Psychological Medicine, 2003, **33**, 1381–1386. © 2003 Cambridge University Press
DOI: 10.1017/S0033291703008547 Printed in the United Kingdom

The effect of a nutritional source of tryptophan on dieting-induced changes in brain 5-HT function

M.-J. ATTENBURROW, C. WILLIAMS, J. ODONTIADIS, J. POWELL, F. VAN DE OUDERAA, M. WILLIAMS AND P. J. COWEN[1]

From the University Department of Psychiatry, Warneford Hospital, Oxford; and Department of Psychology, University of Bangor

ABSTRACT

Background. Dieting in healthy women results in a decrease in the availability of tryptophan (TRP), the amino-acid precursor of serotonin (5-HT), for brain 5-HT synthesis. This is associated with increases in the prolactin response to 5-HT drug challenge suggesting a 'supersensitivity' of 5-HT neuroendocrine responses. The aim of the study was to assess whether increased TRP intake during dieting would prevent the changes in TRP availability and 5-HT neuroendocrine function.

Method. Fifty female subjects underwent a 1000 kcal daily diet for 3 weeks. In the final week of the diet subjects were randomly allocated to receive either nutritionally-sourced TRP (1·8 g daily) or placebo in a double-blind, parallel group, design.

Results. TRP supplementation failed to modify the dieting-induced reduction in fasting TRP availability to the brain. However, in contrast to placebo-treated subjects, subjects receiving additional TRP did not show enhanced prolactin responses to intravenous TRP challenge.

Conclusions. The decrease in TRP availability produced by dieting may be due to increased TRP metabolism rather than decreased TRP intake. While TRP treatment did not increase fasting TRP availability it may have modified the effect of dieting on brain 5-HT function. Further studies will be needed to see if this effect of TRP has consequences for the effectiveness of dieting as means of weight control.

INTRODUCTION

Previous studies from this group have shown that weight loss produced by short-term dieting in healthy female subjects lowers plasma concentrations of the amino acid, L-tryptophan (TRP), the precursor of serotonin (5-HT) (Anderson *et al*. 1990; Cowen *et al*. 1996). This decrease in TRP availability to the brain is accompanied by changes in 5-HT neuroendocrine function, notably an increase in the prolactin response that follows intravenous administration of TRP and the 5-HT releasing agent, D-fenfluramine (Goodwin *et al*. 1987; Anderson *et al*. 1990; Walsh *et al*. 1995). We have proposed that this change in 5-HT neuroendocrine function is a consequence of post-synaptic 5-HT receptor 'supersensitivity', which represents an adaptive response to the decreased brain 5-HT synthesis that accompanies diminished TRP availability (Wurtman *et al*. 1981). In support of this notion we have shown that dieting in women also increases prolactin responses to m-chlorophenylpiperazine, a directly acting post-synaptic 5-HT_{2C} receptor agonist (Cowen *et al*. 1996).

Diminished central 5-HT availability might play a part in dieting-induced disturbances of appetite and mood. In particular, decreased brain 5-HT function in the hypothalamus is associated with a loss of satiety (Blundell, 1984; Tecott *et al*. 1995). This might explain the association between dieting and binge eating (Cooper, 1995) and perhaps account for the limited success of dieting as a strategy to control

[1] Address for correspondence: Professor P. J. Cowen, University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX.

https://doi.org/10.1017/S0033291703008547 Published online by Cambridge University Press

obesity (Wilson, 1995). If this is the case, maintaining TRP availability during dieting could prevent dieting-induced changes in brain 5-HT function and perhaps some of the associated psychological consequences.

One way of maintaining TRP availability is to provide exogenous TRP during the period of dieting. TRP was available for many years as a licensed antidepressant and health food supplement. In 1990, however, TRP treatment was linked to the eosinophilic myalgic syndrome, an autoimmune disorder probably caused by a contaminant produced during TRP manufacture (Kilbourne *et al*. 1996). The medicinal use of TRP was subsequently severely restricted.

It is, however, possible to derive TRP from a safe nutritional source such as milk whey. The aim of the present study was to use a nutritional source of TRP to determine whether TRP treatment would modify dieting-induced changes in plasma TRP availability and 5-HT neuroendocrine reponses in healthy female subjects.

METHOD

Subjects

Eligible volunteers were female between the ages of 18–50 years with a body mass index (BMI) between 25 and 32 with no current or past psychiatric history assessed by the structured clinical interview for the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*). Subjects were medication-free and physically fit as judged by clinical history and examination. All had normal scores on the EAT. Subjects gave informed consent to the study which was approved by the local ethics committee.

Design

All subjects underwent a 3 week protein sparing 1000 kcal diet as previously described (Anderson *et al*. 1990; Cowen *et al*. 1996). The start of the diet was delayed for 1 week after the baseline observations so that all testing would take part at approximately the same stage of the menstrual cycle (the early follicular phase) in the third and final week of the diet. During the final week of the diet subjects were randomly allocated to receive either nutritionally sourced TRP or placebo in a double-blind, parallel group design. At baseline and at the end of the dieting period subjects were weighed after an overnight fast and venous blood taken for amino acid measurements. A subgroup also underwent neuroendocrine testing with TRP. Throughout the diet subjects rated themselves once daily in the evening on visual analogue scales for the following items 'irritability', 'nausea', 'concentration', 'urge to eat', 'preoccupation with food' and 'control over eating'. They were asked to rate how they had felt on that particular day. In addition, subjects rated themselves at baseline and at weekly intervals throughout the diet on the profile of mood states questionnaire (POMS). Subjects were asked to rate how they felt 'right now'.

Nutritionally sourced TRP

The TRP was supplied by Unilever PLC and was prepared from milk whey derived bovine lactalbumin. The lactalbumin was hydrolysed with food grade enzymes to single amino acids and TRP was recovered using reversed HPLC column extraction in ethanol, followed by freeze-drying. The resultant product in the form of a powder averaged 80% TRP. All processes were conducted to food manufacturing standards and the product was passed for microbial safety. The powder was encapsulated with each capsule containing an average of 380 mg of powder. Subjects assigned to active treatment took two capsules three times daily giving an approximate daily dose of TRP of 1·8 g daily. Placebo capsules were identical and contained lactose.

Neuroendocrine testing

Subjects were tested in the week before starting the diet and on the final day of the diet. After an overnight fast an indwelling catheter was inserted at approximately 09.00 h and maintained with heparinized saline. Two baseline venous samples were taken at 15 min intervals, following which TRP 5 g (from a pharmaceutical source) was administered as an intravenous infusion over 30 min. Subsequent venous samples were taken immediately after the end of the infusion and at 15- min intervals for the next 120 min. Samples were taken for measurement of prolactin, TRP and the branched chain amino acids (BCAA) valine, leucine and isoleucine.

Biochemical analysis

Plasma was placed immediately on ice, separated by centrifugation and stored at $-20\ ^{\circ}C$. Plasma

https://doi.org/10.1017/S0033291703008547 Published online by Cambridge University Press

Table 1. *Effect of dieting on weight, BMI and plasma amino acids in subjects treated with tryptophan or placebo in the final week of the diet*

	Placebo ($N=25$)		Tryptophan ($N=25$)	
	Pre-diet	Post-diet	Pre-diet	Post-diet
	Mean (±S.E.M.)	Mean (±S.E.M.)	Mean (±S.E.M.)	Mean (±S.E.M.)
Weight*, kg	79·5 (±1·9)	76·85 (±2·0)	81·49 (±2·0)	78·26 (±1·9)
BMI*	29·2 (±0·6)	28·2 (±0·5)	29·0 (±0·5)	27·9 (±0·5)
Plasma total tryptophan*, μmol/l	60·5 (±2·3)	54·6 (±2·2)	57·8 (±3·1)	48·1 (±3·8)
Plasma-free tryptophan, μmol/l	1·06 (±0·01)	0·99 (±0·01)	0·95 (±0·11)	0·84 (±0·13)
BCAA†, μmol/l	347 (±19)	334 (±16)	344 (±16)	306 (±18)
Total tryptophan/BCAA ratio‡	0·189 (±0·014)	0·173 (±0·012)	0·181 (±0·008)	0·163 (±0·019)

Main effect of dieting: * $P<0·001$; † $P=0·009$; ‡ $P<0·004$ (ANOVA).

prolactin was determined by standard immunoradiometric assay. Inter- and intra-assay coefficients of variation over the range encompassed by the standard curve were $<10\%$ and $<3\%$ respectively. Plasma TRP was measured by high performance liquid chromatography (HPLC) with columetric end-point detection. Samples were deproteinized with 3% trichloracetic and centrifuged, after which the supernatant fluid was injected into the HPLC system. To obtain plasma-free TRP, plasma was spin filtered through a 12K molecular filter. Plasma concentration of BCAA were measured by an automated HPLC system with fluorescence end-point detection and pre-column sample derivatization adapted form the method of Furst *et al.* (1990).

Statistics

Data were analysed with a two-way repeated measures analysis of variance (ANOVA) with 'diet' (before and after the diet) as the main within subject factor and 'treatment' (TRP *v.* placebo) as the main between subjects factor. *Post hoc* testing was carried out with paired or unpaired *t* tests (two-tailed). Prolactin data were analysed both as peak change over baseline (δ prolactin) and area under the curve (AUC) using the trapezoid method with subtraction of baseline extrapolated from time '0'. δ prolactin correlated highly with AUC ($r=0·95$) and in the present report only the AUC values are given. For VAS data we calculated the AUC values for the final 7-day period of the diet during which subjects were taking either placebo or TRP. The VAS values of the day before treatment started were used as baseline and subtracted in the AUC analysis. Differences between the two groups were compared with unpaired *t* tests (two-tailed). POMS data were analysed with a two-way repeated measures ANOVA with 'time' (week of rating) as the within subjects factor and 'treatment' (TRP *v.* placebo) as the between subjects factor.

RESULTS

Subjects and weight loss

The subjects who received TRP supplementation ($N=25$) were well matched for mean ± S.E.M. age with the placebo-treated group ($N=25$) ($36·8\pm2·0$ *v.* $34·8\pm2·0$ years). Their mean BMIs were also similar (Table 1). Dieting significantly lowered body weight ($F=223·1$; df = 1,48; $P=0·001$) but this effect was not significantly influenced by the one week of TRP treatment (diet by treatment interaction, $F=2·2$; df = 1,48; $P=0·14$). Similar results were seen with the BMI data (Table 1). There was no significant difference in the mean weight loss between the two groups (placebo $=2·7\pm0·25$ *v.* TRP $=3·2\pm0·20$ kg, $P=0·20$).

Biochemistry

Dieting caused a significant fall in plasma total TRP ($F=22·3$; df = 1,41; $P=0·001$) but this effect was not prevented by TRP treatment (diet by treatment interaction, $F=1·39$; df = 1,41; $P=0·25$). Dieting produced a small but significant fall in plasma levels of BCAA ($F=7·41$; df = 1,44; $P=0·009$) but again TRP treatment did not influence this effect ($F=1·88$; df = 1,44; $P=0·17$). The ratio of TRP : BCAA was significantly lower after dieting in both treatment groups ($F=9·45$; df = 1,40; $P=0·004$). Dieting

https://doi.org/10.1017/S0033291703008547 Published online by Cambridge University Press

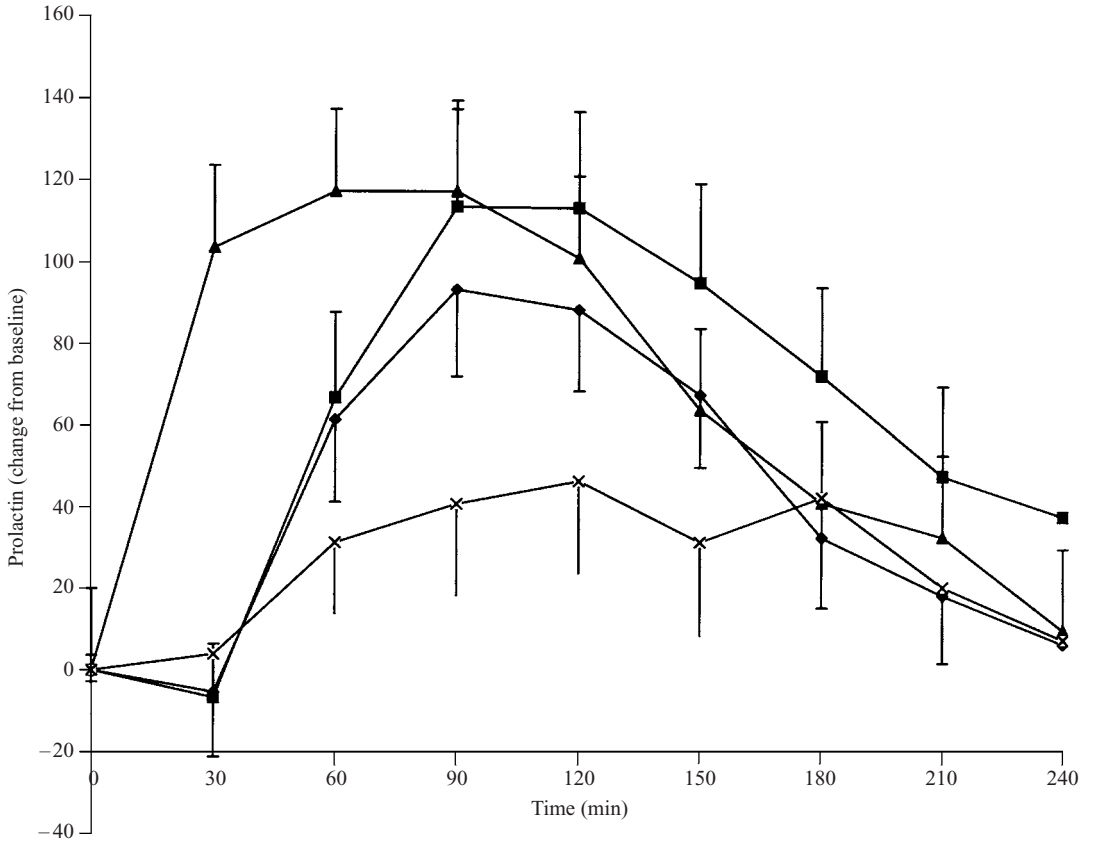


FIG. 1. Mean ± S.E.M. plasma prolactin (PRL) measured as change from baseline following intravenous tryptophan (5 g) at time '0'. Subjects were tested before and after a 3 week 1000 kcal diet, receiving in the final week of the diet either 1·8 g of tryptophan daily orally ($N=11$) or placebo ($N=17$). The difference in area under the curve of prolactin response to intravenous tryptophan is significantly less in the tryptophan-treated group after dieting ($P=0·029$, unpaired t test). (◆, Placebo pre-diet; ■, placebo post-diet; ▲, tryptophan pre-diet; ×, tryptophan post-diet.)

did not alter plasma-free TRP ($F=0·98$; df = 1,41; $P=0·32$) and there was no effect of TRP treatment on plasma-free TRP levels ($F=0·042$; df = 1·421; $P=0·83$) (Table 1).

Neuroendocrine data

Neuroendocrine testing with intravenous TRP was carried out in a subgroup of subjects ($N=28$) who agreed to this additional procedure. Randomization produced somewhat unbalanced subgroups (placebo, $N=17$, TRP treatment, $N=11$). The ANOVA of the AUC prolactin responses showed no significant main effect of diet ($F=0·47$; df = 1,26; $P=0·50$). However, there was a significant diet by treatment interaction ($F=6·41$; df = 1,26; $P=0·018$). The mean (± S.E.M.) AUC prolactin responses in the placebo group tended to increase after dieting (108 ± 30 *v.* 152 ± 29 mIU × h/l, $P=0·18$) while those in the TRP group showed a significant decrease (129 ± 55 *v.* 53 ± 28 mIU × h/l, $P=0·046$). The mean post-treatment AUC prolactin response differed significantly between the two groups (Fig. 1). In previous studies we have found that subjects who fail to lose at least 2 kg during the 3 week diet do not reliably increase their prolactin responses to intravenous TRP (see Smith *et al.* 2000). We therefore repeated the above analysis excluding such individuals giving a comparison of 13 placebo-treated and nine TRP-treated subjects. The ANOVA again showed a significant dieting by treatment interaction ($F=9·28$; df = 1,20; $P=0·006$). *Post hoc* analysis showed that dieting significantly

https://doi.org/10.1017/S0033291703008547 Published online by Cambridge University Press

increased the AUC prolactin response in the placebo-treated group (96 ± 31 *v.* 163 ± 33 mIU × h/l, $P = 0{\cdot}037$). The TRP-treated group showed a trend to a fall in AUC prolactin response (121 ± 67 *v.* 41 ± 33 mIU/l, $P = 0{\cdot}087$). The difference in response between the TRP and placebo-treated groups at the post-dieting test was again significant ($P = 0{\cdot}021$). Baseline prolactin levels (at time '0') were not altered by dieting or TRP treatment (all P values $> 0{\cdot}5$).

The AUC of plasma TRP following infusion was not altered by dieting ($F = 0{\cdot}21$; df = 1,26; $P = 0{\cdot}64$) and there was no effect of TRP treatment ($F = 2{\cdot}79$; df = 1,26; $P = 0{\cdot}11$). The mean ± S.E.M. of the TRP AUC in placebo group before and after dieting was, 1815 ± 103 *v.* 1623 ± 122 nmol × h/l ($P = 0{\cdot}17$). The corresponding values in the TRP-treated subjects were, 1559 ± 98 *v.* 1663 ± 126 μg × h/ml ($P = 0{\cdot}19$).

Psychological measures

VAS scores in subjects treated with TRP for the final week of the diet were no different from those of subjects receiving placebo (all P values $> 0{\cdot}1$). POMS measures of depression, fatigue, concentration and anxiety did not change significantly during the course of the diet and there was no effect of TRP treatment (all P values $> 0{\cdot}1$). Dieting significantly increased ratings of vigour on the POMs ($F = 4{\cdot}55$; df = 4,168; $P = 0{\cdot}002$) but there was no effect of TRP treatment ($F = 1{\cdot}51$; df = 4,168; $P = 0{\cdot}21$). Similarly, a trend effect of dieting to increase POMS ratings of anger ($F = 2{\cdot}63$; df = 4,168; $P = 0{\cdot}07$) was not altered by TRP treatment ($F = 0{\cdot}43$; df = 4,168; $P = 0{\cdot}68$).

DISCUSSION

The main findings of our study are that nutritional supplementation with TRP in the final week of a 3 week diet fails to prevent the dieting-induced decline in fasting TRP availability to the brain. However, TRP addition does appear to prevent dieting-induced increases in the prolactin response to intravenous TRP.

We have previously found that the nutritionally sourced TRP used in the present study has a bioavailability very similar to that of pure medicinal TRP (unpublished observations). The dose we used was designed to be about twice the usual daily intake of TRP (Young, 1986) and we have also found that a single daily dose of this magnitude produced neuroendocrine and neuropsychological changes consistent with increased brain 5-HT function (Attenburrow *et al*. 2003).

As previously, we found that dieting for 3 weeks significantly lowered fasting plasma total TRP (Anderson *et al*. 1990; Walsh *et al*. 1995; Cowen *et al*. 1996). The entry of TRP to the brain depends on its concentration relative to BCAA, which compete with TRP for active transport across the blood–brain barrier (Wurtman *et al*. 1981), dieting also lowered the ratio of TRP : BCAA. In some circumstances the TRP in plasma that is not bound to albumin (plasma-free TRP) is an important determinant of brain TRP level (Curzon, 1979). However, consistent with our previous findings dieting did not alter plasma-free TRP (Anderson *et al*. 1990).

The fact that exogenous oral TRP administration did not prevent the decline in plasma TRP associated with dieting might imply this precursor deficit does not arise through decreased TRP intake. In fact, animal studies suggest that restriction of food intake leads to increased activity of TRP pyrrolase, the hepatic enzyme that metabolizes TRP (Haleem, 1994). Hence, we suggest that the exogenous TRP we supplied was subject to increased metabolism and therefore failed to correct the dieting-induced reduction in fasting plasma TRP levels. However, it should also be noted that even in non-dieting subjects exogenous TRP is metabolized quickly and so it possible that even thrice daily administration provides insufficient TRP exposure to replete body stores (Green *et al*. 1980).

Despite this, the TRP treatment clearly altered the effect of dieting on the prolactin response to intravenous TRP, suggesting that the exogenous TRP may have produced functional effects on brain 5-HT neurotransmission. As noted previously, we found that dieting increased the prolactin response to TRP (Goodwin *et al*. 1987; Anderson *et al*. 1990) and this was indeed prevented by exogenous TRP administration. However, this finding is complicated by the fact that TRP treatment appeared to produce an independent lowering of the prolactin response to intravenous TRP.

https://doi.org/10.1017/S0033291703008547 Published online by Cambridge University Press

Interpretation of this effect is difficult because we did not study a non-dieting control group who were also treated with TRP. However, the ability of exogenous TRP to lower the prolactin response to intravenous TRP does not seem to be exerted through a pharmacokinetic effect on the disposition of TRP after intravenous infusion. To simplify the study design we gave a fixed dose of intravenous TRP. However, the precision of the TRP challenge test would probably have been increased had we corrected for differing body weights.

We think that the most likely explanation for our findings is that repeated administration of exogenous TRP to the dieters increased brain 5-HT function to some extent, thus producing a compensatory down-regulation in the prolactin response to intravenous TRP. If this is the case it would imply that exogenous TRP was able to prevent dieting-induced decreases in brain 5-HT function. Despite this there were no clear consequences of TRP treatment during dieting for weight loss or for the various psychological measures of mood, energy and appetite. It is, of course, possible that a 1 week period of TRP treatment was insufficient for these effects to be apparent.

In conclusion TRP treatment shows promise as a way of modifying dieting-induced changes in brain 5-HT function. Longer-term studies will be needed to determine if such treatment has the ability to decrease the adverse effects of dieting on mood and appetite and improve the effectiveness of dieting as means of weight control.

We thank Alison Reed for technical assistance. The nutritionally sourced TRP was provided by Unilever. The project was supported by an MRC Link award.

REFERENCES

Anderson, I. M., Parry-Billings, M., Newsholme, E. A., Fairburn, C. G. & Cowen, P. J. (1990). Dieting reduces plasma tryptophan and alters brain 5-HT function in women. *Psychological Medicine* **20**, 785–791.

Attenburrow, M.-J., Williams, C., Odontiadis, J., Reed, A., Powell, J., Cowen, P. J. & Harmer, C. J. (2003). Acute administration of nutritionally sourced tryptophan increases fear recognition. *Psychopharmacology* **169**, 104–107.

Blundell, J. E. (1984). Serotonin and appetite. *Neuropharmacology* **23**, 1537–1551.

Cooper, Z. (1995). The development and maintenance of eating disorders. In *Eating Disorders and Obesity: A Comprehensive Handbook* (ed. K. D. Brownell and C. G. Fairburn), pp. 199–206. Guildford Press: New York.

Cowen, P. J., Clifford, E. M., Walsh, A. E. S., Williams, C. & Fairburn, C. G. (1996). Moderate dieting causes 5-HT_{2C} receptor supersensitivity. *Psychological Medicine* **26**, 1155–1159.

Curzon, G. (1979). Relationships between plasma, CSF and brain tryptophan. *Journal of Neural Transmission* (suppl. 15), 81–92.

Furst, P., Pollack, T. A., Graser, H., Godel, H. & Stehle, P. (1990). Appraisal of four pre-column derivatization methods for the high-performance liquid chromatographic determination of free amino acids in biological materials. *Journal of Chromatography* **499**, 557–569.

Goodwin, G. M., Fairburn, C. G. & Cowen, P. J. (1987). Dieting changes serotonergic function in women, not men: implications for the aetiology of anorexia nervosa? *Psychological Medicine* **17**, 839–842.

Green, A. R., Aronson, J. K., Curzon, G. & Woods, H. F. (1980). Metabolism of an oral tryptophan load. 1: Effects of dose and pre-treatment with tryptophan. *British Journal of Clinical Pharmacology* **10**, 603–610.

Haleem, D. J. (1994). Decreases of plasma tryptophan concentrations following restricted feeding do not decrease serotonin and its metabolite in rat brain. *Nahrung* **38**, 606–611.

Kilbourne, E. M., Philen, R. M., Kamb, M. L. & Falk, H. (1996). Tryptophan produced by Showa Denko and epidemic eosinophilic-myalgia syndrome. *Journal of Rheumatology* **23** (suppl. 46), 81–88.

Smith, K. A., Williams, C. & Cowen, P. J. (2000). Impaired regulation of brain serotonin function during dieting in women recovered from depression. *British Journal of Psychiatry* **176**, 72–75.

Tecott, L. H., Sun, L. M., Akanas, F., Strack, A. M., Lowenstein, D. H., Dallman, M. F. & Julius, D. (1995). Eating disorder and epilepsy in mice lacking 5-HT_{2C} serotonin receptors. *Nature* **374**, 542–546.

Walsh, A. E. S., Oldman, A. D., Franklin, M., Fairburn, C. G. & Cowen, P. J. (1995). Dieting decreases plasma tryptophan and increases the prolactin response to d-fenfluramine in women but not men. *Journal of Affective Disorders* **33**, 89–97.

Wilson, G. T. (1995). The controversy over dieting. In *Eating Disorders and Obesity: A Comprehensive Handbook* (ed. K. D. Brownell and C. G. Fairburn), pp. 87–92. Guildford Press: New York.

Wurtman, R. J., Hefti, F. & Melamed, E. (1981). Precursor control of neurotransmitter synthesis. *Pharmacological Reviews* **10**, 315–335.

Young, S. N. (1986). The clinical psychopharmacology of tryptophan. In *Nutrition and the Brain Vol. 7* (ed. R. J. Wurtman and J. J. Wurtman), pp. 49–88. Raven Press: New York.

https://doi.org/10.1017/S0033291703008547 Published online by Cambridge University Press