Effects of intraduodenal infusion of the branched-chain amino acid leucine on ad libitum eating, gut motor and hormone functions, and glycermia in healthy men

Robert E Steinert,²,³,* Maria F Landrock,² Sina S Ullrich,²,³ Scott Standfield,²,³ Bärbel Otto,⁴ Michael Horowitz,²,³ and Christine Feinle-Bisset²,³

²University of Adelaide Discipline of Medicine, Adelaide, Australia; ³National Health and Medical Research Council of Australia, Centre of Research Excellence in Translational Nutritional Science to Good Health, Adelaide, Australia; and ⁴Medizinische Klinik und Poliklinik IV, Klinikum der Universität München–Campus Innenstadt, Munich, Germany

Abstract

Background: Branched-chain amino acids (BCAAs), particularly leucine, act as nutrient signals regulating protein synthesis and degradation as well as glucose metabolism. In addition, leucine has been demonstrated in animal experiments to modulate eating and energy homeostasis.

Objective: We aimed to characterize the effects of physiologic and supraphysiologic loads of intraduodenal leucine on eating, gut hormone and motor functions, and blood glucose in humans.

Design: Twelve lean men were studied on 3 occasions in a randomized, double-blind order. Antropyloroduodenal motility, plasma ghrelin, cholecystokinin, glucagon-like peptide 1, peptide YY, insulin, glucagon, blood glucose, appetite perceptions, and gastrointestinal symptoms were measured during 90-min intraduodenal infusions of leucine at 0.15 kcal/min (total 3.3 g, 13.5 kcal), 0.45 kcal/min (total 9.9 g, 40.5 kcal), or saline (control). Ad libitum eating from a buffet lunch was quantified immediately after the infusions.

Results: Leucine at 0.45 kcal/min inhibited eating (energy intake by ~13%, P < 0.05), increased plasma cholecystokinin, slightly reduced blood glucose and increased plasma insulin, and decreased antral pressures (all P < 0.05). Leucine at 0.15 kcal/min had no effect on food intake, blood glucose, or antral pressures but also slightly increased plasma cholecystokinin (P < 0.05). Neither dose affected plasma ghrelin, glucagon, glucagon-like peptide 1 and peptide YY, or pyloric and duodenal pressures. Plasma leucine concentrations were related to the dose of intraduodenal leucine, with substantial increases during both 0.15 and 0.45 kcal/min.

Conclusions: The effects of intraduodenal infusions of free leucine on eating are probably not primarily mediated by changes in gut motor and hormone functions, with perhaps the exception of cholecystokinin. Instead, increased plasma leucine concentrations may be a potential signal mediating the eating-inhibitory effect of leucine. The study was registered as a clinical trial with the Australia and New Zealand Clinical Trials Registry (www.anzctr.org.au) as ACTRN12613000899741. Am J Clin Nutr 2015;102:820–7.

Keywords: food intake, cholecystokinin, peptide YY, glucagon-like peptide 1, insulin, glucagon, humans

Introduction

There is evidence that branched-chain amino acids (BCAAs), particularly leucine, act as nutrient signals to regulate protein synthesis and degradation as well as glucose metabolism (1–4). In addition, leucine has been shown in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis. Acute administration of leucine either directly into the brain or incorporated in the diet has been demonstrated in animal experiments to modulate eating and energy homeostasis.

The integration of leucine-related signals to regulate energy homeostasis has been attributed to the activation of central signaling cascades, including the mammalian target of rapamycin/S6K kinase and Erk1/2 pathways within the mediobasal hypothalamus (5, 6, 8, 9). More recently, leucine-sensitive neurons have also been identified within the nucleus of the solitary tract (NTS) in the hindbrain (7, 9). Both hypothalamic and NTS neurons are known to integrate gut-derived signals relating to energy availability via endocrine and/or neural pathways, including peptide hormones, such as cholecystokinin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY), which are secreted in response to food ingestion (10, 11). That central leucine and gut-derived signals may interact within the NTS to acutely control meal size has been suggested by recent experiments showing that a subthreshold...
dose of intraperitoneal cholecystokinin enhanced the acute anorectic effect of NTS-administered leucine in rats (7).

The effects of leucine on ad libitum eating, gut hormone function, and upper gut motor function, particularly antropyloroduodenal motility, as an important controller of gastric emptying and, thus, the secretion of gut hormones and postprandial glycemia (12, 13), have not, to our knowledge, been investigated in humans.

In the present study, we hypothesized that intraduodenal infusion of leucine dose-dependently modulates plasma ghrelin, cholecystokinin, GLP-1, PYY, insulin, glucagon, and antropyloroduodenal motility and increases plasma leucine concentrations associated with reductions in food intake and blood glucose. We administered caloric loads of 0.15 and 0.45 kcal leucine/min and used an intraduodenal infusion paradigm to exclude orosensory influences and interindividual variations in gastric emptying to directly characterize the isolated effects of leucine on gut-derived factors.

METHODS

Subjects

Twelve healthy, normal-weight men [mean ± SD age: 25 ± 2 y (range: 18–44 y); BMI (in kg/m²): 21.9 ± 0.4 (range: 18.9–23.9)] participated in the study. Exclusion criteria were smoking, consumption of >20 g alcohol/d, any medical condition, or the use of medications known to affect eating or gastrointestinal function. All subjects were unrestrained eaters [score ≥12 on the eating restraint component (factor 1) of the 3-factor eating questionnaire (14)]. 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 before inclusion in the study.

Study design

All subjects were studied on 3 occasions, separated by 3–10 d, on which they received, in randomized, double-blind fashion, 90-min intraduodenal infusions of leucine at J) 0.15 kcal/min (leucine-0.15), 2) 0.45 kcal/min (leucine-0.45), or 3) control. The lower load was based on our recent study (15), in which L-tryptophan, in that dose, suppressed subsequent energy intake, whereas the higher load corresponded to the solubility threshold for leucine and had been shown previously to affect blood glucose control in humans (16). Leucine solutions were prepared by dissolving 3.3 g CaCl₂·2H₂O and 3.9 g NaCl in distilled water to a final volume of 428 mL. All solutions were isotonic (300 mOsm), infused at a rate of 4.75 mL/min and pH 6–7, and prepared on the morning of the study by a research assistant, so both the investigators performing the studies and the subjects were blinded to the nature of the infusions.

Study protocol

Subjects were instructed to abstain from alcohol and strenuous exercise for 24 h and provided with a standardized meal [beef lasagne (McCain Food); total energy content: 1160 kcal] to be consumed by 1900 on the night before each visit. Subjects then attended the laboratory at 0830 the following morning after an overnight fast (except for water). They were intubated with a small-diameter (3.5-mm), 17-channel manometric catheter (length: 100 cm; Dentsleeve International), which was inserted into the stomach through an anesthetized nostril and allowed to pass into the duodenum by peristalsis (17). The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference between the most distal antral and the most proximal duodenal channels (18). One channel, used for intraduodenal infusion of leucine and control solutions, was located 14.5 cm distal to the pylorus.

Once the catheter was positioned correctly, fasting motility was observed until the occurrence of phase III of the interdigestive migrating motor complex. Immediately after the end of phase III, an intravenous cannula was placed in a forearm vein for blood sampling and a baseline blood sample (t = −10 min) taken, and the subject completed a visual analog scale (VAS) questionnaire to assess appetite perceptions. At t = 0 min, another blood sample was taken, and a VAS completed before an intraduodenal infusion of leucine or control was commenced and continued for 90 min (t = 0–90 min). Antropyloroduodenal motility was recorded continuously, blood samples for measurements of plasma hormones and blood glucose were collected, and a VAS was completed every 15 min. At t = 90 min, the infusion was terminated and the manometric catheter removed. Each subject was then offered a standardized, cold, buffet-style ad libitum test meal (19) and allowed to consume as much food as he wished until he felt comfortably full, for a maximum of 30 min (t = 90–120 min). The meal comprised 4 slices (125 g) of whole-meat bread, 4 slices (125 g) of white bread, 100 g sliced ham, 100 g sliced chicken, 85 g sliced cheddar cheese, 100 g lettuce, 100 g sliced tomato, 100 g sliced cucumber, 20 g mayonnaise, 20 g margarine, 170 g apple, 190 g banana, 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 2825 kcal and the total weight 3425 g, and it contained 95.7 g fat (29.1% of energy), 354.6 g carbohydrate (46.6% of energy), and 136.9 g protein (24.3% of energy) (19, 20), as well as 8560 mg leucine. After ingestion of the meal, at t = 120 min, a final blood sample was taken and a VAS completed, and the subject was then allowed to leave the laboratory.

Measurements

Appetite and food intake

Perceptions of hunger, prospective food consumption, desire to eat, and fullness were quantified with validated 100-mm VAS questionnaires (21). Nausea and bloating were also assessed. Food intake was calculated from the amount of food (g) eaten at the buffet meal. For this purpose, each food item was weighed before and after presentation to the subject. Energy intake (kcal) and macronutrient composition (expressed as g and % of energy) were then calculated with commercial software (Food Works 7.0; Xyris Software). The leucine content of the buffet meal and of the foods consumed by each subject was estimated with online databases (http://www.nutritionvalue.org and http://nutritiondata.self.com).

Antropyloroduodenal pressures

Antropyloroduodenal pressures were recorded and digitized with a computer-based system that ran commercially available
software (MMS Database software, version 8.17; Solar GI). Data were analyzed for 1) number and amplitude of antral and duodenal pressure waves (PWs), 2) number and amplitude of isolated pyloric pressure waves (IPPWs), and 3) basal pyloric pressure (BPP), as described previously (18, 22).

**Blood glucose, plasma hormone, and leucine concentrations**

Blood samples were collected into ice-chilled EDTA-coated tubes. Blood glucose was determined with a portable glucometer (Medisense Precision QLD; Abbott Laboratories). Plasma was separated by centrifugation (~1300 g-force for 15 min at 4°C within 15 min of collection and stored at −70°C until assayed. Plasma total ghrelin (pg/mL) was measured by radioimmunoassay, without peptide extraction (Phoenix Pharmaceuticals). No cross-reactivities with any relevant molecule have been reported. Intra- and interassay CVs were 5.0% and 15.0%, respectively. The detection limit was 44 pg/mL. Plasma cholecystokinin 8 (pmol/L) was measured by radioimmunoassay after ethanol extraction by using an adaptation of the method of Santangelo et al. (23). 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 radioimmunoassay (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 radioimmunoassay (Linco Research). Intra- and interassay CVs were 2.9% and 8.8%, respectively. The detection limit was 1 µg/mL. Plasma insulin (mU/L) was measured by an ELISA assay (Merkodia). Intra- and interassay CVs were 2.9% and 7.0%, respectively. The detection limit was 10 pg/mL. Plasma glucagon (pg/mL) was measured by radioimmunoassay (Millipore). Intra- and interassay CVs were 3.3% and 6.2%, respectively. The detection limit was 20 pg/mL. Plasma leucine (mmol/L) was measured with a precolumn derivatization and reverse-phase HPLC with ultraviolet detection at the Australian Proteome Analysis Facility.

**Data and statistical analysis**

The number of subjects was determined by power calculations based on our previous studies (15). We calculated that with 12 subjects, we would be able to detect a 15% decrease in energy intake at α = 0.05, with a power of 80%. Baseline (“0”) values for all data were calculated as means of values obtained between t = −10 min and t = 0 min. During infusions, the number and amplitude of IPPWs and BPP were expressed as total numbers and mean values over 90 min, respectively. Numbers and amplitudes of antral and duodenal PWs were used to calculate antral and duodenal motility indexes (MIs), respectively (24). VAS, blood glucose, plasma hormone, and leucine concentrations were expressed as means at each time point.

Statistical analysis was performed with SPSS software (version 19.0; SPSS Inc.). VAS scores, BPP, IPPWs, blood glucose, and plasma hormone concentrations were analyzed by repeated-measures 2-factor ANOVA, with time (0–90 min) and treatment (leucine-0.15, leucine-0.45, control) as factors. MIs for antral and duodenal PWs, energy content, weight of food, macronutrient composition, and the amount of leucine 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 correction, were performed where ANOVAs revealed significant effects. Premeal (t = 90 min) and postmeal (t = 120 min) concentrations were compared with Student’s paired t test. The primary focus of this study was to test for the effects of dose compared with control. Correlations among AUCs (calculated with the trapezoidal rule) for BPP, IPPWs, hormones, blood glucose, plasma leucine, and VAS; MIs for antral and duodenal pressures; and energy intake; and the dose of leucine were assessed with linear within-subject correlation analysis corrected for repeated measures (25). R values >0.5 were considered physiologically relevant. All data are reported as means ± SEs. All tests were 2-tailed, and differences were considered statistically significant at P < 0.05.

**RESULTS**

All subjects completed all 3 study visits and tolerated all doses of leucine well.

**Food intake**

There was an effect of treatment on energy intake (kcal), but not the amount (g) eaten (P < 0.05). Leucine-0.45 but not leucine-0.15 decreased energy intake by 13% ± 4% (net reduction 170 ± 48 kcal) compared with control (P < 0.05; Table 1). There was an effect of treatment on the amount (g) consumed of carbohydrate (P < 0.01) and protein (P < 0.05) but not fat (Table 1). There was, however, no effect of treatment on the percentage of energy consumed from carbohydrate, protein, or fat. There was an effect of treatment on the amount of leucine consumed from the buffet meal (P < 0.05), which was less after leucine-0.45 but not leucine-0.15 compared with control (P < 0.05; Table 1), presumably reflecting the reduced energy intake.

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Leucine-0.15</th>
<th>Leucine-0.45</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake, kcal</td>
<td>1214 ± 70 2</td>
<td>1155 ± 52</td>
<td>1044 ± 61*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amount eaten, g</td>
<td>1176 ± 98</td>
<td>1114 ± 76</td>
<td>1049 ± 101</td>
<td>NS</td>
</tr>
<tr>
<td>Fat</td>
<td>33 ± 3</td>
<td>44 ± 3</td>
<td>41 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>128 ± 8</td>
<td>123 ± 7</td>
<td>108 ± 7*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>42 ± 2</td>
<td>42 ± 1</td>
<td>41 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Protein</td>
<td>69 ± 4</td>
<td>64 ± 3</td>
<td>58 ± 4*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Leucine, mg</td>
<td>4546 ± 288</td>
<td>4026 ± 290</td>
<td>3747 ± 276*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

2The ad libitum test meal was consumed immediately after 90-min intraduodenal infusions of leucine-0.15, leucine-0.45, or control. One-factor ANOVA was used to test for differences in energy intake, amount, macronutrient distribution, and leucine content of food eaten. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, determined significant differences between dose and control. Data are from 11 subjects (1 subject was excluded from the analysis for noncompliance with investigator instructions). *P < 0.05 compared with control. Leucine-0.15, 0.15 kcal leucine/min; leucine-0.45, 0.45 kcal leucine/min.

Mean ± SE (all such values).
Appetite perception and gastrointestinal symptoms

There were no differences in baseline ratings for appetite perceptions or gastrointestinal symptoms between study days. There was also no effect of treatment or time on ratings of hunger, desire to eat, prospective food consumption, fullness, nausea, or bloating (data not shown).

Antropyloroduodenal pressures

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

Antral pressures

There was an effect of treatment ($P < 0.05$) on the MI but not the number or amplitude of antral PWs (Table 2). Leucine-0.45 but not leucine-0.15 reduced the MI of antral PWs compared with control ($P < 0.05$).

BPP

There was no effect of treatment on BPP (Table 2).

Isolated pyloric pressure

There was no effect of treatment on the number or amplitude of IPPWs (Table 2).

Duodenal pressures

There was no effect of treatment on the number, amplitude, or MI of duodenal PWs (Table 2).

Plasma hormone and blood glucose concentrations

There were no differences in baseline values between study days for plasma hormones or blood glucose (Figures 1 and 2).

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number, amplitude, and motility index of antral and duodenal pressure waves, basal pyloric pressure, and number and amplitude of isolated pyloric pressure waves during 90-min intraduodenal infusions of leucine-0.15, leucine-0.45, or control*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control</th>
<th>Leucine-0.15</th>
<th>Leucine-0.45</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral pressure waves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>87 ± 13</td>
<td>68 ± 9</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>Amplitude, mm Hg</td>
<td>63.5 ± 9.0</td>
<td>52.7 ± 8.1</td>
<td>42.2 ± 8.5</td>
</tr>
<tr>
<td>Motility index</td>
<td>12.6 ± 4.0</td>
<td>12.1 ± 4.0</td>
<td>10.6 ± 0.8*</td>
</tr>
<tr>
<td>BPP, mmHg</td>
<td>0.0 ± 0.5</td>
<td>0.5 ± 0.6</td>
<td>0.6 ± 0.6</td>
</tr>
<tr>
<td>IPPWs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>41 ± 7</td>
<td>45 ± 7</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>Amplitude, mm Hg</td>
<td>24.0 ± 2.1</td>
<td>24.2 ± 2.0</td>
<td>28.0 ± 5.0</td>
</tr>
<tr>
<td>Duodenal pressure waves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>533 ± 59</td>
<td>606 ± 64</td>
<td>472 ± 73</td>
</tr>
<tr>
<td>Amplitude, mm Hg</td>
<td>32.5 ± 4.2</td>
<td>29.8 ± 2.5</td>
<td>34.1 ± 6.7</td>
</tr>
<tr>
<td>Motility index</td>
<td>15.7 ± 0.2</td>
<td>16.0 ± 0.2</td>
<td>15.3 ± 0.3</td>
</tr>
</tbody>
</table>

*Number and amplitude of antral and duodenal pressures were used to calculate a motility index. One-factor ANOVA was used to test for differences in motility index of antral and duodenal pressures. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, determined significant differences between dose and control. *$P < 0.05$ compared with control. BPP, basal pyloric pressure; IPPW, isolated pyloric pressure wave; leucine-0.15, 0.15 kcal leucine/min; leucine-0.45, 0.45 kcal leucine/min.

1Mean ± SE (all such values).

Ghrelin

There was an effect of time ($P < 0.05$) but not treatment on plasma ghrelin (Figure 1A). After the test meal, plasma ghrelin was suppressed with control ($P < 0.01$), and there was a trend for a suppression with leucine-0.15 ($P = 0.08$) but no suppression with leucine-0.45.

Cholecystokinin

There was an effect of treatment ($P < 0.05$) on plasma cholecystokinin (Figure 1B). Both leucine-0.45 ($P < 0.01$) and leucine-0.15 ($P < 0.05$) increased plasma cholecystokinin compared with control ($P < 0.01$). After the test meal, plasma cholecystokinin increased substantially with all treatments ($P < 0.05$) above concentrations achieved during the infusion.

GLP-1

There was no effect of treatment on plasma GLP-1 (Figure 1C). After the test meal, plasma GLP-1 increased substantially with all treatments ($P < 0.001$).

PYY

There was no effect of treatment on plasma PYY (Figure 1D). After the test meal, plasma PYY increased substantially with all treatments ($P < 0.001$).

Glucagon

There was no effect of treatment on plasma glucagon (Figure 2A). After the test meal, plasma glucagon increased with all treatments ($P < 0.01$).

Insulin

There was a treatment × time interaction ($P < 0.05$) for plasma insulin. Both leucine-0.45 and leucine-0.15 increased plasma insulin slightly compared with control, leucine-0.45 between 15 and 90 min, and leucine-0.15 between 15 and 30 min (all $P < 0.05$; Figure 2B). After the test meal, plasma insulin increased with all treatments ($P < 0.001$).

Blood glucose

There was a treatment × time interaction ($P < 0.05$) for blood glucose. Leucine-0.45 but not leucine-0.15 reduced blood glucose slightly compared with control between 75 and 90 min ($P < 0.05$; Figure 2C). After the test meal, blood glucose increased with all treatments ($P < 0.001$).

Plasma leucine concentrations

Baseline plasma leucine concentrations did not differ between study days. There was a treatment × time interaction ($P < 0.001$) for plasma leucine (Figure 3). Both leucine-0.15 and leucine-0.45 increased plasma leucine substantially between 15 and 90 min ($P < 0.001$). After the test meal, plasma leucine increased with control ($P < 0.001$) but decreased with both leucine-0.45 and leucine-0.15 ($P < 0.001$).
FIGURE 1 Plasma ghrelin (A), CCK (B), GLP-1 (C), and PYY (D) concentrations during 90-min ID infusions of leucine at 0.15 or 0.45 kcal/min or of control and after an ad libitum test meal at 120 min. Data were analyzed by using repeated-measures ANOVAs, with treatment and time as factors. In case of significant differences, post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction, were performed to determine significant differences between dose and control. Comparisons between premeal (t = 90 min) and postmeal (t = 120 min) meal values were done with a paired t test. (A) Time effect (P < 0.05) and (B) treatment effect (P < 0.05). *Leucine-0.15 compared with control, P < 0.05; †leucine-0.45 compared with control, P < 0.05. Data are means ± SEs (n = 12). CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; ID, intraduodenal; PYY, peptide YY.

Relations between leucine dose, energy intake, BPP, IPPWs, hormones, blood glucose, plasma leucine, appetite, and nausea

There were inverse correlations between energy intake (r = −0.65, P = 0.001) and the MI of antral PWs (r = −0.53, P < 0.05), as well as a trend for an inverse correlation between the AUC of blood glucose (r = −0.35, P = 0.08) and the dose of leucine administered. There were positive correlations between the AUCs of plasma cholecystokinin (r = 0.69, P < 0.001), plasma insulin (r = 0.77, P < 0.001), and plasma glucagon (r = 0.53, P < 0.01), as well as a trend for a positive correlation between the number of IPPWs (r = 0.36, P = 0.07) and the dose of leucine administered. There was also a positive correlation between the AUC of plasma leucine and the dose of leucine administered (r = 0.97, P < 0.001).

There were positive correlations between the AUCs of plasma cholecystokinin (r = 0.71, P < 0.001), plasma glucagon (r = 0.58, P < 0.01), and plasma insulin (r = 0.75, P < 0.001); inverse correlations between energy intake (r = −0.53, P < 0.01), amount eaten (r = −0.42, P < 0.05), and the MI of antral pressures (r = −0.47, P < 0.05); and trends for inverse correlations between the AUCs of blood glucose (r = −0.38, P = 0.058) and plasma ghrelin (r = −0.35, P = 0.08) with the AUC of plasma leucine.

There were inverse correlations between energy intake with the AUCs of plasma insulin (r = −0.62, P < 0.01) and plasma glucagon (r = −0.42, P < 0.05).

DISCUSSION

The outcomes of recent studies in animals suggest that leucine acts as a nutrient signal modulating eating and energy homeostasis (5–7). In humans, we found that 90-min intraduodenal infusions of leucine at 0.45 kcal/min inhibited eating (reduction in energy intake by 13%), increased plasma cholecystokinin, slightly reduced blood glucose and increased plasma insulin, and modestly decreased antral pressures. Leucine at 0.15 kcal/min had no effect on food intake, blood glucose, or antral pressures but also slightly increased plasma cholecystokinin. Neither dose significantly affected plasma ghrelin, glucagon, GLP-1 and PYY, or pyloric or duodenal pressures. Plasma leucine concentrations were related to the dose of intraduodenal leucine, with substantial increases during both 0.15 and 0.45 kcal/min. These observations suggest that the eating-inhibitory effect of intraduodenal leucine is not primarily mediated by changes in gut motor and hormone functions (except for perhaps cholecystokinin) and that the increase in plasma leucine is a potential mechanism (9). Studies in which leucine is infused intravenously at doses that result in comparable plasma concentrations as intraduodenal infusions would be required to test this hypothesis.

The effects of leucine on eating and the potential involvement of changes in gut motor and hormone functions have not been investigated previously in humans, and there is limited information about the effect of BCAA ingestion on body weight. For example, in a patient with schizophrenia, prolonged dietary supplementation of BCAAs (135 g/d) reduced body weight by 13.5 kg over 5.5 mo (26); in a study of elite wrestlers, the combination of moderate caloric restriction with BCAA supplementation for 19 d (~0.35 g/kg body weight per day) reduced body weight by 4 kg (27); and finally, in an epidemiologic study, higher dietary BCAA intakes were associated with a lower prevalence of obesity among healthy middle-aged adults from East Asian and Western countries (28). Our data demonstrate an acute effect of intraduodenal administered leucine to suppress food intake in men, suggesting that the
control, control. Comparisons between premeal (post hoc comparisons, adjusted for multiple comparisons by Bonferroni correction), were performed to determine significant differences between dose and control. Comparisons between premeal (t = 90 min) and postmeal (t = 120 min) values were done with a paired t test. Treatment × time effect (P < 0.001): *leucine-0.45 compared with control, P < 0.001; †leucine-0.15 compared with control, P < 0.001. Data are means ± SEs (n = 12). ID, intraduodenal.

There are several possible mechanisms underlying the observed eating-inhibitory effect of intraduodenal leucine: 1) intraduodenal leucine infusions were accompanied by substantial increases in plasma leucine, suggesting that circulating leucine is a potent satiation signal. This would be in line with recent studies in rodents showing potent eating-inhibitory effects of acute administration of leucine either directly into the brain or incorporated in the diet (5–9). In humans, Mellinkoff et al. (31) first reported relations between serum amino acids and fluctuations in appetite (often referred to as “the aminostatic theory”), and subsequent studies in humans support this concept (32, 33), including our recent study in healthy subjects, in which we found a potent eating-inhibitory effect of intraduodenal l-tryptophan that correlated closely with plasma l-tryptophan concentrations (15). However, it is important to recognize that, to further pursue this hypothesis, plasma concentrations of other related amino acids, including isoleucine, valine, tryptophan, and tyrosine, will need to be quantified in future studies. 2) Intraduodenal leucine infusions promptly increased plasma cholecystokinin and modestly suppressed antral motility, suggesting that although the overall effect on gut motor and hormone functions was small, cholecystokinin, at least in part, may be involved in leucine-induced inhibition of eating. A role for cholecystokinin in the effect of amino acids on eating has been suggested also by studies with oral l-phenylalanine and intraduodenal l-tryptophan in humans and non-human primates (15, 34, 35). The lack of an effect of leucine on plasma ghrelin, GLP-1, and PYY suggests that leucine is a poor stimulus for these peptides, although a larger number of subjects may have allowed detecting a significant effect for plasma ghrelin. Because in humans, most t-cells appear to be expressed on enteroendocrine cells in distal parts of the gut (36, 37), an alternative explanation for the lack of effect of leucine on plasma GLP-1 and PYY may be that leucine was absorbed fully before reaching distal gut segments to stimulate t-cells. Although indirect “proximal-to-distal loops” (i.e., stimulation of secretion without reaching distal parts of the gut) have been suggested to account for the prompt increase in plasma GLP-1 after glucose ingestion (30), our previous study with l-tryptophan suggests that this may not be the case with amino acids (15). 3) An additional mechanism that may be involved in the eating-inhibitory effect of intraduodenal leucine in the present study is a potential
interaction between cholecystokinin and plasma leucine. This is suggested by the recent demonstration that a subthreshold dose of intraperitoneal cholecystokinin enhanced the acute anorexigenic effect of NTS-administered leucine in rats (7). This hypothesis could be pursued in humans by concomitant administration of leucine with a cholecystokinin receptor antagonist.

The effect of leucine on blood glucose homeostasis is complex. Under acute conditions, leucine slightly decreases blood glucose despite stimulation of endogenous glucose production when given intravenously or orally in large doses of 300–750 mg/kg body weight to healthy humans (38–43). This effect is probably secondary to a modest increase in insulin secretion (38–43) and can be enhanced when leucine is coadministered with glucose (16, 40). Our data are in line with earlier findings indicating a small blood glucose–lowering, insulinotropic effect of leucine, which appears to be independent of GLP-1, particularly because the insulinotropic effect of the latter is dependent on hyperglycemia (44). Whether these findings indicate a potential use for leucine as a therapeutic approach to lower blood glucose in patients with type 2 diabetes, who are characteristically obese, however, requires further investigation, particularly in light of recent findings linking an elevation of plasma BCAA concentrations to insulin resistance (45–47).

Potential limitations of our study require consideration. We used an intraduodenal infusion paradigm to exclude “gastric” signals. It is thus unknown whether the observed effects fully reflect those when leucine is ingested orally. Placement of the infusion port 14.5 cm distal to the pylorus, which is required to avoid retrograde movement of small intestinal content into the stomach, may have bypassed a small number of chemosensors on duodenal I cells, and thus bypassed cholecystokinin secretion. We studied normal-weight subjects, and thus effects in type 2 diabetes and obesity remain to be established. We report effects of leucine only on fasting blood glucose. Determination of the effects of leucine on postprandial glycemia requires concomitant administration of carbohydrate. Although it would be of interest to study the effect of leucine on food choice, our buffet meal is not effective in evaluating this aspect of food intake (19). Finally, it should be recognized that under physiologic conditions, when protein is ingested orally, approximately two-thirds of protein absorption occurs as di- and tripeptides, not free amino acids, in the upper small intestine, and thus the effect of di- and tripeptides on eating and gut functions warrants investigation.

In conclusion, intraduodenal leucine inhibits ad libitum eating, albeit in doses that are higher than would be consumed with a normal meal. This effect may be mediated via increases in plasma leucine and/or cholecystokinin. Intraduodenal leucine had no effect on plasma ghrelin, GLP-1, and PYY and only small effects on antropyloroduodenal motility, indicating that leucine is a poor stimulus for gut hormone and motor functions. Leucine lowered blood glucose, presumably secondary to an insulinotropic effect independent of GLP-1.

We thank Penelope Fitzgerald for her assistance on study days; Kylie Lange, a professional biostatistician, for statistical support; and Minerva Petrovitsch for her excellent technical assistance in measuring gut hormones. The authors’ responsibilities were as follows—RES and CF-B: conceived and designed the study; RES, MFL, SS, and BO: collected the data; RES, MFL, SSU, and CF-B: analyzed and interpreted the data; RES, MH, and CF-B: drafted and revised the manuscript; and all authors: read and approved the final version of the manuscript. None of the authors declared any conflicts of interest.

REFERENCES


34. Ballinger AB, Clark ML. L-phenylalanine releases cholecystokinin (CCK) and is associated with reduced food intake in humans: evidence for a physiological role of CCK in control of eating. Metabolism 1994;43:735–8.


