

Impaired regulation of brain serotonin function during dieting in women recovered from depression

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Background Amino acid mixtures that lower brain availability of the serotonin (5-HT) precursor tryptophan produce acute depressive relapse in women with a history of major depression. Dieting also lowers brain tryptophan availability, but its effects on brain 5-HT function in recovered depressed women have not been studied.

Aim To test the hypothesis that women with a history of major depression would show impaired regulation of brain 5-HT function during a period of dieting-induced tryptophan depletion.

Method Women with and without a history of major depression were placed on a daily 1000 kcal (approximately 4200 kJ) diet for three weeks. Before the diet and in the final week we measured fasting plasma tryptophan levels and the prolactin response to an intravenous tryptophan challenge.

Results Dieting lowered plasma tryptophan levels equivalently in women with and without a history of depression. In women without a history of depression, dieting also increased the prolactin response to tryptophan. This increase did not occur in women with a history of depression.

Conclusions Women with a history of depression showed impaired regulation of brain 5-HT function in response to dieting.

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In healthy women a three-week calorie-reducing diet lowers plasma concentration of the 5-HT precursor tryptophan and increases the prolactin response to intravenous tryptophan challenge, a measure of brain 5-HT function (Anderson *et al*, 1990). We have suggested (Cowen *et al*, 1996) that the increased endocrine response to tryptophan challenge following dieting is caused by an adaptive up-regulation of post-synaptic 5-HT mechanisms in response to impaired presynaptic 5-HT release. Women who have recovered from depression show an abnormal sensitivity to the mood-lowering effects of acute tryptophan depletion induced by a tryptophan-free amino acid mixture, suggesting that they may have abnormalities in the regulation of brain 5-HT function (Smith *et al* 1997a,b). The aim of the present study was to test the hypothesis that, unlike women without a history of major depression, women recovered from depression who dieted would fail to show an increase in the prolactin response to intravenous tryptophan.

METHOD

Subjects

We recruited, by advertisement, women who were interested in taking part in a study of dieting. All subjects were assessed by using the Structured Clinical Interview for DSM-IV (SCID; First *et al*, 1997) to assess current and lifetime diagnoses according to DSM-IV (American Psychiatric Association, 1994). From the responders who were willing to take part in the study we identified 19 women with a history of at least one episode of DSM-IV major depression who were fully recovered and medication-free for at least six months. We also identified 23 women with no current or lifetime history of any Axis I psychiatric disorder who also reported no family history of such disorder. All subjects gave

informed consent to the study, which was approved by the local ethics committee.

Study design

Subjects were placed on a three-week calorie-controlled diet of 1000 kcal (approximately 4200 kJ) per day, as previously described in Anderson *et al* (1990). During the diet, subjects completed a daily diary recording what they ate, and rated three 100 mm visual analogue scales (VAS), 'happy', 'sad' and 'irritable' (on a scale of 0–100: 0, not at all; 100, extremely). Neuroendocrine testing was carried out before dieting began and at the end of the third week of dieting. The onset of dieting was delayed by one week after the first neuroendocrine test to ensure that both tests took part in the early- to mid-follicular phase of two consecutive menstrual cycles. We have previously found that subjects who fail to lose more than 2 kg in weight during this diet do not reliably increase their prolactin response to tryptophan (details available from author on request). Subjects who failed to achieve this weight reduction were therefore excluded from the present study and did not undergo the second tryptophan test.

Neuroendocrine testing

Neuroendocrine testing was carried out after an overnight fast. Subjects were brought to the research unit at 09.00 and an indwelling venous cannula was inserted and heparinised. A baseline period of 30 minutes elapsed during which three venous samples were taken for plasma prolactin and total tryptophan measurement. Following this, 5 g of intravenous tryptophan was infused over a 15-minute period, and further blood samples were taken for the following 105 minutes. Every 30 minutes subjects rated themselves for 'nausea' (the main adverse effect of tryptophan infusion) on a 100 mm VAS. During the second neuroendocrine test, the dose of intravenous tryptophan given was reduced in proportion to the weight loss that had been achieved during the diet. This was to ensure that individual subjects received the same mg/kg dose of tryptophan in the post-dieting test as they had in the pre-diet test. For administrative reasons it was not possible to blind the staff carrying out the neuroendocrine testing to subject status.

Biochemical measures

Blood samples for measurement of total tryptophan and prolactin levels were separated immediately after collection and stored at -30°C . All assays were carried out blind to subject status. Plasma tryptophan levels were determined by high-performance liquid chromatography with amperometric detection. Plasma prolactin levels were measured by using a standard immunoradiometric assay (reagents provided by Netria, London), with inter- and intra-assay coefficients of variation of 4.8% and 2.4% respectively.

Statistical analysis

Baseline plasma tryptophan levels were measured by taking the mean of the three baseline values before tryptophan infusion. Plasma tryptophan levels after the infusion were measured as areas under the curve (AUCs) with subtraction of baseline values extrapolated from Time '0'. Nausea ratings were measured as peak increase over baseline. Plasma prolactin levels after the tryptophan infusion generally showed a brief increase followed by a decline. For this reason peak prolactin change from baseline was taken as the key response measure, although the AUC was also calculated. The peak values were log transformed in order to approximate to a normal distribution. All the above measures were analysed with a repeated-measures analysis of variance (ANOVA) with 'diet' (pre-diet *v.* post-diet) as the main within-subjects factor and diagnosis (recovered from depression *v.* control) as a between-subjects factor. The prolactin data were also plotted against time and analysed by repeated-measures ANOVA with time as a further within-subject factor. The daily VAS scales were measured as change from baseline, and the resulting AUC values of controls and of those recovered from depression over the three-week dieting period were analysed with an unpaired *t*-test (two-tailed). Body weight and weight loss of the two subject groups were also compared using unpaired *t*-tests.

RESULTS

Of the 43 subjects who entered the study, 25 successfully completed the protocol (14/23 controls, 11/19 of those recovered from depression). The reasons for failure to complete were as follows: 14 subjects (nine controls, five recovered) failed to

achieve the minimum weight loss of 2 kg required by the protocol; one person who had recovered from depression could not be cannulated prior to the second neuro-endocrine test; and two people recovered from depression experienced significant stress-related side-effects during the second tryptophan infusion (vomiting and a vaso-vagal reaction requiring intervention). The characteristics of the subjects who completed the protocol are shown in Table 1.

The recovered patients tended to be heavier than controls, but the two groups achieved similar absolute and percentage weight losses (Table 1). There was a main effect of dieting on mean baseline plasma tryptophan levels ($F=11.8$, $P=0.002$) but no main effect of diagnosis ($F=0.93$, $P=0.35$) or interaction of diet with diagnosis ($F=0.26$, $P=0.61$). Plasma tryptophan levels fell in both groups after dieting (recovered from depression 10.5 (s.e.=0.60) to 9.30 (s.e.=0.72) $\mu\text{g/ml}$; controls 11.0 (s.e.=0.47) to 10.1 (s.e.=0.43) $\mu\text{g/ml}$). Following tryptophan infusion there was a substantial increase in plasma tryptophan values. The ANOVA of the AUC of tryptophan post-infusion levels showed no main effect of diet ($F=0.21$, $P=0.65$) or diagnosis ($F=0.71$, $P=0.41$) and no significant diet-by-diagnosis interaction ($F=0.004$, $P=0.95$). An ANOVA of the peak nausea ratings showed no main effect of diet ($F=0.78$, $P=0.39$) or diagnosis ($F=0.01$, $P=0.92$), and no interaction between diet and diagnosis ($F=1.0$, $P=0.33$). Mean peak nausea ratings for controls during the pre-diet tryptophan infusion were 13.1 mm (s.e.=5.3), compared with 13.6 mm (s.e.=4.3) during the post-diet infusion. The corresponding values for the recovered depression group were 10.0 mm (s.e.=4.2) and 18.2 mm (s.e.=9.2) respectively.

The ANOVA of the peak prolactin values showed no main effect of diet ($F=0.69$, $P=0.42$) or diagnosis ($F=2.9$, $P=0.1$), but there was a significant

interaction between diet and diagnosis ($F=5.7$, $P=0.025$). The prolactin responses to tryptophan of the two groups were very similar prior to dieting. After dieting, however, peak prolactin responses were increased in the controls but not in the recovered group (Fig. 1). The repeated-measures ANOVA including time as a within-subject factor showed no main effect of diet ($F=0.74$, $P=0.40$) or diagnosis ($F=2.12$, $P=0.16$). However, there were significant interactions between diet and diagnosis ($F=7.46$, $P=0.012$) and diet and time and diagnosis ($F=1.95$, $P=0.040$) (Fig. 2). The AUC analysis of prolactin response showed no significant effect of diet ($F=3.30$, $P=0.082$) or diet-by-diagnosis interaction ($F=3.24$, $P=0.085$). However, the mean (s.e.) of the AUC prolactin (over the 120 min infusion period) response was increased by dieting in controls (10 495 group (3728) to 23 320 (8836) $\text{mIU} \times \text{min/l}$, $P=0.045$) but not in the recovered group (6495 (2729) to 6553 (2207) $\text{mIU} \times \text{min/l}$, $P=0.99$).

VAS ratings showed a significant reduction in 'happy' scores over the three-week period of the diet in the recovered group relative to controls (-233.6 ± 80.7) $\text{mm} \times \text{day}$ *v.* -26.8 (s.e.=30.8) $\text{mm} \times \text{day}$, $P=0.03$). However, no significant differences were found between the two groups on ratings of 'sad' (recovered group 45.5 (s.e.=25.4) $\text{mm} \times \text{day}$ *v.* controls 36.3 (s.e.=45.5) $\text{mm} \times \text{day}$, $P=0.86$) or of 'irritable' (-3.6 (s.e.=77.8) $\text{mm} \times \text{day}$ *v.* 37.2 (59.0) $\text{mm} \times \text{day}$, $P=0.68$).

DISCUSSION

5-HT response to dieting in patients and controls

This study confirms the findings of previous investigations by our research group showing that in female volunteers a three-week period of dieting lowers plasma tryptophan

Table 1 Demographic data and weight loss during dieting

	Controls (n=14) (mean (s.e.))	Patients recovered from depression (n=11) (mean (s.e.))	P
Age	38.8 (2.7)	38.9 (2.0)	0.99
Body mass index	28.9 (0.8)	30.5 (0.8)	0.17
Initial weight (kg)	75.3 (2.9)	81.8 (1.2)	0.07
Weight loss during diet (kg)	3.4 (0.3)	3.5 (0.4)	0.73
Percentage weight loss	4.5% (0.4%)	4.3% (0.4%)	0.77

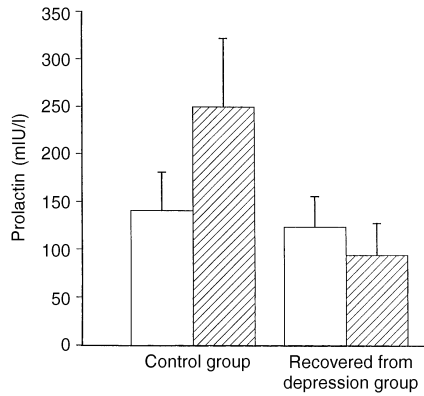


Fig. 1. Mean (s.e.) peak prolactin response (antilog of log transformed data) following intravenous tryptophan infusion (5 g) in healthy controls ($n=14$) and women with a history of depressive disorder ($n=11$), before (open bars) and at the end of a three-week calorie-controlled diet (hatched bars). Dieting increased the prolactin response in controls but not in those recovered from depression (ANOVA: $F=5.7$; $P=0.025$).

levels and increases endocrine responses to 5-HT challenge (Anderson *et al*, 1990; Walsh *et al* 1995; Cowen *et al*, 1996). In women with a history of major depression, dieting also lowered plasma tryptophan levels but did not increase the prolactin response to tryptophan infusion. This suggests that women with a vulnerability to major depression do not respond to decreased precursor availability by up-regulating the responsiveness of central 5-HT pathways.

Baseline 5-HT function in depressed women

The women who had recovered from depression did not differ from the controls in their pre-dieting prolactin response to tryptophan challenge. Although the presence of major depression is associated with blunted endocrine responses to tryptophan (Power & Cowen, 1992), our previous work has indicated that these responses normalise with clinical recovery (Upadhyaya *et al*, 1991). This finding suggests that, if there is a trait abnormality in brain 5-HT function in women vulnerable to major depression, it may be apparent only at times when 5-HT function is under some form of biochemical stress, such as decreased precursor availability.

It is worth noting, however, that women with acute major depression who have particularly marked weight loss can exhibit enhanced prolactin response to tryptophan (Cowen & Charig, 1987). This suggests that the difference between recovered women and controls found in the present study is one of degree, and that with sufficient restriction of tryptophan availability women recovered from depression might also show some up-regulation of 5-HT-mediated endocrine responses.

Mechanism of dieting-induced 5-HT up-regulation

The precise adaptive mechanisms that underlie the increased prolactin response

to tryptophan following dieting in healthy women are not established. We have obtained evidence for a functional up-regulation of post-synaptic 5-HT₂ receptors following dieting (Cowen *et al*, 1996), but the prolactin response to tryptophan appears under normal circumstances to be mediated by post-synaptic 5-HT_{1A} rather than 5-HT₂ receptors (Smith *et al*, 1991). Moreover, in animal experimental studies a low tryptophan diet causes an up-regulation in 5-HT₂ but not 5-HT_{1A} receptor function (Franklin *et al*, 1999). It is possible that, during dieting, receptors other than the 5-HT_{1A} receptor play a role in tryptophan-induced prolactin release. Another possibility is that compensatory changes in presynaptic 5-HT mechanisms may be involved. For example, rats maintained on a low tryptophan diet exhibit changes in 5-HT synthesis consistent with activation of tryptophan hydroxylase (Gil-Ad *et al*, 1976).

5-HT adaptation and psychological response to tryptophan deficit

The lack of neuroadaptive changes in 5-HT pathways during dieting in women recovered from depression might lead them to experience a greater decline in brain 5-HT function than controls. In this respect it is interesting that dieting produced a greater lowering of mood, judged by decreases in VAS ratings of 'happy', in the recovered group. However, the changes seen were not of clinical significance.

While dieting produces a modest (about 10%) but presumably sustained decrease in plasma tryptophan levels, the procedure of tryptophan depletion with a tryptophan free amino acid mixture causes an immediate but much larger decrease (at least 80%) in plasma tryptophan levels (Smith *et al*, 1997a,b). In women with a history of major depression who are recovered and have ceased drug treatment, acute tryptophan depletion causes a striking but temporary relapse in clinical symptoms. By contrast, acute tryptophan depletion fails to cause clinical depressive symptoms in women without a history of depression (Smith *et al*, 1997a,b). It is conceivable that the vulnerability of women recovered from depression to experience acute affective symptoms following the administration of tryptophan-free amino acid mixtures may be a consequence of a failure in 5-HT adaptation in response to precursor deficit. Studies which are able to measure changes in brain 5-HT

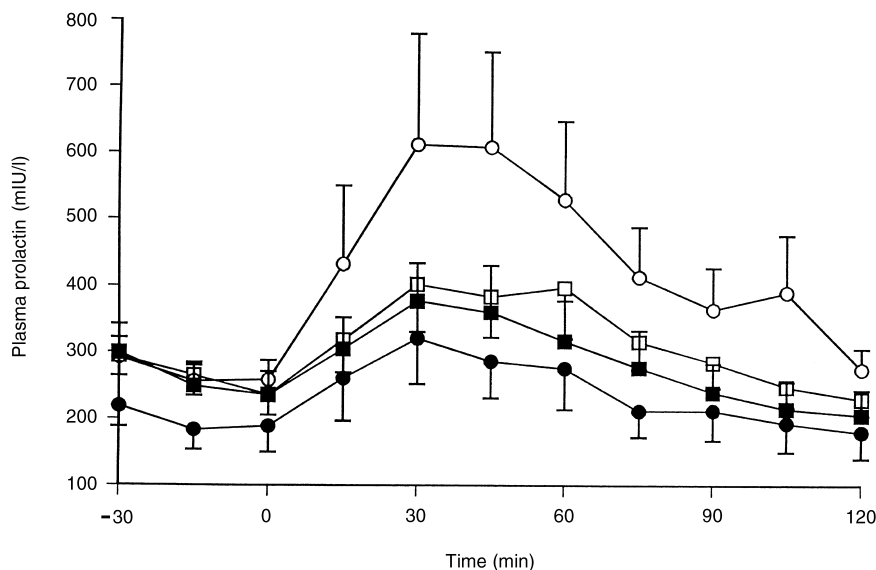


Fig. 2. Mean (s.e.) plasma prolactin concentration in healthy controls (□, ○) and women with a history of depressive disorder (■, ●) before and after infusion of 5 g tryptophan at time '0'. Subjects were tested before (□, ■) and after (○, ●) a three-week diet. Prolactin levels in the healthy subjects after dieting were significantly higher than in those recovered from depression (ANOVA: $F=7.46$, $P=0.012$).

neurotransmission more directly will be needed to test this hypothesis adequately.

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CLINICAL IMPLICATIONS

- Women with a vulnerability to major depression show impaired regulation of brain 5-HT function during a weight-reducing diet.
- This impaired regulation may be associated with a greater liability to mood changes during dieting.
- Impaired adaptation of brain 5-HT pathways in response to precursor deficit may be a vulnerability factor that predisposes to major depression.

LIMITATIONS

- The number of patients recovered from depression in the study was small.
- Impaired regulation of brain 5-HT function may be a consequence of previous depressive episodes rather than a marker of vulnerability.
- The findings cannot be generalised to men.

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