A number of studies have shown reduced 5-HT activity in acutely underweight patients with AN: decreased cerebrospinal fluid (CSF) levels of the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA) [12, 32], and blunted plasma prolactin response to 5-HT agonists [7, 40]

could be secondary to a diet-induced reduction of tryptophan, the precursor amino acid of 5-HT [2, 4]. In order to eliminate the effects of acute malnutrition, it may be useful to study long-term weight-recovered AN patients. In contrast to patients with acute AN, CSF 5-HIAA levels were found to be elevated in recovered AN patients when compared to healthy controls [9]. Subsequently, increased 5-HT transmission has been suggested as a trait marker of AN [33]. Decreased platelet MAO-B activity [14] and changes in 5-HT_{2A} receptor binding as shown by positron emission tomography (PET) in recovered AN are in line with this hypothesis [18]. However, other studies have been contradictory (for reviews see [7, 31]).

The 5-HT transporter (5-HTT) is the major regulating determinant of the extracellular 5-HT concentration in the brain [41]. Food restriction in rats has been associated with decreased 5-HTT in the frontal cortex and raphe nucleus [29, 30]. Platelet cell membranes also contain 5-HTT proteins, which are encoded by the same single-copy gene [36]. Along with the vesicular transporter they are responsible for the uptake and accumulation of 5-HT released from gastrointestinal enterochromaffine cells into the blood. 5-HT uptake in platelets and brain synaptosomes were found to be significantly correlated after controlling for the confounding effects of gender [48]. In line with that, a recent SPECT study found a significant correlation between 5-HT uptake in platelets and specific binding of [^{123}I]-ADAM in the midbrain of female participants [52]. Hence, it seems appropriate to use platelets as a peripheral indicator of central 5-HT metabolism [10].

Two major methodological approaches are commonly used to study platelet 5-HTT: first, kinetic parameters of platelet 5-HT uptake may be described by examining V_{max} (maximum uptake rate) and K_m (affinity constant) in intact platelets, providing information on the functional status of the platelet 5-HTT. Second, binding studies using [^{3}H]-imipramine or [^{3}H]-paroxetine can be performed on isolated platelet membranes. The outcome parameters of the latter methods are B_{max} (maximum binding sites) and K_d (dissociation constant) [20, 49].

To date, only two studies have investigated the functional status of 5-HT uptake (V_{max}, K_m) in platelets of patients with AN. They are limited by small sample sizes ($n = 17$, $n = 15$) and were unable to demonstrate significant differences between patients and control participants [53, 56]. Nevertheless, Weizman et al. [53] found reduced [^{3}H]-imipramine binding in AN. Binding studies with [^{3}H]paroxetine in platelet membranes yielded somewhat inconsistent results. Hassanyeh et al. [25] found no differences between AN patients and healthy controls, whereas others reported reduced [^{3}H]paroxetine binding in patients [8, 46].

Another peripheral marker for 5-HT disturbances frequently used in psychiatric research is the 5-HT content measured in whole blood, serum, plasma or platelets. To date, only three studies have investigated whole blood 5-HT in underweight patients with AN. No changes [3, 37] or an increase in 5-HT content have been reported [25].

Furthermore, a 5-HTT-linked polymorphic region (5-HTTLPR) insertion/deletion polymorphism with long (l) and short (s) forms affects 5-HT transporter expression and function in lymphoblasts [27] but investigations of the influences of 5-HTTLPR genotypes on the expression of the 5-HTT in human platelets and neurons yielded heterogeneous results (see [42] for a comprehensive discussion).

In view of the small number of published studies, the inconsistency of their findings and the proposed role of platelet 5-HT uptake activity as an indicator of central 5-HTT, reassessment of platelet 5-HT uptake in AN seems to be of major interest. No study that we are aware of has addressed the question whether malnourished AN patients differ from weight-recovered AN patients with respect to platelet 5-HTT activity. Therefore, we investigated underweight as well as short-term/partially weight-restored and long-term weight-recovered patients. 5-HTT functionality in platelets was characterised by apparent V_{max} and K_m. In addition, we determined 5-HT uptake with very low substrate concentrations and platelet 5-HT content.

In line with the concept of increased 5-HT transmission in weight-recovered AN patients [33] we hypothesised that 5-HT uptake activity in platelets would be particularly low in this subgroup. Therefore, we expected the two main measures of 5HT uptake (V_{max} and K_m) to be decreased in weight-recovered AN patients. We controlled for confounding factors, such as 5-HTTLPR polymorphism, age [1, 11], hormonal contraceptives [54] and menstrual cycle [55], which have been shown to contribute to variability in platelet measures. We also determined plasma leptin concentrations as a biological marker for the degree of malnutrition. Leptin is a nutritionally regulated hormone primarily secreted by adipocytes that affects food intake and energy expenditure [57]. Hypoleptinaemia is indicative of progressed semi-starvation, an exceedingly low fat mass and impaired functioning of the hypothalamic-pituitary–gonadal axis [26].

Methods

Participants

The sample population consisted of female patients with acute AN, short-term/partially weight-restored AN, recovered AN and healthy control woman as described previously [13, 14]. In short, 58 subjects (14–29 years old) with acute anorexia nervosa (acAN) according to DSM-IV were

admitted to eating disorder programs of a university child and adolescent psychiatry and psychosomatic medicine department. During the study period, all acAN patients were enrolled in a behaviourally orientated nutritional rehabilitation program and were encouraged to gain a minimum of 500 g body weight weekly. Following a stable (approximately 2 weeks) weight gain of between 10 and 30% of the weight at baseline assessment, 26 of the 58 patients were reassessed at the end of their inpatient treatment phase. These patients are referred to as "short-term/partially weight-restored AN".

We also included 36 female subjects (15–29 years old), who were previously treated for AN and had successfully recovered from their illness (recAN). To be considered "recovered", subjects had to (1) maintain a BMI >18.5 (if older than 18 years) or a BMI >10th BMI percentile (if younger than 18 years, [34]) for at least 3 months prior to the study, (2) menstruate and (3) have not binged, purged, or engaged in significant restrictive eating patterns. The majority of the recAN patients in this study had been recovered for more than 12 months ($n = 21$, 58.3%) while all other recAN patients ($n = 15$, 41.7%) had been recovered for at least 3 months.

The control group consisted of 58 normal-weight, eumenorrheic, healthy female control subjects (HCW, 14–26 years old), who were recruited through advertisement among middle school, high school and university students.

Exclusion criteria and possible confounding variables, such as menstrual cycle or contraceptive medication, were obtained using a semi-structured research interview, the SIAB-EX interview (see below) and by physical examination. Smoking habits were also assessed but they have not been reported to affect 5-HT uptake or 5-HT concentration in platelets. HCW were excluded if they had any history of psychiatric illness. Patients were excluded if they had a lifetime history of any of the following clinical diagnoses: organic brain syndrome, schizophrenia, substance dependence, bipolar illness, bulimia nervosa or binge eating disorder. Further exclusion criteria for all participants were: IQ less than 85, current inflammatory, neurological or metabolic illness, chronic bowel diseases, cancer, anaemia, pregnancy, breast feeding, current use of aspirin, cortisone, antibiotics, antihypertensive medication and use of antidepressants or any other psychotropic medications or substances within the past 6 weeks.

This study was approved by the Institutional Review Board, and all subjects (and if underage their guardians) gave written informed consent.

Clinical measures

Current and/or past eating disorders were assessed in all participants using the expert form of the Structured Interview of Anorexia Nervosa and Bulimic Syndromes (SIAB-EX [16]). The SIAB-EX for participants aged between 12 and 65 years is a semi-structured interview that assesses the prevalence and severity of specific eating-related psychopathology over the past 3 months according to DSM-IV diagnostic criteria. It consists of 87 items and provides diagnoses of eating disorders according to ICD-10 and DSM-IV. Internal consistency was good and the interrater reliability ranged from 0.86 to 0.96 [17]. Interviews were conducted by clinically experienced and trained research assistants under the supervision of an attending child and adolescent psychiatrist.

Eating disorder-specific psychopathology was assessed with the short version of the Eating Disorders Inventory (EDI-2), a self report questionnaire comprising eight subscales [47]. Response categories range from 1 'never' to 6 'always'. The three core subscales "drive for thinness", "body dissatisfaction" and "Bulimia" were part of the confirmatory analyses in this study.

General levels of psychopathology were determined using the depressive symptoms subscale, the anxious symptoms subscale, the obsessive–compulsive symptoms subscale as well as the Global Severity Index of the Symptom Checklist 90 Revised (SCL-90-R) [19].

Blood collection and biochemical assessments

To avoid possible confounds of season of testing, patients and controls were recruited and assessed in parallel. Venous blood samples of 9 ml were collected into vacutainer tubes containing EDTA between 7:30 and 9:30 am after overnight fasting. Preparation of platelet-rich plasma (PRP) and assays of 5-HT uptake and 5-HT concentration were performed as described elsewhere. In brief, 5-HT uptake kinetic was examined by incubation of 100 µl PRP ($3.5–4.5 \times 10^7$ platelets) in Krebs phosphate buffer (pH 7.4) at seven different concentrations of [^{14}C]-5-HT/5-HT ranging from 0.1 to 2.5 µM at 37°C for 5 min (final volumen 500 µl). After incubation, platelets were collected by filtration on Whatman GF-B filters and washed [20]. K_m and V_{max} were calculated using the hyperbolic function of the software GraFit version 3.1. To obtain data on the 5-HTT activity under physiologically relevant conditions with very low substrate concentration, the assay of 5-HT uptake was also performed in the presence of 15 nM [^{14}C]-5-HT [21]. In the text, we will refer to the latter parameter as 5-HT^{low} uptake.

For technical reasons not all 5-HT parameters could be assessed in all participants. Assays of kinetic characteristics of 5-HT uptake (V_{max}, K_m) were performed in 51 of 58 HCW, 25 of 58 acAN, and 15 of 36 recAN patients.

Plasma leptin concentrations were measured by Enzyme Linked Immunosorbent Assay with a commercial kit

(Human Leptin "Dual Range" ELISA, Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Depending on the protocol, sensitivity was 0.125–20 ng/ml or 0.5–100 ng/ml.

Genotyping

Genotyping of the 5-HTTLPR was performed using polymerase chain reaction as described previously [43]. Allele frequencies were s/s $n = 19$, s/l $n = 66$, l/l $n = 63$. We detected no evidence for deviation from Hardy–Weinberg equilibrium.

Statistical analyses

If not indicated otherwise, all values are presented as mean ± standard deviation (SD). Histograms, box plots, normal probability plots and Levene statistics were employed to verify the underlying statistical assumptions. Due to violations of homogeneity of variances for plasma leptin concentrations, comparisons including these indicators were performed with nonparametric methods such as Kruskal–Wallis one-way analysis of variance, Mann–Whitney *U* Tests or Wilcoxon tests. All other variables were compared using one-way analysis of variance (ANOVA) with subsequent Scheffé post-hoc tests or paired *T* Tests. Multivariate analysis of covariance (MANCOVA) with a priori contrasts were used to control for the effects of possible confounding variables. Because of unequal cell sizes and the factorial design Type III, Sums of squares and Pillai's criterion were employed for multivariate testing. Age served as covariate. Contraceptive medication and menstrual cycle were entered as cofactors. The variable used for menstrual cycle had three levels according to the presumed hormonal situation: follicular phase (high estradiol), luteal phase (high estradiol and progesterone) and amenorrhoea or irregular cycle (>45 days, low estradiol and progesterone).

Correlations were calculated using Spearman correlation coefficients. All tests were performed with SPSS statistical software version 16.0 (SPSS, Chicago, IL, USA).

Results

Table 1 summarizes general and psychopathological characteristics for acAN, recAN and HCW. RecAN patients were slightly older than acAN patients but not HCW. BMI and plasma leptin concentrations were significantly lower in acAN patients relative to recAN and HCW. 5-HTTLPR genotype distribution was equal in acAN, recAN and HCW. ANOVA indicated significant differences between the three groups for the three EDI-2 core subscales and the selected SCL-90-R sub- and summary scales. Scheffé post-hoc tests revealed that acAN patients scored significantly higher than recAN and HCW on all aforementioned scales except anxiety.

Table 1 Sample characteristics

	acAN ($n = 58$)	recAN ($n = 36$)	HCW ($n = 58$)	F/χ^2	P
Age	17.4 ± 2.7^{a}	19.5 ± 3.3^{a}	18.4 ± 2.9	5.54	0.005
BMI	$15.2 \pm 1.4^{a,b}$	20.7 ± 2.2^{a}	21.6 ± 2.0^{b}	188.47	<0.001
Leptin	$1.4 \pm 2.1_{a,b}$	$13.7 \pm 13.3_{b}$	$14.9 \pm 8.7_{b}$	83.69	<0.001
5-HTTLPR s/s genotype *n* (%)	9 (15.8)	4 (11.1)	6 (10.9)		
5-HTTLPR s/l genotype *n* (%)	24 (42.1)	16 (44.4)	26 (47.3)		
5-HTTLPR l/l genotype *n* (%)	24 (42.1)	16 (44.4)	23 (41.8)	0.838	0.933
EDI-2 drive for thinness	$9.3 \pm 6.5^{a,b}$	3.5 ± 5.5^{a}	1.6 ± 3.2^{b}	33.44	<0.001
EDI-2 bulimia	$1.5 \pm 2.4^{a,b}$	0.4 ± 0.9^{a}	0.2 ± 0.6^{b}	11.48	<0.001
EDI-2 body dissatisfaction	$12.8 \pm 7.2^{a,b}$	6.0 ± 7.0^{a}	4.5 ± 6.1^{b}	23.53	<0.001
SCL-90-R depressive symptoms	$59.0 \pm 11.9^{a,b}$	46.8 ± 8.6^{a}	44.5 ± 7.8^{b}	35.29	<0.001
SCL-90-R anxiety	50.4 ± 11.9^{a}	45.4 ± 9.5	44.4 ± 8.5^{a}	5.59	0.005
SCL-90-R obsessive compulsive symptoms	$55.6 \pm 12.7^{a,b}$	44.7 ± 8.9^{a}	45.9 ± 8.3^{b}	17.01	<0.001
SCL-90-R Global Severity Index	$56.1 \pm 11.9^{a,b}$	44.5 ± 9.1^{a}	42.6 ± 8.8^{b}	28.05	<0.001

Descriptive statistics [mean ± standard deviation or number (%)], results of Kruskal–Wallis one-way analysis of variance (leptin), chi-square test (5-HTTLPR) and one-way analysis of variance (all other variables) of clinical, genetic and psychological variables in the crossectional analyses

Mean with the same letters in their subscripts differ on Mann–Withney *U* tests at the $P < 0.001$ level

Mean with the same letters in their superscripts differ on Scheffé post-hoc tests for multiple comparisons at the $P < 0.05$ level or less. The following units apply: age (years), BMI (kg/m^2), plasma leptin (ng/ml), EDI-2 (adjusted raw scores), SCL-90-R (*T* scores)

AcAN acute anorexia nervosa, *recAN* weight-recovered anorexia nervosa, *HCW* healthy control women

Cross-sectionally determined 5-HT platelet variables of acAN, recAN and HCW are shown in Table 2: the means of the main outcome variables V_{max} and K_m were significantly higher in recAN subjects in comparison to HCW. Although recAN subjects seemed to have higher mean 5-HT^{low} uptake activity, the differences were not statistically significant. Mean platelet 5-HT content was similar in all subgroups. Neither V_{max} and K_m nor 5-HT^{low} uptake activity and 5-HT content differed by 5-HTTLPR genotype ($F = 0.03$, $p = 0.971$; $F = 0.44$, $p = 0.646$; $F = 0.70$, $p = 0.496$; $F = 1.91$, $p = 0.152$) or by the interaction between genotype and diagnostic group ($F = 0.13$, $p = 0.971$; $F = 0.51$, $p = 0.729$; $F = 0.26$, $p = 0.901$; $F = 0.71$, $p = 0.585$).

To address possible confounds related to age, hormonal contraceptives and phase of menstrual cycle, we confirmed the aforementioned significant differences in our main outcome variables (V_{max} and K_m) using MANCOVA (see "Methods"). We found a significant multivariate main effect of our question variable 'diagnostic group' ($F = 3.14$, $p = 0.016$). Age ($F = 2.39$, $p = 0.098$), hormonal contraceptives ($F = 1.55$, $p = 0.219$) and menstrual cycle ($F = 0.50$, $p = 0.735$) did not influence our results. Subsequent univariate testing of the relevant between-subject factors confirmed the significant effect of 'diagnostic group' on V_{max} and K_m ($F = 4.99$, $p = 0.009$ and $F = 4.72$, $p = 0.011$). Our a priori defined contrast analyses verified that recAN had significantly higher V_{max} and K_m in comparison to HCW. In contrast, acAN and HCW had similar V_{max} and K_m. Further models controlling for the effects of depressive, anxious and obsessive–compulsive symptoms (SCL-90-R) or including two-way interactions between 'diagnostic group' and the remaining cofactors were in line with the simpler main effects models.

According to bivariate correlational analyses, which were carried out in each diagnostic subgroup (acAN, recAN, HCW) separately, none of the 5-HT parameters were associated with BMI, leptin or duration of illness.

Twenty-six acAN inpatients were reinvestigated at a second timepoint (T2, 16.3 ± 11.0 weeks after the first assessment) following a short-term/partial weight restoration (longitudinal analysis). We observed a significant increase of BMI, plasma leptin and platelet 5-HT content (Table 3). V_{max} and K_m and 5-HT^{low} uptake activity remained unchanged.

Discussion

Many researchers have emphasised the role of the 5-HT system in the neurobiology of AN. We found similar platelet 5-HTT functionality and 5-HT content in patients with acute AN and healthy control woman. In contrast V_{max} and K_m in weight-recovered AN patients were significantly higher compared to healthy controls. Weight-recovered AN patients showed a substantial freedom from eating disorder-specific and general psychopathology. The 5-HTTLPR insertion/deletion polymorphism was not related to 5-HT platelet variables.

Our results underline the significance of malnutrition and hypoleptinaemia for the interpretation of abnormalities in neurotransmitter systems. Assuming an increased 5-HT transmission in patients recovered from AN as suggested by Kaye et al. [33], we had hypothesised that 5-HT uptake

Table 3 Longitudinal study

	acAN T1	acAN T2	*T/Z*	*P*
Age	16.4 ± 1.4	16.7 ± 1.5	−5.43	<0.001
BMI	14.7 ± 1.1	17.3 ± 0.9	−17.32	<0.001
Leptin	0.4 ± 0.4	5.3 ± 4.7	−4.02	<0.001
V_{max}	1.83 ± 0.36	1.84 ± 0.41	−0.15	0.881
K_m	340 ± 94	328 ± 68	0.39	0.705
5-HT^{low} uptake	53.5 ± 11.2	54.2 ± 9.4	−0.28	0.783
5-HT-content	459.6 ± 131.0	524.7 ± 195.7	−2.25	0.034

Descriptive statistics before and after short-term/partial weight restoration, results of Wilcoxon tests (leptin) and paired *T* tests (all other variables). The following units apply: age (years), BMI (kg/m^2), plasma leptin (ng/ml), 5-HT-uptake (pmol ^{14}C-5-HT/10^9 platelets 5 min), 5-HT-content (ng 5-HT/10^9 platelets), V_{max} (nmol/10^9 platelets min), K_m (nmol)

Table 2 5-HT parameters

	acAN ($n = 58$)	recAN ($n = 36$)	HCW ($n = 58$)	*F*	*P*
V_{max}	1.83 ± 0.31	2.05 ± 0.62[a]	1.66 ± 0.40[a]	5.29	0.007
K_m	362 ± 117	432 ± 215[a]	315 ± 136[a]	3.86	0.025
5-HT^{low} uptake	53.7 ± 11.8	58.1 ± 11.1	55.4 ± 11.4	1.63	0.200
5-HT-content	482.2 ± 187.2	464.8 ± 210.6	472.0 ± 162.2	0.11	0.899

Descriptive statistics and results of one-way analyses of variance for the cross-sectionally acquired 5-HT parameters

Mean with the same letters in their superscripts differ on Scheffé post-hoc tests for multiple comparisons at the $P < 0.05$ level or less. The following units apply: plasma leptin [ng/ml], 5-HT-uptake [pmol ^{14}C-5-HT/10^9 platelets 5 min], 5-HT-content [ng 5-HT/10^9 platelets], V_{max} [nmol/10^9 platelets min], Km [nmol]. For the variables V_{max} and K_m the sample sizes were acAN = 25, recAN = 15 and HCW = 51

activity in platelets would be particularly low in recovered AN. However, the upregulation of 5-HTT functionality in this subgroup could represent an adaptation or possible counter regulation to elevated 5-HT release into the synaptic cleft. Stable weight recovery may unmask intrinsic abnormalities of the 5-HT system, e.g. increased 5HT synthesis, that exist independently of active eating disorder symptoms but could mediate certain core behavioural or temperamental underpinnings of risk and vulnerability. Disturbances of the 5-HT system in recAN patients could also represent lasting 'injuries' or 'scars', associated with a prolonged period of malnutrition. Unfortunately our current data of longitudinally assessed AN inpatients ($n = 26$) are not suitable to confirm the rather speculative hypothesis about the role of high 5-HTT activity as a trait marker. Patients had to gain 10% or more weight and maintain it for at least 2 weeks. These criteria are insufficient to exclude persisting state-related effects. Plasma leptin was still low and psychopathology measures as well as 5-HTT measures of these patients were comparable to acAN rather than to recAN.

Despite the lack of change in the main outcome parameter 5-HTT activity, the short-term/partially weight-restored AN patients showed a significant increase in platelet 5-HT content relative to the baseline values (Table 3). Platelet 5-HT content is regulated by 5-HTT activity and the availability of 5-HT in the bloodstream. Since 5-HT uptake was unchanged, the increase in platelet 5-HT content could be due to an elevated 5-HT synthesis rate. This may be explained by a predisposition of AN patients to high 5-HT synthesis in conjunction with elevated plasma TRP concentrations. A moderate increase of plasma tryptophan concentrations has been observed in AN patients during inpatient short-term weight restoration [4, 13]. In contrast, the long-term weight-recovered AN patients in our study showed normal platelet 5-HT content. However, as reported elsewhere, they were recruited from their homes and showed lower plasma tryptophan when compared to HCW [13].

The comparison of our findings with previous studies on 5-HT uptake in patients with AN is complicated as none of them attempted to differentiate by nutrition status. Two previous investigations also found no differences in V_{max} and K_m between underweight AN patients and healthy controls [53, 56]. More recently [^{3}H]paroxetine binding studies suggested a decreased 5-HTT density (B_{max}) in patients with acute AN, bulimia nervosa and recovered bulimia nervosa [8, 46, 51]. In contrast, V_{max} is thought to reflect both, the number of 5-HTT and their catalytic activity. Studies in which both, functional and binding characteristics of the platelet 5-HTT have been analysed, found no correlations between V_{max} and B_{max} [24, 28, 50].

Multivariate analysis confirmed that the findings of our study could not be attributed to 5-HTTLPR genotype, age, use of hormonal contraceptives or menstrual cycle, depressive, anxious or obsessive–compulsive symptoms. To date, only two other studies controlled for the influence of contraceptive medications on 5-HTT functioning in patients with eating disorders. In line with our results, they found no influence on 5-HTT variables in platelets or brain tissue [5, 8].

The lack of a genotype–phenotype effect corresponds to an increasing body of literature on 5-HTTLPR. Well-designed studies involving reasonably sized samples have failed to detect an association between platelet 5-HT variables and 5-HTTLPR variants among healthy volunteers [44] and patients with affective disorders [39], premenstrual dysphoria [38], alcoholism [45] or cocaine-dependence [42]. Furthermore, an association between 5-HTTLPR and AN (as an illness) remains controversial [23], whereas typical personality characteristics such as neuroticism might be related to the short form of the polymorphism [22].

In comparison to previous studies on platelet 5-HT parameters in AN patients, strength of this investigation include the differentiation of acutely underweight from weight-recovered patients, the exclusion of patients taking antidepressants or any other psychotropic medications, the simultaneous measurement of platelet 5-HTT activity and 5-HT content and the assessment of possibly confounding factors. A limitation of the study is the lack of V_{max} and K_m data for a significant proportion of the patient groups, which was a result of restricted laboratory capacity. However, acAN, recAN and HCW with or without V_{max} and K_m data were not different regarding age, nutritional parameters, usage of contraceptive medications, menstrual cycle, 5-HT content and 5-HT uptake at low substrate concentration (data not shown). Furthermore, a more conservative definition of weight recovery might have yielded more pronounced results.

In summary, the interpretation of findings related to 5-HTT activity can be difficult in AN, if the extent of semi-starvation is not taken into account. Our finding of higher mean V_{max} and K_m after long-term weight recovery could represent an adaptive alteration in 5-HTT activity as a response to increased 5-HT synthesis. Large prospective studies using neuroimaging techniques along with platelet measures are needed to verify our findings and clarify the role of 5-HTT activity before the onset and after recovery from AN.

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Conflict of interest statement All authors declare that they have no conflicts of interest.

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