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Protein source tryptophan versus pharmaceutical grade tryptophan as an efficacious treatment for chronic insomnia

CRAIG HUDSON[1], SUSAN PATRICIA HUDSON[1], TRACY HECHT[2], & JOAN MACKENZIE[3]

[1]*Biosential Inc., 1543 Bayview Avenue, Suite 346, Toronto, Ontario M4G 3B5, Canada,* [2]*Concordia University, 1455 de Maisonneuve Blvd., Montreal, Quebec H3G 1M8, Canada, and* [3]*Department of Psychiatry, Stratford General Hospital, 46 General Hospital Drive, Stratford, Ontario N5A 2Y6, Canada*

Abstract
Background: Intact protein rich in tryptophan was not seen as an alternative to pharmaceutical grade tryptophan since protein also contains large neutral amino acids (LNAAs) that compete for transport sites across the blood–brain barrier (BBB). Deoiled gourd seed (an extremely rich source of tryptophan—22 mg tryptophan/1 g protein) was combined with glucose, a carbohydrate that reduces serum levels of competing LNAAs which was then compared to pharmaceutical grade tryptophan with carbohydrate as well as carbohydrate alone. *Method*: Objective and subjective measures of sleep were employed to measure changes in sleep as part of a double blind placebo controlled study where subjects were randomly assigned to one of three conditions: (1) Protein source tryptophan (deoiled gourd seed) in combination with carbohydrate; (2) pharmaceutical grade tryptophan in combination with carbohydrate; (3) carbohydrate alone. *Subjects*: Out of 57 subjects 49 of those who began the study completed the three week protocol. *Results*: Protein source tryptophan with carbohydrate and pharmaceutical grade tryptophan, but not carbohydrate alone, resulted in significant improvement on subjective and objective measures of insomnia. Protein source tryptophan with carbohydrate alone proved effective in significantly reducing time awake during the night. *Conclusion*: Protein source tryptophan is comparable to pharmaceutical grade tryptophan for the treatment of insomnia.

Keywords: *Blood–brain barrier, insomnia, protein-bound tryptophan, LNAAs*

Introduction

The hypnotic effects of the amino acid tryptophan are well studied and follow a fairly flat dose-response curve with a plateau at approximately 1000 mg (for review see Schneider-Helmut and Spinweber 1986). When given alone, as little as 250 mg of tryptophan has produced improved sleep in people with chronic insomnia (Hartmann and Spinweber 1979, Hartmann 1982). Dosages of 1000 mg are associated with more consistent results (Schneider-Helmut and Spinweber 1986) but higher dosages (2000–12,000 mg) offer little extra benefit and, indeed, the highest dosages (12,000 mg) are associated with disrupted sleep architecture despite a reduction in sleep latency. In disease states where sleep architecture is often disrupted, tryptophan can aid in the restoration of slow wave sleep (Levitan et al. 2000) and conversely, diets that deplete tryptophan can disrupt sleep architecture (Riemann et al. 2002). Although efficacious pharmaceutical grade tryptophan has been associated with eosinohpilia myalgia syndrome (EMS), a serious medical condition that can result in fatality and its sale is restricted in most countries. The reasons for the association of tryptophan with EMS are not clear with most cases arising from L-tryptophan supplied by Showa Denka K.K. in 1989 but many people who consumed this product were

unaffected and cases of EMS and related disorders occurred prior to and after the 1989 epidemic (Hertzman et al. 1990). The risk of EMS may arise from impurities within pharmaceutical grade tryptophan (Williamson et al. 1997) or different patterns of xenobiotic metabolism (Flockhart et al. 1994) with immune response genes conferring increased susceptibility of 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 Food and Drug Administration (FDA) recall and restrictions (FDA communication 1989).

A normal healthy diet contains 1000–1500 mg of protein source tryptophan per day with a minimum requirement of 250 mg/day to maintain nitrogen balance (Young 1986). Tryptophan in food sources exists in protein as part of chains of amino acids, which 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 (Fernstrum and Faller 1978). This apparent contradiction arises because the transport mechanism utilized by tryptophan to cross the blood–brain barrier (BBB) is shared with other large neutral amino acids (LNAAs) such as tyrosine, valine, isoleucine, leucine and phenylalanine. Since tryptophan is the rarest of all essential amino acids, most proteins contain comparatively small amounts of tryptophan compared to competing LNAAs. This prevents tryptophan from entering the brain and metabolizing to serotonin and melatonin.

Fernstrom and Wurtman (1971) were the first to recognize that insulin lowers all serum amino acid levels except tryptophan since tryptophan is largely protein bound, which is unique amongst amino acids (Young et al. 1986). Subsequent investigations revealed that insulin, and carbohydrates which induce insulin, result in higher serum tryptophan levels relative to other LNAAs which allowed tryptophan a competitive advantage for the transport sites to cross the BBB. Further studies indicated that diets that were comprised of all carbohydrate increased brain tryptophan levels whereas diets that were rich in protein either reduced or affected brain tryptophan levels (Fernstrom and Faller 1978).

The present study was designed to test the premise that it may be possible to replace pharmaceutical grade tryptophan with protein rich in tryptophan, if it was combined with a carbohydrate that would induce sufficient serum insulin levels to reduce LNAA competition for transport sites across the BBB. The challenge was to identify a protein sufficiently rich in tryptophan to provide approximately 250 mg, in combination with sufficient carbohydrate to induce a rapid and sustained increase in serum insulin levels, in a quantity and form reasonable for someone to eat at one time. Sleep parameters were chosen as the dependent variables since the relationship between sleep and tryptophan is well studied and may be measured in a sensitive, valid and reliable fashion (Morin 1993a).

Carbohydrate

The diabetes literature provides extensive research into the inherent characteristics of insulin induction of various carbohydrates (Jenkins et al. 1981; 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 to other LNAAs. Healthy controls nil per os (NPO) from the previous night were given various dosages of pure glucose from 0 to 50 g as breakfast. Subsequent measurement of serum amino acid levels determined that dosages of both 25 and 50 g of glucose resulted in a significant increase in tryptophan relative to the competing LNAAs. On an average, when serum insulin levels rise from 15 to 60 microunits/ml there is a resultant 35% increase in the tryptophan/LNAA ratio (Lyons and Truswell 1988).

Protein identification

A review of the US department of agriculture data base (www.nal.usda.gov) determined that pumpkin seeds contain significant amounts of tryptophan. Pumpkin seeds as well as other gourd seed varieties were screened for tryptophan content with second derivative spectroscopy (Balestrierl et al. 1978) at the Guelph Food Technology Centre (GFTC), Guelph, Ontario, Canada with results later verified at an independent laboratory (Maxxam Analytics Inc., Toronto, Ontario, Canada) utilizing high pressure liquid chromatography (HPLC) analysis (Strydoma et al. 1993). Butternut squash seeds contain approximately 22 mg of tryptophan/1 g of protein, which is consistent with other gourd seeds reviewed in the US department of agriculture data base. For this study deoiled butternut squash seed meal (10 mg of tryptophan/1 g of deoiled seed meal) was used as the protein source of tryptophan.

Materials and methods

Functional food preparation

For this study three separate food bars were prepared:

(1) **Food1** contained 25 g of deoiled butternut squash seed meal and 25 g of dextrose.
(2) **Food2** contained 250 mg of pharmaceutical tryptophan, 25 g of dextrose and 25 g of rolled oats.
(3) **Food3** contained 50 g of rolled oats.

The tryptophan content of Food1 and Food2 was 250 mg/food bar and 0 mg for Food3. All food bars contained 25 mg of vitamin B_3 and 5 mg of Vitamin B_6.

Additional vitamins

Vitamin B_3 suppresses the activity of tryptophan pyrrolase (oxygenase), one of the key enzyme in the conversion of tryptophan to nicotinic acid (Boman 1988). Given that an excess of 90% of ingested tryptophan can be converted to nicotinic acid in the human body and, as a result, would not be available as a precursor to serotonin in the brain, vitamin B_3 was included in the test foods for this study (Boman 1988). Vitamin B_6 was included as there is a theoretical increased requirement for vitamin B_6 as one consumes increased amounts of tryptophan (Young 1986).

Hypotheses

The following set of hypotheses was formulated based on three food formulations:

IA: Food1 and Food2 will result in improved objective sleep parameters (i.e. total sleep time, sleep efficiency, total wake time, time awake— middle of the night and overall perceived quality) in the treatment week as compared to the baseline week; there will be no differences in objective sleep parameters between baseline week and treatment week for Food3.

1B: Food1 and Food2 will result in improved objective sleep parameters (i.e. total sleep time, sleep efficiency, total wake time, time awake— middle of the night and overall perceived quality) in the treatment week as compared to the post-treatment week; there will be no differences in objective sleep parameters between treatment week and post-treatment week for Food3.

2A: Food1 and Food2 will result in improved subjective overall sleep quality in the treatment week as compared to the baseline week; there will be no differences in subjective overall sleep quality between baseline week and treatment week for Food3.

2B: Food1 and Food2 will result in improved subjective overall sleep quality in the treatment week as compared to the post-treatment week; there will be no differences in subjective overall sleep quality between treatment week and post-treatment week for Food3

Study design

The three arm study was a parallel design with subjects randomly assigned to one of three groups: Food1 or Food2 or Food3.

Both the subject and the research nurse who conducted the study were blind to the assignment of each subject. The subjects met the nurse on a weekly basis for a three-week period in order to turn in their sleep diaries, to review any possible side effects from that week and to receive the sleep diary for the next week. Each subject received instructions in good sleep hygiene practice at the commencement of the study and reviewed again at the beginning of the second and third week of the study. Subjects met for a final time with the research nurse at the conclusion of the final week of the study to hand in sleep diaries from that week as well as to review possible side effects.

The sleep diaries used in this study are well validated with excellent measures of reliability and validity (Coates et al. 1982). Dependent variables derived from this dairy included the following objective measures: Total sleep time; sleep efficiency (time asleep/total time in bed × 100); time awake during night; time awake during middle of night. Subjective measures, which were combined to create an overall subjective sleep quality index, which included perception of rejuvenation in morning (1 = Exhausted, 2 = Fair, 3 = Refreshed) and perception of sleep quality (1 = Restless, 2 = Fair, 3 = Very sound). Sleep diaries were completed daily (as described below) and these data were averaged to create weekly scores for each week of the three-week protocol. The internal consistency reliability of the subjective sleep quality index was high for each of the three weeks (Week 1: $\alpha = 0.80$; Week 2: $\alpha = 0.95$; Week 3: $\alpha = 0.85$).

Subjects

Ethical approval was received from Ethics Review Committee at the site of the study, Stratford General Hospital, Stratford, Ontario, Canada. Criteria for selection included men and women over the age of 18 with DSM IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) criteria for primary insomnia. The severity of insomnia was determined by specifying subjects with difficulty initiating or maintaining sleep three or more nights/week for a duration of three months or more. Exclusion criteria included heart disease, mental health disease, pregnancy, food allergies, diabetes, sleep apnea, and shift workers.

A sample of volunteers was selected from Perth County region, Ontario, Canada. One hundred and fifty eight (158) subjects were recruited through letters to family doctors, newspaper and radio advertisement. Fifty-one subjects were rejected after a short (approximately 10 min) structured telephone interview indicating evidence of health issues, medication or lack of desire to commit to the three-week protocol. A further 50 subjects were rejected after a detailed structured personal interview with a research nurse for the following reasons: Health (20); failure to appear for the interview (3); medication contraindicated with tryptophan (12), unwillingness to stop sleep medications (3), food allergies (5), unwillingness to

commit to three week protocol (3), shift workers (3) and inability to meet criteria for insomnia (1).

A total of 57 subjects (44 females, 13 males) were randomly assigned to one of the three treatment conditions: 20 to Food1; 20 to Food2 and 17 to Food3.

Subjects enrolled for three weeks. The first week was a baseline week in which they completed structured sleep diaries to record their sleep patterns. Sleep patterns were assessed utilizing a reliable and valid sleep diary (Morin 1993a). In the second week they continued to complete sleep diaries but were also instructed to eat the food they were given for their treatment condition one-half hour prior to bedtime. In the third week they completed sleep diaries as in the previous two weeks, without any treatment food.

After initiation into the study, eight subjects failed to complete the protocol for the following reasons: Time commitment (2), failure to attend weekly interviews (1), death of close family member (1), relationship stress (1), nausea (3: 1, Food1; 2, Food3). All data from subjects who dropped out of the study were excluded from the final analysis and the final distribution of subjects at the end of the study was as follows: 18 in Food1 (2 drop outs), 16 in Food2 (4 drop outs) and 15 in Food3 (2 drop outs). The average ages (53.3, 52.1, 50.1 years) and weights (71.0, 71.6, 71.8 kg.) did not significantly differ between groups for those subjects that completed the three week protocol. Patients were monitored for side effects on a weekly basis and had access to a 7 day per week 24 hour per day support. The only report of side effects was nausea [2 from Food3 (placebo) and 1 from Food2 (squash seed meal and carbohydrate)].

No subject in Food1 (pharmaceutical grade tryptophan) reported any ill effects.

Twin case study

Coincidentally two identical twin brothers enrolled in the study were apparently unaware that each other had enrolled. These twin subjects were randomly assigned to one of the two treatment conditions of Food1 and Food2.

A sample size of two does not allow for any meaningful statistical analysis but it was determined that twin data was of interest since there is greater control for biological and environmental differences between subjects. Therefore, it was decided that the twin data would be reviewed separately at the conclusion of the study and differences reported if they were consistent with overall trend in differences between Food1 and Food2 groups.

Results

Five variables of sleep (four objective and one subjective) were each analyzed initially with a 3×3 split plot multivariate analysis of variance (MANOVA) which found no significant week by condition interaction. Although in each case the analysis demonstrated no significant week by condition interactions, simple main effects for the three treatment conditions were examined based on the *a priori* hypotheses listed. The results are described below. In two instances, the simple main effects were not significant but the pairwise comparisons were reported for the sake of completeness. For overview of all results please see Table I.

Table I.   Summary table of pairwise comparisons.

Total sleep time (minutes)	Baseline week	Treatment week	Post-treatment week
Food1	$339 \pm 15.1^{y,z}$	358 ± 15.1^{y}	361 ± 13.3^{z}
Food2	$314 \pm 16.0^{a,b}$	356 ± 16.0^{a}	342 ± 14.1^{b}
Food3	359 ± 16.6^{a}	376 ± 16.5^{b}	$400 \pm 14.6^{a,b}$
Sleep efficiency (time asleep/time in bed) %	Baseline week	Treatment week	Post-treatment week
Food1	$68.8 \pm 3.06^{a,b}$	74.0 ± 2.77^{a}	75.0 ± 2.63^{b}
Food2	$67.4 \pm 3.24^{a,b}$	74.7 ± 2.94^{a}	72.8 ± 2.79^{b}
Food3	74.8 ± 3.35^{a}	76.9 ± 3.03	81.0 ± 2.89^{a}
Total awake time (min/night)	Baseline week	Treatment week	Post-treatment week
Food1	$154 \pm 16.1^{a,b}$	124 ± 14.6^{a}	121 ± 14.1^{b}
Food2	$159 \pm 17.1^{a,b}$	124 ± 15.5^{a}	130 ± 14.9^{b}
Food3	122 ± 17.7^{a}	114 ± 16.0	93.7 ± 15.4^{a}
Time awake middle of night (min/night)	Baseline week	Treatment week	Post-treatment week
Food1	$59.0 \pm 9.75^{a,b}$	35.8 ± 7.26^{a}	36.9 ± 6.64^{b}
Food2	$57.7 \pm 10.3^{a,b}$	42.9 ± 7.70^{a}	44.6 ± 7.04^{b}
Food3	50.9 ± 10.7	52.6 ± 7.95	44.2 ± 7.27
Sleep quality index (1 = low 2 = average 3 = high)	Baseline week	Treatment week	Post-treatment week
Food1	$1.80 \pm 0.082^{a,y}$	$2.02 \pm 0.096^{a,z}$	$1.92 \pm 0.096^{y,z}$
Food2	$1.86 \pm 0.086^{a,b}$	2.08 ± 0.102^{a}	2.02 ± 0.102^{b}
Food3	1.63 ± 0.089^{a}	1.78 ± 0.106	1.88 ± 0.106^{a}

Note: All statistics expressed as means followed by standard error. Means denoted with the same superscript of either a or b are significantly different from one another ($p < 0.05$); means denoted with the same superscript of either y or z are marginally different from one another ($p < 0.10$); means that are not denoted by superscripts are not significantly different from one another.

Total sleep time

A multivariate test of the week by condition interaction was not significant (Pillai's Trace = 0.127, F[4, 92] = 1.56, n.s.). The simple main effect of week is significant for Food2 (Pillai's Trace = 0.220, F[2, 45] = 6.36, $p < 0.01$) and Food3 (Pillai's Trace = 0.192, F[2, 45] = 5.35, $p < 0.01$) but not for Food1 (Pillai's Trace = 0.085, F[2, 45] = 2.10, n.s.).

Pairwise comparisons between weeks demonstrates that Food1 has a trend toward an increase in total sleep time of 18.66 min/night (5.5% increase) during the treatment week ($p < 0.10$) with a further increase in the post-treatment week for a total of 21.91 min (6.5% increase) ($p < 0.10$) which is consistent with hypothesis 1A but not 1B. Food2 increases (13.3% increase) sleep time by 41.86 min/night ($p < 0.01$) during the treatment week but then there is a non-significant reduction of 14.67 min (4.1%) decrease in the post-treatment week which is consistent with hypothesis 1A but not 1B. For Food3 there was no significant increase during the treatment week which is consistent with hypothesis 1A but a significant 6.6% increase in the post-treatment week for a total of 24.78 min ($p < 0.01$) which is contrary to hypothesis 1B.

Sleep efficiency

A multivariate test of the week by condition interaction was not significant (Pillai's Trace = 0.079, F[4, 92] = 0.94, n.s.). The simple main effect of week is significant for Food1 (Pillai's Trace = 0.171, F[2, 45] = 4.65, $p < 0.05$), Food2 (Pillai's Trace = 0.199, F[2, 45] = 5.91, $p < 0.01$) and Food3 (Pillai's Trace = 0.129, F[2, 45] = 3.34, $p < 0.05$).

Consistent with hypothesis 1A, pairwise comparisons between weeks demonstrate that Food1 significantly increases sleep efficiency by 5.19% during the treatment week ($p < 0.05$) but contrary to hypothesis 1B, there is a non-significant 1% increase in the post-treatment week. Similarly, Food2 significantly increases sleep efficiency by 7.36% during the treatment week ($p < 0.01$) with a non-significant 1.9% decrease in the post-treatment week. For Food3 there was a non-significant 2.1% increase in sleep efficiency in the treatment week and a further non-significant 4.1% increase in the post-treatment week which is consistent with hypotheses 1A and 1B, although there is a significant improvement (6% increase) over the entire three week period which was not expected ($p < 0.05$).

Time awake at night (total)

A multivariate test of the week by condition interaction was not significant (Pillai's Trace = 0.079, F[4, 92] = 0.942, n.s.). The simple main effect of week is significant for Food1 (Pillai's Trace = 0.212, F[2, 45] = 6.05, $p < 0.01$) and Food2 (Pillai's Trace = 0.199, F[2, 45] = 5.60, $p < 0.01$) but not for Food3 (Pillai's Trace = 0.112, F[2, 45] = 3.11, $p < 0.10$).

Consistent with hypothesis 1A, pairwise comparisons between weeks demonstrate that both Food1 and Food2 resulted in a significant reduction to time awake at night during the treatment week of 29.53 min (reduction of 19.2%) and 35.10 min (reduction of 22.1%), respectively ($p < 0.01$). But contrary to hypothesis 1B there was a non-significant reduction in the post-treatment week as compared to treatment week. For Food3 there was a non-significant decrease of 7.5 min (reduction of 6.1%) during the treatment week but a significant reduction (16.7%) in the post-treatment week of 20.48 min ($p < 0.05$) which is consistent with hypotheses 1A but not 1B.

Time awake—middle of the night

A multivariate test of the week by condition interaction was not significant (Pillai's Trace = 0.092, F[4, 94] = 1.130, n.s.). The simple main effect of week was significant for Food1 (Pillai's Trace = 0.177, F[2, 46] = 4.94, $p < 0.05$) but not Food2 (Pillai's Trace = 0.050, F[2, 46] = 1.21, n.s.) or Food3 (Pillai's Trace = 0.034, F[2, 46] = 0.821, n.s.).

Consistent with hypothesis 1A, pairwise comparisons between weeks demonstrate that Food1 significantly decreases (39.3%) time awake at night by 23.24 min ($p < 0.01$) but contrary to hypothesis 1B there was a non-significant increase in the post-treatment week. Food2 resulted in no significant reduction time awake due to sleep interruption, which is contrary to hypotheses 1A. Food3 also resulted in no significant reduction in time awake due to sleep interruptions in any week which is consistent with hypotheses 1A and 1B.

Sleep quality index

Improved sleep quality is a subjective measure of both the perception of sleep quality as well as the perception of restedness the next day.

A multivariate test of the week by condition interaction was not significant (Pillai's Trace = 0.068, F[4, 94] = 0.80, n.s.). Simple main effect of week is significant for Food1 (Pillai's Trace = 0.124, F[2, 46] = 3.29, $p < 0.05$), Food2 (Pillai's Trace = 0.133, F[2, 46] = 3.54, $p < 0.05$) and Food3 (Pillai's Trace = 0.185, F[2, 46] = 5.26, $p < 0.01$).

Consistent with hypotheses 2A but not 2B, pairwise comparisons between weeks demonstrate that Food1 significantly increased perceived sleep quality with a 12.2% increase in sleep quality index in the treatment week (0.22 on a 1 to 3 scale, $p < 0.05$) and a non-significant 4.95% (0.1) reduction in the post-treatment week. Similarly, Food2 significantly increases sleep

quality index by 11.8% (0.22) during the treatment week ($p < 0.05$) which again is consistent with hypothesis 2A and a non-significant reduction of 2.88% (0.06) in the post-treatment week which is not consistent with hypothesis 2B. Food3 results in a non-significant 9.2% (0.15) increase in the sleep quality index during the treatment week and a further non-significant 5.62% (0.1) increase in the post-treatment week which is consistent with hypotheses 2A and 2B.

Twin case study

Pairwise comparisons for data from the twin study on the one variable that was different between Food1 and Food2 is consistent with the trend in the larger group study. To facilitate a paired *t*-test for the twin case study, each night was considered a separate data point.

Time awake—middle of the night

The twin treated with Food1 had a reduction (48.8%) in time awake at night due to a sleep interruption from 93.6 to 45.7 min/night from baseline week to treatment week which was significant ($p = 0.02$) whereas his twin had an increase (12.1%) from 42.9 to 47.9 min/night which was not significant ($p = 0.34$).

Discussion

On the specific dependent measures of this study, a food containing a tryptophan-rich protein in combination with a high glycemic carbohydrate performed in a comparable fashion to pharmaceutical grade tryptophan on both subjective and objective measures of improved sleep patterns in subjects with prolonged and severe insomnia. Although relatively modest, this consistent pattern of improvement is similar to improvements seen with benzodiazepines, which are associated with side effects including disruption in sleep architecture and daytime somnolence (Morin 1993b). There are no reports of such side effects associated with protein containing tryptophan.

The only parameter that Food1 is different from Food2 is suppression of time awake during the middle of the night, a finding which is further reinforced by a consistent pattern seen in identical twins, one treated with Food1 and the other treated with Food2. This difference may be explained by the different pharmacokinetics of Food1 and Food2. When pharmaceutical grade tryptophan is given, serum levels rise in tryptophan peaks within the first hour of ingestion well before any carbohydrate-induced insulin peak. As such tryptophan must compete with LNAAs for transport sites across the BBB. In contrast, protein source tryptophan has a delayed peak since tryptophan must first be released from peptide bonds. This allows tryptophan serum levels to peak after the insulin peak allowing tryptophan to be released into an insulin rich environment where tryptophan/LNAA

ratio is already increased by 35%, on an average (Lyons and Truswell 1988).

On the more general question of this study, it seems that protein rich in tryptophan can function in a similar fashion to pharmaceutical grade tryptophan when combined with a high glycemic index carbohydrate. Obviously, the measure of sleep parameters is an indirect measure on increased CNS tryptophan levels but allows for direct application of future human investigation whereas animal studies would allow for direct measure of CNS tryptophan levels in post-mortem tissue but could not be generalized to changes in human behavior without speculation. Future areas of study, where pharmaceutical grade tryptophan has proven effective in the past, most notably anxiety states, should now be repeated with protein-rich tryptophan in combination with carbohydrate.

The results of improved sleep parameters on relatively low dosages of tryptophan are consistent with much earlier studies that demonstrated the potential efficacy of tryptophan in the dose range of 250 mg (Hartmann and Spinweber 1979) which suggests that food that contains high amounts of protein bound tryptophan has potential to offer an alternative to pharmaceutical grade tryptophan. A food designed as a functional food can deliver other key ingredients within a delivery vehicle that reduce some of the pharmacokinetic issues that make treatment with pharmaceutical grade tryptophan problematic: Vitamin B_3 can be added to suppress activity of tryptophan pyrrolase and thus reduce "loss" of tryptophan to niacin; various high glycemic index carbohydrates can be added to suppress competition from competing LNAAs; the fact that tryptophan is in an amide form as part of a polypeptide chain results in a "time release" so that tryptophan serum concentration increase after serum insulin has increased not before an increase in serum insulin which ensures a suppression of LNAAs at precisely the time tryptophan competes for the transport sites across the BBB.

It is interesting that those treated with placebo demonstrated an improvement in sleep in the last week which was contrary to the proposed hypotheses whereas those treated with Food1 or Food2 responded during the treatment week which is consistent with the hypotheses. It seems that subjects with chronic insomnia treated with sleep hygiene alone will improve and this is consistent with an extensive literature of treatment of insomnia (Morin et al. 1999). What is interesting is that those treated with Food1 or Food2 got better faster and reported an improvement in their subject sense of sleep quality whereas those treated with Food3 (placebo) reported some improvement in objective measures over the entire three week protocol but the improvement was so gradual that it failed to alter their subjective sense of sleep quality in any specific week.

The sustained improvement in the post-treatment week was not anticipated and may have several plausible explanations. From a biochemical perspective, the nightly supplement of tryptophan in combination with vitamins B_3 and B_6 may affect the overall metabolism of tryptophan for a more protracted period of time and therefore, more of the normal daily dietary tryptophan is available for CNS metabolism to serotonin and melatonin. On the other hand, since all three groups improve over time a psychological perspective is equally compelling where a few good nights sleep allows subjects to gain a greater association between their bed and restful sleep and follow though with the sleep hygiene principles which were reviewed in each of the three weeks of the study.

These data are based on a relatively small sample size and therefore, further study is required. Future studies may wish to employ polysomnography measures which would allow for testing with well validated objective measures of sleep parameters and sleep architecture. The results of this study are encouraging, however, to those who view functional foods as a possible replacement for some pharmaceuticals.

The twin data cannot be over interpreted since it is simply a sample size of 2 but the notion that identical twins appeared with a similar pattern of insomnia over a similar period of time suggests that even those with an inherited predisposition to insomnia can be aided with the protein source tryptophan although further research is again required.

Acknowledgements

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

Craig Hudson, Susan Hudson, and Joan MacKenzie

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.

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C. Hudson.[2] Whitby Mental Health Centre, 700 Gordon Street, Whitby, ON L1N 5S9, Canada; Biosential Inc., 1543 Bayview Avenue, Suite 346, Toronto, ON M4G 3B5, Canada.
S. Hudson. Biosential Inc., 1543 Bayview Avenue, Suite 346, Toronto, ON M4G 3B5, Canada.
J. MacKenzie. Stratford General Hospital, Department of Psychiatry, 46 General Hospital Drive, Stratford, ON N5A 2Y6, Canada.

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. 1991a). 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. 1991b).

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 2×2 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.

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