High-glycaemic index and -glycaemic load meals increase the availability of tryptophan in healthy volunteers

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Abstract
The purpose of the present study was to determine the influence of the glycaemic index (GI) and glycaemic load (GL) on the ratio of tryptophan (TRP) relative to other large neutral amino acids (LNAA). Ten healthy men (age 22·9 (SD 3·4) years; BMI 23·5 (SD 1·6) kg/m2) underwent standard GI testing, and later consumed each of a mixed-macronutrient (1915 kJ; 66·5 % carbohydrate (CHO), 17 % protein and 16·5 % fat) high-GI (MHGI), an isoenergetic, mixed-macronutrient low-GI (MLGI) and a CHO-only (3212 kJ; 90 % CHO, 8 % protein, 2 % fat) high-GI (CHGI) meal on separate days. The GI, GL and insulin index values (e.g. area under the curve) were largest after the CHGI meal (117, 200, 158), followed by the MHGI (79, 59, 82) and MLGI (51, 38, 56) meals, respectively (all values were significantly different, \( P < 0·05 \)). After the MHGI and MLGI meals but not after the CHGI meal, TRP was elevated at 120 and 180 min (\( P < 0·05 \)). After the CHGI meal, LNAA was lower compared with the MLGI (\( P < 0·05 \)); also the rate of decline in LNAA was higher after CHGI compared with MHGI and MLGI (both comparisons \( P < 0·05 \)). The percentage increase from baseline in TRP:LNAA after CHGI (23 %) was only marginally higher than after the MHGI meal (17 %; \( P = 0·38 \)), but it was threefold and nearly significantly greater than MLGI (8 %; \( P = 0·05 \)). The present study demonstrates that the postprandial rise in TRP:LNAA was increased by additional CHO ingestion and higher GI. Therefore, the meal GL appears to be an important factor influencing the postprandial TRP:LNAA concentration.

Key words: Glycaemic index; Glycaemic load; Tryptophan; Amino acids; Carbohydrate

The availability of dietary tryptophan (TRP) to the brain depends on its concentration relative to other large neutral amino acids (LNAA), which compete for a common transport mechanism across the blood–brain barrier(1). Increased consumption of carbohydrate (CHO) elicits a marked demand in insulin secretion, which enhances peripheral, skeletal muscle uptake of LNAA. However, TRP is largely albumin bound and therefore protected from this absorption(2). Previous studies have shown that the postprandial concentration of TRP:LNAA increases between 20 and 50 % compared with baseline after predominately CHO-rich meals(3–5). In one study, consumption of a sucrose-based food elicited a larger postprandial increase in TRP:LNAA compared with a raw starch-based food(3). Although the glycaemic index (GI) of the foods was not measured, these authors hypothesised that a high-GI CHO-based meal compared with a low-GI CHO-based meal would elicit a greater insulin release, and therefore a larger postprandial rise in plasma TRP:LNAA levels(3,6,7).

The proportion of CHO relative to either protein or fat may similarly influence the TRP:LNAA response after mixed-macronutrient meals. Berry et al.(8) suggested that a meal containing a CHO:protein ratio of approximately 5:1 would neither raise nor lower postprandial LNAA, given that the LNAA-lowering effect of the insulin demand would be offset by the contribution of LNAA provided by the protein source. Since TRP is the least abundant amino acid found in protein, a protein-rich, low-CHO meal lowers TRP:LNAA due to a greater contribution of LNAA relative to TRP in the meal(3). Conversely, the addition of fat to a meal retards gastric emptying(9), and lowers the peak glycaemic and insulin response(10). Thus, increased fat content in a meal may attenuate the postprandial
TRP:LNAA response after mixed-macronutrient meals when compared with a CHO-only meal.

Recently, the GI of a CHO-rich meal has been shown to significantly influence sleep initiation in healthy sleepers. Afaghiet al.\(^{(11)}\) reported a 50% reduction in the time required to fall asleep after a CHO-rich, high-GI meal compared with an isoenergetic low-GI meal when consumed 4 h before bedtime. This improvement in sleep initiation after a high-GI CHO meal was probably due to an increased plasma TRP:LNAA concentration and increased serotonin; however, biochemical data were not collected to support this claim.

Therefore, the purpose of the present study was to investigate the influence of the GI and glycaemic load (GL) on the TRP:LNAA response after a CHO-only high-GI (CHGI) meal compared with two isoenergetic, mixed-macronutrient high- and low-GI (MHGI and MLGI) meals. We hypothesise that the glycaemic and insulin response to the CHGI meal will be larger than the MHGI and MLGI meals, and the corresponding increase in TRP:LNAA will be proportional to the GL of the meals due to differences in insulin demand.

### Methods and materials

#### Participants

Ten healthy mixed-ethnicity men (age 22·9 (SD 3·4) years) of normal weight (BMI 23·5 (SD 1·6) kg/m\(^2\)) were recruited from a university student population. Exclusion criteria included a self-reported current or past history of medical, psychiatric or sleep disorder, current use of prescribed medication, recreational drug use, allergy related to the study foods or habitual use of a restrictive diet. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Research Ethics Committee of the University of Sydney. Written informed consent was obtained from all subjects before participation.

#### Meats

The energy and macronutrient composition of the meals are summarised in Table 1. The CHGI meal, 3212 kJ, was replicated from a previous study\(^{(11)}\) and consisted of a large portion of rice (Jasmine GI approximately 109; Riviana Foods, Sydney, NSW, Australia) served with a tomato-based vegetable purée. The mixed-macronutrient meals (MHGI and MLGI) were isoenergetic, approximately 1915 kJ, and consisted of rice (MHGI: Jasmine GI approximately 109; MLGI: Doongara GI approximately 46; Riviana Foods) served with a sachet of chicken with sun-dried tomato sauce (965 kJ; 7·9 g fat, 14·1 g protein and 24·7 g CHO; Lean Cuisine\(^{TM}\); Nestlé Australia Limited, Rhodes, NSW, Australia). Meals were prepared in the University of Sydney Nutrition Unit kitchens. Uncooked rice (raw weight of CHGI, 200 g; MHGI, 64·7 g; MLGI, 64·5 g) was prepared using an electric rice cooker before the testing day, with a rice:water ratio of 1:1·5. Cooked rice was frozen (−20°C) in individual portions and reheated in a microwave before serving. Frozen Lean Cuisine\(^{TM}\) sachets were heated in the microwave according to the manufacturer’s recommendations and poured over the rice immediately before serving. All meals were presented to the participants with 250 ml of cool water and consumed within 15 min. Participants were required to consume the entire meal, which was assessed visually by a researcher (C. P. H. and K. S.) and by weighing the plate before and after eating.

#### Procedure

Participants presented to the testing facility in the morning by at least 10.00 hours, having fasted overnight for a period of at least 8 h. Participants were required to avoid vigorous exercise and abstain from alcohol for at least 24 h before testing, and were instructed to avoid over- or under-eating. Smokers were instructed to abstain on the morning before testing. On the night before testing, participants were instructed to consume a high-CHO, low-fat evening meal devoid of legumes in order to avoid extreme hunger and variation in basal blood glucose concentration. Self-reported compliance with these instructions was evaluated by a researcher (C. P. H. and K. S.) each morning before testing.

Participants initially completed three independent reference glucose tests separated by at least 48 h apart. Each reference test used a standard glucose drink (77·1 g Glucodin\(^{TM}\) powder, dissolved in 250 g of cold water), providing 75 g available CHO, in order to calculate the average glucose and insulin response. A glucose load of 75 g was required to appropriately determine the GI of the MHGI and MLGI meals\(^{(12)}\); however, the GI of the CHGI meal would be approximate as it was considered unethical to use a glucose

### Table 1. Energy and macronutrient composition of the carbohydrate (CHO) high-glycaemic index (CHGI), mixed-macronutrient high-glycaemic index (MHGI) and mixed-macronutrient low-glycaemic index (MLGI) meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Energy (kJ)</th>
<th>Fat g</th>
<th>Energy (%)</th>
<th>Protein g</th>
<th>Energy (%)</th>
<th>CHO g</th>
<th>Energy (%)</th>
<th>GI*</th>
<th>GL†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHGI‡</td>
<td>3212</td>
<td>0·4</td>
<td>1·6</td>
<td>16·8</td>
<td>8</td>
<td>171·4</td>
<td>90·4</td>
<td>117</td>
<td>200</td>
</tr>
<tr>
<td>MHGI</td>
<td>1916</td>
<td>7·9</td>
<td>16·2</td>
<td>18·2</td>
<td>17·2</td>
<td>75</td>
<td>66·6</td>
<td>79</td>
<td>59</td>
</tr>
<tr>
<td>MLGI</td>
<td>1913</td>
<td>7·9</td>
<td>16·1</td>
<td>18·6</td>
<td>17·5</td>
<td>75</td>
<td>66·4</td>
<td>51</td>
<td>38</td>
</tr>
</tbody>
</table>

Gl, glycaemic index; GL, glycaemic load.

\* GI was determined using the average glucose response (n 9) and the incremental area under the curve method\(^{(12)}\).

† GL was calculated by multiplying each meal GI by the available CHO (g).

‡ GI for the CHGI meal was approximate due to a larger CHO content of this test meal compared with the reference glucose drink providing 75 g CHO\(^{(12)}\).
reference drink of 171.4 g CHO. All meals were administered in a randomised order at least 48 h apart. Participants were blinded to the GI of the MHGI and MLGI meals, which were identical in appearance. A questionnaire comprising multiple, 10 cm visual analogue scales was administered immediately after meal consumption to assess palatability (0, poor; 10, good), satiety (0, still hungry; 10, completely full) and participant sleepiness (0, alert; 10, sleepy). The validity of visual analogue scales to assess subjective ratings of palatability and mood states after meal consumption is well established\(^{13,14}\).

**Calculation of glycaemic index, insulin index and glycaemic load**

GI and insulin index were calculated according to published guidelines using mean participant data and the following ratio: the 120 min incremental area under the curve of the test meal compared with the mean incremental area under the curve of the reference food, multiplied by 100\(^{12}\). GL was calculated using the equation, GL = (GI/100) \times g available CHO\(^{15}\). It can be highlighted again that despite identical macronutrient and energy composition, the MHGI and MLGI meals were designed to elicit differences in postprandial GL response. This was accomplished by the selection of either a 75 g portion of a high-GI (109) rice for the MHGI meal or a 75 g portion of a low-GI (49) rice for the MLGI meal (see meals above). Thus, the overall glycaemic response of these meals will reflect not only the amount and type of CHO, but also the relative contributions of the fat and protein components\(^9\). This meal design illustrates the importance of investigating the GI of an entire mixed-macronutrient meal\(^{16}\).

**Biochemical analysis**

Finger-prick blood samples were collected into separate heparin-coated eppendorf tubes at baseline (5 and 1 min before meal consumption) and after 15, 30, 45, 60, 90 and 120 min for the analysis of glucose (glucose hexokinase enzymatic assay, Hitachi 912 automatic analyser; Boehringer, Mannheim, Germany) and insulin (solid-phase antibody-coated tube RIA, ‘Coat-A-Count’ Insulin RIA kit; Diagnostic Products Corporation, Los Angeles, CA, USA). All samples were immediately centrifuged at 7900 rpm for 45 s, with plasma stored at −20°C for the subsequent analysis.

After test meals but not after reference tests, additional (2 ml) blood samples were collected by venepuncture from a forearm vein into a Li-heparinised tube at 0 (baseline), 120, 180 and 240 min, and immediately centrifuged (2500 rpm for 15 min at 4°C) to separate plasma for subsequent analysis of amino acids: TRP and five LNAA (valine, leucine, isoleucine, phenylalanine and tyrosine) to calculate the TRP:LNAA ratio\(^9\). Plasma samples were collected into separate eppendorf tubes and stored at −80°C until analysis of amino acids by reverse-phase HPLC (Shimadzu, Kyoto, Japan). All samples were analysed in duplicate following established methodology\(^{17}\).

**Statistics**

Data are reported as means and standard deviations. All data were tested for normality of distribution, and statistical procedures were completed using SPSS for Windows (SPSS version 15.0, Inc.; Cary, NC, USA). Student’s paired-sample \(t\) tests were used to identify differences between the meals in palatability, satiety, sleepiness (visual analogue scales), the GI, GL, insulin index and the incremental area under the curve of the TRP:LNAA response. A two-factor (meal \(\times\) time), repeated-measures ANOVA was used to test for the postprandial effect of time, meal type and their interaction on TRP; LNAA and TRP:LNAA concentrations. In ANOVA, normality was satisfied using Mauchly’s test of sphericity unless reported.

**Results**

**Palatability, satiety and sleepiness (visual analogue scales)**

Palatability of the MHGI and MLGI meals was identical and indicated a ‘good taste’; both mean positive scores were 8.5 (SD 1.4) cm; \(P=0.956\). Both MHGI and MLGI meals were significantly more palatable than the CHGI meal (3.2 (SD 2.2) cm; both comparisons \(P<0.001\)). Satiety was slightly and almost significantly (\(P=0.05\)) higher after the MHGI meal (6.2 (SD 1.6) cm) compared with the MLGI meal (4.9 (SD 2.4) cm). Both these ratings were significantly lower than the CHGI meal (8.7 (SD 1.1) cm; \(P<0.001\)). There were no significant differences between subjective ratings for sleepiness (CHGI 6.2 (SD 1.7) cm; MHGI 5.6 (SD 1.9) cm; MLGI 4.8 (SD 2.4) cm; \(P>0.05\)).

**Glycaemic index, glycaemic load and insulin index**

Postprandial concentrations for blood glucose and insulin are shown in Fig. 1. Data were omitted from one participant due to an abnormal fasting glucose concentration\(^{12}\). The GI, GL and insulin index values were all significantly different between the meals (all \(P<0.05\). The estimated GI for the CHGI (116.6 (SD 30.1)) meal and exact value for the MHGI (78.6 (SD 17.8)) meal were in the high (GI ≥70) range, whereas the GI for the MLGI (50.8 (SD 20.4)) meal was in the low range (GI ≤55\(^18\)). Calculated GL values were greatest after the CHGI (200) meal, followed by the MHGI (58.9) and MLGI (38.2) meals. Insulin index was greatest after the CHGI (158 (SD 59.9)) meal, followed by the MHGI (81.9 (SD 37.2)) and MLGI (55.7 (SD 25.6)) meals, respectively.

**Amino acid concentrations**

Postprandial TRP and LNAA concentrations are presented in Table 2. Data were from seven participants; two participants failed to complete all venepuncture procedures. All basal amino acid concentrations were not statistically different between the meals.

TRP after the MHGI and MLGI meals was elevated compared with baseline at 120 (\(P=0.02\)) and 180 min.
area under the curve was greatest after the CHGI (4.4 (SD 2.6)) meal, followed by the MHGI (2.8 (SD 3.7)) and MLGI (1.4 (SD 1.4)) meals; these values were not statistically significant between the CHGI and MHGI meals (P = 0.38) or between the MHGI and MLGI meals (P = 0.43); however, there was a trend present between the CHGI and MLGI (P = 0.05) meals, and the one-tailed t test was significant (P = 0.03).

Discussion

The present study examined the influence of the GI and GL on the postprandial concentration of TRP:LNAA. The study results indicate that the MHGI and MLGI meals resulted in a lower glycaemic and insulin response compared with the CHGI meal. In addition, there was an initial increase in TRP after the MHGI and MLGI meals, whereas after the CHGI meal, changes in TRP were unremarkable. Conversely, the rate of decline in LNAA was markedly higher after the CHGI meal compared with the MHGI and MLGI meals. As a result, there was a substantial postprandial rise in TRP:LNAA after the CHGI meal, which was slightly attenuated after the MHGI meal and relatively small after the MLGI meal.

The decrease in the glycaemic and insulin response of the MHGI and MLGI meals confirms our initial hypothesis and demonstrates substantial clinical improvement compared to the CHGI meal. The GI of the MHGI (GL 59) and MLGI (GL 38) was significantly lower than the CHGI meal (GL 200). Epidemiological studies have linked high-GI and -GL diets to increased risk of CVD and for the development of type 2 diabetes. A GL value of > 120 over a single day is regarded

<table>
<thead>
<tr>
<th>Meals</th>
<th>TRP (μmol/l)</th>
<th>LNAA* (μmol/l)</th>
<th>TRP:LNAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHGI†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>64</td>
<td>59</td>
<td>0.1077</td>
</tr>
<tr>
<td>120 min</td>
<td>63</td>
<td>480†</td>
<td>0.1328†</td>
</tr>
<tr>
<td>180 min</td>
<td>61</td>
<td>468†</td>
<td>0.1308†</td>
</tr>
<tr>
<td>240 min</td>
<td>58</td>
<td>439†</td>
<td>0.1326†</td>
</tr>
<tr>
<td>MHGI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>59</td>
<td>559</td>
<td>0.1075</td>
</tr>
<tr>
<td>120 min</td>
<td>67†</td>
<td>558</td>
<td>0.1215‡</td>
</tr>
<tr>
<td>180 min</td>
<td>65†</td>
<td>528†</td>
<td>0.1235‡</td>
</tr>
<tr>
<td>240 min</td>
<td>61</td>
<td>516†</td>
<td>0.1198†</td>
</tr>
<tr>
<td>MLGI†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>66</td>
<td>560</td>
<td>0.1179</td>
</tr>
<tr>
<td>120 min</td>
<td>73†</td>
<td>591†</td>
<td>0.1233</td>
</tr>
<tr>
<td>180 min</td>
<td>70†</td>
<td>554</td>
<td>0.1269†</td>
</tr>
<tr>
<td>240 min</td>
<td>63</td>
<td>501†</td>
<td>0.1255‡</td>
</tr>
</tbody>
</table>

* LNAA: valine, isoleucine, leucine, phenylalanine and tyrosine.
† Incremental area under the curve was significantly greater after the CHGI meal compared with the MLGI meal (two-tailed t test, P = 0.05; one-tailed t test, P = 0.03).
‡ Mean values were statistically significant compared with baseline (P < 0.05).
The postprandial decline in LNAA was largely dependent on the meal GL and not on the ratio of CHO:protein, as suggested earlier to be responsible for the change in LNAA [20].

The present data further indicate that the increase in postprandial TRP:LNAA was inversely proportional to the magnitude and rate of decline in LNAA. In fact, the TRP:LNAA response after the CHGI meal is in line with those from previous studies [3, 4]. Importantly, the postprandial concentration of TRP:LNAA dictates the synthesis of central nervous system serotonin [25], a key neurotransmitter involved in the regulation of sleep [26]. In a separate study, the CHGI meal was previously shown to improve sleep initiation when provided 4 h before bedtime compared with an isoenergetic low-GI version [11]. Furthermore, Blum et al. reported that the consumption of a similar-sized CHO breakfast meal resulted in a 461% increase from baseline in the postprandial insulin response and a 3.5-fold increase in platelet poor plasma serotonin in healthy subjects [27]. Thus, the peak percentage change from baseline in insulin after the CHGI meal (650%) indicates that postprandial serotonin was probably increased, which may therefore explain the improvement in sleep initiation after the CHGI meal [11]. In addition, given the peak insulin response to the MHGI meal (450%), it remains a possibility that such a mixed-macronutrient meal may similarly improve sleep initiation [4, 28]. However, given the reduced TRP:LNAA response, we may speculate that this improvement would be present only when sleep propensity is high, such as in the post-lunch period or in the late evening [29–31].

There are several limitations with the present study. It was difficult to estimate a priori the sample size required to identify meal difference in amino acid concentration due to a limited number of published data using mixed-macronutrient meals. Future studies should inflate sample size estimates, which will also help to account for common difficulties with venepuncture. Moreover, since the study was conducted in the morning and the room was abundant with natural light, postprandial serotonin was not measured due to the risk of platelet contamination [32]. Indeed, inadequate satiety and low levels of serotonin are linked to clinical disorders such as insomnia [33], seasonal affective disorder or severe premenstrual syndrome [34]. However, since the participants in the present study were all healthy, young, male adults, it is unknown how these results would generalise to such clinical groups, or women and older participants.

In summary, the present study demonstrates that the postprandial change in LNAA is largely dependent on the meal GL and not on the ratio of CHO:protein, as suggested earlier to be responsible for the change in LNAA [20].

The present data further indicate that the increase in postprandial TRP:LNAA was inversely proportional to the magnitude and rate of decline in LNAA. In fact, the TRP:LNAA response after the CHGI meal is in line with those from previous studies [3, 4]. Importantly, the postprandial concentration of TRP:LNAA dictates the synthesis of central nervous system serotonin [25], a key neurotransmitter involved in the regulation of sleep [26]. In a separate study, the CHGI meal was previously shown to improve sleep initiation when provided 4 h before bedtime compared with an isoenergetic low-GI version [11]. Furthermore, Blum et al. reported that the consumption of a similar-sized CHO breakfast meal resulted in a 461% increase from baseline in the postprandial insulin response and a 3.5-fold increase in platelet poor plasma serotonin in healthy subjects [27]. Thus, the peak percentage change from baseline in insulin after the CHGI meal (650%) indicates that postprandial serotonin was probably increased, which may therefore explain the improvement in sleep initiation after the CHGI meal [11]. In addition, given the peak insulin response to the MHGI meal (450%), it remains a possibility that such a mixed-macronutrient meal may similarly improve sleep initiation [4, 28]. However, given the reduced TRP:LNAA response, we may speculate that this improvement would be present only when sleep propensity is high, such as in the post-lunch period or in the late evening [29–31].
Acknowledgements

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References