Effects of Intraduodenal Infusion of L-Tryptophan on ad Libitum Eating, Antropyloroduodenal Motility, Glycemia, Insulinemia, and Gut Peptide Secretion in Healthy Men

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Context: Changes in gut motor and hormonal function contribute to the eating-inhibitory and glucose-lowering effects of protein. The effect of amino acids, the digestive products of protein, on gastrointestinal function, eating, and glycemia has not been investigated comprehensively.

Objective: We tested the hypothesis that L-tryptophan (L-Trp) stimulates gastrointestinal motor and hormonal functions, inhibits eating, and modulates glycemia.

Design, Settings, Participants, and Intervention: Ten healthy, normal-weight men were studied in randomized, double-blind fashion, each receiving a 90-minute intraduodenal infusion of L-Trp at 0.075 (total 6.75 kcal) or 0.15 (total 13.5 kcal) kcal/min or saline (control).

Main Outcome Measures: Antropyloroduodenal motility, plasma ghrelin, cholecystokinin, glucagon-like peptide-1, peptide tyrosine tyrosine, insulin, glucagon, blood glucose, and appetite perceptions were measured. Food intake was quantified from a buffet meal after the infusion.

Results: Intraduodenal L-Trp suppressed antral pressures ($P < .05$) and stimulated pyloric pressures ($P < .01$) and markedly increased cholecystokinin and glucagon (both $P < .001$). Glucagon-like peptide-1 and peptide tyrosine tyrosine increased modestly (both $P < .001$), but there was no effect on total ghrelin. Insulin increased slightly ($P < .05$) without affecting blood glucose. Plasma L-Trp increased substantially ($P < .001$). All effects were dose-related and associated with increased fullness and substantially decreased energy intake ($P < .001$). There was a strong inverse correlation between energy intake and plasma L-Trp ($r = -0.70; P < .001$).

Conclusions: Low caloric intraduodenal loads of L-Trp affect gut motor and hormonal function and markedly reduce energy intake. A strong inverse correlation between energy intake and plasma L-Trp suggests that, beyond gut mechanisms, direct effects of circulating L-Trp mediate its eating-inhibitory effect. (J Clin Endocrinol Metab 99: 3275–3284, 2014)
Protein ingestion modulates the release of gut hormones relevant to appetite and glycemic control, including ghrelin, cholecystokinin (CCK), peptide tyrosine tyrosine (PYY), glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (1–4), and slows gastric emptying (5), which occurs, at least in part, as a result of the stimulation of contractions located to the pylorus (6). The latter controls the exposure of enteroendocrine cells to chyme and thereby regulates glucose absorption and the neural and hormonal feedback that triggers satiation.

Inhibitory feedback in response to food ingestion arises primarily from interactions of nutrients with the small intestine, although cephalic or gastric phase signals may modulate some of the intestinal reflexes (7). Importantly, the effects of lipid on gastrointestinal (GI) function and food intake depend on fat digestion and the release of fatty acids with a chain length ≥ 12 carbon atoms (8, 9). Whether, in analogy, the release of amino acids (AAs) is required for the effects of protein on GI function, food intake, and glycemia remains unknown. AAs, including L-leucine (10, 11), L-glutamine (12, 13), or L-phenylalanine (14), indeed modulate appetite and/or glycemia in lean, obese, or type 2 diabetic subjects. The aromatic AA, L-tryptophan (L-Trp), is of particular interest; previous studies have reported a potent effect on gastric emptying (15), antropyloroduodenal (APD) motility (15), and food intake (16), although in a recent study in healthy volunteers (17), no effect was observed on plasma GLP-1, ghrelin, appetite, or food intake when L-tryptophan (L-Trp) was added to a breakfast high in gelatin. No studies have investigated the isolated effects of L-Trp on ad libitum eating and glycemia concurrently with a comprehensive assessment of gut motor and hormone functions.

We hypothesized that intraduodenal (ID) infusion of L-Trp dose-dependently modulates appetite, food intake, APD motility, plasma ghrelin, CCK, GLP-1, PYY, insulin, glucagon, and blood glucose in healthy men at the low caloric doses of 0.075 and 0.15 kcal/min. We used an ID infusion paradigm to bypass orosensory influences and interindividual variations in gastric emptying and studied healthy subjects, rather than type 2 diabetic patients, because food intake represented the primary study outcome.

Subjects and Methods

Subjects

Twelve healthy, normal-weight men were included in the study. Ten subjects (mean age, 26.6 ± 8.6 [range, 18–42] y; BMI, 22.5 ± 2.1 [range, 18.7–24.7] kg/m²) completed all three study visits, and generally tolerated the studies well. Only one subject experienced “lightheadedness” between 90 and 100 minutes after L-Trp-0.15. The remaining two subjects withdrew from the study—one because of nausea (maximum 70/100 mm VAS) and an episode of vomiting at 45 minutes during L-Trp-0.15, and another because of “dizziness” and “faintness” at 105 minutes after L-Trp-0.075. In all cases, symptoms resolved promptly.

Data analysis is based on the 10 subjects who completed all three study visits, but data relating to APD pressures include only eight subjects, due to technical problems with the manometry system. The number of subjects was determined by power calculations based on our previous studies (18). We calculated that with 10 subjects we would be able to detect a 15% decrease in energy intake at α = 0.05, with a power of 80%. Exclusion criteria were smoking, consumption of > 20 g of alcohol per day, any medical condition, or the use of medications known to affect eating or GI function. All subjects were unrestrained eaters (score ≤ 12 on the eating restraint component of the three-factor eating questionnaire) (19). The study protocol was approved by the Royal Adelaide Hospital Research Ethics Committee and performed in accordance with the Declaration of Helsinki. Each subject provided informed, written consent.

Study design and protocol

Study design

Each subject was studied on three occasions, separated by 3–10 days, at which they received, in randomized double-blind fashion, 90-minute ID infusions of L-Trp at 0.075 kcal/min (L-Trp-0.075), 0.15 kcal/min (L-Trp-0.15), or control. L-Trp solutions were prepared by dissolving 1.6 g or 3.3 g crystalline L-Trp (PureBulk), 118.3 mg CaCl₂ × 2H₂O and NaCl (4.0 g and 4.2 g, respectively) in 405 mL distilled water. The control solution contained isotonic 118.3 mg CaCl₂ × 2H₂O and 4.6 g NaCl in 405 mL distilled water. All solutions were isotonic (300 mOsm) and were administered at a rate of 4.5 mL/min.

Preliminary study

In a preceding “pilot” study, we tested ID L-Trp loads of 0.05, 0.1, and 0.2 kcal/min in two subjects (0.2 kcal/min was chosen because it is close to the limit of solubility for the chosen infusion rate of 4.5 mL/min). Although L-Trp at 0.05 and 0.1 kcal/min was well tolerated without adverse effects, both subjects reported nausea and/or slight dizziness, and one vomited during infusion of L-Trp at 0.2 kcal/min. Thus, we selected the doses of 0.075 and 0.15 kcal/min.

Study protocol

Subjects were instructed to abstain from alcohol and strenuous exercise for 24 hours and were provided with a standardized meal (beef lasagna, total energy content, 1160 kcal; McCain Food) to be consumed at 7 pm the night before each visit. Subjects then fasted overnight, except from water, until they attended the laboratory at 8:30 AM the following day. On arrival, a small-diameter (3.5 mm), 17-channel manometric catheter (length, 100 cm; Dentsleeve International, Mui Scientific) was inserted into the stomach through an anesthetized nostril and allowed to pass into the duodenum by peristalsis (20). The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuous measurement of the tranmucosal potential difference between the most distal antral and the most proximal duodenal channel (20). One channel, used for ID in-
fusion of L-Trp and control solutions, was located 14.5 cm distal to the pylorus.

Once the catheter was positioned, fasting motility was observed until the occurrence of phase III of the interdigestive migrating motor complex. Immediately after the end of phase III, an iv cannula was placed in a forearm vein for blood sampling, and a baseline blood sample (t = –10 min) was taken. The subject completed a visual analog scale (VAS) questionnaire to assess appetite perceptions. At t = 0 minutes, another blood sample was taken, and ID infusion of L-Trp or control commenced for 90 minutes (t = 0–90 min). APD motility was recorded continuously, blood samples for measurements of plasma hormones and blood glucose were collected, and a VAS questionnaire was completed every 15 minutes. At t = 90 minutes, the infusion was terminated, and the manometric catheter was removed. All subjects were then offered a standardized, cold, buffet-style ad libitum test meal and allowed to consume as much food as they wished until they felt comfortably full for a maximum of 30 minutes (t = 90–120 min). The meal comprised four slices (125 g) of whole-meal bread, four slices (125 g) of white bread, 100 g sliced ham, 100 g sliced chicken, 100 g sliced cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 20 g mayonnaise, 20 g margarine, 170 g apple, 200 g strawberry yogurt, 150 g chocolate custard, 140 g fruit salad, 600 mL iced coffee, 500 mL orange juice, and 600 mL water. The total energy content of the buffet meal was 11 800 kJ (21). After ingestion of the meal, at t = 120 minutes, a final blood sample was taken, and a VAS completed.

Measurements

Appetite and food intake

Perceptions of hunger, fullness, desire-to-eat and prospective consumption, nausea, and bloating were quantified using validated 100-mm VAS questionnaires (22). Food intake from the test meal was calculated from the amount of food (grams) eaten at the buffet meal. For this purpose, each food item was weighed before and after presentation to the subject. Energy intake (kcal) was then calculated using commercial software (FoodWorks 7.0; Xyris Software).

APD motility

APD motility was recorded and digitized using a computer-based system that ran commercially available software (Solar GI, MMS Database software, version 8.17). Data were analyzed for: 1) number and amplitude of antral and duodenal pressure waves (PWs); 2) number and amplitude of isolated pyloric PWs (IP-PWs); and 3) basal pyloric pressure (BPP), as described previously (20, 23).

Blood glucose, plasma hormone, and L-Trp concentrations

Blood samples were collected into ice-chilled EDTA tubes. Blood glucose was determined using a portable glucometer (Medisense Precision QLD; Abbott Laboratories). Plasma was separated by centrifugation at 3200 rpm for 15 minutes at 4°C within 15 minutes of collection and stored at −70°C until assayed. Plasma total ghrelin (pg/mL) was measured by RIA, without peptide extraction (Phoenix Pharmaceuticals). Five cross-reactivities with any relevant molecule have been reported. Intra- and interassay coefficients of variation (CVs) were 5.0 and 15.0%, respectively. The detection limit was 44 pg/mL. Plasma CCK-8 (pmol/L) was measured by RIA after ethanol extraction using an adaptation of the method of Santangelo et al (24). Intra- and interassay CVs were 8.3 and 12.6%, respectively. The detection limit was 1 pmol/L. Plasma total GLP-1 (pmol/L) was measured by RIA (Millipore). The antibody used did not cross-react with glucagon, gastric inhibitory polypeptide, or other gut or pancreatic peptides. Intra- and interassay CVs were 4.8 and 6.8%, respectively. The detection limit was 3 pmol/L. Plasma total PYY (pg/mL) was measured by RIA (Linco Research). Intra- and interassay CVs were 5.3 and 7.0%, respectively. The detection limit was 10 pg/mL. Plasma insulin (mU/L) was measured by an ELISA assay (Mercodia). Intra- and interassay CVs were 2.9 and 8.8%, respectively. The detection limit was 1 mU/L. Plasma glucagon (pg/mL) was measured by RIA (Millipore). Intra- and interassay CVs were 3.3 and 6.2%, respectively. The detection limit was 20 pg/mL. Plasma L-Trp (mmol/L) was measured using a precolumn derivatization and reversed-phase HPLC with UV detection at the Australian Proteome Analysis Facility.

Data and statistical analysis

Baseline values were calculated as means of values obtained between t = –10 and t = 0 minutes. During infusions, the number and amplitude of IPPWs and BPP were expressed as mean values over 15-minute periods (ie, 0–15, 75–90 min). The number and amplitude of antral and duodenal PWs were used to calculate antral and duodenal motility indices (MIs) (25). Number, amplitude, and MI of antral and duodenal PWs were expressed as mean values over the 90-minute infusion period, and VAS, blood glucose, plasma hormone, and L-Trp concentrations were expressed as means at each time point.

Statistical analyses were performed with SPSS software (version 19.0; SPSS Inc). VAS scores, BPP, IPPWs, blood glucose, and plasma hormone concentrations were analyzed by repeated-measures two-factor ANOVA, with time (0–90 min) and treatment (L-Trp-0.075, L-Trp-0.15, control) as factors. Number, amplitude, and MI of antral and duodenal PWs, and energy content and weight of food consumed at the test meal were analyzed by one-factor ANOVA. Sphericity of the time effect for all models was evaluated by Mauchly’s test, and when violated, the adjusted Greenhouse-Geisser P value was reported. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed where ANOVAs revealed significant effects. The primary focus of this study was to test for the effects of either dose vs control. Correlations among areas under the curve (AUCs) (calculated by using the trapezoidal rule) for BPP, IPPWs, hormones, blood glucose, plasma L-Trp, VAS, and energy intake, as well as the dose of L-Trp, were assessed using linear within-subject correlation analysis corrected for repeated measures (26). R values > 0.5 were considered physiologically relevant. All data are reported as means ± SE. All tests were two-tailed, and differences were considered statistically significant at P < .05.

Results

Meal size

There was an effect of treatment on energy intake (kcal) and amount eaten (grams) from the test meal (both P <
.01). L-Trp-0.15, but not L-Trp-0.075, decreased energy intake by 19 ± 6% (net reduction, 206 ± 68 kcal), and the amount eaten, compared with control (Table 1). There was a negative correlation between both energy intake (r = −0.65; P = .001) and the amount eaten (r = −0.51; P < .05) with the dose of L-Trp.

**Appetite perception, nausea, and GI symptoms**

There were no differences in baseline ratings for appetite perceptions between study days. There was a treatment × time interaction for fullness and prospective food consumption ratings (both P ≤ .01). L-Trp-0.075 increased fullness at 90 minutes, and L-Trp-0.15 between 45 and 90 minutes, compared with control (Figure 1A). Likewise, L-Trp-0.075 and L-Trp-0.15 reduced prospective food consumption between 60 and 90 minutes compared with control, although the effect of L-Trp-0.15 was not statistically significant (Figure 1B). There was a positive correlation between the AUC of fullness, but not prospective food consumption, and the dose of L-Trp (r = 0.62; P < .01).

There was a treatment × time interaction for nausea ratings (P = .001). L-Trp-0.15, but not L-Trp-0.075, increased nausea slightly at 75 minutes when compared with control (Figure 1C). There was no effect of treatment on ratings of bloating.

**APD pressures**

Baseline values for antral, duodenal, and pyloric pressures did not differ between study days.

**Antral pressures**

There was an effect of treatment on the total number (P < .05), mean amplitude (P < .05) and MI (P < .05) of antral PWs (Table 2). Both, L-Trp-0.075 and L-Trp-0.15 reduced the total number and MI of antral PWs, and L-Trp-0.15, but not L-Trp-0.075, reduced the mean amplitude of antral PWs, compared with control. There were negative correlations between the total number (r = −0.71; P = .001), mean amplitude (r = −0.60; P = .01), and MI (r = −0.56; P < .05) of antral PWs with the dose of L-Trp.

**Basal pyloric pressure**

There was a treatment × time interaction for BPP (P < .001) (Figure 2A). L-Trp-0.15, but not L-Trp-0.075, increased BPPs between 45 and 75 minutes when compared with control. The AUC for BPP was positively correlated with the dose of L-Trp (r = 0.68; P < .01).

**Isolated pyloric pressure**

There was a treatment × time interaction for the number (P < .05), but not the amplitude, of IPPWs (Figure 2, B and C). The number of IPPWs was higher with L-Trp-0.15 than with control between 60 and 75 minutes. The AUC for the number, but not the amplitude, of IPPWs was positively correlated with the dose of L-Trp (r = 0.59; P < .05).

**Duodenal pressures**

There was an effect of treatment on the mean amplitude (P < .05), but not the total number or MI of duodenal PWs (Table 2). Post hoc comparisons revealed, however, no difference between L-Trp-0.075 or L-Trp-0.15 and control. In three of eight subjects, L-Trp-0.15 was associated with duodenal phase-III-like activity that occurred within 50–70 minutes after the start of infusion (data not shown). Exclusion of periods of duodenal phase-III-like activity from the analysis did not change the overall effect of L-Trp on duodenal PWs.

**Plasma hormone and blood glucose concentrations**

There were no differences in baseline values between study days for plasma hormones or blood glucose (Figure 3, A–F).

**Ghrelin**

There was no effect of treatment or time on plasma ghrelin concentrations; plasma ghrelin did not decrease

### Table 1. Energy Content of the ID Infusion and Energy Content and Compensation and Weight of Food Eaten at the ad Libitum Test Meal

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-Trp-0.075</th>
<th>L-Trp-0.15</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy ID infusion, kcal</td>
<td>0</td>
<td>6.75</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Amount eaten at buffet meal, g</td>
<td>1238 ± 75</td>
<td>1278 ± 100</td>
<td>1051 ± 87a</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Energy intake at buffet meal, kcal</td>
<td>1215 ± 107</td>
<td>1155 ± 109</td>
<td>996 ± 122a</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Net energy intake suppression, kcal</td>
<td>0</td>
<td>−53 ± 40</td>
<td>206 ± 68</td>
<td></td>
</tr>
</tbody>
</table>

The ad libitum test meal was consumed immediately after 90-minute ID infusions of L-Trp at 0.075 kcal/min (L-Trp-0.075), 0.15 kcal/min (L-Trp-0.15), or control. One-factor ANOVAs were used to test for differences in energy content, weight, and macronutrient composition. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, determined significant differences between either dose and control. a P < .05 vs control.

a P < .05 vs control.
significantly in response to the test meal with either treatment (Figure 3A).

**Cholecystokinin**

There was a treatment × time interaction (P < .001) for plasma CCK (Figure 3B). L-Trp-0.15 promptly increased plasma CCK compared with control at 15 minutes and between 45 and 90 minutes, whereas L-Trp-0.075 did not have a significant effect. The AUC for plasma CCK was positively correlated with the dose of L-Trp (r = 0.84; P < .001).

**Glucagon-like peptide-1**

There was a treatment × time interaction for plasma GLP-1 (P < .001). L-Trp-0.15, but not L-Trp-0.075, increased GLP-1 between 75 and 90 minutes when compared with control (Figure 3C). There was a positive correlation between the AUC for plasma GLP-1 and the dose of L-Trp (r = 0.75; P < .001).

**Peptide YY**

There was a treatment × time interaction for plasma PYY (P < .001). L-Trp-0.075 increased PYY between 60 and 90 minutes, and L-Trp-0.075 between 75 and 90 minutes, when compared with control (Figure 3D). There was a positive correlation between the AUC for plasma PYY and the dose of L-Trp (r = 0.76; P < .001).

**Glucagon**

There was a treatment × time interaction for plasma glucagon (P < .001). L-Trp-0.15, but not L-Trp-0.075, increased plasma glucagon gradually between 30 and 90 minutes when compared with control (Figure 3E). The AUC for plasma glucagon was positively correlated with the dose of L-Trp (r = 0.78; P < .001).

**Insulin**

There was a treatment × time interaction for plasma insulin (P = .05). L-Trp-0.15, but not L-Trp-0.075, in-
creased plasma insulin slightly at 75 minutes when compared with control (Figure 3F).

**Blood glucose**

There was no effect of treatment or time on blood glucose (data not shown).

**L-Trp concentrations**

Baseline plasma L-Trp concentrations did not differ between study days. There was a treatment × time interaction for plasma L-Trp (P < .001) (Figure 4A). Both L-Trp-0.075 and L-Trp-0.15 increased plasma L-Trp gradually between 15 and 90 minutes. The AUC for plasma L-Trp was directly correlated with the dose of L-Trp (r = 0.94; P < .001).

**Correlations among energy intake, BPP, IPPWs, hormones, blood glucose, plasma L-Trp, appetite, and nausea**

Energy intake from the test meal was correlated inversely with AUCs for plasma L-Trp (r = −0.70; P < .001) (Figure 4B), CCK (r = −0.68; P = .001), glucagon (r = −0.60; P < .01) and PYY (r = −0.55; P = .01).

The AUC for fullness ratings was correlated with AUCs for plasma L-Trp (r = 0.59; P < .01), GLP-1 (r = 0.55; P = .01), and BPP (r = 0.50; P < .05). The AUC for prospective food consumption ratings was correlated with the AUC for plasma GLP-1 (r = −0.53; P < .05).

The AUC for plasma L-Trp was correlated with AUCs for plasma glucagon (r = 0.83; P < .001), CCK (r = 0.79; P < .001), BPP (r = 0.74; P = .001), PYY (r = 0.72; P < .001), GLP-1 (r = 0.70; P < .001), and the number of IPPWs (r = 0.54; P < .05).

**Discussion**

We investigated the acute effects of 90-minute ID infusions of L-Trp at 0.075 and 0.15 kcal/min on ad libitum eating, gut motor and hormonal functions, and insulin, glucagon, and blood glucose concentrations in healthy normal-weight men.

Based on our previous findings (4, 23) and those of others (1, 2) of a marked effect of protein on GI and pancreatic hormone release, we hypothesized that L-Trp affects eating via changes in the secretion of these hormones. We found that L-Trp potently stimulated CCK and glucagon, both of which, when infused peripherally, suppress meal size in humans (27, 28). L-Trp also stimulated the secretion of two other putative satiety signals, GLP-1 and PYY, although modestly, particularly when compared with studies that employ ID glucose, fat, or protein infusions (8, 23, 29). Differences in the secretory patterns of these hormones, ie, a prompt increase in plasma CCK and later increases in GLP-1 and PYY, although modestly, particularly when compared with studies that employ ID glucose, fat, or protein infusions (8, 23, 29). Differences in the secretory patterns of these hormones, ie, a prompt increase in plasma CCK and later increases in GLP-1 and PYY, are most likely attributable to the regional distribution of proximally located I-cells and more distally located L-cells, respectively. The later rises in plasma GLP-1 and PYY also suggest that L-Trp stimulated L-cells directly after transport into the distal small intestine, rather than initially, via indirect “proximal-to-distal loops” that have been suggested to account for the rapid peaks in plasma GLP-1 after glucose ingestion (30). That the distal small intestine can be exposed to L-Trp after ID administration to stimulate L-cell-
bearing mucosa is supported by a study in healthy men showing increases in AAs in ileal aspirates after ingestion of a protein-rich meal (31). Although the lack of a clear effect of ID L-Trp on plasma total ghrelin concentrations suggests that the ghrelin-suppressant effect of L-Trp may be weak, the effect of L-Trp on physiologically active, acylated ghrelin, which was not measured here, remains to be established.

Consistent with a previous report (32), L-Trp suppressed antral pressures and stimulated pyloric pressures. Pyloric pressures are central to the slowing of gastric emptying (6), which controls the rate of delivery of nutrients to the small intestine and thus, at least in part, the secretion of gut hormones relevant to appetite and glycemia. Although we did not find a direct correlation between changes in pyloric pressures and energy intake, our recent work provided evidence for a role of pyloric pressures as independent predictors of acute energy intake in response to ID lipid and carbohydrate (33).

Plasma L-Trp concentrations in response to ID L-Trp infusion increased gradually with substantial elevations before the test meal and correlated with increases in fullness and the suppression of energy intake, suggesting that circulating L-Trp, or its metabolites, may have directly reduced meal size. In fact, Mellinkoff et al (34) observed, as early as 1956, that elevated plasma AA concentrations correlated with decreases in appetite and proposed that plasma AAs may serve as a direct signal to inhibit eating (now often referred to as the “aminostatic theory”). Subsequent studies have confirmed a relationship between satiation and elevations in plasma AA concentrations in both humans (35, 36) and rats (37). Evidence for direct central effects of AAs comes from Cota et al (38), showing that branched-chain AAs activate the mammalian target of rapamycin, a potential cellular fuel sensor, whose hypothalamic activity is linked to the control of energy intake. In the present study, L-Trp may have affected eating via modulation of the central serotonergic system (39), which is well known for its potency to modulate eating, and 5-hydroxytryptamine (5-HT) agonists, such as fenfluramine and sibutramine, provided effective antiobesity treatments by modifying appetite and reducing energy intake (39). Although we did not measure 5-HT, we hypothesize that the marked increase in plasma L-Trp (particularly compared with physiological increases after the test meal on the control day) increased availability in the brain and, thus, enhanced central 5-HT synthesis.

Protein improves postprandial glycemia via the slowing of gastric emptying and the stimulation of incretin hormones that potentiate glucose-stimulated insulin secretion (5, 40). Moreover, circulating AAs, including L-glutamine or L-leucine, may directly trigger the release of insulin from pancreatic β-cells, particularly in type 2 diabetes (12,

**Figure 3.** Plasma ghrelin (A), CCK (B), GLP-1 (C), PYY (D), glucagon (E), and insulin (F) concentrations during 90-minute ID infusions of L-Trp at 0.075 kcal/min (L-Trp-0.075), 0.15 kcal/min (L-Trp-0.15), or control, and after an ad libitum test meal at 120 minutes. Repeated-measures ANOVAs, with treatment and time as factors, were used to assess differences in ghrelin, CCK, GLP-1, and PYY. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were used to determine significant differences between either dose and control. Comparisons between premeal (t = 90 min) and postmeal (t = 120 min) values were performed using a paired t test. B, Treatment × time effect (P < .001); C, treatment × time effect (P < .001); D, treatment × time effect (P < .001); E, treatment × time effect (P < .001); F, treatment × time effect (P < .05). #, P < .05, L-Trp-0.075 vs control; *, P < .05, L-Trp-0.15 vs control. Data are expressed as means ± SE; n = 10.
greater length of small intestine, interacting with a larger number of receptors, resulting in a greater physiological response (41, 42).

Some potential limitations of our study require consideration. First, ID infusion of L-Trp at 0.15 kcal/min was associated with a slight increase in nausea; however, this is unlikely to have substantially affected food intake, because mean scores were very low; moreover, there was no relationship between nausea and energy intake. Two subjects did not complete the study; one vomited, and another experienced dizziness, suggesting that some individuals may be particularly sensitive to L-Trp. Second, we used an ID infusion paradigm requiring nasoduodenal intubation, a somewhat invasive, but frequently used and well-tolerated procedure, and necessary to avoid the potentially confounding effects of interindividual variations in gastric emptying. However, it excludes gastric signals and may interfere with normal physiological function; thus, we can only speculate to what extent our observed effects may reflect those when food is ingested orally. For example, although initial reports in rats (43) and man (44, 45) show that the stomach is not involved in the control of ghrelin secretion, more recently, a number of chemosensors have been identified in gastric mucosa of rodents (46) and humans (47) that may affect ghrelin secretion. This may provide an alternative explanation for the missing effect of L-Trp on ghrelin secretion in our study. Although placement of the infusion port approximately 14.5 cm distal to the pylorus minimizes the potential of retrograde movement of small intestinal content into the stomach, this may have also bypassed some chemosensors in the duodenum. Nevertheless, stimulation of CCK from duodenal enteroendocrine I-cells was prompt (ie, plasma CCK rose within 15 min), indicating that the ID infusion was an effective stimulus. Third, we did not measure other factors that may mediate the eating-inhibitory effect of protein, including thermic effects, intestinal gluconeogenesis, or ketone body production. Finally, we only studied normal-weight subjects; thus, effects in type 2 diabetes and obesity remain to be established.

In conclusion, L-Trp suppresses energy intake substantially, in clear excess of its own energy content, and stimulates CCK and glucagon secretion, whereas effects on ghrelin, GLP-1, PYY, and APD motility appear to be modest, suggesting that other mechanisms, including direct effects of circulating L-Trp, which rose markedly during the ID infusion, or its metabolites may predominantly mediate its eating-inhibitory effect. Given the effect of L-Trp on the endocrine pancreas, the effects of L-Trp on blood glucose control, eg, after a carbohydrate-containing meal, require further investigation. Moreover, studies in obese, type 2 diabetic patients are warranted to establish the util-

Figure 4. Plasma L-Trp concentrations during 90-minute ID infusions of L-Trp at 0.075 kcal/min (L-Trp-0.075), 0.15 kcal/min (L-Trp-0.15), or control, and after an ad libitum test meal at 120 minutes (A), and relations between the AUC of plasma L-Trp concentrations and energy intake (kcal) (B). Repeated-measures ANOVAs, with treatment and time as factors, were used to assess differences in plasma L-Trp concentrations. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were used to determine significant differences between either dose and control. Comparisons between premeal (t = 90 minutes) and postmeal (t = 120 minutes) values were performed using a paired t test. Correlation between the AUC for plasma L-Trp concentrations and energy intake was assessed using linear within-subject correlation analysis corrected for repeated measures. A, Treatment × time effect (P = .001); B, within-subject correlation (r = -.70; P < .001). #, P < .05, L-Trp-0.075 vs control; *, P < .05, L-Trp-0.15 vs control. Data are expressed as means ± SE; n = 10.

13). We can comment only to a limited extent on potential glucoregulatory effects of L-Trp because the experimental design, employing an ad libitum meal, did not allow us to directly address this question. It is, however, noteworthy that L-Trp stimulated insulin, although slightly. To what extent the increase in plasma glucagon may counteract a beneficial glucoregulatory effect requires further research, also in light of a recent study (10) in which L-leucine when ingested with glucose synergistically stimulated insulin secretion and lowered blood glucose.

The effects of L-Trp on eating and gut functions were dose-dependent, in keeping with our previous observations with dietary protein, glucose, or fatty acid (18, 23, 29), suggesting that the effects are dependent on the amount of nutrient in the small intestinal lumen. Thus, the larger L-Trp load is likely to have been transported over a
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