Intragastric administration of leucine or isoleucine lowers the blood glucose response to a mixed-nutrient drink by different mechanisms in healthy, lean volunteers1,2

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ABSTRACT
Background: The branched-chain amino acids leucine and isoleucine lower blood glucose after oral glucose ingestion, and the intraduodenal infusion of leucine decreases energy intake in healthy, lean men.
Objective: We investigated the effects of the intragastric administration of leucine and isoleucine on the gastric emptying of, and blood glucose responses to, a physiologic mixed-macronutrient drink and subsequent energy intake.
Design: In 2 separate studies, 12 healthy, lean subjects received on 3 separate occasions an intragastric infusion of 5 g leucine (leucine-5g) or an intragastric infusion of 10 g leucine (leucine-10g), an intragastric infusion of 5 g isoleucine (isoleucine-5g) or an intragastric infusion of 10 g isoleucine (isoleucine-10g), or a control. Fifteen minutes later, subjects consumed a mixed-nutrient drink (400 kcal, 56 g carbohydrates, 15 g protein, and 12 g fat), and gastric emptying (13C-acetate breath test) and blood glucose, plasma insulin, C-peptide, glucagon, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), cholecystokinin (leucine study only) were measured for 60 min. Immediately afterward, energy intake from a cold, buffet-style meal was assessed.
Results: Compared with the control, leucine-10g decreased the blood glucose area under the curve (AUC) (P < 0.05) and tended to reduce peak blood glucose (P = 0.07), whereas effects of leucine-5g were NS. Leucine-10g, but not leucine-5g, increased plasma insulin and C-peptide AUCs (P < 0.01 for both), but neither dose affected glucagon, GLP-1, GIP, cholecystokinin, gastric emptying, or energy intake. Compared with the control, isoleucine-10g reduced the blood glucose AUC and peak blood glucose (P < 0.01), whereas effects of isoleucine-5g were NS. Neither load affected insulin, C-peptide, glucagon, GLP-1, or GIP. Isoleucine-10g, but not isoleucine-5g, slowed gastric emptying (P < 0.05), but gastric emptying was not correlated with the blood glucose AUC. Isoleucine did not affect energy intake.
Conclusions: In healthy subjects, both leucine and isoleucine reduced blood glucose in response to a mixed-nutrient drink but did not affect subsequent energy intake. The mechanisms underlying glucose lowering appear to differ; leucine stimulated insulin, whereas isoleucine acted insulin independently. These trials were registered at www.anzctr.org.au as 12613000899741 and 12614000837628.

INTRODUCTION
The ingestion of protein, particularly whey, lowers postprandial blood glucose and reduces energy intake in healthy individuals and in those with type 2 diabetes (1, 2). Mechanisms that are thought to be involved in the mediation of these effects include increased insulin and glucagon release (3), increased plasma amino acids (4), and changes in gastrointestinal motor and hormone functions (5). The latter mechanism includes increases in plasma gut-hormone concentrations, including the incretin hormones glucagon-like peptide-1 (GLP-1)4 and glucose-dependent insulinotropic polypeptide (GIP) and cholecystokinin, as well as changes in motility (1, 2, 5, 6), with the latter effects underlying the slowing of gastric emptying. The rate of gastric emptying is an important determinant of postprandial blood glucose regulation both in healthy individuals and in those with type 2 diabetes (7–9).

The branched-chain amino acids leucine and isoleucine, which are abundant in whey protein, have gained increasing interest as...
potential mediators of the effects of protein on energy intake and blood glucose (10–12). For example, in humans, when co-ingested with 25 g of glucose, leucine and isoleucine, in amounts of ~5–10 g, attenuated the increase in blood glucose in response to oral glucose alone (13, 14). Only leucine, but not isoleucine, increased plasma insulin, which suggested that the underlying mechanisms for glucose lowering differ and may include gastric emptying or the release of incretin hormones. Moreover, to our knowledge, it is not known whether the blood glucose–lowering effects of leucine and isoleucine are also apparent when carbohydrate is co-ingested with other macronutrients or whether their presence would interfere with the effects of those amino acids.

Leucine has also been reported to decrease energy intake in both animals and humans (15, 16). For example, the intracerebroventricular administration of leucine in rats decreased food intake for 24 h, and this effect was associated with the activation of the hypothalamic mammalian target of rapamycin, which is a cellular sensor of nutrient availability (15). In our recent study in healthy, lean men (16), leucine, when infused intraduodenally for 90 min to bypass the potential interindividual variations in gastric emptying, reduced subsequent energy intake in excess of its own caloric content, which was associated with the stimulation of plasma cholecystokinin concentrations. Moreover, we recently reported a close inverse relation between the increase in plasma isoleucine in response to intraduodenal protein with subsequent energy intake (11), which suggested that isoleucine may also play a role in the regulation of energy intake. However, to our knowledge, it is not known whether leucine or isoleucine reduce energy intake when ingested orally.

Therefore, the aim of the current studies was to evaluate the hypothesis that leucine and isoleucine lower blood glucose in response to a mixed-nutrient drink and reduce subsequent energy intake, potentially by different mechanisms. The high amino-acid dose (10 g) was based on our study on intraduodenal leucine (16); the lower dose was used to assess potential dose-dependent effects. Intragastric administration was chosen to avoid the unpleasant taste of the amino acids. We measured effects on key variables that are involved in blood glucose and energy intake regulation, including insulin, C-peptide, and glucagon, and gastrointestinal factors, including gastric emptying and the gut hormones GLP-1, GIP, and cholecystokinin.

METHODS

Subjects

Two separate randomized crossover studies were performed, each of which included 12 healthy, lean subjects [7 men and 5 women; study A: mean ± SEM age, 24 ± 2 y (range: 19–37 y); BMI (in kg/m²), 22.4 ± 0.6 (range: 18.5–24.9); study B: age, 27 ± 2 y (range: 20–42 y); BMI, 21.8 ± 0.5 (range: 19.7–24.6)] (Supplemental Figure 1; for baseline data of individual subjects, see Supplemental Table 1) Both studies were run concurrently, and none of the subjects participated in both studies. Subjects were unrestrained eaters [score ≤12 on the eating-restrained component of the 3-factor eating questionnaire (17)]. Subjects who smoked, had gastrointestinal symptoms or surgery, had low ferritin or iron concentrations, consumed >2 standard drinks/ wk, were lactose intolerant, or were vegetarians or high-performance athletes were excluded from the study. Women who used hormonal contraception or were pregnant were also excluded. A pregnancy test was performed before each study day. Women were studied during the luteal phase of their menstrual cycles (18); they were asked to report day 1 of their menstrual cycles as well as their usual cycle lengths, and study visits were only conducted between day 15 and the last day of the respective cycle. Once subjects were included (by SSU, PCEF, GS), they were assigned to a treatment order of balanced randomization that was generated with the use of an online tool (www.randomization.com) by an investigator who was not involved in data analysis. The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee and was performed in accordance with the Declaration of Helsinki. All subjects provided written informed consent before inclusion in the study. The studies were registered as clinical trials at www.anzctr.org.au as 12613000899741 (study A) and 12614000837628 (study B). The data described in the current article represent the complete set of data collected in these 2 studies, each of which was designed as an independent study within larger projects that were registered under these 2 identifiers. The objectives and endpoints of the studies are outlined in Study Design and also at www.anzctr.org.au (see the link “view full record”).

Study design

The studies evaluated the dose-related effects of the intragastric administration of leucine (study A) or isoleucine (study B) on blood glucose and gut (cholecystokinin, GLP-1, and GIP) and pancreatic (insulin, C-peptide, glucagon) hormone responses to, and the gastric emptying of, a mixed-nutrient drink as well as appetite responses and subsequent energy intake. Cholecystokinin was measured in study A only because of our previous finding that the intraduodenal infusion of leucine increased plasma cholecystokinin concentrations and suppressed energy intake (16).

Intragastric infusions

Two hundred–milliliter intragastric infusions were delivered with 1) 0 g leucine or isoleucine (control) or 2) 5 g or 3) 10 g of either leucine (study A) or isoleucine (study B). Amino acids (particularly leucine) were administered as suspensions because of their low water solubility. For this purpose, 5 or 10 g crystalline leucine or isoleucine (PureBulk Inc.) and 58 mg CaCl₂ × 2H₂O were incorporated in 100 mL of a suspending agent [ORA-Plus (Perrigo)], and isotonic saline was used to adjust to a final volume of 200 mL. Control infusions consisted of 100 mL suspending agent, 58 mg CaCl₂ × 2H₂O, and 100 mL isotonic saline. The suspensions were prepared on the morning of each study day by a researcher (PCEF) who was not involved in the data analysis and administered at room temperature into the stomach via a nasogastric catheter. Syringes were covered to blind the study subject and investigators who performed the study (SSU, PCEF, GS).

Protocol

Each subject was studied on 3 occasions. For men, study visits were separated by 2–7 d, and for women, study visits were separated by ≥2 d within a luteal phase and ≤36 d if study visits were scheduled across menstrual cycles.
Subjects were provided with a standardized meal (Beef Lasagna; McCain Food; total energy content: 1160 kcal) that was to be consumed at 1900 the night before each study. Subjects were instructed to refrain from strenuous exercise and alcohol for 24 h before each study and from solids and liquids, except water, after the evening meal until the subjects arrived in the laboratory in the Discipline of Medicine, Royal Adelaide Hospital, at 0930 the next morning. Subjects were seated in an upright position on a hospital bed, and an intravenous cannula was placed into a right forearm vein for regular blood sampling. At $t = -20$ min, which was defined as the baseline, a blood sample for blood glucose and plasma hormone measurements and a baseline breath sample to measure gastric emptying were taken, and the subject completed a visual analog scale (VAS) questionnaire to assess appetite-related perceptions and gastrointestinal symptoms (Figure 1). Subjects were intubated with a nasogastric soft silicon feeding tube (outer diameter: 4 mm; Dentsleeve), which was inserted through an anesthetized nostril into the stomach. The correct position of the feeding tube was checked by auscultating the stomach with the use of a stethoscope while pushing an 8-mL air bolus through the tube. Immediately thereafter ($t = -18$ min), subjects received the 200-mL intragastric infusion of a (i) control or (ii) 5 g or (iii) 10 g of either leucine or isoleucine within 2 min. The tube was removed, and 15 min later ($t = -1$ min), subjects completed a VAS questionnaire and consumed, within 1 min, 300 mL mixed-nutrient drink (Ensure plus; Abbott) (total energy content: 400 kcal; 56 g carbohydrates, including corn syrup, maltodextrin, and sucrose, 15 g protein, and 12 g fat) labeled with 100 mg $^{13}$C-acetate for the measurement of gastric emptying with the use of a breath test (19, 20). Immediately after consumption of the drink ($t = 0$ min) and for the next hour ($t = 0-60$ min), breath samples were collected every 5 min, and appetite-perception ratings and blood samples were collected every 15 min. At $t = 60$ min, subjects were presented with a standardized, cold, buffet-style test meal to assess energy intake (21). Subjects were instructed to freely consume food until they were comfortably full and were given $\leq 30$ min ($t = 60-90$ min). The meal consisted of 4 slices each of whole-meal bread and white bread, 85 g sliced cheddar cheese, 100 g sliced ham, 100 g sliced chicken breast, 100 g sliced tomato, 100 g sliced cucumber, 100 g iceberg lettuce, 20 g margarine, 20 g mayonnaise, 120 g fruit salad, 175 g yogurt, 190 g custard, 170 g apple, 375 mL iced coffee, 300 mL orange juice, and 600 mL water with a total energy content of 2822 kcal (21). The amount of food offered was in excess of what each subject was expected to consume. At $t = 90$ min, a final blood sample was taken, and a VAS questionnaire was completed. The intravenous cannula was removed, and subjects were free to leave the laboratory.

Measurements

Blood glucose and plasma hormone analysis

Blood samples were collected into ice-chilled tubes containing EDTA. Plasma was obtained by centrifugation at 3200 × g ($\sim 1832$ g-force) for 15 min at 4°C within 15 min of collection and stored at $-80$°C until the subsequent analysis. Blood glucose concentrations (expressed as mmol/L) were determined immediately after sampling with the use of a portable glucometer (FreeStyle OptimumH; Abbott Laboratories).

Plasma insulin concentrations (expressed as mU/L) were measured with the use of an ELISA (10-1113; Mercodia). The minimum detectable limit was 1.0 mU/L; intra-assay and interassay CVs were 2.8% and 10.3%, respectively, in study A and 2.8% and 10.8%, respectively, in study B.

Plasma C-peptide concentrations (expressed as nmol/L) were measured with the use of an ELISA (10-1136-01; Mercodia). The minimum detectable limit was 15 pmol/L; intra-assay and interassay CVs were 2.4% and 4.9%, respectively, in both studies.

Plasma glucagon concentrations (expressed as pg/mL) were measured with the use of a radioimmunoassay (GL-32K; Millipore). The minimum detectable limit was 20 pg/mL; intra-assay and interassay CVs were 3.8% and 8.2%, respectively, in study A and 3.7% and 8.1%, respectively, in study B.

Plasma GLP-1 concentrations (expressed as pmol/L) were measured with the use of a radioimmunoassay (GLPIT-36HK; Millipore). No cross-reactions of the antibody with glucagon, GIP, or other gut or pancreatic hormones have been reported. The minimum detectable limit was 3 pmol/L; intra-assay and interassay CVs were 4.7% and 7.9%, respectively, in study A and 4.6% and 8.1%, respectively, in study B.

Plasma GIP concentrations (expressed as pmol/L) were measured with the use of a radioimmunoassay with some modifications of a previously published method (22). The standard curve was prepared in buffer rather than in extracted, charcoal-stripped serum, and the radio-iodinated label was supplied by Perkin Elmer. The minimum detectable limit was 2 pmol/L; intra-assay and interassay CVs were 4.1% and 9.8%, respectively, in study A, and 5.2% and 9.6%, respectively, in study B.

Plasma cholecystokinin-8 concentrations (expressed as pmol/L) were measured with the use of a radioimmunoassay after ethanol extraction according to an adaption of the method of Santangelo et al. (23). The antibody used binds all cholecystokinin peptides that contain the sulfated tyrosine residue in position 7, showing 26% cross-reactivity with unsulfated cholecystokinin-8 and <2% cross-reactivity with human gastrin I, and does not bind to structurally unrelated peptides. The minimum detectable limit was 1 pmol/L; intra-assay and interassay CVs were 5.2% and 12.7%, respectively.

![Figure 1](https://example.com/figure1.jpg)
Gastric emptying

$^{13}$CO$_2$ concentrations in exhaled air of end-expiratory breath samples were analyzed with the use of infrared spectroscopy (FANci2 Breath Test Analyzer; Fischer Analysen Instrumente GmbH). Breath-sample data were expressed as the percentage of recovery of $^{13}$CO$_2$ in the breath per hour.

Appetite perceptions and gastrointestinal symptoms

Appetite perceptions, including hunger, fullness, the desire to eat, and prospective food consumption, and gastrointestinal symptoms, including nausea and bloating, were assessed with the use of validated 100-mm VAS questionnaires (24).

Food intake

The amount of food consumed (in grams) was obtained by recording the weight of the foods in the buffet meal before and after being offered to the subjects with subjects unaware of the measurement. Energy intake (in kilocalories) was subsequently calculated with the use of commercially available software (Foodworks 7.0; Xyris Software) (21).

Data and statistical analysis

The number of subjects in both studies was determined with the use of power calculations on the basis of our previous studies (16). We calculated that $n = 12$ subjects would allow for the detection of a 15% decrease in energy intake, and $n = 11$ subjects would allow for the detection of a 1.0-mmol/L reduction in blood glucose, both at $t = 0.05$, with a power of 80%. Both studies were designed independently and were powered to detect treatment effects within each study.

Blood glucose and plasma hormone concentrations were expressed as raw data, and gastric emptying and VAS data were expressed as changes from baseline (i.e., $t = -20$ min). To assess the effects of amino acids alone, changes from baseline at $t = 0$ min were calculated for blood glucose and hormones. In response to the mixed-nutrient drink, blood glucose, plasma hormones, gastric emptying, and VAS data were expressed as the percentage of change from control, were assessed expressed as the magnitude of change from control, were assessed with the use of the Pearson correlation. Correlations between the AUC$_{0-60min}$ of insulin and the AUC$_{0-60min}$ of C-peptide, with the use of pooled data from all 3 study visits, were calculated with a linear within-subject correlation analysis that was corrected for repeated measures. To evaluate responses to the buffet meal, paired $t$ tests were performed to compare blood glucose and plasma hormones at $t = 90$ min (i.e., after the buffet meal) with those at $t = 60$ min (i.e., before the buffet meal), and a 1-factor repeated-measures ANOVA was used to analyze effects at $t = 90$ min. For all ANOVAs, the sphericity was evaluated with the use of Mauchly’s test. When violated, the Greenhouse-Geisser $P$ value was reported. Post hoc comparisons, which were adjusted for multiple comparisons with the use of Bonferroni correction, were performed when ANOVAs revealed significant effects to evaluate differences between treatments and the control. Differences were considered statistically significant at $P \leq 0.05$. All data are reported as means ± SEMs.

RESULTS

All subjects completed all 3 study visits in each study and tolerated all treatments without adverse effects. In study A, 1 study subject (a man) was excluded from the analysis because blood glucose and insulin responses were outside the normal range; plasma cholecystokinin data were not available in 2 subjects (both men) because of technical problems with the analysis. The insulin-secretory response could not be calculated in 1 subject (a woman) in study A and in 2 subjects in study B (1 man and 1 woman).

Study A

Blood glucose and plasma hormone concentrations

There were no differences in baseline ($t = -20$ min) values of blood glucose or plasma hormone concentrations between study days (Figures 2 and 3).

Blood glucose

Response to leucine. At $t = 0$ min, the changes from baseline did not differ between treatments and the control (Figure 2A).

Response to the mixed-nutrient drink. Blood glucose increased modestly on all 3 study days. There were effects of treatment on blood glucose AUC$_{0-60min}$ and peak blood glucose ($P < 0.05$ for both) (Table 1); compared with the control, the intragastric infusion of 10 g leucine (leucine-10g), but not the intragastric infusion of 5 g leucine (leucine-5g), decreased the blood glucose AUC$_{0-60min}$ ($P < 0.05$) and tended to lower peak blood glucose ($P = 0.072$).

Response to the buffet meal. Blood glucose was less on the control day ($P < 0.05$) than before the meal ($t = 60$ min), with no differences between either leucine dose and the control.

Insulin

Response to leucine. At $t = 0$ min, the change from baseline was significantly greater with leucine-10g and leucine-5g than with the control ($P < 0.01$ for both) (Figure 2B).

Response to the mixed-nutrient drink. Plasma insulin increased on all study days, and there was an effect of treatment on the insulin AUC$_{0-60min}$ ($P < 0.001$); compared with the control, leucine-10g, but not leucine-5g, increased the insulin AUC$_{0-60min}$ ($P < 0.001$).
FIGURE 2  Mean ± SEM blood glucose (A and E) and plasma insulin (B and F), C-peptide (C and G), and glucagon (D and H) concentrations at baseline (t = −20 min) and in response to the oral ingestion of a mixed-nutrient drink (at t = 0 min) after the IG administration (at t = −18 min) of leucine (A–D) or isoleucine (E–H) and after the consumption of a standardized ad libitum buffet-style meal (t = 90 min). (A) *The AUCsub0–60min of leucine-10g differed significantly from that of the control (P < 0.05); †for the control, blood glucose was lower at t = 90 min than at t = 60 min (P < 0.05). (B) †The change from baseline at t = 0 min was greater after both leucine loads than after the control (P < 0.01); *the AUCsub0–60min of leucine-10g differed significantly from that of the control (P < 0.001). (C) †The change from baseline at t = 0 min was greater after both leucine loads than after the control (P < 0.01); *the AUCsub0–60min of leucine-10g differed significantly from that of the control (P < 0.001). (D) †The change from baseline at t = 0 min was greater after leucine-5g than after the control (P < 0.001); ‡for the control, glucagon significantly increased at t = 90 min compared with t = 60 min (P < 0.05). (E) *The AUCsub0–60min and peak blood glucose were significantly different from control values after isoleucine-10g (P < 0.01 for both); ‡at t = 90 min, isoleucine-10g was significantly different from the control (P < 0.05). (F) †At t = 90 min, insulin significantly increased after the control and isoleucine-10g (P < 0.05 for both) compared with at t = 60 min. (G) †At t = 90 min, C-peptide significantly increased after the control and isoleucine-10g (P < 0.05 for both) compared with at t = 60 min. (H) †At t = 90 min, glucagon tended to increase after the control (P = 0.055) and isoleucine-5g (P < 0.05) compared with at t = 60 min. Data were analyzed with the use of a 1-factor repeated-measures ANOVA. Post hoc comparisons with Bonferroni correction were conducted when the ANOVA revealed significant effects. Paired t tests were used to compare blood glucose and plasma hormone concentrations before (t = 60 min) and after (t = 90 min) the buffet meal. n = 11 in the leucine study, and n = 12 in the isoleucine study. AUCsub0–60min, AUC calculated from t = 0–60 min; IG, intragastric; isoleucine-5g, intragastric infusion of 5 g isoleucine; isoleucine-10g, intragastric infusion of 10 g isoleucine; leucine-5g, intragastric infusion of 5 g leucine; leucine-10g, intragastric infusion of 10 g leucine.
Response to the buffet meal. Plasma insulin did not differ significantly from values before the meal, and there were no differences between either leucine dose and the control.

Insulin-secretory response. There was a trend for an effect of treatment ($P = 0.06$); mean values for leucine-5g and leucine-10g tended to be higher than for the control (Table 1).

C-peptide

Response to leucine. At $t = 0$ min, the change from baseline was significantly greater with leucine-10g and leucine-5g than with the control ($P < 0.01$ for both) (Figure 2C).

Response to the mixed-nutrient drink. Plasma C-peptide increased on all study days, and there was an effect of treatment on the C-peptide AUC$_{0-60\text{min}}$ ($P < 0.01$); compared with the control leucine-10g, but not leucine-5g, increased the C-peptide AUC$_{0-60\text{min}}$ ($P < 0.01$).

Response to the buffet meal. Plasma C-peptide did not differ significantly from values before the meal, and there were no differences between either leucine dose and the control.

Glucagon

Response to leucine. At $t = 0$ min, the change from baseline was significantly greater with leucine-5g, but not with leucine-10g, than with the control ($P < 0.001$) (Figure 2D).

Response to the mixed-nutrient drink. Glucagon decreased slightly on all study days, but there was no effect of treatment on the glucagon AUC$_{0-60\text{min}}$.

Response to the buffet meal. Plasma glucagon increased significantly only after the control ($P = 0.05$), but not after leucine doses, and did not differ between either leucine dose and the control.

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**FIGURE 3** Mean ± SEM plasma GLP-1 (A and D), GIP (B and E), and CCK (C) concentrations at baseline ($t = -20$ min) and in response to the oral ingestion of a mixed-nutrient drink ($t = 0$ min) after the intragastric administration ($t = -18$ min) of leucine (A–C) or isoleucine (D and E) and after the consumption of a standardized ad libitum buffet-style meal ($t = 90$ min). (A) $^1$At $t = 90$ min, GLP-1 increased after leucine-5g and the control, but not after leucine-10g, compared with at $t = 60$ min ($P < 0.001$ for both). (B) $^*$The change from baseline at $t = 0$ min was greater after leucine-5g and leucine-10g than after the control ($P < 0.05$ for both). (C) $^*The change from baseline at $t = 0$ min was greater after leucine-5g, but not after leucine-10g, than after the control ($P < 0.05$). (D) $^1$At $t = 90$ min, GLP-1 increased on all study days compared with at $t = 60$ min ($P < 0.001$ for all). (E) There were no differences in GIP concentrations between either isoleucine dose and the control. Data were analyzed with the use of a 1-factor repeated-measures ANOVA. Post hoc comparisons with Bonferroni correction were conducted when the ANOVA revealed significant effects. Paired $t$ tests were used to compare plasma hormone concentrations before ($t = 60$ min) and after ($t = 90$ min) the buffet meal. $n = 11$ in the leucine study (except for CCK, for which $n = 9$), and $n = 12$ in the isoleucine study. CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IG, intragastric; leucine-5g, intragastric infusion of 5 g leucine; leucine-10g, intragastric infusion of 10 g leucine.
GLP-1

Response to leucine. At \( t = 0 \) min, the change from baseline did not differ significantly between treatments and the control (Figure 3A).

Response to the mixed-nutrient drink. GLP-1 increased on all study days with a peak at \( t = 15 \) min, and there was no effect of treatment on the GLP-1 AUC\(_{0-60\text{min}}\).

Response to the buffet meal. GLP-1 increased significantly with the control and leucine-5g, but not with leucine-10g (\( P < 0.001 \) for both), and there were no differences between either leucine dose and the control.

GIP

Response to leucine. At \( t = 0 \) min, the change from baseline was significantly greater with leucine than with the control (\( P < 0.05 \) for both) (Figure 3B).

Response to the mixed-nutrient drink. GIP increased promptly on all study days. There was no effect of treatment on the GIP AUC\(_{0-60\text{min}}\).

Response to the buffet meal. Plasma GIP concentrations did not differ from values before the meal, and there were no differences between either leucine dose and the control.

Cholecystokinin

Response to leucine. Cholecystokinin appeared to increase on all study days although, at \( t = 0 \) min, the change from baseline was only significantly greater with leucine-5g, but not with leucine-10g, than with the control (\( P < 0.05 \)) (Figure 3C).

Response to the mixed-nutrient drink. Plasma cholecystokinin increased slightly, but there was no effect of treatment on the cholecystokinin AUC\(_{0-60\text{min}}\).

Response to the buffet meal. Plasma cholecystokinin concentrations did not differ from values before the meal, and there were no differences between either leucine dose and the control.

Gastric emptying

Leucine did not affect the gastric emptying of the mixed-nutrient drink (Figure 4A).

Appetite perceptions, gastrointestinal symptoms, and meal size

Leucine did not affect hunger, fullness, the desire to eat, prospective food consumption, nausea or bloating (data not shown), or energy intake (Table 1). There was an effect of treatment on the amount eaten (\( P < 0.05 \)), but post hoc comparisons did not reveal any significant differences.

Relations between plasma hormones, gastric emptying, and blood glucose

There was a strong positive correlation between the plasma insulin and plasma C-peptide AUC\(_{0-60\text{min}}\) (\( r = 0.85, P < 0.001 \)). The magnitude of the change from control in blood glucose after either leucine dose did not correlate with the magnitude of the change in gastric emptying after the respective dose (leucine-5g: \( r = 0.529, P = 0.094 \); leucine-10g: \( r = 0.206, P = 0.543 \)).

Study B

Blood glucose and plasma hormone concentrations

There were no differences in baseline (\( t = -20 \) min) values of blood glucose or plasma hormone concentrations between study days (Figures 2 and 3).

Blood glucose

Response to isoleucine. At \( t = 0 \) min, there was a trend for an effect of treatment on the change from baseline (\( P = 0.072 \)) (Figure 2E).

Response to the mixed-nutrient drink. Blood glucose modestly increased on all study days, and there were effects of treatment on the blood glucose AUC\(_{0-60\text{min}}\) (\( P < 0.05 \)) and peak blood glucose concentration (\( P < 0.01 \)) (Table 1); compared with control, the intragastric infusion of 10 g isoleucine (isoleucine-10g), but not the intragastric infusion of 5 g isoleucine (isoleucine-5g), decreased the blood glucose AUC\(_{0-60\text{min}}\) and peak blood glucose (\( P < 0.01 \) for both).

Response to the buffet meal. Blood glucose did not differ from values before the meal but remained lower after isoleucine-10g, but not after isoleucine-5g, than after the control (\( P < 0.05 \)).

Insulin

Response to isoleucine. At \( t = 0 \) min, the change from baseline did not differ between treatments and the control (Figure 2F).
Response to the mixed-nutrient drink. Plasma insulin increased on all study days with no effect of treatment on the insulin AUC\textsubscript{0–60min}.

Response to the buffet meal. Plasma insulin increased after the control and isoleucine-10g (P < 0.05 for both) with a trend for an increase after isoleucine-5g (P = 0.071) but with no differences between either isoleucine dose and the control.

Insulin-secretory response. There was no significant effect of treatment (Table 1).

C-peptide

Response to isoleucine. At t = 0 min, the change from baseline did not differ between treatments and the control (Figure 2G).

Response to the mixed-nutrient drink. Plasma C-peptide increased on all study days with no effect of treatment on the C-peptide AUC\textsubscript{0–60min}.

Response to the buffet meal. Plasma C-peptide increased after the control and isoleucine-10g (P < 0.05 for both) with a trend for an increase after isoleucine-5g (P = 0.084) but with no differences between either isoleucine dose and the control.

Glucagon

Response to isoleucine. At t = 0 min, the change from baseline did not differ between treatments and the control (Figure 2H).

Response to the mixed-nutrient drink. Plasma glucagon did not change, and there was no effect of treatment on the glucagon AUC\textsubscript{0–60min}.

Response to the buffet meal. Glucagon increased slightly after the control (P = 0.055) and isoleucine-5g (P < 0.05), but not after isoleucine-10g, with no difference between either isoleucine dose and the control.

GLP-1

Response to isoleucine. At t = 0 min, there was a trend for an effect of treatment on the change from baseline at t = 0 min (P = 0.072) (Figure 3D).

Response to the mixed-nutrient drink. Plasma GLP-1 increased, with a peak at \( t = 15 \) min, and with no effect of treatment on GLP-1 AUC\textsubscript{0–60min}.

Response to the buffet meal. Plasma GLP-1 increased substantially on all study days (P < 0.001 for all), with no differences between either isoleucine dose and the control.

GIP

Response to isoleucine. At t = 0 min, the change from baseline did not differ between treatments and the control (Figure 3E).

Response to the mixed-nutrient drink. GIP increased promptly, but there was no effect of treatment on the GIP AUC\textsubscript{0–60min}.

Response to the buffet meal. Plasma GIP did not differ from values before the meal, and there were no differences between either isoleucine dose and the control.

Gastric emptying

There was an effect of treatment on gastric emptying (P < 0.05); compared with the control, isoleucine-10g, but not isoleucine-5g, slowed the gastric emptying of the mixed-nutrient drink (P < 0.05) (Figure 4B).

Appetite perceptions, gastrointestinal symptoms, and meal size

Isoleucine did not affect hunger, fullness, the desire to eat, prospective food consumption, or nausea or bloating (data not shown), and there was no effect of treatment on energy intake or the amount eaten (Table 1).

Relations between plasma hormones, gastric emptying, and blood glucose

There was a positive correlation between the plasma insulin AUC\textsubscript{0–60min} and plasma C-peptide (r = 0.93, P < 0.001). There was a trend for a correlation between the magnitude of the change from control in blood glucose after isoleucine-5g and the magnitude of the change in gastric emptying after isoleucine-5g (r = −0.533, P = 0.074) but not after isoleucine-10g (r = −0.020, P = 0.951).

DISCUSSION

Our study shows that leucine and isoleucine, when administered intragastrically, acutely lower blood glucose in doses of 10 g in response to a drink of mixed macronutrients as opposed to pure glucose (13, 14) in healthy subjects. These effects appear to be mediated by different mechanisms. Leucine increased insulin and C-peptide and had no effect on gastric emptying, whereas isoleucine had no effect on insulin, but at the higher load, isoleucine slowed gastric emptying. Neither amino acid...
affected energy intake, and leucine had no effect on cholecystokinin.

Previous studies have reported marked blood glucose–lowering effects of both leucine and isoleucine in response to a glucose drink in healthy subjects (13, 14). In the current study, both amino acids attenuated the blood glucose response to a drink with mixed macronutrients, which more closely represented the macronutrient composition of a normal meal by ~1.1 mmol/L. This effect occurred in a cohort of healthy subjects with good blood glucose control after only a modest increase in blood glucose in the control condition. Hence, these amino acids appear to be potent in lowering blood glucose, and it is likely that these effects may be even more pronounced in patients with type 2 diabetes who have elevated blood glucose concentrations, which, hence, warrants further evaluation in this patient group. In support of this possibility, the consumption of a preparation of casein hydrolysate with added leucine after each main meal had stronger blood glucose–lowering effects in patients with type 2 diabetes than in healthy control subjects over a 24-h period (25).

We measured the gastric emptying of the nutrient drink and, thus, were able to evaluate whether the blood glucose–lowering effects of leucine and isoleucine were related to the slowing of gastric emptying. Leucine did not appear to slow the gastric emptying of the drink, and the changes in blood glucose were not correlated with the changes in gastric emptying. In contrast, the 10-g isoleucine load slowed the gastric emptying of the drink and reduced blood glucose, but the changes in blood glucose and gastric emptying were not correlated. Thus, our data suggest that leucine and isoleucine affect gastric emptying differently, with the leucine data being in line with our recent finding of a lack of an effect of intraduodenal leucine on pyloric pressures (16); but overall, gastric emptying only makes a small, if any, contribution to the blood glucose–lowering effects of leucine and isoleucine, and the 2 amino acids probably do not underlie the effects of whey to slow gastric emptying (2).

GLP-1 and GIP reduce blood glucose by slowing gastric emptying and stimulating insulin release (26). Moreover, a whey protein preload before the consumption of a carbohydrate meal enhanced plasma GLP-1 and, to an extent, GIP, which was associated with greater insulin and a markedly reduced blood glucose response to the meal (2). In contrast, in the current study, neither of the 2 amino acids enhanced GLP-1 or GIP in response to the mixed-nutrient drink. However, because blood glucose concentrations did not rise >8 mmol/L, an insulinotropic effect of GLP-1 and GIP would not have been expected (27). Thus, the enhanced insulin release in response to leucine was independent of an incretin action, and our findings are in line with a direct effect of leucine to stimulate insulin release from β cells as has been reported previously (28). Moreover, leucine and isoleucine do not appear to mediate the effect of whey on incretin release, at least when blood glucose concentrations remain <8 mmol/L.

Our data also establish that the insulin-enhancing effect of leucine, which has been described in relation to pure glucose ingestion (14), is maintained after the ingestion of mixed nutrients. With 10 g leucine, plasma insulin was increased relative to blood glucose concentrations, compared with the control condition, which indicates that leucine enhances insulin release, which may represent the major blood glucose–lowering mechanism of leucine. In contrast to leucine, but in line with the findings in response to pure glucose (13), isoleucine ingestion did not increase insulin in response to the mixed-nutrient drink. Hence, the effect of isoleucine on blood glucose appears to be independent of insulin. Mechanisms by which isoleucine may affect blood glucose include enhanced glucose uptake into muscle by the enhanced translocation of the glucose transporter type 4 to the muscle cell membrane (29) and decreased gluconeogenesis in the liver (30, 31). Although there was no correlation, it is likely that a slowing of gastric emptying after isoleucine-10g might have contributed perhaps not to the early rise in blood glucose but to the later stages of blood glucose control. Taken together, leucine and isoleucine appear to lower blood glucose via different mechanisms despite their structural similarity. These mechanisms may include specific metabolites of either amino acid or perhaps some stereospecific proteins (i.e., transporters or enzymes) that only recognize either amino acid.

Neither leucine nor isoleucine affected energy intake in our studies. Although the effects of isoleucine have essentially not been investigated, there is evidence, albeit inconsistent data (14, 32, 33), that leucine reduces energy intake. Experiments in rodents have suggested a role for central effects of leucine because energy intake was not affected by oral supplementation (32, 33) but was reduced after cerebroventricular administration (15, 34), which suggests that high plasma leucine concentrations may be required to raise central concentrations across the blood-brain barrier to an amount that is high enough to trigger effects on energy homeostasis. During the intraduodenal infusion of leucine, which reduced subsequent energy intake in our recent study (16), plasma leucine concentrations were substantially increased beyond postprandial concentrations. When ingested with glucose, plasma leucine and isoleucine concentrations did not reach the same plasma concentrations as when ingested alone (13, 14); thus, the presence of other nutrients may affect amino acid absorption. Hence, the mixed-nutrient drink in our current study may have prevented either amino acid from immediately being absorbed and thereby reaching plasma concentrations that are required to induce acute central anorexic effects or, in the small intestine, to stimulate cholecystokinin release. In addition, the time interval between amino acid ingestion and energy-intake assessment may have been too long; a shorter interval (~30 min) has been shown to be associated with a stronger suppression of food intake (35). Moreover, because of the relatively long time interval, maximal plasma concentrations may have been reached well before energy intake was assessed. Thus, the assessment of energy intake closer to amino acid ingestion and, therefore, to the peak amino acid concentration is warranted.

Some limitations of our study should be noted. We studied only healthy subjects during euglycemia with a modest glycemic response to the mixed-nutrient drink, and the high dose we used (10 g) was higher than the usual intake of the amino acids with a meal (16). However, this study was designed to be a proof-of-principle trial, and lower doses may be effective in patients with impaired glucose tolerance or type 2 diabetes. For regular oral ingestion, the unpleasant taste of the amino acids would need to be concealed to avoid taste aversions. The calories, as well as the additional nutrients, that were ingested with the mixed-nutrient drink may have masked energy-intake–lowering effects of leucine or isoleucine. We were unable to assess plasma amino acid concentrations, which warrant investigation in future studies. Finally, both studies were not formally compared with each
other to evaluate the relative magnitude of effect because of the lack of statistical power.

In conclusion, we have established that both leucine and isoleucine decrease blood glucose in response to a mixed-nutrient drink. Both amino acids appear to lower blood glucose by different mechanisms either dependently (leucine) or independently (isoleucine) of insulin. The incretin hormones GIP and GLP-1 and gastric emptying appear to play no, or only a minor, role in the blood glucose regulation by both amino acids although gastric emptying is affected differentially. Whether either amino acid can affect energy intake after oral ingestion warrants further investigation with the use of a different study design. Additional studies are warranted to evaluate the blood glucose-lowering effects of leucine and isoleucine in type 2 diabetic patients and whether the concept of amino acid ingestion before a meal is suitable for the long-term improvement of blood glucose control.

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The authors’ responsibilities were as follows—SSU: analyzed the data and conducted the statistical analysis; SSU, PCEF, and GS: conducted the hormone assays; and Biljana Kovacevic, Medical Diagnostics Australasia, for assistance with the breath-sample analyses.

REFERENCES


35. Little TJ, Luscombe-Marsh ND, Gentilecore D, Brook EJ, Feinle-Bisset C. Effects of varying the inter-meal interval on relationships between antral area, gut hormones and energy intake following a nutrient drink in healthy lean humans. Physiol Behav 2014;135:34–43.