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Leucine, when ingested with glucose, synergistically stimulates insulin secretion and lowers blood glucose

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Abstract

Our laboratory is interested in the metabolic effects of ingested proteins. As part of this research, we currently are investigating the metabolic effects of ingested individual amino acids. The objective of the current study was to determine whether leucine stimulates insulin and/or glucagon secretion and whether, when it is ingested with glucose, it modifies the glucose, insulin, or glucagon response. Thirteen healthy subjects (6 men and 7 women) were studied on 4 different occasions. Subjects were admitted to the special diagnostic and treatment unit after a 12-hour fast. They received test meals at 8:00 AM. On the first occasion, they received water only. Thereafter, they received 25 g glucose or 1 mmol/kg lean body mass leucine or 1 mmol/kg lean body mass leucine plus 25 g glucose in random order. Serum leucine, glucose, insulin, glucagon, and α-amino nitrogen concentrations were measured at various times during a 2.5-hour period after ingestion of the test meal. The amount of leucine provided was equivalent to that present in a high-protein meal, that is, that approximately present in a 350-g steak. After leucine ingestion, the leucine concentration increased 7-fold; and the α-amino nitrogen concentration increased by 16%. Ingested leucine did not affect the serum glucose concentration. When leucine was ingested with glucose, it reduced the 2.5-hour glucose area response by 50%. Leucine, when ingested alone, increased the serum insulin area response modestly. However, it increased the insulin area response to glucose by an additional 66%; that is, it almost doubled the response. Ingested leucine stimulated an increase in glucagon. Ingested glucose decreased it. When ingested together, the net effect was essentially no change in glucagon area. In summary, leucine at a dose equivalent to that present in a high-protein meal, had little effect on serum glucose or insulin concentrations but did increase the glucagon concentration. When leucine was ingested with glucose, it attenuated the serum glucose response and strongly stimulated additional insulin secretion. Leucine also attenuated the decrease in glucagon expected when glucose alone is ingested. The data suggest that a rise in glucose concentration is necessary for leucine to stimulate significant insulin secretion. This in turn reduces the glucose response to ingested glucose.
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1. Introduction

We have previously reported that when people with untreated type 2 diabetes mellitus (DM) ingest 25 g of protein with 50 g of glucose in a single meal, there is an increase in insulin area response and a smaller plasma glucose response when compared with ingestion of 50 g of glucose without protein [1].

Seven different protein sources were used. All resulted in an increased insulin response and a reduced glucose response. However, the magnitude of the response varied greatly. The latter suggested that a difference in the amino acid composition of the proteins may be responsible for this variation.

Therefore, we have begun a systematic evaluation of the insulin and glucose response to individual amino acids ingested with and without glucose. Because protein ingestion also stimulates an increase in glucagon concentration, the glucagon response to individual amino acids is being determined. Young nondiabetic subjects are being studied before doing similar studies in people with type 2 DM. In the present study, the effect of leucine on the circulating insulin, glucose, and glucagon concentrations when ingested with or without glucose is reported.

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2. Methods

Thirteen healthy subjects (6 men and 7 women) were studied. All subjects gave informed consent before participating in the study, which was approved by the Minneapolis Department of Veteran Affairs Medical Center and the University of Minnesota Committees on Human Subjects. Volunteers do not have DM according to the National Diabetes Data Group Criteria. The mean age of the subjects was 24 years (range, 18-33). The mean body mass index was 24 (range, 21-27). The average body weight was 70.9 kg (range, 54-102), with mean lean body mass of 51 kg (range, 40-75) and mean percentage of lean body mass of 75.6% (range, 68%-86%). Lean body mass was determined using a portable body impedance analyzer (RJL Systems, Clinton Township, MI). Thyroid, liver, and kidney function and lipid profiles were within the respective reference ranges.

Subjects were studied in a special diagnostic and treatment unit, which is similar to a clinical research center. Subjects were asked to fast for 12 hours before the study. The next morning, an indwelling catheter was placed into an antecubital or forearm vein and kept patent with intravenous saline. Baseline blood samples were obtained at 7:30, 7:40, and 7:50 AM. At 8:00 AM, subjects ingested only water on the first visit. At subsequent visits, one of the 3 test meals was ingested in random order. The meals consisted of (1) 1 mmol leucine per kilogram lean body mass, mean 7 g (53 mmol) with a range of 5 to 9 g (38-69 mmol); (2) 1 mmol leucine per kilogram lean body mass plus 25 g glucose; and (3) 25 g of glucose alone. Glucose was given as Glutol (Paddock Laboratories, Minneapolis, MN), a D-glucose solution (25 g/45 mL). Leucine was given as L-leucine (Ajinomoto, Raleigh, NC). All subjects were given all 4 test meals. Blood was obtained every 10 minutes for 120 minutes and then at 150 minutes.

After the study period, subjects were asked to complete a satiety index. The satiety index consisted of the following 4 questions: (1) How strong is your desire to eat? (2) How hungry do you feel? (3) How full do you feel? (4) How much food do you think you can eat? In addition, they were asked how pleasant they found the test substances. Answers were quantified on a linear scale of 1 to 100, with 1 being the least and 100 being the most. Subjects then were served a regular mixed meal with more food energy than the subjects could eat, and the amount of food energy (in kilocalories) ingested was calculated. Data from the questionnaires were only available in 10 of 13 subjects. Generally, the 4-day study was conducted over a 2-week period.

Serum glucose concentrations were determined by a hexokinase method using an Abbott Architect analyzer (Abbott Laboratories, Abbott Park, IL). Serum immunoreactive insulin was measured using an automated chemiluminescent assay on DPC's IMMULITE analyzer (Diagnostic Products, Los Angeles, CA). Glucagon was determined by radioimmunoassay using kits purchased from Linco Research (which was subsequently purchased by Millipore, Billerica, MA). α-Amino nitrogen (AAN) was determined by an *O*-phthaldialdehyde dye binding method (Gusmer, Waupaca, WI). Individual amino acid concentrations were measured by high-performance liquid chromatography using precolumn online derivitization with *O*-phthalaldehyde and 3-mercaptopropionic acid and 9-fluorenylmethylchloroformate followed by UV detection. Caloric value of food consumed was calculated using the computer software Nutritionist Pro (Hearst purchased by Axxya Systems, Stafford, TX) or the Veterans Affairs' VISTA energy analysis program. The net integrated 150-minute area responses were calculated using a computer program based on the trapezoid rule.

Statistics were determined using Student *t* test for paired variates with the StatView 512+ program (Abacus Concepts, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA). A *P* value less than .05 was criterion for significance. Data are presented as means ± SEMs.

3. Results

The mean fasting glucose concentration was 4.7 ± 0.1 mmol/L (84± 2.3 mg/dL) (Fig. 1A). After ingestion of glucose, the fasting glucose concentration increased to 7.3

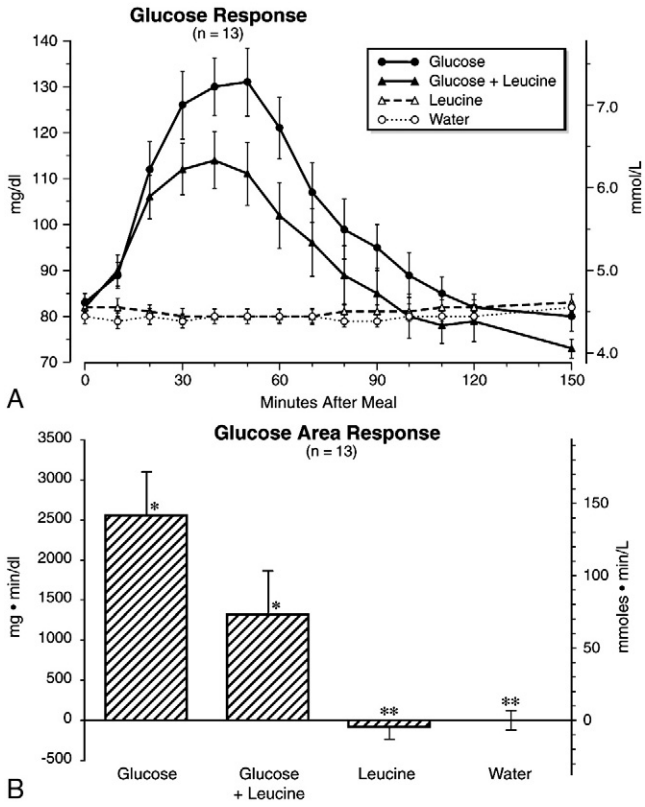

Fig. 1. (A) Mean (±SEM) time course of serum glucose concentrations and (B) net integrated areas under the curve after ingestion of test meals. * *P* = .019, ** *P* = .614.

mmol/L (131 mg/dL) at 50 minutes. It then gradually returned to the fasting concentration after 120 minutes. When the subjects ingested glucose plus leucine, the maximal glucose concentration was considerably less (6.3 mmol/L [114 mg/dL]). In addition, the glucose concentration returned to the fasting concentration by 100 minutes. Leucine, ingested independently, had little effect on the serum glucose concentration. The glucose concentration varied little after ingestion of water.

The glucose area response (Fig. 1B) was markedly attenuated when leucine was ingested with glucose (~50% less, $P = .019$) compared with glucose ingestion alone. The glucose area response to leucine alone was slightly less than that to water alone. This did not reach statistical significance.

The mean fasting serum insulin concentration was 32 ± 3 pmol/L (5.4 ± 0.5 μU/mL) (Fig. 2A). After ingestion of glucose, the mean serum insulin concentration reached a maximum of 186 pmol/L (31 μU/mL) at 50 minutes. It then gradually decreased and reached a fasting concentration at 150 minutes. After ingestion of glucose plus leucine, the mean serum insulin concentration reached a maximum that was 72% greater than that after the ingestion of glucose alone (318 pmol/L [53 μU/mL]); this occurred 10 minutes earlier. It then rapidly returned to the fasting level by 150 minutes. Ingestion of leucine alone resulted in only a modest increase in insulin concentration.

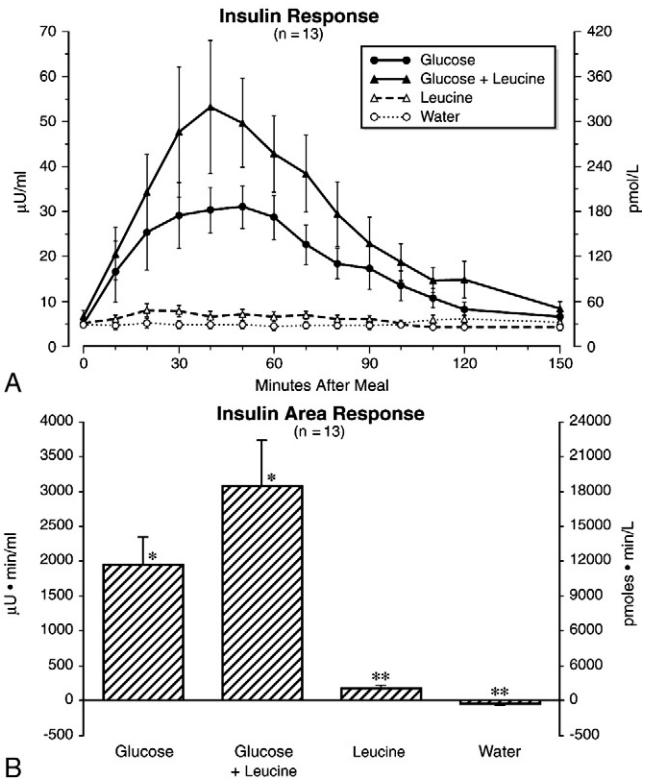

Fig. 2. (A) Mean (±SEM) time course of serum insulin concentrations and (B) net integrated areas under the curve after ingestion of test meals. *P = .003, **P = .0001.

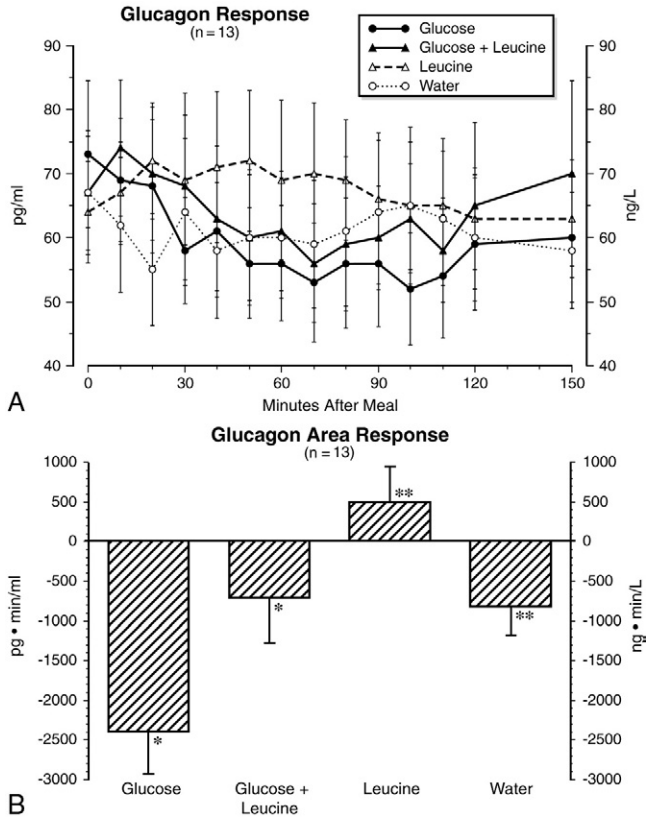

Fig. 3. (A) Mean (±SEM) time course of plasma glucagon concentrations and (B) net integrated areas under the curve after ingestion of test meals. *P = .04, ** P = .006.

The insulin area response (Fig. 2B) was 66% greater after subjects ingested glucose plus leucine compared with when glucose was ingested alone ($P = .003$). Leucine ingestion alone resulted in a modest but significantly higher insulin area response than after water ingestion alone ($P = .0001$).

The mean fasting glucagon concentration was 68 ± 5 pg/mL (Fig. 3A). After subjects ingested glucose alone, the glucagon concentration decreased as expected and remained below the initial fasting value for the duration of the study. After the ingestion of glucose plus leucine, the glucagon concentration decreased in a similar fashion; but the nadir reached was less (56 pg/mL). It then increased to the fasting concentration at 150 minutes. Ingestion of leucine alone resulted in an increase in mean glucagon concentration, and it remained elevated for the duration of the study.

The glucagon area response (Fig. 3B) was positive with leucine ingestion. It was negative after ingestion of the other study meals. Despite the wide variance, the glucagon area after ingestion of leucine with glucose was significantly less negative when compared with the area after glucose ingestion alone ($P = .04$). The glucagon area after leucine ingestion was significantly higher when compared with the area after water ingestion ($P = .006$).

The mean fasting AAN concentration was 3.7 ± 0.1 mg/dL (Fig. 4A). The AAN concentration decreased after glucose

ingestion. It increased after ingestion of leucine and glucose; but the response was reduced compared with when only leucine was ingested, as expected. There was no change in the AAN concentration with water ingestion.

The AAN area response (Fig. 4B) was negative after glucose ingestion and positive after ingestion of leucine with or without glucose. When the glucose was ingested with leucine, the AAN area response was only 38% of the area measured when only leucine was ingested. However, the difference was not statistically significant.

The mean fasting leucine concentration was 148 μmol/L (Fig. 5A). It increased 7-fold after leucine ingestion. The mean increase was reduced by 32% when glucose was ingested with leucine. The leucine concentration remained elevated at the end of the study (150 minutes) when leucine was ingested with or without glucose.

The leucine area response (Fig. 5B) was decreased by 22% after leucine was ingested with glucose (P = .03). Glucose ingestion resulted in a significant decrease of the leucine area when compared with water ingestion (P = .0001).

The subjects reported a greater degree of fullness after ingestion of leucine (P = .08). However, the food energy consumption was comparable after all test meals. Subjects ingested approximately a mean of 626 kcal (range, 590-700) of meals served.

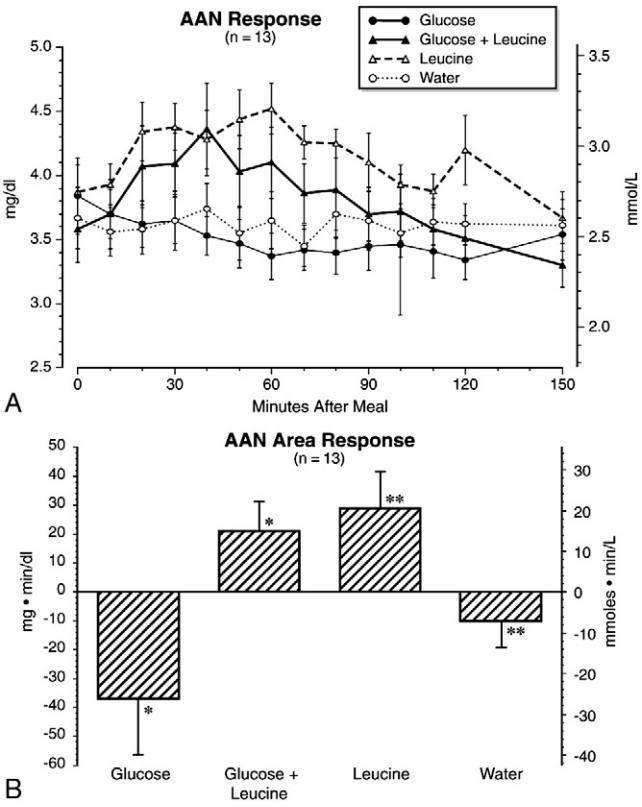

Fig. 4. (A) Mean (±SEM) time course of plasma AAN concentrations and (B) net integrated areas under the curve after test meal ingestion.* P = .006, ** P = .037.

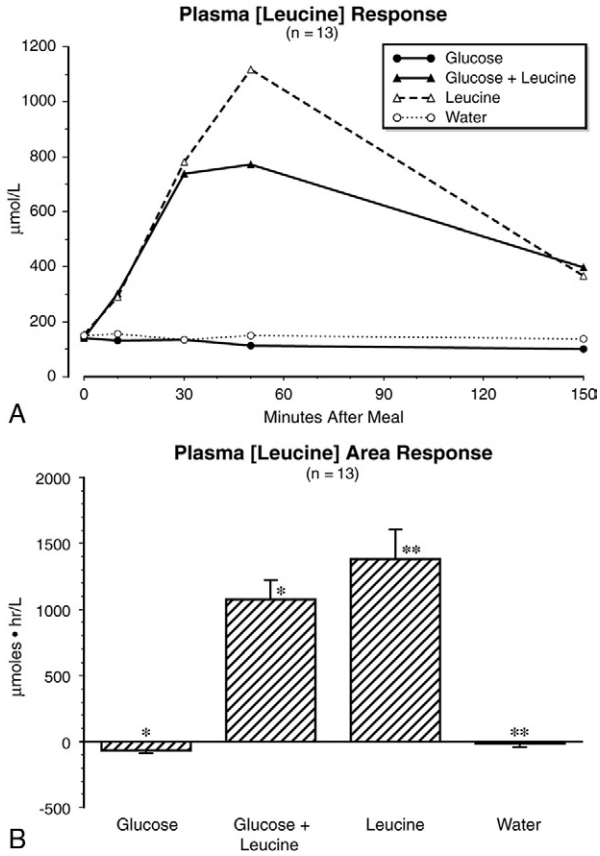

Fig. 5. (A) Mean (±SEM) time course of plasma leucine concentrations and (B) net integrated areas under the curve after test meals. * P = .0001, **P = .0001.

The subjects reported that the leucine taste was unpleasant. Even when given with glucose, the taste was significantly less pleasant compared with glucose (P = .03). The leucine taste was variously described as “bitter” or “sour” and as having an unpleasant “gritty” texture. The subjects did not experience any gastrointestinal or constitutional symptoms after ingesting leucine.

4. Discussion

Leucine is a hydrophobic, branched amino acid that on a molar basis constitutes approximately 8% of the amino acids present in beef protein [2]. As with other amino acids, it is absorbed into the portal vein and is carried to the liver. However, it largely passes through the liver into the peripheral circulation. It then is used for protein synthesis or is taken up and oxidized in skeletal muscle. It is the only amino acid that cannot contribute to gluconeogenesis. Leucine also functions as a nutrient-signaling molecule.

A rise in circulating amino acids after digestion of a protein meal stimulates protein synthesis particularly in skeletal muscle [3]. The mechanism is not well understood but probably is mediated by a rise in dietary indispensable

amino acids. All dietary required amino acids individually are capable of stimulating protein synthesis including leucine [4]. The concentration of these molecules intracellularly regulates translation of messenger RNA. This is mediated through an effect on translation initiation activity, and leucine appears to play a particularly important role [5]. Fujita et al [6] showed that a leucine-enriched essential amino acid–carbohydrate mixture increased protein synthesis in humans both by increasing translation initiation and by promoting translation elongation.

The effect of leucine on protein synthesis is via insulin-independent mechanisms, although basal insulin levels are important for maximal effect [7,8]. How leucine brings about its metabolic effects has not been fully elucidated. Several kinases have been shown to be activated postfeeding and after leucine administration [9,10].

It also has been known for many years that leucine is an insulin secretagogue when given intravenously or orally in very large amounts to adults and children [11-13]. An exaggerated insulin response in obese hyperinsulinemic children also has been reported [14]. It also can result in symptomatically significant hypoglycemia in sensitive individuals [13,15].

The hypoglycemic response appears to be dose dependent [13]. In normal-weight healthy adults and children, ingestion of amounts in the range of 150 to 200 mg/kg body weight, which represents an amount that is modestly greater than might enter the circulation after ingestion of a very large protein meal, has resulted in little change in insulin or glucose concentration [11,16]. If changes were present, they were very modest. Giving a bolus of intravenous infusion of leucine (15 mg/kg body mass) that raised the leucine concentration 10-fold either did not raise the insulin concentration or resulted in a very small increase in healthy adults [17].

Thus, in the presence of a normal fasting glucose concentration, a direct stimulatory effect of leucine on insulin secretion appears to be a pharmacologic effect and not a physiologic one in most but not all people. The sensitivity to administered leucine varies widely. Its effect also can be potentiated by the use of a sulfonylurea type of insulin secretagogue [18].

In the present study, the leucine dose ingested was 1 mmol/kg lean body mass (mean, 7 ± 0.68 g). This amount represents a high physiologic dose. That is, it is equivalent to the amount of leucine present in a 350-g steak. At this dose, it did not decrease the glucose concentration. Thus, these results were similar to those reported previously when similar high physiologic amounts were ingested. Leucine did increase the insulin concentration significantly, but the area response was small and considerably less (9%) than that when 25 g glucose was ingested by the same subjects. On a molar basis, the insulin area response to leucine ingestion was approximately 13% of the response seen with glucose ingestion.

Nevertheless, when leucine was ingested with glucose, there was a striking attenuation in glucose response compared with that when the same amount of glucose was ingested without leucine. This was associated with an increase in insulin area response that was 66% greater than that when glucose was ingested without leucine.

Our data do not provide mechanistic information that could explain this phenomenon. However, a synergistic effect of ingested glucose with leucine on insulin secretion or a threshold effect of glucose concentration on a leucine-stimulated insulin secretion is a possible explanation. This could be a direct effect of leucine or could be mediated, at least in part, by a leucine-stimulated release of a gut incretin hormone.

In this regard, intravenous administration of 30 g leucine with 30 g glucose has been reported to synergistically increase the plasma insulin concentration and reduce the glucose rise resulting from the infused glucose alone [19]. In the present study, a much lower leucine dose was ingested; but the increase in the insulin observed was greater compared with the increase seen in the study by Floyd et al [19]. The attenuation in glucose rise was much smaller. Interestingly, in the same study by Floyd et al [19], an infusion of 30 g histidine, which itself did not stimulate an increase in insulin, still synergistically stimulated an increase in insulin when administered with intravenous glucose. Arginine (30 g) also synergistically stimulated an insulin rise when infused with glucose, indicating that synergism with glucose is not a characteristic unique to leucine, at least when infused in pharmacologic amounts.

It should be noted that we were unable to demonstrate a stimulatory effect of arginine on serum insulin concentration in normal subjects at a dose of 1 mmol/kg lean body mass when ingested alone or with glucose using the same protocol as used here [20]. Thus, although arginine is considered to be the most potent of the amino acids in stimulating insulin secretion when given intravenously in large amounts [12], it is not likely to be a physiologically important insulin secretagogue in humans; nor does it synergize with glucose in stimulating a rise in insulin [20]. In addition, our preliminary data indicate that ingested histidine does not stimulate insulin secretion or lower the glucose concentration (unpublished data).

The present data indicate that orally administrated leucine increases the glucagon concentration [21], although intravenous leucine even when administered in very large amounts (30 g) was reported to have no effect on the plasma glucagon concentration. This suggests that the increase in glucagon was due to stimulation of a gut hormone. We are not aware of the glucagon response to ingested leucine being reported previously.

In summary, ingestion of leucine in an amount likely to be present in a large protein–containing meal weakly stimulates a rise in peripheral insulin concentration, increases the glucagon concentration, but results in no change in glucose concentration. It strikingly reduces the rise in glucose concentration when ingested with glucose. This is likely to be due, at least in part, to a strong synergistic effect of leucine with glucose on insulin

secretion. Whether a similar effect will be observed with smaller doses of leucine remains to be determined.

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