# Protein-source tryptophan as an efficacious treatment for social anxiety disorder: a pilot study[1]

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**Abstract:** Until recently, intact protein that is rich in tryptophan was not seen as an alternative to pharmaceutical-grade tryptophan because protein also contains large neutral amino acids (LNAAs) that compete for transport sites across the blood–brain barrier. Recent evidence indicates that when deoiled gourd seed (a rich source of tryptophan with approximately 22 mg/g protein) is combined with glucose (a carbohydrate that reduces serum levels of competing LNAAs) a clinical effect similar to that of pharmaceutical-grade tryptophan is achieved. Objective and subjective measures of anxiety in those suffering from social phobia (also known as social anxiety disorder) were employed to measure changes in anxiety in response to a stimulus as part of a double-blind, placebo-controlled, crossover study with a wash-out period of 1 week between study sessions. Subjects were randomly assigned to start with either (*i*) protein-source tryptophan (deoiled gourd seed) in combination with carbohydrate or (*ii*) carbohydrate alone. One week after the initial session, subjects returned for a follow-up session and received the opposite treatment of that received at the first session. All 7 subjects who began the study completed the 2-week protocol. Protein-source tryptophan with carbohydrate, but not carbohydrate alone, resulted in significant improvement on an objective measure of anxiety. Protein-source tryptophan combined with a high glycemic carbohydrate is a potential anxiolytic to those suffering from social phobia.

*Key words:* blood–brain barrier, anxiety, protein-bound tryptophan.

**Résumé :** Encore tout récemment, la protéine intacte riche en tryptophane n'était pas considérée comme une solution de substitution au tryptophane de qualité pharmaceutique étant donné que la protéine contient aussi de grands acides aminés neutres (LNAAs) qui entrent en compétition pour traverser la barrière hémato-encéphalique. De récentes données indiquent que l'association graine de courge déshuilée (une très riche source de tryptophane – 22 mg de tryptophane/1 g de protéine) et glucose, un glucide qui abaisse les taux sériques des LNAAs compétitifs, a un effet clinique similaire à celui du tryptophane de qualité pharmaceutique. Nous avons utilisé des mesures objectives et subjectives de l'anxiété chez des sujets atteints de phobie sociale pour apprécier les modifications de l'état d'anxiété en réponse à un stimulus, dans le cadre d'un essai croisé contre placebo, à double insu, dont les sessions ont été séparées par une période de lavage d'une semaine. Nous avons réparti les sujets de façon aléatoire dans deux groupes : (*i*) tryptophane de source protéique (graine de courge déshuilée) associé à un glucide ou (*ii*) glucide seulement. Une semaine après le début de l'expérience, à la session de rappel, les sujets ont reçu le traitement opposé à celui reçu lors de la première session. Les 7 sujets ont complété le protocole de deux semaines. Le tryptophane de source protéique associé au glucide, mais pas le glucide uniquement, a amélioré considérablement la mesure objective de l'anxiété. Ainsi, le tryptophane de source protéique associé à un glucide hyperglycémiant est un anxiolytique potentiel pour les personnes atteintes de phobie sociale, connue aussi sous le nom de trouble d'anxiété sociale.

*Mots-clés :* barrière hémato-encéphalique, anxiété, tryptophane de source protéique.

[Traduit par la Rédaction]

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[1]This article is one of a selection of papers published in this special issue (part 1 of 2) on the Safety and Efficacy of Natural Health Products.
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## Introduction

The anxiolytic benefits of the amino acid tryptophan have been described in animal models for some time (Bliss et al. 1968) and humans as well (Pecknold et al. 1982). When tryptophan is available in the central nervous system (CNS), it undergoes hydroxylation and then decarboxylation to become serotonin, which aids in the treatment of depression, anxiety, and emotional lability (Boman 1988). In low light conditions, it is further metabolized to melatonin, which induces a natural sleep (Schneider-Helmert and Spinweber 1986) with preserved sleep architecture (Levitan et al. 2000).

The study and clinical use of pharmaceutical-grade tryptophan declined precipitously in 1989 after pharmaceutical-grade tryptophan was associated with a syndrome known as

eosinophilia myalgia syndrome (EMS), a serious medical condition that can result in fatality. The reasons for the association of tryptophan with EMS are not clear, although most cases arose from the use of L-tryptophan supplied by Showa Denko K.K in 1989. Many people who consumed this product, however, were unaffected, and cases of EMS and related disorders occurred before and after the 1989 epidemic (Hertzman et al. 1990). The risk of EMS may arise from impurities in the preparation of pharmaceutical-grade tryptophan (Williamson et al. 1997) or from different patterns of xenobiotic metabolism (Flockhart et al. 1994) with immune response genes conferring increased susceptibility to the syndrome (Okada et al. 1994). Despite the lack of certainty over the safety of pharmaceutical grade tryptophan, it is clear that tryptophan as part of intact protein was not associated with these difficulties and was specifically excluded from the US Food and Drug Administration recall and restrictions (FDA Communication 1989).

A normal healthy diet contains 1000 to 1500 mg of protein-source tryptophan per day with a minimum requirement of 250 mg per day to maintain nitrogen balance (Young 1986). Food sources contain tryptophan in protein as part of chains of amino acids that are bound together in their amide form. When tryptophan is ingested as part of a protein meal, serum tryptophan levels rise but brain tryptophan levels decline (Fernstrom and Faller 1978). This paradoxical relationship is due to the mechanism of transport used by tryptophan to cross the blood–brain barrier (BBB). The transporter sites for tryptophan are shared with other large neutral amino acids (LNAAs) such as tyrosine, valine, isoleucine, leucine, and phenylalanine. Tryptophan is the rarest of all essential amino acids and most proteins contain comparatively small amounts of tryptophan compared with competing LNAAs. This low tryptophan level relative to other LNAAs prevents tryptophan from entering the brain and metabolizing to serotonin and melatonin and, as such, protein-source tryptophan has not been seen as having a role as an anxiolytic.

A recent study (Hudson et al. 2005) demonstrated, however, that it is possible to affect changes in CNS function when intact protein rich in tryptophan is combined with a high glycemic index carbohydrate. The rationale behind this particular combination arises from carbohydrate research as well as the identification of a protein source rich in tryptophan, both of which are described below.

### Carbohydrate

Fernstrom and Wurtman (1971) were the first to recognize that insulin lowers all serum amino acid levels except for tryptophan since tryptophan is largely protein bound, which is unique among amino acids (Young 1986). Jenkins et al. (1981) first described inherent characteristics of insulin induction by various carbohydrates (for review tables see Foster-Powell and Brand-Miller 1995). Glycemic index tables indicate that glucose has a high enough glycemic index to induce a rapid rise in serum insulin levels. Martin-Du Pan and colleagues (1982) determined dose–response curves for glucose to increase the serum concentration of tryptophan in comparison with other LNAAs. In this study, dosages of both 25 g and 50 g of glucose resulted in a significant increase in tryptophan relative to the competing LNAAs. On average, when serum insulin levels rise from 15 μU/mL to 60 μU/mL, there is a resultant 35% increase in the tryptophan/LNAA ratio (Lyons and Truswell 1988).

### Protein identification

As described in a previous study (Hudson et al. 2005), pumpkin seeds and other gourd seed varieties were screened for tryptophan content with second derivative spectroscopy (Balestrieri et al. 1978) at the Guelph Food Technology Centre (GFTC), Guelph, Ontario, with results later verified at an independent laboratory (Maxxam Analytics Inc., Toronto, Ont.) utilizing high pressure liquid chromatography (HPLC) analysis (Strydoma et al. 1993). The tryptophan content of butternut squash seeds is 22 mg/g of protein, which is consistent with other gourd seeds reviewed in the US Department of Agriculture database. For this study, deoiled butternut squash seed meal (tryptophan content, 10 mg/g of deoiled seed meal) was used as the protein source of tryptophan.

The present pilot study was designed to test the premise that it may be possible to treat social phobia, also known as social anxiety disorder, with a functional food that incorporates both protein rich in tryptophan and sufficient high glycemic index carbohydrate to reduce LNAA competition for transport sites across the BBB.

## Materials and methods

For this study, 2 separate food bars were prepared.

(*i*) Food 1 (tryptophan bar) contained 25 g of deoiled butternut squash seed meal and 25 g of dextrose. The tryptophan content of food 1 was 250 mg/food bar.

(*ii*) Food 2 (placebo bar) contained 50 g of carbohydrate composed of dried fruit and dextrose. The tryptophan content was 0 mg for food 2.

### Dependent variables

Increases in heart rate from baseline as well as heart rate variation were chosen as objective measures of anxiety. Heart rate and heart rate variation (ratio of maximum to minimum R–R wave interval variation) are well-described objective measures of anxiety (Watkins et al. 1998). They were measured via a heart rate monitor that digitized the signal, allowing for later statistical analysis.

The subjective measure of anxiety was the perception subscale of the Endler Multidimensional Anxiety Scale (EMAS-P), a reliable and valid measure of the subjective sense of anxiety (Endler et al. 1991*a*). Factor analysis of the EMAS supports the empirical relation between 2 domains of anxiety (state and trait) and allows reliable and valid measurement of either domain (Endler et al. 1991*b*).

### Hypotheses

The following set of hypotheses was formulated on the basis of the 2 food formulations:

Hypothesis I: food 1 but not food 2 will diminish a subjective anxiety parameter in response to a clinical stressor.

Hypothesis II: food 1 but not food 2 will diminish an objective anxiety parameter in response to a clinical stressor.

## Study design

Seven subjects (2 males and 5 females) were recruited into a placebo-controlled, double-blind study in which they were randomly assigned to either an active trial (food 1) followed by a placebo trial (food 2), or a placebo trial followed by an active trial. There was a 7-day washout period between trials. Both the subject and the research nurse who conducted the study were blind to the assignment of each subject.

During the initial meeting, subjects were screened for the presence of an anxiety disorder following DSM IV criteria for social phobia (American Psychiatric Association 1994). The subjects were screened and excluded if there was evidence of a coexisting physical or mental health illness. The dependent study variables were measured with the EMAS-P as well as by heart rate, measured with a heart rate monitor capable of assessing individual heart beats. All diagnostic interview findings were confirmed by a psychiatrist (C.J.H.).

## Subjects

A sample of volunteers was selected from the Perth County region, Ontario. Eight subjects were recruited through local advertisement for subjects suffering from social phobia. One subject was rejected after a short (approximately 10 min) structured telephone interview indicating evidence of other health issues.

All subjects were between the ages of 18 to 65 years of age and were informed about the purpose, risks, and benefits associated with the study. Written, signed consent was obtained and a copy was given to the participant approved by the Stratford General Hospital Ethics Review Committee.

## Anxiety protocol

Each subject completed 2 sessions of study. In the 1st session, 2 of the 7 subjects ingested a placebo bar 1 h before their first assessment, whereas the remaining 5 subjects received the tryptophan bar 1 h before their assessment. During the session, the participants completed an initial full EMAS-P (perception subscale) to measure their subjective sense of stress. They were then connected to a heart rate monitor that relayed the digitized signal to a data storage device. A baseline assessment of heart rate was performed for 5 min before participants were exposed to the anxiety-provoking stimulus, then re-measured during the next 5 min of stress and again at 5 min after the end of the stimulus. Subjects also completed a second EMAS-P self-evaluation after exposure to the clinical stressor.

In session 2, the treatments were reversed: 2 of the 7 subjects ingested an active bar 1 h before their assessment, whereas the remaining 5 subjects had the placebo bar 1 h before their assessment. Otherwise, the same objective and subjective measures were employed in both sessions.

The anxiety-provoking stimulus was the same in each instance. The subjects were requested to read a 1-page excerpt from a passage (Myer 2000) that contained complicated words, sentence structure, and concepts. The reading selections for each session were new, unrehearsed, and introduced just before they were to be read. The subjects were seated in front of a video camera and told that their performance would be recorded and later evaluated by a group of 30 volunteers and scored for accuracy, speed, clarity of diction, and cadence. The camera was recording but the subjects were not told that the tape holder was empty. The only dependent measures utilized were those described above.

## Statistical analysis

A $2 \times 2$ repeated measures analysis of variance procedure (using regression procedures as suggested by Judd and McClelland 1989) was used to analyze the effect of treatment, and a matched $t$ test was used on Endler scores. A value of $p < 0.05$ was considered statistically significant.

## Results

A summary of results is presented in Table 1.

### Subjective measure of anxiety: Endler measure

We analyzed the effect of the treatment on the subjective perception of anxiety and found the mean Endler score was higher after the placebo bar (11.83) than after the treatment bar (10.08), but this difference was not significant ($F = 4.55$; $p > 0.05$). A matched $t$ test for the Endler scores did, however, indicate a trend towards a reduction in subjective experience of anxiety with protein-source tryptophan treatment as measured by the EMAS-P ($p = 0.077$), which was consistent with our first hypothesis.

### Objective measure of anxiety: baseline versus acute stress heart rate

We analyzed the effect of tryptophan treatment on heart rate before the stressful event and heart rate during the experience of acute stress. As expected, across treatment conditions, the mean heart rate during acute stress (91.50 beats/min) was significantly higher than mean heart rate during the baseline (80.53 beats/min; $F = 16.05$, $p < 0.01$). The difference in heart rate between acute stress and the baseline when subjects took the placebo and when subjects took the tryptophan bar was not statistically significant ($F = 4.55$; $p > 0.05$).

### Objective measure of anxiety: baseline versus acute stress heart rate variation ratio

We analyzed the effect of the tryptophan treatment on heart rate variance (a ratio of maximum to minimum R–R interval) before the stressful event and heart rate variance during the experience of acute stress. As expected, across treatment conditions, the mean ratio of heart rate variation during acute stress (1.31) was significantly lower than mean ratio of heart rate variation during the baseline (1.48; $F = 9.50$, $p < 0.025$). The difference in heart rate ratio between acute stress and the baseline when subjects took the placebo (0.07) and when subjects took the tryptophan bar (0.26) was also statistically significant ($F = 17.20$; $p < 0.01$). Moreover, the variance measured before and after the stressful event was greater with tryptophan treatment. This provides evidence to support our second hypothesis, namely, that the tryptophan bar diminishes an objective anxiety parameter in response to a clinical stressor.

## Discussion

The results of this study indicate that a functional food combining a tryptophan-rich protein with a carbohydrate

**Table 1.** Descriptive statistics for subjective and objective measures of anxiety, $n = 7$.

|  | Placebo bar | Tryptophan bar |
|---|---|---|
| EMAS-P score | 11.83 (7.25) | 10.08 (7.40) |
| Heart rate, beats/min |  |  |
|   Baseline | 78.96 (10.88) | 82.10 (11.90) |
|   Acute stress | 90.80 (16.77) | 92.19 (15.72) |
|   Post stress | 80.03 (11.76) | 80.29 (11.74) |
| R–R interval, ms |  |  |
|   Baseline | 771.43 (105.05) | 753.43 (115.08) |
|   Acute stress | 678.29 (112.35) | 667.86 (119.44) |
|   Post stress | 779.71 (123.22) | 762.71 (125.42) |
| Heart rate variation, max/min |  |  |
|   Baseline | 1.42 (0.09) | 1.53 (0.17) |
|   Acute stress | 1.35 (0.19) | 1.2 (0.14) |
|   Post stress | 1.41 (0.16) | 1.48 (0.26) |

**Note:** EMAS-P, Endler Multidimensional Anxiety Scale (perception); R–R, time interval between successive R waves of the heart beat. The EMAS-P is an 8-item subscale with a minimum raw score of 5 and a maximum of 25. Data are means (SD).

may exert a positive effect on CNS function. This corroborates a previous study by Hudson et al. (2005), which also demonstrates that protein rich in tryptophan can relieve insomnia provided it is combined with a high glycemic index carbohydrate.

Before these studies, a change in the composition of intact dietary protein was not seen as a possible option for the treatment of common psychological disorders associated with low serotonin levels. In fact, previous experiments would suggest that intact protein should not increase brain tryptophan levels and not affect a response to stress (Fernstrom and Wurtman 1972). Investigations in nonhuman primates parallel those from rat studies in finding that only conditions that favoured an increased ratio of serum tryptophan and competing amino acids resulted in increased brain tryptophan. Leathwood and Fernstrom (1990) demonstrated a dose-dependent increase in tryptophan in subcortical regions of the brain, in concert with a dose-dependent reduction in competing amino acids, when groups of adult cynomolgus monkeys were fed various combinations of carbohydrate (maltodextrin) and 20, 90, or 400 mg/kg of synthetic tryptophan.

The current study indicates that a functional food containing protein rich in tryptophan can offer a benefit to those who experience social phobia or social anxiety disorder by mitigating both subjective and objective responses to stress provided the functional food also contains a high glycemic index carbohydrate that will suppress serum levels of large neutral amino acids.

## Acknowledgements

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