Tryptophan supplementation and serotonin function: genetic variations in behavioural effects

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The neurotransmitter serotonin has a role in affective disorders such as depression and anxiety, as well as sleep, cognitive function and appetite. This review examines the evidence that serotonin-related genotypes may moderate the behavioural effects of supplementation with the serotonin precursor amino acid l-tryptophan (TRP), on which synthesis of serotonin (or 5-hydroxytryptamine; 5-HT) depends. However, 95 % of serotonin is synthesised and used in the periphery, and TRP is also metabolised via non-5-HT routes such as the kynurenine pathway. Moreover, understanding of genotypes involved in regulation of serotonin raises questions over the generalisability of TRP effects on behaviour across individuals with varied serotonergic genotypes. To date, only differences between variants of the 5-HT transporter-linked promoter region (5-HTTLPR) have been investigated in relation to behavioural effects of TRP supplementation. Effects of 5-HTTLPR genotypes are usually compared between the alleles that are either high (L/L) or low (S/S) expressing of mRNA for the 5-HT transporter receptor. Yet, another key genetic variable is sex: in women, the S/S genotype predicts sensitivity to improved mood and reduced cortisol by TRP supplementation, during stressful challenges, whereas the L/L genotype protects against stress-induced mood deterioration. In men, the L/L genotype may confer risk of stress-induced increases in negative affect; there are insufficient data to assess effects on male S/S genotypes. However, better-powered studies to detect sex by genotype by stress by TRP interactions, as well as consideration of more genotypes, are needed before strong conclusions and recommendations for behavioural effects of TRP treatment can be reached.

Tryptophan supplementation: Serotonin and behaviour: 5-HT transporter-linked promoter region: Genetic polymorphism: Stress

Dietary tryptophan and the pathways to serotonin function

Serotonin, or 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter in the central nervous systems (CNS) of the majority of animals, including human beings. Its synthesis depends on the supply of the essential amino acid, l-tryptophan (TRP), which cannot be biosynthesised by human beings and so must be obtained from dietary sources. Moreover, serotonin synthesis rate depends on the availability of the precursor TRP. The scope of this review is to consider recent findings from research involving effects of supplementing TRP supply on behaviour and their interaction with genetic susceptibility, including indirect evidence that TRP supplementation likely alters affective states via effects on central serotonin function.

An important consideration for understanding effects of TRP administration is that only about 5% of endogenous serotonin is found in the brain; the

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HTTLPR, 5-HT transporter-linked promoter region; ATD, acute tryptophan depletion; CNS, central nervous system; CSF, cerebrospinal fluid; LNAA, large neutral amino acids; MAO, monoamine oxidase; TDO, tryptophan 2,3-dioxygenase; TPH, TRP hydroxylase; TRP, l-tryptophan.

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1. Tryptophan loading, genes and behaviour

The remainder is in the gut (about 90%), principally released by enterochromaffin cells, and in peripheral tissue or in the blood, where it is taken up into blood platelets\(^1\). Indeed, the name serotonin derives from its discovery in blood 70 years ago and the observation that it caused contraction of vascular smooth muscle\(^6\); thus, one function of serotonin is to regulate local blood flow. This imbalanced distribution between brain and periphery needs to be borne in mind when considering the possible impact of dietary manipulation of central serotonin by TRP, and the potential influence of alternative metabolic pathways as well as probable moderating effects on these metabolic routes. These issues are considered further later; nevertheless, serotonin is a widely distributed and important CNS neurotransmitter, arising from neuronal cell bodies located in the higher and lower raphe nuclei of the brainstem, and acting at multiple receptor subtypes with a range of behavioural effects\(^6\). Serotonin's established importance in affective disorders and appetite, as well as sleep and cognition\(^6\), make understanding who might benefit most from therapeutic use of TRP an important goal of research.

**Metabolic pathways for L-tryptophan**

As with other essential amino acids, TRP can contribute to hepatic biosynthesis of proteins; however, TRP is typically incorporated into proteins at only 1–2% of total amino acids, making it the scarest of amino acids in dietary proteins\(^3,7\). Nevertheless, if TRP is acutely deficient, incorporation into protein synthesis will contribute to a substantial lowering of plasma TRP levels\(^8,9\). However, in the absence of TRP deficiency, the majority of consumed TRP is metabolised via other pathways, including for synthesis of 5-HT, melatonin and niacin (vitamin B3). Indeed, it has been estimated that only 1% of dietary TRP is used for brain 5-HT synthesis\(^10\). TRP use for synthesis of niacin is via the oxidative kynurenine pathway, which has also been termed the TRYCAT pathway\(^11\). This pathway is becoming increasingly recognised as having important implications for health, including neuropsychiatric conditions such as depression\(^11,12\). A further route for TRP metabolism is via degradation by gut microbiota, which can lead to production of both positive and detrimental active metabolites, including quinolinic acid\(^4\); therefore, individual variation in the gut microbiome may have implications for TRP metabolism and thus brain health and psychological wellbeing\(^13\).

The kynurenine, or TRYCAT, pathway involves an initial rate-limiting metabolism of TRP to kynurenine catalysed by the hepatic enzyme, tryptophan 2,3-dioxygenase (TDO), which can be induced by glucocorticoid hormones\(^14\). However, under inflammatory conditions, the extrahepatic enzyme, indole 2,3-dioxygenase becomes increasingly important in metabolising TRP to kynurenine, due to induction by pro-inflammatory cytokines\(^11\). These inductive influences on diversion of TRP metabolism away from 5-HT synthesis have been proposed as mechanisms underlying the link between stress, inflammation, deficient 5-HT function and depression\(^11,12\).

The metabolism of TRP for the synthesis of 5-HT is catalysed by the rate-limiting enzyme, TRP hydroxylase (TPH), which converts TRP into 5-hydroxytryptophan. In turn, 5-hydroxytryptophan is decarboxylated to 5-HT by the enzyme aromatic amino acid decarboxylase. The key observation for this pathway is that TPH is not fully saturated by its substrate TRP under normal conditions, so that raising brain TRP levels could increase serotonin synthesis. However, brain TRP levels are buffered from plasma TRP by the blood–brain barrier: to be transported into the brain, TRP has to compete for uptake across the blood–brain barrier against other amino acids, in particular a group known as the large neutral amino acids (LNAA), especially the branched chain amino acids, leucine, isoleucine and valine, but also phenylalanine and tyrosine (the precursors for catecholamine, dopamine, adrenaline, noradrenaline, transmitter synthesis). Thus, the ratio of plasma or serum TRP to LNAA (TRP:LNAA) is recognised as the best peripheral biomarker of uptake of TRP into the brain\(^7\). Some 90% of TRP in blood is typically bound to the blood protein albumin, and it is often assumed that the remaining free unbound fraction of TRP should be taken to be the best predictor of TRP entry across the blood–brain barrier. However, it has been shown that TRP binding to albumin is very labile, such that TRP can easily be released in cerebral circulation. Furthermore, TRP can be displaced from or prevented from binding to albumin by NEFA, which also bind readily to albumin\(^7,9\). Therefore, factors that alter NEFA levels in blood will affect the availability of free TRP for entry into the brain: for example, sympathetic activation by stress or exercise will induce lipolysis, increase plasma NEFA and so release more TRP from albumin. This acute stress-induced increase in availability of TRP for serotonin synthesis might contribute to the observation that even mild stress can increase 5-HT release in rat brain\(^15\). It also suggests that caution is required in interpreting correlations between single measures of plasma-free TRP and personality traits such as anxiety or aggression, as these may interact with the experimental procedure and perceived stressful nature of the study to modify TRP levels. In contrast, food or drink that stimulates insulin release, and so promotes uptake of NEFA into tissue, will tend to reduce the availability of free plasma TRP, but at the same time will remove competing LNAA from plasma into tissue\(^9\). Thus, measuring both free and total TRP may ensure better prediction of TRP entry into the brain and its behavioural associations\(^9,16\).

However, 95% of 5-HT is synthesised and used in the gut, blood and peripheral tissue\(^11,14\). Although the synthesis of 5-HT from TRP follows a similar biochemical path in brain and periphery, the form of the enzyme TPH, by and large, differs slightly between these regions; these isoforms are known as TPH1 and TPH2 respectively, indicating the order of characterisation\(^17,18\). To be precise, in the brain the principal isoform, TPH2, shown to depend on expression of a different gene form
from TPH1\(^{(18)}\), was found to be highly expressed by measuring mRNA specific to the brainstem raphe nuclei, where brain serotonin is primarily synthesised, whereas TPH1 was found to be responsible for 5-HT synthesis, and ultimately melatonin, in the pineal gland\(^{(19)}\) and gut\(^{(18)}\). However, this classification is oversimplified, as TPH1 mRNA has also been shown to be more highly expressed in the amygdala and hypothalamus than TPH2\(^{(20)}\), although its precise role in those sites is uncertain.

### Serotonin and behaviour

Serotonin has long been associated with several fundamental aspects of behaviour, including sleep, appetite, cognition, and social and emotional behaviours such as anxiety, depression, empathy and aggression\(^{(21,22)}\). These influences of serotonin on behaviour will be briefly reviewed prior to consideration of the impact of TRP supplementation and its interaction with 5-HT-related genotypes.

Early neurophysiological and lesion work on the function of CNS 5-HT demonstrated a clear role in regulating sleep\(^{(23)}\), whereas the therapeutic use of monoamine oxidase (MAO) inhibitors (which prevent serotonin, and other monoamine, metabolism by the enzyme MAO), as well as development of tricyclic antidepressants (which inhibit synaptic reuptake of monoamine neurotransmitters), such as imipramine, to treat depression, led to the ‘serotonin hypothesis’ of depression, in which depression is seen primarily to result from a deficit in 5-HT function\(^{(24,25)}\). The theory expanded to consider a role for CNS 5-HT in associated clinical affective disorders as well as regulation of mood in healthy people\(^{(25)}\). However, this pharmacotherapeutic evidence was non-specific to serotonin, and ironically, notwithstanding the risk of oversimplifying neural bases to complex disorders, the best evidence for a major role for CNS 5-HT in control of affect has come from studies that manipulate TRP entry to the brain\(^{(26)}\). Furthermore, whilst recent studies combining neuroimaging with administration of selective serotonin reuptake inhibitors have also strengthened the evidence for a role for central 5-HT in depression\(^{(27)}\), other evidence is emerging for the importance of peripheral metabolic pathways for TRP, including roles in inflammatory processes and melatonin synthesis, underlying major depression\(^{(28)}\), seasonal affective disorder and bipolar disorder\(^{(29,30)}\).

Central serotonin is known to be involved in cognitive function, especially memory, attention, decision making and information processing, as well as in the processing of emotionally relevant stimuli\(^{(26,29,30)}\). However, cognition and emotion, or affect, are not entirely separable, and are often strongly interdependent\(^{(31–33)}\). Emotions, via their neural substrates, influence memory and attention for example, and depression and anxiety are associated with cognitive impairments and biases that can contribute to the affective disorder and its maintenance\(^{(32,34)}\).

### Effects of acute tryptophan depletion

This review is mainly concerned with genetic susceptibility to effects of forms of TRP administration that may lead to increased serotonin synthesis in the brain; however, by way of comparison, and given the scientific influence, a brief overview is included of findings, and their implications, on deficits in central 5-HT induced by acute TRP depletion (ATD) methods\(^{(33,35)}\). ATD is usually induced by ingestion of amino acid loads devoid of the precursor amino acid TRP to suppress 5-HT synthesis, and can be preceded by a low-TRP diet for a few days\(^{(29)}\). This results in a substantial (e.g. >70 %) and rapid drop in plasma TRP, and TRP:LNAA ratio (>80 %) that may last 4–6 h\(^{(36,37)}\); similar effects have been found with a more palatable low-TRP collagen protein mixture\(^{(38)}\), and more recently a gelatin-derived TRP-free protein/carbohydrate mixture has been used\(^{(39)}\). Moreover, the serotonin metabolite, 5-hydroxyindole acetic acid measured in cerebrospinal fluid (CSF) declined by about one-third at 12 h, after which measurements stopped\(^{(37)}\). ATD methods have provided the most consistent evidence for serotoninergic involvement in cognition, including impairment of memory consolidation\(^{(40,41,42)}\) and aspects of cognitive flexibility including learning\(^{(40)}\) and decision-making\(^{(41)}\). Moreover, evidence in animal models is persuasive of opposing effects of both ATD and TRP supplementation on brain 5-HT\(^{(42–44)}\).

In support of a key role for serotonin in affective disorders, ATD also alters emotional processing and regulation\(^{(45–47)}\). Reducing TRP access to the brain by ATD tends to mimic the cognitive biases seen in depressed populations, such as impaired memory for, attention to, or recognition of positive v. negative information including facial expressions\(^{(34,48,49)}\). However, positive effects of ATD on cognition, for example on decision making and focused attention have also been reported\(^{(50–52)}\), albeit interacting with a history of depression\(^{(53)}\). One explanation has been that serotonin may affect ‘hot’ cognitive tasks that include an affective component, but not ‘cold’ cognitive tasks that do not obviously involve emotional stimuli\(^{(49)}\).

Neuroimaging techniques show that activity of brain regions involved in emotion regulation such as the limbic system and prefrontal cortex is sensitive to ATD\(^{(46)}\). The evidence is consistent with a normally inhibitory role of serotonin on any tendency for negative emotional bias\(^{(54,55)}\). Importantly, family or personal history of depression, sex and at-risk genotypes, have been reported to moderate effects of ATD on brain activity to emotional stimuli\(^{(46,47,56)}\).

Despite a history of use of anorexigenic drugs with serotoninergic agonist activity such as d-fenfluramine\(^{(57)}\), and reductions in food intake established for high doses of TRP\(^{(58)}\), and thus an expectation that ATD might increase appetite, the few studies addressing this directly in human beings suggest little effect of ATD on appetite despite concurrent mood effects\(^{(59–61)}\). Two studies comparing ATD in women with bulimia nervosa v. healthy controls found conflicting results\(^{(62,63)}\), although both studies found increased negative affect in bulimic.
women, only one reported increased energy intake in these women(62), although the other did find an increased desire to binge eat(60). However, curiously, another study reported a concurrent increase in both nausea and hunger in healthy women(63). These findings also need to be considered in the context of opposing relationships between depression and appetite across patients(60).

Two other behaviours that appear to be sensitive to serotonin depletion are aggression and impulsivity(33,65). ATD has resulted in increased aggressive behaviour in the majority of studies where measured(34), and aggressive traits have correlated with plasma levels of TRP and CSF indices of serotonin turnover(65). However, gene by environment interactions, including stressful life events, and sex differences, are likely to moderate findings(66,67), and a meta-analysis of associations between 5-HT function and aggression in human beings revealed only a weak negative relationship(68). It may be that stronger associations will be found when genetic variants influencing serotonin function, such as in enzymes involved in synthesis and metabolism, or polymorphisms in transporter systems (see later), are taken into account(69,70). Indeed, a key criticism put forward is the observation that ATD lowers TRP quite universally across participants, and yet the behavioural effects differ considerably depending on a propensity to dysfunction of mood or emotional regulation, or poor stress coping(9).

**Effects of L-tryptophan administration and supplementation**

In contrast to ATD, which is a research tool to investigate serotonergic processes in human beings, and for which most effects are not beneficial, administration of TRP (and its first-stage metabolite, 5-hydroxytryptophan) has a long history of being studied for potential clinical benefit in depression, as well as for basic research, as a means to facilitate entry of TRP into the brain and thus elevate 5-HT synthesis and release(26,33). The methods can vary from intravenous administration of TRP to oral supplementation of TRP, or use of TRP-rich proteins or peptide preparations, either acutely or chronically(26,29,71). It is also possible to increase the TRP:LNAA ratio, and so enhance TRP entry across the blood–brain barrier, by feeding a carbohydrate-rich, very low-protein meal, since the rise in insulin removes more LNAA into surrounding tissue. This dietary method has been shown to benefit cognitive and emotional function, and reduce the cortisol response to stress, in more stress-prone, neurotic participants(72,73). This mechanism has also been suggested to underlie dietary effects on mood and performance, such as calming after high-carbohydrate meals v. arousal after protein-rich meals(76,77). Recently, using data from the US National Health and Nutrition Examination Survey for nearly 30,000 adults, dietary intake of TRP was found to be inversely associated with self-reported levels of depression, and positively related to sleep duration (more strongly in women; adjusted for protein intake)(78).

Thus, even in complex whole diets, TRP intake appears to provide psychological benefits.

TRP supplementation has been employed as a potential treatment for depression and sleep disturbance since the early 1960s(24,79), although a Cochrane Review of 108 trials (including for 5-hydroxytryptophan) for antidepressant effects in 2002 found that only two trials were of sufficient quality to be included(80). Nevertheless, on that limited evidence, TRP was considered to be better than placebo in alleviating depression, at least in mild to moderately depressed people. Moreover, for more than a decade prior to that review, the US Food and Drug Administration had banned over-the-counter sales of TRP following an outbreak in 1989 of the harmful eosinophilia–myalgia syndrome in users of TRP supplements. The cause was eventually traced to impurities in TRP supplements from one Japanese manufacturer, after which the ban was lifted in 2001(10,26). Thus, for at least five decades, TRP has been used pharmacologically, i.e. at daily doses sometimes well in excess of ten times the RDA (5 mg/kg) for this essential amino acid. There was early evidence for probable enhancement of brain 5-HT function: after 50 mg/kg TRP (3.5 g per 70 kg subject) was consumed in a milk drink, plasma TRP increased 8-fold, TRP in CSF increased 6-fold after 6–8 h, and the metabolite 5-hydroxyindole acetic acid increased almost 2-fold in CSF by 8 h, suggesting increased turnover of brain 5-HT(81). This 2-fold increase in 5-HT turnover was replicated in a later study of CSF 5-hydroxyindole acetic acid changes, using 3 and 6 g TRP, with no further increase at the higher dose, although the level was sustained for longer, i.e. 12 v. 8 h(82).

In a review of potential side-effects, Fernstrom(26) concluded that such use of TRP appears to be largely safe from adverse events, although the evidence is limited and not systematic. There are some reports of symptoms such as nausea, tremor or dizziness when high doses are used (although these are also common symptoms reported in placebo-treated subjects). However, the greatest risk of side-effects occurs when TRP is combined with other drugs that enhance 5-HT availability, such as antidepressant selective serotonin reuptake inhibitors or MAO inhibitors: then a toxic ‘serotonin syndrome’ may occur that can include hyperthermia and coma(26). A more common effect of high doses of TRP is fatigue or drowsiness, which has led to TRP being used to aid sleep, in which case sedation is not an unwanted side-effect(26). However, a complication of oral TRP at higher doses is that it increases the release of several hormones, including growth hormone, cortisol and prolactin(83) (the latter thought to indicate increased central serotonin, and dopamine, activity). A recent study also reported that intragastric administration of 1.56 g TRP increased plasma cholecystokinin and glucagon-like peptide 1, as well as slowing gastric emptying(84): although subjective appetite was not affected, it is likely that these mechanisms contribute to reduced food intake reported after higher doses of TRP(38). Even so, food intake might be reduced merely due to TRP-induced drowsiness.
There is also concern that excess metabolism through pathways such as TRYCAT could lead to high levels of neuronally active metabolites such as kynurenic acid and quinolinic acid. However, a recent review did not find evidence for adverse side-effects via these routes, although it was acknowledged that more systematic research is needed(11). Furthermore, it has been argued that the modest antidepressant effect of TRP loading is due to accelerated hepatic degradation of TRP in depressives, probably via stress-related neuroendocrine enhancement of the catabolic hepatic enzyme TDO(89,90).

As would be expected in a treatment with antidepressant potential, there is considerable evidence for beneficial effects of TRP on mood and social behaviour, and these findings have recently been reviewed(22,33). There is some evidence that TRP can reduce aggression in schizophrenic patients(33), and reduce quarrelsomeness is some evidence that TRP can reduce aggression in children, compared with placebo(86). This was interpreted as possibly re-expressing the development of more control in social interactions at home.

Effects of L-tryptophan-rich protein preparations

Bearing in mind such concerns about loading with high doses of TRP as the single amino acid, in recent years methods have been developed to enhance TRP availability to the brain by administering TRP-rich dietary proteins: the most published example is the whey protein α-lactalbumin. The effects of this protein are usually compared with responses after ingestion of another protein, typically casein hydrolysate (another milk protein), which has lower levels of TRP but greater amounts of the competing LNAA(29).

Similarly to a high-carbohydrate meal, α-lactalbumin has been shown to enhance (or correct) serotonin function (indexed by prolactin release) and cognition, and to reduce cortisol release, in stress-prone (more anxious) participants(67,88). α-lactalbumin attenuated deficits in delayed memory in women suffering from premenstrual syndrome(89) and in recovered depressives and healthy subjects(90). This TRP-rich protein also improved the perception of emotional faces within women(91); however, effects on emotional face processing tend to be weaker relative to a casein control treatment(98).

Role of genetics in moderating effects of L-tryptophan supplementation or challenge on serotonin-related behaviours

Gene polymorphisms involved in the metabolism of TRP and regulation of serotonin could have a substantial influence on behavioural effects of manipulations of TRP availability. There is potential for moderation of TRP effects by polymorphisms in each of the key enzymes influencing TRP metabolism and thus serotonin synthesis, i.e. TPH1, TPH2, TDO, indole 2,3-dioxygenase and also by polymorphisms of the MAO-A enzyme that metabolises central serotonin (Fig. 1). These various 5-HT-related polymorphisms may form an interactive system that determines the aetiology and prognosis of various forms of affective disorder(77,99–102). However, the most evidenced serotonergic genetic influence on behaviour is the 5-hydroxytryptamine transporter-linked promoter region (5-HTTLPR) polymorphism of the serotonin transporter gene (SLC6A4)(103–104). The recommended classification of 5-HTTLPR genotypes is a functional combination of variable number tandem repeats of short or long length of the gene promoter amplicon and SNP variants, LA and LG, where LG is the most evidenced serotonergic genetic in...
Brain 5-HT synthesis rates are reportedly 50% lower in women than men (105), and ATD causes greater lowering of mood in women than men (106). In some studies, women also appear to be more sensitive to, or to benefit more from, TRP supplementation; indeed, some researchers chose to study women only for these reasons (97,98). Furthermore, sex interacts with serotonergic gene polymorphisms in several systems, including 5-HTTLPR, TPH1, TPH2 and MAO-A (67,107), and these interactions can be further moderated by stress (108–111). Therefore, the sex of participants needs to be considered when interpreting findings in this area.

Tryptophan administration and 5-hydroxytryptamine transporter-linked promoter region genotypes

Only a few studies have investigated whether these 5-HT- and TRP-related genotypes alter the effects of TRP loading (or challenge or supplementation), and these appear to be limited to a comparison of 5-HTTLPR genotypes: these studies are summarised in Table 1. In the earliest published study (108) to examine moderation of TRP loading by the 5-HTTLPR tri-allelic genotype, forty-one men and thirty-one women were infused intravenously with a high dose of TRP (100 mg/kg), while aspects of mood were assessed (Profile of Mood States). Far from improving mood, this procedure generally increased negative affect, but the effects were moderated by genotype and sex: in men, only those with the high-expressing L/L′ polymorphism showed increased negative mood, whereas in women, only the L/L′ group showed no increase in negative mood. This opposing interaction between sex and 5-HTTLPR genotype is in line with evidence based on the impact of social stressors on negative affect in adolescents (111). However, sample sizes were small, especially in the S/S′ groups (seven men; nine women).

Using a far lower dose, and oral administration, Markus and Firk (112) examined potential interactions between acute TRP supplementation, stress and 5-HTTLPR genotype on mood, cortisol and cognition. They hypothesised that the TRP challenge would ameliorate the effects of stress on mood and cortisol in subjects homozygous for the tri-allelic S/S′ genotype compared to those with the L/L′ genotype. In a cross-over design, thirty student participants (sixteen S/S′-allele; fourteen L/L′-allele; only one man in each group) received either TRP (2 × 0.4 g) or placebo (lactose), prior to a stressful challenge, with baseline and post-stress measures of...
Table 1. Summary of studies investigating interactions between \(L\)-tryptophan (TRP) supplementation or challenge and tri-allelic 5-HT transporter-linked promoter region (5-HTTLPR) genotypes on behaviour

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Design and intervention</th>
<th>Measures</th>
<th>Main findings</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Brummett et al.</td>
<td>Healthy adults; thirty-one females, forty-one males; 54 % Mean (\pm) SD age = 33.5 ± 9.1</td>
<td>Single blind. Overnight fast. TRP (10 mg/kg body weight) i.v. infusion. Saline infusion day 1, followed by TRP on day 2</td>
<td>Negative affect assessed by POMS prior to and 1 h after start of infusion.</td>
<td>Scores for Depression-Dejection increased 3-fold from pre- to post-TRP infusion for L/L' males, but did not change for S/L or S/S females. In females, L/L' scores did not change, but increased moderately for S/S' genotypes. TRP reduced depression and fatigue scores, and increased vigil, only in S/S' genotypes. No interaction with pre/post-stress</td>
<td>Small sample size for S/S' groups (seven males, nine females). No saline infusion on same day as TRP. No significant effects on fatigue, anxiety and anger</td>
</tr>
<tr>
<td>Markus &amp; Firk</td>
<td>Twenty-eight female students. Mean (\pm) SD age = 19 ± 2</td>
<td>Double-blind cross-over design. Overnight fast. Oral TRP (2 × 0.4 g) or lactose placebo capsules, then stressful challenge (cold pressor and Serial-7 tasks in front of the camera)</td>
<td>POMS at baseline and post-stress. Cortisol in saliva</td>
<td>TRP prevented the modest increase in negative affect seen after placebo for S/S' but not L/L' allele group. Stress weakened ability to inhibit negative information in S/S' allele group but enhanced it in L/L' group. No effect of TRP on this measure</td>
<td>No stress-free condition. Single cortisol samples pre- and post-stress. No effect of TRP or genotype on cortisol</td>
</tr>
<tr>
<td>Markus &amp; De Raedt</td>
<td>Twenty-eight female students. Mean (\pm) SD age = 19 ± 2</td>
<td>Double-blind cross-over design. Overnight fast. Oral TRP (0.8 g) v. cellulose placebo, then stressful challenge (cold pressor and Serial-7 tasks in front of the camera)</td>
<td>NAP using pictures with positive or negative valence – assesses tendency to inhibit negative emotional information. Positive and negative affect by questionnaire (PANAS)</td>
<td>No effect of TRP or genotype on stress-induced rise in cortisol. Stress increased anger in both TRP and placebo conditions, except for L/L' group who did not increase anger after TRP. This same L/L' group showed a reduced liking for high-fat sweet foods after stress in the TRP condition only. Overall, TRP reduced food intake v. placebo</td>
<td>No stress-free condition. Despite NAP being sensitive to stress and genotype, no effect of TRP on this measure</td>
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<tr>
<td>Markus et al.</td>
<td>Forty-two female students (nineteen S/S', twenty-three L/L'). High or low restrained eaters. Mean (\pm) SD age = 19 ± 2</td>
<td>Double-blind cross-over design, counterbalanced for genotype and restraint level. TRP-rich protein hydrolysate drink (235 mg TRP) or placebo (casein hydrolysate), then stress: adapted</td>
<td>Baseline, pre- and post-stress measures of salivary cortisol (3 before stress, one after), mood (POMS), urge for food, snack food intake</td>
<td>No interactions with restrained eating, but this is not a good measure of emotional eating tendencies. Snack food intake during the study may have modified impact of TRP treatment, but note that L/L' showed the greatest increase in plasma TRP: LNAA after TRP treatment v. placebo</td>
<td>No interactions with restrained eating, but this is not a good measure of emotional eating tendencies. Snack food intake during the study may have modified impact of TRP treatment, but note that L/L' showed the greatest increase in plasma TRP: LNAA after TRP treatment v. placebo</td>
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| Cerit et al.       | Twenty-two females, twenty-four males; approx. half of each were S/S' or L/L'. Mean \(\pm\) SD age = 20.4 ± 3 | Double-blind between-subjects, stratified by genotype. Subchronic oral TRP (2.8 g/d as 7 × 0.4 g capsules taken morning, afternoon and evening) for 6 d, then TSST on day 7 | Anxiety and depression (HADS); positive and negative affect (PANAS); tension, anxiety, sadness, annoyance by single-item MSS | Cortisol results suggest that S/S' show greater stress responsiveness that in turn is reduced by TRP. Cortisol AUC not analysed. Sex analysed as a covariate, but significance not reported | }
### Capello & Markus (123)

<table>
<thead>
<tr>
<th>Ninety-nine female, nineteen male students; sixty in S/S’ and fifty-eight in L/L’ groups. Mean ± SD age = 24.0 ± 1.7</th>
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<tr>
<td>Double-blind between-subjects, stratified by genotype and neuroticism (N) trait (DPQ-N). Subchronic oral TRP (3 g/d as 2 × 0.5 g capsules taken three times/d) for 7 d, then stress (Maastricht Acute Stress Test) after lunch on day 8</td>
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<td>Salivary cortisol (one baseline, two post-stress), mood (POMS), anxiety (state scale of STAI), appetite ratings, pre- and post-stress. Snack food intake after stress</td>
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<td>Stress-induced rise in cortisol was reduced by TRP only in the S/S’ group. TRP treatment also reduced the stress-induced rise in anxiety (STAI) only in the S/S’ group. Negative affect (POMS) was increased by stress but not altered by genotype or treatment. For S/S’ only, high N subjects showed a stress-induced increase in appetite after placebo but not after TRP. Curiously, low N subjects ate more high-fat sweet snacks than did high N</td>
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### Van Dalfsen & Markus (127)

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<thead>
<tr>
<th>S/S’ allele group: forty-six women, eleven men; L/L’ allele: forty-six women, eight men. Mean ± SD age = 23.9 ± 1.7</th>
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<tr>
<td>Double-blind between-subjects, stratified by genotype and neuroticism trait (median split on DPQ-N). Subchronic oral TRP (3 g/d as 2 × 0.5 g capsules taken three times/d) for 7 d</td>
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<td>Prior to treatment: subjective sleep quality (1 month; adapted PSQI), neuroticism (DPQ-N), depression (BDI), (SLE; Dutch Life Events Questionnaire). During treatment: Daily Hassles Checklist. After treatment: PSQI sleep quality for 1 week</td>
</tr>
<tr>
<td>More neurotic participants had lower general sleep quality, unrelated to genotype, and also reported more SLE. Following treatment, only S/S’ genotype together with higher neuroticism was associated with poorer sleep quality for the placebo group, but with better sleep quality for the TRP-treated group</td>
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Relatively large sample but not enough males to examine sex effects. Parallel effects of TRP in S/S’ subjects for cortisol, anxiety and appetite. Lunch intake, sex and BMI controlled for by covariance. Avoidance of high-fat sweet snacks in high N subjects may be related to health/weight concerns.
mood (Profile of Mood States) and salivary cortisol. The stressor consisted of repeated unpredictable cold pressor stress (hand on a 1.5°C cold plate) interspersed with a Serial-7 subtraction task (repeatedly subtracting 7 from a variable starting number), performed in front of a camera and researcher; errors were recorded. The design did not include a stress-free condition, and only a single baseline measure of cortisol, so interpretation of the observed decline in cortisol after the stress is difficult, as this decline is anyway typical for cortisol during the morning. However, neither TRP treatment nor genotype significantly altered this decline in cortisol. Nevertheless, the stressor caused mood to deteriorate, with increases in feelings of anger, depression and fatigue, but a decrease in vigour. A key finding of this study is that the TRP treatment reduced depression and fatigue, while increasing vigour, specifically in the S/S allele group only. However, these effects were pooled across stress condition, so presumably were not significantly altered by stress (data for a pre/post-stress × genotype × treatment interaction were not presented). Genotype also influenced performance on the subtraction task: the S/S group performed worse than the L/L group after placebo, but after TRP, performance was the same for both allele groups; again, this result was independent of stress. Even so, pre-stress results were not presented, so stress may have contributed somewhat to the findings. For example, the fact that the S/S group made more mistakes in the subtraction task under placebo may indicate that subjects with this genotype were not coping as well.

Subsequently, Markus et al.\(^{114}\) established a larger student cohort screened for 5-HTTLPR genotype, and studied nineteen female S/S and twenty-three female L/L homozygous allele groups, with about half of each group selected to be either high or low on restrained eating (Three Factor Eating Questionnaire\(^{115}\)). This study investigated potential interactions between TRP treatment, 5-HTTLPR genotype, stress, restraint and emotional eating, in a double-blind placebo-controlled crossover design. Stress was elicited using a modified Trier Social Stress Test\(^{116}\); TRP challenge was accomplished using an egg-white protein hydrolysate enriched with TRP (4-g dose given as a 200-ml drink, containing 0.24 g TRP; DSM, Delft; see earlier), \(v\) a casein hydrolysate placebo (0.03 g TRP). Blood samples were taken for amino acid analysis 90 min after consuming the drinks, and four salivary samples were taken during the study to assess cortisol levels. Interestingly, there was a significantly greater increase in plasma TRP:LNAA ratio following TRP treatment for the L/L group (70 % increase) compared with the S/S group (50 % increase). However, although stress resulted in a rise in cortisol, there were no significant effects of either TRP treatment, genotype or restrained eating on cortisol in this study.

Mood generally deteriorated from before to after the stress; of particular interest, the increase in anger after stress occurred in all groups except the L/L genotype group who had received TRP supplementation, in whom there was no change in anger following stress.

Liking (pleasantness of taste) for a variety of foods of different sensory categories (sweet or savoury, low- or high-fat) was assessed using ratings of images of the foods. Only the high-fat sweet food liking ratings showed significant effects: in the L/L allele group, liking for high-fat sweet foods declined following stress only when given the TRP supplement, whereas there were no significant changes to liking ratings for the S/S allele group. Actual food intake was assessed by offering several snack foods (mini chocolate bars, pretzels and nuts) both before and after stress. The only significant result was a 38 % reduction in snack intake after TRP treatment (averaged across stress pre/post-measures); no effects of genotype, stress or restrained eating were seen. An overall appetite-suppressant effect of TRP may be expected, given that ATD tends to increase appetite\(^{63}\), and higher doses of TRP (at least 2 g) have long been known to suppress appetite and reduce food intake by 10–20 %\(^{58}\); nevertheless, the dose of TRP effective here is considerably smaller (0.24 g), so the size of this effect is remarkable.

There are several intriguing findings in this study, not least the weaker increase in plasma TRP:LNAA ratio in the S/S subjects. The authors point out that this difference between genotypes is a unique finding, and speculate that it may be due to increased diversion of peripheral TRP to metabolism via the kynurenine pathway, due to induction of the hepatic TDO and peripheral indole 2,3-dioxygenase enzymes, which are known to be stress-sensitive\(^{14}\). However, direct evidence for such a mechanism reducing the TRP:LNAA ratio in S/S allele subjects after TRP supplementation is lacking. One
study that measured 5-HTTLPR genotypes and administered 50 mg/kg TRP did assess the plasma kynurenine:TRP ratio as an index of TDO activity; however, this was in male patients with alcohol use disorder, and the study did not assess behavioural effects of TRP \cite{117}. Those patients who experienced ‘blacked-out violent impulsive behaviour’ during binge drinking showed a higher kynurenine:TRP ratio than those who did not, suggesting that less TRP would be available to the brain. Nevertheless, no differences were reported for 5-HTTLPR genotype subgroups, although sample sizes may have been too small (nine cases, nine alcohol-dependent controls, received oral TRP) for meaningful statistics in this pilot study, and polymorphisms in the enzymes themselves were not measured. This may be important as there is evidence for example that the TPH1 218AA polymorphism is a risk factor for alcoholism and bipolar disorder \cite{118}. Anyhow, this impaired effect of TRP treatment on the plasma ratio in this S/S group \cite{114} may explain the lack of behavioural effects seen for this group in this study, in contrast to some effects that were specific to the L/L' genotype. Conversely, the most likely explanation for a lack of stress-induced, or emotional, eating is the probability that few of the participants had emotional eating tendencies. Participants were selected on the basis of scores on the Three Factor Eating Questionnaire restrained eating scale, which, unlike some items on the disinhibition or hunger scales of this questionnaire, does not explicitly assess emotional eating and is usually orthogonal to it. We have argued previously that cognitive restraint per se is not a good predictor of stress eating tendencies \cite{119,120}. Furthermore, in a more recent study from this group, S/S' allele subjects (both male and female) were shown to be more likely to eat sweet fatty foods after mild stress than L/L' genotypes, an effect that was reduced by a sucrose preload \cite{121}. However, in that study, there was no manipulation by TRP load. Another study from this group investigated whether examination stress would differentially affect appetite for these two genotype groups \cite{122}, findings confirmed that the S/S' genotype group was more likely to show stress-induced eating of sweet snacks, although again there was no manipulation of TRP.

Nevertheless, the interaction between genotype, stress, emotional eating and effects of subchronic TRP supplementation was investigated in mainly female participants (ninety-nine women, nineteen men) asked to self-administer 3 g TRP daily for 7 d (or placebo cellulose), before undergoing an acute stress test (repeated cold pressor and Serial-17 subtraction task known as the Maastricht Acute Stress Test) \cite{123,125}. Changes in appetite ratings, snack intake, mood and cortisol were assessed. Subchronic TRP treatment reduced the cortisol response to stress only in the S/S' allele group. Similarly, the TRP treatment resulted in a significantly less stress-induced increase in anxiety only in the S/S' group, but independently of trait neuroticism. Stress increased rated appetite, but interestingly TRP reduced this increase specifically in S/S' subjects who also scored highly on neuroticism. The parallels across these TRP by genotype interactions are notable. By comparison, the only significant finding reported for post-stress snack intake was a greater intake of sweet fatty snacks by the low neuroticism v. the high neuroticism group, perhaps due to health concerns in the latter group. The interaction of genotype with neuroticism on the stress-induced change in rated appetite is similar to the results of an earlier study in which mainly female participants with low or high trait anxiety were subjected to stress (mental arithmetic during loud noise) and treated acutely with either TRP-rich α-lactalbumin or casein \cite{134}. Food liking and preference was assessed by responses to food images displayed via a computer program \cite{125}. While appetite ratings increased for all groups after stress, both liking and preference for sweet foods increased specifically for highly anxious participants, and these increases were prevented by α-lactalbumin treatment, implying that the increased desire for sweet food induced by stress in high-anxious participants was related to impaired 5-HT function. However, in this study, genotypes were not measured. Moreover, in the case of actual eating \cite{122}, it seems that other factors influenced the behaviour, although differences in timing between stress and food intake could be involved, and in this subchronic treatment design, no treatment was given on the test day.

Another group also examined effects of a similar subchronic TRP treatment (2-8 g/d for 6 d) on responses to stress (Trier Social Stress Test) in relation to 5-HTTLPR genotype \cite{126}. In this study, about half the participants were female (twenty-two women, twenty-four men), although sex was included as a covariate in analyses, rather than reporting interactions with sex. There was a clear interaction between stress, genotype, and treatment on salivary cortisol: S/S' allele subjects on placebo (cellulose) showed the largest rise in cortisol induced by the stress, supporting a stress sensitivity of this genotype, but this effect was substantially reduced by prior TRP treatment (even though no TRP was taken on the test day): the lower cortisol response in L/L' participants was not further reduced by TRP. However, while mood deteriorated after the stress, this was not differentially influenced either by treatment or genotype, contrary to Capello and Markus \cite{123}.

Subsequently, a recent study investigated whether a similar subchronic treatment with TRP (3 g/d for 7 d) could benefit the quality of sleep, and whether this might depend on 5-HTTLPR genotype \cite{127}. Thus, this study compared effects between S/S' allele subjects (forty-six women, eleven men) and L/L' allele subjects (forty-six women, eight men). Potential effects of neuroticism were investigated using a median split of questionnaire scores into high and low neuroticism groups. General sleep quality was assessed prior to treatment, then sleep quality after the week of treatment was measured for a further week. Higher neurotic participants tended to report lower general sleep quality, unrelated to genotype. However, following treatment, specifically S/S' genotype together with higher neuroticism was associated with poorer sleep quality for the placebo group, but with better sleep quality for the TRP-treated group.
Finally, there is recent evidence of differential impact of 5-HTTLPR genotypes on mood changes during challenging tasks in the context of two intervention studies that had found beneficial effects of acute and chronic treatment with a TRP-rich egg-white protein hydrolysate (DSM) on mood, emotional processing, and cognition in Caucasian women aged 45–65 years. Participants were genotyped for the tri-allelic 5-HTTLPR polymorphism, and distributions of genotypes were in accordance with Hardy-Weinberg equilibrium (allele sample sizes; acute study: SS/SL_G = 11, SL_G/L_A = 36, L_A/L_A = 13; chronic study: SS/SL_G = 13, SL_G/L_A/L_A = 36, L_A/L_A = 10).

We planned to compare the two homozygous groups (SS/L_G (designated S/S) vs. L_A/L_A (designated L/L)) on behavioural outcomes; however, with several different treatment groups, cell sizes would be too small for meaningful analyses of treatment by genotype effects. Therefore, we examined outcomes on the pre-treatment baseline day, when the participants completed the same set of tests as during treatment, which allowed us to pool the outcome data for all participants within each genotype group. The series of cognitive and behavioural tests lasted for 3·5 h from the baseline (pre-test) mood measure to the final post-test mood measure, with 1 h of rest in between, so represented a challenging and potentially ego-threatening process for the participants. Furthermore, we compared pre-test to post-test changes only in those emotions that had proved responsive to subsequent TRP supplementation treatment. Specifically, these were wellbeing and fatigue in the acute study, and a positive feeling of high energy (stimulated, buzzing, impulsive) in the chronic study (emotions were derived by factor analyses of ratings on twenty-eight items presented on a computer, known as the Mental and Physical Sensations Scale). For the acute study, we found that well-being declined from pre- to post-test in the S/S group, but not in the L/L group, whereas fatigue increased significantly only for the S/S group. For the chronic study, high-energy mood increased from pre- to post-test for the L/L group, but did not change for the S/S group.

These differences in genotypes for mood changes during challenging and potentially stressful tasks are in line with evidence that the S/S genotype would confer greater risk of affective disorders such as anxiety or depression, or conversely a protective effect of the L/L allele, in women. Moreover, the known sensitivity of these changes in mood to TRP treatment supports mediation via changes in serotonin function.

Conclusions

The main theme emerging from the literature on TRP supplementation and genotypes is the observations of interactions between TRP and genotypes, sex and stress on changes in mood, cognition, cortisol and appetite. It is particularly important to consider the influence of a key ‘genotype’, sex. For example, in women, the 5-HTTLPR S/S genotype predicts sensitivity to improvements in the mood by TRP supplementation, especially during stressful challenges, whereas the L/L genotype tends to be protective against stress-induced mood deterioration and rise in cortisol, but may differ in sensitivity to TRP administration. In men, if anything, the L/L genotype confers risk of stress-induced increases in negative affect; however, there are insufficient studies with adequate power to detect sex × genotype × stress × TRP in the literature to draw strong conclusions.

Since the 5-HTTLPR genotypes may influence neurodevelopment and/or tonic 5-HT adaptive responsiveness at least as much as acute functioning of the brain serotonin system, it would be advantageous to assess extent of early life stress and/or stressful life events, as well as personality traits predictive of affective disorders, in studies of TRP effects on behaviour. However, when measuring multiple influences on behaviour, as well as sex differences, investigators need to ensure sufficiently large sample sizes to increase the likelihood of reliable findings. Routinely screening for genetic polymorphisms in suitable populations would be helpful.

There is a need to broaden studies on the potential benefits of TRP supplementation to include a greater range of serotonin-related genotypes, including enzymes involved in key metabolic pathways. This may involve in key metabolic pathways (Fig. 1). This may eventually lead to clear predictions as to who is likely to benefit most from this relatively simple nutrient-based treatment. Until then, although there is preliminary evidence that individuals with some genotypes, particularly the 5-HTTLPR S/S allele in women, may benefit from TRP supplementation as an aid to stress coping and emotional regulation including comfort eating, further research is needed before reliable recommendations can be made on targeted use of TRP treatment, or adjustment of dietary TRP intake, for beneficial behavioural outcomes.

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Conflicts of Interest

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